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Jews

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13. ABSTRACT (Maximum 200 Words) This project will test the basic hypothesis that a given microsatellite marker allele occurs with greater frequency among the individuals affected with prostate cancer than among the controls. These studies will take advantage of the fact that two populations of Ashkenazi Jewish men are readily available for a case-control study. The first is a group of men at high heritable risk based on their having early-onset prostate cancer. The second is a group of men at low heritable risk who have no personal or family history of prostate cancer. Thus, we expect to observe predisposition alleles in the men at high risk that are not present in the men at low risk. The predisposition genes are likely to be within chromosomal regions in which loss of heterozygosity has occurred. Because these regions have remained identical by descent since the high-risk mutations occurred, they can be recognized by the presence of specific alleles of microsatellite markers in the high-risk group that are not present in the low-risk group.

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INTRODUCTION

This study uses several observations about the genetic basis of prostate cancer to enhance the efficiency of identifying susceptibility genes. 1) Prostate cancer is a multi-step genetic disorder in which some of the observed genetic alterations in prostate cancer cells were acquired through the germline. 2) The chromosomal locations of some of these genes can be identified readily in prostate cancer cells on the basis of their demonstrating loss of heterozygosity. 3) Historically, certain populations have been endogamous causing them to have more genetic homogeneity and to have prevalent founder mutations in some of their disease susceptibility genes. As a result of the population's endogamy, short chromosomal regions have remained identical by descent, leading to recognizable associations of the founder mutations with linked marker alleles (*linkage disequilibrium*). Ashkenazi Jews represent such a population.

BODY

Task 1. Subject identification. Months 13-24

Samples from 200 high-risk subjects were identified during the first year. *To increase the power of the study, samples from an additional 100 cases were identified during the second year.* The medical histories of each of these subjects were reviewed, confirming ethnicity and diagnosis of prostate cancer, and noting family history, age at diagnosis and Gleason score at time of diagnosis. For each subject, tissue blocks were obtained from non-cancerous tissues (usually lymph nodes) and thick (50 micron) sections were cut. DNA was purified from these sections using a protocol optimized in our laboratory and then quantified. To extend the utility of these sections, a technique for whole genome amplification using primer extension preamplification (PEP) was optimized. This technique reproducibly provides approximately 50-fold amplification of the DNA samples. *From the pool of low-risk subjects, an additional 200 samples were chosen and added to the 200 samples already obtained for subsequent analysis. These were amplified using PEP for subsequent analysis. Methods of drying down these amplified DNAs in 96-well microtiter plates for subsequent use were developed. This will enable efficient high throughput marker analyses to be performed.*

Task 2. Development of markers. Months 13-24

A. Markers from regions associated with loss of heterozygosity (LOH) in prostate cancer will be identified and fluorochrome-labeled primers will be synthesized. We have identified microsatellite markers for each of the following chromosomal regions 1q24-q25, 7q31, 8p21-p22, 10q23-q25, 16q22, 17p, 17q21-q22, Xq11-q13. *We have added markers for additional pertinent genomic regions and optimized reagents for their analysis.* Because of uncertainties about relative map positions, we have confined our markers to those which have shown (LOH) in a high proportion of subjects in a single report, to those which show (LOH) in more than one report, or to those whose map positions are known with a high degree of confidence from the GeneMap99 (<http://www.ncbi.nlm.nih.gov/GeneMap99>) and which are tightly linked to markers that show LOH. In addition, we have added markers for the following chromosomal regions that have shown linkage to prostate cancer susceptibility in families with multiple affected members, 1q24-25, 1q42-43, and Xq27-28 (Smith, et al., 1996, Cooney, et al., 1996, Gronberg, et al., 1997, Xu, et al., 1998, Berthon, et al., 1998) (figure 1)

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Oddoux, Carole

B. Standard PCR conditions will be developed for each of these markers. The primer sequences for each of these markers was identified using standard databases (<http://www.gdb.org>). The predicted sizes of the PCR product alleles were noted and markers yielding products of different predicted sizes were grouped and labeled with one of three different fluorescent dyes (tet, fam, hex). The net effect of this grouping is that multiple markers can either be amplified simultaneous and/or pooled from separate amplifications to minimize the number of electrophoretic runs. Procedures for pooling separate amplification reactions have been optimized.

The methods for multiplex analysis were developed to enhance the throughput of marker analysis. For each fluorochoime, three markers (small, medium, and large) were coamplified in the same well. In turn, these multiplex amplifications for each of the fluorochoimes were pooled for subsequent fragment length analysis. This led the analysis of up to 9 markers simultaneously in a single run.

Task 3. Data analysis

Procedures for organization, archiving, and efficient analysis of data have been put into place. Data analysis commenced for a small number of markers. Data analysis was delayed pending collection of additional cases and controls and development of multiplex analysis methods to improve throughput. As noted above, these have been accomplished. Data analysis was also delayed by recurring technical issues with the major analytical instruments (ABI 310 DNA analyzers). These have been remedied.

KEY RESEARCH ACCOMPLISHMENTS:

Development of DNA databases from cases and controls for genomic analysis.

Development of high-quality, reproducible methods for microsatellite typing

Development of high-quality, reproducible methods for whole genome amplification

REPORTABLE OUTCOMES:

Proposal, "Genetic Susceptibility to Prostate Cancer in the Netherlands Cohort Study," (PC99-1496) was funded by USARMC.

Proposal, "Mentorship Program in Prostate Cancer Genetics" K24 (CA85326-01A1), was funded.

CONCLUSIONS

This work demonstrates the feasibility for high-throughput multiplex microsatellite marker analysis and the feasibility for extending small samples of DNA 50-fold for genetic analysis. It creates the foundations for the analyses that will be performed in the remainder of this study.

REFERENCES

Berthon P, Valeri A, Cohen-Akenine A, Drelon E, Paiss T, Wohr G, Latil A, Millasseau P, Mellah I, Cohen N, Blanche H, Bellane-Chantelot C, Demenais F, Teillac P, Le Duc A, de Petriconi R, Hautmann R, Chumakov I, Bachner L, Maitland NJ, Lidereau R, Vogel W, Fournier G, Mangin P, Cussenot O (1998) Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2-43 Am J Hum Genet 62:1416-1424

Cooney KA, McCarthy JD, Lange E, Huang L, Miesfeldt S, Montie JE, Oesterling JE, Sandler HM, Lange K. (1997) Prostate cancer susceptibility locus on chromosome 1q: a confirmatory study. J. Natl Cancer Inst. 89:955-959.

Gronberg H, Xu J, Smith JR, Carpten JD, Isaacs SD, Freije D, Bova GS, Danber JE, Bergh A, Walsh PC, Collins FS, Trent JM, Meyers DA, Isaacs WB. (1997) Early age at diagnosis in families providing evidence of linkage to the hereditary prostate cancer locus (HPC1) on chromosome 1q24-25. Cancer Res 57:4707-4709.

McIndoe RA, Stanford JL, Gibbs M, Jarvik GP, Brandzel S, Neal CL, Li S, Gammack JT, Gay AA, Goode EL, Hood L, Ostrander EA. (1997) Linkage analysis of 49 high-risk families does not support a common familial prostate cancer-susceptibility gene at 1q24-25. Am J Hum Genet 61:347-353.

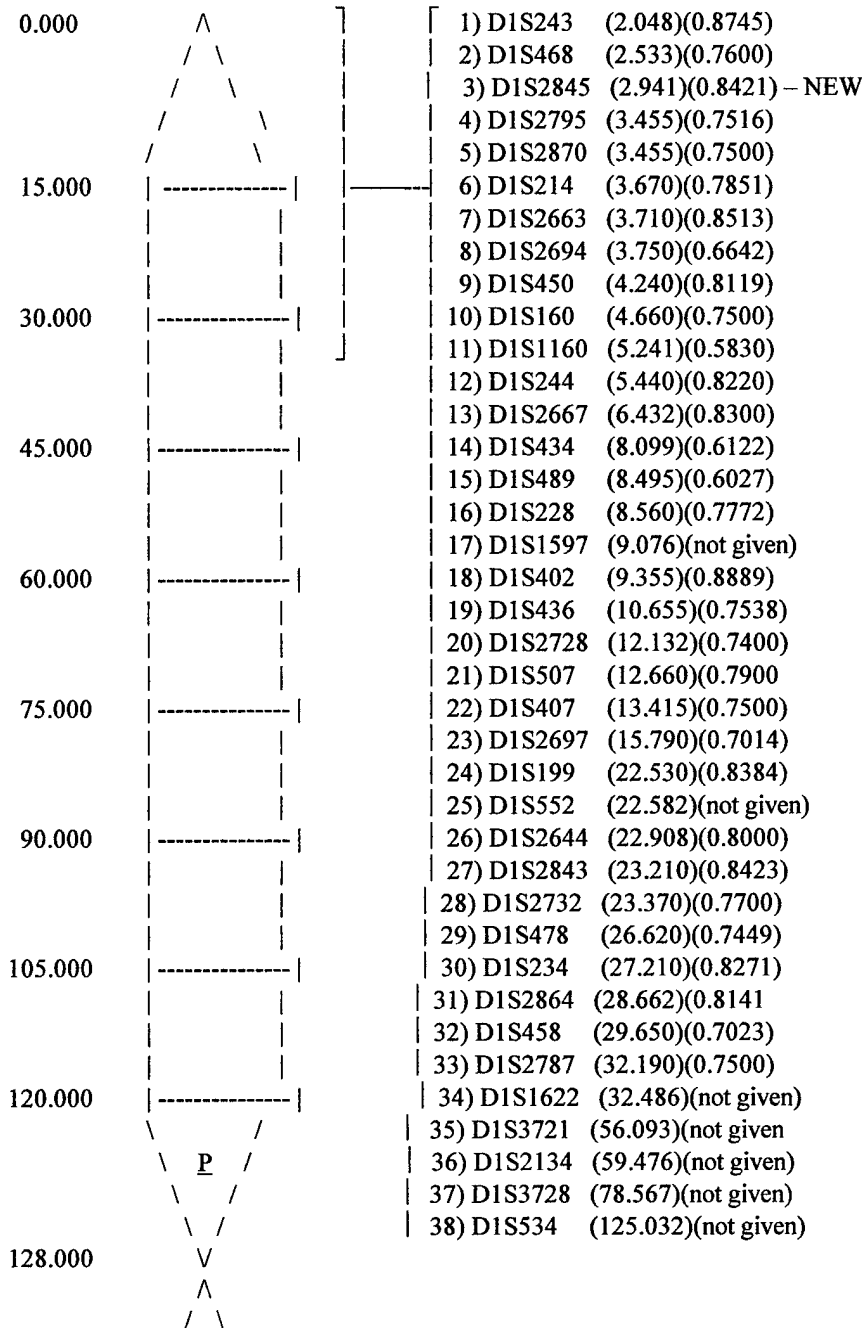
Smith JR, Freije D, Carpten, JD, et al. (1996) Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 274:1371-1374.

Xu J, Meyers D, Freije D, Isaacs S, Wiley K, Nusskern D, et al. (1998) Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet 20:175-9.

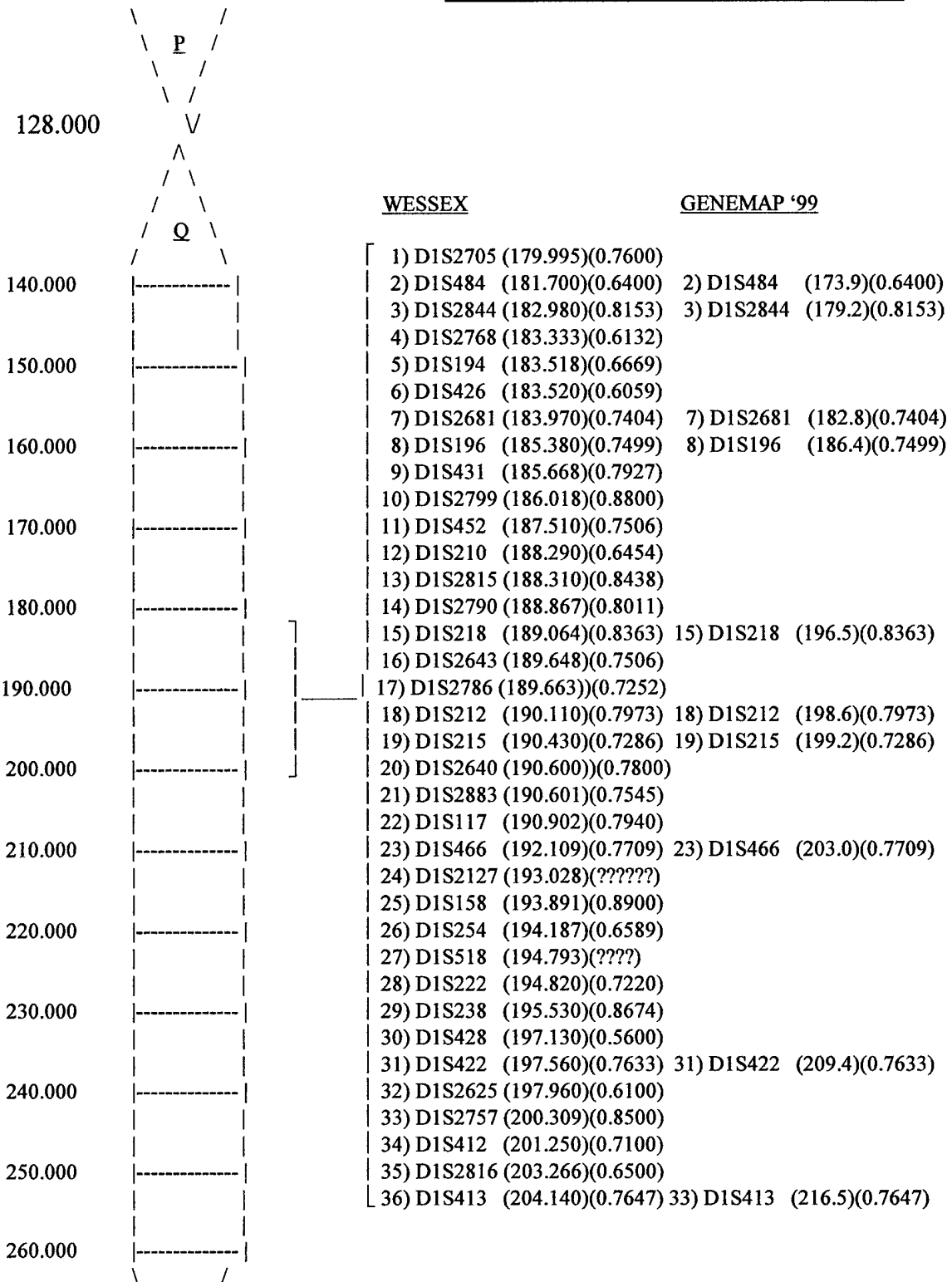
FIGURES: MARKERS FOR USE IN PROSTATE CANCER MAPPING STUDY

MARKERS ON CHROMOSOME 1p36

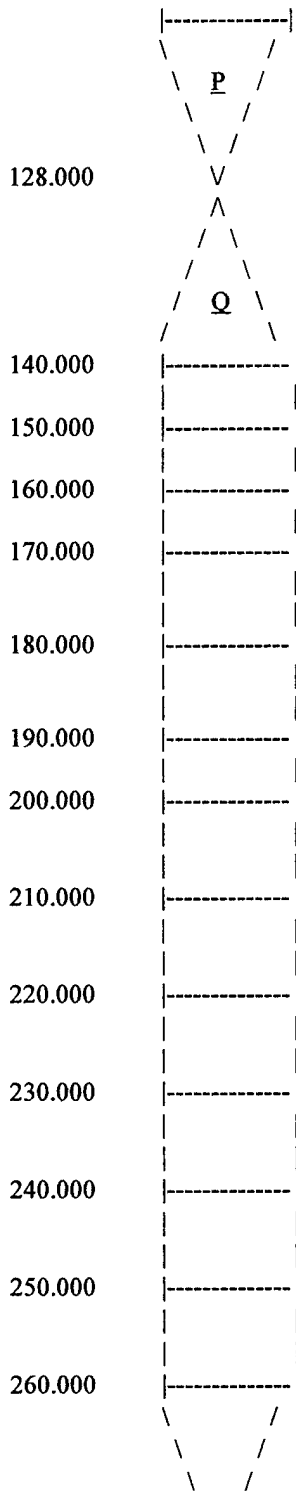
WESSEX



MARKERS ON CHROMOSOME 1q24-25



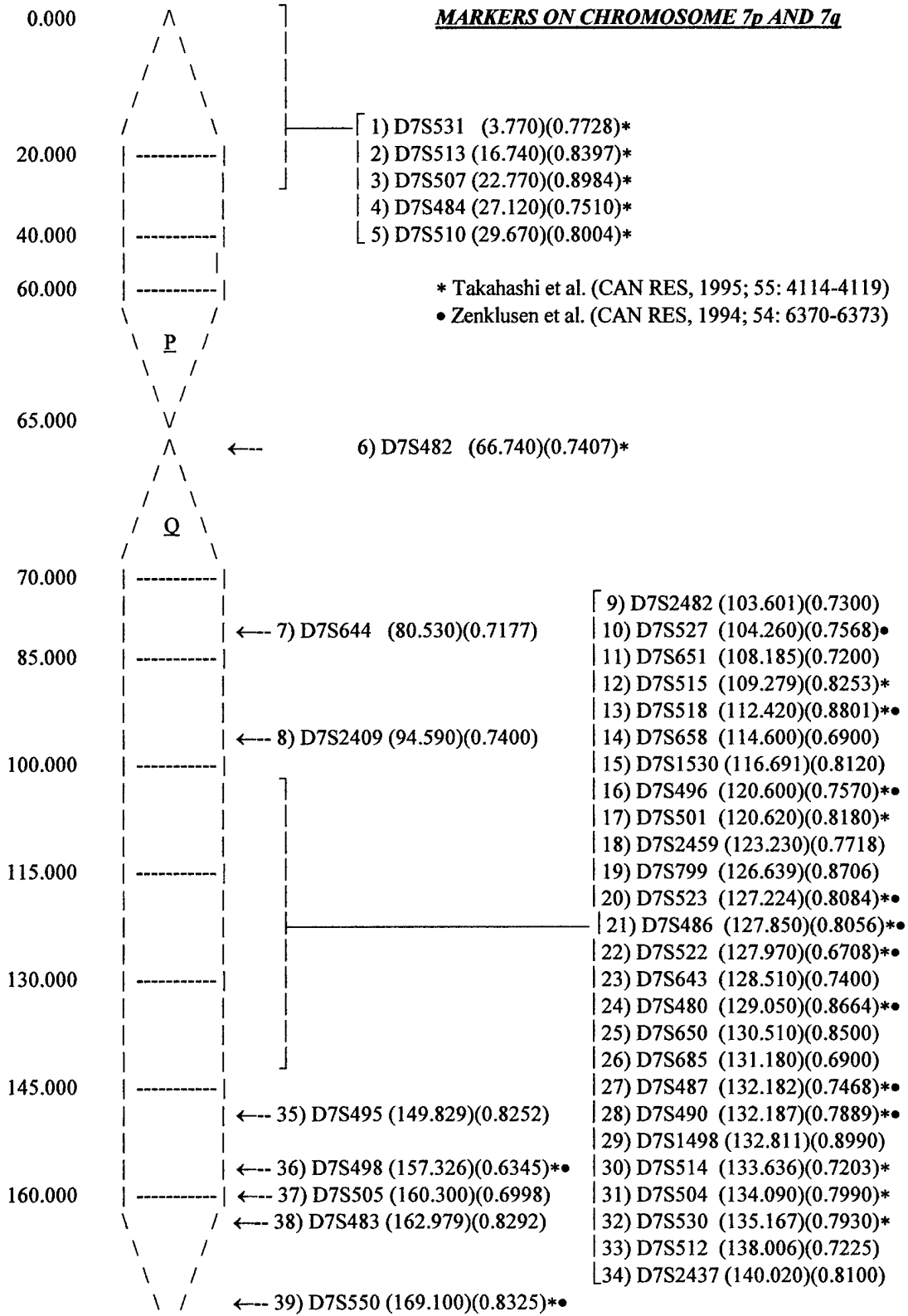
MARKERS ON CHROMOSOME 1q42.2-43



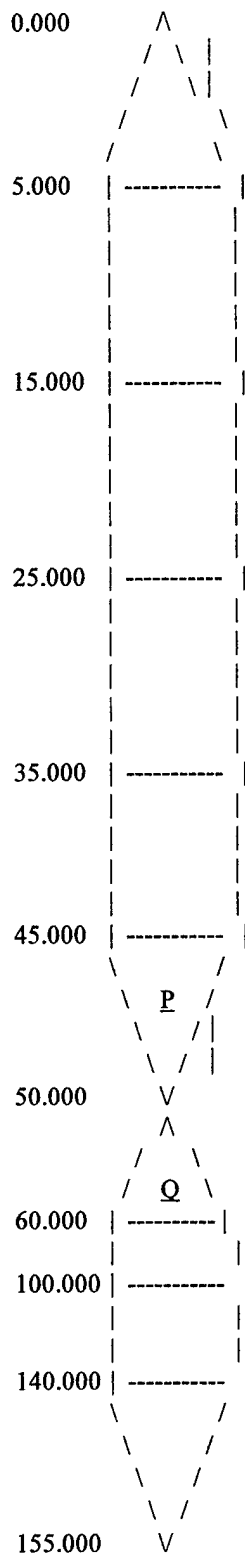
WESSEX

GENEMAP '99

	1) D1S2827 (235.407)(0.7900)	
	2) D1S229 (236.700)(0.7794)	
210.000	3) D1S490 (236.942)(0.7500)	3) D1S490 (241.6)(0.7500)
	4) D1S2758 (239.751)(0.6900)	
	5) D1S2880 (239.942)(0.7600)	
220.000	6) D1S2641 (240.061)(0.6900)	
	7) D1S2871 (241.750)(0.8403)	
	8) D1S213 (243.020)(0.8661)	8) D1S213 (246.2)(0.8661)
230.000	9) D1S251 (244.141)(0.8276)	9) D1S251 (249.0)(0.8276)
	10) D1S2709 (245.616)(0.7300)	
	11) D1S459 (245.670)(0.7800)	
240.000	12) D1S437 (245.994)(0.6600)	
	13) D1S446 (250.840)(0.6939)	12) D1S446 (256.1)(0.6939)
	14) D1S235 (252.310)(0.6883)	13) D1S235 (258.7)(0.6883)
250.000	15) D1S2850 (253.500)(0.6520)	
	16) D1S2785 (254.934)(0.7619)	15) D1S2785 (269.7)(0.7619)
	17) D1S304 (260.160)(0.6142)	
	18) D1S180 (260.620)(0.9000)	
260.000	19) D1S2842 (261.449)(0.7624)	18) D1S2842 (277.3)(0.7624)
	20) D1S2811 (261.729)(0.8925)	
	21) D1S2836 (262.267)(0.8000)	



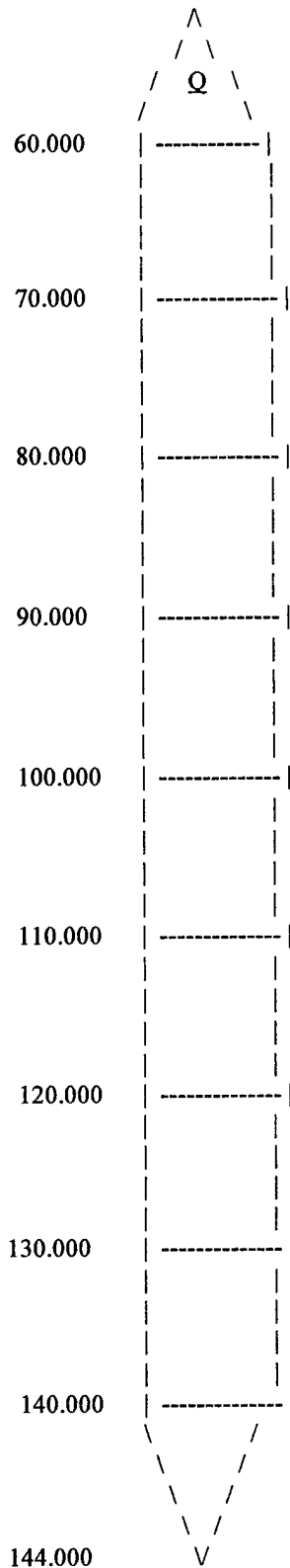
MARKERS ON CHROMOSOME 8p AND 8q



- | | |
|---|--|
| <ul style="list-style-type: none"> 1) D8S264 (1.346)(0.8450)* □ @ 2) D8S262 (1.604)(0.7193)• 3) D8S201 (1.960)(0.9200)♣ ♦ ♥ 4) D8S1742 (3.537)(0.7900) 5) D8S277 (4.054)(0.7375)* • □ | <ul style="list-style-type: none"> 6) D8S503 (5.692)(0.7400) 7) D8S520 (7.957)(0.7800) 8) D8S351 (8.818)(0.8400)* 9) D8S265 (9.094)(0.7921)• 10) D8S1130 (9.908)(not given) □ 11) D8S552 (11.412)(0.7908)* □ 12) D8S1106 (12.24)(not given)□ 13) D8S511 (14.201)(0.68)* ♥ □ |
| <ul style="list-style-type: none"> 14) D8S1827 (16.521)(0.6714) 15) D8S549 (17.169)(0.6400) □ 16) D8S1731 (17.242)(0.8400) 17) D8S133 (18.192)(0.7750)* • ♦ ♥ 18) D8S254 (20.342)(not given) ⊕ 19) D8S1145 (21.807)(not given) □ 20) D8S261 (22.408)(0.7760)* • ⊕ □ 21) LPL (22.883)(0.8430)♣ ♦ ⊕ 22) D8S258 (24.614)(0.7139)* ♥ ⊕ □ ▽ | <ul style="list-style-type: none"> 23) D8S136(25.5)(0.88)* • ♦ ♥ ♣ 24) D8S282 (26.337)(0.7303)• □ 25) D8S560 (26.390)(0.7600) □ 26) D8S298 (27.159)(0.70)* • ♥ 27) D8S1786 (27.722)(0.8622) 28) D8S1752 (28.324)(0.7700) 29) D8S1734 (28.355)(0.6700) 30) D8S137 (32.705)(0.6760)* • 32) D8S339 (34.670)(0.8300)* • 33) D8S283 (35.167)(0.799)* • 34) D8S87 (35.549)(0.679)* • ♦ 35) D8S1750 (37.629)(0.7900) 36) D8S1722 (41.325)(0.8800) 37) D8S255 (43.922)(0.7364) 38) D8S268 (47.116)(0.6101) 39) D8S587 (50.230)(not given)@ |

* Vocke, et al. (CAN RES, 1996; 56: 2411-2416)
 • Trapman, et al. (CAN RES, 1994; 54: 6061-6064)
 ♣ Bova, et al. (CAN RES, 1993; 53: 3869-3873)
 ♦ Chang, et al. (AJPathology, 1994; 144: 1-6)
 ♥ Kagan, et al. (Oncogene, 1995; 11: 2121-2126)
 ♠ Emmert-Buck, et al. (CAN RES, 1995; 55: 2959-2962)
 ⊗ Haggman, et al. (Urology, 1997; 50: 643-647)
 ⊕ Ishii, et al. (PNAS, 1999; 96(7): 3928-3933)
 □ Kalapurakal, et al. (Int. J. Rad. Onc. Biol. Phys., 1999; 45(4):835-840)
 ▽ Lutchman, et al. (Can. Gen. Cyto., 1999; 115: 65-69)
 @ Virgin, et al. (The Prostate, 1999; 41: 49-57)

MARKERS ON CHROMOSOME 10q



1) D10S195	(80.352)(0.6939)(1)
2) D10S202	(81.332)(0.6803)(0)
3) D10S532	(82.608)(0.7100)(0)*@
4) D10S201	(82.646)(0.8331)(0)*
5) D10S219	(82.723)(0.7800)(2)•
6) D10S1658	(83.438)(0.7329)(1)
7) D10S1644	(84.120)(0.8700)(0)□
8) D10S541	(86.580)(0.7844)(2)*•@□
9) D10S1739	(88.363)(0.6400)(0)
10) D10S583	(93.508)(0.8400)(3)*@□
11) D10S185	(93.935)(0.7661)(2)*
12) D10S571	(96.348)(0.7900)(0)*
13) D10S1709	(98.803)(0.7428)(0)
14) D10S198	(101.451)(0.7000)(2)*
15) D10S192	(102.020)(0.7839)(0)*
16) D10S222	(103.031)(0.7164)(0)
17) D10S1697	(103.567)(0.7740)(0)
18) D10S1671	(104.653)(0.6726)(2)
19) D10S205	(104.823)(0.9048)(0)
20) D10S597	(107.400)(0.6420)(2)*
21) D10S1682	(110.648)(0.6300)(0)*
22) D10S562	(112.932)(0.7400)(0)

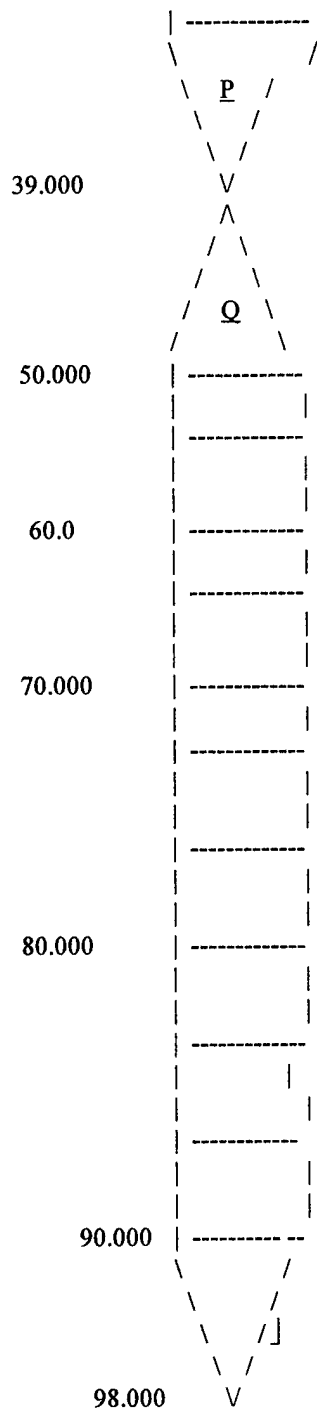
* Gray, et. al.; Can Res: 55, 4800-4803, 11/1/95.

• Feilolter, et. al. ; Oncogene: 16, 1/43 – 1/48, 1998.

@ Rubin, et. al.; Human Path.: 31,4,504 – 508, 4/2000.

□ Hamilton, et. al.; Brit. J. of Can.: 82,10, 1671 – 1676, 2000.

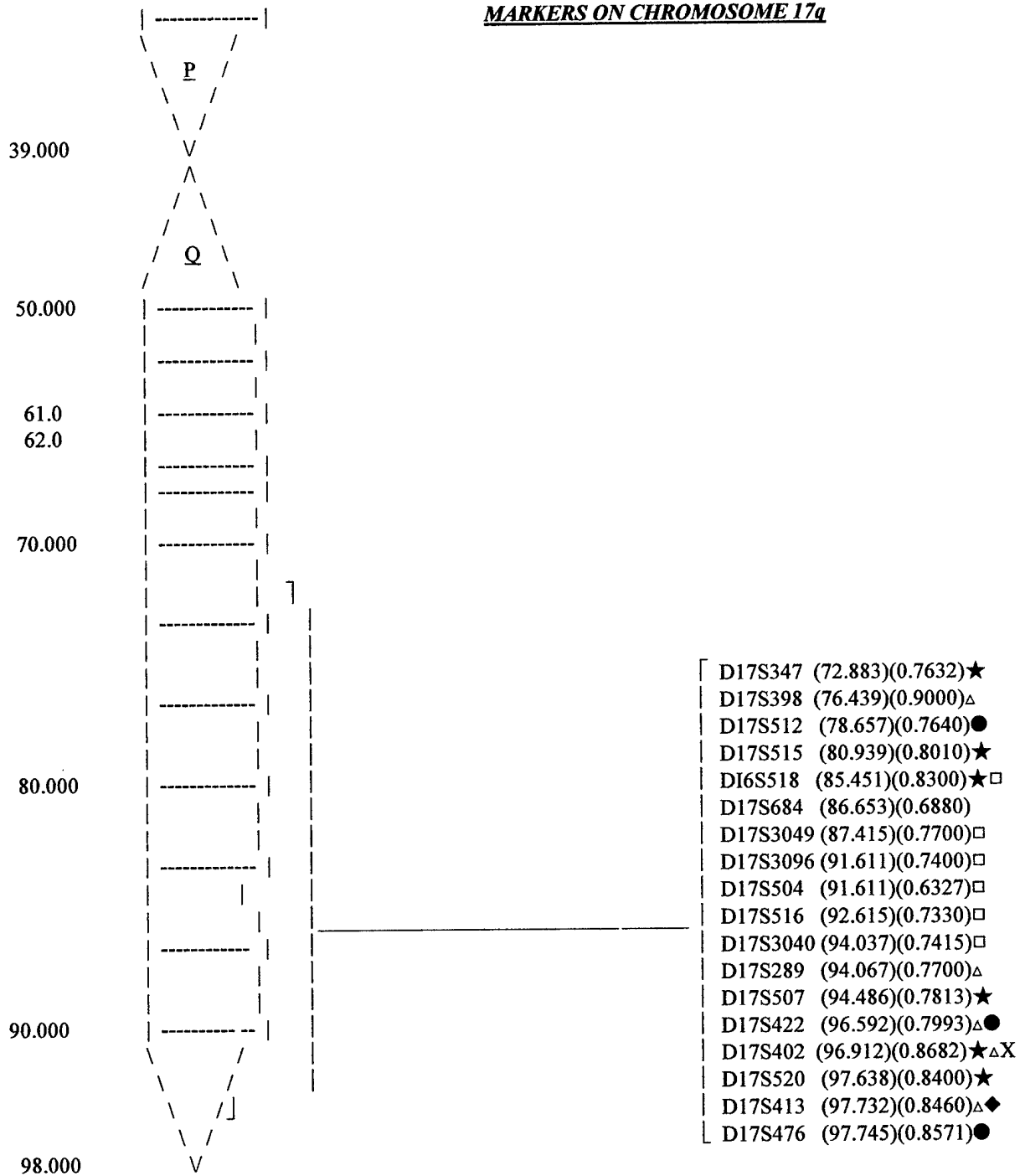
MARKERS ON CHROMOSOME 16q



- 1) D16S347 (72.883)(0.7632)★
- 2) D16S398 (76.439)(0.9000)△
- 3) D16S512 (78.657)(0.7640)●
- 4) D16S515 (80.939)(0.8010)★
- 5) D16S518 (85.451)(0.8300)★□
- 6) D16S684 (86.653)(0.6880)
- 7) D16S3049 (87.415)(0.7700)□
- 8) D16S3096 (91.611)(0.7400)
- 9) D16S504 (91.611)(0.6327)□
- 10) D16S516 (92.615)(0.7330)□
- 11) D16S3040 (94.037)(0.7415)□
- 12) D16S289 (94.067)(0.7700)△
- 13) D16S507 (94.486)(0.7813)★
- 14) D16S422 (96.592)(0.7993)△●
- 15) D16S402 (96.912)(0.8682)★△X
- 16) D16S520 (97.638)(0.8400)★
- 17) D16S413 (97.732)(0.8460)△◆
- 18) D16S476 (97.745)(0.8571)●

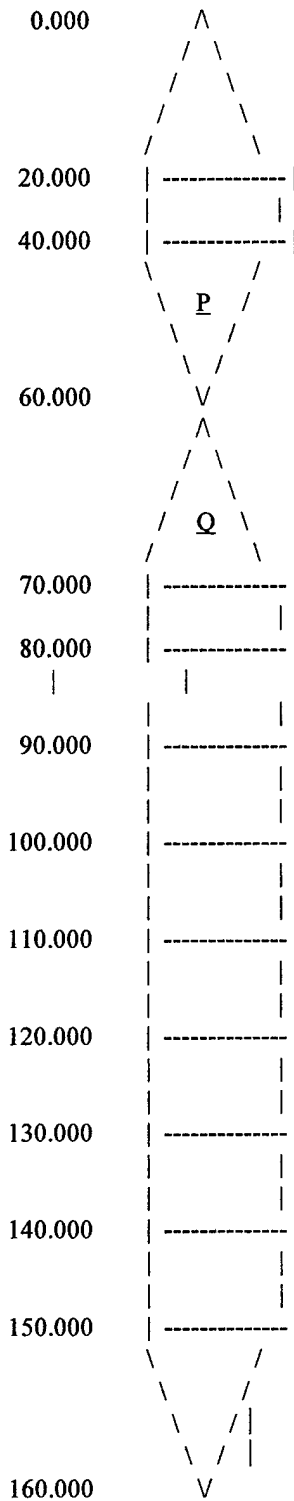
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 △ Saric, T. et al. (INT. J. CAN, 1999;81:219-224)
 ● Elo, J.P. et al (CAN. RES, 1997;57:3356-3359)
 □ Paris, P.L. et al (CAN. RES, 2000;60:3645-3649)
 X (J. of PATH, 1999;188:271-277)
 ◆ Visakorpi, T. et al. (CAN. RES, 1995;55:342-347)

MARKERS ON CHROMOSOME 17q



- ★Lidereau, R. et al (CAN. RES, 1997;57:1058-1062)
- △ (INT. J. CAN, 1999;81:219-224)
- Vinko, P. et al (CAN. RES, 1997;57:3356-3359)
- Casey, G. et al (CAN. RES, 2000;60:3645-3649)
- X (J. of PATH, 1999;188:271-277)
- ◆ (CAN. RES, 1995;55:342-347)

MARKERS ON CHROMOSOME Xq27-28



WESSEX

GENEMAP '98

- | | |
|-------------------------------|----------------------------------|
| 1) DXS1047 (150.3)(0.8100) | |
| 2) DXS1232 (139.500)(0.7400) | |
| 3) DXS1062 (153.7)(0.7429) | |
| 4) DXS984 (140.500)(0.7231) | |
| 5) DXS1192 (155.8)(0.8447) | |
| 6) DXS1205 (141.500)(0.7200) | |
| 7) DXS1227 (142.000)(0.7336) | ----- 7) DXS1227 (164.7)(0.7336) |
| 8) DXS8106 (142.500)(0.6917) | ----- 8) DXS8106 (173.6)(0.6917) |
| 9) DXS8043 (145.800)(0.8100) | |
| 10) DXS8028 (146.800)(0.8317) | |
| 11) DXS1200 (148.500)(0.6500) | ----- |
| 12) DXS548 (149.800)(0.6460) | |
| 13) DXS6687 (150.545)(0.7826) | |
| 14) DXS8091 (151.312)(0.7600) | |
| 15) DXS1193 (152.398)(0.7500) | ----- |
| 16) DXS1113 (152.615)(0.7610) | |
| 17) DXS8069 (153.267)(0.6200) | |
| 18) DXS8103 (153.375)(0.8020) | |
| 19) DXS8011 (153.484)(0.8112) | |
| 20) DXS8061 (155.656)(0.6903) | |
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| 23) DXS1108 (159.695)(0.7500) | |
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