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13. ABSTRACT *(Maximum 200 Words)*

We have developed a series of allele-specific PCR amplification procedures that allow us to amplify the flanking sequences from the most recent subfamilies of Alu elements in the human genome. There are approximately 1000 elements amplified in these experiments, and we have developed several strategies for amplifying specific subsets of these elements. The goal is to identify subsets of elements that can be amplified and 'displayed' on a gel-based or subtractive method that will allow us to detect differences in these recent elements in breast tumor vs. normal tissue from a patient. This will allow us to detect either insertion of a new Alu element and assessment of the rate of gene damage from retrotransposition, as well as detect major sequence losses that encompass one of these elements.

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(4) Table of Contents

(5) Introduction:

This project was based on the hypothesis that early cellular transformation events involved in breast cancer formation might influence the amplification of human Alu repeats. Any increases in Alu amplification, might contribute to further destabilization of the human genome and inactivation of tumor suppressors that could contribute to the progression of breast cancer. At least in sporadic cases, Alu insertions have been shown to contribute to a number of cancers, including at least one case of breast cancer due to inactivation of BRCA2 [1]. We have previously shown that only a specific set of subfamilies of Alu elements are actively amplifying in the human genome [2;3]. This project combines this information with an anchored PCR procedure we have developed to form displays of the most recently amplified Alu elements. We have demonstrated that this Allele-Specific Alu PCR (ASAP) will effectively display the members of the smallest of the recent Alu subfamilies as bands on an acrylamide gel (5). Our goal is to generalize these procedures to the larger subfamilies and explore various procedures to deal with the larger number of bands expected. We will then use these procedures to compare breast cancer and normal DNA from a number of individuals to determine whether there are new, tumor-specific Alu inserts. This will allow us to determine whether this form of genetic instability plays a role in human breast cancer.

Because of some difficulties with initial implementation of the ASAP assay, we also designed approaches to use an L1 retrotransposition reporter gene system (Moran) to study the specific influences on retrotransposition of genetic changes associated with tumorigenesis, as well as environmental influences that may contribute to breast cancer. This will allow. Because it is thought that Alu elements utilize the same retrotransposition machinery as L1, this system should allow an alternate assessment of the primary question of whether retroelement insertions are likely to contribute to breast cancer genomic instability.

(6) BODY

Original Goals:

First Six Months:

- Optimization of ASAP. Our primary goal will be to optimize the Allele-Specific PCR further. We will work to identify the very best PCR primers to allow the most effective allele-specific amplification of the Alu inserts and flanks. This will allow us to develop a procedure with both minimal steps and minimal background in the later experiments.
- No patient samples will be needed at this stage.

First Year:

- Optimization of Displays. We will utilize the ASAP procedure to generate test samples from all three relevant Alu subfamilies, which can then be utilized to improve the display procedures, in particular the subdivision with PCR into 16 subdivisions. We will begin to explore ways to utilize subtraction procedures on these samples.
- No patient samples will be needed at this stage

Second Year:

- Refinement of Subtraction Technology. Technical development will continue with refinement of the subtraction procedures and tests of the sensitivity of detection of bands and the ability to pool samples in the PCR reactions.
- Preliminary work on tumor samples. Work will begin with existing technology to carry out analysis on tumor samples. We expect to have carried out analysis of the first 10-20 samples in this year. We will use this experience to determine the best approach to generate data in a production mode. This will provide an initial feel for the level of diversity in the displays and a basic characterization of any diversity to determine whether it is caused by insertions. Any evidence of other forms of genomic instability influencing the assay will be assessed at this point and procedures optimized to compensate.

Third Year:

- Completion of Tumor Samples. During the previous year, we expect to have optimized the ASAP procedures and their display completely. This will allow us to have determined the most effective approach for analysis of large numbers of samples. We will utilize this year solely to generate data on as many tumors as possible. We will focus our efforts initially on late stage tumors, but will move progressively towards earlier stage tumors, particularly if we detect extensive Alu amplification at late stages.
- We expect to complete 100 samples by the end of the third year. It is our hope that the subtraction of pooled samples will increase the data flow and we can carry out experiments on enough samples to be able to analyze subgroups based on tumor stage, ethnic origin of tumor or other correlations with clinical features or treatment.

By the Second year it became clear that there were more technical difficulties getting the displays fully optimized and implementable on a large number of samples and our goals had to be scaled back to a more pilot level. In addition, last year we reported in our progress report an alternative approach to address the critical issue of whether retrotransposition played a critical role in breast cancer progression. The approach was to use a reporter system for L1 retrotransposition and test whether genetic alterations associated with tumorigenesis altered retrotransposition rates.

Accomplishments of the three year period:

(This includes a summary of the first two year's work, although without the detail placed in those reports).

During the first two years we explored a wide range of approaches for optimizing displays of the most recently inserted Alu inserts. Year 1 focused primarily on the PCR-based display itself, utilizing a number of variations to both increase the resolution of the technique, as well as ways to deal with the large numbers of elements in some of the more active subfamilies which gave rise to too many elements to allow our assay to work. We were successful at generating quality displays for the very smallest subfamilies of elements. We also had some success utilize various less frequent restriction digestions to allow us to display a limited subset of the more abundant subfamilies. Our biggest difficult at this point was to figure out how to display the 2000 Ya5 subfamily members (which are responsible for the majority of Alu inserts causing disease), without the massive number of bands obscuring the variant signals. We had limited success with the use of PCR primers that added two bases to the end of the primer that went into the genomic flanking sequence to allow us to display one sixteenth of the group of bands at a time. Several primers gave use decent, although not crisp displays. I believe that our biggest problem with this approach was that some of the primers could sit down on sites in which the last two bases base-paired using non Watson-Crick pairing (i.e. G-T pairing), resulting in weaker bands that created background. In our efforts, although several primers worked pretty well, others worked very poorly. A number of variants (include perfect match, altering stringency, etc) did not improve these displays ultimately. Perhaps our biggest disappointment was that several attempts to utilize subtraction strategies to eliminate the common bands did not work at all. Our only observation was that the bands all got lighter, but even attempts to spike a unique band in the mix did not allow us to enrich the unique band. These studies may have been influenced by the presence of a small segment of common repetitive DNA sequence on the end of each fragment, and they may have also been made more difficult by the very high A+T content of the sequences adjacent to Alu elements.

As more human genomic sequence was made available in GENBANK, we were able to identify new subfamilies of Alu elements. More importantly, we found that some of the subfamilies showed very high levels of polymorphism in the human genome. Using a combination of bioinformatics with measurements of the polymorphism associated with these different subfamilies, we were able to determine the relative age and copy number of each of their subfamilies and provide estimates of their likelihood of current activity. Although these data did provide some new, smaller subfamilies that we could adapt to our display technique, by far the majority of Alu elements that had inserted recently to cause disease still remained as part of the larger Ya5 and Yb8 subfamilies. Thus, our original plan of displaying the majority of potential Alu inserts in tumor DNA was not going to work with this approach.

As we approached year 3, we also began to tackle some of the issues associated with adapting this technique to a number of tumor tissues to allow a reasonable sampling. If anything the tumor tissues were even more intractable, partly because the DNA was not always of as high a quality as the tissue culture DNA, and blood DNAs, that we were using in the pilot experiments. Furthermore, our display would be seriously handicapped by any heterogeneity in the tumor tissue that might weaken the signals, while not lessening the background. Therefore, although we worked out the ability to display distinct subsets of the recent Alu inserts, we were never able to adapt the technique to be able to display a significant portion of these inserts in a manner which convinced us that we would be able to see any significant portion of new inserts. Given that new inserts may have been as low as one in 100 tumors, we began to explore alternative approaches for addressing the potential role of retrotransposition in breast cancers.

Although the ideal was to look at authentic tumor tissues and look for authentic Alu inserts, we would obtain a pretty good picture of the relative impact by using a reporter system introduced into tumor cells and measuring the rate of retrotransposition of the reporter system in normal versus transformed cells. The development of an L1 element that activated a neomycin selection cassette upon retrotransposition, provided a potential method to quantify L1 retrotransposition rates in tumors [4]. Furthermore, as most of us believe that Alu retrotransposes with the L1 machinery, using the L1 system should provide insight into both L1 and Alu rates.

Our initial experiments using p53 transformation as a model were very promising and were reported in the last report. However, as we have learned more about the L1 assay, we believe that those preliminary results were an artifact caused by the stimulatory influence of the mutant p53 causing the cells to grow faster. To some extent this is also a function of cell plating density and whether the G418 selection for neomycin resistance is able to be effective before the cells approach confluence. Ultimately, after many repetitions, we can see no influence of p53 mutation on the L1 retrotransposition rate. However, we also wanted to look at the effect of cell cycle in general and we have been able to demonstrate that slowing cell growth by a factor of two by lowering the growth temperature results in an order of magnitude decrease in retrotransposition rates. Furthermore, this effect correlates with growth rate and not just temperature. If the temperature is lowered just at the beginning of the assay, the rate does not change. Thus, the L1 enzymes are not susceptible to temperature, instead, lowering the temperature for a prolonged period has a secondary effect that greatly lowers retrotransposition rates. We have utilized fluctuation analysis on long-term transformants for all of these assays and have also created a transient transfection-based assay. At this point we are gearing up to look at various breast cancer cell lines for their retrotransposition potential, as well as cells with various genetic defects associated with tumorigenesis and DNA repair. Thus, although we cannot yet answer the question of whether transformation alters retrotransposition and therefore retrotransposition may contribute to the progression in cancer, we now have the tools and should be able to test a number of model systems soon.

(7) Key Research Accomplishments

Year 1

- Establishment of optimum conditions for amplification of the most recent subfamilies of Alu inserts
- Obtaining clear displays of the Ya8 subfamily on acrylamide and agarose gels which allow the isolation of insertion polymorphisms between different individuals.
- Demonstrating the use of modified primers that display subsets of the Ya5 elements that will allow at least a substantial portion of Ya5 inserts to be studied.

Year 2

- Identification of the youngest, most active Alu subfamilies that can be amplified and displayed directly without the use of subtraction protocols.

Year 3

- Development of a complete understanding of the recent amplification of Alu elements in the human genome based on the fusion of bioinformatics on the complete human genome sequence and laboratory-based studies.
- Development of approaches to use retroposition reporter gene systems for studies of the role of various genes and environmental influences on the retrotransposition frequency.

(8) Reportable Outcomes

Astrid Roy-Engel was supported by this grant.

- **Deininger, P**. and Batzer, M. (1999) *Alu repeats and human disease.* Mol Gen and Metab **67**, 183-193.
- Roy, A.M., M. Carroll, D.H. Kass, Sun, MA. Batzer, **P.L. Deininger** (1999) *Recently integrated human Alu repeats: Finding needles in the haystack.* Genetica **107**, 149-61.
- Roy, A.M., M.L. Carroll, S.V. Nguyen, A.-H. Salem, M. Oldridge, A.O.M. Wilkie, M.A. Batzer, and **P. L. Deininger** (2000) *Potential gene conversion and source gene(s) for recently integrated Alu elements.* Genome Research **10**, 1485-1495.
- Roy-Engel, ML Carroll, E. Vogel, RK Garber, SV Nguyen, A-H Salem, MA Batzer and **P. Deininger** (2001) *Alu insertion polymorphisms for the study of human genomic diversity.* Genetics (in press)
- ML. Carroll, A. Roy-Engel, SV. Nguyen, A-H Salem, E. Vogel, B.Vincent, J. Myers, Z. Ahmed, L. Nguyen, M. Sammarco, WS. Watkins, J. Henke, W. Makalowski, LB. Jorde, **P. Deininger**, and MA. Batzer. (2001) *Large-scale analysis of the Alu Ya5 and Yb8 subfamilies and their contribution to human genomic diversity.* J. Mol. Biol. (in press).

(9) Conclusions

We were able to develop a PCR procedure that can selectively amplify the subset of most recently inserted Alu elements. Although we were able to display a subset of these elements, we were unable to overcome sufficient technical difficulties to allow an assessment of the number of Alu insertions occurring in breast tumors.

We developed quantitative approaches to measure the retrotransposition capability of different cell types using a reporter-gene approach. Using this approach we showed that dominant negative p53 mutations did not alter retrotransposition rates, but that major changes to cells influencing growth rates had a tremendous influence. We are currently gearing up for a full assessment of breast cancer cell lines, and a number of genes associated with tumorigenesis using this quantitative assay.

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   Ref Type: Abstract

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APPENDIX

one reprint for:

- **Deininger, P**. and Batzer, M. (1999) *Alu repeats and human disease.* Mol Gen and Metab **67**, 183-193.
- Roy, A.M., M. Carroll, D.H. Kass, Sun, MA. Batzer, **P.L. Deininger** (1999) *Recently integrated human Alu repeats: Finding needles in the haystack.* Genetica **107**, 149-61.
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Potential Gene Conversion and Source Genes for Recently Integrated Alu Elements

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Alu elements comprise >10% of the human genome. We have used a computational biology approach to analyze the human genomic DNA sequence databases to determine the impact of gene conversion on the sequence diversity of recently integrated Alu elements and to identify Alu elements that were potentially retroposition competent. We analyzed 269 Alu Ya5 elements and identified 23 members of a new Alu subfamily termed Ya5a2 with an estimated copy number of 35 members, including the de novo Alu insertion in the *NF1* gene. Our analysis of Alu elements containing one to four (Ya1–Ya4) of the Ya5 subfamily-specific mutations suggests that gene conversion contributed as much as 10%–20% of the variation between recently integrated Alu elements. In addition, analysis of the middle A-rich region of the different Alu Ya5 members indicates a tendency toward expansion of this region and subsequent generation of simple sequence repeats. Mining the databases for putative retroposition-competent elements that share 100% nucleotide identity to the previously reported de novo Alu insertions linked to human diseases resulted in the retrieval of 13 exact matches to the *NF1* Alu repeat, three to the Alu element in *BRCA2*, and one to the Alu element in *FGFR2* (Apert syndrome). Transient transfections of the potential source gene for the Apert's Alu with its endogenous flanking genomic sequences demonstrated the transcriptional and presumptive transpositional competency of the element.

Alu elements belong to a class of retroposons termed SINEs. SINEs are Short INterspersed Elements usually ~100–300 bp in length commonly found in introns, 3′ untranslated regions of genes, and intergenic genomic regions (Deininger and Batzer 1993). Alu is the most abundant class of SINEs in primate genomes, reaching a copy number in excess of one million/haploid genome (Jelinek and Schmid 1982; Jurka et al. 1993, Smit 1999). Alu elements increase their genomic copy number by an amplification process termed retroposition (Rogers and Willison 1983; Weiner et al. 1986).

Alu elements appear to have arisen in the last 65 million years (Deininger and Daniels 1986). The human Alu family of repeats is composed of a small number of distinct subfamilies characterized by subfamily-specific diagnostic mutations (Slagel et al. 1987; Willard et al. 1987; Shen et al. 1991; Batzer et al. 1996b). The source Alu gene(s) for each of the subfamilies has been retropositionally active during different periods of primate evolution. The rate of Alu amplification (mostly Sx subfamily) appears to have reached its peak between 60 and 35 million years, and subsequently decreased several orders of magnitude to the present amplification rate (Shen et al. 1991). Only a limited number of SINEs, termed master or source genes, appear to be capable of retroposition (Deininger and Daniels 1986; Batzer et al. 1990; Deininger et al. 1992), although the critical factor(s) defining functional source genes are not understood. A variety of factors influence the retroposition process (Schmid and Maraia 1992). All of the recently integrated young Alu subfamilies appear to be retropositionally active. Almost all of the recently integrated Alu elements within the human genome belong to one of four closely related subfamilies (Y, Ya5, Ya8, and Yb8), with the majority being Ya5 and Yb8 subfamily members (Batzer et al. 1990, 1995; Deininger and Batzer, 1999).

Previously, analysis of individual Alu elements from the different subfamilies involved laborious procedures, such as cloning, library screening, and subsequent sequencing (Batzer et al. 1990, 1995; Arcot et al. 1995a). However, the availability of large-scale human

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genomic DNA sequences as a result of the Human Genome Project facilitates genomic database mining for Alu elements (Roy et al. 1999). We have taken advantage of these databases and have analyzed a significant portion of the Alu Ya5 subfamily, as well as intermediates between the Ya5 subfamily and the ancestral Alu Y subfamily. In addition, we searched the databases for putative retroposition-competent source Alu genes that generated the de novo Alu inserts associated with a number of human diseases (Deininger and Batzer 1999).

RESULTS

Computational Analyses

To search for subfamilies unidentified previously within the Ya5 Alu subfamily, we selected all of the Alu family members that matched our Ya5 consensus query sequence from the human genome nonredundant (nr) database. Only Ya5 elements found randomly within other sequences were included in our analysis, thereby eliminating Alu elements that had been identified previously in directed Alu-specific projects. In addition, truncated Alu elements were eliminated from the analysis. Ya4 elements that did not contain the first Ya5-specific diagnostic mutation #11 (Fig. 1) (Shen et al. 1991), which is a CpG dinucleotide in the Ya5 subfamily, were considered as Ya5 Alu family members. We obtained a total of 269 matches to the Ya5 query sequence that met our criteria. Of these, 47 shared 100% nucleotide identity with the subfamily consensus sequence and 83 were near perfect matches (aside from a few CpG mutations).

Analysis of the 269 Ya5 Alu elements resulted in the initial identification of two subsets of potential subfamilies containing two diagnostic mutations each, one with six members and the other with four. These subfamiles will be referred to as Ya5a2 and Ya5b2, respectively, in compliance with the standard Alu subfamily nomenclature (Batzer et al. 1996a). Each consensus sequence with the two diagnostic mutations specific to each new Alu subfamily is shown in Figure 1. Interestingly, the de novo Alu Ya5 insert present within an intron of the *NF1* gene (Wallace et al. 1991) is an exact match to the Ya5a2 consensus. The nr database contained 16.0% of human DNA sequences for a total of 515,596,000 bases on the date of the search. The estimated size of the Ya5a2 subfamily is (3 × 10^9 bp/515,596,000 bp) × 6 unique Ya5a2 matches = 35 subfamily members. In comparison, the estimated size of the Ya5b2 subfamily is (3 × 10^9 bp/515,596,000 bp) × 4 unique Ya5b2 matches = 22 subfamily members. We utilized only the randomly found Ya5a2 elements for the calculations to avoid overestimating the size of the subfamilies. However, these numbers may be underestimations, because some specific polymorphic elements of these subfamilies may not be represented in the database.

```
                 .         .         .         .         .         .
Ya5     GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGA 60
Ya5a2   ............................................................ 60
Ya5b2   ............................................................ 60
Ya5a1   ............................................................ 60
Ya5b1   ............................................................ 60
Ya5c1   ............................................................ 60

                 .         .       11.     12  .         .         .
Ya5     TCACGAGGTCAGGAGATCGAGACCATCCCGGCTAAAACGGTGAAACCCCGTCTCTACTAA 120
Ya5a2   ...........A................................................ 120
Ya5b2   .........................A...C.............................. 120
Ya5a1   .....................G...................................... 120
Ya5b1   ............................................................ 120
Ya5c1   ............................................................ 120

                 .         .    13   .         .         .   14    .
Ya5     AAATACAAAAAA-TTAGCCGGGCGTAGTGGCGGGCGCCTGTAGTCCCAGCTACTTGGGAG 179
Ya5a2   ............A............................................... 180
Ya5b2   ............-............................................... 179
Ya5a1   ............-............................................... 179
Ya5b1   ............-............................G.................. 179
Ya5c1   ............-............................................... 179

                 .         .         .         .         .      15 .
Ya5     GCTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAGATCCCG 239
Ya5a2   ............................................................ 240
Ya5b2   ............................................................ 239
Ya5a1   ............................................................ 239
Ya5b1   ............................................................ 239
Ya5c1   ......................................................G..... 239

                 .         .         .         .
Ya5     CCACTGCACTCCAGCCTGGGCGACAGAGCGAGACTCCGTCTC 281
Ya5a2   .......................................... 282
Ya5b2   .......................................... 281
Ya5a1   .......................................... 281
Ya5b1   .......................................... 281
Ya5c1   .......................................... 281
```

Figure 1 Consensus sequence alignment of Ya5, and the potential new subfamily members identified. Nucleotide substitutions at each position are indicated with the appropriate nucleotide. Deletions are marked by dashes (-). The Ya5 diagnostic nucleotides are indicated in bold with the corresponding diagnostic number above as defined by Shen et al. (1991).

To derive a second estimate of the copy numbers of the Ya5a2 and Ya5b2 Alu subfamilies, we used their consensus sequences as queries for the high throughput genome sequence (htgs) and genomic survey sequence (gss) databases. Seventeen additional Alu Ya5a2 elements were found in these searches. Of the 23 total Ya5a2 elements, 13 shared 100% nucleotide identity with the subfamily consensus sequence. No additional Ya5b2 elements were found in the other databases, therefore the Ya5b2 subfamily was not subjected to further analysis. Three additional potential subfamilies, Ya5a1 (five members), Ya5b1 (four members), and Ya5c1 (four members) with only one specific diagnostic mutation were identified (Fig. 1). Because of the small copy number, and the possibility that some of those represent parallel mutations rather than new subfamilies, no further analyses were performed.

To determine the age of the Ya5a2 subfamily, we divided the nucleotide substitutions within the elements into those that have occurred in CpG dinucleotides and those that have occurred in non-CpG positions. The distinction between types of mutations is made because the CpG dinucleotides mutate at a rate that is ~10 times faster than non-CpG (Labuda and Striker 1989; Batzer et al. 1990), as a result of the deamination of 5-methylcytosine (Bird 1980). A total of five non-CpG mutations and seven CpG mutations occurred within the 23 Alu Ya5a2 subfamily members identified. By use of a neutral rate of evolution for primate-intervening DNA sequences of 0.15%/one-million years (Miyamoto et al. 1987) and the non-CpG mutation rate of 0.092% (5/5382 bases using only non-CpG bases) within the 23 Ya5a2 Alu elements, yields an estimated average age of 0.62 million years for the Ya5a2 subfamily members with a predicted 95% confidence level in the range of 0.28–1.08 million years, given that the mutations were random and fit a binomial distribution. The Ya5a2 subfamily appears to be much younger than Ya5, Ya8, or Yb8 Alu subfamilies with estimated ages of 2.8 million years (Batzer et al. 1990), 2.75 million years (Roy et al. 1999), and 2.7 million years (Batzer et al. 1995), respectively (Fig. 2).

Determination of the number of elements that perfectly match the subfamily consensus sequence can also give an indirect estimate of Alu subfamily age and recent rate of mobilization. Recently transposed Alu elements share higher levels of nucleotide identity with their source copies because they have not resided in the genome long enough to accumulate random mutations. In contrast, older Alu elements that have resided in the genome for longer periods of time tend to have less nucleotide identity with their source genes as a result of the accumulation of random mutations subsequent to integration into the genome. We compared our search results for the Ya5a2 subfamily with parallel searches from the Ya8 and Ya5 Alu subfamilies. Our BLAST searches from the nr database yielded one perfect match of 12 elements for Ya8, 47 of 269 for Ya5, and 3 of 6 for Ya5a2 (Fig. 2). Searching all three databases (nr, gss, and htgs) yielded 5 perfect matches of 27 for Ya8 and 13 of 23 for Ya5a2. These results are in good agreement with the previous estimates, indicating that Ya5a2 is the youngest Alu subfamily reported to date, as it also has the highest proportion of elements that share 100% nucleotide identity with the consensus sequence.

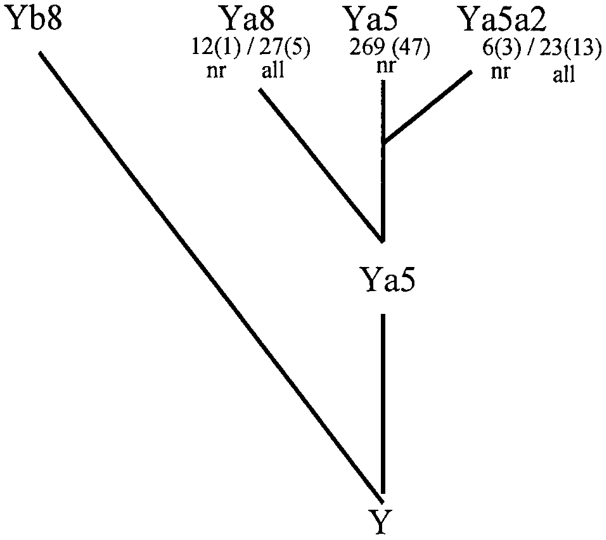

Figure 2 Schematic for the evolution of recently integrated Alu subfamilies. The origin of the Ya5a2 Alu subfamily is shown after the divergence of Ya5 and Yb8 elements. The total number of elements found in the nr-database (perfect matches in parenthesis) are shown first separated by a slash from the total number of elements found in all three databases (nr, gss, htgs). For the Ya5 elements only the nr-database results are shown.

Table 1. Alu Middle A-Rich Region

Ya5-middle A rich region	A_n							
	4	5	6	7	8	9	10	11
$T(\mathbf{A}_n)TACA_6TT$[a]	0	269[c]	9	1	0	1	—	—
$TA_5TAC(\mathbf{A}_n)TT$[b]	0	2	269[c]	37[d]	11	7	3	0

[a] n = 5 in Ya5 consensus.
[b] n = 6 in Ya5 consensus.
[c] Data from the non-redundant database only.
[d] All 23 Ya5a2 members are included.

Stability of the Middle A-Rich Region in Alu Ya5 Members

The oligo-dA-rich tails and middle A-rich regions of Alu elements have been shown previously to serve as nuclei for the genesis of simple sequence repeats (Arcot et al. 1995b). In the autosomal recessive neurodegenerative disease, Friedreich ataxia, the most common mutation, is the hyperexpansion of a GAA within the middle A-rich region of an Sx Alu element (Montermini et al. 1997). Because these regions appear unstable, we analyzed the middle A-rich region of Alu elements retrieved from the databases to detect expansions/contractions of this sequence.

To evaluate potential expansions/contractions, we performed a BLAST query of three databases (nr, htgs, and gss) using the Alu Ya5 consensus sequence with varying numbers of A nucleotides within the middle A-rich region (TA_nTACA_nTT). Our results demonstrate that the majority of the elements identified matched the consensus sequence. However, there is a trend for an A expansion at both positions (Table 1). In contrast, very few sequence contractions were detected for any of the positions.

Human Genomic Variation

To determine the human genomic variation associated with the Ya5a2 Alu subfamily members, we selected the 13 Ya5a2 elements identical to the subfamily consensus sequence as well as 2 others and determined the degree of fixation associated with the elements using PCR-based assays of a panel of diverse human DNA samples with the primers shown in Table 2. The panel is composed of 20 individuals of European origin, African-Americans, Greenland natives, and Egyptians for a total of 80 individuals (160 chromosomes). The Alu elements were classified as fixed absent, fixed present, and high, intermediate, or low frequency insertion polymorphisms (see Table 3 for definitions). By use of this approach, 3 of the 14 elements tested (Ya5NBC206, Ya5NBC207, and Ya5NBC235) were always present in the human genomes that were surveyed, suggesting that these elements became fixed in the genome prior to the radiation of modern humans from Africa. Five of the elements (Ya5NBC208, Ya5NBC240, Ya5NBC241, Ya5NBC242, and Ya5NBC220) are intermediate frequency Alu insertion polymorphisms. The remaining six elements are low-frequency Alu insertion polymorphisms (Table 3). The population-specific genotypes and levels of heterozygosity for each element are shown in Table 4. The high proportion of polymorphic elements is in good agreement with our other observations, indicating that the Ya5a2 subfamily is younger than any of the other Alu subfamilies identified previously in the human genome.

Gene Conversion and Alu Sequence Diversity

In our query of the human genome (nr) database, 91 of the Alu elements identified contain one to four of the five Ya5 diagnostic nucleotides (Fig. 1). Of these 91 intermediate elements, 4 are Ya1, 1 Ya2, 7 Ya3, and 79 Ya4 Alu elements (Fig. 3). Surprisingly, not all of the Alu elements with different numbers of subfamily mutations had the same combination of mutations. To facilitate identification of the individual elements with different diagnostic mutation combinations, the diagnostic nucleotides were numbered consecutively in order of abundance (Ya3.1, Ya3.2, etc., see Fig. 3). Seventeen Alu elements (Ya4.4) did not contain the first diagnostic mutation (#11), but were still classified as Ya5 for the analyses outlined above.

Previous evolutionary analyses of the Ya5 founder element with different primate DNA samples demonstrated the sequential accumulation of the Ya5 diagnostic mutations with diagnostic positions #13/#14 first, followed by #12/#16, and finally position #11 (Shaikh and Deininger 1996). Our data are not consistent with a sequential order in the accumulation of the diagnostic mutations. The elements classified as Ya1, Ya2, Ya3.4, Ya3.5, and Ya4.4 (26 total) fit the proposed order (Fig. 3). However, the remaining 65 elements represent almost every other permutated order. Several mechanisms could explain the occurrence for mosaic

Table 2. Alu Ya5a2 PCR Primers, Chromosomal Locations, and PCR Product Sizes

					Product size[c]	
Name	5′ Primer sequence (5′–3′)	3′ Primer sequence (5′A–3′)	A.T.[a]	Chromosome[b]	filled	empty
Ya5NBC206	TCCTTAGCTATCTCACAAGCTACAT	ACACATTTCCTTCAAGAGGTCAAAG	60°C	4	734	424
Ya5NBC207	CAGTTTTATACACTGGCCTGTTTTC	TTGTAGGAGAAAGAGGGGAAATACT	50°C	6	443	122
Ya5NBC208	AATACCTTGTACATCTTCACCCCTA	TCTCTCTGCTGCACAGTTTGTT	50°C	14	441	115
Ya5NBC240	CAGGAGATAAATATGTTCGGAGAGT	TAACTGGGACAGTGAGTTTTACCTG	55°C	9	505	202
Ya5NBC241	GGTTCCAATAGAGAGCAACAGAA	ACCTTAAGCTTTCCCCCAGA	55°C	15	392	66
Ya5NBC242	AACAAAATTCCCTTTCCTCCA	GGCAATCTGACCTTGGGTAA	55°C	7	503	192
Ya5NBC7	TGATGGATATTTGGGTTGGTTC	GGACTGTAAACTAGTTCAACCATTGTG	60°C	7	522	216
Ya5NBC205	ACATGAAGGGCCGACTGTAT	TGCTGCTGCATTATCAACTG	50°C	21	435	81
Ya5NBC209	GTCTATGGGAAGATGAAGAATAGGA	GATGGAGTCACTCATGTGAAAAGTA	55°C	14	447	116
Ya5NBC239	CAGCTGAGAACTGTCACAAATAGAA	ATCAATGACTGACTTGTGCTGAGT	55°C	9	531	198
Ya5NBC243	CCATGATTCGTCATTCACCA	AGGAGACCTGCCAATGAATG	60°C	21	406	86
Ya5NBC220	AAATCAAGCTGCCATACCTCA	GAAACCATCCTTCACAGTGG	60°C	1	463	141
Ya5NBC235	CCCAAGGCACTTGCTGTTA	CCCTTCGAGAAAGAGGAAGG	50°C	2	391	76
Ya5NBC244	CCTATGGCTGAAACTTCTGAAACT	ATATCTTGGTCCACTAGACAAGCAC	60°C	18	453	130
Ya5NBC237[d]	CCCATGGAGGGTCTTTCCTA	CTGGAAACCATCCTTCACAGT	60°C	1	410	88

[a] Amplification of each locus required 2.5 min at 94°C initial denaturing, and 32 cycles for 1 min 94°C, 1-min annealing temperature (A.T.) and 1-min elongation at 72°C. A final extension time of 10 min at 72°C was also used.

[b] Chromosomal location determined from accession information or by PCR analysis of NIGMS monochromosomal hybrid cell line DNA samples.

[c] Empty product sizes calculated by removing the Alu element and one direct repeat from the filled sites that were identified.

[d] Alu Ya5a2 element of the *FGFR2* gene.

Table 3. Alu Ya5a2 (*NF1*)-Associated Human Genomic Diversity

Ya5a2 elements	Accession no. (duplicates)	Position	Allele frequency[a]
Ya5NBC206	AC004057	76767–77048	fixed present
Ya5NBC207	AL118555 (AL132992)	9981–9700 (40728–41009)	fixed present
Ya5NBC208	AL109919	70170–69889	intermediate
Ya5NBC220	AC007611	136715–136434	intermediate
Ya5NBC240	AC133410 (AL135841)	34800–35081 (49829–49548)	intermediate
Ya5NBC241	AC018924	144017–144298	intermediate
Ya5NBC242	AC009517	161301–161582	intermediate
Ya5NBC7	AC004848	24522–24241	low
Ya5NBC205	AL011328	204488–204207	low
Ya5NBC209	AC00808	147056–146775	low
Ya5NBC239	AL133284	115867–115586	low
Ya5NBC244	AC026839	64885–64604	low
Ya5NBC243	AJ011929	151192–151473	low
Ya5NBC235[b]	AQ748733	458–739	fixed present
Ya5NBC237[c]	AL031274	33175–33501	intermediate

[a]Allele frequency was classified as fixed present, fixed absent, low, intermediate, or high frequency insertion polymorphism. (Fixed present) every individual tested had the Alu element in both chromosomes; (low frequency insertion polymorphism) the absence of the element from all individuals tested, except for one or two homozygous or heterozygous individuals; (intermediate frequency insertion polymorphism) the Alu element is variable as to its presence or absence in at least one population; (high frequency insertion polymorphism) the element is present in all individuals in the populations tested, except for one or two heterozygous or absent individuals.

[b]Several Ns.

[c]Ya5NBC237 is the exact match to the *FGFR2* Alu insertion.

Alu elements, which are addressed in the discussion section. However, we believe the most likely explanation for the existence of these mosaic elements is through gene conversion events. A limited amount of gene conversion between Yb8 Alu elements has been reported previously (Batzer et al. 1995; Kass et al. 1995). In theory, gene conversion may change the sequence of all or part of any Alu element in either an evolutionarily forward (Ya5 subfamily in this case) or backward (Y subfamily) direction by changing the di-

Table 4. Alu Ya5a2-Associated Human Genomic Diversity

	African American				Greenland natives				European				Egyptian				
Elements	genotype[a]			*f*Alu[b]	genotypes			*f*Alu	genotypes			*f*Alu	genotypes			*f*Alu	het.[c]
Ya5NBC206	20	0	0	1.000	20	0	0	1.000	20	0	0	1.000	20	0	0	1.000	0.000
Ya5NBC207	20	0	0	1.000	20	0	0	1.000	20	0	0	1.000	20	0	0	1.000	0.000
Ya5NBC208	4	1	7	0.375	3	0	4	0.429	13	0	6	0.684	7	0	5	0.583	0.482
Ya5NBC236	5	6	2	0.615	5	8	6	0.474	15	5	0	0.875	6	8	1	0.667	0.422
Ya5NBC240	5	1	9	0.367	11	0	4	0.733	5	1	10	0.344	5	3	3	0.591	0.464
Ya5NBC241	3	9	5	0.441	6	11	2	0.605	0	7	11	0.194	3	8	4	0.467	0.459
Ya5NBC242	2	13	1	0.531	7	4	3	0.643	3	4	11	0.278	3	3	1	0.643	0.474
Ya5NBC7	0	0	19	0.000	0	0	20	0.000	0	0	20	0.000	0	0	20	0.000	0.000
Ya5NBC205	0	0	20	0.000	0	0	20	0.000	0	0	20	0.000	0	0	20	0.000	0.000
Ya5NBC209	0	1	17	0.028	0	0	17	0.000	0	0	19	0.000	0	0	19	0.000	0.000
Ya5NBC239	0	0	20	0.000	0	0	20	0.000	0	0	20	0.000	0	0	20	0.000	0.000
Ya5NBC243	0	0	20	0.000	0	0	20	0.000	0	0	20	0.000	0	0	20	0.000	0.000
Ya5NBC220	0	14	5	0.368	1	15	2	0.472	0	18	1	0.474	0	9	2	0.409	0.502
Ya5NBC244	0	0	12	1.000	—	—	—	—	0	0	10	0.000	0	0	8	0.000	0.000
Ya5NBC235	20	0	0	1.000	20	0	0	1.000	20	0	0	1.000	20	0	0	1.000	0.000
Ya5NBC237[d]	18	1	0	0.974	15	4	0	0.895	20	0	0	1.000	18	1	0	0.974	0.075

[a]Genotypes: +/+ Alu, +/− Alu, −/− Alu.

[b]Frequency of the presence of the Alu.

[c]Average heterozygosity.

[d]Ya5NBC237 is the exact match to the *FGFR2* Alu insertion.

— not determined.

```
                       Diagnostic site
                   1    2     3   4     5
          Y        T    C     G   C     G
(4)       Ya1      ...........A..........    f
(1)       Ya2      ...........A...T......    f
(1)       Ya3.1    C....A.....A..........    -
(1)       Ya3.2    C..............T.....C    b
(1)       Ya3.3    C....A...............C    b
(2)       Ya3.4    ...........A...T.....C    -
(2)       Ya3.5    .....A.....A...T......    f
(6)       Ya4.1    C..........A...T.....C    b
(11)      Ya4.2    C....A.....A.........C    b
(13)      Ya4.3    C....A.........T.....C    b
(17)      Ya4.4    .....A.....A...T.....C    -
(32)      Ya4.5    C....A.....A...T......    -
          Ya5      CpG  A     A   T     C
```

Figure 3 Evolution of the diagnostic nucleotide positions from Y to Ya5 Alu elements. Alignment of the five Alu Ya5 diagnostic nucleotides as defined by Shen et al. (1991) and the different Ya1, Ya2, Ya3, and Ya4 elements found in the nr database. For easy reference, individual elements containing different combinations of the diagnostic mutations were numbered consecutively in order of abundance (Ya3.1, Ya3.2, etc.). Ya4.4 elements were considered as Ya5 elements in the first Ya5 subfamily analysis in this paper. The total number of elements found for each subgroup is indicated at *left* in parenthesis. Potential forward (f) or backward (b) gene conversions are indicated at *right*. The previously reported order of appearance of Ya5 diagnostic mutations (Shaikh and Deininger 1996) is indicated below. Elements with diagnostic mutations that follow the stepwise hierarchical accumulation are circled.

agnostic mutations. In addition, double gene conversions would be extremely rare, making the direction of the gene conversion clear in some elements. We classified the 91 mosaic Alu element sequences as gene converted forward (f), backward (b), or could not be determined (-), (see Fig. 3) If the Alu elements that fit the proposed sequential evolution are ignored in the analysis, all of the other elements may be classified as backward gene conversion (32 total) or could not be determined (33 total), and none were clearly gene-converted forward. Therefore, backward gene conversion may have contributed to between 10% and 20% (32 to 65/269 Ya5 + [91–17] Ya1–Ya4) of the Alu Ya5 sequence diversity. Interestingly, evaluation of the five random Ya5a2 non-CpG mutations shows that one mutation in position #13 is a backward mutation to the Y subfamily, another putative example of a reverse gene conversion.

In Search of Retroposition-Competent Alu Repeats

Sixteen different Alu insertions have been linked to human diseases (Deininger and Batzer 1999). Four belong to the Alu Y subfamily, one to the Ya4 subfamily, eight to the Ya5 subfamily, and three to the Yb8 subfamily. Closer inspection of the nucleotide sequences of these Alu elements show that they have some mutations that are different from their respective subfamily consensus sequences. Because these Alu insertions are very recent in origin, they are likely to be identical to their source genes aside from rare mutations introduced during reverse transcription of the Alu element. Therefore, sequence database queries utilizing each Alu element along with its individual mutations (away from the subfamily consensus sequence) may facilitate the identification of the source Alu element that generated the copy. This strategy is similar to that used previously in the identification of active LINE elements from the human genome (Dombroski et al. 1993).

A database query using the sequence of the individual Alu elements responsible for each disease to mine three databases (nr, htgs, and gss) identified exact complements to four of the disease-associated Alu repeats. Thirteen of the identified elements were exact matches to the *NF1* Alu insertion (Ya5a2 subfamily, Table 3; Wallace et al. 1991); three were exact matches to the *BRCA2* Alu element (Miki et al. 1996) (accession nos. AL121964, AL136319, and AL135778); one matched the *FGFR2* Alu repeat (Oldridge et al. 1999) (accession no. AL031274); and one matched the Alu repeat in the *IL2RG* gene (Lester et al. 1997) (accession no. AC010888).

Potential Source Gene for the Ya5 Insert in *FGFR2*

As mentioned above, our BLAST query only detected one exact match (accession no. AL031274 or Ya5NBC237) to the Ya5 Alu found in the *FGFR2* gene that caused Apert syndrome. We estimated the level of human genomic variation associated with Ya5NBC237 using the same human DNA panel and determined that it was an intermediate frequency Alu insertion polymorphism (Table 4).

Mobilization-competent Alu elements must be capable of transcription, the first step in the retroposition process. To evaluate Alu Ya5NBC237 as a potential source gene for the de novo insert in the patient with Apert syndrome, we determined its transcription capability. Constructs with the genetic loci containing the Ya5NBC237 Alu and the de novo Apert syndrome Alu element were made. Transcription levels from the two constructs were evaluated by Northern blot analysis relative to a control plasmid in which the Alu element is flanked immediately upstream by vector sequence.

Transient transfections (Fig. 4) of the constructs into rodent cell line C6 (rat glial tumor) were performed. Although the Alu element in the control plasmid has an intact internal Pol III promoter, Alu transcripts are barely detectable from the control plasmid. In contrast, the transcription from the Apert's Alu element and its potential source gene were elevated three- to fourfold, as expected for putative mobilization-competent Alu repeats. This result suggests that the genomic flanking sequence of Ya5NBC237 probably makes the Alu transcription competent, one of the several requirements of a source gene. The same results

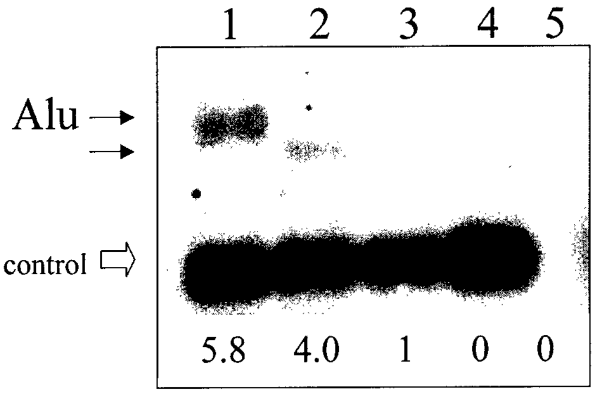

Figure 4 Evaluation of transcriptional capability of the potential *FGFR2* source Ya5 Alu element. The transcriptional efficiency of the de novo *FGFR2* Alu repeat and its putative source gene were evaluated by Northern blot analysis from transient transfection studies. The following constructs were evaluated: (lane *1*) p^{-290}Ap, (lane *2*) p-^{416}Ya5NBC237, and (lane *3*) p^{NP}Ya5NBC237. Lanes *4* and *5* are internal control only, and no DNA controls, respectively. Small arrows indicate the Alu transcripts and the open arrow indicates the internal control transcript. The ratio of the Alu transcript/control transcript (numbers below) was normalized to the p^{NP}Ya5NBC237 transcription ratio, which was assigned the arbitrary value of 1.

were obtained from transfections in the human embryonic kidney cell line 293 (data not shown).

DISCUSSION

Our computational and experimental analyses of the Ya5 subfamily of Alu repeats provides an overall picture of the most active of the recently integrated young Alu subfamilies from the human genome. The analysis of Alu Ya5 repeats allowed us to address a number of questions about the biology of these elements, such as the potential impact of gene conversion events, and the identification of Alu family members from the human genome that may be capable of retroposition.

Alu elements spread throughout the genome by retroposition in the last 65 million years. The master/source gene model (Batzer et al. 1990; Shen et al. 1991; Deininger et al. 1992) posits that a very small subset of the >1,000,000 Alu elements within the human genome are capable of high levels of retroposition; although a much larger number may make a few copies. The formation of Alu subfamilies may be explained by the sequential accumulation of mutations within the active source gene(s) followed by proliferation of the mutated source elements. A number of studies indicate that relatively few source Alu genes have played a dominant role in the amplification and evolution of Alu elements (Shen et al. 1991; Deininger et al. 1992; Deininger and Batzer 1993; Kapitonov and Jurka 1996). Although retroposition is the primary mode of SINE mobilization and sequence evolution through mutations in the source gene(s), our analysis suggests that gene conversion and genetic instability of Alu-based simple sequence repeats have also had a significant impact on the sequence architecture of this major family of human genomic sequences.

There are several alternatives that could explain the occurrence of mosaic Alu elements. First, some of the mosaic Alu elements with a single mutation could be explained by the occurrence of parallel mutations. However, this seems unlikely unless there were selection for these specific mutations, possibly through a post-transcriptional selection process (Sinnett et al. 1992). It is also difficult to envision a selection process that would only select for mutations at adjacent diagnostic positions, such as we see here. Also, recombination between different Alu elements could have generated some of these intermediate Alu elements that contain a mosaic of diagnostic mutations. However, in many cases, multiple recombination events would be required to obtain this outcome, making it highly unlikely. Although there are alternative mechanisms, we believe gene conversion is the most likely explanation for the occurrence of mosaic Alu elements.

The mechanisms of genome-wide gene conversion between mobile elements are not well understood in humans (see Kass et al. 1995, and references therein). Our data show that even the very short, dispersed Alu elements appear to be capable of high levels of gene conversion, which usually involve only short sequence stretches. In addition, our data show that reverse or backward gene conversions may be more favored. It seems likely that higher levels of the Y element copy number (Shen et al. 1991) or transcription (Shaikh et al. 1997) may play a role in determining the directionality of the gene conversion events. Although older Alu subfamilies, such as J and Sx are present in higher copy numbers in the genome, they diverged greatly from their consensus sequences due to mutations that have accumulated throughout evolution. Gene conversion would not be favored between such divergent sequences. However, Alu Y elements tend to be more conserved (better matches to Ya5) and with high copy number (Batzer et al. 1995). Therefore, both abundance (genomic copy number and/or transcript levels) and sequence identity appear to be influential in the Alu gene conversion events observed.

There are multiple examples of gene conversion events in literature. Genetic exchange between exogenous and different endogenous mouse L1 elements has been demonstrated previously to readily occur (Belmaaza et al. 1990). Kass et al. (1995) reported previously a gene conversion event in which one of the oldest Alu family members was converted to one of the youngest Alu subfamilies, Yb8. In addition, a partially converted Yb8 Alu element was also reported previously by Batzer et al. (1995). In yeast, some types of mobile elements spread through the genome by gene converting pre-existing elements (Hoff et al. 1998). When we combine this type of mobilization in the yeast genome with the Alu gene conversions reported previously, as well as those in this paper, one could argue that gene conversion may represent a second type of amplification mechanism for short interspersed elements in the human genome. These observations suggest that evolutionary studies of all types of interspersed elements that ignore gene conversion events may lead to biased conclusions.

Variations in the length of the middle A-rich region and oligo-dA-rich tails of Alu elements are not uncommon (Economou et al. 1990; Arcot et al. 1995b; Jurka and Pethiyagoda 1995). Microsatellite repeats have been found to be associated with the 3′ oligo (dA) tails and the middle A-rich region of Alu elements. In the case of Friedreich ataxia, the most common mutation is the hyperexpansion of a GAA trinucleotide repeat within the middle A-rich region of an Sx Alu (Montermini et al. 1997). However, microsatellites in the middle of Alu elements are not as common because of the much shorter initial length of the middle A-rich region. Arcot et al. (1995b) reported previously that only about one-fourth of the Alu elements containing $(AC)_n$ repeats had them as a part of their middle A-rich region. The one specific example they studied in detail had an evolutionary expansion of the A-rich region (orangutan and gibbon) before the genesis of the AC repeat; suggesting the requirement for an initial expansion. Interestingly, our large-scale analysis of the middle A-rich regions of Ya5 elements demonstrates a trend toward expansion of the A region, providing additional support for this region of the Alu elements to act as a potential nucleus for the genesis of simple sequence repeats.

From our subset of 269 AluYa5 elements, we were able to identify a new Alu subfamily termed Ya5a2. The estimated average age of 0.62 million years (0.28–1.08 million years with 95% confidence) makes Ya5a2 the youngest subfamily of Alu repeats identified in the human genome to date. It is as abundant as the Ya8 subfamily (Roy et al. 1999) and its higher level of insertion polymorphism suggests a higher level of current retroposition. The Ya5a2 subfamily may have originated from a Ya5 Alu element that inserted in a genomic region that favored transcription and corresponding retroposition activity of the element, thereby generating a source gene. The subsequent accumulation of the two specific mutations facilitated the differentiation of the copies made by the Ya5a2 source gene from the larger background of several hundred genomic Ya5 Alu family members. As new Alu elements integrate into the genome in favorable genomic locations, they can occasionally remain retropositionally competent and generate copies of themselves. However, the frequency of fortuitous insertions of new Alu elements into favorable genomic locations for subsequent mobilization is still a rare event because the continuity of the hierarchical subfamily sequence structure of the Alu elements is largely conserved throughout primate evolution.

Alu elements that are polymorphic for insertion presence/absence have been proven previously to be useful for the study of human population genetics and forensics (Batzer et al. 1991; Jorde et al. 2000; Perna et al. 1992; Batzer et al. 1994; Tishkoff et al. 1996; Stoneking et al. 1997). The identification of a very young Alu subfamily with a high proportion of polymorphic members provides a new source of Alu insertion polymorphisms for the study of human population genetics. However, it is important to note that theYa5a2 subfamily is extremely small (~35 copies total in a background of >1,000,000) comparable with Ya8, so that an exhaustive analysis of a single human genome would only generate ~20 polymorphic Ya5a2 elements.

Because our analysis of Alu elements related to the Apert's insertion only included ~40% of the human genome (both finished and draft sequence included), there are possibly one or two other perfect complements in the human genome that have not yet been sequenced and may be the actual source gene for these elements. The transcriptional potential of this element would be consistent with its role as the potential source Alu gene. This confirms the existence of minor active source genes that differ from the source gene that generated almost all of the Alu elements present in the human genome today. In addition, the de novo Apert's Alu element was also transcriptionally active. There are two possible explanations for this result. First, the transcriptional capacity of the elements was evaluated by transient transfections in tissue culture. This system does not reflect the influence of chromatin structure and methylation patterns (position effects) on the transcription and presumably retroposition potential of the two Alu repeats. Alternatively, the de novo Apert's Alu element may have inserted in a region of the *FGFR2* gene that fortuitously enhances its own transcription capability. Although further studies will be required to make more definitive statements in this regard, the transcriptional capability of Ya5NBC237 is consistent with one of the many requirements a source gene possesses, making it a plausible candidate source gene for the de novo Apert's insertion.

In summary, the computational analyses of a subset of recently integrated Alu elements demonstrate that Alu sequence evolution is affected by a number of dynamic events. New retroposition-competent Alu source genes, gene conversion, and genetic instability each play an important role in Alu sequence evolution and proliferation within the human genome.

METHODS

Computational Analyses

Screening of the GenBank nr, the htgs, and the gss databases were performed by use of the Advanced Basic Local Alignment Search Tool 2.0 (BLAST) (Altschul et al. 1990) available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). For the Ya5 subfamily analysis, the database was searched for matches to the 281 bases of the Ya5 consensus sequence with the following advanced options: -e 1.0 e-120, -b 1000, and -v 1000. A region composed of 500 bases of flanking DNA sequence directly adjacent to the sequences identified from the databases that matched the initial GenBank BLAST query were subjected to annotation by use of either RepeatMasker2 from the University of Washington Genome Center server (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker) or Censor from the Genetic Information Research Institute (http://www.girinst.org/Censor_Server-Data_Entry_Forms.html) (Jurka et al. 1996). These programs annotate the repeat sequence content of DNA sequences from humans and rodents. The sequences were then subjected to more detailed analysis by use of MegAlign (DNAStar version 3.1.7 for Windows 3.2). The following parameters were used to select the Ya5 elements to be analyzed: (1) Ya5 had to have all five diagnostic nucleotides (except for the first position, as it is a highly mutable CpG). (2) No truncated Alu elements were included in the analysis. (3) No Alu elements identified as a result of directed cloning strategies designed to identify Alu repeats were included (only those randomly found within larger data sequence). (4) Duplicate Alu elements were eliminated on the basis of flanking sequences. The consensus sequences of the Yb8 and Ya8 subfamilies were used for parallel searches of the three GenBank databases mentioned above. A complete list of the Alu elements identified from the GenBank search is available from M.A.B. or P.L.D. and at http://www.genome.org/cgi/doi/10.1101/gr152300.

To search for putative source genes of the Alu elements that have been associated previously with different diseases, the three GenBank databases were searched by use of the sequence of each individual repeat to identify exact complements (Deininger and Batzer 1999).

DNA Samples

Human DNA samples from the European, African-American, Egyptian, and Greenland native population groups were isolated from peripheral blood lymphocytes (Ausubel et al. 1996) that were available from previous studies (Roy et al. 1999).

Oligonucleotide Primer Design and PCR Amplification

A region composed of ~500 bases of flanking unique DNA sequences adjacent to each Alu repeat were used to design primers for 14 Ya5a2 Alu elements (13 exact matches to consensus, Table 2). PCR primers were designed with the Primer3 software (Whitehead Institute for Biomedical Research) (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The resultant PCR primers were screened against the GenBank nr database for the presence of repetitive elements by use of the BLAST program, and primers that resided within known repetitive elements were discarded and new primers were designed. PCR amplification was carried out in 25-µL reactions with 50–100 ng of target DNA, 40 pM of each oligonucleotide primer, 200 µM dNTPs in 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.4), and Taq DNA polymerase (1.25 units) as recommended by the supplier (Life Technologies). Each sample was subjected to the following amplification cycle: an initial denaturation of 2:30 min at 94°C, 1 min of denaturation at 94°C, 1 min at the annealing temperature, 1 min of extension at 72°C, repeated for 32 cycles, followed by a final extension at 72°C for 10 min. Twenty microliters of each sample was fractionated on a 2% agarose gel with 0.25 µg/ml ethidium bromide. PCR products were directly visualized by UV fluorescence. The human genomic diversity associated with each element was determined by the amplification of 20 individuals from each of 4 populations (African American, Greenland native, European, and Egyptian; 160 total chromosomes). The chromosomal location for elements identified from randomly sequenced large-insert clones was determined by PCR analysis of National Institute of General Medical Sciences (NIGMS) human/rodent somatic cell hybrid mapping panels 1 and 2 (Coriell Institute for Medical Research, Camden, NJ).

Construction of Plasmids

The following constructs were made: p^{-416}Ya5NBC237 (416 bp upstream genomic – Alu – 223 bases downstream); p^{-290}Ya5Ap (290 bp upstream genomic – Alu – 293 bases); and p^{NP}Ya5NBC237 (no upstream vector flank–Alu – 223 bases). Unless otherwise noted, PCR was performed in 20-µL reactions by use of an MJ Research PTC 200 thermal cycler with the following conditions: 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.25 µM primers, 1.5 units of *Taq* polymerase (Promega) at 94°C for 2 min; 94°C for 20 sec, 55°C (annealing temperature) for 20 sec, 72°C for 1 min, for 30 cycles; 72° C for 3 min. To PCR amplify and clone the 864-bp fragment containing the de novo Alu Ya5 from Apert syndrome patient 1 (accession no. AF097344), the following primers were used: forward, 5′-GGTGTGGCCAAAGTGGAGGATGTGTAC-3′ and reverse, 5′-TTATTCAAGGATAAAAGGGGCCATTTC-3′ with an annealing temperature of 50°C; and for the 920-bp fragment containing AluYa5NBC237 (accession no. AL031274) the primers used were: forward, 5′-TTATTCCATTG GTCCTTTCCACCAG-3′ and reverse, 5′-CAGGCAGGGAGG TACTTGTCTCTTG-3′ with an annealing temperature of 55°C.

For the p^{NP}Ya5NBC237, PCR amplification from the clone was done with the same reverse primer and the FAlu5 primer 5′-GGCCGGGCGCGGTGGCTCA-3′.

The final PCR product of the complete construct was cloned into pGEMTeasy Vector System I (Promega). Constructs were subjected to DNA sequence analysis to verify their sequence context. Purified plasmids from the constructs were prepared by alkaline lysis of bacterial cells followed by banding in a CsCl gradient twice. DNA concentrations were determined spectrophotometrically by use of A_{260} and verified by visual examination of ethidium bromide-stained agarose gels.

Alu Transcription in Cell Lines and RNA Analysis

Transient transfections were carried out in the rodent cell line C6 glioma (ATCC CCL107). Monolayers were grown to 50%–70% confluency and transfected with 3 µg of the construct-containing plasmid and 1 µg of control plasmid (p^{7SL}BC1) by use of LipofectAmine Plus (GIBCO Life Sciences) following the manufacturer's recommended protocol. Total RNA was isolated 16–20 h post-transfection.

RNA was extracted from cell lines utilizing the Trizol Reagent (Life Technologies, Inc.) according to the manufacturer's protocol. Equal amounts of RNA were fractionated on a 2% agarose–formaldehyde gel and then transferred to a nylon membrane, Hybond-N (Amersham). Northern blots were hybridized utilizing the following end-labeled oligonucleotide probes: unique-1 5′-TGTGTGTGCCAGTTACCTTG-3′ (complementary to the 3′ end of the control plasmid) and AluYA5-1 5′-ACCGTTTTAGCCGGGAATGGTC-3′ (complementary to Ya5 Alu RNA, but not to 7SL) in 5× SSC, 5× Denhardt's, 1% SDS, and 100 µg/mL herring sperm DNA. Oligonucleotides were end labeled by incorporating [γ-^{32}P]ATP (Amersham) with T4 polynucleotide kinase (New England BioLabs), and subsequently separated from free label by filtration through a Sephadex G-50 column. Blots were washed three times at 45°C with a low stringency buffer (2× SSC and 1% SDS) and subjected to autoradiography or quantified with a FujiFilm FLA-2000 fluorescent image analyzer (Fuji Photo Film Co. LTD). Statistical analysis was performed with the Jandel SigmaStat Statistical Software Version 2, (Jandel Corporation).

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MINIREVIEW

Alu Repeats and Human Disease

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Alu elements have amplified in primate genomes through a RNA-dependent mechanism, termed retroposition, and have reached a copy number in excess of 500,000 copies per human genome. These elements have been proposed to have a number of functions in the human genome, and have certainly had a major impact on genomic architecture. Alu elements continue to amplify at a rate of about one insertion every 200 new births. We have found 16 examples of diseases caused by the insertion of Alu elements, suggesting that they may contribute to about 0.1% of human genetic disorders by this mechanism. The large number of Alu elements within primate genomes also provides abundant opportunities for unequal homologous recombination events. These events often occur intrachromosomally, resulting in deletion or duplication of exons in a gene, but they also can occur interchromosomally, causing more complex chromosomal abnormalities. We have found 33 cases of germline genetic diseases and 16 cases of cancer caused by unequal homologous recombination between Alu repeats. We estimate that this mode of mutagenesis accounts for another 0.3% of human genetic diseases. Between these different mechanisms, Alu elements have not only contributed a great deal to the evolution of the genome but also continue to contribute to a significant portion of human genetic diseases. © 1999 Academic Press

Key Words: **Alu repeats; recombination; insertion mutation; human disease; genetic diversity.**

THE SPREAD OF Alu ELEMENTS IN THE HUMAN GENOME

Alu elements represent a sequence of approximately 300 nucleotides (nt) in length that are transcribed by RNA polymerase III. The RNA transcript is then reverse-transcribed and inserted into a new location in the genome. This RNA-mediated process for making new copies of the element is termed retroposition (1). Different Alu elements in the genome are not identical to one another. It appears that Alu elements that have integrated recently within the genome are quite homogeneous, and almost exact copies of one another (2). However, the older copies have accumulated random mutations, making them typically divergent by 20% or more from one another at the sequence level (3).

Alu elements began inserting early in primate evolution, approximately 65 mya (3). Although there are some related elements in mammals outside of the primate order, they do not have the specific structure of Alu elements. The rate of Alu amplification appears to have reached a maximum between 35 and 60 mya, and is currently amplifying at only 1% of the maximum rate. There are probably only about 2000 Alus specific to the human genome, and not found in chimpanzee and gorilla. Thus, about 99.8% of the 500,000 Alus in the human genome can

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TABLE 1
Alu Insertions and Disease

Locus	Distribution	Subfamily	Disease	Reference
CaR	Familial	Ya4	Hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism	(51)
Mlvi-2	*De novo* (somatic?)	Ya5	Associated with leukemia	(52)
NF1	*De novo*	Ya5	Neurofibromatosis	(53)
PROGINS	About 50%	Ya5	Linked with ovarian carcinoma	(54)
IL2RG	Familial	Ya5	XSCID	(55)
ACE	About 50%	Ya5	Linked with protection from heart disease	(35)
Factor IX	A grandparent	Ya5	Hemophilia	(56)
EYA1	*De novo*	Ya5	Branchio-oto-renal syndrome	(57)
2 × FGFR2	*De novo*	Ya5 & Yb8	Apert's syndrome	(41)
Cholinesterase	One Japanese family	Yb8	Cholinesterase deficiency	(58)
APC	Familial	Yb8	Hereditary desmoid disease	(59)
Btk	Familial	Y	X-linked agammaglobulinaemia	(55)
C1 inhibitor	*De novo*	Y	Complement deficiency	(60)
BRCA2	*De novo*	Y	Breast cancer	(61)
GK	?	Y	Glycerol kinase deficiency	(62)

be found at the same locus in all of the great apes, and 85% of the elements at specific loci can be found in all monkeys. Our best estimates of Alu amplification in the human genome are that there is one new insert in about every 200 new births (4). Although this is well below the peak rate, it is still high enough to represent a significant factor in human mutagenesis.

In addition to random mutations, which occur to Alu elements after their insertion in the genome, there are specific base changes that allow separation of Alu elements into different subfamilies (5–10). The different subfamilies were all inserted at different stages of primate evolution. Almost all of the insertions that have occurred specifically in the human genome come from four closely related subfamilies, Alu Y, Ya5, Ya8, and Yb8. Ya5 and Yb8 inserts represent the majority of the inserts and Alu Y inserts are relatively rare. All of the new inserts belong to a small group of the most recently created subfamilies (see Table 1). This demonstrates that only a small subset of Alus is capable of amplification (11).

Several explanations for the selective amplification of specific subfamilies have been proposed. One likely explanation is that a few specific loci are capable of active amplification, while almost all other loci are not, and that there are almost no such loci in the older subfamilies (11). Alternatively, one has to propose that loci from all subfamilies express, but that the RNAs expressed from the newer subfamilies interact with the retroposition apparatus much better than the older subfamily RNAs (12,13).

Alus AND L1 ELEMENTS

The other major mobile element in the human genome is the L1 element. Alu elements are RNA polymerase III-derived transcripts that have no coding capacity. Thus, they do not code for any proteins that might be involved in the retroposition process. L1 repeats, on the other hand, are much longer and have two open-reading frames (reviewed in (14)). One open-reading frame apparently codes for an RNA-binding protein whose exact function is unknown. The other open-reading frame codes for a protein that includes domains for reverse transcriptase, as well as for an endonuclease that apparently nicks the genome at the site of insertion (15–17). An assay that allows rapid L1 retroposition in cultured cells has been devised recently (18). This assay facilitates the dissection of the details of the L1 retroposition mechanism.

Alu elements must obtain the enzymes for their retroposition from somewhere. In addition, there are striking similarities between the mechanisms of Alu and L1 retroposition that make it very attractive to think that L1 elements may supply the necessary components for Alu retroposition (15,16,19,20). This idea is certainly very attractive, and thus the rate of Alu retroposition may be very dependent on the rate and evolution of L1 elements.

Alu ELEMENTS: FUNCTIONAL ROLE OR A PARASITE'S PARASITE

Alu repeats represent over 5% of the mass of the human genome. They are also spread throughout the entire genome, at varying densities. These observations, along with other specific properties of the Alu elements have led to a number of hypothetical functions for the Alu elements that might explain their ubiquitous presence in primate genomes. Some of the proposed roles involve an everyday function for the cell, while others are of a more sporadic nature.

The first role ever proposed for Alu elements was that they might be origins of DNA replication (21). This role is consistent with their high copy number and dispersed nature, but has not been substantiated by direct experimentation and seems like too important a function to be served by an element that is not found outside of primates.

More recently, evidence has been presented that Alu RNAs may stimulate protein translation by inhibiting a RNA-dependent protein kinase, PKR (22–24). Because Alu RNAs from many loci are stimulated by a number of cellular stresses, such as viral infection and heat shock, this would provide a mechanism by which dispersed sequences may contribute to a cellular process as a group. If this is a function of Alu elements, then it is likely to represent only a slightly modified regulation seen in nonprimate species that is filled by other RNAs or molecules in those species.

Evidence has been presented in yeast that retrotransposable elements may aid in healing chromosomal breaks (25,26). This suggests the possibility that Alu and L1 elements may provide the same role in the human genome.

There are several thoughts concerning the possible roles of Alu elements in the evolution of the human genome. As discussed below, Alu elements can lead to unequal recombination that results in deletion or duplication of sequences. These events could allow duplication of exons and therefore formation of new protein variants. They can also contribute to interchromosomal recombination that may lead to cytogenetic alterations that are involved in human speciation.

There are also several ways in which Alu repeats have been proposed to influence the evolution of gene expression. Because Alu elements are rich in CpG dinucleotides that represent the substrate for genomic methylation, Alu elements represent CpG-rich islands that make up about 30% of the methylation sites in the human genome (24). When an Alu element inserts in a new location in the genome, it introduces a CpG island at that new location. CpG islands have been associated with gene regulation, as well as imprinting of genes, and therefore Alu elements may contribute to the evolution of gene expression and imprinting in the human genome. In addition, Alu elements have been found to carry functional promoter elements for several of the steroid hormone receptors (27,28). Thus, insertion of a new Alu element in the vicinity of a gene may introduce new transcription factor-binding sites that could alter the regulation of gene expression. There are a number of cases where elements that influence gene expression have been mapped to within an Alu repeat (29), demonstrating that the introduction of these sequences can at least occasionally contribute to gene expression and regulation.

Although, there are numerous cases where individual Alu elements have had a positive impact on the human genome, it might be argued that none of them has been confirmed as a function. In this sense we would not define something that happens in a positive sense every few thousand years as being a function, because it would be occurring too sporadically to apply a positive selection for the presence of Alu elements. In addition, studies of individual Alu elements demonstrate that there is essentially no selective pressure on any given Alu repeat, although it is possible that selection does exist for a handful of master elements. Thus, it has been argued that Alu and L1 elements may both represent "selfish" DNA, or DNA that is only working to replicate itself. Selfish DNA may often have negative impacts on the host, but can be tolerated if it does not have too strong an adverse affect. Selfish DNA may also occasionally have positive benefits, but only by chance, and not by functional design. If L1 elements are essentially a parasite within the human genome, and if Alu relies on L1 elements for their amplification process, then one might describe Alu as a "parasite's parasite."

Alus AS MARKERS FOR HUMAN DIVERSITY

Although there is still a question as to whether there is a true functional role for Alu elements in the human genome, Alu elements have proved to be useful in studies of human DNA. The presence of Alu repeats located ubiquitously throughout the human genome, but not in nonprimate species, has allowed detection of human DNA sequences that have been transfected into the cells of other organisms, such as mice. This has been useful in marker-rescue experiments in isolating a number of genes, including the first examples of oncogenes isolated by transforming rodent cell lines with human tumor DNAs (30). More recently, inter-Alu PCR (31,32) has found a broad range of uses in isolating specific human DNA regions from mouse/human hybrid cell lines and other complex sources containing large segments of human DNA.

Recent Alu insertions have also proven useful in a number of human population studies. In particular, there are over 1000 Alu insertions that occurred recently enough to be present only in a subset of human chromosomes. Because there does not seem to be any specific mechanism for removing Alu elements from the genome, once inserted they make a very stable genetic marker (33,34). This observation, along with the extremely low probability that any two recently integrated elements have inserted independently in the same chromosomal location, makes Alu insertions one of the best identical-by-descent (IBD) markers for human evolution studies. Any two individuals sharing an Alu insert almost certainly do so because they share a common ancestor in which the insertion occurred. Table 1 includes an example of an Alu insertion in the angiotensin-converting enzyme (ACE) locus that shows a useful association with protective advantages from heart disease (35). Many other Alu insertion polymorphisms have been identified either in random genomic loci or in specific genes, but without any known disease association. These Alu insertions are easy to assay for their presence or absence in a chromosomal location and have been found to be very powerful markers for human forensic and molecular anthropology studies (36,37).

RETROPOSITION OF Alu ELEMENTS AND DISEASE

Alu elements are located throughout the genome and in almost any location within a gene except those in which they would totally disrupt the function of that gene. Figure 1 illustrates some of the positions relative to a typical gene structure in which Alu may land. Alus landing far enough upstream of a gene may have no influence on that gene's expression. However, Alus landing in or near the promoter/enhancer regions of a gene have been found to influence the expression of specific genes (reviewed in (29)), as well as to have the general potential to add transcription elements, like steroid hormone receptor elements (27,28), to the upstream gene region.

Very few Alu elements are found within the 5′ noncoding or coding regions of exons, presumably because insertions in those locations are too disruptive to gene function. There are a number of instances where Alu elements have been found to be part of the region coding for the carboxy-terminus of a protein product (38,39). Presumably these Alus insert far enough downstream in the coding sequence to result in a new carboxy-terminus that does not disrupt the structure of the protein.

Insertions into the 3′ noncoding regions of genes are found commonly and appear to have few negative affects. Similarly Alus are commonly found in introns, demonstrating that Alu insertions in much of the intronic region do not alter gene function significantly.

The vast majority of Alu insertions that have led to human disease insert into coding exons, or into introns relatively near an exon and presumably alter splicing. Table 1 is a list of the genetic defects that are thought to be caused by Alu insertion events. Not all of these cases have been demonstrated to be directly causative for the disease, but the rarity of Alu insertion events, coupled with the lack of other detectable mutations in these cases, strongly indicates that these are the causative events. The ACE insertion (35,40) is likely to be one example, however, that shows association with disease, but is highly unlikely to be the causative event.

The above examples demonstrate that Alu insertions are capable of causing genetic defects which lead to human disease. Examples of this type are being found at an increasing frequency as the tools for genetic analysis allow more mutations to be detected. Finding 16 Alu-based insertion mutations in the Human Genetic Mutation Database that contains 14374 characterized human mutations suggests that Alu elements contribute to approximately 0.1% of human genetic diseases. This number agrees well with a previous calculation based on a similar dataset of mutations where Alu and L1 insertions were estimated to each contribute approximately 0.075% of human mutations (16). In some cases, the insertional mutagenesis may make detection of mutations easier, biasing the results in favor of the

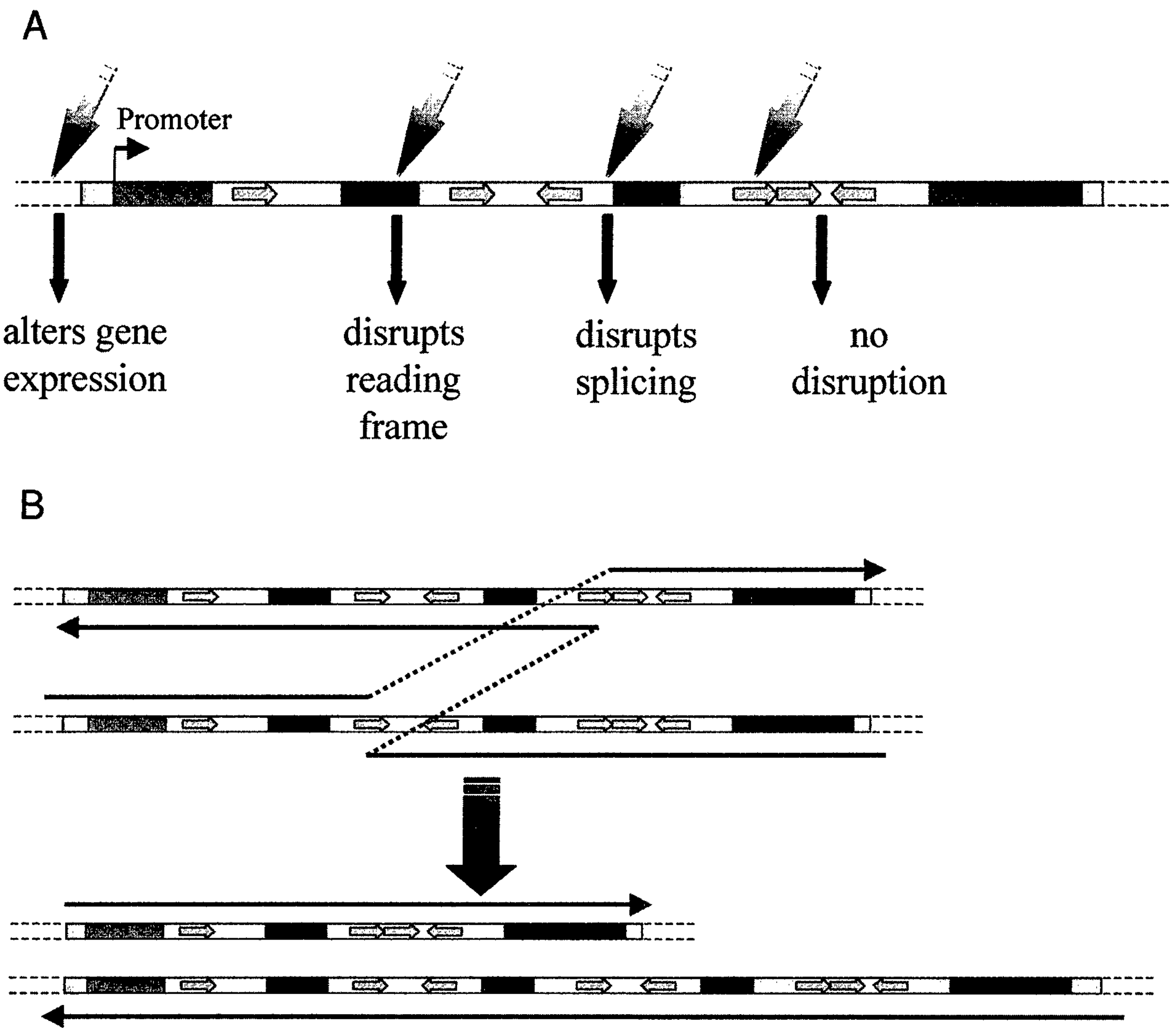

FIG. 1. Schematic of Alu-induced damage to the human genome. Panel A illustrates some of the potential consequences of insertion of a new element in the vicinity of a gene. The colored boxes represent various exons of the gene. The red arrows show existing Alu elements oriented in different directions in the introns of the gene. Depending on the site of insertion, the Alu element has varied probability of impact on the genome as shown. Panel B illustrates an unequal, homologous recombination occurring between two Alu elements in different introns of a gene. The arrows broken by dotted lines show the path of the recombination event. The genes below show that one copy will have a deletion while the other will duplicate gene sequences. Either is likely to be deleterious.

detection of Alu insertions. However, many mutation detection strategies are designed to identify point mutations, particularly in coding regions, and may overlook insertions, particularly if they occur in introns. In addition, many new mobile element insertions may be lethal during embryogenesis. Therefore, it is likely that these estimates of insertion frequencies are underestimates of the true contribution of new Alu insertions to human disease.

We expect that with increasing study of mutations, it will be found that some genetic diseases are more likely than others to result from retroposon insertion. It has certainly been observed that some genes have a much higher Alu repeat content, making it reasonable that they will have a higher frequency of disabling Alu insertions. It has been observed that 2 out of 258 mutations in the FGFR2 gene were caused by Alu insertions (41). This is the first case of multiple Alu insertion mutations being detected associated with a single disease, suggesting that this genetic locus may be more susceptible to retroposon insertions than other regions of the ge-

TABLE 2
Alu/Alu Recombination and Germ-Line Disease

Locus	Distribution	Disease	Reference
8 × LDLR	Kindreds	Hypercholesterolemia	(63–67)
5 × α-globin	Kindreds	α-thalassaemia	(68–71)
5 × C1 inhibitor	Kindred	Angioneurotic adema	(60,72)
Lys Hydrox.	Kindreds	Ehlers-Danlos syndrome	(73)
DMD	Kindred	Duchenne's muscular dystropy	(74)
ADA	One patient	ADA deficiency-SCID	(75)
Apo B	One patient	Hypo-betalipoproteinemia	(76)
Ins. Rec. β	One patient	Insulin-independent diabetes	(77)
α-gal A	One patient	Fabry disease	(78)
HPRT	One patient	Lesch-Nyhan syndrome	(79)
Plat. Fibrinogen Receptor	Kindred	Glanzmann thrombasthenia	(80)
Phosphorylase kinase	One patient	Glycogen storage disease	(81)
GALNS	One patient	Mucopolysaccharidosis type IVA	(82)
Antithrombin	One patient	Thrombophilia	(83)
XY	One patient	XX male	(84)
β-HEXA	Classic form of disease	Tay Sachs	(85)
C3	Kindred	C3 deficiency	(86)
HEXB	27% of patients	Sandhoff's disease	(87)

nome. However, the number of insertions found so far is still fairly low making more definitive conclusions difficult.

RECOMBINATION BETWEEN Alu ELEMENTS ASSOCIATED WITH DISEASE

In addition to the potential impact of Alu element insertions in causing human disease, their dispersion throughout the genome provides ample opportunity for unequal homologous recombination which leads to a much higher level of mutations. Figure 1B illustrates how this unequal recombination can cause insertion or deletion mutations. When recombination occurs between Alu elements on the same chromosome, the result is that there is either duplication or deletion of the sequences between the Alus. Recombination may also occur between Alu elements on different chromosomes, resulting in chromosomal translocations or more complex chromosomal rearrangements.

Table 2 presents a compilation of Alu/Alu recombination events that have contributed to germ-line disease with Alu-based recombination events associated with cancer shown in Table 3. There are many more recombination than insertion events contributing to disease and the table of recombination events is not intended to be exhaustive in presenting all of the Alu/Alu recombinations that have contributed to human disease. In addition, there are many recombination events that occurred between an Alu element and some other non-Alu-related sequence which may have been influenced by the presence of the Alu element (42). Although single Alu elements may contribute specifically to such recombination events, we have made no efforts to collect those data. The mutations resulting from Alu/Alu recombination include 33 mutations that are the result of germ-line recombination and 16 mutations that are the result of somatic events that led to cancer. Based on the calculations in the previous section, the germ-line recombination mutants would represent about 0.3% of mutants characterized. We expect that this number is an underestimate as mutation schemes aimed at detecting point mutants would often be expected to overlook large duplication and deletion events, and we have probably not reported all known Alu/Alu recombinations in the tables.

The data in Tables 2 and 3 show that Alu/Alu recombination events are highly biased towards specific genes. The first to show evidence for this was the LDLR gene, which has at least eight independent cases. It was also reported that these recombination events appeared to take place in a preferred location within the Alu element (42,43). These data suggested that Alu elements may represent hot spots for recombination by a mechanism that was more than simple homologous recombination. Multiple Alu/Alu recombination events have also occurred in the germ line involving two other genes.

TABLE 3
Alu/Alu Recombination and Cancer

Locus	Distribution	Disease	Reference
10 × ALL-1 (MLL)	Somatic	Acute myelogenous leukemia	(88–90)
2 × BRCA1	Somatic and kindreds	Breast cancer	(91,92)
MLH1	Two kindreds	HNPCC	(93)
TRE	Somatic	Ewing's sarcoma	(94)
RB	Common	Association with glioma	(95)
EWS	Subset of Africans	Protective against Ewing sarcoma?	(96)

Even more striking is the preferential recombination seen in somatic recombination. The All-1 gene which participates in a high proportion of acute leukemias is another hotspot for Alu/Alu recombination. This includes intragenic recombination which is the major cause of acute myelogenous leukemia in individuals without a cytogenetic defect, as well as a possible contribution to recombination between the All-1 gene and other chromosomal loci in causing more complex cytogenetic defects associated with leukemia (44–46).

The genes that show high levels of Alu/Alu recombination tend to have a large number of Alu sequences. Although Alu density may help contribute to this recombination, the correlation does not seem to hold up upon analysis of other Alu-rich genes. Therefore, it seems likely that some other factor contributes to the high recombination rates seen in these genes and that the Alu elements are likely to help in that process rather than to be the primary cause.

It has generally been found that longer stretches of sequence identity allow more efficient homologous recombination and that 300 bp of imperfect sequence identity would represent a relatively inefficient target (47). Therefore, as Alu elements accumulate random mutations after integration in the genome their recombination potential gradually decreases. Thus, early in primate evolution when a high proportion of Alu elements were closer matches to one another, Alu/Alu recombination may have contributed even more to the evolution and reshaping of primate genomes.

Based on the above considerations, one might expect the much longer L1 family of elements to contribute significantly to recombination, as well. Surprisingly, we are familiar with only two L1/L1 recombination events in the human genome (48). Therefore, it would appear that: (1) L1 elements are located in less recombinogenic regions of the human genome; (2) the approximately 10-fold lower copy number of L1 elements is more than enough to offset their larger size in terms of probabilities of recombination; (3) some basic property of the Alu elements themselves makes them recombinogenic; or (4) the larger average spacing between L1 elements causes the vast majority of L1/L1 recombination events to be lethal. It is possible that all of these factors may contribute to this observed difference. Transient transfection experiments suggest that the third possibility may not be true since Alu sequences did not recombine more frequently than other control sequences (49). However, in their native chromatin environment, or in specific cell types or cell stimuli *in vivo,* Alus may still respond with higher recombination rates. We believe that the fourth possibility may be the dominant factor, however. The vast majority of Alu/Alu recombination events listed in the tables represent recombination between Alu elements within the same gene. This limits the effect of the recombination to a single gene defect. With their lower copy number and tendency to be located between genes rather than in genes, L1/L1 recombination events are likely either to involve only intergenic regions or to involve a much larger region that may cause defects in several genes simultaneously, resulting in loss of viability.

There is growing evidence that repetitive DNAs contribute to disease either through the mutations they cause during the retroposition process that forms them (16,50) or through recombination processes involving unequal cross-overs of repetitive elements. These recombination events may involve repetitive sequences of various repetition frequencies with the likelihood that longer and more perfect repeats that are near one another probably recombine well, while short, mismatched repeats (like Alu) recombine relatively poorly. However, the extremely high copy number of Alu elements makes them a major factor in the molecular basis of human diseases.

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Recently integrated human Alu repeats: finding needles in the haystack

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Abstract

Alu elements undergo amplification through retroposition and integration into new locations throughout primate genomes. Over 500,000 Alu elements reside in the human genome, making the identification of newly inserted Alu repeats the genomic equivalent of finding needles in the haystack. Here, we present two complementary methods for rapid detection of newly integrated Alu elements. In the first approach we employ computational biology to mine the human genomic DNA sequence databases in order to identify recently integrated Alu elements. The second method is based on an anchor-PCR technique which we term Allele-Specific Alu PCR (ASAP). In this approach, Alu elements are selectively amplified from anchored DNA generating a display or 'fingerprint' of recently integrated Alu elements. Alu insertion polymorphisms are then detected by comparison of the DNA fingerprints generated from different samples. Here, we explore the utility of these methods by applying them to the identification of members of the smallest previously identified subfamily of Alu repeats in the human genome termed Ya8. This subfamily of Alu repeats is composed of about 50 elements within the human genome. Approximately 50% of the Ya8 Alu family members have inserted in the human genome so recently that they are polymorphic, making them useful markers for the study of human evolution.

Introduction

Alu repeats are the most successful class of mobile elements in the human genome. Alu elements spread through the genome via an RNA mediated amplification mechanism termed retroposition and reviewed in Deininger and Batzer, 1993. There are over 500,000 Alu elements in the human genome, which have clearly played a major role in sculpting and/or damaging the genome. Alu elements have contributed to genetic disease, both by the disruption of genes through the insertion of newly retroposed elements and by recombination between Alu elements (reviewed in Deininger & Batzer, 1999). Previous estimates indicate that retroposition of Alu elements contributes to approximately 0.1% of human genetic diseases and recombination between Alu repeats contributes to another 0.3% of genetic diseases (Deininger & Batzer, 1999). Therefore, the spread of the Alu family of mobile elements has generated a significant amount of human genomic variation as well as diseases through recombination-based fluidity as well as insertional mutagenesis.

Alu repeats are distributed rather haphazardly throughout the human genome. Alu elements began expanding in the ancestral primate genomes about 65 mya (Shen, Batzer & Deninger, 1991) reaching a peak amplification between 35 and 60 mya. Presently, Alu elements amplify at a rate that is 100 fold lower than their peak rate, with an estimate of one new Alu insert in every 100–200 births (Deininger & Batzer, 1993, 1995). Evolutionary studies have demonstrated that the majority of evolutionarily recent Alu inserts have specific diagnostic sequence mutations (Deininger & Batzer, 1993, 1995). These mutations have accumulated in Alu elements throughout primate evolution resulting in a hierarchical subfamily structure, or lineage, of Alu repeats. The mutations facilitate the classification of Alu elements into different subfamilies, or clades, of related elements that share common diagnostic mutations (reviewed in Batzer, Schmid & Deninger, 1993; Batzer & Deininger, 1991; Batzer et al., 1996a). Almost all of the recently integrated Alu elements within the human genome belong to one of four closely related subfamilies: Y, Ya5, Ya8, and Yb8, with the majority being Ya5 and Yb8 subfamily members. Collectively, these subfamilies of Alu elements comprise less than 10% of the Alu elements present within the human genome with the Ya5/8 and Yb8 subfamilies collectively accounting for less than half of a percent of all Alu elements. These evolutionarily recent Alu insertions are useful for human population studies, since there appears to be no specific mechanism to remove newly inserted Alu repeats, and the Alu elements are identical by descent with a known ancestral state (Batzer et al., 1991, 1994a, 1996a; Stoneking et al., 1997; Perna et al., 1992).

Previously, it has been technically impossible to determine the full impact of mobile elements on the human genome. The identification of newly inserted Alu elements has been very difficult due to the complexity of detecting one new Alu insertion in a cell that already has 500,000 pre-existing Alu elements. We have previously utilized laborious library screening and sequencing strategies to isolate relatively small numbers of Alu insertion polymorphisms (Arcot et al., 1995a, b, c; Batzer & Deininger 1991a; Batzer et al., 1990, 1991b; 1995), as well as investigating rare 300 bp restriction fragment length polymorphisms (Kass et al., 1994). This makes these studies the genomic equivalent of the search for needles in the haystack. In this paper, we discuss two alternative methods that overcome the inherent difficulties in these experiments, making these studies manageable. First, the availability of large quantities of human genomic DNA sequence provided by the Human Genome Project facilitates genomic database mining for recently integrated Alu elements. This approach should prove useful in determining the chromosome-specific and genome wide dispersal patterns of mobile elements, as well as for the identification of polymorphic mobile element fossils to apply to the study of human population genetics and primate comparative genomics. Secondly, we have developed a PCR-based method that we term Allele-Specific Alu PCR (ASAP). This technique allows us to take advantage of the subfamily-specific diagnostic mutations within Alu mobile elements to isolate and display recently integrated Alu repeats from different DNA samples, allowing for direct comparisons of the Alu content of different genomes or different cells from an individual.

Materials and methods

Cell lines and DNA samples

The cell lines used to isolate human DNA samples were as follows: human (*Homo sapiens*), HeLa (ATCC CCL2); chimpanzee (*Pan troglodytes*), Wes (ATCC CRL1609), gorilla (*Gorilla gorilla*), Ggo-1 (primary gorilla fibroblasts) provided by Dr. Stephen J. O'Brien, National Cancer Institute, Frederick, MD, USA. Cell lines were maintained as directed by the source and DNA isolations were performed using Wizard genomic DNA purification (Promega). Human DNA samples from the European, African American and Greenland native population groups were isolated from peripheral blood lymphocytes (Ausubel et al., 1996) that were available from previous studies (Stoneking et al., 1997). Egyptian samples were collected from throughout the Nile river valley region and DNA from peripheral lymphocytes was prepared using Wizard genomic DNA purification kits (Promega). Human DNA used for ASAP was isolated from peripheral lymphocytes utilizing the super-quick gene method (Analytical Genetic Testing Center).

Computational analyses

A schematic overview summarizing the computational analyses of recently integrated Alu elements is shown in Figure 1. Initial screening of the GenBank non-redundant and high throughput genomic sequence (HTGS) databases was performed using the basic local

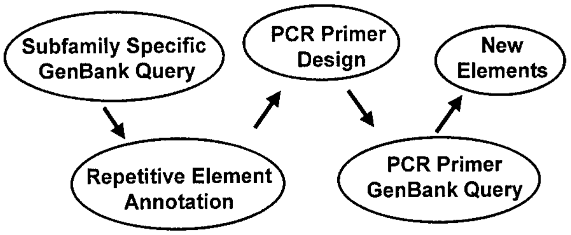
Subfamily Specific GenBank Query
PCR Primer Design
New Elements
Repetitive Element Annotation
PCR Primer GenBank Query

Figure 1. Computational analysis of repetitive elements. The flow chart shows the computational tools utilized for the identification and analysis of recently integrated Ya8 Alu family members. The process begins with BLAST searches of the non-redundant and high-throughput genomic sequence databases. Subsequently sequences (about 1000 nucleotides) adjacent to the matches with 100% identity to the query sequence are annotated using the RepeatMasker2 or Censor server. Following sequence annotation, oligonucleotide primers complementary to the unique DNA sequences adjacent to each element are designed using the Primer3 web server. The oligonucleotides designed using Primer3 are then subjected to a second BLAST search to determine if they reside in other repetitive elements, and subsequently they are used for PCR based analyses of individual mobile elements.

alignment search tool (BLAST) (Altschul et al., 1990) available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The database was searched for exact complements to the oligonucleotide 5′-ACTAAAACTACAAAAAATAG-3′ that is an exact match to a portion of the Alu Ya8 subfamily consensus sequence containing unique diagnostic mutations. Sequences that were exact complements to the oligonucleotide were then subjected to more detailed annotation. A region composed of 1000 bases of flanking DNA sequence directly adjacent to the sequences identified from the databases that matched the initial GenBank BLAST query were subjected to annotation using either RepeatMasker2 from the University of Washington Genome Center server (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker) or Censor from the Genetic Information Research Institute (http://www.girinst.org/Censor_Server-Data_Entry_Form_s.html) (Jurka et al., 1996). These programs annotate the repeat sequence content of DNA sequences from humans and rodents.

Primer design and PCR amplification

PCR primers were designed from flanking unique DNA sequences adjacent to individual Ya8 Alu elements using the Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The resultant PCR primers were screened against the GenBank non-redundant database for the presence of repetitive elements using the BLAST program, and primers that resided within known repetitive elements were discarded and new primers were designed. PCR amplification was carried out in 25 μl reactions using 50–100 ng of target DNA, 40 pM of each oligonucleotide primer, 200 μM dNTPs in 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl pH 8.4 and Taq® DNA polymerase (1.25 U) as recommended by the supplier (Life Technologies). Each sample was subjected to the following amplification cycle: an initial denaturation of 2:30 min at 94°C, 1 min of denaturation at 94°C, 1 min at the annealing temperature, 1 min of extension at 72°C, repeated for 32 cycles, followed by a final extension at 72°C for 10 min. Twenty microliters of each sample was fractionated on a 2% agarose gel with 0.25 μg/ml ethidium bromide. PCR products were directly visualized using UV fluorescence. The sequences of the oligonucleotide primers, annealing temperatures, PCR product sizes and chromosomal locations are shown in Table 1. Phylogenetic analysis of all the Alu elements listed in Table 1 was determined by PCR amplification of human and non-human primate DNA samples. The human genomic diversity associated with each element was determined by the amplification of 20 individuals from each of four populations (African–American, Greenland Native, European and Egyptian) (160 total chromosomes). The chromosomal location of Alu repeats identified from clones that had not been previously mapped was determined by PCR amplification of National Institute of General Medical Sciences (NIGMS) human/rodent somatic cell hybrid mapping panel 2 (Coriell Institute for Medical Research, Camden, NJ).

Allele-Specific Alu PCR (ASAP)

We used a modification of the IRE-Bubble PCR method (Munroe et al., 1994), utilizing the same amplification (anchor) primer, but altering the annealed anchor/linker primers. The annealed linkers formed a Y instead of a bubble to avoid end-to-end ligation. Also, instead of blunt-end digestion, genomic DNA was digested with *Mse*I; that cuts 5′-T′TAA-3′ and does not cut in the Alu consensus. Otherwise the genomic-anchor ligations were prepared according to (Munroe et al., 1994). The annealed linker primers are: MSET: 5′-TAGAAGGAGAGGACGCTGTCTGTCGAAGG-3′ and MSEB: 5′-GAGCGAATTCGTCAACATAGCATTTCTGTCCTCTCC TTC-3′. The amplification (linker) primer is: LNP:

Table 1. Ya8 accession numbers, primers, location, and product sizes

Name	Accession #	5′ Primer sequence (5′-3′)	3′ Primer sequence (5′-3′)	A.T.[1]	Chromosomal location[2]	Product size[3]	
						Filled	Empty
Ya8NBC1	AC006959	CCTGCTGACATTTAGAAATGACTCT	ATATACAAGTCATCAGATGGGGACAC	60°C	5	504	293
Ya8NBC2	AC006556	GCCTGTGTACCTCCTTTAAATATCTTG	CTCAAAAACTGGAGCAGGAGTAA	50°C	21	503	242
Ya8NBC3	AC006989	GGTGGTCATCCATATACTATCTCATAGG	AGAGTTCTGGAAAAGTTGACAGGAT	55°C	Y/X[4]	498	178
Ya8NBC4	AL049871	CATTCCACCCTGTCAGCATT	GCTTTGGAAGTAGGCAGGTTAC	60°C	14	536	204
Ya8NBC6	AC004066	ACTTAGCTTTGAGTATTTTTCTGAACTATC	CTAAAATGGAGGTACCGATATACTTTATTA	60°C	4	470	132
Ya8NBC8	AL034422	GGATCACAAACCTAAATGAAAGAGGTAA	CCGTCTCAAAACAAACAGACAAATA	60°C	20	501	155
Ya8NBC10	AC004893	GGATTACTTTGATGAAAATATCTTAGTAGG	AACTGGATTGTACTTTGAAGACCAC	60°C	7	757	371
Ya8NBC11	AC007688	GAGTGCCTATTATGTGTTAGGTACTTTGCT	ACTCTCACTAGATTATAAGCCCCATAAGGA	60°C	12	419	105
Ya8NBC12	AL022302	CATCTTAAAAGACATTAGAAAAGTACACAG	CTGGCCACTTAGTATATTTTCAATCAG	60°C	22	530	211
Ya8NBC13	AL008722	CCATTTTCTATAAGAAGGCTTCACC	AAAGTAATGTGAAAGTATTGGAGAAGAGAT	60°C	22	402	77
Ya8NBC14	AF094481	GAATCTCTATCTCTGACACTAGCCACT	GGCAACAAGTCTGATGAATACTTAAAGGAG	60°C	3	500	189
Ya8NBC15	AF179296	CTCTACAGTACAGATGAGAAAGTACAGACA	CGCCTTGCTAGATTTCTTTCTAATG	60°C	8	620	299
Ya8NBC17	AC005205	CTAGTTCCCACATACCGAAAACAC	CCTGTCTCGTTCAGTCTTCTTTG	58°C	19	501	155
Ya5NBC60	AC006553	CAGTCCATAGCAGTCATGGTAAATAAG	AAGTCTATACCGGTTACCTCTTTCTT	58°C	4	456	149

[1] Amplification of each locus required 2:30 min @ 94°C initial denaturing, and 32 cycles for 1 min 94°C, 1 min Annealing Temperature (A.T.) and 1 min elongation at 72°C, with a final extension time of 10 min at 72°C.

[2] Chromosomal location determined from Accession information or by PCR analysis of monochromosomal hybrid cell lines.

[3] Empty product sizes calculated by removing the Alu element and one direct repeat from the filled sites that were identified.

[4] Ya8NBC3 is located in the pseudoautosomal region of the X and Y chromosome.

5′GAATTCGTCAACATAGCATTTCT-3′. We placed an *Eco*RI site at the 5′ end of the primer for the option of cloning PCR products into cloning sites of common vectors. No bands are observed on a gel when this primer is used alone with the anchored template at an annealing temperature of 55°C.

Unless otherwise noted, PCR conditions (for all ASAP reactions) were performed in 20 μl using a Perkin-Elmer 9600 thermal cycler with the following conditions: 1 × Promega buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.25 μM primers, 1.5 U Taq polymerase (Promega) at 94°C – 2 min, 94°C – 20 s, 62°C – 20 s, 72°C – 1 min, 10 s, for 5 cycles; 94°C – 20 s, 55°C – 20 s, 72°C – 1 min, 10 s, for 25 cycles; 72°C – 3 min. Nested Alu primers were used that move along the Alu in an upstream direction as follows: ASII (Ya5-specific): 5′-CTGGAGTGCAGTGGCGG-3′; HS18R (Ya8-specific): 5′-CTCAGCCTCCCAAGTAGCTA-3′; HS16R (Ya8-specific): 5′-CGCCCGGCTATTTTT-GTAG-3′.

The ASII primer has Ya5 diagnostic nucleotides (present in both Ya5 and Ya8 subfamilies). In the first round of PCR, stock genomic DNA (2.4 ng anchored DNA) was used as the template. For subsequent rounds of amplification, PCR products were purified through microcon-30 (Amicon) columns using two centrifuge spins following the addition of 400 μl of water. For the second round of amplification, 1 μl of microcon-purified first round PCR reaction was used as the template, and for the third round 1 μl of microcon-purified second round PCR products was used. For display analysis (see below) the PCR products were ‘equalized’ in volume following microcon purification.

Display of anchor-Alu PCR products

Third round PCR was performed utilizing a 5′ end-labeled primer incorporating [γ-^{32}P] ATP (Amersham) with T4 polynucleotide kinase (New England BioLabs). PCR conditions were as above with the exception of using 0.188 μM of each Ya8 and LNP cold primers and 0.075 μM of end-labeled Ya8 primer. Anchor-PCR and end-labeled molecular weight markers (ϕX174 DNA digested with *Hinf*I; Promega) were separated by electrophoresis on denaturing 5% long ranger (AT Biochem) gels, and examined by autoradiography following exposure to Amersham Hyperfilm at room temperature. DNA samples from different ethnic groups were utilized in the display to identify variants that resulted from recent Alu insertion events (polymorphism).

Verification of PCR generated DNA fragments as Ya8 products

Gels were aligned to autoradiographs by either small cuts in various parts of the gel, or placement of low-level radioactive dye on the gel prior to re-exposure. Bands were then sliced out of the gels, placed in 200 μl of water and eluted by heating at 65°C for 15 min. Samples were re-amplified with third round PCR primers, cloned and sequenced as described above. Following verification these bands were amplified by the third round primer pair, new nested oligonucleotides based on the flanking unique sequences were designed to move, by PCR, downstream through the Alu element to the opposite flank. Annealing temperatures were adjusted to reflect the Tm of the oligonucleotide primers. Generally two or three rounds of PCR were utilized to obtain the 3′ flanking sequences of the Alu. These PCR products were also cloned and sequenced in the same manner.

Results

We present two complementary approaches that facilitate rapid detection of newly inserted Alu elements from the human genome. First, computational analyses of human genomic DNA sequences from the GenBank database are used in the identification of recently integrated Alu elements. Second, allele-specific PCR amplification is used for the selective enrichment of young Alu elements. To compare and contrast these two approaches, we present the data obtained when these methods are applied to the identification of members of the Ya8 Alu subfamily, the smallest previously reported subfamily of Alu repeats in the human genome.

Copy number and sequence diversity

In order to estimate the copy number of Ya8 Alu family members, we determined the number of exact matches to our subfamily specific oligonucleotide query sequence as a proportion of the human genome that had been sequenced in the non-redundant database. We obtained 27 matches to the subfamily specific query sequence from the non-redundant database. Upon further sequence annotation using the RepeatMasker2 web site, five matched the Ya8 Alus previously sequenced in our laboratories (Batzer et al., 1990; Batzer & Deininger, 1991; Batzer et al., 1995). Eight of the elements identified in the search were classified as Alu Sx subfamily members, and two matched the TPA 25 Ya8 Alu family member. A total of 13 independent Ya8 Alu elements were identified from the search of the non-redundant database that were not sequenced as part of a project to specifically identify recently integrated Alu elements. The non-redundant database contained 45.3% human DNA sequences for a total of 590,140,703 bases of human sequence on the date of the search. The estimated size of the Ya8 subfamily is (3 × 10^9 bp/590, 140, 703 bp) × 13 unique Ya8 matches = 66 Ya8 subfamily members. This estimate compares favorably with that of 50 previously reported based upon library screening, restriction digestion or Southern blotting (Batzer et al., 1995). An additional six matches to the Ya8 subfamily query sequence were identified in the HTGS. One of these elements was an Alu Sq subfamily member, while a second element was a duplicate copy of Ya8NBC60. PCR analyses of two elements identified in the high throughput database, Ya8NBC7 and Ya8NBC16 (GenBank accession numbers AL109937 and AC008944), were inconclusive and these elements were eliminated from further analysis. These two elements were identified from low pass first sequence runs in the HTGS database. It is not surprising that the PCR analyses failed, since the DNA sequences are of presumably lower quality than finished DNA sequences contained in the non-redundant database. However, two additional Ya8 Alu repeats (Ya8NBC8 and Ya8NBC15) were identified in the HTGS database and subjected to further analysis.

A comparison of the nucleotide sequences of all of the Ya8 Alu family members is shown in Figure 2. In order to determine the time of origin for the Ya8 subfamily we divided the nucleotide substitutions within the elements into those that have occurred in CpG dinucleotides and those that have occurred in non-CpG positions. The distinction between types of mutations is made because the CpG dinucleotides mutate at a rate that is about 10 times faster than non-CpG positions (Labuda & Striker, 1989; Batzer et al., 1990) as a result of the deamination of 5-methylcytosine (Bird, 1980). A total of 14 non-CpG mutations and 8 CpG mutations occurred within the 14 Alu Ya8 subfamily members reported. Using a neutral rate of evolution for primate intervening DNA sequences of 0.15% per million years (Miyamoto, Slightom & Goodman, 1987) and the non-CpG mutation rate of 0.413% (14/3388 using only non-CpG bases) within the 14 Ya8 Alu elements yields an estimated age of 2.75 million years old for the Ya8 subfamily members. This estimate of age is somewhat higher than the 660,000 years previously reported (Batzer et al., 1995). However, the previous study of Ya8 Alu family members involved only four elements making the calculated age more subject to random statistical fluctuation. This estimate is also consistent with the expansion of a family of mobile elements that began around the time humans

```
AluYa8 Con   GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGG  59
AluYa8NBC1   ...........................................................
AluYa8NBC2   ..-----------------------------------------................
AluYa8NBC3   ...........................................................
AluYa8NBC4   ...........................................................
AluYa8NBC6   ...........................................................
AluYa8NBC8   ...........................................................
AluYa8NBC10  ...........................................................
AluYa8NBC11  .---------------------------------.........................
AluYa8NBC12  ...........................................................
AluYa8NBC13  ...........................................................
AluYa8NBC14  .......G.....T...........G.................................
AluYa8NBC15  ...........................................................
AluYa8NBC17  ...........................................................
AluYa8NBC60  ...........................................................

AluYa8 Con   ATCACGAGGTCAGGAGATCGAGACCATCCCGGCTAAAACGGTGAAACCCCGTCTCTACT  118
AluYa8NBC1   .................................................T.........
AluYa8NBC2   ...........................................................
AluYa8NBC3   .............................A.............................
AluYa8NBC4   .......................................A...................
AluYa8NBC6   ...........................................................
AluYa8NBC8   ...........................................................
AluYa8NBC10  ...........................................................
AluYa8NBC11  ...........................................................
AluYa8NBC12  ...........................................................
AluYa8NBC13  ...........................................................
AluYa8NBC14  ...........................................................
AluYa8NBC15  .......................................T...................
AluYa8NBC17  ...........................................................
AluYa8NBC60  .....A.....................................................

AluYa8 Con   AAAACTACAAAAAATAGCCGGGCGTAGTGGCGGGCGCCTGTAGTCCTAGCTACTTGGGA  177
AluYa8NBC1   ...........................................................
AluYa8NBC2   ...........................................................
AluYa8NBC3   ...........................................................
AluYa8NBC4   ...........................................................
AluYa8NBC6   ...........................................................
AluYa8NBC8   ..............................................C............
AluYa8NBC10  ...........................................................
AluYa8NBC11  ...........................................................
AluYa8NBC12  ..............................................C............
AluYa8NBC13  ...........................................................
AluYa8NBC14  ...........................................................
AluYa8NBC15  ...........................................................
AluYa8NBC17  ...........................................................
AluYa8NBC60  ...........................................................

AluYa8 Con   GGCTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAGATCCC  237
AluYa8NBC1   ............................................................
AluYa8NBC2   ............................................................
AluYa8NBC3   ............................................................
AluYa8NBC4   ............................................................
AluYa8NBC6   ............................................................
AluYa8NBC8   ............................................................
AluYa8NBC10  ..........................................................G.
AluYa8NBC11  ............................................................
AluYa8NBC12  .............................A............A.................
AluYa8NBC13  ............................................................
AluYa8NBC14  ............................................................
AluYa8NBC15  ..........................................................G.
AluYa8NBC17  ............................................................
AluYa8NBC60  ............................................................

AluYa8 Con   GCCACTGCACTCCAGCCTGGGCGACAGAGCGAGACTCCGTCTCAAAAAAAAAA        290
AluYa8NBC1   ............................................--.......
AluYa8NBC2   ..G...........................A.............--.......
AluYa8NBC3   A...........................................GA.......
AluYa8NBC4   ............................................--.......
AluYa8NBC6   ............................................--.......
AluYa8NBC8   ............................................--.......
AluYa8NBC10  ............................................--.......
AluYa8NBC11  ............................................--.......
AluYa8NBC12  ............................................--.......
AluYa8NBC13  ..............C.............................--.......
AluYa8NBC14  ............................................--.......
AluYa8NBC15  ............................................--.......
AluYa8NBC17  ......................................A.....--.......
AluYa8NBC60  ............................................--.......
```

Figure 2. Multiple alignment of Ya8 subfamily members. The Ya8 subfamily consensus (con) is derived from the most common nucleotide found at each position within the subfamily members. Nucleotide substitutions at each position are indicated with the appropriate nucleotide. Deletions are marked by '–'.

Ya8NBC1	AAGAGGGGGAGAG	[Alu]	A_{18}	AAGAGGGGGAGAG
Ya8NBC2	GGA	[Alu]	$A_{16}CA_{4}$	TGGA
Ya8NBC3	GAAGAAGTTTTGC	[Alu]	$ACA_{21}CA_{2}$	GAAGAAGTTTTGC
Ya8NBC4	CGACAATTT	[Alu]	$A_{17}CA_{13}CA_{10}$	CCGACAATTT
Ya8NBC6	AAATTTAAAATATT	[Alu]	A_{44}	AAATTTAAAATATT
Ya8NBC8	AAGAAAATATAGGCATA	[Alu]	$A_{11}CA_{14}CA_{23}$	AAGAAAATATAGGCATA
Ya8NBC10	AAAAATAAAATA	[Alu]	A_{4}	AAAAATAAAATA
Ya8NBC11	AAGGAATGAGACTG	[Alu]	A_{20}	AAGGAATGAGACTG
Ya8NBC12	AAAGTTCTTTGCA	[Alu]	A_{27}	AAAGTTCTTTGCA
Ya8NBC13	AAGAAGGCTTCACCAG	[Alu]	A_{30}	AAGAAGGCTTCACCAG
Ya8NBC14	ATCCC	[Alu]	A_{26}	ATCCC
Ya8NBC15	AGAACCACCAGGAA	[Alu]	A_{27}	AGAACCACCAGGAA
Ya8NBC17	AAGGAATCTC	[Alu]	A_{17}	AAGGAATCTC
Ya8NBC60	GGTAAATAAGCTTTCTT	[Alu]	A_{25}	GGTAAATAAGCTTTCTT

Figure 3. Nucleotide sequences flanking Ya8 subfamily members. Nucleotide sequences flanking the Ya8 Alu family members are shown. Nucleotides encompassed in the direct repeats are underlined. The length of the oligo-dA rich tail is denoted by an (A) and a subscript indicating the number of adenine residues.

and African apes diverged, which is thought to have occurred 4–6 million years ago (Miyamoto, Slightom & Goodman, 1987).

Inspection of the nucleotide sequences flanking each Ya8 Alu family member shows that all of the elements were flanked by short perfect direct repeats (Figure 3). The direct repeats ranged in size from 3–17 nucleotides. These direct repeats are fairly typical of recently integrated Alu family members. Two of the Alu Ya8 Alu family members contained 5′ truncations (Ya8NBC2 and Ya8NBC11). Since Ya8NBC2 and Ya8NBC11 are both flanked by perfect direct repeats the truncations in these elements probably occurred as a result of incomplete reverse transcription or improper integration into the genome rather than by post-integration instability. All of the Ya8 Alu family members had oligo-dA rich tails that ranged in length from a minimum of four nucleotides to over 40 bases in length. It is also interesting to note that the 3′ oligo-dA rich tails of several of the elements (Ya8NBC2, Ya8NBC3, Ya8NBC4, and Ya8NBC8) have accumulated random mutations beginning the process of the formation of simple sequence repeats of varied sequence complexity. The oligo-dA rich tails and middle A rich regions of Alu elements have previously been shown to serve as nuclei for the genesis of simple sequence repeats (Arcot et al., 1995b).

Phylogenetic distribution, and chromosomal location

The phylogenetic distribution of each Ya8 Alu element was determined by amplifying genomic DNA from two non-human primates (common chimpanzee and gorilla). All of the Ya8 Alu family members were absent from the genomes of non-human primates. This suggests that the majority of these elements dispersed within the human genome sometime after the human and African ape divergence. The chromosomal location of each Ya8 Alu element was taken directly from the GenBank database entry or determined by PCR amplification of human/rodent monochromosomal hybrid cell line DNA samples (Table 1).

Human genomic diversity

In order to determine the human genomic variation associated with each of the Ya8 Alu family members we subjected a panel of human DNA samples to PCR amplification (Table 2). The panel was composed of 20 individuals of European origin, African Americans, Greenland Natives and Egyptians for a total of 80 individuals (160 chromosomes). Using this approach four of the 14 (Ya8NBC8, Ya8NBC10, Ya8NBC14 and Ya8NBC15) Alu Ya8 subfamily members were monomorphic for the presence of the Alu element suggesting that these elements integrated in the genome prior to the radiation of modern humans from Africa. Three of the elements (Ya8NBC2, Ya8NBC13 and Ya8NBC17) appeared heterozygous in all of the individuals that were analyzed, suggesting that they had integrated into previously undefined repetitive elements within the human genome as previously described (Batzer et al., 1991). However, the remaining seven elements were polymorphic for the presence of an Alu repeat within the genomes of the test panel individuals (Table 2). The unbiased heterozygosity values (corrected for small sample sizes) for these polymorphic Alu insertions were variable, and approached the theoretical maximum in several cases. This is quite interesting since the maximum uncorrected heterozygosity for these biallelic elements is 50% and suggests that these Alu insertion polymorphisms will make excellent markers for the study of human population genetics. In addition, 50% of the randomly identified Ya8 Alu family members are polymorphic. These results suggest that the Ya8 subfamily is younger than either the Ya5 (from which Ya8 was derived) or Yb8 Alu subfamilies, since only 25% of the members of these Alu subfamilies are polymorphic in the human genome (Batzer et al., 1995).

Allele-Specific Alu PCR (ASAP)

Although database screening is extremely efficient for identifying recent Alu elements, it will not allow identification of new elements from genomes not included in the sequencing efforts. Our primary objective with the ASAP technique is to rapidly identify newly inserted Alu elements from a background of 500,000 older Alus. To accomplish this feat, we utilized a

Table 2. Alu Ya8 associated human genomic diversity

Elements	African American					Greenland natives					European					Egyptian					
	Genotypes			*f* Alu	Het	Genotypes			*f* Alu	Het	Genotypes			*f* Alu	Het	Genotypes			*f* Alu	Het[1]	Avg Het[2]
	+/+	+/−	−/−			+/+	+/−	−/−			+/+	+/−	−/−			+/+	+/−	−/−			
Ya8NBC1	10	2	7	0.58	0.50	5	0	9	0.36	0.35	10	5	1	0.78	0.48	8	0	10	0.44	0.51	0.46
Ya8NBC3	0	12	7	0.32	0.44	0	6	14	0.15	0.44	0	12	7	0.32	0.26	0	9	10	0.24	0.51	0.41
Ya8NBC4	1	4	13	0.17	0.29	6	0	7	0.46	0.51	8	5	6	0.55	0.52	18	0	1	0.95	0.10	0.35
Ya8NBC6	8	2	6	0.56	0.51	11	0	3	0.85	0.00	16	0	0	1.00	0.35	12	2	3	0.76	0.37	0.31
Ya8NBC11	13	2	0	0.93	0.13	12	0	0	1.00	0.09	10	1	0	0.95	0.00	13	3	0	0.91	0.18	0.10
Ya8NBC12	17	0	0	1.00	0.00	19	0	0	1.00	0.05	18	1	0	0.97	0.00	17	0	0	1.00	0.00	0.01
Ya8NBC60	6	9	3	0.58	0.50	6	7	5	0.53	0.51	5	9	3	0.56	0.51	10	5	4	0.66	0.46	0.49

[1] This is the unbiased heterozygosity.

[2] Average heterozygosity is the average of the population heterozygosity.

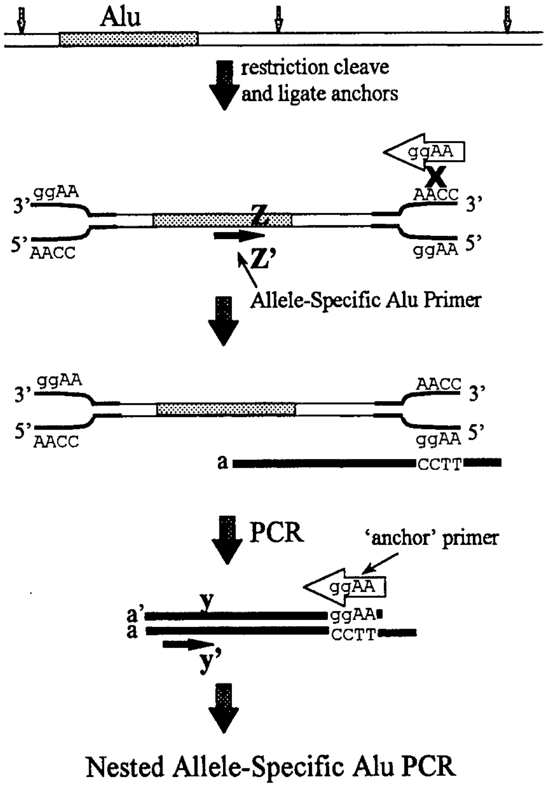

Figure 4. The Allele-Specific Alu PCR (ASAP) anchor strategy. Schematic diagram of the technique for the isolation of a designated subset of Alu repeats based on a modification of the IRE-bubble PCR technique (Munroe et al., 1994). The shaded rectangle represents an Alu sequence in genomic DNA. The *Mse*I (or an alternative restriction enzyme) cleaves in unique sequences flanking the Alu repeat (small arrows). The anchors with the complementary *Mse*I site are ligated. The anchors are designed so that the two oligonucleotide strands base-pair only at the *Mse*I site end, but not at the other end (represented here schematically with four arbitrary bases). PCR is initiated using an allele-specific Alu primer (Z′). The anchor primer will not be able to base pair preventing anchor-to-anchor amplification. Only those fragments (a) generated by the Alu primer are available for amplification by the anchor primer. The amplified product (a and a′) provides a template for nested PCR (primer y′) to further decrease the background.

modification of the IRE-bubble PCR technique (Munroe et al., 1994). The procedure utilizes an anchored PCR strategy (Figure 4) in which genomic DNA is cleaved with an enzyme that does not cleave within the Alu repeat. The modified anchor is then ligated to the fragment ends. This anchor will only allow PCR amplification if a primer first primes within the fragment and replicates across the linker eliminating any problems with amplification from anchor to anchor. We take advantage of the base changes that identify the younger Alu subfamily members (Batzer et al., 1996b; Batzer & Deininger, 1991). In addition, this allows the selective enrichment for a smaller fraction of the Alu elements from the genome, as there are only 1000 Ya5 and 1000 Yb8 Alu repeats and approximately 50 Ya8 Alu family members in the human genome (Batzer et al., 1995). We gain the specificity for the recent inserts by using a PCR primer that matches the particular Alu subfamily with the diagnostic positions at its 3′ end. Each amplification will extend from a specific Alu subfamily member through its upstream flanking sequences to the randomly located flanking restriction site. The numerous older Alu repeats have accumulated many mutations and may compete for the PCR primers with the Ya5/8 elements. Therefore, although the first amplification provides a great deal of subfamily specificity, we then carry out a 'nested' reaction using a second allele-specific primer to improve the specificity, followed by a third round with another allele-specific primer. In theory, we can utilize primers for each of the 5–8 diagnostic mutations in a subfamily.

In the example presented in this paper, we focused our attention on the identification and display of the lower copy number Alu Ya8 subfamily. Also, to better display the results, we used nested primers in the upstream direction of Ya8 to avoid amplification problems through the A-rich tail. Using the primers described in the Materials and methods section, by the third round of PCR, we were able to visualize discrete DNA fragments on an agarose gel (data not shown). The size range of these fragments appeared to be between 150 bp and 800 bp. To enhance this display, we chose an alternative method of electrophoretic separation and end-labeled the nested primer to further minimize background (see below). To verify these were Ya8 repeats, we directly cloned the third round PCR products and sequenced them. Partial or complete sequences of these products, using vector primers in both directions, demonstrated all 12 clones to be amplified by the Alu-anchor primer pair, although in one case the unique linker sequence was imprecise. All these elements contained the Ya5/8 diagnostic nucleotides (There were no further upstream diagnostics to declare these as Ya8 elements.).

For eight of the 12 isolated clones, there were between 12 and 18 unique nucleotides between the linker and the Alu (or truncated Alu) sequences. Since Alu elements preferentially insert into A-T rich regions (Daniels & Deininger, 1985) and *Mse*I cuts at the sequence TTAA, then this result is not surprising. The advantage of using *Mse*I for the restriction digestion is that most of the Alu-linker products are small enough to be amplified. Although it would be difficult to perform nested PCR in the opposite direction with those few A-T rich nucleotides, searching GenBank using the BLAST program with the obtained flanking unique DNA sequences as the query may in some cases identify the rest of the genomic sequence for each Alu element. This will provide the Alu location with both its flanking sequences. Flanking unique sequence primers can then be designed and the Alu polymorphism can then be confirmed using other human DNA sources. Once the polymorphism is confirmed subsequent population studies can be performed.

Display and rapid identification of Ya8 associated variants

To alleviate the need for testing every Ya8 element obtained by this assay, we chose to end-label the third round nested PCR primer to enable a display of individual Ya8 repeats following electrophoretic separation and autoradiography. Observed variations may be due to primer mismatch, genomic rearrangements, small insertion/deletions or Alu based insertion/deletions (I/D).

We carried out the procedure with four different individuals to discern which bands represent variants (Figure 5), and to effectively display variants as DNA fingerprints. We obtained about 40 bands per individual from a single reaction. Among the four individuals analyzed, about one half of the bands appeared variant (Figure 5). We have developed a potent method for the generation of Ya8 associated DNA fingerprints that is in reasonable agreement with the database mining approach and seems to display the majority of Alu subfamily members. This necessitated addressing what proportion of the fragments generated were the result of the presence of a Ya8 Alu element and whether the lack of the same band in another individual represented an Alu insertion polymorphism. We chose 12 bands to re-amplify and verify as Ya5/8 elements. Those bands that appeared variant were analyzed for Alu insertion polymorphisms. Other bands were selected for future testing of dimorphisms as these individual Ya8 elements may display variation among other people/populations. Occasionally, upon re-amplification from the isolated band, we obtained background products and therefore, generally more than one clone was sequenced. Of the 12 isolated bands (Figure 5) nine were verified as precisely amplified HS16R-LNP products. Two others each contained a Ya5/8 Alu, one randomly amplified by HS16R (anc-8) in lieu of the linker primer, while anc-3 contained sequences downstream of HS16R. Anc14 apparently was an amplified J (PS) Alu element (data not shown). Therefore, this demonstrates the majority of the bands visualized on the autoradiograph are AluYa5/8 repeats and most probably Ya8. The numerous bands at about 178 nt coincide with our previous finding that many of the products will have between 12 and 18 unique sequences. Of the nine bands where we attempted to obtain the opposite flank by nested anchored PCR, we reached the opposite (downstream) flank of the Alu for

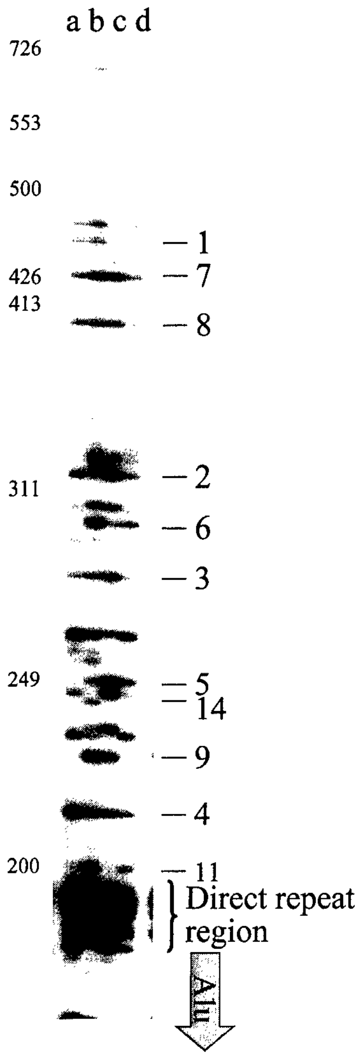

Figure 5. DNA fingerprints of unrelated individuals based on anchored-Alu PCR. Individual bands are numbered for identification purposes. Fragment lengths are shown in nucleotides to the left. DNA samples used are of Caucasian (lane a), Hispanic (lane b), Hindu–Indian (lane c) and Chinese (lane d) descent.

three of them (anc-5, anc-6, anc-4). In some cases the amount of unique sequence was too small to employ nested primers, and in some cases there was a high level of A-T richness. In one case we merely got a non-specific product. All three sequences obtained were authentic Ya8 Alu elements based on the diagnostic nucleotide positions and the high level of conservation of the sequence in relation to the consensus. This demonstrates the successful nature of our protocol to select for this subfamily of repeats amongst a large background of Alu repeats.

When 'crossing' the anc-5 Alu by nested PCR using four individuals (not all identical to Figure 5), we found a correspondence between the generation of a distinct band among the individuals that also had the anc-5 band on an autoradiograph. However, we obtained a short 3′ flank of 12 nucleotides that proved difficult in amplifying DNA from various individuals with unique flanks. It is still possible that this variant represents an I/D event. Besides anc-5, anc-6 also appeared polymorphic on the autoradiograph, although anc-4 did not. However, since we had both flanks, for these Alu elements, we developed primers to rapidly assess various individuals for an insertion variant. For anc-6, one of a few different primer sets worked well, yielding the band of expected size, although also generating a few non-specific bands. However, a band was present for 11 unrelated individuals analyzed (data not shown), including those observed on the autoradiograph, suggesting that the anc6 polymorphism was not the result of an I/D variant. In addition, this band was absent in the chimpanzee, possibly indicating the absence of the Alu or perhaps primer mismatch due to nucleotide divergence. Although anc-4 was not variant on the autoradiograph, we tested 13 individuals of various ethnic backgrounds for an I/D event and observed it to be monomorphic. Although we have not verified any of the displayed variants to be the result of an Alu insertion, this potential remains, as we observed Ya8 elements to be highly polymorphic, and all the bands, but one, analyzed were Ya8 repeats.

Discussion

In this manuscript we present an analysis of the smallest defined subfamily of Alu elements located within the human genome termed Ya8. This subfamily of Alu elements was derived from the Ya5 subfamily of Alu elements. The Ya5 subfamily is composed of approximately 1000 members and has largely integrated into the human genome sometime after the human-African ape divergence. The main reasons that supported the more recent origin of the Ya8 subfamily are the accumulation of three additional diagnostic mutations as compared to the Ya5 subfamily and the lower copy number for the Ya8 subfamily. It is also important to note that a higher percentage of the Ya8 Alu family members (50%) are polymorphic for insertion presence/absence as compared to only 25% polymorphism in the Yb8 and Ya5 Alu subfamilies. These data also suggest a recent origin for the Alu Ya8 subfamily within the human genome. However, it is still possible that the Ya8 Alu subfamily may have amplified from an allelic variant of the Ya5 subfamily that was not as efficient at mobilization as the Ya5 source gene.

The ability to detect a handful of Alu repeats from the background of several hundred thousand Alu elements in the human genome is impressive. The application of computational biology to the analysis of large multigene families such as Alu repeats offers the potential to address a number of new questions in comparative genomics as an increasing proportion of the human genome is sequenced. Studies of the present, as well as ancient, integration patterns of mobile elements in the human genome may begin to be addressed. In addition, the patterns of diversity generated by the integration of mobile elements into the human genome may be analyzed at a scale that was previously unimaginable. These types of studies will shed new insight into the relationships between different types of mobile elements in the human genome, integration site preferences, impact, and the biological properties of these elements.

The development of the ASAP technique facilitated the display of a subset of Ya8 Alu elements from a large and complex background. The preferential isolation of the young Alu elements, as demonstrated here, enhances the identification of recent Alu insertion events in the genome. We focused our efforts on the smallest known defined subfamily of Alu repeats to best address issues of sensitivity of the display of individual elements. One of the advantages of this technique is its flexibility. Altering the restriction enzyme used for digestion of genomic DNA selects for distinct subsets of Alu elements within a particular subfamily, since this technique preferentially amplifies products that range from 200 and 800 bp in size. In addition, modifications to the ASAP technique, such as the use of a less frequent restriction endonuclease, may allow for a display of subsets of the larger groups of Alu repeats such as Ya5 elements. Alternatively, the use of primers that select for subfamily 'subgroups' may also be used to reduce the complexity of the resultant display by decreasing the number of PCR products. Although we focused on Ya8 Alu elements due to their low copy number, the young Yb8 Alu subfamily is another alternative for ASAP with an estimated copy number of only 1000 elements (Batzer et al., 1995; Zietkiewicz et al., 1994) and some polymorphic members (Hutchinson et al., 1993; Hammer 1994; Arcot et al., 1998). We have previously demonstrated the isolation of young Alu elements (based on sequence identity to a consensus) using a Yb8 diagnostic primer, and a generic Alu as an anchor in the amplification reaction, that can be profiled with minimal background (Kass, Batzer & Deininger, 1996). It is conceivable that variations on the anchored-Alu PCR technique can be employed to rapidly localize individual elements from all three subfamilies of young Alu elements.

Once the flanking sequences of the young Alu elements are obtained, the PCR strategy can be employed to trace polymorphisms that have resulted from recent Alu insertions and are not yet fixed in human populations. The anchored-Alu PCR approach not only facilitates rapid identification of young elements by displaying the amplification products, but will also increase the potential for selecting only those mobile element fossils that exhibit presence/absence variation. Selection in this manner also shifts the spectrum for new elements toward the elements that are lower frequency and less likely to be held in common between individuals or populations. Therefore, this approach should prove to be quite useful for the ascertainment of mobile element fossils to address questions about more recent human diversifications. In contrast, the identification of mobile element fossils using computational biology affords the opportunity to identify multiple frequency classes of Alu elements that are shared at different geographic levels within the human population.

The ASAP method's strength comes from its ability to isolate a subset of interspersed repeat sequences from different DNA sources and compare them at the same time. In other words, this approach is not limited to Alu elements, but may be used with other SINEs (from other organisms) or even long interspersed elements (LINEs) or for that matter any repeated DNA sequence family that has a defined subfamily structure. A second potential application would be the use of ASAP to monitor genomic instability associated with different forms of cancer by providing a multi-locus monitoring system. Due to its high flexibility the ASAP technique has an enormous range of potential applications.

Mobile element fossils have proven to be simple powerful tools for tracing the origin of human populations (Perna et al., 1992; Batzer et al., 1994a,b, 1996a; Stoneking et al., 1997). These elements should also prove quite useful to the forensic community as paternity identity testing reagents (Batzer & Deininger, 1991; Novick et al., 1993). Some Alu insertion polymorphisms have been identified by chance (Deininger & Batzer, 1995) while others have been identified by library screening in a directed approach (Batzer & Deininger, 1991; Batzer et al., 1995; Arcot et al., 1995a, b, c; Batzer et al., 1996a; Arcot et al., 1998). Here, we have presented two complementary methods involving computational biology and PCR based displays that will enhance our ability to identify the genomic fossils of recently integrated mobile elements from complex genomes. These approaches will contribute to a new era in biological sciences that will increasingly rely upon informatics/computational biology as well as hard-core bench molecular biology to answer global questions in comparative genomics.

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Large-scale Analysis of the Alu Ya5 and Yb8 Subfamilies and their Contribution to Human Genomic Diversity

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We have utilized computational biology to screen GenBank for the presence of recently integrated Ya5 and Yb8 Alu family members. Our analysis identified 2640 Ya5 Alu family members and 1852 Yb8 Alu family members from the draft sequence of the human genome. We selected a set of 475 of these elements for detailed analyses. Analysis of the DNA sequences from the individual Alu elements revealed a low level of random mutations within both subfamilies consistent with the recent origin of these elements within the human genome. Polymerase chain reaction assays were used to determine the phylogenetic distribution and human genomic variation associated with each Alu repeat. Over 99 % of the Ya5 and Yb8 Alu family members were restricted to the human genome and abse⌐t from orthologous positions within the genomes of several non-human primates, confirming the recent origin of these Alu subfamilies in the human genome. Approximately 1 % of the analyzed Ya5 and Yb8 Alu family members had integrated into previously undefined repeated regions of the human genome. Analysis of mosaic Yb8 elements suggests gene conversion played an important role in generating sequence diversity among these elements. Of the 475 evaluated elements, a total of 106 of the Ya5 and Yb8 Alu family members were polymorphic for insertion presence/absence within the genomes of a diverse array of human populations. The newly identified Alu insertion polymorphisms will be useful tools for the study of human genomic diversity.

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Abbreviations used: myr, million years old.
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Introduction

Alu elements are the most abundant Short INterspersed Elements (SINEs), reaching a copy number of over one million in the human genome,[1] making them the mobile element with the highest copy number. Alu repeats compose greater than 10% of the mass of the human genome. Full-length Alu elements are approximately 300 bp in length and commonly found in introns, 3′ untranslated regions of genes, and intergenic genomic regions.[2–4] Amplification of Alu elements occurs through the reverse transcription of RNA in a process termed retroposition.[5] However, Alu elements have no open reading frames, so they are thought to parasitize the required factors for their amplification from Long Interspersed Elements (LINEs).[6–8] Although the human genome contains over one million Alu elements, only a few Alu elements, termed "master" or source genes, are retroposition competent.[9–13] The crucial factor(s) that determine an Alu as a functional source gene are not fully known. Several factors have been suggested to influence the amplification process, including transcriptional capacity, priming or self-priming for reverse transcription and others.[14]

Alu elements first appeared in the primate genomes over 65 million years (myr) ago.[11] Since then, the amplification of Alu elements within the human genome has been punctuated, with the current rate being at least 100-fold slower than the initial rate of Alu expansion within primate genomes.[15] Throughout Alu evolution, the source gene(s) accumulated mutations that were incorporated into the new copies made, creating new Alu subfamilies. Therefore, the Alu family is composed of a number of distinct subfamilies characterized by a hierarchical series of mutations that result in a series of subfamilies of different ages.[15–20] Of these subfamilies, almost all of the recently integrated Alu elements within the human genome belong to one of several closely related "young" Alu subfamilies: Y, Yc1, Yc2, Ya5, Ya5a2, Ya8, Yb8, and Yb9 with the majority being Ya5 and Yb8 subfamily members.[9,18,21,22]

The availability of a draft human genomic DNA sequence as a result of the Human Genome Project[23] facilitates the *"in silico"* identification of recently integrated Alu elements from the human genome.[17,18] This method proves to be less demanding in comparison to older approaches, such as cloning and library screening.[9,21,24] These recently integrated Alu elements serve as temporal landmarks in the evolution of our genome, and many of them will prove to be useful in the study of human evolution and in the study of the natural history of different regions of the genome. Here, we present an analysis of the human genomic diversity associated with 475 members of the Alu Ya5 and Yb8 subfamilies in the human genome.

Results

Subfamily copy number and sequence diversity

In order to determine the copy number of each subfamily of Alu elements, we searched the draft sequence of the entire human genome for the presence of Alu repeats using oligonucleotide sequences complementary to each of the subfamilies (outlined in the Materials and Methods). Our query of the draft human genome sequence identified 2640 Alu Ya5 subfamily members and 1852 Alu Yb8 subfamily members. Both of these copy numbers are in good agreement with previous estimates of the sizes of these Alu subfamilies based upon high-resolution restriction mapping and computational biology.[18,21]

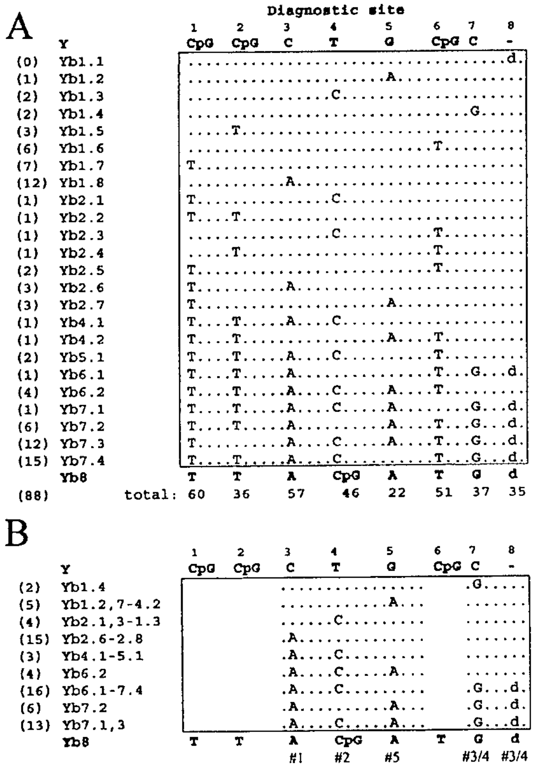
A comparison of the nucleotide sequences of all of the Ya5 and Yb8 Alu family members can be found at our website (http://129.81.225.52). In order to determine the time of origin for the respective Ya5 and Yb8 subfamilies, we divided the nucleotide substitutions within the elements in each family into those that occurred in CpG dinucleotides and those that occurred in non-CpG positions. The distinction between types of mutations is made because the CpG dinucleotides mutate at a rate that is about ten times faster than non-CpG positions[9,25] as a result of the deamination of 5-methylcytosine.[26] In addition, all insertions, deletions and 5′ truncations were excluded from our calculations. A total of 441 non-CpG and 241 CpG mutations occurred within the 231 Alu Ya5 subfamily members used in this analysis. For the 244 Alu Yb8 subfamily members analyzed, a total of 478 non-CpG and 275 CpG mutations were observed. Using a neutral rate of evolution for primate intervening DNA sequences of 0.15% per million years[27] and the non-CpG mutation density of 0.799% (441/55,209) within the 231 Ya5 Alu elements yields an estimated age of 5.32 million years for the Ya5 subfamily members. Using only non-CpG mutations in the 244 Yb8 sequences yields an estimate of 5.30 million years old for the Yb8 subfamily (478/60,024). This estimate of age is somewhat higher than the 2.7-4.1 million years previously reported.[21] However, the previous study of Ya5 and Yb8 Alu family members involved only a small number of elements making the calculated subfamily ages more subject to random statistical fluctuation. Alternatively, the new estimated age based upon non-CpG mutations may be artificially inflated due to sequencing errors in the human draft sequence that may account for an increase in the number of mutations observed.

We can also estimate the ages of each Alu subfamily using CpG-based mutations. The only difference in the estimate is to multiply the CpG mutation density by a mutation rate that is approximately ten times the non-CpG rate as previously described.[9,25] In this case we calculate an average CpG mutation density for the Ya5 subfamily (241 mutations/11088 CpG bases) or 2.17%, and (275 mutations/11,224 CpG bases) 2.45% for the Yb8 subfamily. Using a neutral rate of evolution for CpG based sequences of 1.5%/million years yields estimates of 1.44 and 1.63 million years old for the Ya5 and Yb8 Alu subfamilies, respectively. Both estimates are consistent with the initiation of the expansion of the Ya5 and Yb8 Alu subfamilies that is roughly coincident with the divergence of humans and African apes.

Inspection of the nucleotide sequences flanking each Ya5 and Yb8 Alu family member shows that most of the elements are flanked by short perfect direct repeats. The direct repeats range in size from 3-23 nucleotides. The observed direct repeats are fairly typical of recently integrated Alu family members.[7,9] The appearance of truncations within a number of these elements probably occurred as a result of incomplete reverse transcription or improper integration into the genome rather than by post-integration instability. All of the Ya5 and Yb8 Alu family members analyzed have oligo(dA)-rich tails that range in length from six nucleotides to over 60 nucleotides in length. It is also interesting to note that the 3′ oligo(dA)-rich tails of many of the elements have accumulated random mutations beginning the process of the formation of simple sequence repeats of varied sequence complexity. The oligo(dA)-rich tails and middle A-rich regions of Alu elements have previously been shown to serve as nuclei for the genesis of simple sequence repeats.[28]

Alu Y to Yb8 sequence evolution

In our query of the human genome, we identified 88 Alu elements containing one to seven of the eight Yb8 diagnostic nucleotides. These 88 "mosaic" elements were subdivided into Yb1, Yb2, Yb4, Yb5, Yb6 and Yb7 depending on the number of diagnostic changes present (Figure 1(a)). To facilitate identification of the individual elements with different diagnostic mutation combinations, the mosaic elements were numbered consecutively in order of abundance (Yb1.1, Yb1.2, etc., see Figure 1(a)). No evident sequential order of accumulation of the Yb8 diagnostic mutations can be easily discerned. Interpretation becomes complicated due to the fact that four out the eight diagnostic mutations are CpG changes (positions 1, 2, 4 and 6 Figure 1(a)). The Alu Y has three CpG sites (positions 1, 2 and 6) that become TpG in Yb8, and Alu Yb8 has one (position 4). CpG dinucleotides mutate at a rate that is about 9.2 times faster than non-CpG,[9,25] as a result of the deamination of 5-methylcytosine.[26] Therefore, it is difficult to know if the presence of a TpG diagnostic mutation is due to a change in the Alu source gene or in the particular individual Alu element being evaluated. Because CpG dinucleotides represent hot spots for mutation, a high proportion of CpG positions in the Y subfamily might have mutated to TpG. This makes discrimination between source gene changes and parallel forward mutations occurring in multiple Y elements at these loci difficult. Therefore, we have eliminated these sites (positions 1, 2 and 6) from our analysis (Figure 1(b)). Position 4 represents a different situation. Because the TpG to CpG mutation occurs at the normal evolutionary rate, it was not eliminated from the analysis. However, some variations may be observed where individual copies might have mutated the position back to a TpG that need to be taken into consideration. Now, a sequential evolution of the appear-



```
A                                Diagnostic site
                         1    2     3    4     5    6   7   8
      Y                  CpG  CpG   C    T     G    CpG C   -
(0)   Yb1.1              .................................d.
(1)   Yb1.2              ......................A............
(2)   Yb1.3              ................C..................
(2)   Yb1.4              .............................G.....
(3)   Yb1.5              .....T.............................
(6)   Yb1.6              ..........................T........
(7)   Yb1.7              T..................................
(12)  Yb1.8              ...........A.......................
(1)   Yb2.1              T...............C..................
(1)   Yb2.2              T....T.............................
(1)   Yb2.3              ................C.........T........
(1)   Yb2.4              .....T....................T........
(2)   Yb2.5              T.........................T........
(3)   Yb2.6              T..........A.......................
(3)   Yb2.7              T.....................A............
(1)   Yb4.1              T....T.....A....C..................
(1)   Yb4.2              T....T................A...T........
(2)   Yb5.1              T....T.....A....C.........T........
(1)   Yb6.1              T....T.....A..............T..G...d.
(4)   Yb6.2              T....T.....A....C.....A...T........
(1)   Yb7.1              T....T.....A....C.....A......G...d.
(6)   Yb7.2              T....T.....A..........A...T..G...d.
(12)  Yb7.3              T..........A....C.....A...T..G...d.
(15)  Yb7.4              T....T.....A....C.........T..G...d.
      Yb8                T    T     A    CpG   A    T   G   d
(88)           total:    60   36    57    46   22   51  37  35

B
                         1    2     3    4     5    6   7   8
      Y                  CpG  CpG   C    T     G    CpG C   -
(2)   Yb1.4                         ................    .G.....
(5)   Yb1.2,7-4.2                   ............A...    .......
(4)   Yb2.1,3-1.3                   ......C.........    .......
(15)  Yb2.6-2.8                     .A..............    .......
(3)   Yb4.1-5.1                     .A....C.........    .......
(4)   Yb6.2                         .A....C.....A...    .......
(16)  Yb6.1-7.4                     .A....C.........    .G...d.
(6)   Yb7.2                         .A..........A...    .G...d.
(13)  Yb7.1,3                       .A....C.....A...    .G...d.
      Yb8                T    T     A    CpG   A    T   G    d
                                    #1   #2    #5       #3/4 #3/4
```

Figure 1. Evolution of the diagnostic nucleotide positions from Y to Yb8 Alu elements. (a) Alignment of the eight Alu Yb8 diagnostic nucleotides and the different Yb1, 2, 3, 4, etc. elements found in the databases. The eight diagnostic nucleotides are indicated in bold at the top for Alu Y, and for Alu Yb8 at the bottom. At position 8, – or d represents the absence or presence of the seven nucleotide duplication, respectively. For easy reference, individual elements containing different combinations of the diagnostic mutations were numbered consecutively in order of abundance (Yb1.1, Yb1.2 , etc.). The total number of elements found for each subgroup is indicated on the left in parenthesis. Note that no Yb1.1 was found (0). The total number of the Yb8 individual diagnostic sites found in all the intermediate elements is indicated at the bottom. (b) Alignment of the same elements after eliminating the diagnostic sites in Alu Y elements involving CpG to T changes. Commas separate elements within the same Yb group and dashes between different groups, i.e. Yb1.2,7-4.2 represents Yb1.2, Yb1.7 and Yb4.2. The suggested evolutionary order of the occurrence of the changes at the diagnostic sites are indicated at the bottom (#1, #2...).

ance of the diagnostic sites can be obtained, starting with position 3, then 4, 7 and/or 8, and finally position 5 (Figure 1(b)). The mutation at position 3 appears to have occurred first, being the most common single nucleotide change with 15 Yb8 mosaic elements. The other Alu Yb8 mosaic elements with only one diagnostic nucleotide change occur in lower frequencies and may be explained by parallel mutations, post-transcriptional selection,[8] or by a forward gene conversion event. The order in which the mutation at positions 7 and 8 (the seven nucleotide duplication) occurred cannot be resolved with these data. Four of the elements (Yb6.2 in Figure 1(b)) do not fit the proposed sequential evolutionary pattern. In this case multiple recombination events would be required to obtain this outcome or some selection occurring at the retroposition process, both highly unlikely. Alternatively, position 5 may be explained by gene conversion events or parallel mutations. The possibility of gene conversion between Alu repeats has been suggested previously.[29] In addition, limited amounts of gene conversion between Yb8 Alu elements[21,30] and extensive levels of short gene conversions in the Ya5 subfamily[18] have been previously reported.

Phylogenetic origin

In order to determine the approximate time of origin of each Alu subfamily member (Ya5 and Yb8) in the primate lineage, we amplified a series of human and non-human primate DNA samples using the polymerase chain reaction (PCR) and the oligonucleotide primers shown in Tables 1 and 2. In this assay, genomes that are homozygous for the presence of an Alu element amplify a PCR product about 400 bases in length. Genomes that do not contain the Alu element at a particular chromosomal location amplify a 100 bp fragment, while heterozygous genomes amplify both fragments. Using this approach we investigated the phylogenetic origin of each Alu element. All 231 Ya5 Alu family members were subjected to this analysis and only one element (Ya5NBC42) was present in the orthologous locus from the common chimpanzee genome. For the Yb8 subfamily, 244 elements were assayed with none being present in the common chimpanzee genome. This suggests that almost all of these Alu elements dispersed within the human genome sometime after the human and African ape divergence and that less than 0.21% (1/475) of the Ya5 and Yb8 Alu subfamily members in the human genome also reside in non-human primate genomes. In fact, this is only the second Ya5 Alu element ever reported that is also found in the genome of a non-human primate.

Human genomic diversity

In order to determine the human genomic variation associated with each of the Ya5 and Yb8 Alu family members, each element was subjected to PCR amplification (outlined above) on a panel of human DNA samples. The panel was composed of 20 individuals of European origin, 20 African Americans, 20 Greenland Natives or Asians and 20 Egyptians for a total of 80 individuals (160 chromosomes). Using this approach 134 Alu Ya5 (Table 1) and 160 Yb8 (Table 2) subfamily members were monomorphic for the presence of the Alu element, suggesting that these elements integrated in the genome prior to the radiation of extant humans. A total of 28 Ya5 and Yb8 Alu family members appeared heterozygous in all of the individuals that were analyzed, suggesting that they had integrated into previously undefined repeated regions within the human genome as reported previously.[31] In the PCR-based assay these elements generate a pre-integration site size product from the duplicate copies of the pre-integration site located throughout the genome along with an Alu filled site from the one pre-integration site sequence that contains the new Alu insertion. These elements were not subjected to any further analysis. An additional six elements were located in other repetitive regions of the genome that were identified computationally and discarded from further analysis. The remaining elements were polymorphic for the presence of an Alu repeat within the genomes of the test panel individuals (Tables 3 and 4). Loci that were polymorphic for the presence/absence of individual Alu insertions were subsequently classified as high, low or intermediate frequency insertion polymorphisms (defined in Tables 1 and 2). The unbiased heterozygosity values (corrected for small sample sizes) for these polymorphic Alu insertions were variable, and approached the theoretical maximum of 50% in several cases. This suggests that many of these Alu insertion polymorphisms will make excellent markers for the study of human population genetics. Approximately 25% (58/231) of the randomly identified Ya5 and 20% (48/244) of the Yb8 Alu family members are polymorphic for insertion presence/absence within the human genome. These results are in good agreement with previous estimates of the percentages of insertion polymorphisms within these two Alu subfamilies.[21]

The Alu inserts that have been in the genome longest are more likely to approach fixation. Therefore, we might expect to find different levels of sequence divergence for the Alu elements from each insertion frequency class. Using this approach the average number of non-CpG/CpG-based mutations for the Ya5 Alu family was 1.62/1.06, 2.83/0.67, 2.16/0.66 and 2.53/1.0 for the fixed present, high frequency, intermediate frequency and low frequency Alu insertion polymorphisms, respectively. In the case of the Yb8 subfamily the average number of non-CpG/CpG mutations was 1.86/1.16, 5.0/0.6, 2.2/0.66 and 1.7/1.2 for the fixed present, high frequency, intermediate frequency and low frequency Alu insertion polymorphisms, respectively. In all cases the standard deviations for each average were as large or larger than the average number of mutations reflecting the heterogeneity in the dataset. No detectable difference in the mutation density within each frequency class of Alu insertions was observed. Therefore, our data suggest that any sequence differences between the polymorphic elements and those with fixed presence may be obscured because of the small number of total mutations and sequencing errors (see Discussion).

Discussion

Alu elements account for more than 10% of the mass of the human genome. The majority of Alu elements integrated into the genome early in primate evolution. Only a small number of elements (a few thousand) have amplified in the human genome after the divergence of humans and African apes. Here, we report an investigation of the dispersion and insertion polymorphism of the two largest subfamilies of recently integrated Alu repeats within the human genome. Our copy number estimates of 2640 Ya5 and 1852 Yb8 Alu elements within the draft sequence of the human genome are in fairly good agreement with previous estimates of the sizes of these Alu subfamilies although they both exceed the previously published figures.[21]

Using the mutation density and a neutral mutation rate we were able to estimate the ages of each subfamily as 5.32 million years (myr) old for Ya5 and 5.30 myr old for Yb8 using non-CpG-based estimates and 1.44 myr (Ya5) and 1.71 myr (Yb8) using the CpG mutation density. Each of these reported average ages based upon non-CpG mutation density is substantially higher than those reported previously of about 1 myr and 2.7 to 4.1 myr for the Ya5 and Yb8 subfamilies, while the estimates based upon CpG mutation density compare favorably to those previously reported.[21,32] If we assume a linear amplification of these Alu subfamilies in the human genome, the oldest elements would be no greater than 10.64 myr old for Ya5 and 10.6 myr old for Yb8 using non-CpG mutation density, or 2.88 myr old for Ya5 and 3.42 myr old for Yb8 using the CpG mutation density. The non-CpG based estimates for the oldest subfamily members appears to be somewhat higher than expected for a group of repeated DNA sequences that largely amplified within the human genome after the divergence of humans and African apes which is thought to have occurred within the last 4-6 myr.[27] This discrepancy between the two estimates can be explained by considering sequencing errors as a potential factor influencing our current calculations. In the determination of the non-CpG mutations for the estimation of the Alu subfamily age, sequencing errors would be included in the count as mutations, making the estimated age higher than the actual age for the subfamily. If we assume that the sequencing errors are distributed evenly across the entire Alu sequence, then the number of sequencing errors would be higher in the non-CpG-based estimates than the CpG-based estimates, since there are more non-CpG (242-246) than CpG (only 44-48) nucleotides in the subfamily consensus sequences. Our observation that the levels of sequence divergence from the subfamily consensus sequences do not effectively correlate with polymorphism levels in the human genome also argues that it will not be beneficial to use sequence divergence from the subfamily consensus sequences as a method for the identification of additional polymorphic members of these Alu subfamilies.

We can also compare the calculated ages of each Alu subfamily based upon non-CpG mutation density as a whole to the estimated percentages of Alu insertion polymorphisms and copy number to evaluate the contribution that these elements make to human genomic diversity. Here, we report estimated ages of 1.44 myr for the Ya5 subfamily and 1.71 myr for the Yb8 subfamily. The percentage of Alu insertion polymorphisms in each of the subfamilies was 25% for the Ya5 subfamily and 20% for the Yb8 subfamily. The copy numbers of the two subfamilies of Alu elements were also different with 2640 Ya5 Alu elements and 1852 Yb8 elements. When considered together these data indicate that the Ya5 Alu subfamily with both a higher copy number and more insertion polymorphisms has been more successful at amplification within the human genome. In fact, if we assume that the ages of the two subfamilies are about the same the Ya5 subfamily has been about 40% more efficient at amplification in terms of both copy number and the generation of new Alu insertion polymorphisms within the human genome. Although the sample size is presently small, this is also in good agreement with the number of previously reported Ya5 (six) and Yb8 (three) Alu repeats associated with different human diseases (reviewed in ref. 22). In addition, these data also provide compelling support for the simultaneous expansion of multiple Alu subfamilies within the human genome. The reasons for the differential amplification of the two Alu subfamilies remain unknown. However, they likely reside in the ability of each subfamily to produce RNA for retroposition or at some other point in the process of retroposition itself such as the reverse transcription step. Further experiments will be required to determine the precise molecular mechanism(s) leading to the differential expansion of these two Alu subfamilies within the human genome.

Using the non-CpG-based average ages of the Ya5 and Yb8 Alu subfamilies along with a linear amplification rate we can also estimate the number of members from each Alu subfamily that should be present within the orthologous loci of the non-human primate genomes. Using this approach the oldest Alu repeats from each subfamily would be approximately twice the average age. In other words, the Ya5 subfamily would have begun to expand 10.64 myr ago with the Yb8 subfamily hav-

Table 1. Alu Ya5 accession numbers, locations, human diversity, oligonucleotide primers and PCR parameters

							Product size[b]
Name	Accession	5′ Primer sequence (5′-3′)	3′ Primer sequence (5′-3′)	A.T.[a]	Human diversity[b]	Chr.[c] Loc.	Filled-empty
Ya5NBC2	M28713	CTTTAGACTACAGTTGTGTTAGCCTCTTG	CTGCACTTTCCAAATTTTCTACCAC	55	FP	22	710-384
Ya5NBC3	AL023807	ACTGCTTGAAGCTAGAACTTAAGAGACC	CTCTTGTCTGCTTCTAGACTTGTGAATAAC	60	FP	6	564-243
Ya5NBC4	AL008629	CTGATGAGAAATCTGCTGCTATTG	GCAAACCTCAAACAGGATAAACAC	60	R/R	X	483-154
Ya5NBC5	AC007363	TAGGATATTACTGTACAAAGCCGTAGATTT	GTTTTAAGCTAAGCGTTATTACAAAAGAGT	60	IF	2	476-163
Ya5NBC6	AC006344	GATTACATCCTGTGATCCTGAAACT	GAACATTTGTTCTTTTGTGACTGCT	60	FP	6	539-189
Ya5NBC8	AC006478	ATTAAGCACCAGGAAATTGCCATAC	CTAGTAAGGCTAGTCCCATAATTTGAAGTG	55	FP	7	513-196
Ya5NBC9	AC006382	CTTCCCTAGGATTTAAGTCACCATAAAGAC	TTTTCAACTTGTAACTGTAGAGGACAGGAC	60	FP	Y	415-102
Ya5NBC10	AC008725	AAAGCATAAAGAAAAGTACGCCAAC	CAATGAAGATATAGAACAGCCCCTA	60	FP	20	449-141
Ya5NBC12	AC009307.6	GGAGTCAAAGGTACTTACAACGTCT	CTCCCTGTCTTCCTAACGTAATTTT	60	FP	2	470-129
Ya5NBC13	AL031302	CTTCCTGTGTATACTTCTCTGCAC	GTCTGTGACCTGCAACACAAG	60	FP	22	604-291
Ya5NBC14	AL050342	Alu flanked by other repeats	Alu flanked by other repeats	-	-	1	-
Ya5NBC15	AC008676	CTTTCCTCAGCTTGGTTTATTCTACTG	GAAGATTCAGGTGGACAGAAAAAC	60	-	5	502-192
Ya5NBC16	AC008608	CTTAACCAAAATAGTGGACGAGGTT	CAGAAGTATTTACTACTCGAACAGTGAGC	65	HF	5	539-229
Ya5NBC17	AC007076	TATGCTCTGAGAGGTTTTCTAGATCTCTG	GAATAGGAGCATCATTCAAGTTCAG	65	FP	7	553-232
Ya5NBC18	AC008433	AGACCATCTTTAAGAGGAATACCATGT	GAAACGTAAATTTGTTAATAAAGTGGTGAC	65	HF	5	495-180
Ya5NBC19	AL109948.1	AATCACTGTTACTCATGGGGTATCT	AGACCTACGTGCCTTACTCACTGT	60	FP	1	433-116
Ya5NBC21	AC008482	AGCTGTCGTTCAAATGAGACTTTCT	AAGCTCACTCATCAATAAGAACACC	60	FP	5	512-177
Ya5NBC22	AC004519	TCTGTGTTCTTTGAATGTGTATTACTCTTA	GAATGTAAAGCTGTAACTTTCCTTTTCAAT	55	IF	7	471-156
Ya5NBC24	AJ011932	AAAATTGAGAGACGAGGAGAAGGT	CCTCATCAATACTGTAACTGTCACAAC	60	LF	21	595-286
Ya5NBC25	AC004220	GTGAAGGACATGAACAGACACTTCT	CACCAACAGTGTAAAAGTGTTCCTA	55	FP	5	538-218
Ya5NBC26	AP000311	GGGCTATTCTGATTTTCTTCTTCTC	AGAAGAGACATCACTACAGATACTACAGAC	55	FP	21	476-158
Ya5NBC27	AC003691	CTGAATACAGGTATCACTGAACAGAAC	ACAGTGTAAAGTCTAACCTACCAGAGGAT	55	IF	11	591-265
Ya5NBC28	AC005862	GGGTACATGTGCAGGTTTCTTATAC	GCTAACTGATGAGAACACACAGATACATAG	55	LF	7	474-191
Ya5NBC30	AC007159a	CTGGCACATAGTAGGTGTTCAATAA	GAGTAGTTTGAGTCTGTTTGTAGCAGAG	60	FP	?	502-191
Ya5NBC31	AL033543	GTATCTTGTGTGTTTCCTAACAAGACTGAG	CTCATTTTCACTTATCAGGTCTGTCTC	60	FP	22	523-238
Ya5NBC33	AC006288	GCAATTGCTATCCTTGAGTGTTTC	CTCCTAGTCTAGAGTTTTTCCCATTGTATC	60	FP	9	543-226
Ya5NBC34	AL031575	CACTCTGATACTTATCTCTGTGCCTGTAT	TGAGAGACATCAAACCAGAAATCC	60	FP	X	494-150
Ya5NBC35	AC004534	GAGAAGTACTCAGAGAGGACATCATTT	GTAGTCATGGAGGTAAGAAAAGAAGACAC	60	IF	7	515-179
Ya5NBC36	AC004006	ATGAAATAACTCCTAGATTCAGGCTTC	AGTTTCTTGTGTTAGTTTCCTTAAATACCT	60	R/R	7	515-200
Ya5NBC37	AC002476	GCTTGAGGTTTTCATACTACTCTTATCTTT	ACTGTATAAGCATTTTCCTCTTTATCTTTC	60	IF	X	497-184
Ya5NBC38	AC006033	GTACCCTCTAATTTACAGTCATCTCATACC	GAACTTCTCTGGCTTGAAAAATCAG	60	LF	7	487-170
Ya5NBC39	AC005533	TGGGACTTAGCTGTTTTGGTATCTA	CTAAACACAGGTTACAGCACCTCTT	60	FP	14	469-152
Ya5NBC40	AC008887	ATTGATCTCCAACTGATGCCCTA	GACAACAGACTTACCCTGCCTATACTATT	58	-	5	417-105
Ya5NBC41	AC008828	CTCTTTATGGGACTTGACAAGCA	GTTCTACATTGCCATAATAGTGTAGGG	55	FP	5	441-128
Ya5NBC42	AL078621	AGTAAGTCCCTCCCCATATGCT	GGTCTTTCTAACCCAAAGGTCAC	55	FP	22	486-185
Ya5NBC43	AL096867.7	CCTTTCCTTACTAGACAGTGACAACAT	CTTTTAGCCATCTTCTTGGTGTTTG	55	FP	6	539-218
Ya5NBC44	AL096840	CATTCTCTCCTTTGGTCCTCTTTAT	GTGAGTTTGGGGATATGGTGAG	55	FP	1	525-202
Ya5NBC45	AL049868	TAGGGTAAGGAATATGTGCTGCTTTAG	GTCTCTGAACGACTATGTGAGCAG	60	IF	20	591-265
Ya5NBC46	AC009466	GATGTGTGAATACTGTGTAGATTCCAG	GTAAAGCTTTTGTAGTGCCTAGCTCTTAGT	55	FP	11	405-94
Ya5NBC47	AC007227	CTCAAGATTGGCCTATAGTCGTTAT	AGACACAGGTATCCAGTGAAAGAGT	55	FP	1	526-200
Ya5NBC48	AC002290	ACTGTTAAGATAGTGAATTTTACTGCTCCA	AACTCACACGTGATACAAAATACTCACAGA	55	FP	11	481-176
Ya5NBC50	AL096829	AGCCTGGCCCATGATAAAAA	GTTTCATAGATAAGACACTGGCATGTTACT	-	-	1	465-155
Ya5NBC51	AC008249	ATATTCCAGAAGTTTCCTTACATCTAGTGC	AAAGCTTTAAGTCTCCACCATCTCT	60	IF	3	437-140
Ya5NBC52	AC009094	AAGTTGAAGTCCAAAGTCTCATCTG	CTTCTTCCCCACTTAAATATCAAGG	53	-	16	688-371
Ya5NBC54	AL024507	GTTTATGTCAGTAGGAGTTTTCTCGTGTAG	TCATTGTATCATCTGCTGTACCTGT	60	LF	6	433-130

Ya5NBC56	AL109767	TCATTGTATCATCTGCTGTACCTGT	AGTCCAACATAGATGTAATTGGAGTTCAGG	60	FP	14	469-148
Ya5NBC57	AC009107	GACGTAAAGAGATGTTGTTAAGTGAAAAAT	ACTGTAGGAGGTAAATGGAAAGTCAACAGA	60	IF	16	444-126
Ya5NBC58	AC008376	TGCCTCTTTAACCAATTTCTCTTATTTTCA	TATTTGGCTGGATTTGAGTTATCTCTTAGG	60	FP	5	481-141
Ya5NBC61	AC009594	TGAAATAATCCAGTTGGGGAAG	GTATATCTCTACCGAGACTCAGTTTTTAGC	55	IF	4	493-180
Ya5NBC66	AC006210	ATGGTAATTTCCCTCATTTGTCA	GTAATGTCCTCCATTGTTCATTTG	61	FP	X	448-115
Ya5NBC67	AC006005	CACACACCCCGTATTTTCT	TGCATTCTCTTGGAGTTTG	58	FP	7	424-131
Ya5NBC69	AC004053	GGGATCAGTTACAGTGCTTC	ATGCAACGCAACTTAGAACT	50	FP	4	359-42
Ya5NBC70	AC004454b	ATCAACGTGGGACATAACCA	TTCAGAAGGCACATTAGTGCT	60	FP	4	391-116
Ya5NBC72	HS234H5	CCTTGCTGCATAAAAACCTA	TATGACTAATGTGGGGCTTT	52	FP	6	416-102
Ya5NBC73	AC004454a	GACTATTAATACGAATCCAAAGTACACG	TTTAACTTGGTCCTACCTGTGTGTC	51	FP	4	465-129
Ya5NBC76	M96868	ACTCTTTAGTTTGTAAGATGGCAAG	GGTGGAGGGAAGTAGCAGAA	60	FP	2	735-417
Ya5NBC77	AL008629	TTTTTGCCGTTAGTTTTCAGAAG	GGGCAAACCTCAAACAGGAT	60	-	X	408-83
Ya5NBC78	AC006155	AACTCCAACAGCCACATCCT	TGGTGGGTCAGTATTGAGTGA	60	-	7	382-66
Ya5NBC80	HS960017	CTCTCCTGTGTCCATACTTCTT	CTGGCATGGAGATTTCTTAC	60	FP	X	368-47
Ya5NBC81	HU95742	GTGGCAGTGGAGATAGAAAA	ACACTCAATCCATCACCTTT	60	-	16	352-42
Ya5NBC82	AC005217	AGTGCTGGGAAGACCAGT	GCTGGTGCTCTTGACAAA	60	-	5	348-37
Ya5NBC83	AL022101	AGGGAAGTCAGGCAGAAC	CCTTCCCTAGGAGCACAT	55	FP	1	384-106
Ya5NBC87	AL109830	AGTAAATACTGTAAGAGATGTGGAGAGCAC	CACAGCATTAGAGAGAGTTGATGATAG	55	R/R	20	434-201
Ya5NBC89	AC009807	ATCTTCCCGGCATAAACCTC	GAGGCCCAAATTTGCTTACA	55	FP	11	516-195
Ya5NBC91	AL034378.2|	GGTCATGGTTCTTTGCTATTCATTC	GTATTGTAACCCATAGAGCAACCAT	55	R/R	1	531-214
Ya5NBC93	AC010086.1	CCTCTGCCATATGGTATGTTTAGAA	TAGGATGAGGTCAAAAGTGAGAAAC	60	-	Y	610-294
Ya5NBC94	AC008788.1	ATTGTCTTTCCTGTGCTACTCTCAT	CACTTTAGTGGATGCTTATCTTTG	57	FP	5	531-201
Ya5NBC95	AC009962	ATTATAGTAATAAATCCTGGAATTGGACCT	GAAATGAATCTATGGGATATGTTTCT	60	FP	2	489-148
Ya5NBC96	AC004547	TAGATGAGATAGAGCCATCAAACACTC	GTATTGTAACCCATAGAGCAACCAT	60	IF	7	509-169
Ya5NBC97	AC004453	GCTCTTTTCTTGTTTTCTGGAAGTG	TGTGAGTGTAAGAACACGTGTAAGAG	55	FP	7	442-147
Ya5NBC98	AL049591	TATAGCTAGTAAATGGTAGAGCCAGGA	CTGTCTAAGATAGTGATTGGACCTACTATG	62	HF	X	504-209
Ya5NBC99	AL031312	TATACACACACACACAGAGAATGACTG	CCTGACTCGAAAGTACTGTTTTCTAAG	55	FP	X	515-198
Ya5NBC100	AL035683	TGAACAGTTCTCTTAGGTGGTTAGTAG	TAATATACAGTTGTGCTCACTAGCATACCC	50	-	20	477-153
Ya5NBC101	AC006030	CTCACTGACACTTTTGGTCAGACT	ATTTACTGAGCACAATGCCTCATAC	55	FP	2	519-204
Ya5NBC102	AF118569	TCCCATTTCTCTAGACCTGCTG	CCCATAACAGGTCTTCATATTTCC	55	IF	17	483-194
Ya5NBC103	AL034408	ACTCTCTCTCCTACATCACTGACTTCTC	GTAAGCTTTGAGTTCAGAGGACAGATA	58	FP	X	556-237
Ya5NBC104	AC07065	GGGCATAGCTGTAGATATAAGCACTACAA	AGAAGAATAGAGGACTATGTCTGTGTCTC	58	FP	12	508-188
Ya5NBC105	AC006040	GTATACATTCTGCAACCAGTGGAG	AGAGGTAGAGAGCTTGCATTTCAG	62	R/R	Y	598-281
Ya5NBC106	AC005532	CTTACAGTTACGGAGGCTAGAAAGTC	GTTATCATGGGAAGGGAACTGT	60	R/R	7	509-207
Ya5NBC107	AC004884	GTAATGAATAGCCGTGCAACTGTC	CACCCAGCCATTTCCTAGTTAT	60	FP	7	556-236
Ya5NBC108	AC007092	CATATGAGTGCCTGACTTTTACTACTTCTC	CTAAATACAGGATGAAAGGACTGGTAG	60	FP	2	567-215
Ya5NBC109	AC005745	GTGCCTGGTACTCTAGAAATAAAACTCTCT	AGAATGAAACTCCGGCTCAAA	58	IF	22	561-251
Ya5NBC110	AC004761	GAGTCTTTGTTCTGTTAACTTAGTGGTGAG	CTAGAAGGTCACACATATGTCAAGGAT	60	FP	5	558-170
Ya5NBC112	AC008032	GGTTATTAGTTTTGGGGTGGTAGTC	GGGATACCCAATTCAGTTGTACTAGA	60	FP	3	396-93
Ya5NBC114	AC007782	GAGGATTTTAGCAGATAGTATTGTGTTACAG	CAAAGTCTTATCAAATACAGCACACTG	60	-	12	524-223
Ya5NBC115	AC009316	AGAACAAACTGCACATCGAGTATCT	ACCTTCAAATTTCTCCTTTGAGGAC	60	FP	2	574-240
Ya5NBC116	AC006344.2	GATCCTGAAACTATTTAAAATCAAGAAGAC	TCTAACCATATGTAGAGTTAATCTCTTTGAC	60	FP	7	575-237
Ya5NBC117	AJ010770	GGGAGGAGAAAGGAGAAACATCTAGT	CTCTCCAGCTATAACCCCAACTACT	60	FP	7	515-188
Ya5NBC118	AC005913	AATACGTGTGTCTGTGTGTATATGTTT	TGCATACCTTCCCAGAGATAATG	60	FP	X	533-235
Ya5NBC119	AC006002	TGTTAATAAACAAGAACACTACTCCAAGG	CTTTTGTTATATATACTGAGGAGAAAATGG	60	FP	7	482-167
Ya5NBC120	AC005863	GGACCACATGACTGAGTGTAAAGT	GAGGTGGCCTCTTAACCATAATTC	55	IF	17	518-199
Ya5NBC121	AJ011932	AGGGGGAAAACATCAAAAACTC	CCTCATCAATACTGTAACTGTCACAAC	60	FP	21	510-202
Ya5NBC122	AC005747	CCATTCATTCTATTTGGGGAGTTAG	GACTAAACCAGGATGTGAGCTTTT	53	FP	17	527-217
Ya5NBC123	AC005739	ATCAAGTTGACACTCAGTATTCACCAC	CTAGTCTGCAGAACTGTGAGAAATGTA	55	IF	5	490-180
Ya5NBC124	AL022310	CTAGACAGTGCAACAGTTCCTAAATACAG	CATAATGGAAAACTCCATGTGCTAC	60	FP	1	457-131

Table 1. *(continued)*

							Product size[b]
Name	Accession	5′ Primer sequence (5′-3′)	3′ Primer sequence (5′-3′)	A.T.[a]	Human diversity[b]	Chr.[c] Loc.	Filled-empty
Ya5NBC125	AC004206	AGTATTTTGCACTTCTCTAAGGGTGTC	CTGGTCTTATGTTTCATCTGGATTC	60	FP	6	507-223
Ya5NBC126	AC005144	GTCTGCTGAATGATTAAACCAACAC	GTGCCATTTCTACTACTGAAACCTAAC	60	FP	17	480-171
Ya5NBC128	AC004808	GGGTGGGACAAAGAAATACTCA	GCTTATGGCTTGCAGTTTCACT	55	-	7	648-293
Ya5NBC129	AL008635	TACATGGAGTTAGAGCCCGTTC	ACAAGTGGCTGTCACYCAACAC	60	FP	22	486-180
Ya5NBC130	AC004629	GTTGTGTCCACTCTTTGACTAGTATGA	GACAGTTTTACTGACTACACAGGATTTCAG	60	FP	5	602-287
Ya5NBC131	AF002996	CCCAAGATCTAGGTGATGGACAC	GCACTTGAGATAACCTAGTTAGAATGC	60	IF	X	495-174
Ya5NBC132	U91328	CTCGTGATTCACAGAAGTGTTGTAAG	CGGGGTTCATCCTTAATACATACAT	60	IF	6	458-228
Ya5NBC133	AC000355	TGTTATCATTACACAAATACAGCACTTTAG	TCTTTGGCTATAAGGATATGAAAACTTAAC	60	FP	7	692-374
Ya5NBC135	U01102	ATTAAGCTCATGGTAACCAGCAC	GACTCTCCTCTCTGGATTAGAAACAG	60	LF	11	436-117
Ya5NBC136	AC008124	CAGCAACAATCAAAGTTTATAATGC	GGAAAATTGAATGATGGCAAA	60	FP	12	749-439
Ya5NBC137	AC005002	GTTGCTGTTTTCTGCTGCAC	GCATAAGAGACCAATCCTGGAG	55	R/R	?	521-197
Ya5NBC139	AL031650	TGAAAGCTCTTAAGGTCTTCTCTCT	TAAGTAGACCAGAAACAGGGAACAG	60	FP	20	851-634
Ya5NBC140	AC007877	GCAGCCCCAAGTGTTAAATTACTAT	GGTTGTGGTAATGTCATCATAAACG	60	FP	2	471-135
Ya5NBC141	AL096769	CTGAGAAACCAGCAAAGTAACTGAC	CATGGACCCATATACAGACTACAAA	60	R/R	20	480-139
Ya5NBC142	AC007392	ACATTCTAGGACACCTGTCAGTCAT	GGTCAATAGCATGGGAAAGAATATC	60	FP	2	663-321
Ya5NBC143	AC006374	GCAATGCACATAAGATATGCTC	CTTTTCCCTACCATGGTGTCTTT	60	FP	7	572-251
Ya5NBC145	AL035667	TGCATCCTCTTCTGCTGTTC	AATTGGGTTCACTAGACAAAGG	60	FP	20	500-276
Ya5NBC146	AL022329	CTGTCCCTTCTCAGGCTCATT	CTAGCATGTTGTCACCTCTCAACC	53	R/R	22	604-131
Ya5NBC147	AC007656	TAGCTGGGGGAGGTAGATAATAAAC	AAATATCACCTTATCAGTGGGACCT	60	LF	12	493-155
Ya5NBC148	AL031659	ACAAGATGACAGATGTAAACCCAAC	AAGGTGTTGTCAGACTAATCTATCG	60	IF	20	505-193
Ya5NBC149	AL033525	GTGTTACTGTGGCCAACTATCTCAT	ACTTATATGAGCGGGGTACAGTTCT	60	FP	1	466-155
Ya5NBC150	AF135028	AAATGGAGACACAGAGGTGTAAAGA	CCCAAACTGCATATTTAAAGGGTAG	60	IF	19	491-169
Ya5NBC152	AC004953	Alu flanked by other repeats	Alu flanked by other repeats	-	-	7	-
Ya5NBC153	AC005820	CCAATCTGGGAATTATGACAAGTAG	CTTCAGACTTCTGCTTGATTTCTTC	60	FP	Y	496-186
Ya5NBC154	AC006371(B)	AAACACCCTAGATGCTGGGTAA	AGATGAGTGAGCCTCAGAACAAAG	60	IF	Y	501-197
Ya5NBC155	AC006565	TGTCAATATCAGACAGATCCATGAG	ACTTCCAACTATGTGGTCAGTTTTG	60	LF	Y	505-182
Ya5NBC156	AC002531	TGTGGTAAGTGTAGTTTCAAAAGAGTTT	TAATCTCTGGACTGGAAACATAAAA	55	FP	Y	480-148
Ya5NBC157	AC005281	CATACGTTAAATCACTCGGTACTCA	TCAGAAAAGTATACAGGTGATGTGC	60	HF	17	516-207
Ya5NBC158	AC005019	TATCTCCCCCTACCAAATTTCTTTC	GGATGGATTAGAAAGGATGGATTAG	60	FP	7	500-172
Ya5NBC160	AC005245	CTCAGCTGTGCCTGATACTCTATAA	GCCTACTGGATAAGTCACACATTTT	55	IF	17	551-234
Ya5NBC161	AL031978	CCTGTCTAAACTCCAGAATGAAGAA	GCAGTAGAAAGATCACAGGCTCTAA	60	FP	6	491-199
Ya5NBC162	AC003957	ATGAGCAAGTCTACATATTCCTCCA	CTTGTTGCTGTCAAGGGTCTAATA	60	FP	17	481-167
Ya5NBC163	AC004057	CAAACCAAGAGTTCTTATCACCAGT	TAGTAAGAGGTTTCCAAAGTACACG	60	FP	4	624-316
Ya5NBC164	AF042090	CTGCTGACTTTGAACTTAAACTGC	GATGGAAGATGTCTTAGGGTTCTCT	60	FP	21	503-190
Ya5NBC166	AC004040	CCCTTGGCTCTATAGATAAAGTTGG	ACTGCACCAAAACTAGAGAGGAAA	60	FP	1	532-210
Ya5NBC167	AC003980	AGCCCACAGCTAACGTTATACTAGA	GTGGGGTCTTTAAGGTTTCAATAG	60	FP	7	515-239
Ya5NBC168	Z97876	AGTGCTAACCAGAGATGTGTGTGAC	TTAGTGGAATGTTCCAGGACTGTAT	45	FP	1	492-164
Ya5NBC169	AC002456	TATATAATCCCACAGTAAGCCCTCA	ATAGTTGTATACCAAGCCAACGACA	60	FP	7	493-184
Ya5NBC170	Z94722	GCAAGACCTGTGTGTATGCTTAAAT	GAGAGTACACGAAAATACAGGCTTT	60	FP	X	521-195
Ya5NBC171	AL035688	TCTAGAATTACAAGTGCAAGCCATC	CTTCTCATCCCTGCTAACATAACAT	55	LF	6	451-130
Ya5NBC172	AC006371	CCAAACGTAAGATTGAGTGG	AGTGGTGTTCTCGGTATTTC	55	LF	Y	473-155
Ya5NBC173	AC003977	ACACACACGAATGCAGGATAAT	TGCTCACAGTCCTTAGACTTTACAA	53	-	16	508-107
Ya5NBC174	AC006462	TCACTCTTTGTCTTGCTGACTACAG	GCTATAGCTTCTATTTACGGGGAAT	55	IF	Y	526-206
Ya5NBC175	AC000396	CCAGTGTCATACGGTGCTTAAATC	GGACTGGGCTCTTCAGGAC	55	FP	9	483-148
Ya5NBC176	Z74739	GGGGGAGTATGGTTTGATATACAG	CCCTCATGGGAGGTGTTATTT	55	FP	13	666-298
Ya5NBC177	AC000111	TTTTCCAAGCGGTCCCTTAT	TCATGGAAAGCTTGTTTGGT	53	R/R	7	617-300
Ya5NBC178	AC004900	AGAGCCTGGACTCTGATGTTAGAC	GAGCCATGATAGGAGGAATACTAGAC	60	FP	14	583-260
Ya5NBC179	AC006373	GCAGAAGCTTGCAATAACCTCT	GCTGAACACCTAAACACTGCTAGAC	60	FP	7	797-490
Ya5NBC180	AL109618	CTTGAAGATCGCCATGAGTAGA	GGCATTTCTTTGGACTTGTCTC	55	FP	20	525-211
Ya5NBC181	AC008041	GTTACAGTGCCTACTTCTGGTTCTC	AGCCTTCCATCCTCATAGACC	58	FP	3	450-205

Ya5NBC182	AC006365	GAAGGACTATGTAGTTGCAGAAGC	AACCCAGTGGAAACAGAAGATG	60	IF	7	563-287
Ya5NBC183	AC006365b	GGACAGGTAGAGACGATTTCCTAGA	CAAGGGACTCATGTACTCTGTGAAC	60	FP	7	722-410
Ya5NBC184	AC000047	CTTGGATAGAGCTGGAGGTCATTA	ACCCAAGCAGTTTATACTGTACCC	60	LF	9	522-205
Ya5NBC185	AC006552	GAGTTTATTTGCCGTAGGTAGCTC	GGTAGGGGCTAAATGGAAAACA	61	FP	4	513-202
Ya5NBC186	AL035445	CATCTTCTGAACCCATAGGGAAAAT	GCCAATTGCCTGGTATGTTTTA	55	FP	6	649-381
Ya5NBC188	AC004970	GACAAGGACACAGATGTTAGGAATC	ATCTCTTGCAGTTGAATGTCTAAGC	55	FP	7	476-156
Ya5NBC191	AC007191	TGACGGGTGAGATGTATATAGAAGC	ACTCTTCTCATCTGTGTCAATTTGG	60	FP	19	645-330
Ya5NBC192	AC005678	CACTCAGATAAAGATGTGGACTTCA	GCTTTAGAGAGTCTGACTTTGCTTC	60	FP	6	536-238
Ya5NBC193	AC005065	GTTCTTTTTCTCTAAATGCCTCCTC	CCACATTTTTCTGGAACCACTTTAC	60	FP	7	525-206
Ya5NBC194	AC004866	TATTCTTATGCCGTTATGTCCTCAG	CCATGGAATACTACTCAGCTATGAA	55	LF	7	486-169
Ya5NBC196	AL031785	Alu flanked by other repeats	Alu flanked by other repeats	-	-	6	-
Ya5NBC197	AL031785b	CAGAAGTAAGATTGCTGGATCGTAT	CTCAATGAGATATCACCTCACACAT	60	LF	6	461-204
Ya5NBC198	AC004055	GCATAACTCCTAACCCATAATTTCC	GATCTAACACAACCAACTCCATCTT	60	FP	4	530-230
Ya5NBC199	AC005293	CTACCATCAATAACTTGGACACAGA	ATTACAGAGAGCCTGCCATGAT	60	FP	12	500-200
Ya5NBC200	AC005161	GTTTAATGGAAACCACCAAACGTAG	AGGCTGCTAGTTTCAGAAGGATAAT	50	R/R	7	500-174
Ya5NBC201	AC004745	CGCCACTTTCCCCAGTTA	CACCTCCCTAAAAAGCAGGA	50	IF	7	499-188
Ya5NBC202	AC004603	ACGCTCCAAAGTCCTCACCT	TGGAAGCTGGTTCTTCAGTG	60	FP	19	487-154
Ya5NBC203	AC004593	CAGCCTGTAGAAGCTGGAAAAG	ATACAACAGTTCTGGAGGTCTGAAG	55	LF	7	445-128
Ya5NBC204	AC002385	AAGCAAATCAGTCCTACCATGA	TATTTTGGAGAGTTGTAGGCAGGA	55	LF	7	5199-186
Ya5NBC210	AC004848	GAGGGGGTAGGGATAGCATT	GTGTTAATATTGTCCCCACATGTAA	62	IF	7	750-424
Ya5NBC212	AC002074	CATTTGGCGCAAGTGGTATT	ATCCCAAAGAAACCCACGA	60	HF	7	502-190
Ya5NBC213	AL078463	AGGAGAGTGGGAATGGGTGA	GATTCTCATGTACCCATCATGC	60	R/R	1	397-91
Ya5NBC214	AC004948	TGTTGTTGCAAAGGACAGGA	ACGTCCACATTCCCATGTTT	55	LF	7	500-170
Ya5NBC215	AL096710	GCCAATCTAAACGAATAATCA	AGGCAGAATGTAGTTGTTGG	60	FP	6	780-467
Ya5NBC216	AC007245	GATGTGACCCTGGCTTGTAAA	CAGAGTCCCTGTGCAAAATG	55	IF	7	456-141
Ya5NBC217	AC007298	TCCAAACCTTTTTGCTCTGC	GTATTTTGCCCCTGCCCTA	60	-	12	623-308
Ya5NBC218	AC006989	AGCCCAACATCTGGTTTTGT	TCCAGTCTCGTGTAAAATAGCTTG	55	FP	Y	445-109
Ya5NBC219	AC006989b	CCTGGCAACCACCATTCTAC	AAACCTGGAGGGCATTCTTT	58	IF	Y	445-129
Ya5NBC221	AC004019	CAGTTTTCCATATACATGTGGGTTC	TAGTGTTAAGAGGCCCATTTTCTAC	60	IF	22	640-313
Ya5NBC223	AC005006	GTTCTCTGTAAAATGGACCAATCAG	CATAGACCTTCCCAGTGAGTGTTAC	60	LF	22	455-214
Ya5NBC224	BK407F11	ACATGCTTTCCCATTATGTGTG	CCAAGTGGCAGTAATAGACTCTGTC	55	FP	22	502-195
Ya5NBC225	AC002470	Alu flanked by other repeats	Alu flanked by other repeats	-	-	22	-
Ya5NBC226	DJ323M22	CCTCCACGGACTCCTAATTACA	GTGGCCCTGAGAAGGAATTT	55	FP	22	421-130
Ya5NBC228	AC004832	ACTGCATGCCAGCCTCA	GCTAGTTACAATGAAAATGTGCTGT	55	FP	22	842-529
Ya5NBC229	AL096873	Alu flanked by other repeats	Alu flanked by other repeats	-	-	22	-
Ya5NBC230	AC000100	GACAAAGAAAATGTCACAAGGGTAA	GGAAAGAATTATCTAGGACAGCTTTG	60	-	19	418-99
Ya5NBC231	BA422A16	Alu flanked by other repeats	Alu flanked by other repeats	-	-	22	-
Ya5NBC301	AC007682	TCATGCCTGAACATCTGCAT	ACCTACAGCTGTGCCTACCA	60	-	2	795-677
Ya5NBC302	AL035665	CCTGCATACCCACACATACC	GGCAGTCAGCTTTTGACCTC	65	FP	20	395-72
Ya5NBC303	AL0136295	CTCCTCAAGGTCCCATGTTC	GGTGCCTCTGGGAATGAGTA	62	FP	14	426-111
Ya5NBC304	AL132642	GAGCTACTGGCACCTTCCAC	TTTTGACTCACCCTGCTTTTT	60	FP	14	368-60
Ya5NBC305	AP000966	CACATGGAGCTGTTTGCTGT	TGAGGGTTCTGTGAGAATTCAA	62	R/R	21	493-190
Ya5NBC307	AL133289	TCCCTGAAACAAAACCCATT	AAGACCACAACCCCCATACA	65	FP	1	450-144
Ya5NBC308	AL133404	CAACAGAGAAGAAATGATCAGTGG	TGGGCCCTATATTTGAACAGA	60	FP	6	429-147
Ya5NBC309	AC020663	CCTCTACCTGCTGGGTTCAA	CCCAGGGACTCTCCAGAAA	55	FP	16	425-114
Ya5NBC310	AC008372	ATTGCAAATTGGCGATGTTC	CACCACTGAAGCATGCTAGG	62	FP	14	535-207
Ya5NBC311	AC008843	TCTTGGCAAGGAGATGTGAA	AATCACATCCGAGGGTGTCT	60	IF	5	584-279
Ya5NBC312	AC01069	CACTCAGCATCCAGTTCACG	GGCCTCTGGTTTCAATTGTC	60	FP	3	365-54
Ya5NBC313	AL121823	CACTTGCCATTGACTCCAAA	GGCTGGGTTGTGTGAGTTCT	60	IF	X	481-174
Ya5NBC314	AC016025	GTTCCAGGGGGAAATGAAAT	GTGGGGCACTGTGTGATTC	60	FP	22	392-70

Table 1. *(continued)*

Name	Accession	5′ Primer sequence (5′-3′)	3′ Primer sequence (5′-3′)	A.T.[a]	Human diversity[b]	Chr.[c] Loc.	Product size[b] Filled-empty
Ya5NBC315	AP000474	GTAGACACCGCAGGCAACTC	AAAAGGATCCGTAAGAAGGAGA	62	FP	21	444-134
Ya5NBC317	AL132985	CCAAGTCAGGCCACCAATAG	GATGGATAACCTTTTTCCTGGT	60	FP	14	384-64
Ya5NBC319	AC007395	TTGCTGGTCCACAAACCATA	CCTTTGTCATCATGGTGCTG	60	FP	2	358-77
Ya5NBC320	AC009498	CCATCTCCCTCATTATTGTTCA	CCATTTGGGAGAAGGTTCAA	60	FP	2	478-161
Ya5NBC321	AL121748	GGAGATCCTTCTTTTTCAGCAA	GGAGGTGTCATCCTGGTACA	60	FP	10	455-145
Ya5NBC322	AL132800	AGTGCGTCAGATCCTGTTCA	GGGTCTTTGAAAAGTTCATGG	60	FP	14	451-129
Ya5NBC323	AC007076	TTGAAAGAGGAAGCCCAAGA	TCTCTGCTCCCCAACTCTTC	60	FP	7	556-268
Ya5NBC324	AC008268	TGTCTCAAGGGTCATCCTCA	TCCCCATCCCTAACTCTTTCTT	60	IF	2	486-164
Ya5NBC325	AC009479	CTTCTCTCTCTGAAATGCCAAT	CAGTTGAAAGGTTTGACAATACACC	60	IF	Y	501-184
Ya5NBC326	AL133500	CCAAGAGACCACTTCCTATTTCA	AATGGGGGAGAGGACAGTCT	60	FP	X	539-216
Ya5NBC327	AL132799	AGGCAGGTTCAATGTTCAAA	TTGTCTTATTGTGCTGGCTAGA	60	IF	6	668-339
Ya5NBC329	AL121892	TTTTTCCCCTGTAGTTGGACA	TTGTTCAGGAGAGGGAAGGA	60	FP	20	465-154
Ya5NBC330	AL133399	ATGCTGTGGGTTGCTAAGGA	CTGTCCCTTGTTTGGCTTGT	60	FP	11	402-88
Ya5NBC331	AL121593	TTCATGGCGAAAGCTTGATA	AGCTCCTGGCCAGATTAACA	62	FP	20	414-92
Ya5NBC332	AL050342	TGGAAACAGAGCAATGGACA	ACACAGGTCCTTGAATATGAGC	65	FP	1	631-416
Ya5NBC333	AL117356	GGCATGCTATCATTCCCAAA	CCAAACTTCTGTTTGAGAGAATACG	60	IF	14	588-281
Ya5NBC334	AL132708	ACACTGTCTTGGAGGCATTC	CCTCCATCCCAGTACCATGA	60	FP	14	435-117
Ya5NBC336	AC007151	AGGCCCACATCACTGTAAGG	TGATCCATAGCTCTTTTTGTGC	60	FP	16	486-172
Ya5NBC338	AC009510	TCAAGAAGCTAAAGGCACCAA	AGGGGAAGAGGAAAAGATGC	60	FP	12	564-271
Ya5NBC340	AL109985	TCCATATCCCTTGTCTGGTTC	CCTGACCAGGTCCAAATGAC	60	FP	14	468-145
Ya5NBC341	AC007899	ATGCAATTGCTGAACACCAG	GGTGGACCGAGATTTTCTTTC	60	FP	2	494-174
Ya5NBC342	AL049823	TTTTCCACAAATGGCACTGA	TGTCTGTGGCTCGTCATTTC	60	R/R	6	604-285
Ya5NBC343	AC005660	GACCACACTGGTCAGGGACT	CCCTCTTTGGTCTTGAGTGG	60	FP	10	457-154
Ya5NBC344	AL109853	CGTGAGAAAGCATAGGCAAC	TCCTTTCCTTATGCCTGCAA	60	FP	Xq	472-158
Ya5NBC346	AL096776	GGAGAACTAGTGTGGGAGCAG	ACACTCCCCTGTCCATTCCT	60	-	1	396-60
Ya5NBC347	AL035411	CATGCCCATTGCTTTACGTT	TGGGGTAGATGGACTCATCC	60	IF	1	465-140
Ya5NBC349	AC011504	TCAAGAACTGTGGGCCAAAT	GGATGTTGTCACAGCAGCAT	60	HF	19	469-53
Ya5NBC351	AP000459	TTCCTCCCCTTTTTCCTGTT	TGTCAGTATGTAAACCCATGCT	55	IF	21	437-123
Ya5NBC353	AL034549	CCATGTAACCTGGTAGACCTTT	GTTCAGCGGGAACAGTGAGT	60	FP	20	432-119
Ya5NBC354	AC008039	GTAGCTTGGCCTGTGCTCTT	CCTCTGGGCTGAGAAACTCTT	65	IF	7	466-148
Ya5NBC355	AL078477	CATCTCACTTGAAAGCCCATT	TGTGTCTTAATGACCCTGGAAA	60	FP	11	802-481
Ya5NBC356	AF130343	CAGGGTCCTGTGAATCCAAT	GGAGACAGAGAAAAGGGGAGA	62	FP	8	389-84
Ya5NBC359	AC007564	GCAAGTCCTATGCAAGGTCAA	AGGCTTTTCAAGCCAGTGTT	60	FP	12	775-457
Ya5NBC360	AL031121	GAAACAAACATTTGGTAATGATGC	GACCAATGTCACTTATGAAATCCTT	60	FP	6	407-61
Ya5NBC361	AC007270	AATATTTTCTCCCATTCTTTTGG	TGTTAAAAGCGCAAGTCACAA	60	IF	7	423-131
Ya5NBC362	AL050308	CAAGTTTGTTGGCATAGAGGTG	ATCAATCCAGGAGCCGTTTT	60	R/R	X	506-187

[a] Amplification of each locus required 2:30 minutes at 94 °C initial denaturing, and 32 cycles for one minute at 94 °C, one minute at annealing temperature (A.T.), and one minute elongation at 72 °C. A final extension time of ten minutes at 72 °C was also used.

[b] Allele frequency was classified as: fixed present (FP), low (LF), intermediate (IF), or high frequency (HF) insertion polymorphism. Fixed present: every individual tested had the Alu element in both chromosomes. Low frequency insertion polymorphism: the absence of the element from all individuals tested, except for one or two homozygous or heterozygous individuals. Intermediate frequency insertion polymorphism: the Alu element is variable as to its presence or absence in at least one population. High frequency insertion polymorphism: the element is present in all individuals in the populations tested, except for one or two heterozygous or absent individuals. (–) Indeterminable. (R/R) Repeat in repeat.

[c] Chromosomal location determined from Accession information or by PCR analysis of NIGMS monochromosomal hybrid cell line DNA samples.

[d] Empty product sizes calculated by removing the Alu element and one direct repeat from the filled sites that were identified.

ing expanded about 10.6 myr ago. If we assume that humans and African apes diverged from each other only 4 myr ago, then we can calculate that 6.64/10.64 (62%) and 6.6/10.6 (62%) of the Ya5 and Yb8 Alu elements should also be found at orthologous positions within the genomes of non-human primates. If we shift the divergence of humans and African apes to 6 million years ago then the estimates change to 4.64/10.64 (44%) and 4.6/10.6 (43%). However, less than 0.21% of the elements were also located in orthologous positions in the genome of the common chimpanzee. The observed distribution of Ya5 and Yb8 Alu repeats located within the common chimpanzee genome would require a human and non-human primate divergence of greater than 10 myr ago. This is clearly a much older divergence time than is commonly accepted.

Three potential explanations may account for this. One is the selective removal of Alu elements from orthologous positions in non-human primate genomes effectively resulting in an ascertainment bias against elements in the non-human primate genomes because our elements were obtained by scanning a database of human genomic sequences. However, we consider this to be highly unlikely, because there are no known mechanisms to specifically remove Alu elements from primate genomes and even when an element is partially deleted from the genome it leaves behind a signature of itself.[33] A second and more likely explanation is that the amplification rate for these subfamilies has increased recently in the human lineage. Alternatively, the higher average ages for each of the Alu subfamilies than those previously reported may reflect a higher sequencing error rate in the genome database, resulting in an inflated age estimate for the Alu subfamilies. The estimated ages of the subfamilies are also inflated by the faster accumulation of non-CpG based mutations (as a result of the larger number of potential target sites) as compared to CpG nucleotides. Therefore, the use of the CpG-based mutation density for Alu subfamily age estimates will be much more accurate than the use of non-CpG mutation density-based estimates using the current draft sequence of the human genome. The magnitude of the putative sequencing errors can be estimated by comparing the previously reported non-CpG mutation density for these Alu subfamilies of approximately 0.4% for the Ya5 and Yb8 Alu elements to the levels reported here of approximately 0.8% for the same subfamilies. Therefore, the maximum possible error rate would be estimated as 0.8% − 0.4% = 0.4%. In our data analysis, there are a few Alu elements with much higher mutation densities than previously seen. We are not sure whether these represent a small number of authentic, highly divergent subfamily members (approximately 10% divergence), or the concentration of sequence errors in a few elements. Thus, other than the possibility of a few areas where errors may be concentrated, there is a relatively low sequencing error rate across the entire database, demonstrating the reliability of the draft human genomic sequence. Large scale re-sequencing of the Alu elements characterized in this paper would resolve this issue and allow for an accurate estimate of sequencing error rates within the draft human genomic sequence; it would also provide a refined estimation of the average age of the Alu Ya5 and Yb8 subfamilies as well.

SINE retroposition is the primary mode of mobilization of Alu elements, where mutations in the source gene(s) create their sequence evolution. However, previously we reported that gene conversion and genetic instability might have also significantly impacted the Alu sequence architecture.[18] Our analysis of the Yb8 mosaic elements also suggests that gene conversion may have influenced the evolution of the Yb8 Alu subfamily. Among the alternative explanations for the occurrence of mosaic elements, multiple parallel mutations seems unlikely; unless there was selection for these specific mutations, such as the post-transcriptional selection previously proposed.[8] However, a selection process that would only select for these specific mutations would be improbable. Recombination may have generated some of these mosaic elements, but multiple recombination events would be required, making it unlikely. Therefore, we believe gene conversion to be the most likely explanation for the existence of the mosaic Alu elements.

Our analysis of the human genomic diversity associated with the Ya5 and Yb8 Alu elements reported here resulted in the recovery of 106 new Alu insertion polymorphisms. The percentages of Alu insertion polymorphisms recovered from each subfamily were 25% and 20% for the Ya5 and Yb8 subfamilies, respectively. The percentages of Alu insertion polymorphisms in these two subfamilies are in good agreement with previously published insertion polymorphism estimates for these Alu subfamilies.[21] We can also estimate the total number of Alu insertion polymorphisms within the draft sequence of the human genome using our copy number estimates and the percentage of Alu insertion polymorphisms associated with each family. Using this approach we should recover 2640 × 0.25 or about 660 Ya5 Alu insertion polymorphisms and 1852 × 0.20 or about 370 Yb8 Alu insertion polymorphisms through the exhaustive analysis of the draft sequence of the human genome. Therefore, the exhaustive analysis of the entire Ya5 and Yb8 Alu subfamilies from the draft sequence of the human genome should generate a little more than 1000 Alu insertion polymorphisms from these subfamilies.

Additional Alu insertion polymorphisms that are present in diverse human genomes may also be recovered using PCR based display approaches such as those previously reported for Alu and LINE elements.[17,34] Each of the Alu insertion polymorphisms in the genome is a temporal genomic fossil that is identical by descent with a known

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Table 2. Alu Yb8 accession numbers, locations, human diversity, oligonucleotide primers and PCR parameters

Name	Accession	5′ Primer sequence (5′-3′)	3′ Primer sequence (5′-3′)	A.T.[a]	Human diversity[b]	Chr.[c] Loc.	Product size[b] Filled-empty
Yb8NBC1	AL049798	TACCAAGAGGATGTAAAACACAAGG	GGAACCCAAGGCTTATAATTTAGTC	60	FP	1	495-174
Yb8NBC2	U91327	GTAACTCTGTGGGTCCTCATATTATCACT	ATACCTCATCAGCAATAGGCAATAG	60	FP	12	461-130
Yb8NBC3	AC004804	AGATGCGCAAGTCCCTGATA	ATTTTTGGATTCAGCCAACG	61	HF	12	558-236
Yb8NBC4	AC005156	AGTGAGATGGTGGTTGCACA	AAAAACCTAAAGAGGGCAGT	60	FP	7	451-133
Yb8NBC5	AC004027	AAGGTCTAAGCGCAGTGGAA	TGTATGCAGGTTGCTTGCTC	60	LF	7	503-167
Yb8NBC6	AC006150	ATACCAAGACATCACACTGC	TAAAGCTGACACATTGTTGG	60	FP	7	606-203
Yb8NBC7	AC005048	AGGTTCATCCATGTTGTAGG	CTTAGAAGGGAATCCAGGAG	60	HF	7	605-285
Yb8NBC8	Z98950	AAGAAAACTGATGGGGAAAG	CCAACTAGAGAAACGGAGAA	60	IF	X	599-198
Yb8NBC9	AC004825	GTCCCCACCAATCCCTATCT	TGCTCAAAGTCCCACAGCTA	55	IF	14	655-322
Yb8NBC10	AC006352	CACGACAACACGTTTACCTCA	TTTCCTTTCAGGAACGTGGA	60	IF	7	505-165
Yb8NBC11	AL022477	TGGAAAGTGCGTGCCTTAAT	ACCTGAGGGAGAGACATTTCC	60	FP	6	510-188
Yb8NBC12	AC0006241	CCCAGCCAGGGTTTATTCTT	ACCCTGAAATGTCCTAGTGC	60	FP	9	487-160
Yb8NBC13	AC002331	TCTGGGTTTCTCTGGTGGAC	CTGGCAAATGCTACCCAAGT	60	LF	16	510-168
Yb8NBC14	AL08633	AGGAGACATTACAACTGATACTGC	TTGGCCTATTCCAGTCATGG	58	FP	X	499-167
Yb8NBC15	Z82211	CTAATTCCCCTGGCTGCATA	CTCTGGTACGGCCATAAAGC	60	FP	X	481-165
Yb8NBC16	U51244	TCACGAGAGGCCACTTTAGG	CCGACCACAAGCCAGAGTAT	60	FP	2	519-211
Yb8NBC17	AL049642	AGCATACAATTTGGCAAGCA	GCAGGAAGTGATTGTGCTGA	60	FP	X	507-184
Yb8NBC18	AL078476	TGTGGTGTGGGCTAGAGGATG	TGGGACTCAGATTTCTGATAGGA	56	IF	21	431-133
Yb8NBC19	AC007077	TTGGCATGTGAATTGTCTGAG	AAACCGTCAGTTGGGATCAG	55	FP	2	516-191
Yb8NBC20	AC002059	TCTGGGCAAGTCACTCAAAA	ACTGTTTCCATGGGCATGAT	56	R/R	22	502-158
Yb8NBC22	AC004875	CCACCAATGTCCTTTCTTAC	AGTGGAATCAGTCAATTGGT	50	FP	7	598-275
Yb8NBC23	AC005821	CCTCCTTGAGAAGGCAACAG	TGCATCTCCAGGTGTTCATC	60	FP	17	499-177
Yb8NBC24	AC005772	AACACTCCACCCAATAACTG	CATCTCTTCTTTACCTGTTTCC	56	HF	17	661-338
Yb8NBC26	AP000036	GGGTCCCTCTCTGAAGGTAAA	TTTTTCCTGCCAACCAACAC	56	HF	21	499-150
Yb8NBC27	AL021940	TTTTGGGAGGGACACAGTTC	GGGCCACACAAATAAATTTCC	55	FP	1	522-188
Yb8NBC28	AC003998	CTTTTTGATCATGAGCTGTG	ACAACAGAAACACCAGCTTT	60	FP	5	515-197
Yb8NBC29	AL09177	CTCTCCAGTATGGACAGAGG	AGTGCCCAGAACAGATATGA	60	FP	6	519-200
Yb8NBC30	Z95124	TTGCCTTGGATGGCATATCT	AAATGGCCGGAGTAAGTCCT	55	IF	X	497-194
Yb8NBC31	AC005046	TGAGGCAATTTGGAGGAGAG	TGGTGTGCTGTATGTTTTCCA	60	FP	7	518-188
Yb8NBC33	AP000352	GGAGAGCAAGCACACATAGT	GCACAATAGCAGAGGAGAAT	55	FP	22	496-167
Yb8NBC34	AP000168	ATGACCTTTGCATTTCACCA	GTTCAAGCCCCATCACATCT	60	FP	21	513-227
Yb8NBC35	AP000171	CCTTTTTGTCTCCTGGCTGA	CCTGAACACACCAGAGCAGA	60	R/R	21	497-220
Yb8NBC36	AP000156	CTAAGGAGAACGGGACAGAT	TATATTCTGGATCCCAGTCC	60	IF	21	506-183
Yb8NBC37	AP000193	AGAATCGAGTTTTCCGAGCA	GGGATGGTACAGATGGCATT	60	FP	21	486-162
Yb8NBC38	AC002367	CGAGAGAAAGGGGTAGAAAGC	AATGCCTTCCAAGGACATCTT	60	FP	X	480-311
Yb8NBC39	AP000111	CATGTGTCCATGGTGGTCAG	TCACCCCACTTCGGATTAAC	60	FP	21	456-129
Yb8NBC40	AP000080	TCCCAGGAGAAGGATGAGAA	CCTGTGTAGTTTTGGGCAATG	60	FP	8	500-171
Yb8NBC41	AC006011	TGCTTAAGGTTGGTCAGCAG	CAACCAGGAATGCTGTTTTACA	60	R/R	8	482-153
Yb8NBC42	AC005971	TTCCAGCATGTGAGAACATA	ATGCATCTTAGTCTGCTTGG	55	R/R	17	517-189
Yb8NBC43	AC004228	GTTGGACTGGGCTCTACCAG	GACAGCAGTTTGCCATCTCA	60	FP	11	521-201
Yb8NBC44	AC005521	ACCACCCCTGGTACCAATTT	ATGCCTGCTCGTGCTTTACT	55	R/R	7	503-180
Yb8NBC45	AB020874	TGGCTTCAAGAGCATATCCA	GAGAAATGAACGCATTTTGG	55	FP	9	567-274
Yb8NBC46	AL031224	TGCATTTCTAAGCAGTACCAGTG	TCCATTCATTCGTGCCTTCT	60	FP	6	523-203
Yb8NBC48	AC005495	AGGGAACAGGTGATGTTTGG	GAGGATGCAAAAGCATGTGA	58	IF	17	511-178
Yb8NBC49	AL031904	GCAGTGGATTGGTTTTTCTG	GCTGAAAGAGGCATTGAAATC	60	IF	6	542-205

Yb8NBC51	AC005585	GCAGATAAGGGTTAACTGGA	GAAACACTTGGAAGATGCAA	60	FP	22	522-185
Yb8NBC52	AC004984	GGGGTCAGGGGAGTAGAGAC	ACATCGGTGATTGGAAAACC	60	FP	7	515-196
Yb8NBC53	AL050338	GGTGGTTGCTAAAAGCTAGGG	GTGGCAGGTTTTGAGAGGAT	60	R/R	6	499-186
Yb8NBC54	AF165147	TGCCTACCTTTTGCACTTGA	AGCATATCATGACTGGGTTGAA	60	FP	21	454-220
Yb8NBC55	AC006374	CAAGGGGGCTATGCACTTTA	CCACCCAAATCTTTTTGTCA	60	FP	7	494-146
Yb8NBC56	AC007056	TGAACCACATGGACATCAAA	TATGCCCACCCAGATAATCC	50	FP	14	510-405
Yb8NBC57	AC006230	CCTTCACCTGCTTCCTTCAG	TATTCACAACAGGCCGCTTT	60	FP	14	519-191
Yb8NBC58	AC007319	ACACATCCCTGGGGCTTATT	CTTCCACTTGAAGTTTTCACTG	60	FP	4	566-251
Yb8NBC59	Z99758	GCAAAGGCCATTGAGAGAAA	AGGTCAAGCTGATCACTCACAG	60	FP	1	527-211
Yb8NBC60	AC004765	TGGGATGTGGTTCTGTGATT	TCAGACTGTGTTACTACGGGTCA	55	FP	12	528-304
Yb8NBC61	AC005040	GGAACTGCCTGAATGAACAA	CCTCTGCGCCCCTCTACTAT	60	FP	2	539-209
Yb8NBC62	AL031368	TGCCACACATTGTTCTAGGC	TGCCAACTATTGGAGGAGATG	45	-	X	548-307
Yb8NBC63	AC005901	CTCCCCACGACAGAGAATTT	GATGGGTGGCAATCAGAGAT	60	FP	17	609-282
Yb8NBC65	AL031228	ATCTCATCTCCCTGCCTCTG	GGGAGGTCTGGAGATCTGTG	60	IF	6	517-186
Yb8NBC66	AC004981	TTTTAGGAATTGCCCCATTG	TCACTAATGTCCCAGCCAATC	55	FP	7	485-167
Yb8NBC67	AL022170	TCTCTACCCAGCTTTACCAA	GAGGACCAGCTTAGTTTGTG	55	IF	6	503-375
Yb8NBC68	AC004103	AGAAAAGTGCAAAGTGCCTA	TCCAAGCTCCTTAGTGTAGC	60	FP	X	518-188
Yb8NBC69	AC004458	CTTTCCCAAGGCCTCTGTC	GCCACACACCTAAAGCCATC	55	LF	7	557-242
Yb8NBC70	AC002456	CCAAAATCTTCCCAAGCAAA	TGTCAGTTCCTGGTTGGCTA	55	-	2	541-208
Yb8NBC71	AC006222	GTGCTGAGCGCAATCTTGA	GGACCAAGGGGTGCATTTA	61	LF	7	493-167
Yb8NBC72	AC002432	GGTAGTGAAGAGGGCGAGGT	ATGCCAACTGGGTCTGCTAC	45	R/R	7	526-210
Yb8NBC73	U47924	AGCCATTTTTCCCACTTCTGT	TGACCTCCCTTCAGGAATTG	55	FP	12	437-111
Yb8NBC75	Z68328	CCCACTGTGTTTATTGTTCC	GCTAAAGTACCCAGACCAAG	60	FP	X	519-200
Yb8NBC76	AL031770	TGCAGTTAAAGCAACGCAAA	TTCACCTGACGAAGCCTGTA	55	R/R	6	515-197
Yb8NBC77	AC007542	CGGAATGTTCTGAGGATCAAA	GGAAGCTCTGCACAACTCCTA	55	LF	12	547-218
Yb8NBC78	AC005866	GAAAACGCCTCCACCATAGA	TCAAACCTGGCTACACACAGA	60	FP	12	536-215
Yb8NBC80	AC006249	ATTTCACAGTGCCCTGTCCT	TCCAGGCAGATGAATTGACA	60	IF	18	456-123
Yb8NBC81	AC004837	TCCATTACTGAACACTTGGA	GAAGGGCAGTTTTGTGATAC	60	FP	7	502-173
Yb8NBC82	AC023806	TCATTGTTCTCTTGGTGTTG	ATGGAAACAGTGTGAGGAAC	60	FP	6	566-239
Yb8NBC83	AC004741	CTTCCCCCTTCAGTGCCTAT	AACATGCATCCTGTGCAGAC	60	FP	7	528-199
Yb8NBC84	AL008628	TGGTCTGCAGGTTCTCCTCT	TCAGCAAATAAAGCCCAAGG	60	FP	6	517-206
Yb8NBC85	Z98744	CACCGCGTGTTGATAGAGTC	CCACCTCACCATTCCAGAGT	60	FP	6	483-163
Yb8NBC86	AC004595	CTCCCTTTATCCCGGATGTT	GGAAGGCCATGGTGAAGATA	60	FP	7	460-353
Yb8NBC87	AC003048	TACTGAGGCCATCGAGGAAC	ACCGGCAGAGCATAAATCAG	60	FP	X	537-210
Yb8NBC88	AC002524	CAGCTAGGCCTTGGAGATCA	TCAGAACCATGTTCTTGGAATC	60	FP	X	526-199
Yb8NBC90	AF02503	AGCTTTCTGCAAGCAAAGGA	AAAACAGTCTCAGGGCCAGT	55	R/R	3	488-153
Yb8NBC91	AC000083	AGCAACAGGGATTTGTCTGC	CAGTGGCTGACACACACACA	60	FP	22	513-194
Yb8NBC92	Z82189	CCTACCAAGACCGACAGGAA	GCAGGGCTGCACTTTTTATC	60	FP	22	533-183
Yb8NBC93	AL035461	AAGTGAGTCCCAGGGCCTTCT	CACACAGGCACTTGTTTGGT	60	IF	20	601-274
Yb8NBC94	AL049633	GGGTGTGATGAAGGAAATAA	GCACTGCTCTGACCTCTATC	60	FP	20	601-274
Yb8NBC95	AL031346	GTGCTCAGCCAAAATGTCA	GGCACCAGGTTTCCTGAAGT	60	FP	22	532-210
Yb8NBC96	AC006222	GAACCCAAATAGCCAAAGCA	TGTCCTTCCCCCAGTTTATG	60	LF	22	498-224
Yb8NBC98	AC006441	TTGGCTATGTGAGTTAGATTGG	GGGCAATTTCAATAAGCAAGAG	60	FP	17	514-192
Yb8NBC99	Z92542	GATTCCAGTGCCTTCTGCTC	CAACCCATACAGTGCTTGGA	60	FP	X	524-200
Yb8NBC100	AB019438	CGCAGGTGCAATAATATGGA	AATTCCCTCCAGGTTGCTGT	60	IF	14	574-247
Yb8NBC101	AC005104	TTTGGCATCCACAGATTTGA	CTGTGCAGATTCCCCAAGTT	60	FP	?	593-273
Yb8NBC102	AL035088	GCCTGCAGAATGGAAGAAAC	CCAACTGAGTCCCCAGAAAA	60	HF	X	512-323
Yb8NBC103	AL022576	GCCTTTAAAGCCTAGCTCTC	GTGAATGCAAGTGAATGAAA	60	FP	X	531-205
Yb8NBC104	AC004638	GGGAGGAGTGGATGAATAAT	AAAACTGATGTCCAGCCATA	60	FP	16	533-207
Yb8NBC106	AF064865	TCACAGCACAATTCACAACTG	CTGGGTTGCATTTCATGGTA	60	IF	21	558-233

Table 2. *(continued)*

							Product size[b]
Name	Accession	5′ Primer sequence (5′-3′)	3′ Primer sequence (5′-3′)	A.T.[a]	Human diversity[b]	Chr.[c] Loc.	Filled-empty
Yb8NBC107	AC006222	GTTTGGTTTTTCCGCAGTGT	GACTCGTCACTGGGTTGGAG	60	FP	4	527-207
Yb8NBC108	AF164343	TGTCACTTGATTGTCCGCATA	TCAATGGCATCCTGAAAACA	60	IF	Y	550-194
Yb8NBC109	AC006371	GTGCAACTTCAGTTTCTGCTAAGAT	CATGGTTATCTGCAAAGACTATGAC	55	IF	Y	532-212
Yb8NBC110	AC006383	AATAGGCTGAATGCCCCAAT	CTAGCATTGCAATCCCTGCTTT	60	LF	Y	507-186
Yb8NBC111	AC007320	CCAGTGTCATCATCCAGACTTATTC	TACACACACACACATGCATTCTAAG	60	FP	Y	531-192
Yb8NBC112	AC006999	GCATCTTAACCTAAATACCTGATGC	CAGGGACATAGGGTGTGAGTTACTA	60	FP	Y	503-192
Yb8NBC114	AC004617	GGGTGAGATAGCTTAAGGAAAGAGA	AGATCTTCCCAAGAAGCCTTTC	60	FP	Y	510-164
Yb8NBC115	Dj102d24	TCATTCAGCCAACACTGACC	CAGGTTTTACCTCTACCCTTGG	60	FP	22	628-297
Yb8NBC117	Z82189	CAACCACACAGCCTAAAGACAT	GGCTGCACTTTTTATCCACCTA	60	FP	22	461-111
Yb8NBC118	AC006548	GCAGAGACACATAAGACTGATTGAA	ACCTGGGCTATGACCTGATAAATA	60	FP	22	519-200
Yb8NBC119	Z95114	AGACCTTTGTCAGATGGATAGATTG	GTTTTGTGCTGTAAGGCTGAGTAG	60	FP	22	425-110
Yb8NBC120	AC004019	CAGTGGATCTCCATTTTACCTCTC	GGAAAGGTTTCAGGAAGAAAGTG	60	IF	22	532-212
Yb8NBC123	AL031846	TTTGGATGTTTGTTCCCTCT	GGTGAGAGCAGAAGCACGAG	60	-	22	732-412
Yb8NBC125	Dj309I22	AGCCAGAAACCCTGAACAAG	AAAGGCCCCAGAAGTATACCA	60	IF	22	415-97
Yb8NBC126	AC002055	AAAATGTCCCCTTTGTCCTTC	CCTACGCAGAAACACCCTAGA	60	LF	22	438-118
Yb8NBC129	AC004052	CCCAAACCTCCTAGATCTGC	CCCTGATTTCTTTCAGCAGTG	55	R/R	4	528-136
Yb8NBC131	AC002994	TGTGGGTCTATTTCTGACTCCA	TCTACAAAACCGAAGCTGTT	55	FP	17	506-264
Yb8NBC132	AC002458	GTTTCTGTGGGTTGGGATTC	AGCCAGCAAGAACCTGAGTC	60	FP	4	507-187
Yb8NBC133	Z84470	GCCATTGATCCCACAGAAAT	GCTGTGAATTCGTTGGTCCT	55	LF	X	536-232
Yb8NBC134	AC002067	TGAGCAAAGGATTTGAATAGGC	AGGGTTCCAGTTTCCCCATA	60	LF	7	526-206
Yb8NBC135	AC007392	TTCCTCCTCTTCTGGGACAA	GGAACCAAGGAGCAAAGAGA	60	FP	2	659-206
Yb8NBC136	AC007055	CTTGCTCACACTCTGGTGGA	CTGATTTCACCGGTTTTTCTTC	60	-	14	530-196
Yb8NBC137	AL031782	GGGTAAGTGGACAGGCGAAA	TGAAGCTATCTGGACCAGGAGT	55	FP	6	454-126
Yb8NBC138	AL031653	GTTCCTTTCTTCTCCTCAAAAG	TGCCTTTAATGTGCCATCTT	60	FP	20	650-332
Yb8NBC140	AC006012	TTTACTGGACAGTTTGAAGC	CAGAAATGGTTCCTGTGTTT	55	R/R	7	494-180
Yb8NBC141	AC003950	GGGTAAGACAATAGTGGGGATT	TTCACTAGATGTGCAAGGGTTC	60	FP	17	530-219
Yb8NBC142	AL049869	TCCAGTGCCTCAGAAGGTG	CATGGTGTCCTTCCTGTGTG	60	FP	14	487-162
Yb8NBC143	AC009044	GGCTCTCTAAGCTAAGACAATCAA	CGTGCTCAAGGTATTGGTCA	55	FP	16	443-133
Yb8NBC144	AL033531	TCACAGCGTGTGCATTACAA	AGGACTTCATTTTGGGGGATT	60	FP	1	578-255
Yb8NBC145	AL035089	TGGTCCAGAACCTTCTCCAA	CAGGAACATGGGCTGAGTGT	65	FP	20	520-197
Yb8NBC146	AC009028	CTCTTCTCTCCAGGAAACGTC	GGAGCTCTGCCTTACACTCAA	60	IF	16	887-592
Yb8NBC147	AC010340	GAAATCTGTGCCATAGACGAAA	TGTGTGTGTACCACCATTTACA	55	FP	5	516-149
Yb8NBC148	AC010582	CCAGGCCTCCATCTTTGATA	TCACTTTTGGGCATGTCAAG	60	IF	14	537-218
Yb8NBC149	AL135746	TGAGTGAGTTCAGAAAAATCAAGG	TGATTAATTTACTTCATTTGGCAGT	60	FP	14	460-138
Yb8NBC150	AP000855	CTGGCCATAAATTCCCTCAG	TCAGAAACTGCCCAAGAGAGA	55	-	21	474-160
Yb8NBC151	AP000456	GGCACCAGGAGGAGAGAGAT	TGGTACCAAACTGCCTTCCT	60	FP	21	464-138
Yb8NBC152	AC007911a	TGATGTTGACTTTGGCTTGA	GCTCCATAACTGGGTTCAGG	60	FP	18	520-183
Yb8NBC153	AL049776	GGGAGTTAATCACTGTCCTCAA	CAGGCTTTAGAATAAGCAGTGAGA	60	FP	14	569-248
Yb8NBC154	AF172277	GGCCTGAGCACTGGTAGTTT	TGTGACTGGCCTATTTCACG	60	FP	7	469-147
Yb8NBC155	AC010169	GGGAAGAGGGTCCAAGTGA	TTCCCCTCTACTCCCTCATTC	60	FP	3	421-90
Yb8NBC156	AP000566	ACTCAGGCCTTTCATTCTGC	ACTGGCAAGGGAATGTGAGA	60	FP	21	554-234
Yb8NBC157	AL121748	TATGGTTCTCAGCCATCACG	ATTCTTCCCCAAAGGGAGTC	60	IF	10	712-423
Yb8NBC158	AC007671	GCAGAATACACCAAGCTGAGG	TGCCTGACTGTCCTATTTCAGA	60	FP	12	394-69
Yb8NBC159	AC007680	TCACATTGTCCCTTCTCAGC	TATGCAGGGCCTTCAACATA	60	FP	2	448-112
Yb8NBC160	AC007284	CCACACATGGGTACCAGTCC	TTGCTTACCCACAGTCACCTC	60	IF	Y	404-72
Yb8NBC161	AC007100	CCATGTTCCAGGAAGTGTTCA	CACGCAAAGTTAACAGAAGTGC	60	FP	2	418-85
Yb8NBC162	AL132987	GCACTCATTTTAGTGGCTGCT	TGCAGTTCAGCCCATAACAG	60	FP	14	504-185
Yb8NBC163	AL035467	TTTTCAATGCCTCTGTGCTG	TCCACTCACAAAGCTTCACG	60	-	6	462-133
Yb8NBC164	AC009509	TGACAACATCCGTGACAGAAA	TCACAGGCCCAATAAAACAT	60	FP	12	387-76
Yb8NBC165	AC010200	TGGGATGAAGGGAAGATTGT	AACAGTGCCAATTCCTGAGAA	60	FP	12	465-151

Yb8NBC166	AL121852	CTGCTGCCTTCCCTAGACTG	CTCACTCTTAAGTGAACAGACTCAA	55	FP	14	570-248
Yb8NBC167	AL049777	CCTCTGGCTCCACAGGTAAA	ACTGGGTGCTTCAAAAGTGG	60	FP	14	415-95
Yb8NBC168	AL078603	AGATGCCCCCACATATCAAA	ATGGCATTCGTGGGTTTCTA	60	FP	6	469-150
Yb8NBC169	AC006480	TTTGGTAGCACTTCCGGTCT	GCCTCCTCACCCAATAGGA	60	-	7	426-99
Yb8NBC170	AL109653	TCCCCAAAGAAGGAGAGACA	TTCCCCCATTCCACAATTTA	60	FP	X	599-275
Yb8NBC171	AL096771	TGCGTGTATTTTTCAACTGGTC	GACAAAGGGAAAATCCCATC	60	FP	6	537-206
Yb8NBC172	AC010197	GACACTTTGAGTTTACCAGGAAAGA	CACTTCTAAATGAGACTGGCTTGAC	60	FP	12	408-88
Yb8NBC173	AC007250	GTAACTTCTGTCCCCCTTAAAATGT	CATGACCAGAATCACAGTCTCAA	60	FP	18	424-103
Yb8NBC175	AC011493	GCTAGGACCTCCAGTATTATGTTGA	AGCATTCCCTCTGTGAACTGAAAT	55	-	19	420-87
Yb8NBC177	AP000561	GACTACTCCAAAACTGCAAACAAAG	CTCAGTGAAATGCAAACTCTTTGAC	55	FP	21	474-150
Yb8NBC178	AL080286	TGGTTTCTTCTAGGCTGCTTATTAC	TAGGTCCATTTCTCACCCTTTATAC	60	-	1	489-108
Yb8NBC181	AC007917	CATGTACCTTAGAATTCCACTCTCA	CCCCAAAGTTTATAGTCTGTTGTCT	55	FP	3	487-151
Yb8NBC183	AP000497	GGAAGAAATGCAAACTAAATAATGAGAG	CATTGTTACCAGCAACTTATTTACA	55	FP	3	465-140
Yb8NBC184	AP000495	AACTAACATAGCCCTGGTACAGAAA	CATTCCTGGATTACATCCTGTTTTA	55	FP	3	509-179
Yb8NBC185	AC008040	CACTTTGAAATAGTGCAAGGAATTT	CTCATTGACTCCTTTGACTCTTGTG	55	FP	3	500-211
Yb8NBC186	AC008055	ACAGTGGATGCTCCATATTTTTACT	AGGTCTTGGAACTAGGAGCTTTATG	55	FP	12	503-177
Yb8NBC187	AL031905	GTCCATTCCATTTTACTGCTTACTC	TCCTGCATGTTTAACTTAACTTTCC	55	FP	6	491-179
Yb8NBC189	AC007684	GGAAGATTTGAGAGTGAAATACCC	ACATCATGGCCTGAACTAGTTTTTC	67	IF	2	541-220
Yb8NBC191	AL078604	AGTGACCAGAAAGCTCACAGTGTAT	CAGGGTTGCATGTACTGAGATATAG	65	FP	6	687-346
Yb8NBC192	AC006325	CTGCTCTACCCTAGGCTCTTCTATC	GCTCCTCTGCTTTTATGTGTTCTAC	55	HF	7	423-132
Yb8NBC193	AL049836	AGTGTTGTATTTAGGTCGGTGCAA	GCATGCTTGCAGGTGAGTC	55	-	14	536-164
Yb8NBC195	AL109733	CCTTTCTGGAAGGTTTTCAATG	CATGATGGAGAGGTACAAAGAGATT	58	R/R	X	531-201
Yb8NBC198	AL035695	AGGTCTCAAGTAGGATCCAGAGAAG	GTTTGTGCAAGCTGGGAAGTTA	58	FP	6	528-194
Yb8NBC199	AC007377	TAGATGGCTTTAGCAATTATAAGGT	CAATTTCAGGAACACTGTAAAGTCA	60	FP	2	848-522
Yb8NBC200	AC008041	GGAAAGCAGATCTTCTGACTCCTA	AGGCCAATTACGGAATACATAACTC	55	FP	3	426-99
Yb8NBC201	AC007558	GGAGAAAATGTAAGGTTTCTAGCAC	ACCAATGCAACTATCTACACTGACA	60	IF	7	476-145
Yb8NBC202	AC006984	ATGTAGAGAAAGCTGGTCTGTGAAG	CATTTCCTATCTTACTCTCCATGTC	58	FP	7	405-90
Yb8NBC203	AL031655	CAAGATTGTCAGTGACCCTTAAGAA	TCATTCTAACCCGTTCAGATGTACT	55	FP	20	518-200
Yb8NBC204	AC004885	CTTCCTCTTTTCCTATTCAAGCTCT	CAGAAGAAAGTGCATCTCTCAAAAG	55	FP	7	489-181
Yb8NBC205	AC007543	CTGCCCTAAAACTCAGTGACTAAAA	AAGTGGACCTGAAACCTATGTGATA	60	FP	12	419-93
Yb8NBC206	AL033525	CTATTTCCTAGCGTGCCTGAGAT	TGAGGTGATTTACCTTCACTCTACC	60	FP	1	486-153
Yb8NBC208	AP000243	ATCACTAAAGAGACTGTTGGCGTTT	TAATCTAGGCAAAACTGCTTACCC	60	IF	21	357-111
Yb8NBC209	AC006511	AAGTCATTGCTTACAGAAACTGGAG	CCATGGAATGACATCTAGGTTGTT	60	FP	12	548-227
Yb8NBC210	AC007198	TGACGTGCAGACTACCTAATGTAAA	TACTTTTAGAACAGGGCCTCAGAAT	60	FP	?	416-91
Yb8NBC211	AC007165	TGAAAACCAGTTTGCCAGAA	GGGGCTAACTCAGATGTCCA	55	-	18	383-58
Yb8NBC212	AC006561	TGGACTACAACATACCATCCTCA	CAGCGTGTTGTGACATTTGTT	55	FP	12?	418-102
Yb8NBC213	AL033381	CAGCATTTGTGCCTTATCCTT	TTGGTGTTCTTGAAGAGGTGAA	60	FP	6	562-210
Yb8NBC214	AC000159	CCCTGCAAACCATTTCATCT	GGGTGAGAGGGCTGTTAGAA	60	FP	11	719-472
Yb8NBC216	AC005999	TCTTTGTTCCTATCTTACCCAATTC	AGGCACAAAGTGGAAACTGG	55	FP	7	400-84
Yb8NBC217	AC005988	CTTGCCATAGCCCTTTTTGT	GGGTCTTCTTGGGGATGAA	50	FP	17	648-308
Yb8NBC218	AC005099	TGAGGTGAGGCTCTGTTTCC	CTGTTTCTTTTCCTGCACCA	50	-	7	531-215
Yb8NBC219	AC004866	TACCAGCATTGCCTCACATC	GCACATGGCAACTGTCTGAG	62	FP	7	580-231
Yb8NBC220	AL024509	AAAGAGGTTTCTTTGGCTGGA	AAACTCACTGAATGGCTGACAC	60	FP	6	387-65
Yb8NBC221	AL034370	AATTCAAGCCAATGAACCAC	TCAGTGCTCTGAAGAAGCTCA	60	FP	X	431-97
Yb8NBC222	AC005552	AGCTCCCCACTCCCTACTTT	GGGAGAGTTCAGATGGGAAA	60	FP	17	426-102
Yb8NBC223	AC004915	CCATCCACAAATATCACAAAGC	TGGGGAACCATACCTTCTTG	60	FP	7	550-226
Yb8NBC224	AC004861	GGTCACTGTATTTTCCTCAAATCC	GGTGTTTGAGTATATGTAGGTGTGC	58	FP	7	417-102
Yb8NBC225	AC005868	GAGTCCAGCCCATTTTAGCA	CCCAGCACAAACATGTCATT	60	IF	12	449-135
Yb8NBC226	AC004853	GGAAATGCAAATGCCCAATA	CATGATGGTTTGCTGCAACT	60	FP	7	537-189
Yb8NBC227	AC005799	AAGAAAAGGGAAGCCTGGAG	CAGTCATCACCAGCCATGAG	60	IF	17	881-546

Table 2. *(continued)*

Name	Accession	5′ Primer sequence (5′-3′)	3′ Primer sequence (5′-3′)	A.T.[a]	Human diversity[b]	Chr.[c] Loc.	Product size[b] Filled-empty
Yb8NBC228	AC005722	GTGTCCAGACCTGTGGCTCT	CCAGACAGCTGGGGTTTTT	62	FP	17	630-310
Yb8NBC229	AC005754	CCCAGTTTTCTACTTTGCACTG	TGCCAACTGAGCACTTCTTG	60	FP	5	411-90
Yb8NBC230	AB014460	CAAAATGGCCGTGTTCTTTT	GTGTCCACGGATCTTTGCAG	62	IF	16	458-124
Yb8NBC231	AC005618	GGAAGACTCCCTTGTTCAGG	ATGCATTATTTTCCCCCACA	60	FP	5	501-181
Yb8NBC232	AL023875	TGTGAATCCCACAGTCAGAAA	TTCACAGCTGGATCAGTTCAA	55	FP	X	402-83
Yb8NBC233	AC004702	TCCACATGGATGGAAGATGA	GTGGTCTGCAAGGGAACAGT	60	FP	17	446-114
Yb8NBC234	AC005207	ACCTGCAAAAGAGGCGTAGA	CTAATGAGGCCACCACTCAA	60	FP	17	523-198
Yb8NBC235	AC005221	CATTCTGGGCACCTCACTTT	CCATCCAAATTGCCTAAGGT	60	FP	5	473-146
Yb8NBC236	AL021939	CTGCTTTCAGTGTCCAGGAT	CAAAGCCTATGTCTCGCTCA	60	FP	6	775-449
Yb8NBC237	AC004613	GCCAAAATCAACTGCCAAAC	TGCTGAGGATAGAGCTATAGCAGA	60	IF	7	491-164
Yb8NBC238	AC004592	AATGAAGTCACCTGCCCTTG	CCTGAAGAGATGGTGGAAGG	60	FP	5	437-117
Yb8NBC239	AF031078	TTGCTGACAGATCAGGGATG	TCCCCCTTCAAACCTATTCC	55	FP	X	730-419
Yb8NBC240	AC004452	TTCACAGTGATTCCTGCTCA	GGTGTCTTCTGAGAAATGCCTA	60	FP	7	564-257
Yb8NBC241	AC004391	GGACTGTGTCTAAGGGTGTCCT	GGTAATTGGGAGCAGTTGAGA	60	IF	7	424-93
Yb8NBC242	AC002349	ATCCACCATCAGGGAATCAA	TGCAGATCTTATCAGCACATTG	60	FP	X	450-117
Yb8NBC244	AF043945	CGGATGTCCCTTTACCACAT	CACTGCGTGGTTCATCACTT	60	FP	21	403-77
Yb8NBC245	AC004029	AACCCATTGTCTCATGTCTAGC	CTCTCATCCAACAAAAGTCAGTGT	60	FP	7	647-318
Yb8NBC246	AC002981	CACCACCTTTCAACCAGGAA	ATCGCTGGAATGTGGTTCTC	60	FP	X	464-149
Yb8NBC247	AC002366	GCAGCACAAAGTAGTGGTTGG	TGCACCCACTTGATATGCTT	60	FP	X	551-259
Yb8NBC248	AC003088	TTTCTTTCCCTCTCGCATGT	CCCTTTGGTCTCGACACATT	60	FP	7	441-120
Yb8NBC249	Z98049	ATGGGCCCAAATAAAAGGAT	GTGATGGCCTTGACAGCAT	62	FP	6	491-148
Yb8NBC250	AC002462	GGGATCCCAGACATTGATTT	TTGCTCCTCACTTGCTCCTT	60	FP	?	397-63
Yb8NBC251	AC002477	CGGCCCTGATATGTCTTTGA	TCCACAAAGGCAAATGGATA	60	FP	X	838-500
Yb8NBC252	AC002123	GCCCACCATCGAGATCTACT	TCCACATCTCCATCAGAGCTT	62	FP	5	424-107
Yb8NBC253	AF001548	CAAAGGCAATCTTGGAGCTG	CCCCTCCTTCTCCTTTGCTA	60	-	16	473-144
Yb8NBC254	AC002088	GGGGGAAACATTACTACAGAGG	ATATATTTTTGGCCAGGTACGG	55	FP	7	740-413
Yb8NBC255	AC000062	GGAATGAAGTGTCCACAGATGA	CAGAGGCAGGGAGACCAG	55	FP	13	423-93
Yb8NBC256	Z73986	CCCACAATTTCCACTTCAGG	GCATTGCTTCCCTTCTATTTC	55	FP	X	503-24
Yb8NBC257	Z69921	CTGCACCAAAAGAGACACACA	GCAAAAACATAGAAAGCGGTGT	55	FP	4	508-187
Yb8NBC258	AC009429	TTAGTGGTTCCTGCATGTGG	AGCGCAGGGTTAGTAGCAAA	60	-	?	431-103
Yb8NBC259	AC015600	TCCAGGAAAAAGGGAACATT	TTTCAAGAGAAAGGGCAACA	60	-	?	547-227
Yb8NBC260	AC012000	TTTCCACCATCAGTCCCTCT	AGGGACTTAGGAGTGATTTTAGTG	55	-	2	661-327
Yb8NBC261	AC009478	GCAGCACTTAATGCCAATCA	TCATCGTTCTTTAGCTCCTCTG	60	-	2	375-50
Yb8NBC262	AC020728	ATCCAGATTTGCAGGACCAC	CCTCAGCTAAGTGCCAGGAG	60	FP	5	687-361
Yb8NBC263	AC009318	GAAAGAGAGGGCAGCATTGT	AAAGTTTATGCTCCCGCTGA	60	-	12	518-177
Yb8NBC264	AC007619	AAGCAAGACATATGCATGAAAAA	ATCGTTTTTAAATGTTGCATACCA	60	FP	12	781-475
Yb8NBC265	AC025436	TGCCAACTGAGCACTTCTTG	CCCTTTTGGATTCTCTCTGC	60	FP	5	434-114
Yb8NBC266	AC009078	TATTCATTGCCTCCCTTGGA	ATGCTCCCAACCCTTTTAGG	60	FP	16	475-149
Yb8NBC267	AC008925	GGGAAGTTTCAACAAAACCAGA	CACCACTGAATGATACCCTTTT	60	FP	5	777-447
Yb8NBC268	AC016681	TGGGGATAGAGGAAGAAGACAA	CCTTTTCATCCAACTACCACTG	60	IF	Y	517-188
Yb8NBC269	AF241735	CACGCTTAACCTCTACCACCA	TGGACTCCCACTGAGATGTG	60	-	X	587-261
Yb8NBC270	AC007489	CTTCTGCAGCTCCTGACTGA	AGTCTAGGCTTCGGATGCAG	55	-	16	403-72
Yb8NBC271	AC023602	GGAAAAACTGCATGCTAGGC	CAGTGAATGTTTCCCTGTGGT	60	FP	3	493-168
Yb8NBC272	AC023602a	TGCAGAATGTTTGTTCTTGGAG	TTTCCCTAGCTCCTTGAAATG	60	-	3	537-215
Yb8NBC273	AC011284	CTCCTTTGTGGGGGAGAAG	CATGCTCCTTGGGAACTCTC	55	R/R	7	431-112
Yb8NBC274	AL133305	TCAACATCAACCCCACTGAA	TCCGAGGGAGGAATGAGATA	55	FP	14	489-168
Yb8NBC275	AL122000	TTCCTGAAAAAGACCTACACCTG	TTTGGCCTTATGTGACAAGC	60	FP	1	472-146
Yb8NBC276	AL109928	TCTGCTGGGGTCAGAAAAAC	GGCGGTTGTTTAAGTGGAAA	55	-	20	507-183
Yb8NBC277	AL121944	AAACAATGAACTGAAGGGGACT	CCCTGCAGCCTGTATAAATCA	60	-	6	404-82

[a] Amplification of each locus required 2:30 minutes at 94 °C initial denaturing, and 32 cycles for one minute at 94 °C, one minute at annealing temperature (A.T.), and one minute elongation at 72 °C. A final extension time of ten minutes at 72 °C was also used.

[b] Allele frequency was classified as: fixed present (FP), low (LF), intermediate (IF), or high frequency (HF) insertion polymorphism. Fixed present: every individual tested had the Alu element in both chromosomes. Low frequency insertion polymorphism: the absence of the element from all individuals tested, except for one or two homozygous or heterozygous individuals. Intermediate frequency insertion polymorphism: the Alu element is variable as to its presence or absence in at least one population. High frequency insertion polymorphism: the element is present in all individuals in the populations tested, except for one or two heterozygous or absent individuals. (–) Indeterminable. (R/R) Repeat in repeat.

[c] Chromosomal location determined from Accession information or by PCR analysis of NIGMS monochromosomal hybrid cell line DNA samples.

[d] Empty product sizes calculated by removing the Alu element and one direct repeat from the filled sites that were identified.

mb MS 4847 [administrator 28/6/]

Table 3. Alu Ya5 subfamily associated human genomic diversity

	African American					Greenland natives/Asian[c]					European					Egyptian					
	Genotypes					Genotypes					Genotypes					Genotypes					
Elements	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	Avg. Het[b]
A. *Intermediate frequency*																					
Ya5NBC5	2	5	5	0.38	0.49	3	2	8	0.31	0.44	1	6	11	0.22	0.36	2	8	4	0.43	0.51	0.45
Ya5NBC22	3	15	1	0.55	0.51	4	14	0	0.61	0.49	1	16	1	0.50	0.51	19	1	0	0.98	0.05	0.39
Ya5NBC27	0	5	14	0.13	0.24	0	8	11	0.21	0.34	2	7	9	0.31	0.44	2	7	10	0.29	0.42	0.36
Ya5NBC35	9	10	1	0.70	0.43	5	12	2	0.58	0.50	8	12	0	0.70	0.43	7	13	0	0.68	0.45	0.45
Ya5NBC37	2	2	13	0.18	0.30	1	4	12	0.18	0.30	3	2	15	0.20	0.33	4	3	10	0.32	0.45	0.34
Ya5NBC45	7	7	2	0.66	0.47	19	0	0	1.00	0.00	17	0	0	1.00	0.00	8	3	0	0.86	0.25	0.18
Ya5NBC51	4	10	3	0.53	0.51	5	6	8	0.42	0.50	6	7	7	0.48	0.51	3	8	9	0.35	0.47	0.50
Ya5NBC57	10	1	2	0.81	0.32	4	8	3	0.53	0.52	13	2	1	0.88	0.23	9	1	1	0.86	0.25	0.33
Ya5NBC61	10	6	3	0.68	0.44	5	2	10	0.35	0.47	9	7	1	0.74	0.40	8	4	5	0.59	0.50	0.45
Ya5NBC96	17	2	0	0.95	0.10	9	5	3	0.68	0.45	18	1	0	0.97	0.05	16	3	0	0.92	0.15	0.19
Ya5NBC102	3	2	13	0.22	0.36	0	0	6	0.00	0.00	3	4	12	0.26	0.40	2	0	13	0.13	0.24	0.25
Ya5NBC109	7	11	1	0.66	0.46	7	11	2	0.63	0.48	5	13	1	0.61	0.49	7	8	4	0.58	0.50	0.48
Ya5NBC120	7	11	0	0.69	0.44	15	4	0	0.90	0.19	8	12	0	0.70	0.43	14	5	0	0.87	0.24	0.32
Ya5NBC123	5	7	7	0.45	0.51	6	5	4	0.57	0.51	14	5	1	0.83	0.30	11	5	1	0.79	0.34	0.41
Ya5NBC131	0	5	6	0.23	0.37	0	9	8	0.27	0.40	0	11	6	0.32	0.45	0	15	2	0.44	0.51	0.43
Ya5NBC132	4	0	5	0.44	0.52	9	0	0	1.00	0.00	13	0	0	1.00	0.00	11	0	1	0.92	0.159	0.17
Ya5NBC148	7	6	6	0.53	0.51	2	6	12	0.25	0.39	0	0	20	0.00	0.00	0	0	17	0.00	0.00	0.22
Ya5NBC150	17	0	0	1.00	0.00	4	0	14	0.22	0.36	19	0	1	0.95	0.10	17	0	1	0.94	0.11	0.14
Ya5NBC154	0	12	5	0.35	0.47	0	7	9	0.22	0.35	0	12	8	0.30	0.43	3	4	13	0.25	0.39	0.41
Ya5NBC160	2	7	9	0.31	0.44	0	0	19	0.00	0.00	0	0	19	0.00	0.00	0	4	12	0.13	0.23	0.17
Ya5NBC174	0	5	3	0.31	0.46	0	3	8	0.14	0.25	0	12	8	0.30	0.43	2	5	9	0.28	0.42	0.39
Ya5NBC182	2	9	9	0.33	0.45	9	8	0	0.77	0.37	5	6	7	0.44	0.51	1	10	3	0.43	0.51	0.46
Ya5NBC201	6	6	5	0.53	0.51	4	7	6	0.44	0.51	16	3	0	0.92	0.15	8	7	2	0.68	0.45	0.41
Ya5NBC210	0	4	15	0.11	0.19	0	1	15	0.03	0.06	0	4	16	0.10	0.19	0	4	12	0.13	0.23	0.17
Ya5NBC216	5	7	5	0.50	0.52	6	8	5	0.53	0.51	7	12	0	0.68	0.44	0	0	10	0.00	0.00	0.37
Ya5NBC219	0	10	9	0.26	0.40	1	12	7	0.35	0.47	0	11	9	0.28	0.41	0	0	6	0.00	0.00	0.32
Ya5NBC221	5	7	4	0.53	0.51	9	5	3	0.68	0.45	16	0	1	0.94	0.11	13	2	0	0.93	0.13	0.30
Ya5NBC311[c]	12	1	6	0.66	0.46	11	4	2	0.77	0.37	15	1	4	0.78	0.36	11	2	4	0.71	0.43	0.41
Ya5NBC313[c]	9	3	5	0.62	0.49	4	6	6	0.44	0.51	2	8	3	0.46	0.52	5	6	3	0.57	0.50	0.50
Ya5NBC324[c]	0	8	1	0.44	0.52	0	15	1	0.47	0.51	0	14	4	0.39	0.49	0	15	1	0.47	0.51	0.51
Ya5NBC325[c]	0	10	10	0.25	0.39	0	9	9	0.25	0.39	0	11	9	0.28	0.41	0	6	6	0.25	0.39	0.39
Ya5NBC327[c]	2	9	9	0.33	0.45	13	6	1	0.80	0.33	19	0	0	1.00	0.00	7	6	1	0.71	0.42	0.30
Ya5NBC333[c]	5	5	9	0.40	0.49	4	7	8	0.49	0.49	3	8	8	0.37	0.48	5	3	5	0.50	0.52	0.50
Ya5NBC347[c]	17	2	1	0.90	0.19	4	7	8	0.40	0.49	7	8	2	0.65	0.47	11	1	5	0.68	0.45	0.40
Ya5NBC351[c]	3	12	3	0.55	0.51	7	9	3	0.61	0.49	13	3	3	0.76	0.37	11	1	5	0.68	0.45	0.46
Ya5NBC354[c]	0	2	16	0.06	0.11	2	6	10	0.28	0.41	10	4	5	0.63	0.48	2	4	9	0.27	0.41	0.35
Ya5NBC361[c]	0	9	10	0.24	0.37	2	11	5	0.42	0.50	0	5	12	0.15	0.26	3	3	7	0.35	0.47	0.40

B. *High frequency*																				
Ya5NBC16	20	0	0	1.00	0.00	20	0	0	1.00	0.00	10	10	0	0.75	0.39	20	0	0	1.00	0.00	0.10
Ya5NBC18	17	1	0	0.97	0.06	18	1	0	0.97	0.05	20	0	0	1.00	0.00	20	0	0	1.00	0.00	0.03
Ya5NBC98	17	0	1	0.94	0.11	18	2	0	0.95	0.10	17	1	2	0.88	0.22	16	3	0	0.92	0.15	0.14
Ya5NBC157	20	0	0	1.00	0.00	20	0	0	1.00	0.00	20	0	0	1.00	0.00	15	0	1	0.94	0.12	0.03
Ya5NBC212	16	1	0	0.97	0.06	20	0	0	1.00	0.00	20	0	0	1.00	0.00	18	0	0	1.00	0.00	0.01
Ya5NBC349[c]	19	1	0	0.98	0.05	14	2	4	0.75	0.39	18	0	0	1.00	0.00	12	0	2	0.86	0.25	0.17
C. *Low frequency*																				
Ya5NBC24	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0.00
Ya5NBC28	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0.00
Ya5NBC38	0	0	16	0.00	0.00	0	0	15	0.00	0.00	0	0	20	0.00	0.00	0	0	18	0.00	0.00	0.00
Ya5NBC54	0	0	14	0.00	0.00	0	2	12	0.07	0.14	0	1	8	0.06	0.11	0	1	7	0.06	0.13	0.09
Ya5NBC135	0	1	18	0.03	0.05	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	17	0.00	0.00	0.01
Ya5NBC147	0	1	17	0.03	0.06	0	1	19	0.03	0.05	0	0	20	0.00	0.00	0	1	17	0.03	0.06	0.04
Ya5NBC155	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0.00
Ya5NBC171	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	19	0.00	0.00	0	0	20	0.00	0.00	0.00
Ya5NBC172	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	18	0.00	0.00	0.00
Ya5NBC184	0	0	20	0.00	0.00	0	0	18	0.00	0.00	0	0	20	0.00	0.00	0	0	19	0.00	0.00	0.00
Ya5NBC194	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0.00
Ya5NBC197	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	19	0.00	0.00	0.00
Ya5NBC203	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0.00
Ya5NBC204	0	0	19	0.00	0.00	0	0	17	0.00	0.00	0	0	17	0.00	0.00	0	0	15	0.00	0.00	0.00
Ya5NBC214	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	5	15	0.13	0.22	0	0	19	0.00	0.00	0.06
Ya5NBC223	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	19	0.00	0.00	0.00

[a] This is the unbiased heterozygosity.

[b] Average heterozygosity is the average of the population heterozygosity.

[c] The following were tested using DNA samples from Asian individuals.

Table 4. Alu Yb8 subfamily associated human genomic diversity

	African American					Greenland natives/Asian[c]</sup>					European					Egyptian					
	Genotypes					Genotypes					Genotypes					Genotypes					
Elements	+/+	+/−	−/−	*f*Alu	Het$_a$	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	Avg. Het[b]
A. *Intermediate frequency*																					
Yb8NBC3	10	2	0	0.92	0.16	12	4	0	0.88	0.23	4	6	0	0.70	0.44	9	0	0	1.00	0.00	0.207
Yb8NBC7	5	8	0	0.69	0.51	4	14	0	0.61	0.49	1	16	1	0.50	0.51	19	1	0	0.98	0.05	0.39
Yb8NBC8	8	1	0	0.94	0.11	10	4	0	0.86	0.25	11	1	2	0.82	0.30	12	2	3	0.77	0.37	0.26
Yb8NBC9	3	5	10	0.31	0.44	2	3	13	0.19	0.32	5	1	9	0.37	0.48	0	7	8	0.23	0.37	0.402
Yb8NBC10	9	9	0	0.75	0.39	9	11	0	0.73	0.41	12	7	0	0.82	0.31	11	5	0	0.84	0.27	0.344
Yb8NBC18	1	0	15	0.06	0.12	2	9	9	0.33	0.45	0	6	14	0.15	0.26	1	6	11	0.22	0.05	0.22
Yb8NBC30	8	6	0	0.79	0.35	7	11	0	0.69	0.44	5	8	0	0.69	0.44	14	2	0	0.94	0.12	0.338
Yb8NBC36	5	14	1	0.60	0.49	8	0	0	1.00	0.00	10	9	0	0.76	0.37	8	8	0	0.75	0.39	0.312
Yb8NBC48	0	4	6	0.20	0.34	0	1	2	0.17	0.33	0	3	4	0.21	0.36	0	2	3	0.20	0.36	0.347
Yb8NBC49	1	9	10	0.28	0.41	7	8	5	0.55	0.51	5	9	6	0.48	0.51	1	8	9	0.28	0.41	0.46
Yb8NBC65	7	6	5	0.56	0.51	3	10	7	0.40	0.49	7	4	9	0.45	0.51	2	5	9	0.28	0.42	0.481
Yb8NBC67	8	5	5	0.58	0.50	9	6	4	0.63	0.48	13	2	0	0.93	0.13	4	7	4	0.50	0.52	0.406
Yb8NBC71	0	3	13	0.09	0.18	3	3	10	0.28	0.42	0	5	12	0.15	0.26	2	2	9	0.23	0.37	0.304
Yb8NBC77	2	2	16	0.15	0.26	2	0	16	0.11	0.20	0	1	17	0.03	0.06	0	0	16	0.00	0.00	0.13
Yb8NBC80	1	4	15	0.15	0.26	2	5	12	0.24	0.37	3	1	15	0.18	0.31	2	5	8	0.30	0.43	0.344
Yb8NBC93	1	3	10	0.18	0.30	7	5	2	0.18	0.30	7	2	5	0.57	0.51	12	4	1	0.82	0.30	0.35
Yb8NBC96	0	7	9	0.22	0.35	0	14	3	0.41	0.50	0	3	15	0.08	0.16	0	5	7	0.21	0.34	0.338
Yb8NBC106	4	6	7	0.41	0.50	2	8	10	0.30	0.43	0	2	18	0.05	0.10	3	5	11	0.29	0.42	0.362
Yb8NBC108	2	11	7	0.38	0.48	2	10	7	0.37	0.48	0	3	11	0.11	0.20	3	4	10	0.29	0.43	0.396
Yb8NBC109	0	11	8	0.29	0.42	1	11	8	0.33	0.45	4	1	6	0.41	0.51	7	0	11	0.39	0.49	0.467
Yb8NBC120	5	8	5	0.50	0.51	5	6	8	0.42	0.50	8	7	3	0.64	0.48	4	2	6	0.42	0.51	0.499
Yb8NBC125	0	0	20	0.00	0.00	0	3	16	0.08	0.15	0	3	17	0.08	0.14	0	5	14	0.13	0.24	0.132
Yb8NBC146	18	0	2	0.90	0.19	12	1	1	0.89	0.20	16	0	2	0.89	0.20	10	1	6	0.62	0.49	0.268
Yb8NBC148	11	0	2	0.85	0.27	11	1	6	0.64	0.48	6	2	10	0.39	0.49	13	3	4	0.75	0.41	0.411
Yb8NBC157	19	0	1	0.95	0.10	6	5	1	0.71	0.43	3	9	6	0.42	0.50	16	2	2	0.85	0.26	0.322
Yb8NBC160	0	12	8	0.25	0.39	0	13	7	0.33	0.45	0	10	10	0.25	0.39	1	6	13	0.20	0.33	0.387
Yb8NBC189	10	10	0	0.75	0.39	18	2	0	0.95	0.10	9	7	2	0.69	0.44	18	2	0	0.95	0.10	0.254
Yb8NBC201	5	9	0	0.59	0.50	3	8	7	0.39	0.49	9	5	6	0.58	0.50	2	9	8	0.34	0.46	0.488
Yb8NBC208	5	6	5	0.50	0.52	18	2	1	0.91	0.18	10	8	2	0.70	0.43	15	4	1	0.85	0.26	0.346
Yb8NBC225[c]	10	9	1	0.73	0.41	12	2	4	0.72	0.41	11	6	3	0.70	0.43	8	2	5	0.60	0.50	0.4375
Yb8NBC227[c]	10	8	2	0.70	0.43	5	6	5	0.50	0.52	18	2	0	0.95	0.10	15	4	1	0.85	0.26	0.326
Yb8NBC230[c]	1	2	11	0.14	0.25	0	0	19	0.00	0.00	0	2	15	0.06	0.11	1	4	3	0.38	0.50	0.217
Yb8NBC237[c]	13	4	1	0.83	0.29	12	5	2	0.76	0.37	15	2	0	0.94	0.11	10	8	1	0.74	0.40	0.293
Yb8NBC241[c]	0	0	16	0.00	0.00	2	0	14	0.13	0.23	2	3	10	0.23	0.37	1	6	8	0.27	0.41	0.25
Yb8NBC268[c]	0	13	5	0.36	0.48	0	7	12	0.18	0.31	1	9	8	0.31	0.44	0	5	12	0.15	0.26	0.37

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B. *High frequency*																					
Yb8NBC24	12	2	0	0.93	0.14	13	1	0	0.96	0.07	15	0	0	1.00	0.00	12	0	0	1.00	0.00	0.052
Yb8NBC26	12	1	0	0.96	0.08	14	1	0	0.97	0.07	9	1	0	0.95	0.10	13	0	0	1.00	0.00	0.061
Yb8NBC102	16	0	2	0.89	0.20	20	0	0	1.00	0.00	20	0	0	1.00	0.00	18	0	0	1.00	0.00	0.051
Yb8NBC181	14	0	0	1.00	0.00	13	0	0	1.00	0.00	8	9	3	0.63	0.48	13	0	0	1.00	0.00	0.12
Yb8NBC192	20	0	0	1.00	0.00	20	0	0	1.00	0.00	19	1	0	0.98	0.05	20	0	0	1.00	0.00	0.012
C. *Low frequency*																					
Yb8NBC5	3	10	4	0.47	0.01	0	2	10	0.08	0.16	0	0	18	0.00	0.00	0	0	20	0.00	0.00	0.042
Yb8NBC13	15	3	0	0.92	0.08	0	0	17	0.00	0.00	0	0	15	0.00	0.00	0	0	12	0.00	0.00	0.019
Yb8NBC69	0	0	18	0.00	0.00	0	0	17	0.00	0.00	0	0	16	0.00	0.00	0	0	16	0.00	0.00	0
Yb8NBC100	0	1	17	0.03	0.06	0	5	15	0.13	0.22	0	1	18	0.03	0.05	0	0	0	0.00	0.00	0.083
Yb8NBC110	0	0	18	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0
Yb8NBC126	1	6	7	0.29	0.07	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0.018
Yb8NBC133	0	2	18	0.05	0.06	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0	0	20	0.00	0.00	0.016
Yb8NBC134	0	4	18	0.09	0.17	0	0	17	0.00	0.00	0	0	10	0.00	0.00	0	0	19	0.00	0.00	0.042

[a] This is the unbiased heterozygosity.

[b] Average heterozygosity is the average of the population heterozygosity.

[c] The following were tested using DNA samples from Asian individuals.

ancestral state.[35,36] Previously, the analysis of Alu insertion polymorphisms has proved useful for the study of human population genetics.[35–43] The newly identified Alu insertion polymorphisms from the Ya5 and Yb8 Alu subfamilies should prove useful for the study of human population genetics.

Materials and Methods

Cell lines and DNA samples

The cell lines used to isolate primate DNA samples were as follows: human (*Homo sapiens*), HeLa (ATCC CCL2); and chimpanzee (*Pan troglodytes*), Wes (ATCC CRL1609). Cell lines were maintained as directed by the source and DNA isolations were performed using Wizard genomic DNA purification (Promega). Human DNA samples from the European, African American, Asian, Egyptian, and Greenland Native population groups were isolated from peripheral blood lymphocytes[44] available from previous studies.[18]

Computational analyses

Initial screening of the GenBank non-redundant and high throughput genomic sequence (HTGS) databases was performed using the Basic Local Alignment Search Tool (BLAST)[45] available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Copy number estimates were determined using Megablast and the draft human genome sequence database.[46] The database was searched for exact complements to the oligonucleotide 5′-CCATCCCGGCTAAAAC-3′ and 5′-TGCGCCACTGCAGTCCGCAGTCCG-3′ that are exact matches to a portion of the Alu Ya5 and Yb8 subfamily consensus sequences (respectively) that contain unique diagnostic mutations.[21] Sequences that were exact complements to the oligonucleotides were then subjected to more detailed annotation. A region composed of 500-1000 bases of flanking DNA sequence directly adjacent to the sequences identified from the databases that matched the initial GenBank BLAST query were subjected to annotation using the RepeatMasker2 program from the University of Washington Genome Center server (http://ftp.genome.washington.edu/c/s.dll/RepeatMasker) or Censor from the Genetic Information Research Institute (http://www.girinst.org/Censor_Server-Data_Entry_Forms.html).[47] These programs annotate the repeat sequence content of individual sequences from humans and rodents. A complete list of the Alu elements identified from the GenBank search is available from MAB. The copy numbers for each subfamily of Alu elements were determined by screening the draft sequence of the entire human genome with the oligonucleotides shown above.[23] For the Yb8 subfamily analysis, the database was searched for matches to the consensus Yb8 sequence without the seven-nucleotide duplication (287 bases). The sequences were then subjected to more detailed analysis using MegAlign (DNAStar version 3.1.7 for Windows 3.2) selecting only for Yb8 intermediate elements containing between one and seven of the Yb8 diagnostic sites.

Primer design and PCR amplification

PCR primers were designed from flanking unique DNA sequences adjacent to individual Ya5 and Yb8 Alu elements using the Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The resultant PCR primers were screened against the GenBank non-redundant database for the presence of repetitive elements using the BLAST program, and primers that resided within known repetitive elements were discarded and new primers were designed. PCR amplification was carried out in 25 μl reactions using 50-100 ng of target DNA, 40 pM of each oligonucleotide primer, 200 μM dNTPs in 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.4) and *Taq*® DNA polymerase (1.25 units) as recommended by the supplier (Life Technologies). Each sample was subjected to the following amplification cycle: an initial denaturation of 150 seconds at 94 °C, one minute of denaturation at 94 °C, one minute at the annealing temperature, one minute of extension at 72 °C, repeated for 32 cycles, followed by a final extension at 72 °C for ten minutes. For analysis, 20 μl of each sample was fractionated on a 2% agarose gel with 0.25 μg/ml ethidium bromide. PCR products were directly visualized using UV fluorescence. The sequences of the oligonucleotide primers, annealing temperatures, PCR product sizes and chromosomal locations for all Ya5 and Yb8 elements can be found on our website (http://129.81.225.52). Phylogenetic analysis of all the ascertained Alu elements was determined by PCR amplification of human and non-human primate DNA samples. The human genomic diversity associated with each Alu element was determined by the amplification of 20 individuals from each of four populations (African-American, Greenland Native or Asian, European and Egyptian) (160 total chromosomes). The chromosomal location of Alu repeats identified from clones that had not been previously mapped was determined by PCR amplification of National Institute of General Medical Sciences (NIGMS) human/rodent somatic cell hybrid mapping panel 2 (Coriell Institute for Medical Research, Camden, NJ).

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Alu Insertion Polymorphisms for the Study of Human Genomic Diversity

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ABSTRACT

Genomic database mining has been a very useful aid in the identification and retrieval of recently integrated Alu elements from the human genome. We analyzed Alu elements retrieved from the GenBank database and identified two new Alu subfamilies, Alu Yb9 and Alu Yc2, and further characterized Yc1 subfamily members. Some members of each of the three subfamilies have inserted in the human genome so recently that about a one-third of the analyzed elements are polymorphic for the presence/absence of the Alu repeat in diverse human populations. These newly identified Alu insertion polymorphisms will serve as identical-by-descent genetic markers for the study of human evolution and forensics. Three previously classified Alu Y elements linked with disease belong to the Yc1 subfamily, supporting the retroposition potential of this subfamily and demonstrating that the Alu Y subfamily currently has a very low amplification rate in the human genome.

ALU elements have been accumulating in the human genome throughout primate evolution, reaching a copy number of over a million per genome. However, most of these Alu copies are not identical and can be classified into several subfamilies (reviewed in Deininger and Batzer 1993). These different subfamilies of Alu elements were generated once mutations occurred within the "master" or "source" gene that actively retroposed at different rates and time periods of primate evolution (Deininger *et al.* 1992). Currently, the Alu retroposition rate is reduced by 100-fold from its peak early in primate evolution (Shen *et al.* 1991). The vast majority of the Alu elements present in the human genome inserted before the radiation of extant humans and are therefore observed in all individuals in the human population. However, almost all of the recently integrated Alu elements in the human genome are restricted to several closely related "young" subfamilies, with the majority being Ya5 and Yb8 subfamily members (Batzer *et al.* 1994, 1995). Several of these new subfamilies appear to originate from an Alu element that fortuitously inserted into a favorable region of the genome capable of supporting Alu retroposition. Subsequent or concurrent mutations in the new source element(s) result in groups of elements that are identifiable as new subfamilies.

Collectively, the Alu Y, Ya5, Ya5a2, Ya8, and Yb8 subfamilies comprise <10% of the Alu elements present within the human genome, with the Ya5/8 and Yb8 subfamilies together accounting for <0.5% of all Alu elements. Although the human genome contains >1,000,000 copies of Alu (~15% of the genome; Smit 1996), <0.5% are polymorphic. Due to their recent evolutionary introduction into the human genome, many of the young Alu elements are polymorphic between individuals and/or populations. There is an inverse correlation between the age of the Alu subfamily and the percentage of polymorphic elements it contains. Identification of evolutionarily recent Alu subfamilies and their polymorphic insertions is useful for human population studies, forensics, and DNA fingerprinting for two reasons: (i) There is no apparent specific mechanism to remove newly inserted Alu repeats, making inserts identical by descent; and (ii) the Alu insertions have a known ancestral state (Batzer and Deininger 1991; Batzer *et al.* 1994).

The availability of large quantities of human genomic DNA sequence provided by the Human Genome Project facilitates genomic database mining for recently integrated Alu elements. Through this approach we were able to identify the youngest Alu subfamily reported to date, termed (Ya5a2), and determined that the majority of its members are Alu insertion polymorphisms (Roy *et al.* 2000). We expanded our computational analyses to identify other Alu subfamilies derived from the Alu

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Y and Yb8 subfamilies. Here, we present the analysis of three of the most recently formed Alu subfamilies and demonstrate their utility for the study of human genomic diversity.

MATERIALS AND METHODS

Computational analyses: Sequence alignments for the identification of Alu subfamilies were made using MegAlign software (DNAStar version 3.1.7 for Windows 3.2). Screening of the GenBank nonredundant (nr), the high throughput genome sequence (htgs), and the genomic survey sequence (gss) databases was performed using the advanced basic local alignment search tool 2.0 (BLAST; Altschul *et al.* 1990) available from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Database searches for Yb8 consensus Alus showed a common single-base variant termed Yb9. The databases were searched for matches to the 289 bases of the Yb9 consensus sequence (as inferred from the previous Yb8 analysis) or the 281 bases of the Alu Y consensus with the expected value (real) set at $-e\ 1.0e^{-150}$ and $-e\ 1.0e^{-140}$, respectively, in the advanced BLAST options. Only Alu Yb9 elements with all nine diagnostic mutations were selected. A similar type of search procedure was performed with the Yc1 and Yc2 consensus sequences or with an oligonucleotide query sequence complementary to the subfamily diagnostic base positions. Only Alu Yc1/Yc2 elements with 100% identity to the oligonucleotide query sequences or entire subfamily-specific consensus sequnce were utilized for further analysis. To estimate the copy numbers of the Yb9 subfamily we searched the draft sequence of the human genome (Lander *et al.* 2001), using a subfamily-specific probe that contained the Yb9-specific mutation as well as the insertion in the Yb8 subfamily. A complete list of the Alu elements identified from the GenBank search is available from M. A. Batzer or P. L. Deininger.

DNA samples: Human DNA samples from the European, African-American, Alaskan Native, Egyptian, and Asian population groups were isolated from peripheral blood lymphocytes (Ausubel *et al.* 1996) that were available from previous studies (Roy *et al.* 1999).

Oligonucleotide primer design and PCR amplification: Flanking unique DNA sequences adjacent to each Alu repeat were used to design primers for the Yb9, Yc1, and Yc2 Alu elements (Table 1). PCR primers and reactions were performed as previously described (Roy *et al.* 1999). The heterozygosity associated with each element was determined by the amplification of 20 individuals from each of four populations (African American, Alaskan Native, or Asian, European, and Egyptian; 160 total chromosomes). The chromosomal location for elements identified from randomly sequenced anonymous large-insert clones was determined by PCR as previously described (Roy *et al.* 1999).

RESULTS

The Alu Yb9, Yc1, and Yc2 subfamilies: Analysis of a set of 243 Yb8 Alu elements retrieved from the GenBank database allowed us to identify a putative subfamily containing all the known Yb8 diagnostic mutations plus one new mutation, which is referred to as Yb9 in compliance with the standard Alu subfamily nomenclature (Batzer *et al.* 1996). The Yb9 consensus sequence is shown in Figure 1. Searches from the nr, the htgs, and gss retrieved a total of 56 Yb9 elements. Of these, 25 elements were retrieved from the nr database (30.4% of the human genome at the time), giving an estimated size of 82 members for the Yb9 subfamily. This estimate is also in good agreement with a search of the draft human genomic sequence (Lander *et al.* 2001) that identified 79 perfect matches with a Yb9 subfamily-specific query sequence.

Using a different approach, we also retrieved one previously identified subfamily, Yc1 [formerly termed Sb0 (Jurka 1995)], and a new variant, Yc2. GenBank database searches for Alu Y elements that perfectly match the consensus sequence brought several Alu Y elements to our attention that share one or two specific mutations that differ from the Y consensus. Closer inspection facilitated the retrieval of the additional Alu subfamilies. BLAST searches using the consensus sequence for Alu Yc1 and Yc2 will also retrieve a large number of elements that are matches to the Alu Y subfamily as well, making the analysis of the elements identified in this manner impractical. Therefore, we selected only the elements of these subfamilies with 100% identity to the oligonucleotide query sequence that contained the subfamily-specific diagnostic bases. A total of 176 Yc1 (13 perfect matches to the entire subfamily consensus sequence) and 17 Yc2 (11 perfect matches to the entire subfamily consensus sequence) elements were retrieved. A count of all Yc1 elements retrieved by BLAST on a single initial search of the nr database yielded a total of 116 elements, giving an estimated copy number of 381 Yc1 elements in the human genome (the nr database contained 30.4% of the human genome sequence at the time of the search). Interestingly, three of the four elements previously classified as Alu Y elements linked to disease (Deininger and Batzer 1999) belong to the Alu Yc1 subfamily (Figure2): the *de novo* insertion in the C1 inhibitor gene (C1inh; Stoppa-Lyonnet *et al.* 1990), another *de novo* insertion in BRCA2 (BRCA2; Miki *et al.* 1996), and glycerol kinase deficiency (GK; Zhang *et al.* 2000).

About one-half of the 56 total Yb9 elements (29) shared 100% nucleotide identity with the subfamily consensus sequence. To get an approximation of the age of the Yb9 subfamily, we evaluated the number of non-CpG mutations present within the different Alu elements as previously described (Roy *et al.* 2000). A total of 19 CpG mutations, 25 non-CpG mutations, and two 5′ truncations occurred within the 56 Alu Yb9 subfamily members identified. Using a neutral rate of evolution for primate intervening DNA sequences of 0.15% per million years (Miyamoto *et al.* 1987) and the non-CpG mutation density of 0.1908% (25/13,104 bases using only non-CpG bases) within the 56 Yb9 Alu elements yield an estimated average age of 1.27 million years (myr). The age for the Yb9 subfamily members is predicted at a 95% confidence level in the range of 0.8–1.8 myr, given that the mutations were random and fit a binomial distribution. No analysis can be made for the

TABLE 1

PCR primers, chromosomal locations, and PCR product sizes

Name	Accession	Position	5′ primer sequence (5′–3′)	3′ primer sequence (5′–3′)	A.T[a]	Human diversity[b]	Chr.[c] loc.	Product size[d]	
								Filled	Empty
			Alu Yb9						
Yb9NBC1	AC024091	26414–26105	AGTATCTTTAGATCCAGGGTGAAGC	TTCCAGTGGTAAGTCTATGGGAAT	60	FP	12	411	86
Yb9NBC2	AC024896	142649–142362	GCAGACAGTACCCACTTATTTTTGT	TGGTTCTATAAAGCATTTGTTTCTTC	55	FP	7	462	146
Yb9NBC3	AC005342	167963–167121	GAAGCTCACTCTGCCATGTG	CATGTTCGGTCCTGCTTACA	60	FP	12	527	200
Yb9NBC6	AC020900	61455–61742	GCAGACCGTATGTTCAATAAATGAC	CCACTTGGAAAAACACCCAAA	55	FP	5	493	153
Yb9NBC7	AC009062	351148–351457	CAGTAAATGATGGGAACAACCTTC	CTAAATGTCAAGCTATGCCACAGA	58	HF	16	403	83
Yb9NBC8	AC011967	156726–157013	TAACTTTAGTTTTCCATCGCACATT	ACACTAGTTTTACCCTTGTCAGCAC	60	LF	18	419	86
Yb9NBC9	AC022199	71329–71616	AGCTTCCCATTTCTGGTTTGTCTAT	GCCTTGTTAAACCCAACCTTTT	60	FP	17	453	120
Yb9NBC10	AC025961	22060–21773	GTTTTCCTGGTGTGCCCTAAATA	TTTACCTAACTCACAAGACCCAAAG	60	IF	4	524	197
Yb9NBC11	AC019189	172700–172987	AAAGACTTTTCAGGTTCTTGTAGCA	ATGCATGTCTATGCAAACTATCAAA	55	FP	1	392	74
Yb9NBC12	AC011170	158821–159108	AACACCTGAGAAAGGCTCATTG	GCTTTGGAAAATACCTAAGAAGCAC	55	LF	10	414	93
Yb9NBC13	AC003985	36492–36792	TCTAGTTTGGAGTGCCATGC	CTCCCAGTCATGCTTCCTGT	60	FP	7	510	167
Yb9NBC15	AC006036	24671–25059	CCAAGGTTCAGCTTTATGCTC	GCTCAAAACCGCTGAATTGT	60	FP	7	489	159
Yb9NBC16	AC024057	13965–13678	GAAGAAAGAATGGAAAGGTAGTTG	ACCCCCATGACACTAATTTACCTAT	55	—	3	416	117
Yb9NBC17	AC012664	22598–22311	ACTTACCCAAAACGCATGATTC	AACGTAGATGCAGACAACACTCTTT	60	FP	2	709	391
Yb9NBC18	AC005751	35038–35343	CGTTTGAAAGCTACGTTACC	TCCCATGAGGTAGTGATGAT	60	FP	16	531	203
Yb9NBC21	AL136081	33392–33083	TTCATGTAGCCAAAACTACCTGTTC	TTAACAGCTTACAGTTTGGCAGAG	52	FP	6	425	107
Yb9NBC22	AL139193	160587–160874	TGCACAACTATACACCAGACACTG	TTGTCTCCCATCAGTAGAACCTAAG	55	LF	14	435	110
Yb9NBC23	AL356756	80321–80034	CAGGACTTTTATTGAATCCTCACCT	AAAGAGAGACATGGCCCAATTA	58	FP	14	412	83
Yb9NBC25	AC008558	90385–90099	GAGTTGTCAAATTTGGAATGGATAC	ACATCATTAAGCTCTTCCTGACATT	55	FP	5	496	159
Yb9NBC27	AC011966	13523–13810	CATGGATAACATCATAAGGCTTCAG	ACACTAGTTTTACCCTTGTCAGCAC15	55	LF	15	482	149
Yb9NBC28	AC004808	25856–26143	AAAAAGGTGTGCCTGGATATTTA	CTGTGGCATAACTCAAACTGTAATG	55	FP	7	539	208
Yb9NBC29	AC005008	29772–30089	GTAATATGAGGTGATGGGGGTTACT	GGTGAAAGAAGAACCCCTAAGTTAT	60	LF	7	474	138
Yb9NBC30	AC003003	35922–36249	GAACCCCATCCATTCTCTTACA	GTGGCAAAATATTGGCGACT	60	IF	16	508	156
Yb9NBC31	AF107258	58225–58541	TTTCTCAGCACTATCCCTGT	GACAGTGAGTTGGCAGTACC	56	FP	21	457	130
Yb9NBC32	AL121582	154486–154199	CCTAACCCCTACATTTTACCATTTC	GTCATTTGCACTTGTCAAAGAGTGT	55	FP	20	469	141
Yb9NBC34	AL121841	28487–28800	CCAAGTTTCCTCTGCTGGAA	CACAAATACTCCCTGCCTCAG	55	FP	14	489	90
Yb9NBC35	AC040906	166712–167029	TTAACAGCTTACAGTTTGGCAGAG	TTCATGTAGCCAAAACTACCTGTTC	60	FP	6	427	109
Yb9NBC36	AF015725	15626–15909	AAGCAGTCATCATCCATTTT	ACCACAAAAATGCACTTACC	60	FP	21	521	201
Yb9NBC37	AB014460	3311–3621	CAAAATGGCCGTGTTCTTTT	GTGTCCACGGATCTTTGCAG	62	FP	8/16	458	142
Yb9NBC39	AC004542	12799–13104	AGAGGATCTTTGCAGGCACT	GTGCTGTGGGGTTAGGAAGA	55	—	22	509	176
Yb9NBC40	AP000237	60117–60404	AGTGAGTTGGCAGTACCCAAAT	CTCAGCACTATCCCTGTTCTTACAT	60	FP	21	450	124
Yb9NBC41	AC004140	4672–4851	TGTTTCCTCATCTGCCAACTT	AAAAGACTGTTGATGACCACTCAG	55	FP	7	761	389
Yb9NBC42	AC004945	152516–152833	GACATCTCCCTCCTTTCTCT	GAAAACCTGAACATGGGTAA	55	FP	7	521	177
Yb9NBC44	AC006561	13793–13506	GACTACAACATACCATCCTCAAAGG	GTATAGGAAACAGCGTGTTGTGAC	55	FP	12	426	106
Yb9NBC45	AL121978	17555–17268	GGAGAACCACTTGAACATGGAG	AGCCCTGCTATATCCAGCTCTT	60	—	6	486	167
Yb9NBC48	Z95114	30489–30202	GCTGCATACCAGACCTTTGTC	TTGTGCTGTAAGGCTGAGTAGG	60	FP	22	432	117
Yb9NBC49	AC005375	129358–129604	ATCCTTTAGATCAGAGGTCATCAAG	CAACAACTAATCTGCTTTCTGTCAC	58	FP	17	393	134
Yb9NBC50	AL109865	28485–28772	GTTCCACAAGTACAGGAGAAAATGT	GAAGCTCTTTAGGAAACCAAATCTC	55	IF	11	460	138

(continued)

TABLE 1

(Continued)

Name	Accession	Position	5′ primer sequence (5′–3′)	3′ primer sequence (5′–3′)	A.T[a]	Human diversity[b]	Chr.[c] loc.	Product size[d]	
								Filled	Empty
Yb9NBC53	AQ382257	185–472	GGGACTGGGTATAAATGAGGTG	GGACCAATCCTACCTTGTATGG	55	HF	20	454	68
Yb9NBC54	AL050305	39695–40005	TAGGATGAGAATGAACTTTGAGATG	CCATTTATAACCAATGACGACAAAG	58	FP	X	492	172
Yb9NBC55	AQ076355	91–379	CTCAGATAAGGAAACTGAAACACAG	CCTATACCTTAAAACAAGCTTGGAC	60	FP	1	425	108
Yb9NBC58	AC022199	109996–109711	TTGACTGTAACGTCACTTTATTTGC	TGACTAGTGCTTTTGTGTATTGAGAA	60	FP	17	445	128
Yb9NBC59	AL121582	149776–150063	GTTTTCTCAGTCTCTTGCATTTTGG	GGGTGCAGAGACCAAAAACTT	55	FP	20	480	160
Yc1NBC1	AC011296	4067–3787	AGTACGTGAGGTTTCTATGCCTTG	GATTTGTCCATAATAGCCCCTAACT	60	IF	?	481	159
Yc1NBC2	AC006195	139237–139517	TCTCTCATGAACATAGATACAAA	CGTGCATTCTTGAGATAAAT	60	IF	7	443	102
Yc1NBC3	AC010072	48921–49201	GGATACCCCTTGGGAAAAGA	GAACACCATGTAACCCTCACC	63	FP	14	405	92
Yc1NBC6	AC004016	82266–81986	CAAACTCTGTGCACCTTGACA	CACTCGCATTATGGATTTTGG	65	FP	7	1009	677
Yc1NBC8	AC007298	28402–28682	CATCAAACCCCACACACTCA	TCCTTGGAGCCACATGTTTT	63	FP	12	463	115
Yc1NBC9	AL121603	31558–31838	GCCCAGCTGGAAATAGCTT	AGAAATTCTGCATGTGTCTCAG	63	IF	14	490	159
Yc1NBC11	AF123462	93456–93176	GGGAATGTTCATAGGACATGG	TGGAACATGCCAGAAAGAGA	63	FP	14	778	437
Yc1NBC13	AL122006	69774–70054	TCCCAAGTCCCATCCCTTAGAA	GCCATTCCTCACCAGCCATT	60	FP	1	504	165
Yc1NBC14	AL031734	146718–146998	TGAGGTCCTGTGACTTGGTG	TCGCAAAGCATTTCTCAAAG	60	FP	1	464	149
Yc1NBC15	AL031650	85392–85112	GGAATGGCATAGGAAGTGGA	ACCAAATGAAAGGGGAGACA	63	FP	20	418	112
Yc1NBC20	AP001696	246018–246298	GCAAGTAATGAAAGGATTCTAGGG	AGAGCTGCCCTATTTCTT	60	FP	21	486	163
Yc1NBC23	AC004626	28992–29271	TGCTCAGGTTAGGGATGTTAATGC	TTCTGAGCTGCTGGGGGACT	60	LF	16	445	120
Yc1NBC24	AL137013	69320–69041	TCAAAGGGGAATACTGGGGAAA	GGGGAAATGACAATCAAGTGGAA	60	FP	X	408	88
Yc1NBC25	AC018637	72620–72340	GGGCAGGGGATGTAGGGACT	TGCCCCTGTTTCATCTGTGC	60	FP	7	432	108
Yc1NBC26	AC027279	127822–128102	TCACTTCAGAAGGGGAAAAA	TGTGGTCGCCTGGACTTCAA	60	FP	16	472	165
Yc1NBC28	AC017019	30139–29859	TGGTGAGTTCCTGGTCTTGCTG	TGCTCACTCTTTGGGTCCACAC	60	—	Y	414	99
Yc1NBC30	AL157756	37868–38148	GCCGCTAGCCTTTGTGTAAA	CAAAGTCATCTCTGACCCCCAGA	60	FP	14	497	177
Yc1NBC31	AC008062	103843–103563	TTCTGTAAAAGCCTGTTAGGTCCA	CAGCATTTCACTGTCAGCATTGG	60	LF	7	443	110
Yc1NBC32	AC005866	37960–37680	GCGAGGGCAAGCAGACAATAA	GTGGAGCTCACCCCTTCAGA	55	FP	12	425	114
Yc1NBC33	AL132994	40508–40788	CTTTATGGGTCTTACAGTAGAA	TGCATATGTAGCCTCTGATTC	60	FP	14	500	186
Yc1NBC34	AL136382	87933–87653	CCCACAACCCTTTCCCAGAG	CAGCAACCTGGATGGAGTGG	60	—	1	477	165
Yc1NBC35	AC004638	32778–33058	CCCATTCTCCATGCCGTGAT	TGCAAGGCATTGGGGATACA	60	IF	16	481	162
Yc1NBC36	AL121903	24409–24129	CACAGGAACTATTCCCCACAA	GCGAAATTCTTGAAGGAAAAACTGG	60	FP	20	437	101
Yc1NBC37	AL049562	25982–25702	CGTGCATTCCTTCTCATCACA	GGCACTTTTACCTAAAGAGCTTACA	60	FP	X	406	88
Yc1NBC38	AC000118	10509–10789	TCCAAACCTCTCTTGTTTGGAT	TGAAAGGATTTATGCCAGGTG	60	FP	7	435	113
Yc1NBC39	AP001695	126848–127128	TCCAGGAAGCAAACAGAATTCAGAG	TCAACCCCACTCTGATGCTCAA	60	FP	21	623	291
Yc1NBC45	AF218891	1964–1684	TGGCCACATTGGAATTCAAAACTAT	TTCCTGCTGCTAAGTGACACATGA	60	FP	20	401	94
Yc1NBC46	Z86061	56824–57103	CTTTGAAGCATGCAAGGAAAGG	CAGTTTCCAATCTTAGGGACTTGA	60	FP	X	489	172
Yc1NBC48	AC007094	66892–66612	TTCACCACAATTAATAGGAAGGTTT	CAAACGCAGCACAGGATCTGA	60	FP	?	700	392
Yc1NBC49	AC011493	52071–51791	TGTGCTGTTACTATGGAGCCCTAC	CTGGGGAGACATCCCCTTCC	60	—	19	413	94
Yc1NBC50	AC010382	50258–49978	GGTATGGGGCCAAATTTAATCCA	TCCAAGAGAAGCCAAACCTACAGA	60	IF	5	406	101
Yc1NBC51	AC009415	123638–123918	TCATACAAAAGACAGGCTTTGC	CAAGGGAACAGATTCAGAAGAAACA	55	LF	7	521	208
Yc1NBC52	AC002429	141029–140749	GCTTTTGACACATCCCCAGGT	CACAAGATTTGGGGCCAAGAG	62	FP	7	429	111
Yc1NBC53	AC004848	43020–42740	AAAGCTATCAACCATGCCAACA	GAAAATGCTATTTTGGGGAATG	62	IF	7	505	186

(*continued*)

TABLE 1

(Continued)

Name	Accession	Position	5′ primer sequence (5′–3′)	3′ primer sequence (5′–3′)	A.T[a]	Human diversity[b]	Chr.[c] loc.	Product size[d]	
								Filled	Empty
Yc1NBC56	AC006017	155231–154951	TCTGTAAAAGTGCTTCACAT	GGGGGTGTGATATTCGTGCTG	55	—	7	593	287
Yc1NBC58	AL133367	83515–83795	TGCTGCATCAATCAGCCAGA	TCCCAGTCCTTGGCAACCAT	65	FP	14	427	118
Yc1NBC59	AC006213	58483–58763	ACCCTCCCCTCCTTCTGTGG	CCCTGCAGAACGCTGGAAAA	60	FP	19	428	93
Yc1NBC60	AL136319	30378–30658	GAAACCGCCAAGATTCTCACC	TCTCCATCATGATTCCCAACTGA	60	IF	10	522	205
Yc1NBC63	AL121964	57663–57943	GGTACTCAGTAACACATCAAGA	AAGCTGGGTGGTGGGTTCAC	60	IF	6	502	181
Yc1NBC64	AL121904	25022–25262	CAGATCCTGGTTCGTAGGAGGTG	CAAGCTGTGTATTCTTGATACTGC	60	IF	20	600	292
Yc1NBC65	AL049643	46216–45936	TTGGCTGAGGATATCAGATGTGT	TCCAGTGCTTAAGAGTAAAGCAAGC	60	FP	X	456	152
Yc1NBC66	AJ006998	11416–11136	GGCCTAGCAAGGTCTTTTGC	TGATGAGTGTACAAGCCACACTT	60	FP	21	422	110
Yc1NBC69	AB020859	19030–19310	CCCACATTTATCAGTACCTACA	CCTTGCAGAATAGCAATGAT	55	IF	8	524	210
Yc1NBC70	AL133238	24939–24661	AGCAATTGTGGAGCCAGGAA	GAGGGTGCTTAGTGGAGCAAA	60	FP	14	452	137
Yc1RG60	AC019215	161766–162046	TCCCACATTTCAGTGTGAATTT	GGCATTTGGGATAGTTCCTG	60	HF	8	474	159
Yc1RG62	AC007428	139021–139301	GCTCAACATGCATAACCTTGAAC	ATTTCCAAAGAAACCCCTGACT	60	FP	?	522	216
Yc1RG83	AC009004	751–1030	CTGGCTGGGAGATTTTGTTAAA	GTTGGAAACAGTGTATTGCCTGTA	60	FP	19	724	397
Yc1RG64	AC009289	65992–66272	TCCAGTCATCTTAATGTGCCTTAG	GGATAGACCTTGCCTTTCTGATT	60	FP	14	380	67
Yc1RG65	AC019181	63269–63549	GCAGCCTGGATATCAATTAAGG	ATGGGTTAAAACTCCTAGCACTG	60	FP	2	735	413
Yc1RG66	AC009506		CTTTTTCTCAGACTGTGCTTGC	GCAACAAGACAAAACAGCAACTG	60	FP	1	419	109
Yc1RG67	AC008039	178981–179192	AAACTACCTTCCCCAGACTCC	CCCTAAGGACTTTATAATGGGACT	60	FP	7	382	125
Yc1RG68	AC008039	164672–164954	ATGGTGTCCACAAGAAACTGAG	GGAAGGCTCCATTATAGGTCTTG	60	IF	7	480	166
Yc1RG70	AC006323	3461–3741	CTCTGCAGCATGACAAATCAAT	CAGCATCTAAAGCACTCACTTCA	60	FP	17	504	178
Yc1RG71	AC011450	98261–98574	TACTGAAGACCAGTGGGCACAA	TTCCACTCACCTTACCCAGATTA	60	FP	19	435	73
Yc1RG73	AC007739	154145–154426	ATTGCCAAGAACCTTGTGTTTC	GGGGTTGAGAAAAAGTTCAGTG	60	FP	?	463	143
Yc1RG74	AC006038	73850–74014	AACTACCGTAGAATGGGCAAAAT	GAATGGATGGAAAACCAACATAA	60	FP	2	415	226
Yc1RG77	AC005783	19041–19327	GAGAAAGAGCTGCAAGGATGTC	CAAGTAAGGCCCAAATGAGGT	60	FP	19	401	84
Yc1RG78	AC002044	13430–13712	CTCCAGGATCTGCTTTCATCTTA	TCATGGTAACTAGCACAAGATCC	60	FP	16	431	119
Yc1RG79	AC004690	35856–36140	GGGTCTATCATCACCTTAATTTTGA	TGGTTTTTAGATGCCAAACACTAT	60	FP	7	497	158
Yc1RG80	AC004485	74445–74724	CAGAAATTGGTCCTTACAGTTTCC	AGAGGTGAACAGTTATTGCCTGA	60	FP	7	482	134
Yc1RG81	AF088219	1767000–176982	CACACAGCAGCAGTTACAAAAAC	CTTCTAGGCTTTAGTTGGGGAAG	60	FP	17	535	354
Yc1RG82	AF088219	99726–100005	CCTGGACCTTTAGGCATTTTT	CAGTCATCTCATCTTCACAGCAC	60	FP	17	388	91
Yc1RG83	AC005026	82038–82232	GCAGTAATGGTGGCCTGTTATAG	GGAAACTGTTAATGCTTCCCTCT	60	FP	7	389	153
Yc1RG84	AF131217	50031–50317	CCACTTGCCACTCCTATGGTAT	AAAATGCACAGGAATAGCGTTC	60	FP	21	387	60
Yc1RG86	AC005412	78372–78652	ATTGGGTGACCAGTTGTATTGAC	CTTCTGGAGGGGAACTGTTTTTA	60	FP	17	499	188
Yc1RG87	AC008071	84205–84487	GAACATGTGAACACATTGCTAGG	AATGTACCTTCAAAGTCACACAGC	60	FP	7	427	92
Yc1RG88	AC006305	13802–14086	GGTGACTCTCAACCCTAACTTCA	GTGGATTCCCGACAGAAGTATT	60	FP	18	395	74
Yc1RG90	AC004671	68017–68298	CCTTAATAATTTCCCCCGGATT	GCTGTAGGGGCTAAAATACCAAC	60	FP	12	398	100
Yc1RG91	AC005288	37818–38107	AATGGGTGAAAAGAGGTAGAAGG	TGTGTCCTTAACAAAGAGGATGG	60	FP	17	700	391
Yc1RG92	AC004675	78485–78767	ACACTCTATGCAGGCAGTCATCT	CCTGGACCTTTAGGCATTTTT	60	FP	17	402	85
Yc1RG93	AC005324	137294–137574	GGGATTCAAGTGTGGGTAGAAT	AAGGAAGGCAATATGATGTGG	60	LF	17	377	63
YclRG95	AL049537	38717–38997	ACCTAACAGATGACCTGCTGAAA	GAGGTAGAGAAAGGCAAGCATTC	60	IF	20	701	390
Yc1RG96	AF042091	61095–61379	ACACACAACCTGAAAACTCAACC	CCACACCAGCATGTTATTTGAT	60	FP	21	457	128

(continued)

TABLE 1

(Continued)

Name	Accession	Position	5′ primer sequence (5′–3′)	3′ primer sequence (5′–3′)	A.T.[a]	Human diversity[b]	Chr.[c] loc.	Product size[d]	
								Filled	Empty
Yc1RG97	AF042090	42069–42352	AAGTGCACACTTTGACGTTCAC	CCTTGATTGGCATTCAGGTTTA	60	HF	21	441	88
Yc1RG98	U92032	3903–4188	TCTTATCTCTGACACCTGACACG	AAAGAACCCAGAGCTATGACAGA	60	FP	6	442	113
Yc1RG99	AL022163	85835–86116	AAAGCACTTGGTACAGAATCAGC	CCATGGCGAAGTTAATGAGAAGT	60	IF	X	390	64
Yc1RG100	AL354872	86112–86401	ACTTCCATGAGCTAGTGGCTGTA	GATCTCTAAACGATAAAGGCTCAC	60	LF	1	474	143
Yc1RG101	AL031662	26328–26613	CCAGCCAAAGAGATTACCAAAA	GTCCAGTCCATTTCTCAAAGAAG	60	HF	20	541	235
Yc1RG102	AL158040	201136–201376	CTGCCTTTGTAGTAAATGTCAAGG	GTAGACATTCGCTCCACCTTTAT	60	FP	10	414	110
Yc1RG103	AL158157	101226–101505	GGCATTTGCATTCTGATGCTTA	GACATGTTAGAGAAAAGGTGACATC	60	HF	9	383	79
Yc1RG104	AL157384	87495–87786	CTGGAAGGGATCTTTTCTTATGG	CCCTTTTCTGATCCTATTCTCCA	60	FP	9	438	130
Yc1RG107	AL358293	139195–139492	GTTTGATCAGCTGTCCTCAGACT	TGAATGAATTTTTGAGTTGGTGA	60	FP	14	399	76
Yc1RG108	AL035458	29348–29629	GTTATATGAACAAAGCCCGGTA	GACCAAAAGAACCGAGAAGAAAC	60	FP	20	381	71
Yc1RG109	AL137794	36815–37094	GCTAGAATTCATAATGGAACCATCC	TCCAGTTGAGTTGGAGTGATTT	60	FP	1	502	188
Yc1RG110	AL109824	732–1012	CTAGGGTTAAGGAGTCCCTTGG	GTGACCTAGGCCAGAGGTTAATG	60	FP	20	395	85
Yc1RG113	AL163278	90774–91055	CTGTACCGCTAAGAGCTTCTGTG	GATATCTCAGCAGAATGGCAGAC	60	FP	21	376	76
Yc1RG114	Z98051	36444–36724	ATCAGGCATACAGTCTGAAAAGC	AATCTTGGTTAGTGTGAGTCAACC	60	FP	X	426	110
Yc1RG115	Z98046	60991–61271	GTTCTGCTGTTTGGATCTGGAAT	GTGGTGAAGGTACAGACTCATCC	60	FP	X	392	72
Yc1RG116	AL078621	142330–142621	GGTTAAAAGAACACATGGGATGG	GAAAGGTGGGTGTCTAAATGCTA	60	FP	22	419	99
Yc1RG117	AL096861	42260–42540	GAATAACCCAAACTTGGTAGGTG	TGCAATAAAGAGTGTTCCTCTCC	60	FP	X	490	166
Yc1RG118	Z71183	21436–21716	TACACAGACCAATGGGGAAAGTA	TCCAGATCCATGACATAACACT	60	FP	22	389	89
Yc1RG120	AL023283	61027–61306	TCTGCTCTTGCTATACACTGCTG	GAAGGCAGTGAATGAGACACTCT	60	FP	6	499	194
Yc1RG121	AL109760	24171–24451	CATGGACATTTGGGAGAATGTA	CGCCCCTATAATTACTCAGCAG	60	FP	4	398	92
Yc1RG123	AL023882	16690–16970	CACACACACACACAAAATTAGCC	GTGAGTCTTGAAACGGCTTTTAC	60	LF	16	563	234
Yc1RG124	AL022397	18401–18681	AAATCACTGTACCAACCCTGTCA	GCAAACACCACTGAAGCATAAA	60	FP	1	397	79
Yc1RG125	X76070	298–578	TGTTCTCTTCCTGTCTCCATTTC	CTGTTTCTATGATCTTGAAGGATGG	60	IF	2	415	97
Yc1RG126	AP001752	250076–250356	CCCTGTAGTAATGGCTCAGTGAA	GGCGATTTAGGCATAGACATAGA	60	FP	21	415	91
Yc2NBC1	AC002430	108794–109074	ACATAGTGGGCATTCAAGAG	CTTAATGTTTCCATTTCTCCA	55	IF	7	467	131
Yc2NBC5	AC007384	128277–128557	GAAGGAATACAGGGAGGAAT	CTCCCAAACAACTTAAAACC	55	IF	7	461	125
Yc2NBC9	Z98051	36444–36724	GAAAAGCCTGATACTTTTGG	CTTGGTTAGTGTGAGTCAACC	55	FP	X	407	91
Yc2NBC11	Z69666	9696–9416	CGACAAGTGACTAACCTTACG	CTCCTCCAATGATCTATGTGT	55	FP	16	409	82
Yc2NBC13	AC007882	150095–149815	TGGGATAATGATTTGTCTCC	AACATGTGGCAGATGATGA	60	FP	16	407	89
Yc2NBC15	AC007541	129217–129497	GGTAAGGCAAAACCAAGTAA	GTTTTGAGGAAGCTGATGAC	55	FP	12	410	92
Yc2NBC17	AC005541	74313–74593	ATCAAATGGCAGCCTTACT	GGTTTTCCATTCCTGAGTTA	60	FP	7	401	82
Yc2NBC19	AL022163	81833–82113	GCTTAAAGCACTTGGTACAGA	TGGCGAAGTTAATGAGAAGT	55	HF	X	393	67

Perfect matches to the consensus are in italics.

[a] Amplification of each locus required 2 hr 30 min at 94° initial denaturing and 32 cycles for 1 min 94°, 1 annealing temperature (A.T.), and 1 min elongation at 72°. A final extension time of 10 min at 72° was also used.

[b] Allele frequency was classified as fixed present (FP), low (LF), intermediate (IF), or high frequency (HF) insertion polymorphism. Fixed present: every individual tested had the Alu element in both chromosomes. Low frequency insertion polymorphism: the absence of the element from all individuals tested, except for one or two homozygous or heterozygous individuals. Intermediate frequency insertion polymorphism: the Alu element is variable as to its presence or absence in at least one population. High frequency insertion polymorphism: the element is present in all individuals in the populations tested, except for one or two heterozygous or absent individuals. —, indeterminable.

[c] Chromosomal location determined from accession information or by PCR analysis of NIGMS monochromosomal hybrid cell line DNA samples.

[d] Empty product sizes calculated by removing the Alu element and one direct repeat from the filled sites that were identified.

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                 .         .         .         .         .   1 .
Y       GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGA 60
Yb8     ........................................................T... 60
Yb9     ........................................................T... 60

           2     .         .         .      3  .         .         .
Y       TCACGAGGTCAGGAGATCGAGACCATCCTGGCTAACACGGTGAAACCCCGTCTCTACTAA 120
Yb8     ...T..................................A..................... 120
Yb9     ...T..................................A..................... 120

                 .         .  4      .         .         .   9     .
Y       AAATACAAAAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCGGGAGG 180
Yb8     .......................C.................................... 180
Yb9     .......................C.............................G...... 180

                 .         .         .5        .         .    6    .
Y       CTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAGATCGCGC 240
Yb8     ..............................A.........................T.... 240
Yb9     ..............................A.........................T.... 240

               7 .    8    .         .         .
Y       CACTGCACTCCA-------GCCTGGGCGACAGAGCGAGACTCCGTCTC 281
Yb8     .......G...GCAGTCCG............................. 288
Yb9     .......G...GCAGTCCG............................. 288
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FIGURE 1.—Consensus sequence alignment of Y, Yb8, and the potential new subfamily Yb9 identified. Nucleotide substitutions at each position are indicated with the appropriate nucleotide. Deletions are marked by dashes (-). The Yb8 and Yb9 diagnostic nucleotides are indicated in boldface type with the corresponding diagnostic numbers above.

Yc1 and Yc2 Alu elements, because only subfamily members with perfect identity to the subfamily consensus sequence or one mismatch were isolated from the database using one of the database screening procedures.

Phylogenetic distribution and human genomic diversity of the new subfamilies: Amplification of the Yb9, Yc1, and Yc2 elements from nonhuman primate genomes facilitated the analysis of the phylogenetic distribution of these elements, using PCR and the oligonucleotide primers in Table 1. The majority of the elements evaluated were absent from the genomes of the nonhuman primates, suggesting that these elements dispersed and were fixed in the human genome after the human and African ape divergence.

We performed a PCR analysis on a panel of human DNA samples to determine the levels of human diversity associated with the Alu elements from these new subfamilies, using the oligonucleotide primers shown in Table 1. The panel consists of 20 individuals of European origin, African-Americans, Asians, and Egyptians for a total of 80 individuals (160 chromosomes). We were able to analyze 28 out of the 56 Yb9 elements, 97 out of 176 Yc1 elements, and 8 out of 17 Yc2 Alu elements, using this approach. Several factors did not allow for analysis of all the elements. Mainly, we were unable to design appropriate primers due to insufficient flanking unique DNA sequences or because the element analyzed resided within another type of repeat as described previously (BATZER *et al.* 1991). The Alu elements were classified as fixed present and high, intermediate, or low frequency insertion polymorphisms (see Table 1 for definitions). In general, we observed that approximately one-fourth to one-third of the elements analyzed had some degree of insertion polymorphism (Yb9 with 10/28, Yc1 with 24/97, and Yc2 with 3/8). The population-specific genotypes and levels of heterozygosity for each element are shown in Table 2. The high proportion of polymorphic elements in these Alu subfamilies is in good agreement with our previous observations, indicating that these subfamilies are very recent in origin and still actively retroposing within the human genome.

DISCUSSION

From our subset of AluYb8 and Y elements, we were able to retrieve three Alu subfamilies termed Yb9, Yc1, and Yc2. A schematic of the evolutionary relationship of these subfamilies with the previously defined Alu subfamilies is shown in Figure 3. Alu subfamilies arise as a result of mutations occurring in an existing master element or new source elements capable of significant amplification. In this case, the new subfamilies are presumably examples of Alu subfamilies that may have originated from the rare instances when an Alu element fortuitously becomes both transcriptionally and retropositionally active, therefore allowing it to be another Alu source gene.

The young Alu subfamilies are currently active with respect to retroposition, whereas the older Alu subfamilies typically are not. The old Alu subfamilies (Sx, J, and Sg1), which comprise the vast majority (>1,000,000 copies) of the Alu elements present in the human genome, appear completely inactive as none of their members have been associated with *de novo* Alu inserts that result in human diseases (Table 3). When noting the ratio of reported Alu insertions associated with diseases and the estimated size of the Alu subfamily, the younger

```
                  .         .         .         .         .         .
Y        GGCCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCGGGCGGA 60
Yc1      ............................................................ 60
Yc2      ............................................................ 60
Clinh    ............................................................ 60
BRCA2    ............................................................ 60
GK       ----------------------------------------................T... 60

                  .         .         .       2 .         .         .
Y        TCACGAGGTCAGGAGATCGAGACCATCCTGGCTAACACGGTGAAACCCCGTCTCTACTAA 120
Yc1      ............................................................ 120
Yc2      ......................................A..................... 120
Clinh    ......................................T..................... 120
BRCA2    ..........................T................................. 120
GK       ............................................................ 120

                  .         .         .  1      .         .         .
Y        AAA-----TACAAAAAATTAGCCGGGCGTGGTGGCGGGCGCCTGTAGTCCCAGCTACTCG 175
Yc1      ...-----........................A........................... 175
Yc2      ...-----........................A........................... 175
Clinh    ...AAAAA........................A........................... 180
BRCA2    ...-----........................A........................... 175
GK       ...-----........................A........................... 175

                  .         .         .         .         .         .
Y        GGAGGCTGAGGCAGGAGAATGGCGTGAACCCGGGAGGCGGAGCTTGCAGTGAGCCGAGAT 235
Yc1      ............................................................ 235
Yc2      ............................................................ 235
Clinh    ...............G.........................CG................. 240
BRCA2    ............................................................ 235
GK       ...............G............................................ 235

                  .         .         .         .
Y        CGCGCCACTGCACTCCAGCCTGGGCGACAGAGCGAGACTCCGTCTC 281
Yc1      .............................................. 281
Yc2      .............................................. 281
Clinh    .............................................. 286
BRCA2    .............................................. 281
GK       .............................................. 281
```

FIGURE 2.—Consensus sequence alignment of Y, Yc1, Yc2, and three Alu Yc1 elements associated with disease. The diseases linked withYc1 Alu elements are the angioedema caused by a *de novo* insertion in the C1 inhibitor gene (C1inh; STOPPA-LYONNET *et al.* 1990), breast cancer with another *de novo* insertion in BRCA2 (BRCA2; MIKI *et al.* 1996), and glycerol kinase deficiency (GK; ZHANG *et al.* 2000). Nucleotide substitutions at each position are indicated with the appropriate nucleotide. Deletions are marked by dashes (-). The diagnostic nucleotides are indicated in boldface type with the corresponding diagnostic numbers above.

subfamilies Ya5, Yb8, and Yc1 currently appear to be ~1000 times more active than the Alu Y subfamily with 7/2640, 3/1852, and 3/400 compared to 1/200,000 (Table 3). The Alu Ya5a2 subfamily appears to have even a higher current retroposition rate (1/40), but the very young age and small size of the subfamily may be an influencing factor. In general, two independent observations support the current mobility of these young Alu subfamilies within the human genome. First, there are examples of Alu inserts that have caused disease that belong to these young subfamilies. Second, the subfamilies have a high proportion of Alu insertion polymorphisms between individuals/populations (Table 3), indicating the recent proliferative/amplification activity of these Alu elements in the human genome.

Alu elements that are polymorphic for insertion presence/absence have previously proven useful for the study of human population genetics and forensics (BATZER *et al.* 1991, 1994; PERNA *et al.* 1992; NOVICK *et al.* 1993; HAMMER 1994; TISHKOFF *et al.* 1996; STONEKING *et al.* 1997; MAJUMDER *et al.* 1999; COMAS *et al.* 2000; JORDE *et al.* 2000; WATKINS *et al.* 2001). The identification of very young Alu subfamilies with a high proportion of polymorphic members provides new sources of Alu insertion polymorphisms for the study of human population genetics. However, it is important to note that an exhaustive analysis of these small subfamilies will only generate a relatively small number of new Alu insertion polymorphisms.

Master element *vs.* source gene: Alu elements have been proposed to fit an evolutionary model where the copies arose from "master" genes (DEININGER and SLAGEL 1988; LABUDA and STRIKER 1989; SHEN *et al.* 1991; DEININGER *et al.* 1992). A master gene can be defined as an element that is highly active during a long period, therefore generating a lot of copies of itself. However, we demonstrated that recently inserted Alu elements (*de novo*) belong to a variety of Alu subfamilies, indicating the simultaneous presence of multiple active elements in the human genome. These active elements that have a low rate of amplification and are only active for a very short period of time should not be classified as master genes. To distinguish between them, we suggest the use of the nomenclature of "master gene" when

TABLE 2

Alu Yb9, Yc1, and Yc2 associated human genomic diversity

	African American					Asian/Alaska native					European					Egyptian					
	Genotypes					Genotypes					Genotypes					Genotypes					
Elements	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	Avg het[b]
Yb9NBC8	0	0	20	0.000	0.000	0	0	19	0.000	0.000	0	0	17	0.000	0.000	0	0	12	0.000	0.000	0.000
Yb9NBC7	19	0	0	1.000	0.000	19	0	0	1.000	0.000	17	3	0	0.925	0.142	16	0	0	1.000	0.000	—
Yb9NBC10	3	1	4	0.438	0.525	2	0	14	0.125	0.226	3	0	14	0.176	0.299	6	0	9	0.400	0.497	0.369
Yb9NBC12	1	6	12	0.211	0.341	0	14	5	0.368	0.478	0	9	8	0.265	0.401	0	9	5	0.321	0.452	0.418
Yb9NBC22	0	0	14	0.000	0.000	0	0	15	0.000	0.000	0	0	15	0.000	0.000	0	0	13	0.000	0.000	0.000
Yb9NBC27	0	0	15	0.000	0.000	0	0	12	0.000	0.000	0	0	18	0.000	0.000	0	0	11	0.000	0.000	0.000
Yb9NBC29	0	1	9	0.050	0.100	0	7	12	0.184	0.309	0	2	8	0.100	0.189	0	0	3	0.000	0.000	0.199
Yb9NBC30	2	1	11	0.179	0.304	0	3	16	0.079	0.149	0	6	11	0.176	0.299	1	3	14	0.019	0.246	0.250
Yb9NBC50	0	0	15	0.000	0.000	0	6	7	0.231	0.369	1	0	15	0.063	0.121	1	3	14	0.139	0.246	0.184
Yb9NBC53	13	0	2	0.867	0.239	20	0	0	1.000	0.000	15	0	1	0.938	0.121	15	0	2	0.882	0.214	0.144
Yc1NBC1	1	7	12	0.225	0.073	0	2	18	0.050	0.062	0	10	10	0.250	0.068	0	7	13	0.175	0.078	0.070
Yc1NBC2	1	13	6	0.375	0.038	0	15	5	0.375	0.038	1	15	4	0.425	0.023	0	10	10	0.250	0.068	0.042
Yc1NBC9	4	13	3	0.525	0.008	3	13	4	0.475	0.008	3	8	9	0.350	0.045	0	0	14	0.000	0.000	0.015
Yc1NBC23	0	0	18	0.000	0.000	0	0	19	0.000	0.000	0	0	18	0.000	0.000	0	0	19	0.000	0.000	0.000
Yc1NBC31	0	0	18	0.000	0.000	0	0	19	0.000	0.000	0	0	19	0.000	0.000	0	0	19	0.000	0.000	0.000
Yc1NBC35	1	6	7	0.286	0.073	2	10	8	0.350	0.045	2	13	2	0.500	0.000	1	12	2	0.467	0.012	0.032
Yc1NBC50	0	2	18	0.050	0.062	14	4	0	0.889	0.081	4	9	5	0.472	0.009	5	2	10	0.353	0.048	0.050
Yc1NBC51	0	4	18	0.091	0.169	0	0	18	0.000	0.000	0	0	20	0.000	0.000	0	0	9	0.000	0.000	—
Yc1NBC53	8	7	1	0.719	0.070	3	12	1	0.563	0.022	1	13	2	0.469	0.011	4	11	2	0.559	0.020	0.031
Yc1NBC60	6	9	3	0.583	0.027	6	9	5	0.525	0.008	5	11	4	0.252	0.008	2	7	10	0.289	0.062	0.026
Yc1NBC63	0	0	0	—	—	1	5	8	0.250	0.082	3	6	10	0.316	0.056	0	3	10	0.115	0.096	0.078
Yc1NBC64	0	0	5	0.000	0.000	0	5	8	0.192	0.323	0	5	12	0.147	0.258	0	6	12	0.167	0.286	0.216
Yc1NBC69	0	0	13	0.000	0.000	8	4	5	0.588	0.030	2	4	7	0.308	0.070	3	7	5	0.433	0.024	0.031
Yc1RG60	16	0	4	0.800	0.328	19	0	0	1.000	0.000	14	5	1	0.825	0.296	18	0	0	1.000	0.000	0.156
Yc1RG68	1	4	14	0.158	0.273	6	6	8	0.450	0.508	3	7	10	0.325	0.450	3	3	14	0.225	0.358	0.397
Yc1RG93	0	0	20	0.000	0.000	0	0	20	0.000	0.000	0	0	19	0.000	0.000	0	0	14	0.000	0.000	0.000
Yc1RG95	2	17	1	0.525	0.512	4	15	0	0.605	0.491	0	20	0	0.500	0.513	6	12	0	0.67	0.457	0.493
Yc1RG97	19	1	0	0.975	0.050	19	0	0	1.000	0.000	20	0	0	1.000	0.000	18	0	0	1.000	0.000	0.013
Yc1RG99	19	1	0	0.975	0.050	6	14	0	0.650	0.467	8	11	1	0.675	0.450	14	4	1	0.842	0.273	0.310
Yc1RG100	0	0	18	0.000	0.000	0	0	19	0.000	0.000	0	0	18	0.000	0.000	0	0	16	0.000	0.000	0.000

(*continued*)

TABLE 2

(Continued)

Elements	African American					Asian/Alaska native					European					Egyptian					Avg het[b]
	Genotypes					Genotypes					Genotypes					Genotypes					
	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	+/+	+/−	−/−	*f*Alu	Het[a]	
Yc1RG101	20	0	0	1.000	0.000	17	0	0	1.000	0.000	17	2	0	0.947	0.102	16	0	0	1.000	0.000	0.026
Yc1RG103	16	2	2	0.850	0.262	19	0	0	1.000	0.000	18	0	0	1.000	0.000	15	0	0	1.000	0.000	0.065
Yc1RG123	0	0	20	0.000	0.000	0	0	20	0.000	0.000	0	0	20	0.000	0.000	0	0	20	0.000	0.000	0.000
Yc1RG125	0	16	4	0.400	0.492	0	9	11	0.225	0.358	0	17	3	0.425	0.501	0	19	0	0.500	0.514	0.466
Yc2NBC1	1	4	3	0.375	0.061	3	6	5	0.429	0.027	13	1	0	0.964	0.065	10	3	1	0.821	0.093	0.061
Yc2NBC5	3	10	4	0.471	0.010	3	10	1	0.400	0.031	17	3	0	0.925	0.071	13	4	0	0.882	0.085	0.049
Yc2NBC19	15	3	0	0.917	0.077	18	0	0	1.000	0.000	7	10	3	0.600	0.03	14	4	1	0.842	0.081	0.047

[a] This is the unbiased heterozygosity.

[b] Average heterozygosity is the average of the population heterozygosity.

Elements in italics were screened using DNA collected from Alaska natives rather than from the Asian population.

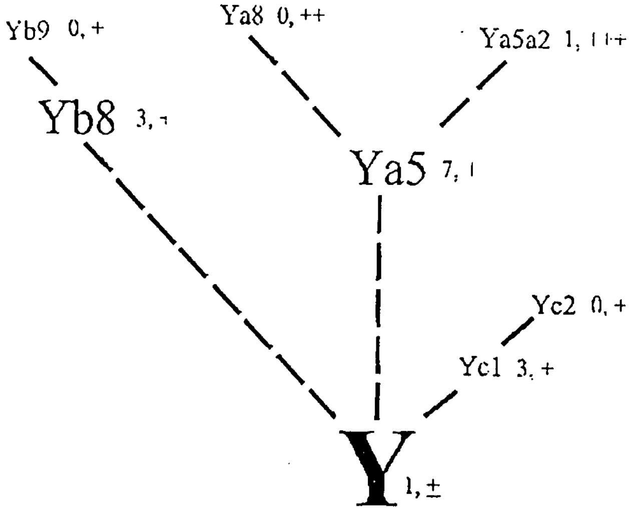

FIGURE 3.—Schematic diagram of the evolution of recently integrated Alu subfamilies. All the origins of the young Alu subfamilies are shown. The origins of the Yb9, Yc1, and Yc2 Alu subfamilies are shown after the divergence of the Yb8 and the Y subfamily, respectively. The size of the font is relative to the number of elements within each subfamily, the largest representing 100,000–200,000 copies; medium, 1000–2000 copies; and the smallest, 50–500 copies. The total number of elements from each subfamily linked to disease is indicated to the right. The proportion of polymorphic elements within each family is represented by the following: ±, rarely polymorphic elements are found; +, low percentage of polymorphic elements; ++, ~50% the elements are polymorphic; and +++, most of the elements are polymorphic.

referring to the highly active genes for long evolutionary periods of time, like the Alu element that generated the majority (>90%) of the Alu elements currently present in the genome today. For those copies, or daughters, that acquired the ability to retropose we propose the use of the term "source genes." However, some of the elements classified as source genes may be potential master genes, and only the progression of time will allow the appropriate distinction to be made.

Evolutionary reduction in the Alu retroposition rate: Our data indicate the existence of several currently active Alu elements that belong to different subfamilies within the human genome. However, the present amplification rate of Alu elements has drastically decreased from when it reached its peak 35 and 60 million years ago (mostly Sx subfamily). The majority of the Alu elements present in the genome of extant humans inserted during this peak amplification period. There are multiple reasons that could explain the reduction in the amplification rate' of Alu elements. First, mutations within or near the master Alu element could reduce its retroposition activity or even totally abolish it by a variety of mechanisms (DEININGER and BATZER 1993; SCHMID 1996). Alternatively, mutations within the master gene or in the LINE elements that affect the ability to "parasitize" LINE element-encoded enzymes necessary for retroposition could also reduce the Alu amplification rate. Furthermore, the host may have also evolved cellular

TABLE 3

Young Alu subfamilies copy number, inserts linked to disease, and polymorphism

Alu subfamily	Estimated copy number	Inserted linked with disease[a]	General subfamily polymorphism[b] (%)	
J, Sx, Sg1	>1,000,000	0	—	
Y	>200,000	1	±	
Ya5	2640	7	+	26
Ya5a2	40	1	+++	80[c]
Ya8	70	0	++	50
Yb8	1852	3	+	20
Yb9	80	0	+	36
Yc1	400	3	+	25[c]
Yc2	ND	0	+	37.5[c]

ND, not determined.

[a] Previously published Alu elements linked with disease (Deininger and Batzer 1999).

[b] The proportion of polymorphic elements within each family is represented by the following: ±, rarely polymorphic elements are found; +, low percentage of polymorphic elements; ++, ~50% the elements are polymorphic; and +++, most of the elements are polymorphic.

[c] Percentage polymorphism was determined using a selected subgroup introducing a bias.

mechanisms to reduce Alu proliferation. Finally, the availability of suitable genomic "insertion sites" may be reduced, since most evolutionarily neutral or positive sites are presumably already "filled" with different types of preexisting repeats. Alternatively, new Alu insertions may result in unacceptable local levels of unequal homologous recombination (Deininger and Batzer 1999).

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