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TITLE: Identification of a genomic signature predicting for recurrence in early stage ovarian cancer

PRINCIPAL INVESTIGATOR: Michael Birrer, MD, PhD

CONTRACTING ORGANIZATION: Massachusetts General Hospital

55 Fruit St
Boston, MA 02114

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14. ABSTRACT The first year of the grant required: IRB approval from all the Consortium collaborative Institutions, specimen collection, inventory, initial specimen processing. All the collaborating Institutions have obtained IRB approval to identify early stage/high grade tumors from their respective pathology service and to ship these tumors together with their clinical annotations to Massachusetts General Hospital (MGH). MGH has obtained IRB approval for using these cancer FFPE samples to identify molecular features that distinguish recurrent and non-recurrent tumors through RNA sequencing. Overall we have: 1) identified 592 early-stage high-grade ovarian cancers with 5-year follow-up, clinical annotation and accurate pathological review (228 recurrent and 364 non-recurrent), 2) established a specimen repository and clinical data inventory at MGH, 3) micro-dissected and isolated RNA from 110 tumors, and 3) optimized the preparation of cDNA libraries using NuGene WT-Ovation FFPE System V2. Throughout the project we have modified the protocol for processing the tumor tissues in order to be able to isolate both RNA and DNA from each sample. This was done in response to a DoD RFA requesting research projects for secondary utilization of collected tumor specimens. The DoD has recently selected for funding the project we have described in response to this RFA.					
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Introduction

All patients with high-risk early stage ovarian cancer are treated with comprehensive surgery followed by chemotherapy over a four to six month period. Yet it is clear that many of these women are cured by surgery alone. The overtreatment results from our inability to accurately identify patients who will not likely recur with surgery alone. This ultimately exposes these patients to both short and long term toxicities from chemotherapy. An objectively measurable characteristic (i.e. biomarker) that could accurately predict for ovarian cancer recurrence would be of great clinical value much like *Oncotype DX* has done for triaging early stage breast cancer patients. This ovarian biomarker would enable health care providers to provide a more tailored approach to ovarian cancer patients. We have identified a preliminary but promising genomic signature (i.e. characteristic expression of a set of genes) that can be applied to surgically attained ovarian specimens and predicts for cancer recurrence. While we do not expect this precise signature to validate, it is proof of principle that this type of genomic tool can be identified. This project proposes to generate and validate a recurrence signature for early stage ovarian cancer. A key bottleneck precluding the validation of cancer-related signatures, in general, lies in the large number of specimens needed to ensure that the signature is clinically valuable. This proposal will utilize a larger number of early stage ovarian cancer specimens obtained from an international consortium of clinical research groups to identify a genomic signature which can accurately identify patients who will suffer tumor recurrence. The stratification of patients according to risk of recurrence will allow those patients at high risk to receive more intense therapy and those at low risk to avoid chemotherapy toxicities. This will provide patients with early stage ovarian cancer a more personalized approach in addition to reducing overall costs of treatment. The identification of a recurrence signature will occur over the three years of the grant and due to our industrial collaborations, we expect the genomic signature to rapidly transition into a commercially available tool. In addition, all specimens will undergo extensive genomic analyses to generate a publically available database of genetic changes within early stage ovarian cancer to help researchers worldwide identify biomarkers that can aid early detection and inform novel targets for therapy. This will provide a unique database which will complement existing publically available genomic data. This project will leverage unique individual banks of stored specimens and associated clinical data present in the collaborating but disparate organizations. This will allow this clinically important question to be addressed and fulfill an important unmet need.

Body

The first year of the grant required: IRB approval from all the Consortium collaborative Institutions, specimen collection, inventory, initial specimen processing. All the collaborating Institutions have obtained IRB approval to identify early stage/high grade tumors from their respective pathology service and to ship these tumors together with their clinical annotations to Massachusetts General Hospital (MGH). MGH has obtained IRB approval for using these cancer FFPE samples to identify molecular features that distinguish recurrent and non-recurrent tumors through RNA sequencing.

Overall we have: 1) identified 592 early-stage high-grade ovarian cancers with 5-year follow-up, clinical annotation and accurate pathological review (228 recurrent and 364 non-recurrent), 2) established a specimen repository and clinical data inventory at MGH, 3) micro-dissected and isolated RNA from 110 tumors, and 3) optimized the preparation of cDNA libraries using NuGene WT-Ovation FFPE System V2. Throughout the project we have modified

the protocol for processing the tumor tissues in order to be able to isolate both RNA and DNA from each sample. This was done in response to a DoD RFA requesting research projects for secondary utilization of collected tumor specimens. The DoD has recently selected for funding the project we have described in response to this RFA.

Key Research Accomplishments:

- **Specimen inventory:** We have developed a large consortium to collect early-stage, high-grade ovarian cancer FFPE tissues through local and international collaboration. We have confirmed collaboration from the following cooperative groups: the Gynecology Oncology Group (GOG), Buffalo, NY, Grupo Español de Investigación de Cáncer de Ovario (GEICO), Barcelona, Spain, and Nordic Society Gynecologic Oncology (NSGO) Copenhagen, DK. At the end of the first year study, we have estimated 228 recurrent and 364 non-recurrent early-stage, high-grade ovarian cancer FFPE specimen for the proposed study. (See Table 1). We could not confirm the collaboration with the International Collaborative Ovarian Neoplasm (ICON) group due to policy changes that restricted shipment of tumor samples to MGH. To compensate for this loss we have identified tissues appropriate for the studies at MGH and are in the process of establishing collaboration with The University of Indiana and The University of Washington.
- **Study approval:** Each Institution of this consortium has obtained IRB approval for the identification, or usage of the required specimen. MGH Protocol #: 2012P001330 (See Attachment #1). Approval of the IRB from NSGO was lengthier than expected which has delayed delivery of the tumors belonging to this repository.
- **Specimen curation and import from GEICO:** We have received all specimens with complete annotation from GEICO, including 30 recurrent and 80 non-recurrent early-stage, high-grade ovarian cancers in FFPE blocks. These samples have undergone full curation including sample identification, selection and pathological reviewing. Bank ID numbers have been confirmed and cross-referenced with the clinical ID numbers. An independent pathologic review has been completed before sample shipment to ensure the pathologic diagnosis. Sections at 10 micron thickness have been prepared from all GEICO samples for RNA extraction.
- **Specimen curation and import from NSGO:** Similar to GEICO, full specimen curation has been completed at the site of Nordic Society Gynecologic Oncology (NSGO) for 130 recurrent and 95 non-recurrent early-stage, high-grade ovarian cancers. The institutional policy of NSGO restricted sending FFPE blocks. Instead, NSGO provides 10 FFPE sections at 10 micron for each sample with full annotation. Samples sectioning and shipment are still on-going and is expected to complete in May 2014.
- **Specimen curation and collection from MGH:** We have identified 30 recurrent and 50 non-recurrent early-stages, high-grade ovarian cancers from the coordinating center, MGH. FFPE blocks of the samples have been obtained for the proposed study. Sections at 10 micron thickness have been prepared from all MGH samples for RNA extraction.
- **Specimen curation and collection from GOG:** All clinical specimens from GOG are located at the GOG tissue bank (Columbus, Ohio). The formalin fixed paraffin embedded (FFPE) specimens have been identified, selected and reviewed. Bank Id numbers have been confirmed and cross-referenced with the clinical ID numbers (located in the GOG statistical Office Buffalo). This has resulted in 378 validated specimens (70 recurrent and

308 non-recurrent) for the DOD grant. An independent pathologic review at the GOG tissue bank was performed to ensure the pathologic diagnosis. This involved reviewing an H&E section of the tissue block adjacent to the sections which will be used for genomic analysis. The H &E section was reviewed by the GOG reference pathologist Nilsa Rameriz, MD. This process is ongoing. Until recently, the pathological reviewing has been finished at 50%, resulting in 38 recurrent and 139 non-recurrent early-stages, high-grade ovarian cancer specimens which will be shipped to the study site (MGH) by the end of June 2014.

- **Initial sample processing:** We have completed sectioning and RNA isolation from all specimens from GEICO (n=110, 30 recurrent and 80 non-recurrent each). Total RNA isolation was done using a QIAGEN AllPrep® RNA/DNA FFPE kit. The estimated mean yield is 1.76 µg per sample (ranges from 0.21 to 11.24 µg). RNA extraction from the MGH samples is currently ongoing.
- **Selection of genomic platform:** The DOD grant proposed using NuGene's WT-Ovarian FFPE System 2.0 V2 to linearly amplify and convert to cDNA for library construction. We have tested the robustness of this kit to properly process FFPE RNA for Affymetrix Human Gene 1.0ST Array system which has more stringent requirement than RNA-seq to generate meaningful data. The results have been written into a manuscript for Clinical Cancer Research (See Attachment #2). In the same study, we also tested the robustness of NanoString nCounter platform using FFPE samples. NanoString nCounter platform eliminates the requirement of amplification for typical low-throughput RNA quantification technologies (such as the Taqman low density array proposed for the validation study in the proposal) and represents a high-sensitive, cost-effective way with precision superior to qPCR. We confirmed the NanoString platform will be a better choice for validating the RNA-seq results than the originally proposed Taqman system.

Reportable outcomes:

- Development of a tissue repository of FFPE specimens of early-stage, high-grade ovarian cancers. (Summarized as Table 1)
- Manuscript for Clinical Cancer Research: Comparing platforms for messenger RNA expression profiling of archival formalin-fixed, paraffin embedded tissues (Tyekucheva et al, Submitted, Michael Birrer as corresponding author) (Attachment #2).

Conclusions:

We have established an active and collaborative consortium through which we have archived the number of specimens required to identify a genomic signature predictive of occurrence of early stage ovarian cancers. We have also validated the possibility to extract RNA and DNA from these samples and perform the genomic analyses described in this grant as well as in a follow-up grant recently funded by DoD.

Appendices:

- Attachment #1: IRB approval for the study
- Attachment #2: A manuscript for Clinical Cancer Research for the test of two systems to be used for the proposed study namely the NuGene WT-Ovarian FFPE System 2.0 V2 and NanoString nCounter platform.

Supporting Data:**Table 1:** Development of a tissue repository of FFPE specimens of early-stage, high-grade ovarian cancers.

TRIAL	RECURRENT	NON-RECURRENT	TOTAL
GOG-175	20	44	64
GOG-157	5	15	20
GOG-252	10	54	64
GOG-136	3	26	29
GEICO	30	80	110
NSGO	130	95	225
MGH	30	50	80
TOTAL	228	364	592

Continuing Review: Notification of IRB Approval/Activation Protocol #: 2012P001330/MGH

Date: September 5, 2013

To: Michael J Birrer, MD, Ph.D
MGH
Medical Services

From: Partners Human Research Committee
116 Huntington Avenue, Suite 1002
Boston, MA 02116

Title of Protocol:	Identification of a genomic signature predicting for recurrence in early stage ovarian cancer
Version Date:	6/30/2012
IRB Continuing Review #:	1
IRB Review Type:	Expedited
Risk:	Minimal Risk
Expedited Category/ies:	(5)
IRB Approval Date:	7/12/2013
Approval Activation Date:	9/5/2013
IRB Expiration Date:	7/12/2014

This project has been reviewed by MGH IRB . During the review of this project, the IRB specifically considered (i) the risks and anticipated benefits, if any, to subjects; (ii) the selection of subjects; (iii) the procedures for obtaining and documenting informed consent; (iv) the safety of subjects; and (v) the privacy of subjects and confidentiality of the data.

Please note that if an IRB member had a conflict of interest with regard to the review of this project, consistent with IRB policies and procedures, the member was required to leave the room during the discussion and vote on this project except to provide information requested by the IRB.

Approved for Continued Secondary Use of Samples/Data (Health/Medical Information).

As Principal Investigator, you are responsible for ensuring that this project is conducted in compliance with all applicable federal, state and local laws and regulations, institutional policies, and requirements of the IRB, which include, but are not limited to, the following:

1. Submission of any and all proposed changes to this project (e.g., protocol, recruitment materials, consent form, status of the study, etc.) to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to

eliminate apparent immediate hazards to subjects must be reported to the IRB as an unanticipated problem.

2. Submission of continuing review submissions for re-approval of the project prior to expiration of IRB approval and a final continuing review submission when the project has been completed.
3. Submission of any and all unanticipated problems, including adverse event(s) in accordance with the IRB's policy on reporting unanticipated problems including adverse events.
4. Obtaining informed consent from subjects or their legally authorized representative prior to initiation of research procedures when and as required by the IRB and, when applicable, documenting informed consent using the current IRB approved consent form(s).
5. Informing all investigators and study staff listed on the project of changes and unanticipated problems, including adverse events, involving risks to subjects or others.
6. When investigator financial disclosure forms are required, submitting updated financial disclosure forms for yourself and for informing all site responsible investigators, co-investigators and any other members of the study staff identified by you as being responsible for the design, conduct, or reporting of this research study of their obligation to submit updated Investigator Financial Disclosure Forms for this protocol to the IRB if (a) they have acquired new financial interests related to the study and/or (b) any of their previously reported financial interests related to the study have changed.

The IRB has the authority to terminate projects that are not in compliance with these requirements.

Questions related to this project may be directed to Krishna Maldonado-Soto, KMALDONADO@PARTNERS.ORG, 617-424-4206.

CC: Wei Wei, MGH - Surgery - Surgical Oncology, Research Assistant



Partners Human Research Committee
Partners Human Research Office
116 Huntington Avenue, Suite 1002
Boston, MA 02116
Tel: (617) 424-4100
Fax: (617) 424-4199

Application: Notification of IRB Approval/Activation

Protocol #: 2012-P-001330/1; MGH

Date: 08/17/2012

To: Michael Birrer, MD, Ph.D
Medical Services

From: Krishna D. Maldonado-Soto
PHS
116 huntington Ave

Title of Protocol: Identification of a genomic signature predicting for recurrence in early stage ovarian cancer
Version Date: 06/30/2012
Sponsor/Funding Support: Department of Defense-Congressionally Directed Medical Research Programs
IRB Review Type: Expedited
Expedited Category/ies:
IRB Approval Date: 08/08/2012
Approval Effective Date: 08/17/2012
IRB Expiration Date: 08/08/2013

This Project has been reviewed and approved by the MGH IRB. During the review of this Project, the IRB specifically considered (i) the risks and anticipated benefits, if any, to subjects; (ii) the selection of subjects; (iii) the procedures for securing and documenting informed consent; (iv) the safety of subjects; and (v) the privacy of subjects and confidentiality of the data.

NOTES: The following protocol has been reviewed and approved by the IRB: Secondary use of Research Samples/Data.

As Principal Investigator you are responsible for the following:

1. Submission in writing of any and all changes to this project (e.g., protocol, recruitment materials, consent form, study completion, etc.) to the IRB for review and approval prior to initiation of the change(s), except where necessary to eliminate apparent immediate hazards to the subject(s). Changes made to eliminate apparent immediate hazards to subjects must be reported to the IRB.
2. Submission in writing of any and all adverse event(s) that occur during the course of this project in accordance with the IRB's policy on adverse event reporting.
3. Submission in writing of any and all unanticipated problems involving risks to subjects or others.
4. Use of only IRB approved copies of the consent form(s), questionnaire(s), letter(s), advertisement(s), etc. in your research. Do not use expired consent forms.
5. Informing all physicians listed on the project of changes, adverse events, and unanticipated problems.



Partners Human Research Committee
Partners Human Research Office
116 Huntington Avenue, Suite 1002
Boston, MA 02116
Tel: (617) 424-4100
Fax: (617) 424-4199

The IRB can and will terminate projects that are not in compliance with these requirements. Direct questions, correspondence and forms (e.g., continuing reviews, amendments, adverse events, safety reports) to Krishna D. Maldonado-Soto.

cc: Wei Wei, Surgery

Title: **Identification of a genomic signature predicting for recurrence in early stage ovarian cancer**

Sponsor Name: **U.S. Army Medical Research Acquisition Activity**

PI Name: **Birrer, Michael**

Protocol #: **2012P001330**

Type: **New Protocol**

Date Received: **June 30, 2012**

Study Staff

Name	Role	Degree	Organization	Citi Certified
Birrer, Michael	Principal Investigator	MD, Ph.D	MGH > Medical Services	6/6/2012
Wei, Wei	Research Assistant		MGH > Surgery > Surgical Oncology	8/5/2012

Signatures

PI Name: Birrer, Michael, J, MD, Ph.D

Authenticated: June 30, 2012

Sponsor Funding: Department of Defense-Congressionally Directed Medical Research Programs

Select the source of funding that will be used to support the proposed research:

- ☒ Government / Foundation / Other Non-Profit
- ☐ Corporate
- ☐ Institutional Award
- ☐ Department Funds
- ☐ None

Indicate application type:

- ☒ Grant / Contract (direct award to an Institution)
- ☐ Subcontract (from another Institution)

Indicate the applicant institution:

- ☐ BWH
- ☒ MGH
- ☐ SRH
- ☐ McLean

- ☐ Faulkner
- ☐ Broad Institute
- ☐ Other

Enter Principal Investigator name (if different):

Birrer, Michael J

Enter title of proposal (if different):

Identification of a genomic signature predicting for recurrence in early stage ovarian cancer

Enter grant number (if known):

Example of NIH grant number:

InfoEd proposal number (read-only field):

2011D002412

Has the project been awarded at the time of this submission?

- ☐ Yes ☒ No

Explain:

A copy of IRB Approval for human subject is needed for funding.

NOTE: For HHS-funded research: The IRB requires investigators to provide a copy of the entire grant application when there is a direct award to a Partners' Institution. Salary information (not % effort) may be redacted. Exception: Copies of HHS cooperative group umbrella grants do not need to be submitted.

For NIH-sponsored cooperative group multi-center trials: The IRB requires a copy of the cooperative group protocol and sample informed consent documents be submitted for review and comparison with the documents submitted for local IRB review.

For guidance, refer to, "IRB Review of Applications for HHS Support."

Medicare Coverage Analysis Requirement

Does the protocol for this study involve any items or services that will be billed to Medicare/private insurance, including study-specific procedures or those considered usual and customary care ("standard of care") outside the trial context?

- ☐ Yes ☒ No

NOTE: If you are unsure how to answer this question, please contact Sarah Bednar at Partners Clinical Research Office at 617-954-9364, or for NWH investigators, please contact Lynne Friedlander at 617-243-5802 for more information.

Is this the primary source of funding?

- ☒ Yes ☐ No ☐ Not applicable

Will the funding cover all subject study-related drugs, devices, procedures, tests, and visits?

- ☐ Yes ☐ No ☒ Not applicable (no subject study-related costs)

Secondary Use of Research Samples / Data

This form may be used for studies limited to the secondary use of research samples or data. Secondary use is the use of existing research samples and/or data for a new research project.

1. Purpose and Description

Enter the purpose and description of the study:

The five-year survival of patients with high grade epithelial ovarian cancer is directly related to tumor stage. Women with early stage disease (stage I and II) have a 5-year survival rate ranging from 65 – 90% compared with 19 - 47% for advanced stage disease (stage III and IV). The standard of care for patients with high-grade early stage ovarian cancer is surgery followed by 6 cycles of adjuvant chemotherapy. However, it is commonly accepted that patients with early-stage ovarian cancer are over treated and many are exposed to the short and long term toxicities of chemotherapy with minimal benefit. Thus, the identification of accurate prognostic markers to stratify patients with early-stage disease into those who will benefit from chemotherapy and spare patients from unnecessary treatment has become an

important translational science effort with cost-effectiveness ramifications. In addition, the specific genomic abnormalities which distinguish recurrent from non-recurrent early stage ovarian cancer may provide a better understanding of their biology and identify potential novel therapeutic targets.

In this study, we propose to utilize Illumina RNAseq technology to accurately characterize the expression profiles of FFPE specimens from recurrent and non-recurrent early stage ovarian cancers. However, for this project to be successful it will require large numbers of fully clinically annotated specimens making it necessary to form an international consortium to link multiple biorepositories to provide sufficient numbers of specimens. This proposal will leverage the resources of the Gynecologic Oncology Group (GOG), International Collaborative Ovarian Neoplasm (ICON), Nordic Society Gynecologic Oncology (NSGO), and Grupo Español de Investigación de Cáncer de Ovario, Spanish Ovarian Cancer Group (GEICO) to provide the needed specimens. Since these specimens were derived from clinical trials they have extensive clinical data. Further, it is important to note that none of these existing resources are sufficient to address this important question individually but combined will provide adequate numbers of specimens.

This project reflects an approach that will provide the needed data to develop clinically relevant biomarkers in the early stage ovarian cancer space that can predict for tumor recurrence, prognosticate patient survival, and perhaps identify early detection markers.

This study is based on international cooperation with four independent tissue repositories: GOG, ICON, NSGO and GEICO. Without this international collaborative effort, along with the genomic technologies proposed here, the clinical need will never be met. The PI of this IRB application will coordinate the multi-site research at MGH.

Will data resulting from this research ever be submitted to the FDA?

☐ Yes ☒ No

2. Samples / Data To Be Used

Briefly describe the samples /data to be used:

While collections of early stage ovarian cancers specimens are rare, we have been able to identify existing collections of FFPE specimens many of which were obtained from prospective clinical trials. As such, these tissue specimens are fully clinically annotated with substantial follow-up. There are two types of collections: 1) clinical trial specimens (GOG 136, 157, 175 & 252, ICON 1& 7 and institutional collections (GEICO and NSGO). In total 1268 formalin fixed paraffin embedded (FFPE) tumor specimens (including 455 recurrent cases) will be used in this study. All specimens are high grade, early stage (Stage I, II) serous or endometrioid cancers with at least 5 years of clinical follow-up.

RNA will be extracted from the obtained FFPE samples for expression profiling studies.

3. Fetal Tissue

Will this study involve the secondary use of fetal tissue?

- ☐ Yes ☒ No
-

4. Source of Samples / Data and Related Information

Indicate where will you obtain the samples / data (check all that apply):

- ☐ Collaborators within Partners
☒ Collaborators at outside Institutions

Specify:

Gynecologic Oncology Group (GOG),
International Collaborative Ovarian Neoplasm (ICON),
Nordic Society Gynecologic Oncology (NSGO),
Grupo Español de Investigación de Cáncer de Ovario, Spanish Ovarian Cancer
Group (GEICO)

- ☐ Other

Do you plan to re-contact subjects?

- ☐ Yes ☒ No

Will the research be limited to the use of existing samples / data?

- ☒ Yes ☐ No

Do the samples / data retain a code linking sample / data to individual human subjects?

- ☒ Yes ☐ No

Will the key to the code or identity of the subjects ever be known to you?

- ☐ Yes ☒ No
-

5. Data To Be Used

Data to be used. Check all that apply.

Administrative

- ☐ Billing data
- ☒ Coded encounter data (diagnoses, procedures, dates)
- ☒ Demographic data (age, gender, vital status)
- ☐ Personal data (name, address, PCP)

Health / Medical

- ☐ Allergies
- ☒ Discharge summary
- ☐ Doctors orders
- ☒ History / Physical
- ☐ Immunizations
- ☒ Medication List
- ☒ Office / Clinic Notes
- ☒ Operative / Procedure Notes (e.g. endoscopy)
- ☐ Pharmacy
- ☐ Problem List

Health / Medical Reports / Results

- ☒ Blood bank
- ☒ Laboratory
- ☒ Pathology
- ☒ Radiology

Sensitive / Personal Information

- ☐ HIV status
- ☐ Mental Health
- ☐ Reproductive health (e.g. abortions)
- ☐ Sexual behavior / sexually transmitted diseases
- ☐ Substance abuse (drug or alcohol abuse)
- ☐ Other potentially stigmatizing behaviors
- ☐ Other Information

6. Protected (Identifiable) Health Information

PHI refers to health/medical information that is accompanied by any of the listed 18 HIPAA identifiers or by a code (where the key to the code is accessible to investigators) that links to the identifiers.

DE-IDENTIFIED DATA (without any identifiers or codes that link back to individuals) are not considered PHI, and are not subject to HIPAA regulations.

Will you be recording any of the identifiers listed above with the samples / data or using a code to link the samples / data to any of the identifiers?

☐ Yes ☒ No

7. Informed Consent / Authorization / Waivers

Were the samples / data collected as part of an IRB approved protocol with informed consent of subjects?

☐ Yes ☒ No

Explain why the risk to subjects, specifically the risk to privacy, is no more than minimal risk.

Research involves the collection and use of existing data, records, and pathological specimens. The information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

Explain why the research could not practicably be carried out without the waiver of consent / authorization.

Many of the patients are deceased or impossible to contact.

“Only in a few research studies would it be impossible to obtain informed consent; however in many studies the financial cost would be prohibitive and a potentially poor use of limited research resources.” Ensuring Voluntary Informed Consent and Protecting Privacy and Confidentiality, National Bioethics Advisory Commission.

Explain why the rights and welfare of the subjects will not be adversely affected by the waiver of consent / authorization.

Considering the individual's right to privacy, the identity of the individual subject will not be known to the investigators of this study.

Many of the subjects are deceased.

The study is to determine the single institution experience with ovarian cancer. The data will have no adverse effects on the study objects.

8. Sending Data Outside Partners

Will any identifiable samples / data be sent to collaborators outside of Partners?

☐ Yes ☒ No

Materials Transfer Agreements (Transferring Material To Research Collaborators)

1. Transfer of samples/data to academic or non-profit entities or collaborators, refer to CSRL Materials Transfer Checklist. For Template Agreements between academic or non-profit entities or collaborators, see below: [Template Agreement for Coded Samples](#) [Template Agreement for Non-Identifiable Samples](#)
2. Transfer of samples/data to for-profit or commercial entities or collaborators, contact Partners Research Ventures & Licensing.

HIPAA and Tracking Disclosures of Identifiable Health Information (PHI)

1. Disclosures of PHI to persons or entities outside Partners without the written authorization of the subject must be tracked in accordance with Partners policy: ["Accounting of Disclosures"](#) (PHS internal link).
2. Tracking is NOT required for disclosure of LIMITED DATA SETS under a DATA USE AGREEMENT. For more information about LIMITED DATA SETS and DATA USE AGREEMENTS, refer to Partners policy ["Limited Data Sets Policy/Data Use Agreements."](#)

NOTE: Partners (PHS) is the HIPAA covered entity. PHS includes BWH, Faulkner, MGH, MCLEAN, PCHI, SRH, NSMC, and NWH, among others. PHS does not include other HARVARD affiliated hospitals, such as BIDMC, DFCI, HSPH, CHB, or MEEI.

Attachments

Name	Mode
GOG218 Letter.of.Support.Birrer.05.04.12(Consent Form)	Electronic
Consent Form ICON(Consent Form)	Electronic
Consent Form_03(Consent Form)	Electronic
Consent Form_NGSO(Consent Form)	Electronic
Response to Review_01(Response to Review)	Electronic

Philip J. DiSaia, M.D.
Group Chair

Administrative Office
Four Penn Center
1600 John F. Kennedy Boulevard, Suite 1020
Philadelphia, Pennsylvania 19103
Phone: 215-854-0770 Fax: 215-854-0716

Laura L. Reese
Executive Director of Operations



Larry J. Copeland, M.D.
Group Vice Chair

Finance/Development Office
2127 Espey Court
Suite 100
Crofton, Maryland 21114
Phone: 410-721-7126 Fax: 301-261-3972

Mary C. Sharp
Chief Financial Officer

May 4, 2012

Michael J. Birrer, M.D., Ph.D.
Professor of Medicine
Harvard Medical School
Director, Gynecologic Medical Oncology
Massachusetts General Hospital Cancer Center
Yawkey 9072
55 Fruit Street
Boston, MA 02114

RE: Letter of Support R01 Grant Application

Dear Dr Birrer:

It is with great pleasure that I write this letter of support for your R01 grant application entitled, "The FGF18/FGFR4 amplicon: novel therapeutic biomarkers for ovarian cancer." This grant focuses on the validation of a genomically derived prognostic biomarker in ovarian cancer. It also characterizes its biologic functions and provides data as to why it is prognostic. As such, this type of study is exactly the type of translational research that the Gynecologic Oncology Group (GOG) should be doing, and wishes to support. The use of GOG clinical trial specimens for validation of important biomarkers is the best use of these precious materials.

As the Group Chair of the GOG, I can assure you that the GOG-0218 specimens will be available for your project, and you will have access to all of the appropriate specimens associated with the trial. I will ensure that the process to obtain the specimens will be simple and efficient.

On behalf of the GOG, I enthusiastically support your application. I wish you the best of luck with the application, and look forward to working with you on this exciting project

Sincerely,

Philip J. DiSaia, M.D.
Group Chair
Gynecologic Oncology Group

cc: Administrative Files

ICON7 Supplementary Patient Consent Form - Biological Samples

Centre Name & Number:

Patient ID Number:

Date and Version: 21/09/2006 v1

Affix patient sticker here

ICON7 - A randomised, two-arm, multicentre Gynaecologic Cancer InterGroup trial of adding bevacizumab to standard chemotherapy (carboplatin and paclitaxel) in patients with epithelial ovarian cancer

Short Title: Bevacizumab in Ovarian Cancer

ISRCTN: 91273375

If you do not wish to participate in the scientific study then do not sign, you can still participate in the clinical part of the trial.

Please initial boxes to agree

1. I have read and understood the patient information sheet for the above study and have had the opportunity to ask questions and discuss it with my doctor. ☐
2. I agree to a *sample of my cancer*, removed during surgery, being used for research in the above project. I understand that I am free to withdraw my approval for use of the sample at any time without giving a reason and without my medical care or legal rights being affected. ☐
3. I agree to give *sample(s) of blood* for research in the above study. I understand how the sample(s) will be collected, that giving sample(s) is voluntary and that I am free to withdraw my approval for use of the sample(s) at any time without giving a reason and without my medical care or legal rights being affected.
 - **Level 1** (no extra blood tests are required)
 - **Level 2** (This will require one extra blood test of 10ml [2 teaspoons] for study purposes taken before treatment starts) ☐
 - **Level 3** (This will require 4 extra blood tests, and 130mls [just under 9 tablespoons] of blood in total) ☐
 - **Level 4** (This will require 10 extra blood tests and 310mls [just under 21 tablespoons] of blood in total) ☐

[Page 1/2]

4. I agree that the samples and information collected about me will be stored on behalf of the Medical Research Council (MRC) for use in future projects. I understand that some of these projects may be carried out by researchers other than the MRC, including researchers working for commercial (including pharmaceutical) companies. ☐
5. I understand that future research using the samples I give may include genetic research aimed at understanding the genetic influences on ovarian or primary peritoneal cancer, but the results of these investigations are unlikely to have any implications for me personally. ☐
6. I understand that I shall not benefit financially if future research leads to the development of new treatments or medical tests. ☐

_____ Name of Patient	_____ Date	_____ Signature
_____ Name of Person taking consent (Principal Investigator or authorised delegate)	_____ Date	_____ Signature

(3 Copies: 1 for patient, 1 for researcher and 1 to be kept with hospital notes)

ICON7 Translational/Laboratory Research - An Explanation

Alongside the clinical part of the study, ICON7 will also run a laboratory based study. This will involve extracting DNA (genetic material) or other material (such as proteins) from the tumour and/or blood samples from patients in the ICON7 trial, to see if we can discover markers in the blood or cancer tissue which will help us to identify and then predict which patients in the future will benefit most from the addition of bevacizumab to their chemotherapy. We are also going to investigate whether we can identify markers in the blood early on which show that the disease is progressing, before we see progression on the scans. This would allow ineffective therapy to be stopped and alternatives prescribed.

Blood and tumour samples from patients participating in clinical trials, such as ICON7, are extremely valuable as accurate clinical information is collected on the same patients. This information, including whether the cancer responds to treatment or whether the disease comes back in the future, can be used together with the information discovered in the laboratory to help answer questions about ovarian cancer and its treatment.

All of the samples will be de-identified. This means the samples will be identified by a code number, not your name, and neither you nor your relatives will be identified or contacted.

These additional studies will not affect your treatment in any way. If you decide not to take part in the collection of samples for laboratory research, this will not affect your participation in the clinical part of ICON7, or your relationship with your doctor.

Any unused parts of the samples collected will be stored for as long as possible, in case further investigations are developed in the future, which may help us understand more about the response of ovarian cancer to these therapies. The samples may also be used for other studies in the future relating to ovarian cancer. Any such studies will have to be approved by the ICON7 Translational Research subgroup and external ethics committees. We cannot describe here all the potential tests which may be done on any samples stored and if you feel uncomfortable about this please do not participate.

Different hospitals will participate in this research to different extents (Levels 1 to 4). The tests required at different levels are summarised below. Please discuss with your doctor which Level your hospital is participating in.

This research will be based in laboratories associated with clinical trial sites, but may involve collaboration with commercial companies (including pharmaceutical/drug companies) or other institutions. You will not benefit financially from any of this research.

These extra blood tests will often, but not always, be able to be taken at the same time as your other blood tests are being taken.

All patients will be asked if extra tests can be done on a sample of their cancer. Specimens from your cancer will have been routinely saved in the hospital pathology laboratory. In asking you to take part in this study we will also request your permission to use this stored material in future scientific studies.

Level 1

No extra blood tests will be required.

Level 2

This will require one extra blood test of 10ml (2 teaspoons) for study purposes taken before treatment starts.

Level 3

This will include 4 extra blood tests in total taken at different times throughout your treatment and follow up. 40mls (just under 3 tablespoons) of blood will be taken for the first test, and 30mls (2 tablespoons) for each of the others. This is 130mls (just under 9 tablespoons) in total. The blood tests will be taken before cycle 1, before cycle 2, before cycle 6 and if the disease comes back, or at 5 years after starting treatment.

Level 4

This will include 10 extra blood tests taken at different times throughout your treatment and follow up. 40mls (just under 3 tablespoons) of blood will be taken for the first test and 30mls (2 tablespoons) for the others. This is 310mls (just under 21 tablespoons) of blood in total. Two blood samples will be taken before treatment starts and then after the 1st cycle of treatment. Blood tests are then required before the 2nd cycle of treatment, before and after the 6th cycle of treatment, at 3, 6 and 9 months after the chemotherapy is finished (first 6 cycles), and if the disease comes back, or at 5 years after starting treatment.

Thank you for your help.

Informed consent for the voluntary donation of biological samples for research

Title: *An integrated genomic and epigenomic signature to predict for recurrence in early stage ovarian cancer.*

IP: Andres Poveda, MD

Location: Fundación Instituto Valenciano de Oncología.

1. Identification and description of procedure

The standard study of malignant tumors is carried out in the department of Pathology anatomy. However, we are aware that the individual molecular event of each ovarian cancer does not always coincide with tumor type. For this reason, one of our main aims in investigation is to find new biomarkers to help us better classify the disease.

Along these lines, *omic* is a methodology that offers high capacity for biomarker identification from different tissues.

In this context, what is requested from you is to have access to the tumor paraffine sample obtained from the testing you underwent for the ovarian cancer that you had.

The donated samples will be stored in the biobank of the IVO (Fundación Instituto Valenciano de Oncología) who will be in charge of making use of them according to the different needs of the project.

2. AIM OF THE STUDY

The aim of the study is to validate molecular reclassification and identify the most characteristic molecular events. This could allow for a more accurate prognostic evaluation of your illness and to establish relevant target therapies in hopes to develop future more precise clinical treatments and actions.

3. DONATION CONDITIONS

You will not receive any economic or other type of compensation for the donated samples. However, if the possible research conducted proves successful, this could help other patients with the same disease or similar disorders in the future.

The samples that you donate will not be sold or distributed to third parties for commercial purposes, but the conservation and shipment costs will be covered on a non-profit basis.

The donation of samples will not restrict your (or your family) access to them, as long as they are available at that given moment if you so wish.

4. PREDICTABLE CONSEQUENCES

You may be contacted you in order to get additional information about your situation or even to request a new sample from you if it proved beneficial for the development of this biomedical research. In this case, you would be informed and you have the option, if you so choose, to continue to participate or withdraw. For this reason, it is important that you inform the Admissions office of any changes regarding your contact information such as address and telephone number as it is the only way we can get in touch with you.

The studies conducted with your samples may provide relevant information for your health, especially relevant genetic information. You have the right to be informed of your genetic data and other personal details attained in the research. Please get in touch with your contact person for this project to provide you with all the information.

5. RIGHT TO REVOKE CONSENT

The decision to donate your samples is completely voluntary, and you are also entitled to refuse to donate

without providing any explanation whatsoever, and this shall have no effect on the medical assistance you receive at the center.

If you revoke this consent, you will be able to decide whether your excess samples not be used for research should be destroyed or rendered anonymous. This decision will not affect data obtained from research carried out prior to your revoked consent.

6. RISKS

The requested sample comes from a surplus of diagnostic processes which have already finished. The samples are stored in the hospital where you were treated; therefore obtaining it, in no way implies a risk to you.

7. DATA PROTECTION AND CONFIDENTIALITY

All your personal and health data collected from your clinical history or equivalent will be included and processed in a data base in accordance with the guarantees established by Spanish health law and the Protection of Personal Data (LOPD 15/1999).

The sharing of samples with other investigation centers, public or private, as well as the information included in the data base linked to you and your state of health will be carried out using disassociated data. This means, that your personal information will be replaced by a code.

During a research project, there may be a surplus of genetic information from the samples. If the results obtained are relevant, the disassociated information may be used and shared for other scientific purposes and published in scientific journals but with restricted use by researchers. However, they will never reveal your identity or information which would identify you.

8. CONSENT DECLARATION

I, Mr/Ms.....confirm that I am years old, and live at
....., DNI..... and SIP nº.....
I, Mr/Ms..... confirm that I am years old, and live at
....., DNI nº.....acting as the representative (in the
event of underage or disabled patients) of the patient....., with
DNI nº..... and SIP nº.....

HEREBY DECLARE

I have read all the information given to me

I have been informed by (Name of the doctor requesting the study) about the study in question entitled **An integrated genomic and epigenomic signature to predict for recurrence in early stage ovarian cancer**

I have understood the explanations given to me in a clear and simple language

I have been able to ask questions about the studies in question

I understand that my participation is voluntary and that I can chose to withdraw from the study:

1. when I want
2. without giving any justification
3. without it affecting my medical care

I give my express consent freely and voluntarily for my participation in the s study **An integrated genomic and epigenomic signature to predict for recurrence in early stage ovarian cancer**

CONSENT

That the hospital or other public or private research centers may use my data and donated samples according to the conditions expressed in the information form.

Signed: Mr/Ms.....DNI nº.....

Signed by the health professional:

Dr/.....DNI nº.....

In on, 20....

9. WITHDRAWAL OF CONSENT

**I, Mr./Ms.....withdraw my consent given on
..... 20..... and do not wish to continue the voluntary donation, which I
terminate on this date**

Inon....., 20.....

Signed: Mr/Ms.....DNI nº.....

Signed by the health professional:

Dr/.....DNI nº.....

You have previously been treated for early ovarian cancer at the Radium Hospital

After such operations, the tumor tissue is evaluated at the department of pathology. Some tissue is left after the evaluation and is stored in the hospitals bio bank.

We hereby ask you, whether you can accept that some of this left over tissue can be used in a new study.

Name of the study:

An integrated genomic and epigenomic signature to predict for recurrence in early stage ovarian cancer. A lab study.

The scope of the study

The intention by the study is to try to find markers that can predict the risk for relapse after treatment for early ovarian cancer.

Patients with early ovarian cancer are treated with surgery and many will afterwards receive chemotherapy. However, only about 20% will suffer a relapse, so most patients receiving chemotherapy does not need it. At the Radium Hospital we use a method with determination of the amount of DNA to identify patients with a greater risk of relapse. We find this method useful, but it is not optimal and is not internationally accepted.

We now wish to participate in an international study using modern laboratory methods to evaluate the DNA of the tumor cells. We want to evaluate abnormalities and state of function of genes in the tumor cells.

What happens with the samples and information about you?

For this study we are using just a small piece of the tumor tissue left in our bio bank. We do not ask for new samples to be taken. The sample will be transferred to a bio bank at Harvard University, Boston, USA under the responsibility of Professor Michael Birrer. The sample will only be used for the study mentioned here.

All information and samples will be handled without name or date of birthday or other directly identifiable information. A code will link your data and sample to a list with names. This code list will be kept at the Radium Hospital and only staff personnel responsible for this study will have access.

All analyses will be performed at Harvard University, Boston, USA. This university works under US legislation which does not comply with European Human Rights Protection legislation.

It will not be possible to identify you in the results of the study when this is published. The results of the study will not have any importance for you and you will not receive information about the result of analysis on your tumor.

The aim of this study is to identify methods to predict special risk of recurrence. You will not benefit in any way in case the study leads to development of new medical tests.

Freedom to participate

You are free to decide whether you want to participate in this lab research. In case you later regret, you can ask your data and samples to be destroyed unless they already are part of a publication.

In case of questions, please contact:

Dr. Gunnar Kristensen Phone: 22 93 40 00

You receive 2 copies of this letter.

In case you accept that your de-identified data and a sample of the tumor tissue can be used in this study, please sign and date the consent sheet and send one copy to us. You keep the other as your copy.

Sincerely yours

Gunnar Kristensen
Consultant PhD
Dept Gynecologic Oncology
The Norwegian Radium Hospital
Oslo

Consent form

An integrated genomic and epigenomic signature to predict for recurrence in early stage ovarian cancer. A lab study.

1. I accept that a sample from my tumor, removed during surgery, is used for research in the above mentioned study. I know that I at any time can withdraw my consent without giving any reason.
2. I understand that I will not have any economic gain in case this research leads to development of new medical tests.

I maintain a copy of the information and consent form

Date _____ ; _____
(Patient's signature)

**PARTNERS HUMAN RESEARCH COMMITTEE
RESPONSE TO REVIEW FORM**

Use this form to respond to questions/concerns raised by the IRB at the time of review.

Principal Investigator: Dr. Michael J. Birrer	Protocol#: 2012-P-001330/1
Study Title: Identification of a genomic signature predicting for recurrence in early stage ovarian cancer	
Response to: ☒ Initial Review ☐ Continuing Review ☐ Amendment ☐ Other	
IRB Review Date: 07/10/2012	

INSTRUCTIONS: Please respond to IRB questions by restating the question and then providing your answer in a different font. This speeds review and makes your file very clear in the event of external review. This format is not required for addressing comments on consent form changes. For consent form changes, make the proposed changes to the consent form and provide a second copy with changes underlined. When complete, this document should be attached in the Attachments section of the pending application in Insight eIRB and resubmitted. The investigator does not need to sign this form. [Learn more...](#)

* Upon preliminary review, the following additional information is needed:

Question #1: Please submit either the model consent forms used at ICON, NGSO, and GEICO, or submit a letter of support from the Steering Committees of those groups approving your use of their samples (similar to the letter from GOG).

Answer: Consent forms from ICON, NGSO and GEICO will be included as an attachment.

Question #2: Do you plan to return results to the 4 tissue repositories?

Answer: Yes. We will return the results to the GOG, ICON, NGSO and GEICO repositories.

Comparing platforms for messenger RNA expression profiling of archival formalin-fixed, paraffin embedded tissues

Svitlana Tyekucheva^{1,2,*}, Neil E Martin^{3,*}, Edward C Stack^{4,5}, Wei Wei⁶, Vinod Vathipadiekal⁶, Levi Waldron⁷, Michelangelo Fiorentino⁸, Rosina T Lis^{4,5}, Meir J Stampfer^{9,10,11}, Massimo Loda^{4,5,12,13,14}, Giovanni Parmigiani^{1,2}, Lorelei A Mucci^{9,10,†}, Michael Birrer^{6,†}.

* These authors contributed equally

† These authors share senior authorship

¹ Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA.

² Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA.

³ Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

⁴ Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA

⁵ Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, Boston, MA, USA

⁶ Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA.

⁷ City University of New York School of Public Health, Hunter College, New York, NY, USA

⁸ Pathology Unit, Addarii Institute, S. Orsola-Malpighi Hospital, Bologna, Italy

⁹ Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

¹⁰ Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA

¹¹ Department of Nutrition, Harvard School of Public Health, Boston, MA, USA

¹² Department of Medical Oncology, Dana Farber Cancer Institute, Boston, MA, USA

¹³ The Broad Institute, Cambridge, MA, USA

¹⁴ Division of Cancer Studies, King's College London, London, UK

Corresponding Author:

Svitlana Tyekucheva

Department of Biostatistics and Computational Biology

Dana-Farber Cancer Institute

450 Brookline Avenue

Boston MA 02115, USA

Phone: (617) 632-3638

Fax: (617) 632-2444

Email: svitlana@jimmy.harvard.edu

Runnning Title: RNA profiling of formalin-fixed paraffin embedded tissues

Word count: XXXX

Number of tables: 1

Number of figures: 3

Number of supplemental tables: 2

Number of supplemental figures: 4

Abstract

Purpose: Archival formalin-fixed, paraffin-embedded (FFPE) tissue specimens represent a readily available but largely untapped resource for gene expression profiling based biomarker discovery. Several technologies have been proposed to cope with the bias from RNA crosslinking and degradation associated with such archival specimens to generate data comparable to RNA from matched fresh frozen materials. Direct comparison studies of these RNA expression platforms remain rare.

Experimental Design: We compared two commercially available platforms for RNA expression profiling of archival FFPE specimens from clinical studies of prostate and ovarian cancer. The first platform was the Affymetrix Human Gene 1.0ST Array following whole-transcriptome amplification using NuGEN WT-Ovation FFPE System V2. The second platform was the Nanostring nCounter without amplification.

Results: We observed that both platforms produced gene expression profiles with high sensitivity and reproducibility through technical repeats from FFPE materials of prostate and ovarian cancers. The detection sensitivity and reproducibility showed no significant association with FFPE block age within cohorts. A strong concordance was shown for the transcript expression values for genes detected by both platforms. We further demonstrated the biological validity of the gene signatures generated by both platforms for both cohorts.

Conclusions: Our study supports the feasibility of gene expression profiling and large-scale signature validation on archival FFPE prostate and ovarian tumor specimens using commercial platforms. These approaches have the potential to drive precision medicine by biomarker discovery and validation.

Translational relevance

One challenge limiting development of clinically useful biomarkers lies in the paucity of well-annotated frozen specimens on which current high-throughput technologies largely relies. Archival formalin fixed paraffin embedded (FFPE) materials represent the only form of clinical specimens in large cohorts with extensive annotation and long-term clinical follow-up. Unfortunately to date no genomic platform has been identified which can accurately interrogate these specimens due to the extensive fixation- and storage-related RNA degradation. In this study, we compared the performance of two platforms with distinct technologies on FFPE samples. We assessed a high-throughput approach ideal for discovery studies and a more economical approach ideal for simultaneous validation of a developed signature consisting of multiple biomarkers. Both platforms generated robust, reproducible data with high-level of intra- and inter-platform concordance, regardless of FFPE block age and RNA integrity. Through this study, we have demonstrated the feasibility of using FFPE materials for biomarker discovery and validation for further integration into clinical practice.

Introduction

With the advent of precision medicine, there is a growing interest in developing prognostic and predictive signatures for the care of patients with cancer (1). Among approaches taken to realize the goal of identifying the ideal treatment for the patient at the correct time are mutation analysis (2), immunohistochemical staining (3), and mRNA expression analysis (4-6).

To allow for morphologic assessment by a pathologist, clinical samples including biopsies and surgical specimens are typically formalin fixed, and embedded in paraffin. While this process preserves morphological features of the tumors, it also makes RNA expression profiling more challenging relative to fresh frozen tissue. RNA in formalin fixed, paraffin embedded (FFPE) samples is subject to degradation, fragmentation and cross-linking, which typically limit library preparation and gene expression assessment (7). Importantly, traditional quality control measurements for RNA, such as RNA integrity number (RIN) are not necessarily predictive of the success of corresponding gene expression assays (8).

The large numbers of well-annotated FFPE tumor tissue samples currently archived remain a vast and under-utilized resource in the genomic study of cancer. Notably, most large clinical and epidemiological cohorts only collect FFPE samples. Given this wealth of archival material from patients with known outcomes and the continued FFPE processing of new clinical specimens, there is a need to develop reliable methods for profiling mRNA expression in FFPE materials.

Several platforms have been developed in recent years to assess mRNA expression from FFPE tissue including whole transcriptome amplification (9, 10) and direct assessment using multiplexed color-coded probes (11). As an initial step to developing prognostic and predictive mRNA signatures from archival tumor specimens, we performed head-to-head comparisons of gene expression profiles from prostate and ovarian cancer FFPE specimens using two

platforms: the NuGen WT-Ovation FFPE System V2 + Affymetrix GeneChip Human Gene 1.0 ST Array (NuGen+Affy) and the Nanostring nCounter Cancer panel (Nanostring).

Materials and Methods

Prostate samples.

Archival formalin fixed, paraffin embedded radical prostatectomy specimens were collected from treating institutions for men diagnosed with prostate cancer who had enrolled in the prospective Physicians' Health Study (12, 13). Pathology was reviewed centrally to provide consistent Gleason scores. Areas of high-density tumor were identified, and 0.6 mm punches were taken from both tumor and adjacent non-tumor prostatic tissue for RNA extraction. The cores were deparaffinized using 800 μ L Citrisolv (Fisher Scientific, Pittsburgh, PA) at 60°C for 20 minutes followed by 1.2 mL Citrisolv:absolute alcohol (2:1) at room temperature for 10 minutes. Cores were subsequently washed with absolute alcohol, dried at 55°C, and incubated overnight at 45°C in 300 μ L lysis buffer (10 mM NaCl, 500 mM Tris [pH 7.6], 20 mM EDTA, 1% sodium dodecyl sulfate) containing 1 mg/mL proteinase K (Ambion, Austin, TX). RNA was extracted using the RecoverAll Total Nucleic Acid Isolation kit (Ambion). Post tissue digestion, following the manufacturer's protocol, samples were incubated in 10x DNase (Ambion) and column purified to elute RNA. Concentration was determined using the Nanodrop 1000 (Fisher Scientific) and RiboGreen RNA Assay Kit (Ambion). Samples and technical replicates were randomized and assigned study identifications to blind them.

Ovarian samples.

Ovarian cancer specimens were obtained from the Gynecologic Oncology Group (GOG) tissue bank (Columbus, OH). All specimens were archival FFPE tissue samples obtained from women with advanced stage epithelial ovarian cancer on clinical trial GOG218. GOG218 was a randomized phase III trial testing the impact of the addition of bevacizumab to standard

chemotherapy for the upfront treatment of advanced stage epithelial ovarian cancer (14). The specimens were obtained at primary debulking surgery.

The pathology was confirmed by central GOG review to ensure the correct histology and percentage of tumor. 10 μ m sections were made on positively charged slides. RNA was extracted from paraffin scrolls using the RNeasy FFPE kit (Qiagen) with modification. In brief, 1 ml of xylene was first added to a 25 μ m paraffin scroll, vortexed vigorously for 10 sec and centrifuged at full speed for 2 min. Supernatant was then removed and 1 ml of ethanol (100%) was added to the pellet, mixed by vortexing and centrifuged at full speed for 2 min. Supernatant was removed and the tube was incubated at room temperature from 10 min with lid opened. Subsequently, RNA was extracted using reagents provided in the RNeasy FFPE extraction kit according to the manufacturer's instruction. Finally, purified RNA was eluted from the RNeasy MinElute spin column using 30 μ l RNase-free water.

Experimental design

For prostate cancer samples we profiled seven paired tumor and adjacent normal prostate tissue samples from three patients with Gleason score 8, one with Gleason score 7 and three with Gleason 6 disease on NuGen+Affymetrix platform, and a subset of five pairs (two Gleason 6 and three Gleason 8) on Nanostring. Eleven of the NuGen+Affymetrix specimens and nine of the Nanostring specimens were assayed with two or three technical replicates. Block ages for prostate cancer specimens ranged from eleven to twenty one years. For ovarian cancer we selected five serous carcinoma and six clear cell carcinoma samples, block ages for this cohort ranged from four to seven years. These samples were profiled on both NuGen+Affymetrix and Nanosring, with either two or three technical replicates. Details of the study design are presented in supplementary table S1.

Messenger RNA Expression Profiling.

Array: For Array-based mRNA profiling, we first performed whole transcriptome amplification using the WT-Ovation FFPE System V2 (NuGEN Inc; San Carlos, CA). This approach initiates amplification at the 3' end as well as randomly throughout the transcriptome improving the performance in severely degraded FFPE samples. Following isothermal amplification, 50 or 100 ng of total RNA was amplified to 4 – 7 µg of biotinylated cDNA complementary to the original mRNA. The amplification step has been optimized for RNA extracted from formalin-fixed, paraffin embedded specimens (15, 16) and has demonstrated comparable differential expression profiles to corresponding fresh frozen tissues (17). Following amplification, we hybridized 3.75 µg of amplified cDNA to the GeneChip Human Gene 1.0 ST Array (Affymetrix; Santa Clara, CA). The 1.0 ST Array profiles expression of >28,000 genes with an average of 26 probes per gene.

NanoString: We used the NanoString nCounter platform (18) (NanoString Technologies, Seattle, WA) to capture and count 230 cancer-related human genes using a pre-built kit supplied by the manufacturer. Following manufacturer's instructions, we aliquoted 100-200 ng total RNA in 5 µL to initiate analysis.

Data preprocessing.

We normalized the Affymetrix data across samples and batches using RMA (19, 20). For the Nasnostring platform the data were processed according to the manufacturer's recommendations. Briefly, background subtracted counts were multiplied by scaling factors proportional to the sum of counts for spiked-in positive control probes to account for individual assay efficiency variation, and to the geometric average of the housekeeping gene probes (CLTC, GAPDH, GUSB, HPRT1, PGK1 and TUBB) to account for variability in the mRNA content. Background signal was calculated as a median value of the negative hybridization control probes. Normalized counts were log-transformed for downstream analysis.

Results

To study feasibility of gene expression profiling from FFPE tissues and compare the NuGen amplified mRNA hybridized on Affymetrix GeneChip Human Gene 1.0 ST arrays and Nanostring nCounter platforms, we designed a pilot study utilizing samples from prostate and ovarian cancer cohorts. Importantly, we selected samples from large-scale epidemiologic studies and clinical trials representative of a wide variety of fixation times, block ages and block storage conditions. Prostate cancer specimens were from the Physicians' Health Study (PHS) cohort and ovarian specimens from the Gynecologic Oncology Group (GOG) clinical trials cohort. We sought to evaluate several potential variables related to RNA expression assays performance. We assessed sensitivity and reproducibility of the gene expression measurements across technical replicates of RNA extracted from the same tissue with respect to (1) RNA input levels; (2) FFPE block age; and (3) tumor grade and histology. We also used tumor grade and histology to assess the biologic validity of the expression profiles.

Sensitivity and Reproducibility Analysis

For each platform and each cohort we calculated percentages of probes detectable above the background. In each platform, background probes comprise sequences not found in human genome. We found no significant association between the proportion of detectable probes and the block age, as quantified by the Pearson correlation coefficients (Table 1). The wider range of in the percent of probes present for the NuGen+Affymetrix data on the prostate cohort is likely to be explained by differences in the instrument calibrations between the two batches in which the samples were assayed. One batch had higher intensities, which were corrected analytically by RMA normalization (see Supplementary Figure S1). As expected, we observed a lower percent of present probes in the samples with smaller RNA input quantity.

The Pearson correlation coefficients between all pairs of technical replicates within each platform ranged from 0.88 to 0.99 (Table1; Figure 1, panels a-d). The consistency of the gene expression levels, as measured by the magnitude of the correlation, between technical replicates did not change with block age, but depended on the input RNA amounts for Nanostring platform (Supplementary Figure S2). We also found lower correlations between replicates among the ovarian samples which presented more extensive tissue necrosis. Necrosis was not measured in the prostate samples. Since correlation coefficients could be driven by outlying values, we also assessed concordance for each pair of the replicates by considering the fraction of genes that are either below or above certain expression intensity for both members of the pair (Figure 1, panels e-h). Lower concordance is observed at lower, less reliably measured expression intensities, as expected. Only a small fraction (<2%) of genes with medium to high expression intensities in one replicate had low expression in the other replicate. As expected, the gene-wise correlations (Figure 1, panels i-l) between the replicate pairs tend to be lower for genes with lower average intensities, suggesting reliable gene expression quantification from FFPE tissues. Scatterplots of gene expression values for each group of technical replicates are shown in Supplementary Figure S3.

Concordance between NuGen + Affy and Nanostring

Using NetAffx annotations, we mapped all 236 genes surveyed on the Nanostring Cancer panel assay to a total of 256 transcript clusters on the Affymetrix GeneChip Human Gene 1.0 ST array. For these genes we calculated correlations between the samples assayed on both platforms. For this analysis, we averaged transcript expression values for technical replicates within platform. Most of the genes had high positive correlations, with lower correlations predominantly found for the genes with lower expression levels (Figure 2). Of the 256 correlations, 77 in prostate and 33 in ovarian were below 0.3. Among these 18 mapping pairs, corresponding to 15 unique gene symbols, were in common to both diseases: BCR, CASP10,

CEBPA, CSF3, CYP1A1, FLT3, GATA1, HRAS, LMO2, MLH1, MLL, MPL, TFE3, WEE1, and WNT10B. Scatter plots for individual genes are presented in Supplementary Figure S4.

Biological validation

To provide evidence that both platforms provide biologically useful information we considered gene signatures to distinguish prostate tumor versus normal tissue, high versus low Gleason grade, and clear cell versus serous ovarian adenocarcinoma. The prostate tumor-versus-normal-tissue signature was obtained from Oncomine, and defined by genes that were significantly up- or down- regulated in tumor versus normal comparison in four prostate cancer studies (21-24) and had a median rank of the differential expression below 150 across these four studies. An mRNA signature related to Gleason grade was taken from Penney et al (25). For ovarian cancer, the signature was retrieved as the union of three gene signatures describing differences between serous and clear cell carcinomas from GeneSigDb(26-29). Gene signatures used for validation are presented in Supplementary Table S2.

We performed Principal Components Analysis using genes from each signature that were represented on Affymetrix and Nanostring platforms (Figure 3). For each comparison we observed meaningful separation of the classes defined by the signatures on the first two principal components.

In addition, for the Gleason signature genes, we calculated log fold changes in the gene expression values between high and low Gleason grade tumors. We compared our results to the log fold changes observed in the Gleason signature. Using NetAffx annotations we mapped 150 of 157 genes from the signature to 157 Affymetrix transcript clusters. Correlation between log fold changes from two studies was 0.57. Only 10 genes from the 157-gene Gleason signature were represented on the Nanostring Cancer Panel. Correlation between log fold changes observed on Nanostring and previously published data was 0.89.

Discussion

The reliable use of archival FFPE samples for mRNA signature development will provide enormous opportunities for precision medicine in oncology. Previous work has established that new approaches for RNA expression profiling in FFPE correlate well with corresponding fresh frozen samples (10, 11, 30). In this study, we sought to determine the reliability of a two approaches for mRNA expression profiling in archival FFPE samples from two representative research cohorts. The Nanostring platform allows for assessment using low RNA input quantity and provides expression data on a pre-specified set of genes. Whole-genome expression data can be obtained using an array-based approach following amplification.

We found that both Nanostring platform and whole-genome amplification followed by array profiling produced highly concordant results across technical replicates. These findings were independent of FFPE block ages up to 21 years old. These results were also independent of the use of suboptimal RNA input amounts highlighting the clinical applicability. There was good correlation of mRNA abundances between the two gene expression profiling platforms. As expected from experience with gene expression profiling from fresh frozen specimens, reproducibility diminishes with the expression levels, and reliable assessment of gene expression remains imperfect for low abundance transcripts (31). Further, while we did not intend to directly compare results at the sample level to mRNA expression data from fresh frozen tissue, we did observe biologic correlations to existing literature from fresh frozen samples suggesting that biologically relevant signatures can be derived from archival FFPE samples.

Concordance between microarray gene expression profiling and NanoString nCounter platform has been previously shown for frozen specimens (32). To our knowledge, our study is the first to compare performance of these two platforms on FFPE specimens across two different diseases. We have demonstrated the feasibility of discovery using larger microarray assays and a follow up validation using targeted Nanostring nCounter approach.

Though the study sample size was relatively modest, we did not find any samples which failed to produce usable results on either platform. This is in contrast to our prior experience with the Illumina DASL platform (33). This study did not aim to compare the DASL platform to these more recent profiling approaches for FFPE.

Our comparative study and several recent studies (32, 34) that used NuGen+Affymetrix and Nanosting platforms show feasibility of these platforms for clinical applications such as discovery of the gene expression signatures for personalized medicine and its applications from FFPE biopsy material. However, development of additional statistical methodologies specifically tailored for preprocessing of the gene expression data from FFPE tissues might still be beneficial, since reproducibility between technical replicates observed in our study allows for further improvement.

With a growing acceptance of the utility of mRNA expression profiles in precision medicine (6, 35, 36) researchers will require reliable methods for assessing gene expression data from archival specimens. In this study, we found that two modern platforms, one for more targeted profiling and one looking across the genome, can produce reliable, biologically relevant signatures. While additional experience is needed, these data should help researchers use FFPE tissue to answer questions central to the care of their patients.

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Acknowledgements:

[Some stuff was provided by affymetrix and nanostring for free.](#)

Tables:

Table 1: Ranges of the percentages of the probes with the signal above the background, correlation of the percent present probes with block age and ranges of correlations between technical replicates for each cohort and gene expression platform.

Platform	Percent present	Correlation with block age	Correlations between technical replicates
<i>Prostate samples</i>			
NuGen+Affymetrix	0.17 -- 0.58	0.02	0.91 -- 0.98
Nanostring	0.40 -- 0.79	-0.11	0.88 -- 0.97
<i>Ovarian samples</i>			
NuGen+Affymetrix	0.40 -- 0.68	0.06	0.94 -- 0.98
Nanostring	0.83 -- 0.93	-0.04	0.95 -- 0.99

Figure legends:

Figure 1: (a-d) Correlations between technical replicates as a function of block age for all platforms and cohorts. (e-h) Percent of the genes that are either above or below the intensity threshold in each sample in a technical replicates pair. Each pair of replicates is plotted with an individual curve. (i-l) Scatterplots of correlations between gene expression values for each gene represented on a platform across technical replicates.

Figure 2: Bubble chart showing relationship between correlations of the gene expression values on NuGen+Affymetrix and Nanostring platform and average expression levels of those genes. Areas of the circles are proportional to the absolute value of the correlations; salmon color represents positive and blue color negative correlations. (a) Prostate specimens. (b) Ovarian specimens.

Figure 3: Genes from the signatures that are represented on each platform were projected onto first two principal components and plotted. Colors represent compared classes and distinct plotting characters represent technical replicates from the same specimen. (a) NuGen+Affymetrix on prostate samples. Tumor samples are in orange and normal in blue. (b) Nanostring on prostate samples. Tumor samples are in orange and normal in blue. (c) NuGen+Affymetrix on prostate tumor samples. Gleason 6 samples are in blue, Gleason 7 in green, and Gleason 8 in orange. (d) Nanostring on prostate tumor samples. Gleason 6 samples are in blue, Gleason 8 in orange. (e) NuGen+Affymetrix on ovarian samples. Clear cell carcinoma samples are in blue and serous carcinoma in orange. (f) Nanostring on ovarian samples. Clear cell carcinoma samples are in blue and serous carcinoma in orange

Supplementary materials:

Supplementary Figure S1 – boxplots of raw and RMA normalized NuGen+Affymetrix prostate samples data

Supplementary Figure S2 – Boxplots of the correlations between technical replicates against input RNA amount

Supplementary Figure S3 -- Scatter plots of gene expression values between technical replicates

Supplementary Figure S4 – Scatterplots of NuGen+Affymetrix vs Nanostring gene expression values for genes represented on both platforms

Supplementary Table S1 – complete information on the study design

Supplementary Table S2 – gene signatures used for biological validation

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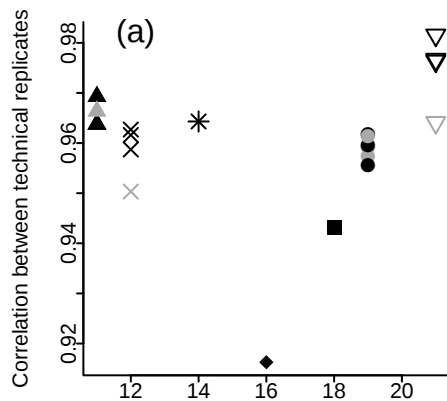
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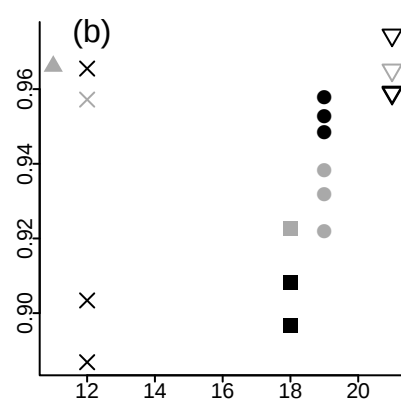
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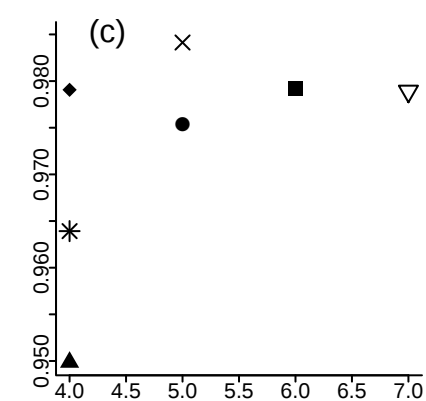
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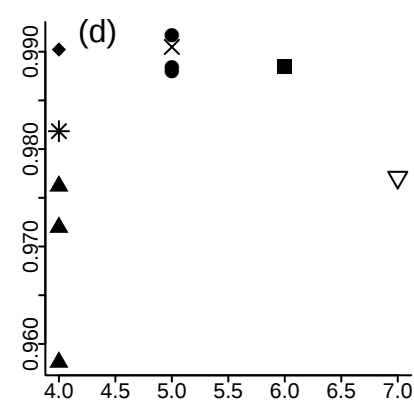
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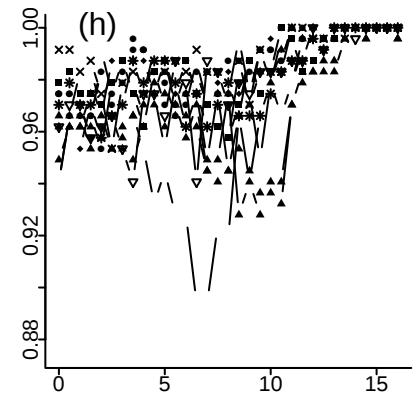
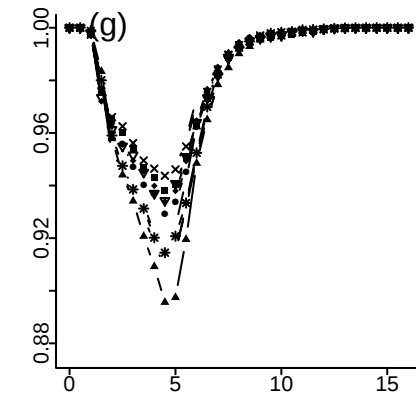
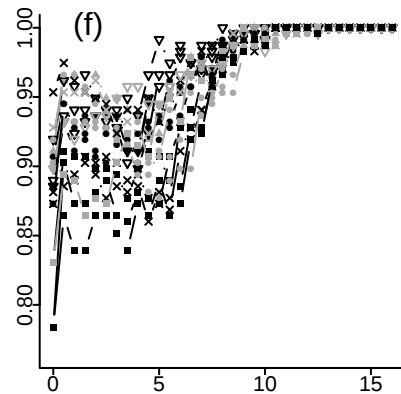
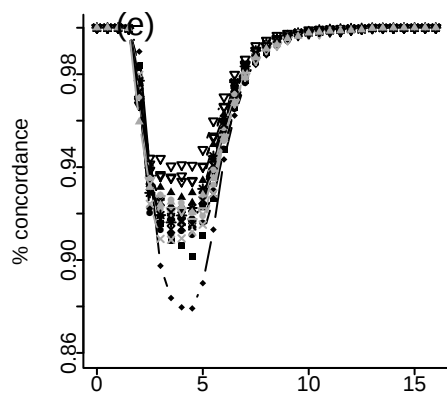
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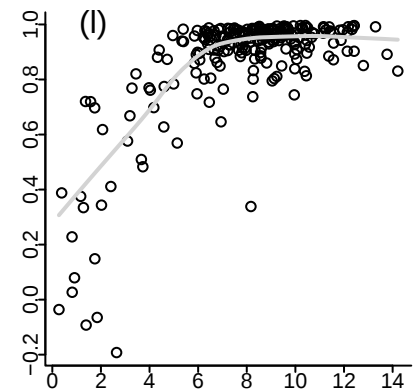
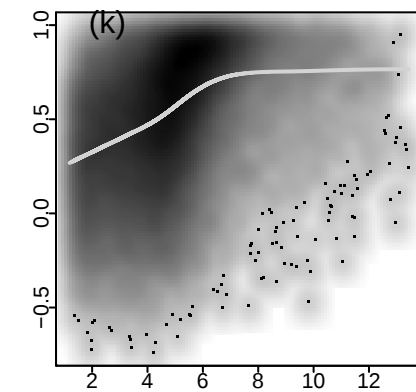
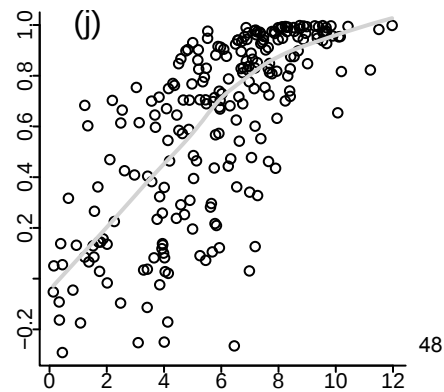
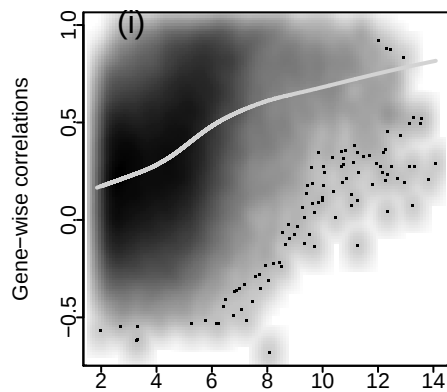
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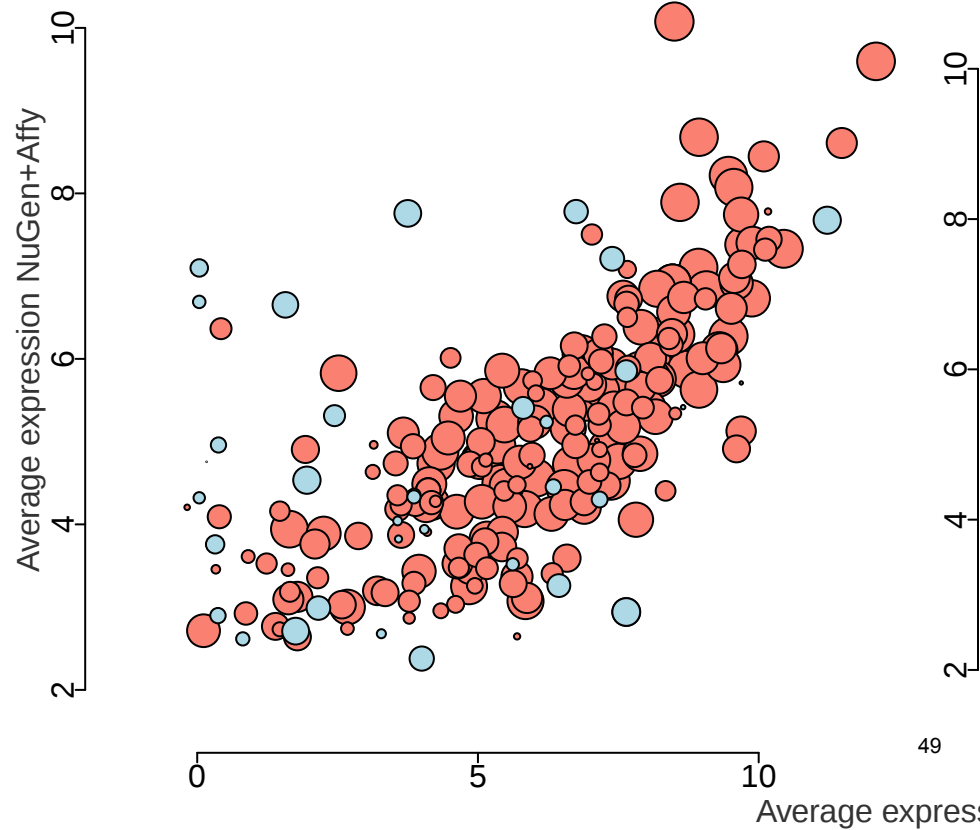


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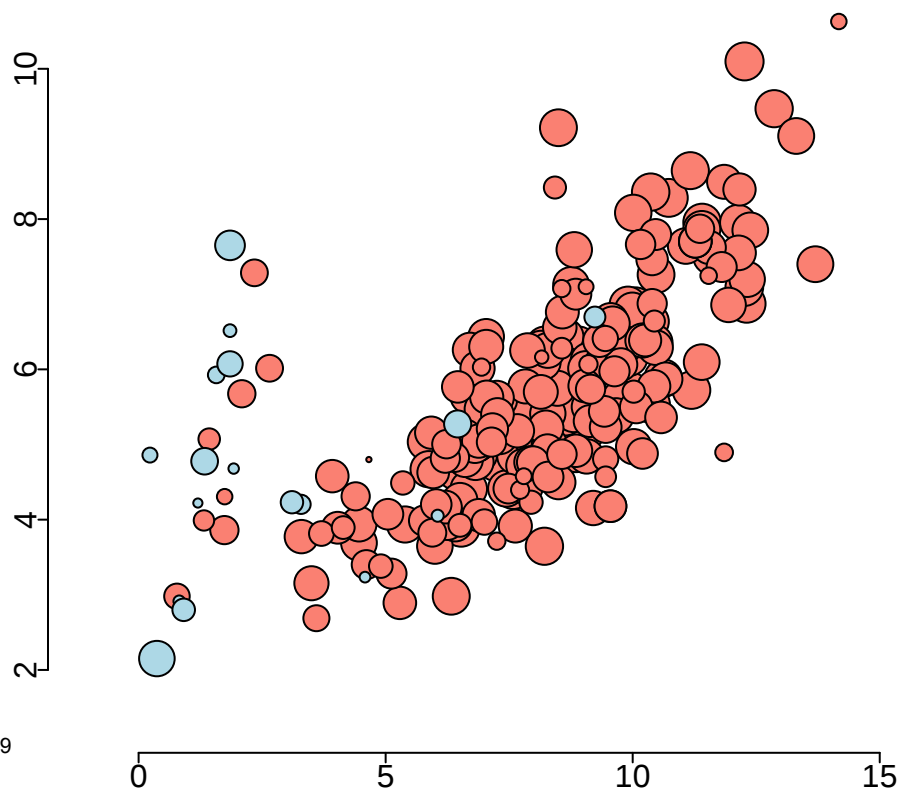


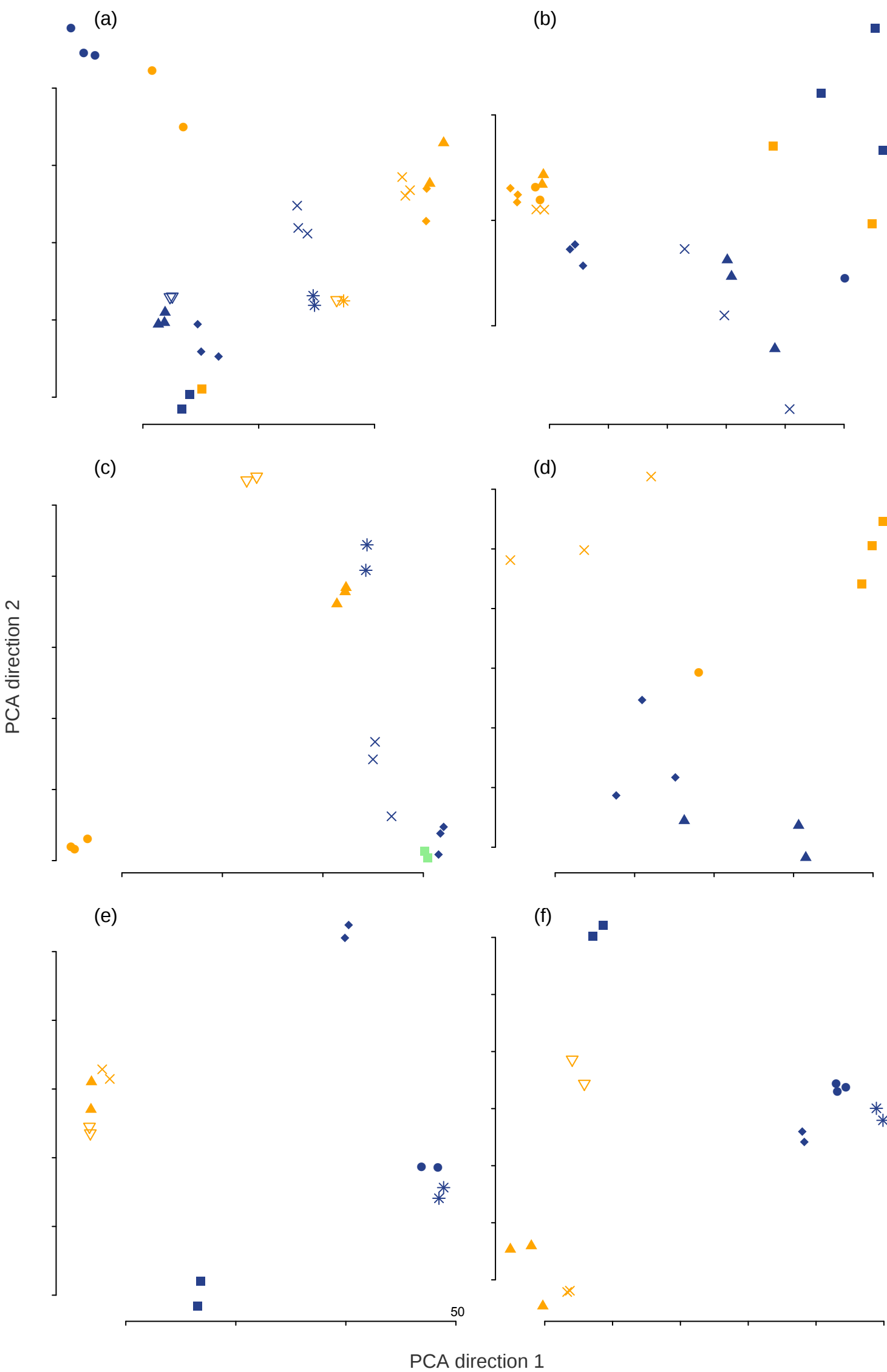
Average intensity

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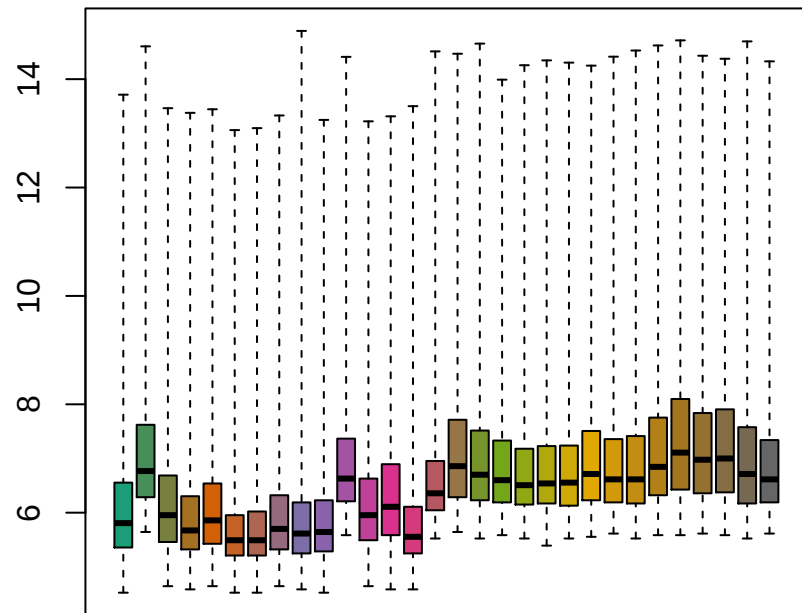


(b) Ovarian

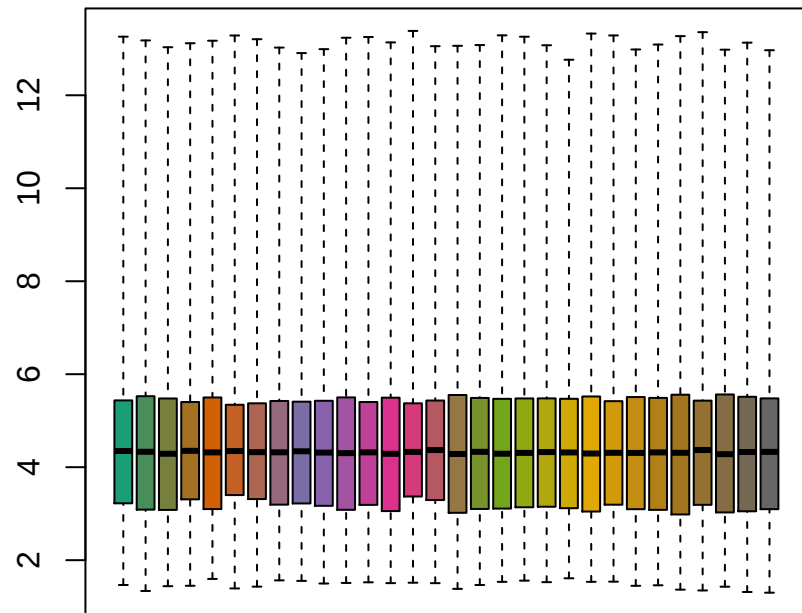




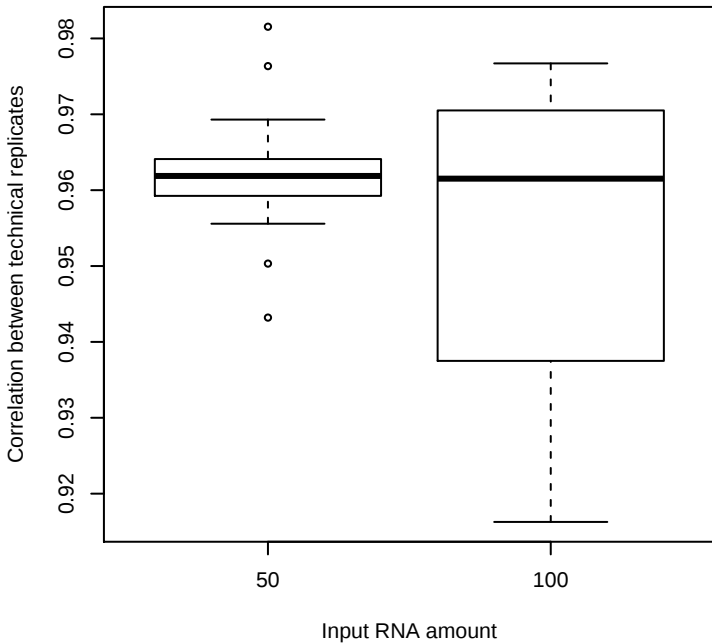
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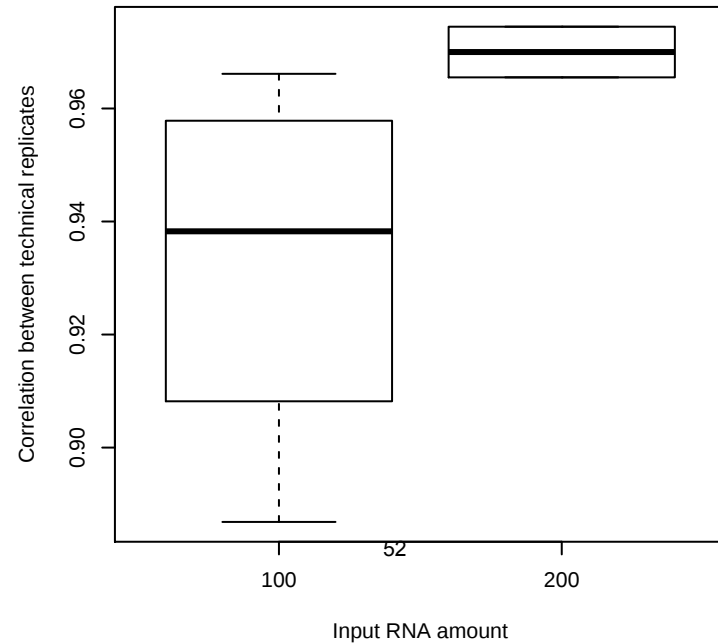
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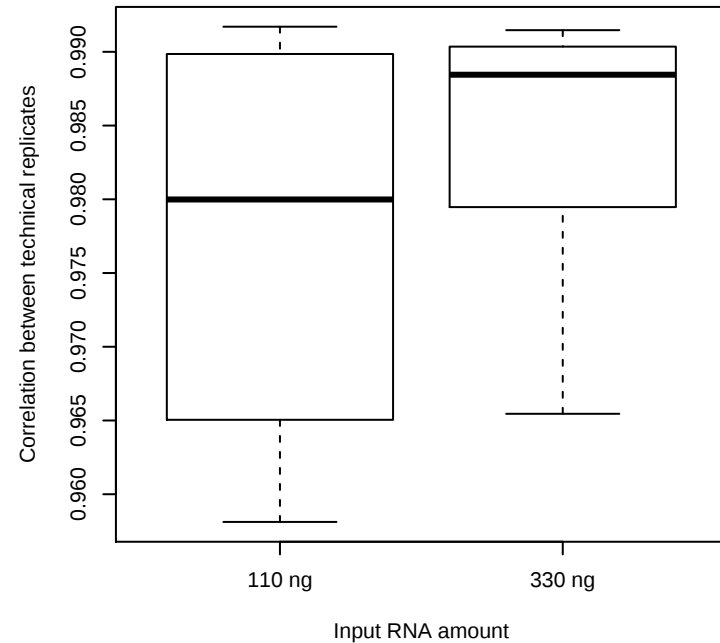
NuGen+Affymetrix – Prostate Samples



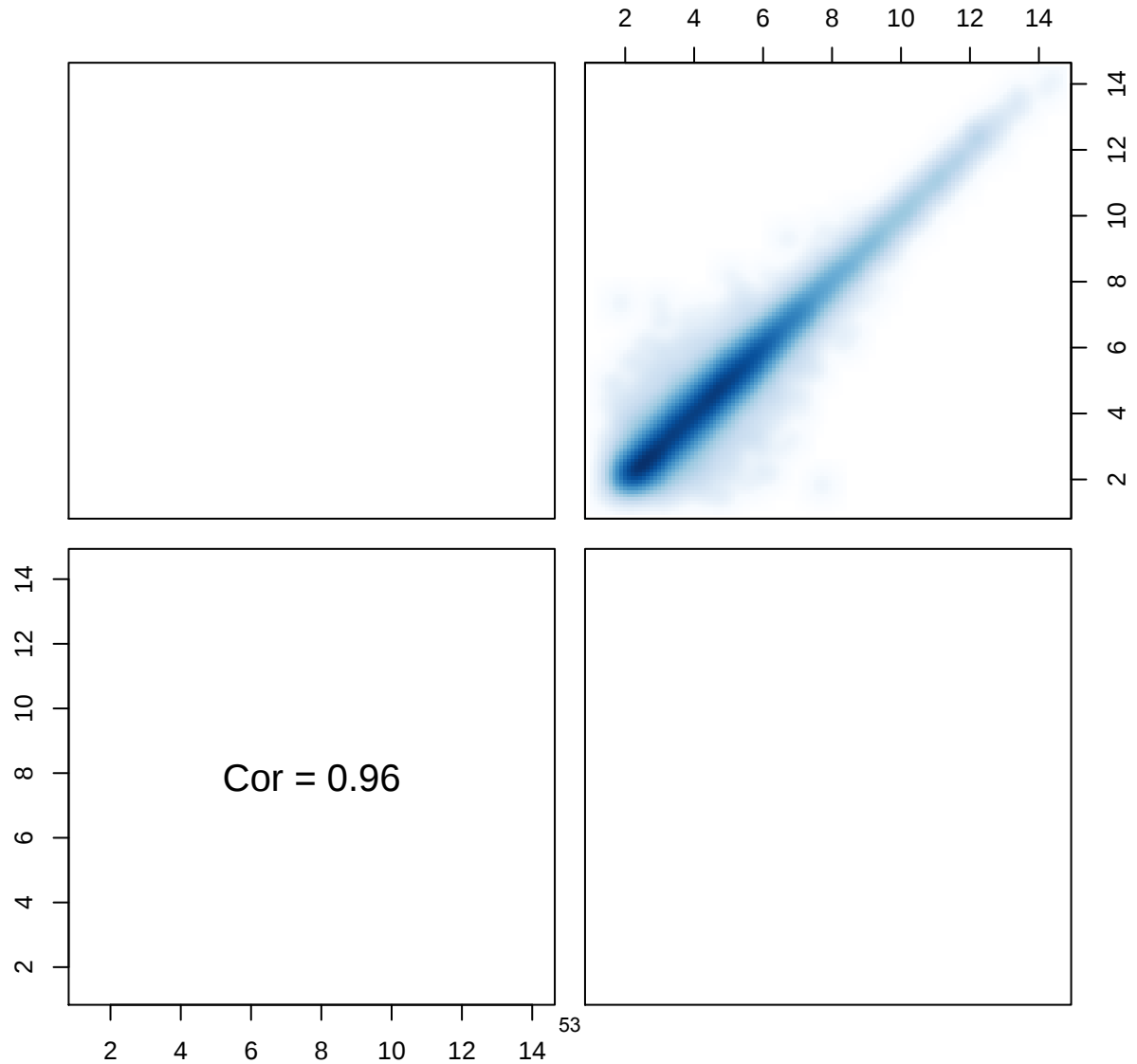
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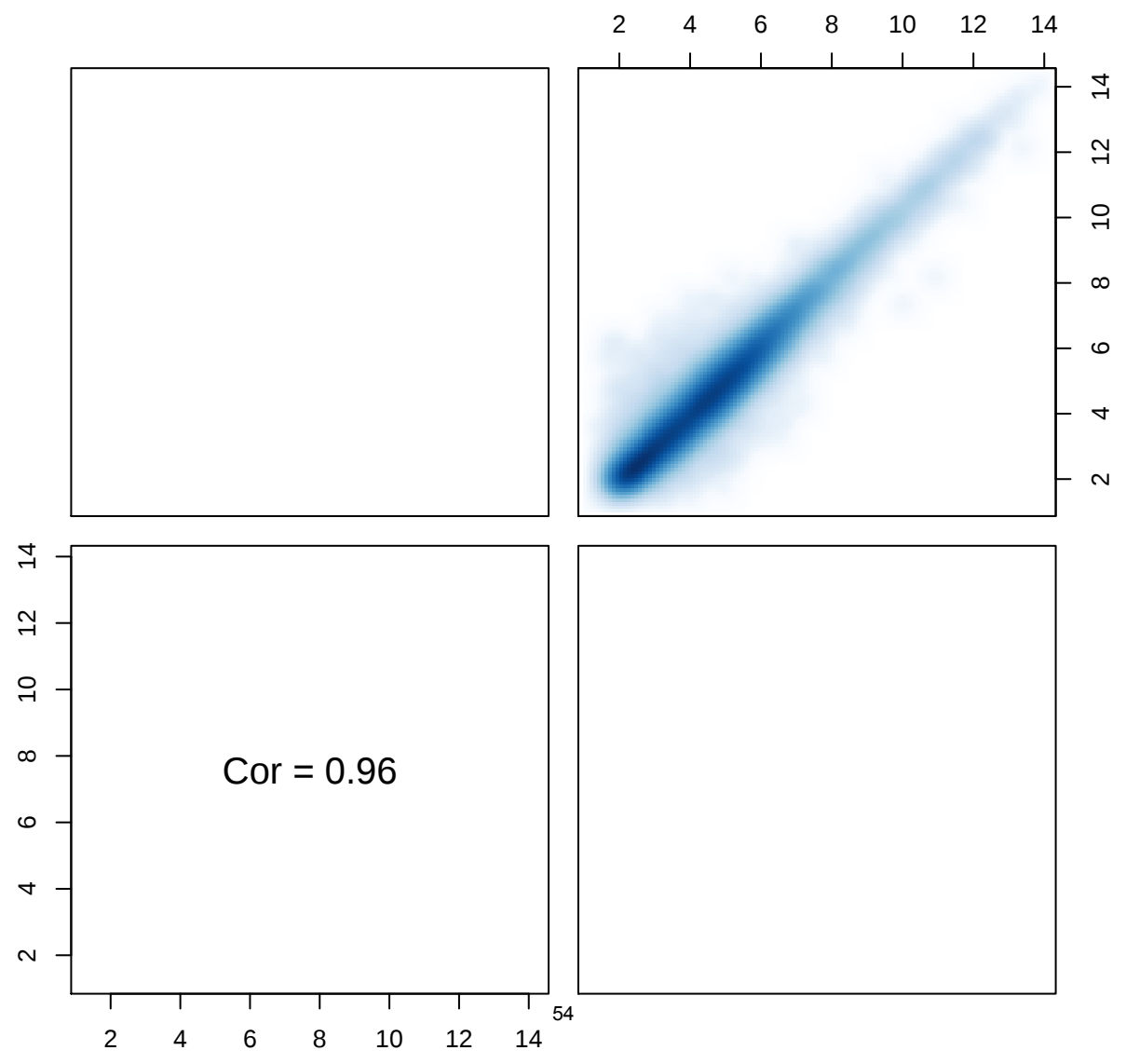
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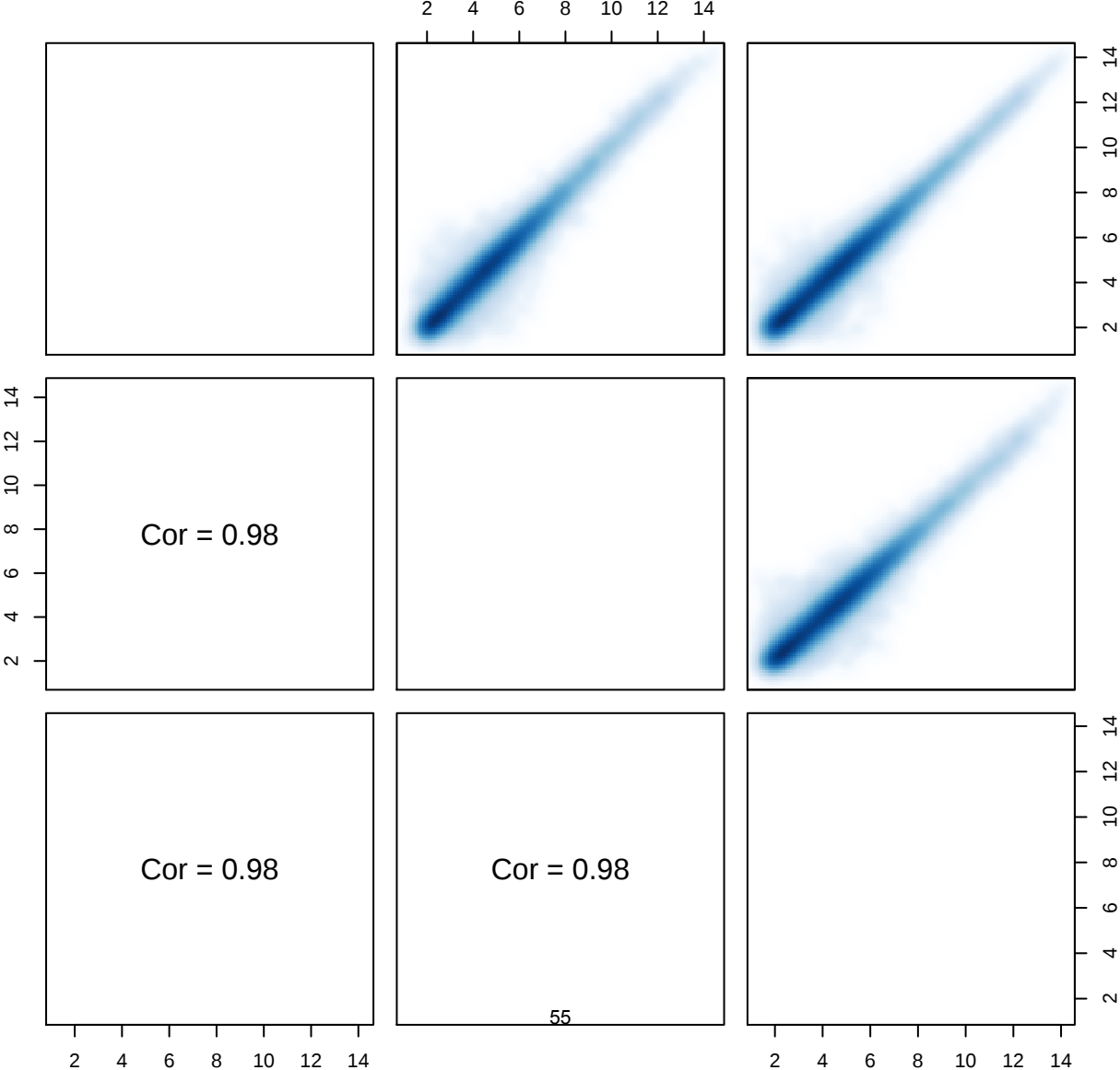
NuGen + Affymetrix -- Prostate samples. Case: p1 Tumor; block age: 14



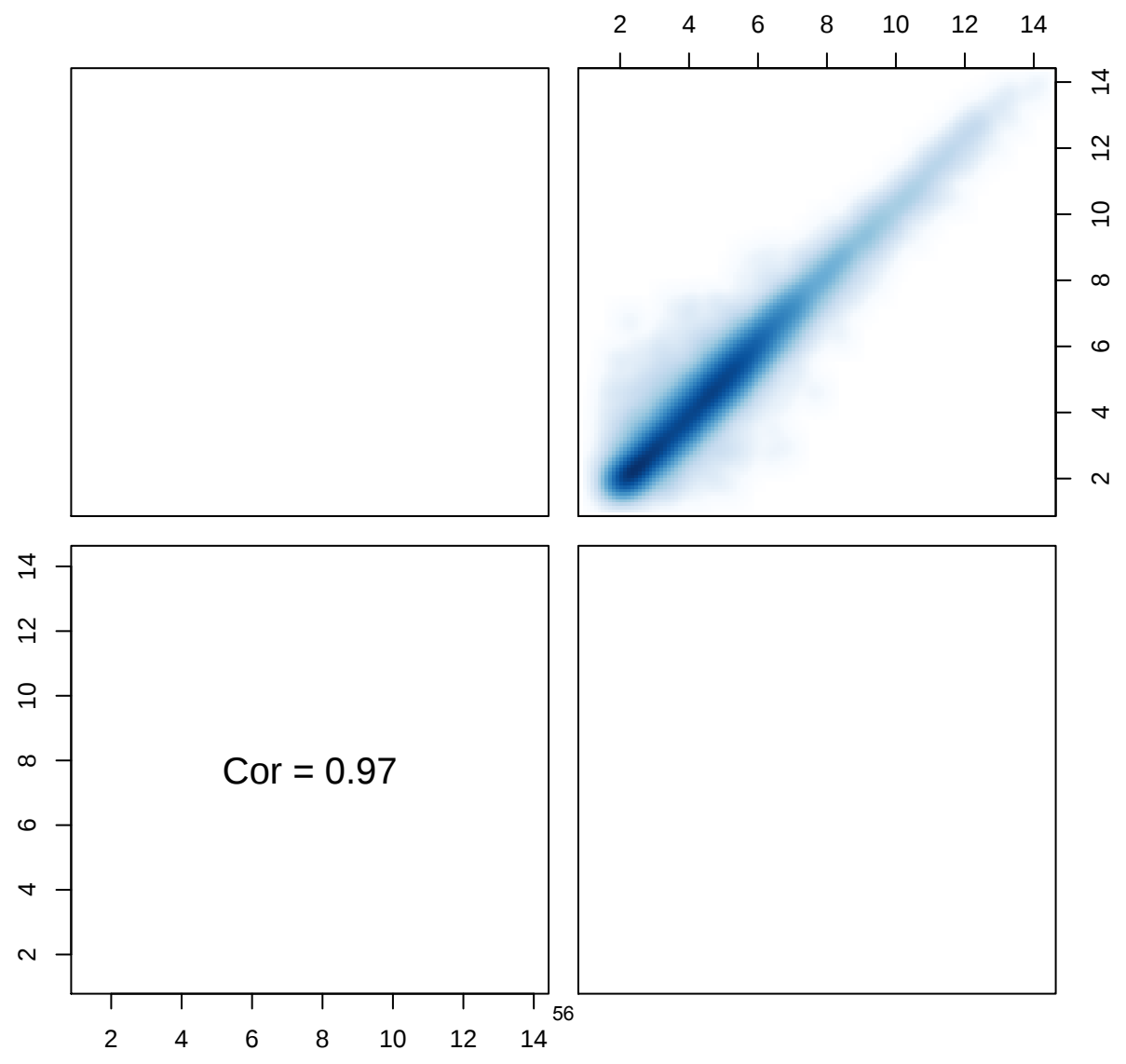
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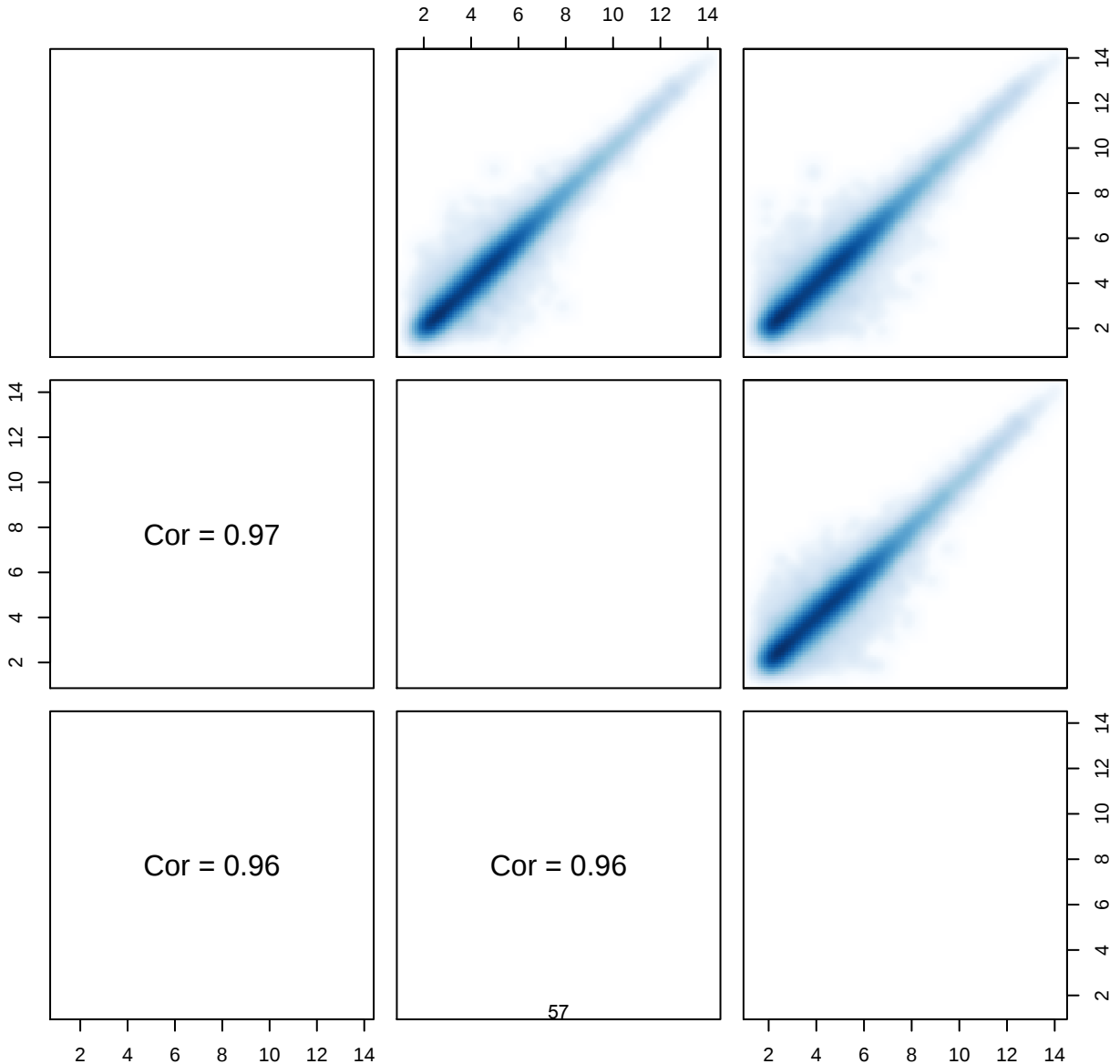
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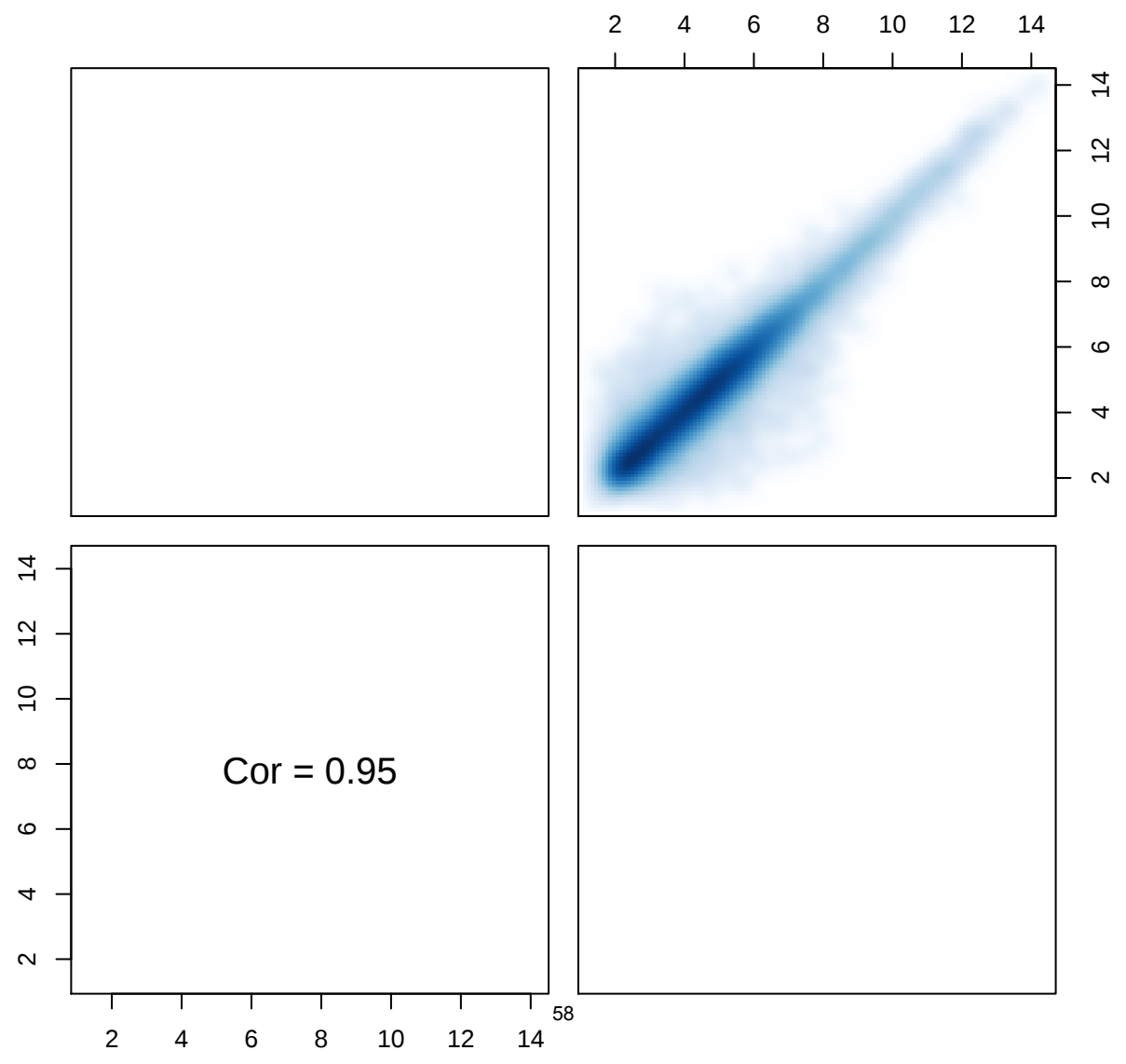
NuGen + Affymetrix -- Prostate samples. Case: p16 Normal; block age: 11



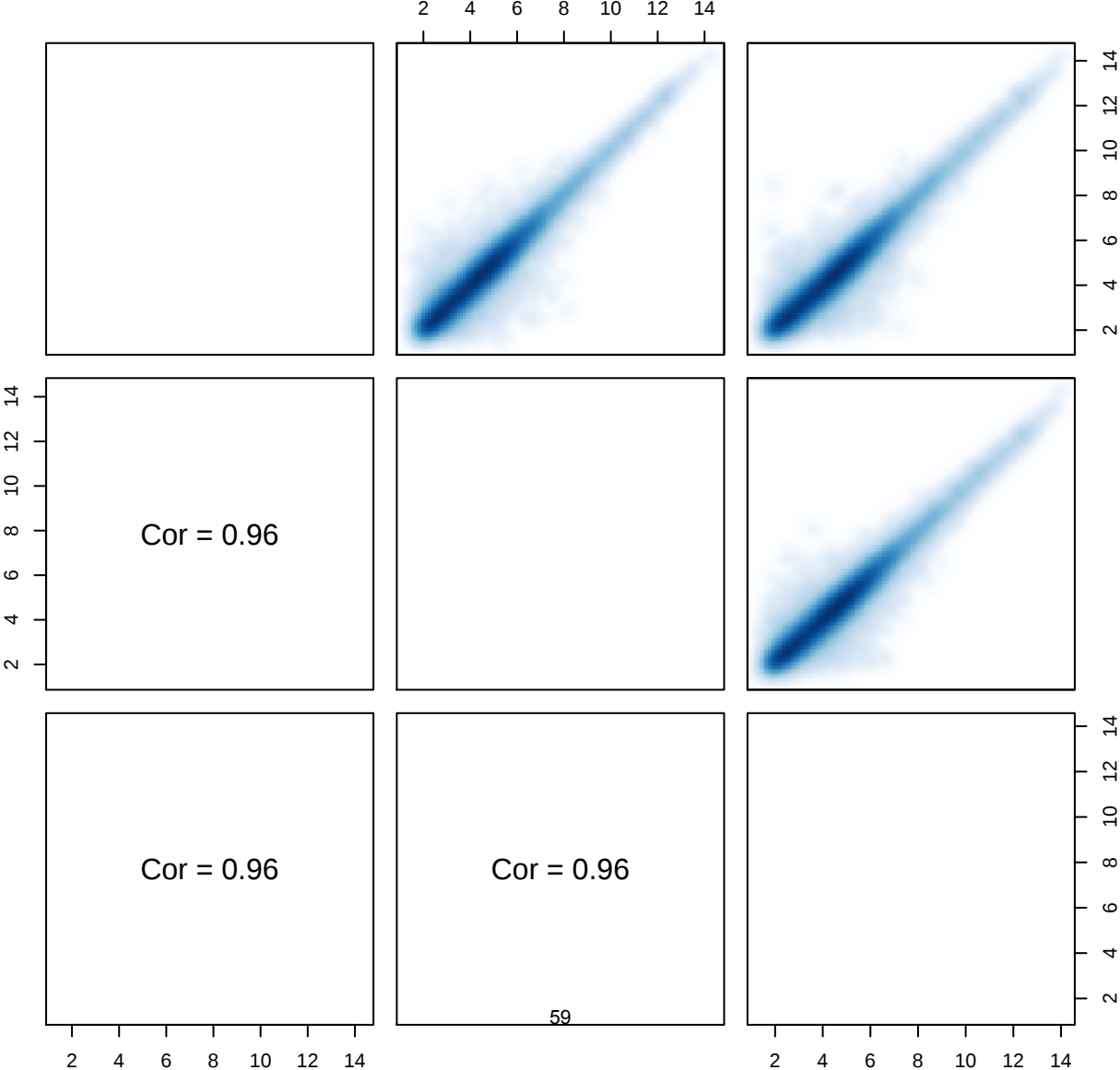
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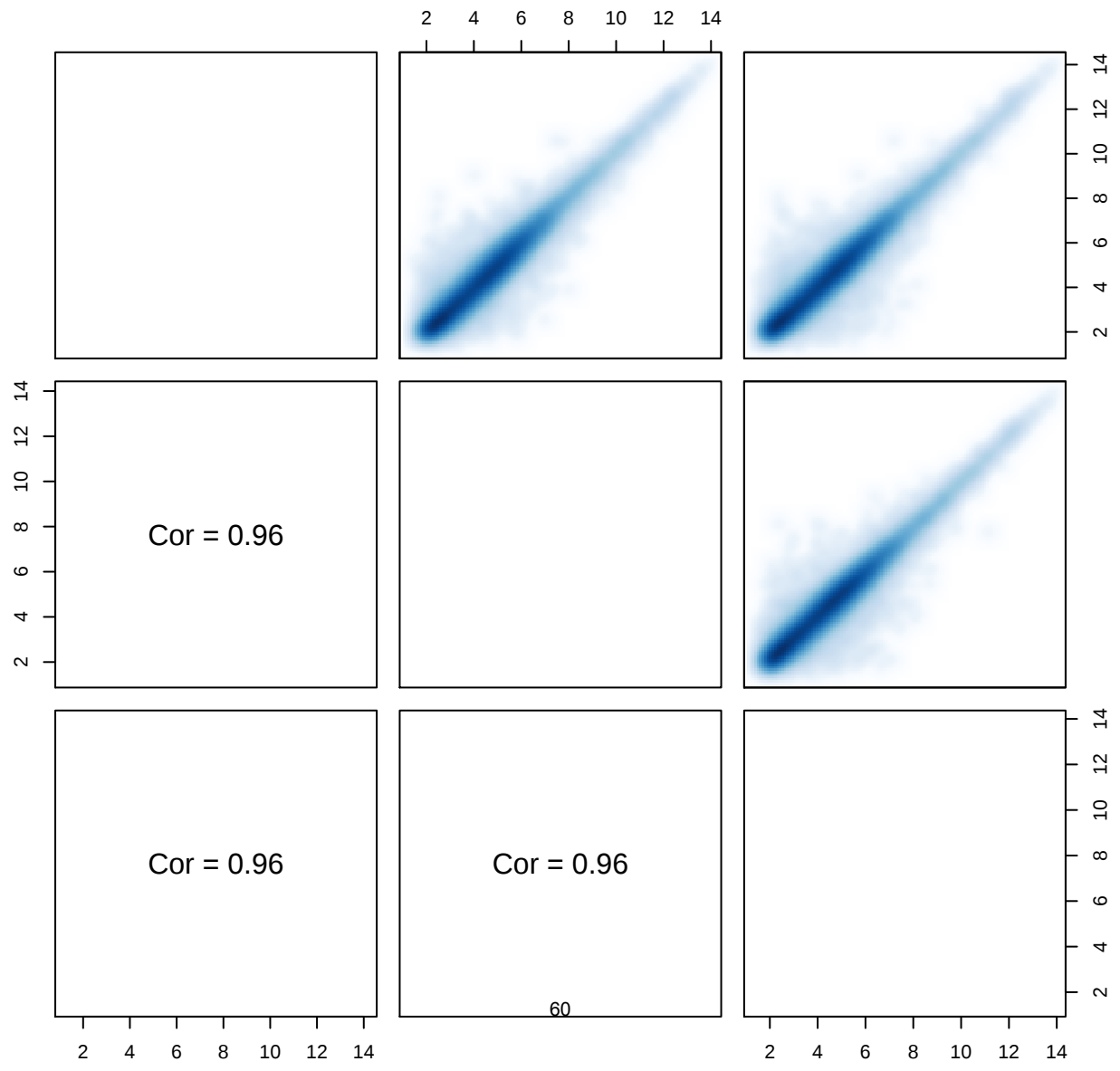
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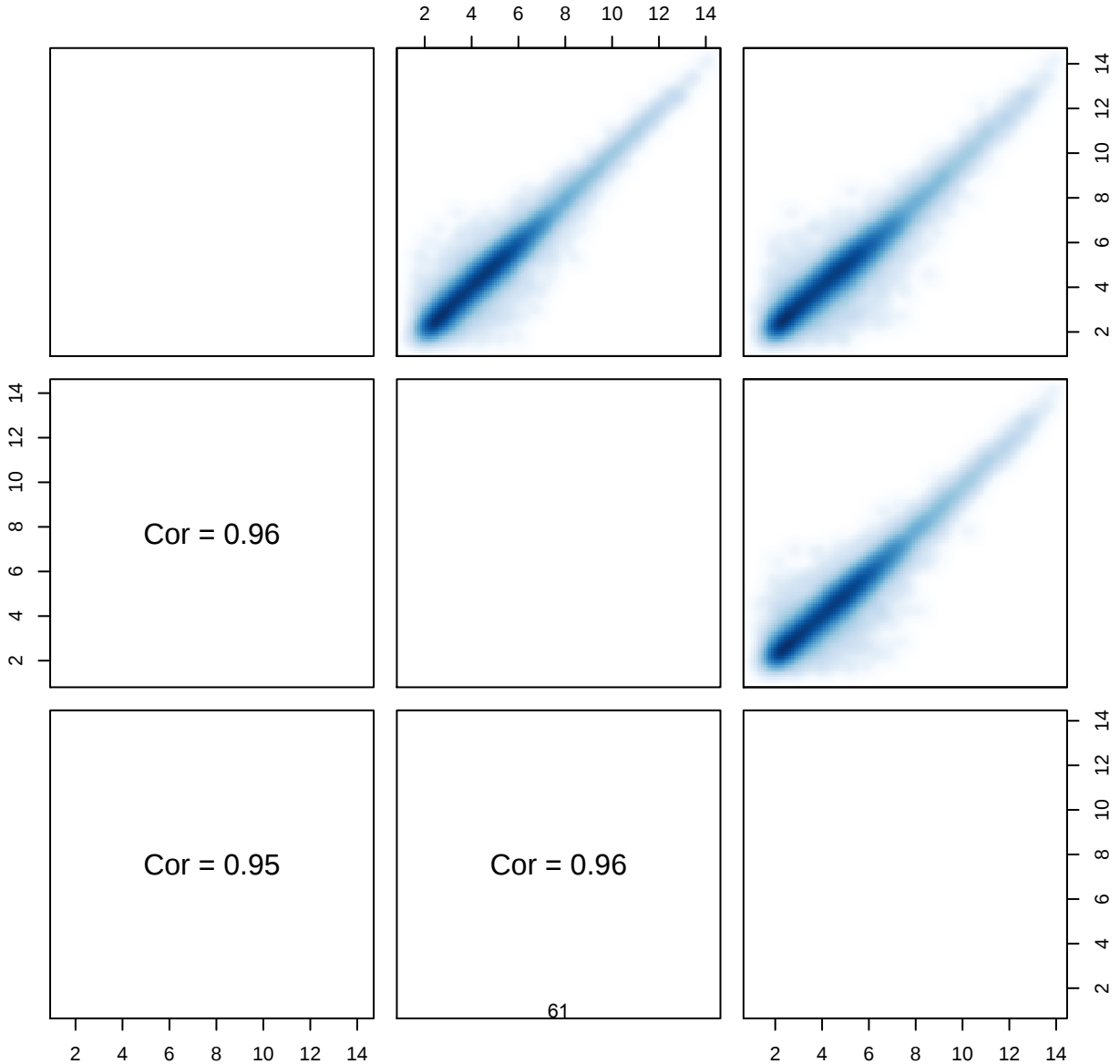
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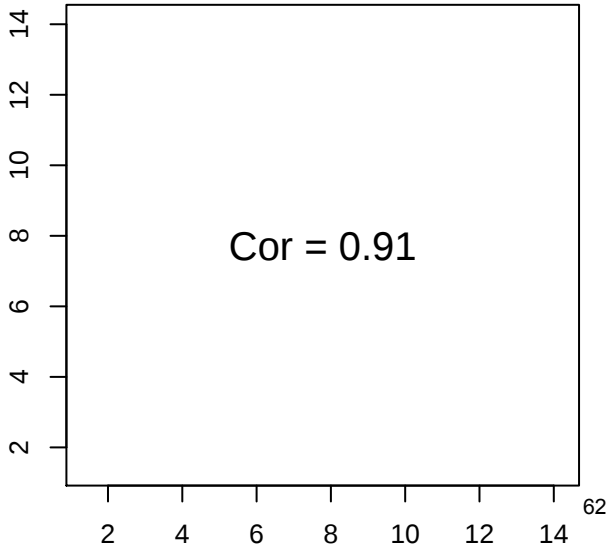
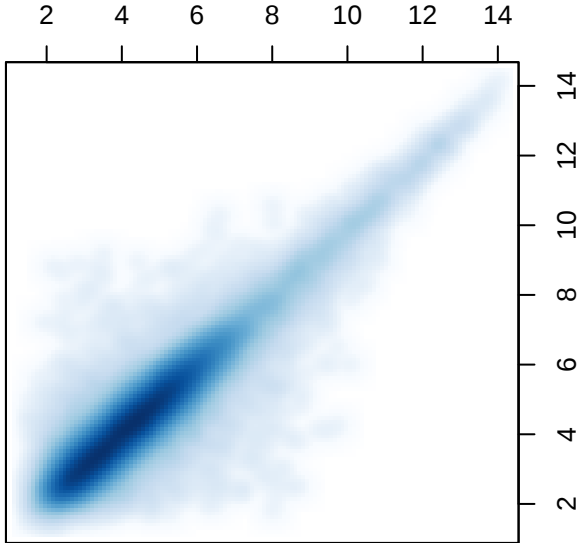
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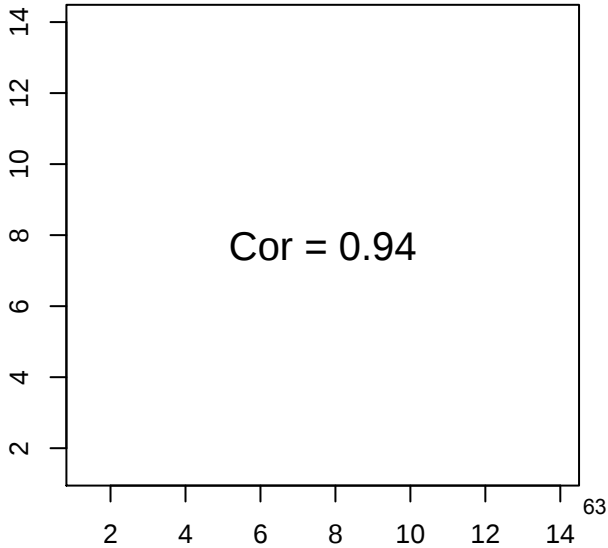
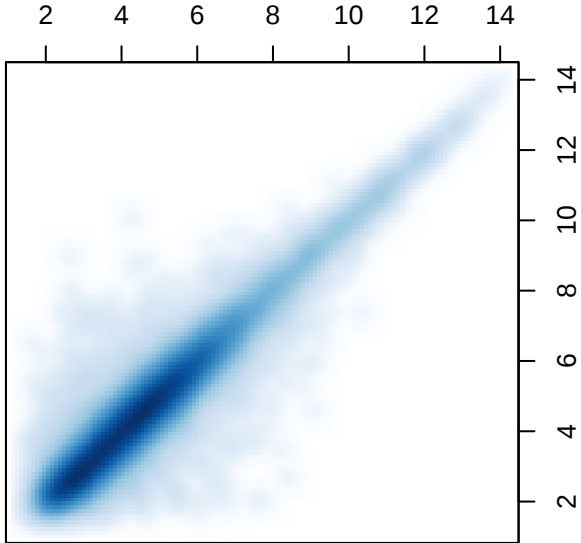
NuGen + Affymetrix -- Prostate samples. Case: p31 Tumor; block age: 19



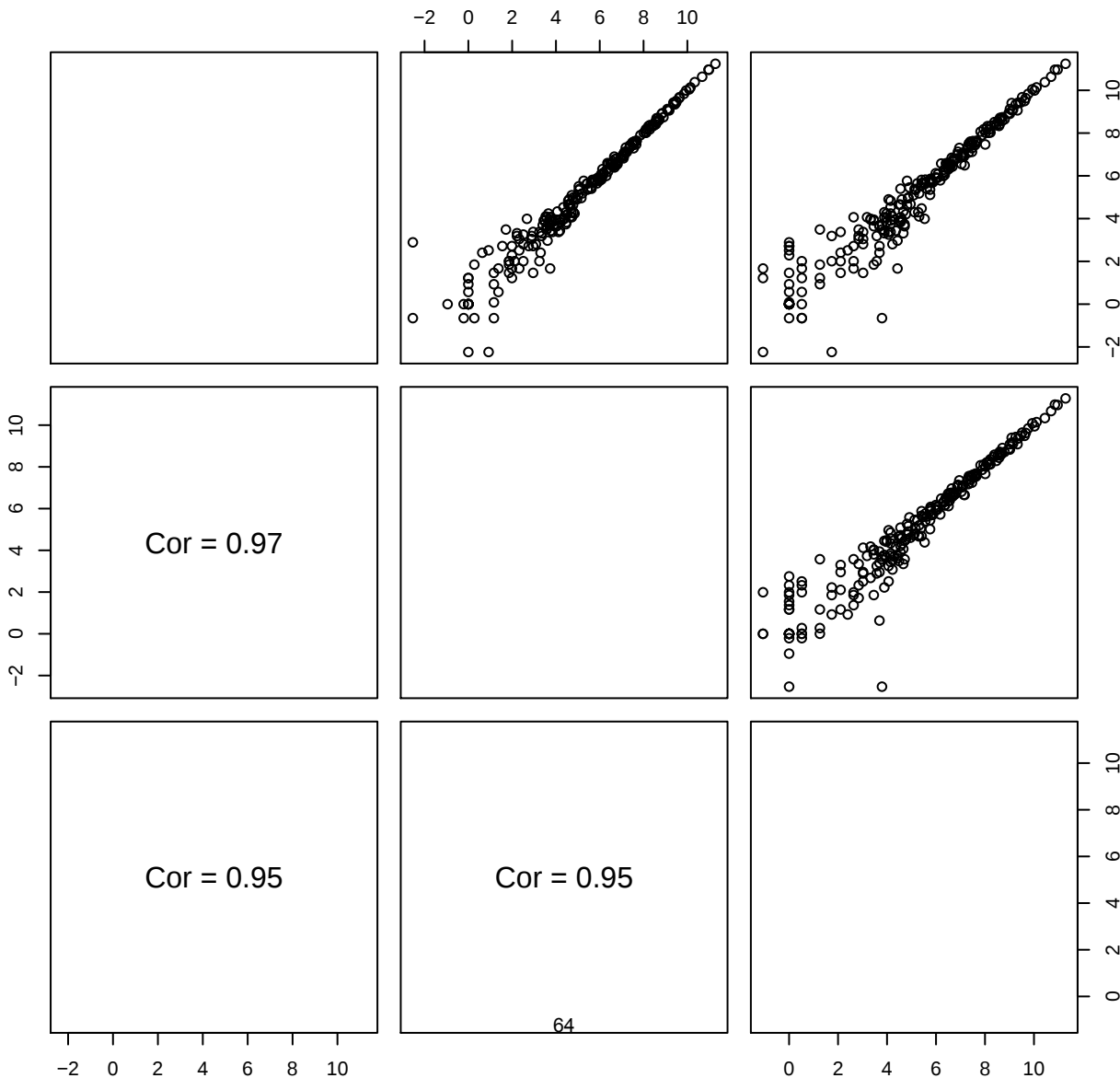
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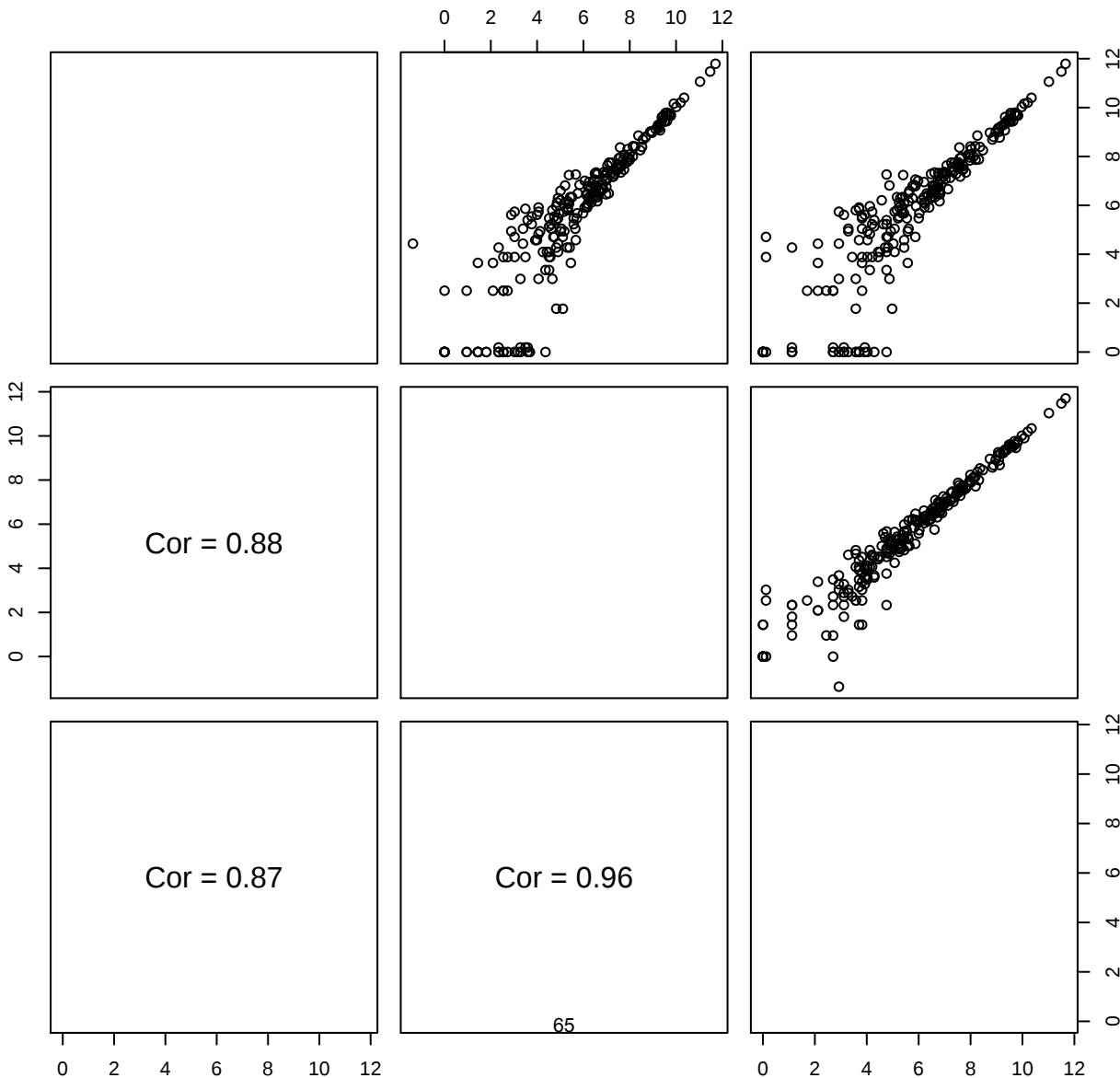
NuGen + Affymetrix -- Prostate samples. Case: p8 Tumor; block age: 18



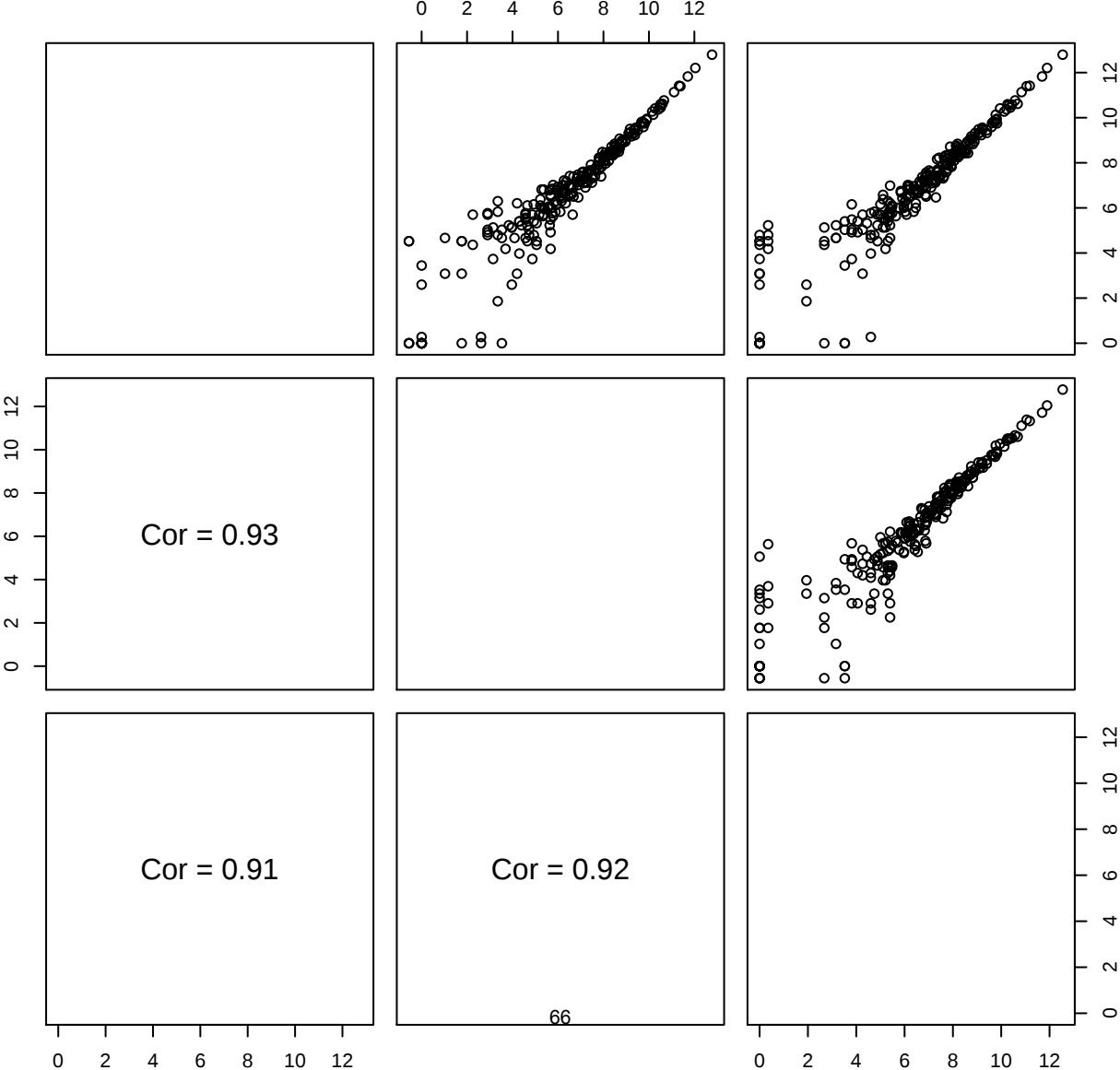
Nanostring -- Prostate samples. Case: p14 Tumor; block age:



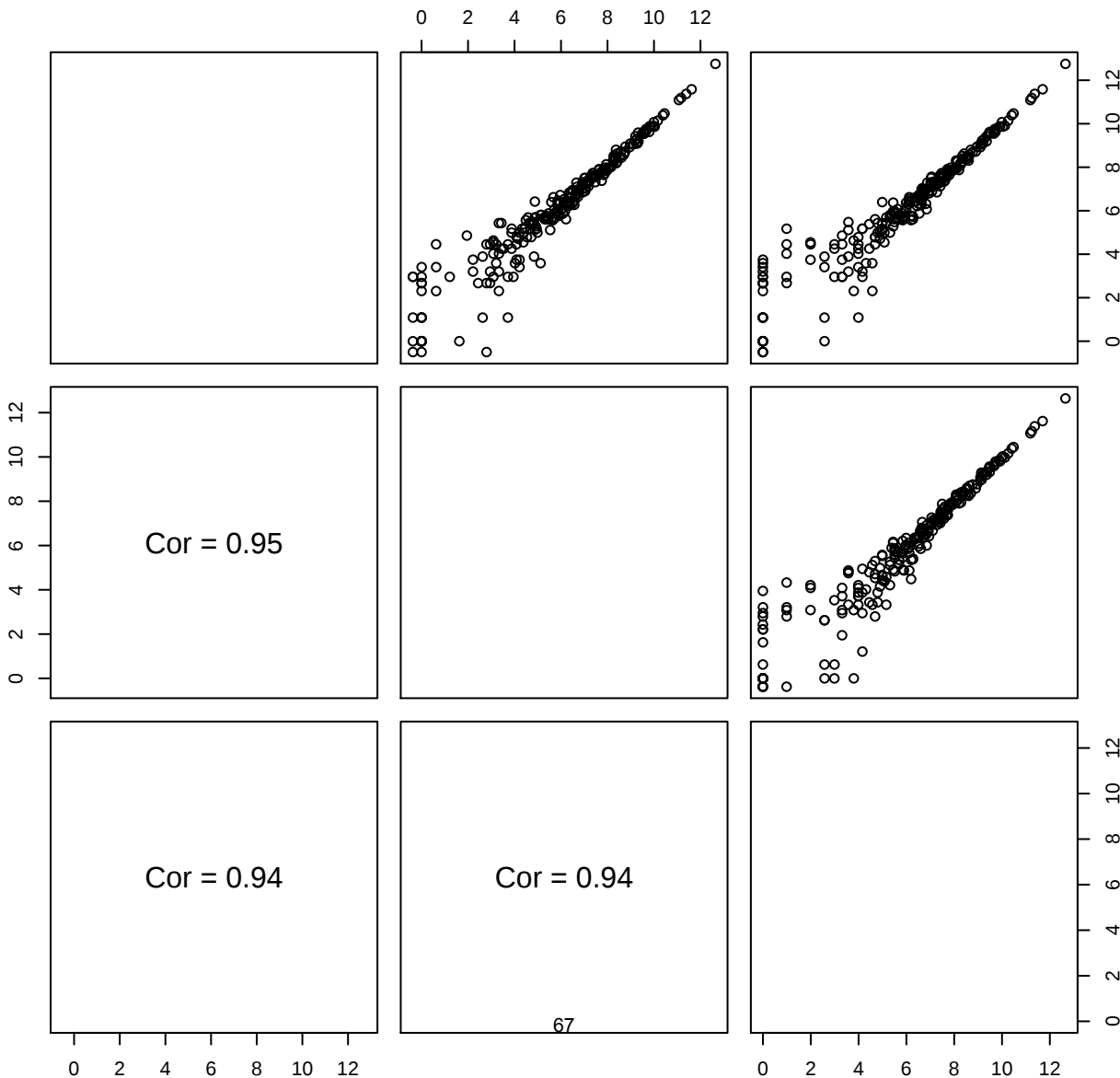
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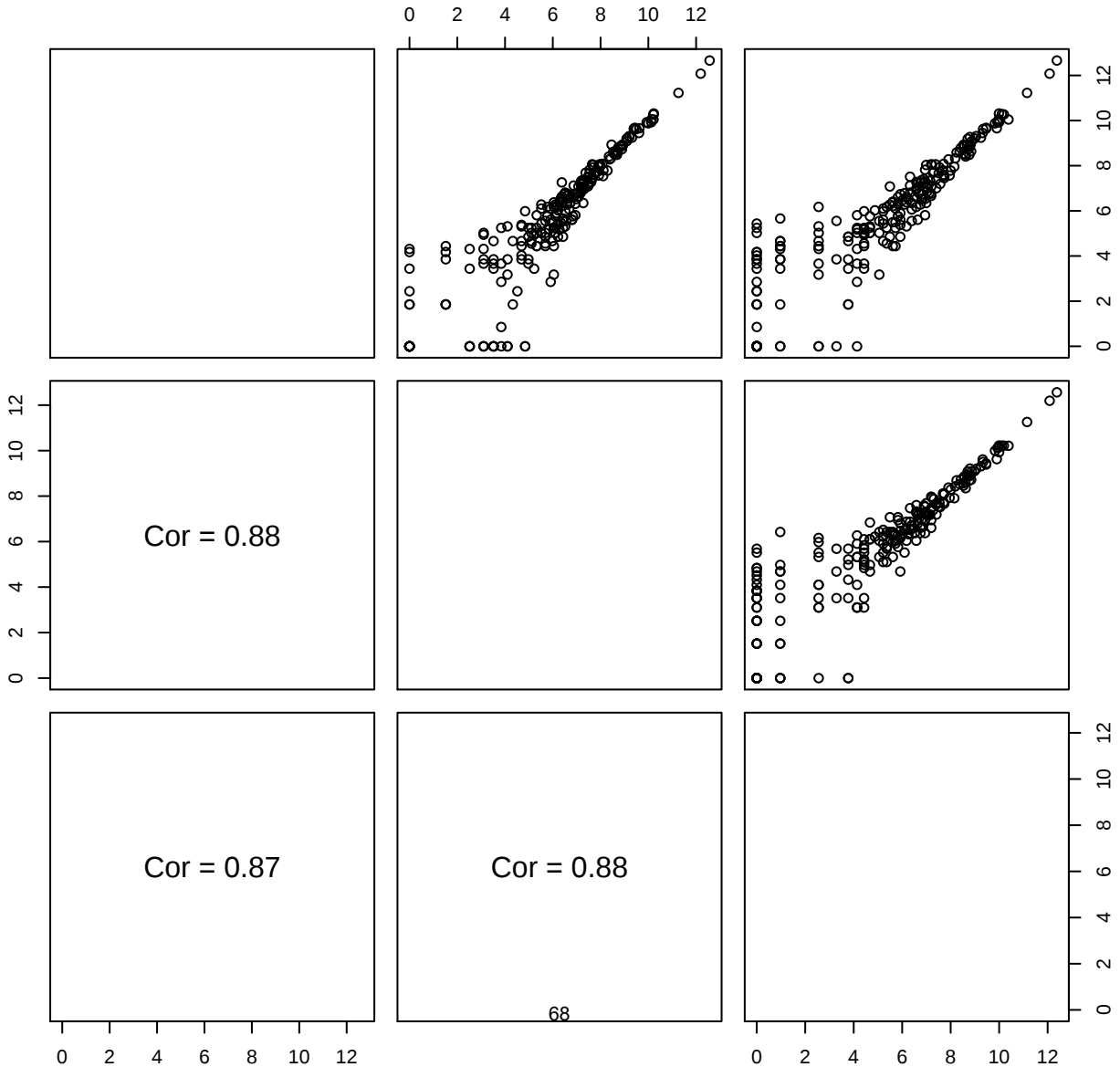
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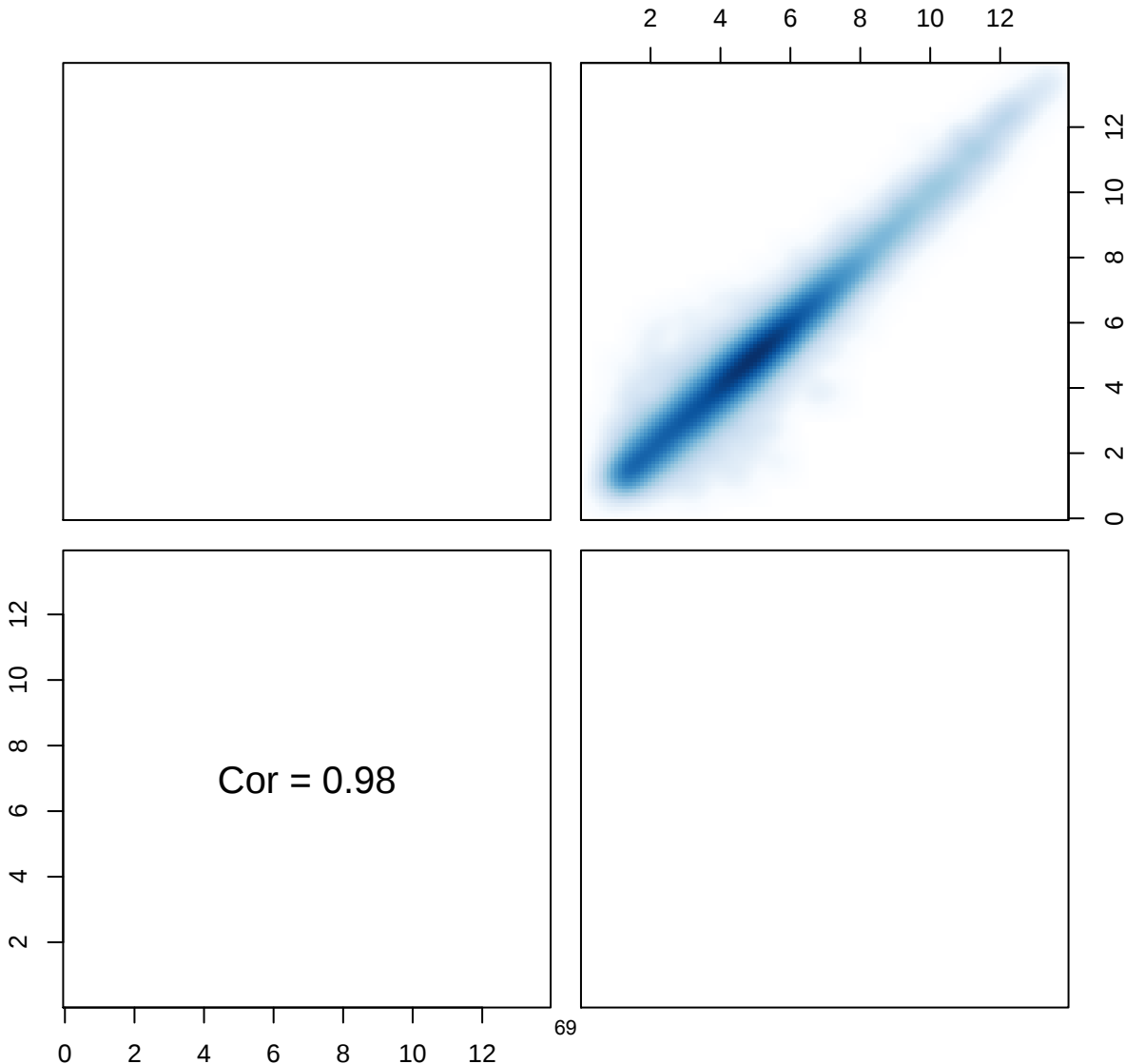
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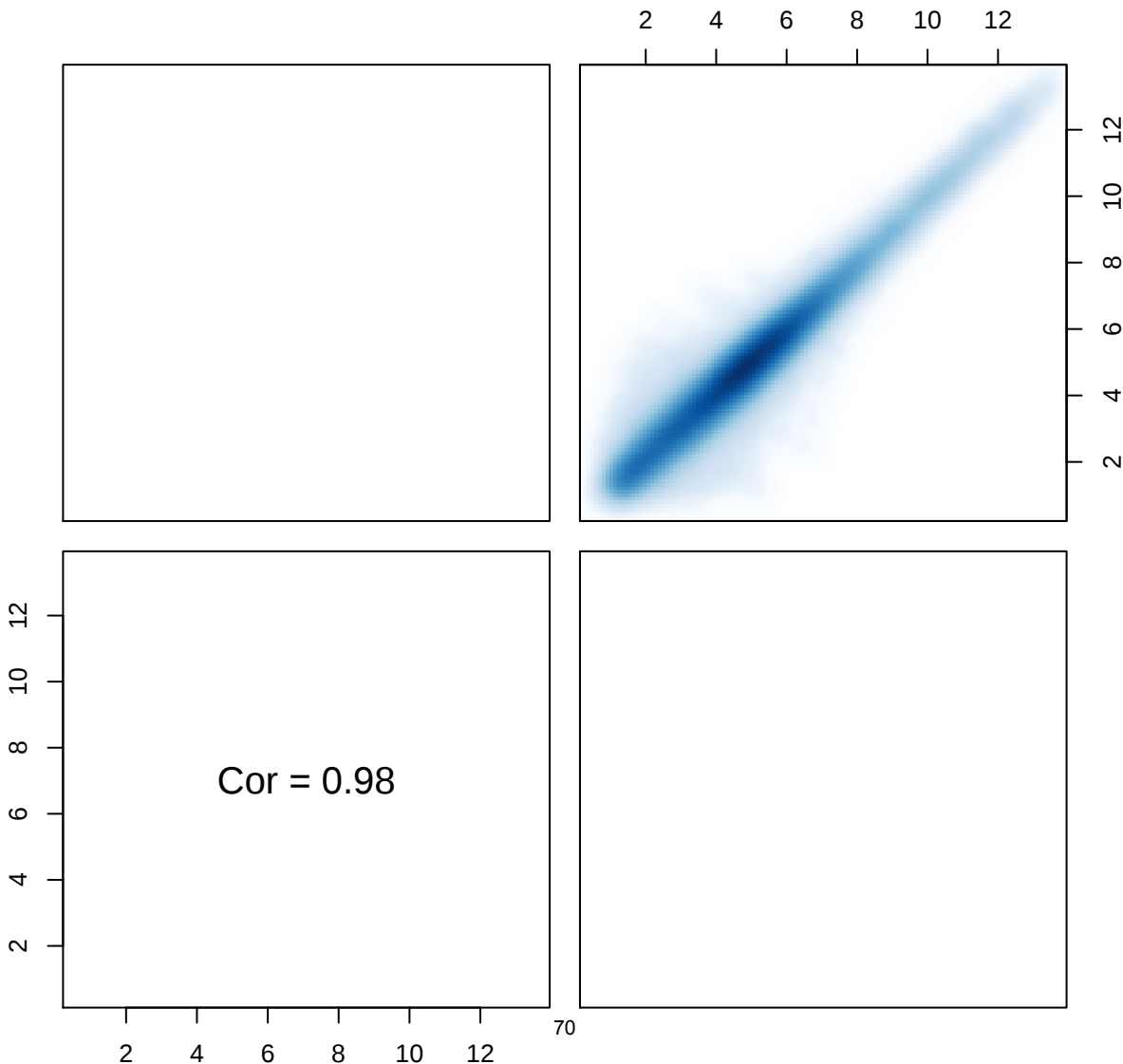
Nanostring -- Prostate samples. Case: p8 Tumor; block age:



NuGen + Affymetrix -- Ovarian samples. Case: G366; block age: 6

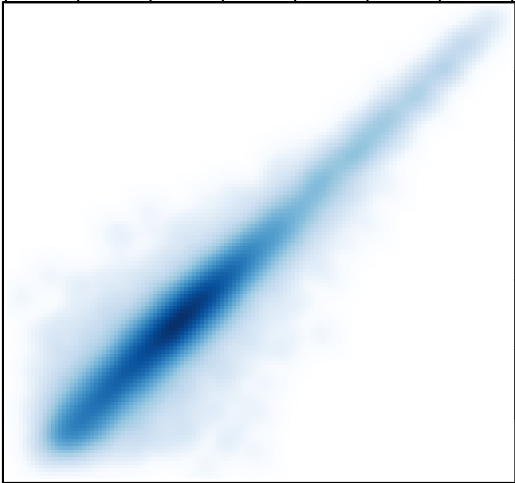
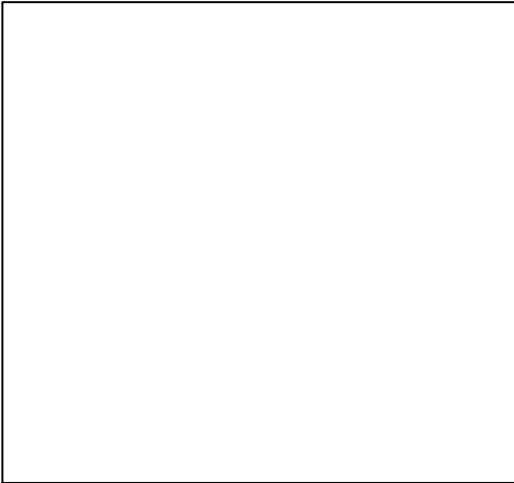


NuGen + Affymetrix -- Ovarian samples. Case: G800; block age: 5



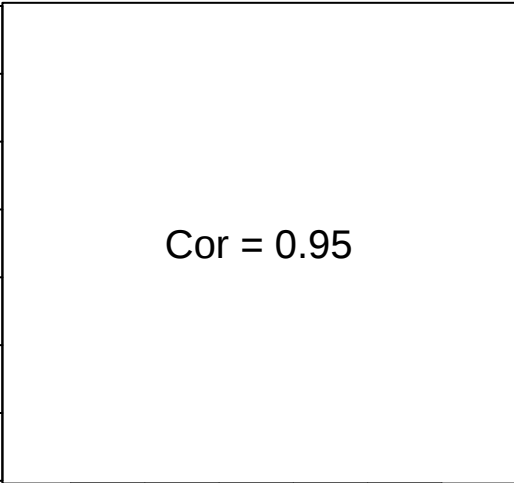
NuGen + Affymetrix -- Ovarian samples. Case: G319; block age: 4

0 2 4 6 8 10 12 14



2 4 6 8 10 12

0 2 4 6 8 10 12 14

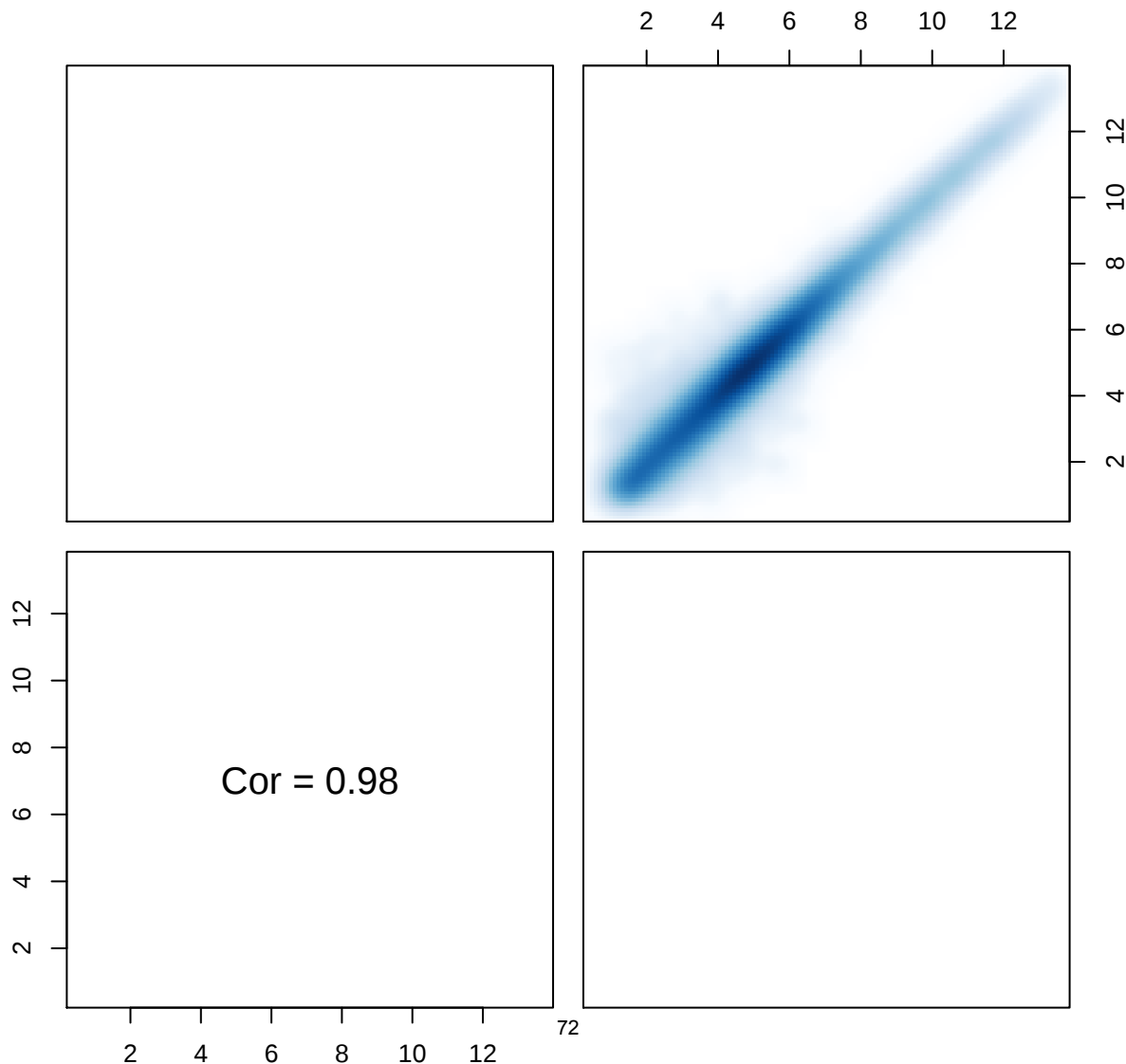


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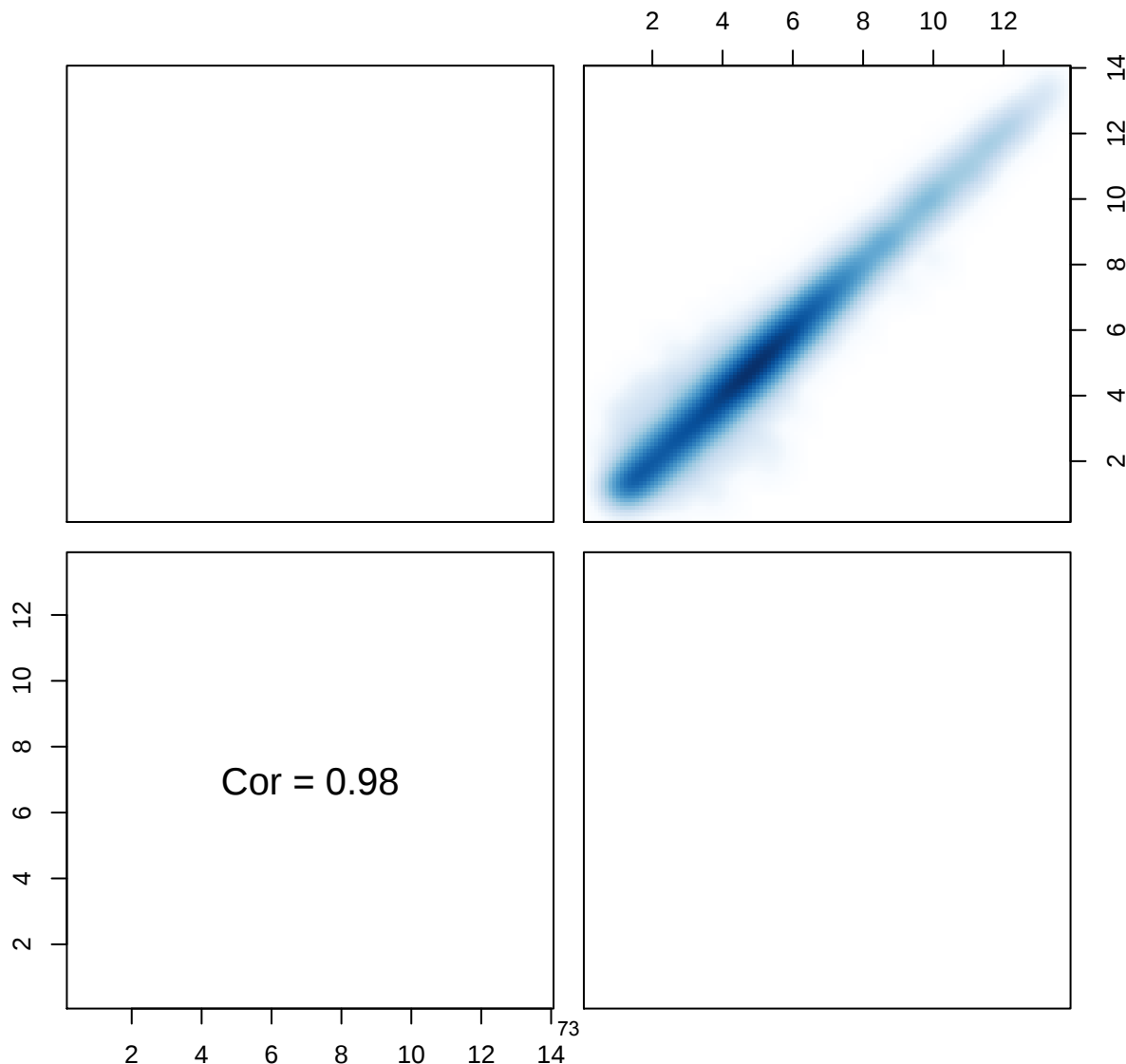
71

2 4 6 8 10 12

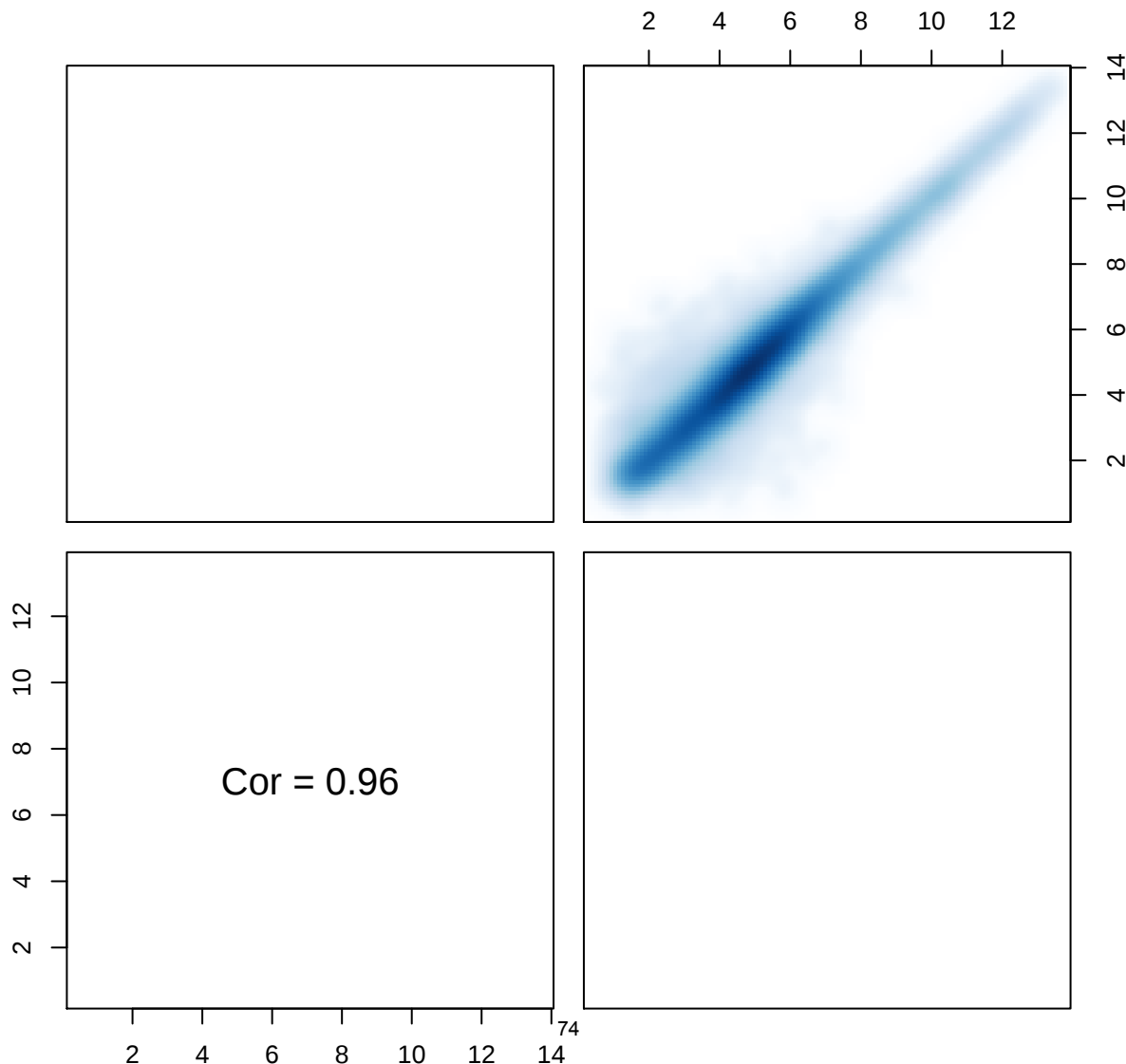
NuGen + Affymetrix -- Ovarian samples. Case: G794; block age: 4



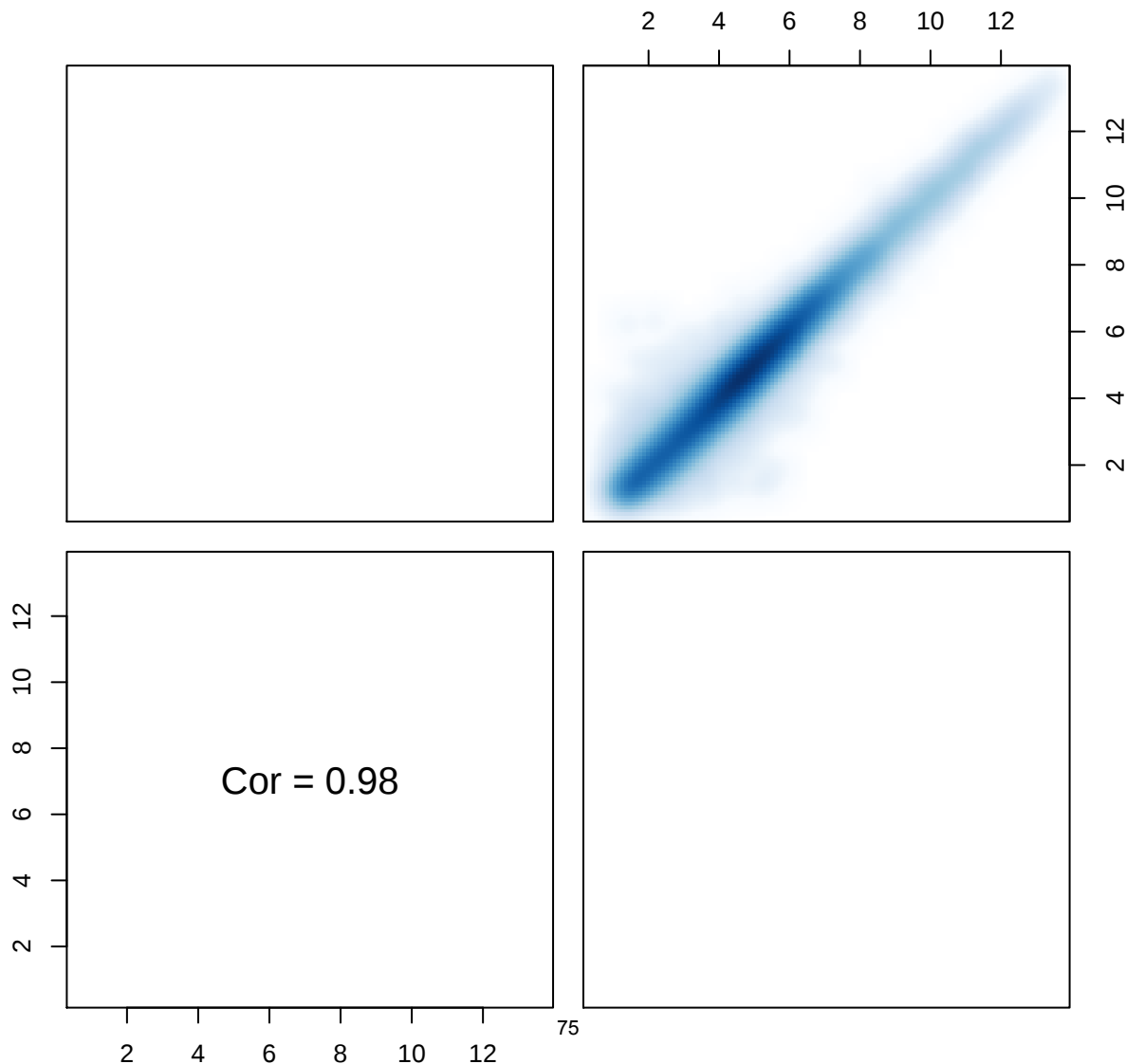
NuGen + Affymetrix -- Ovarian samples. Case: G678; block age: 5



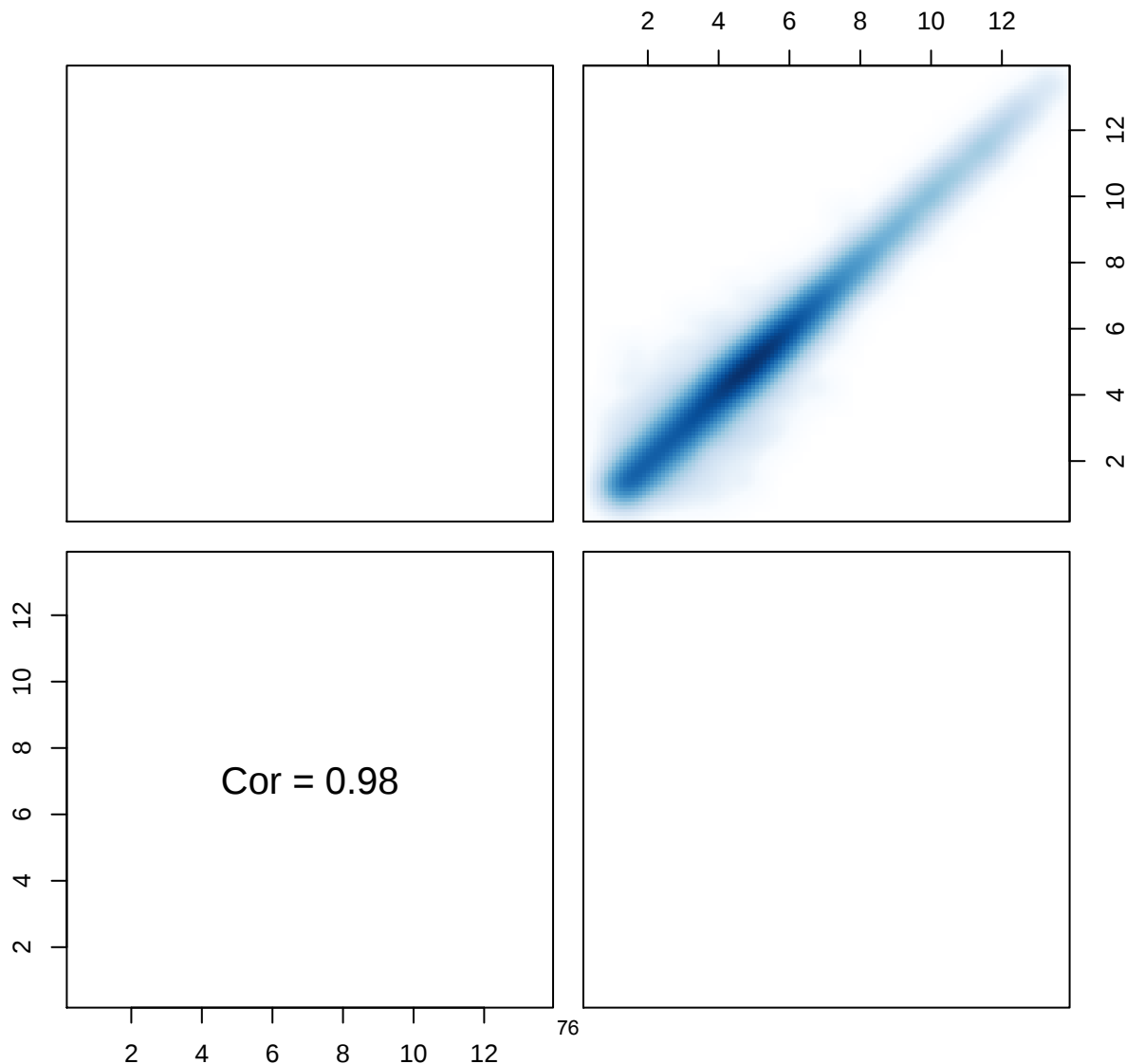
NuGen + Affymetrix -- Ovarian samples. Case: G776; block age: 4



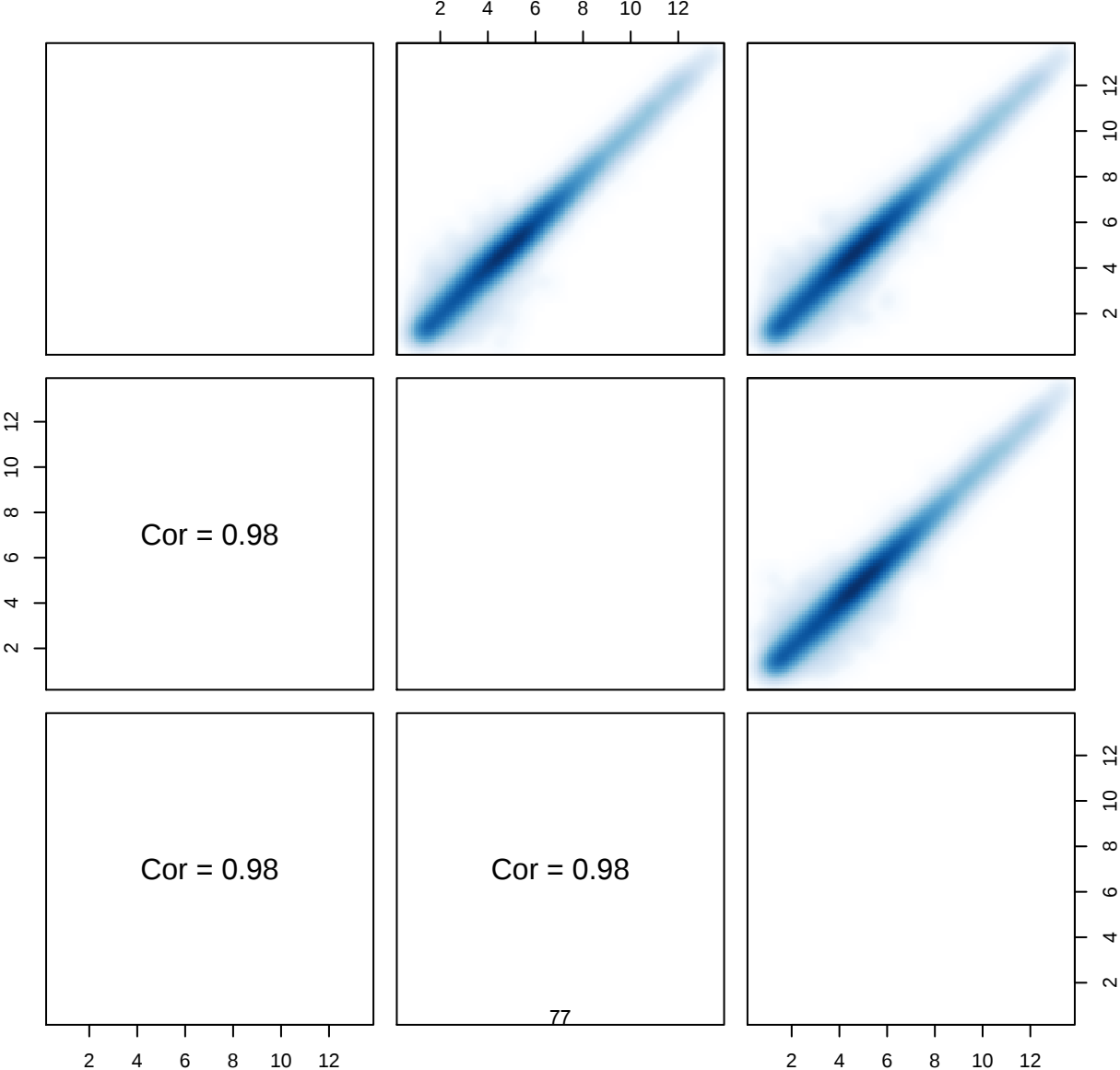
NuGen + Affymetrix -- Ovarian samples. Case: G653; block age: 7



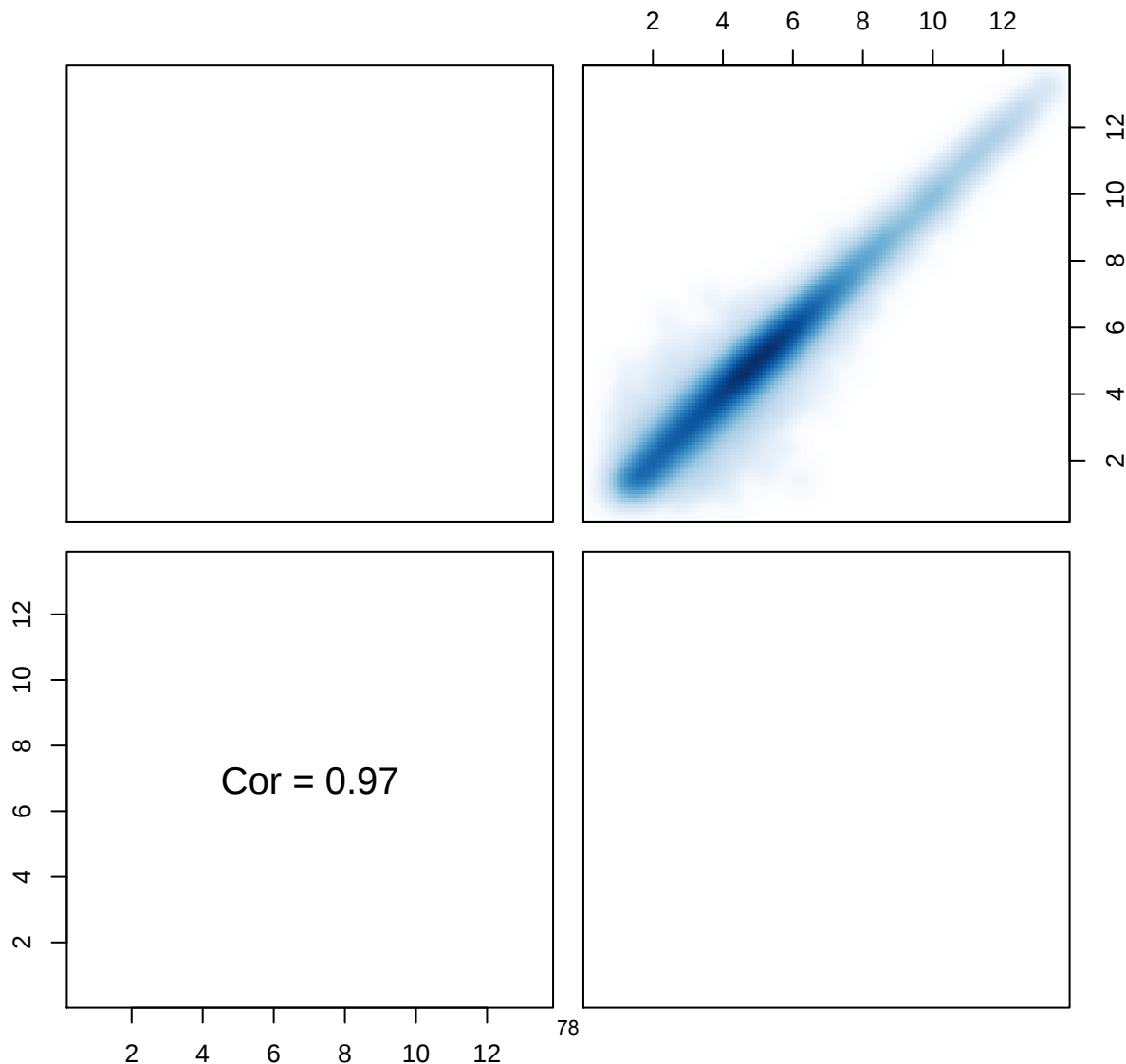
NuGen + Affymetrix -- Ovarian samples. Case: G403; block age: 5



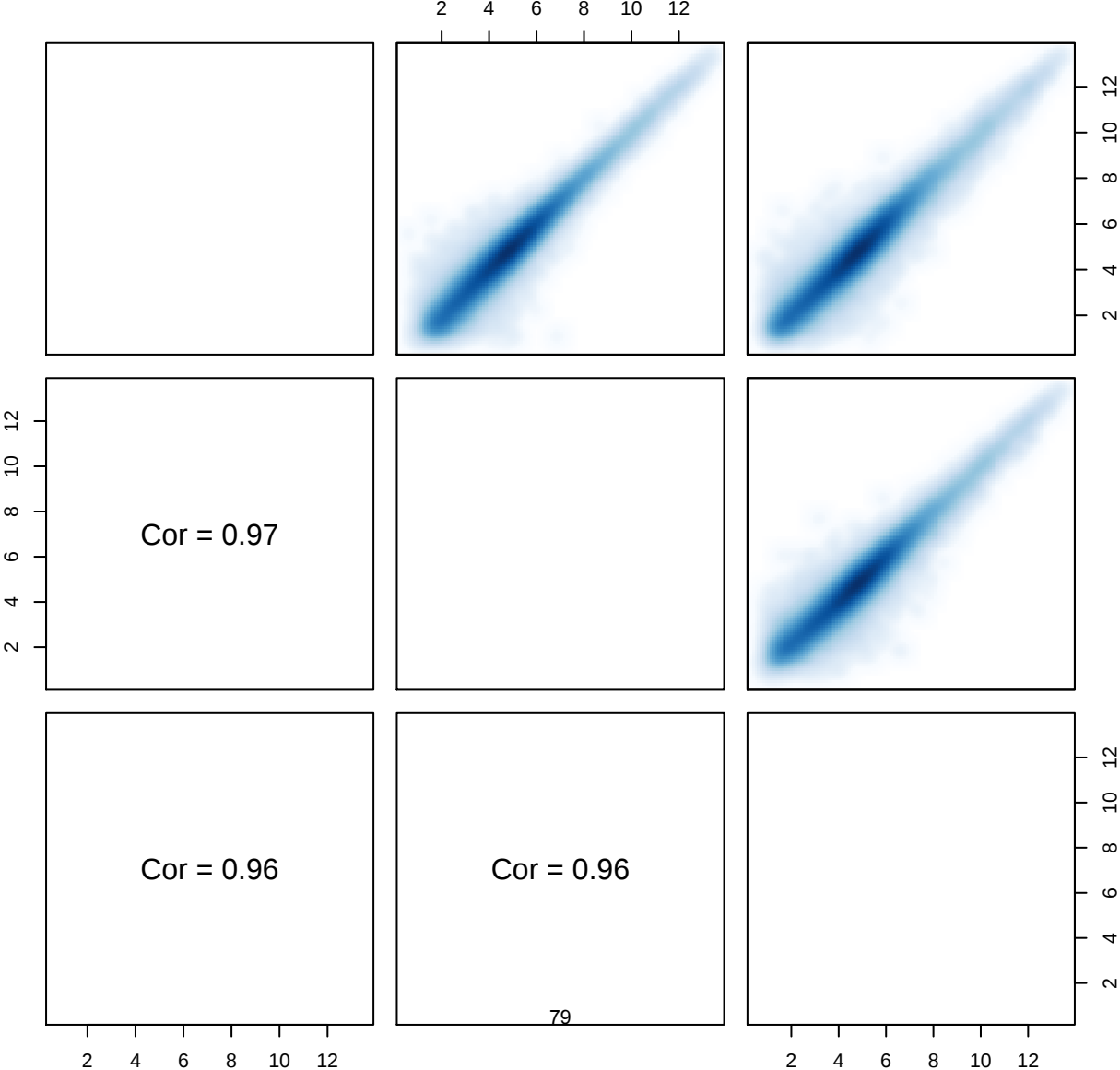
NuGen + Affymetrix -- Ovarian samples. Case: G765; block age: 4



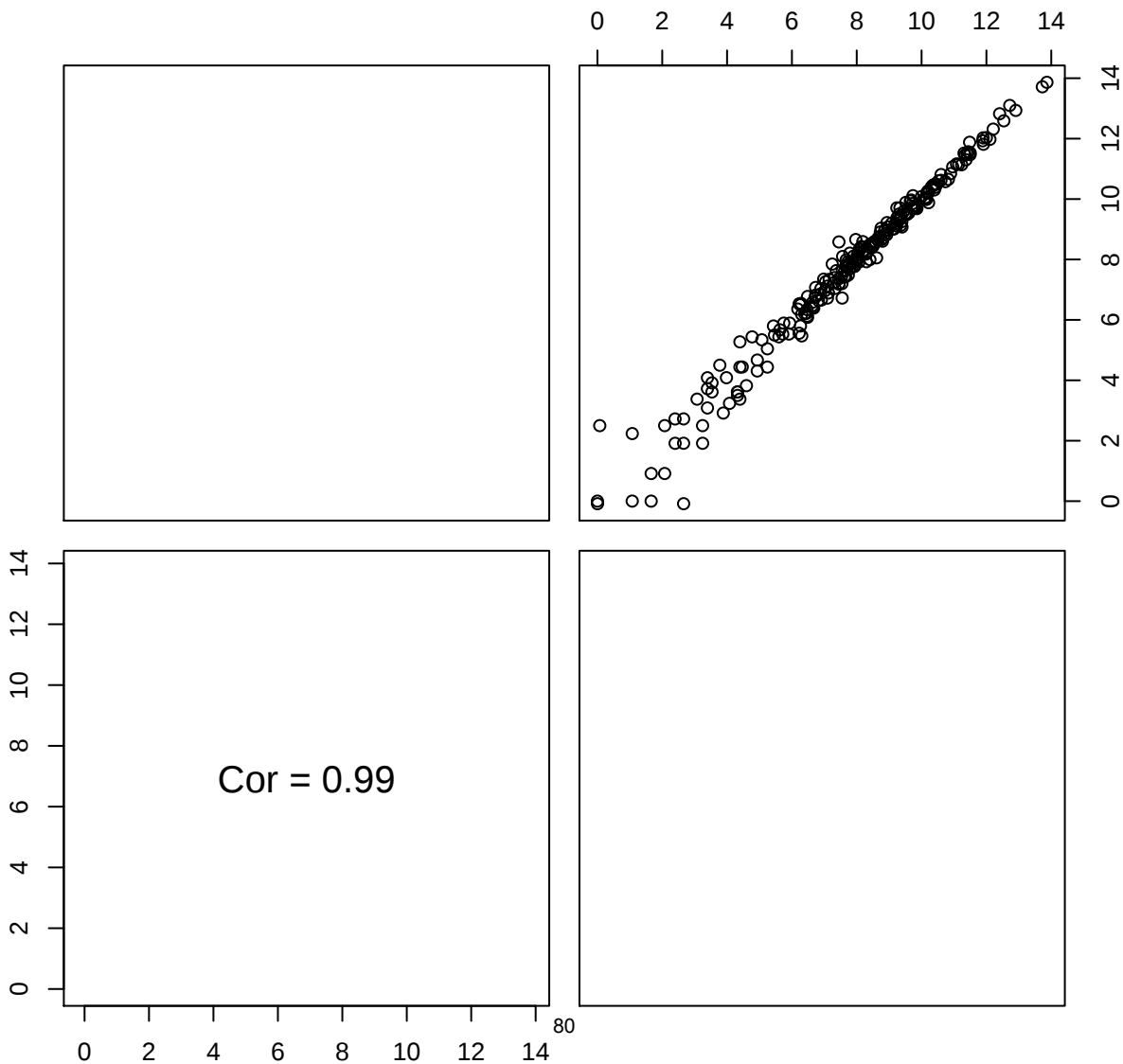
NuGen + Affymetrix -- Ovarian samples. Case: G969; block age: 6



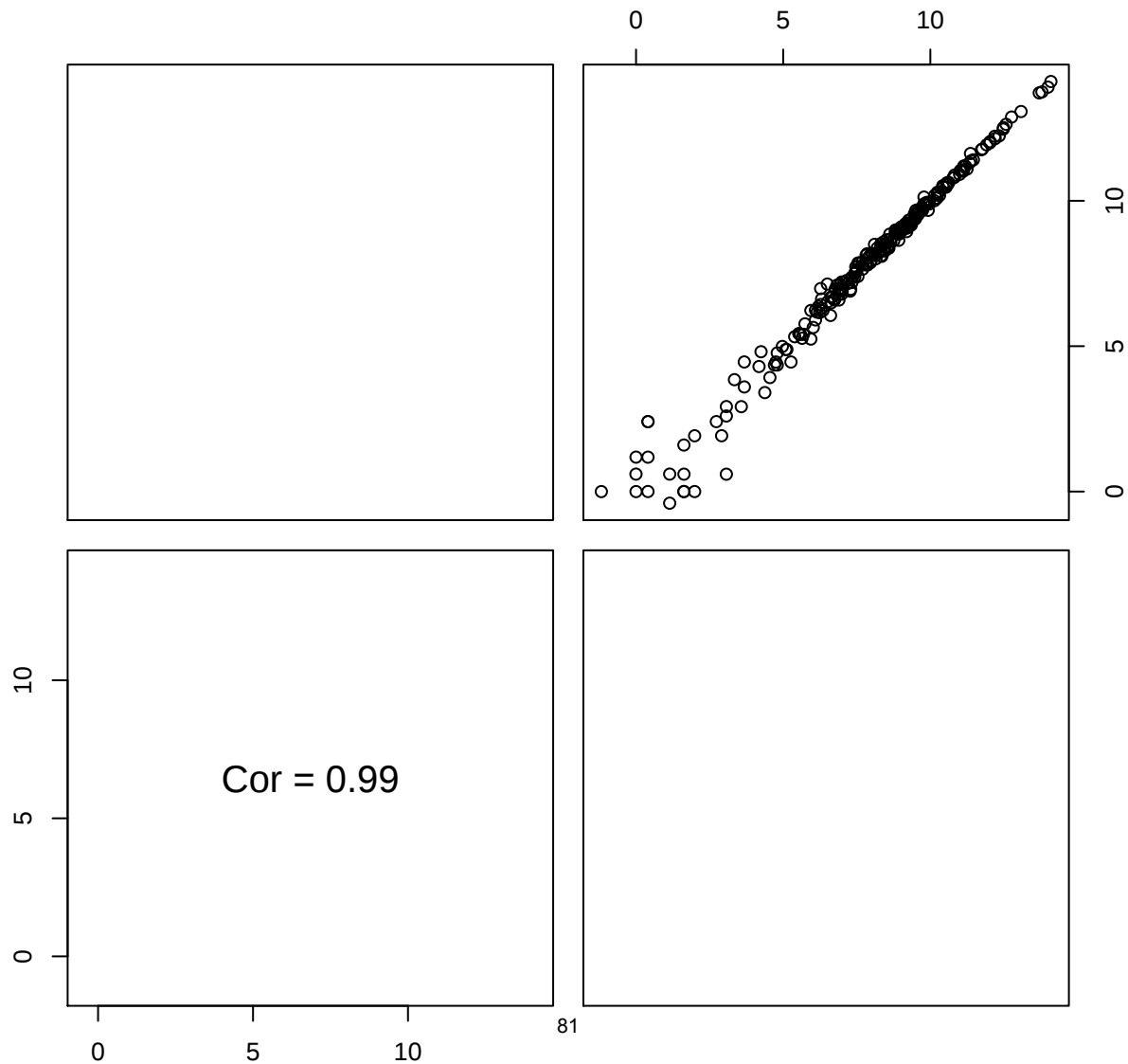
NuGen + Affymetrix -- Ovarian samples. Case: G901; block age: 6



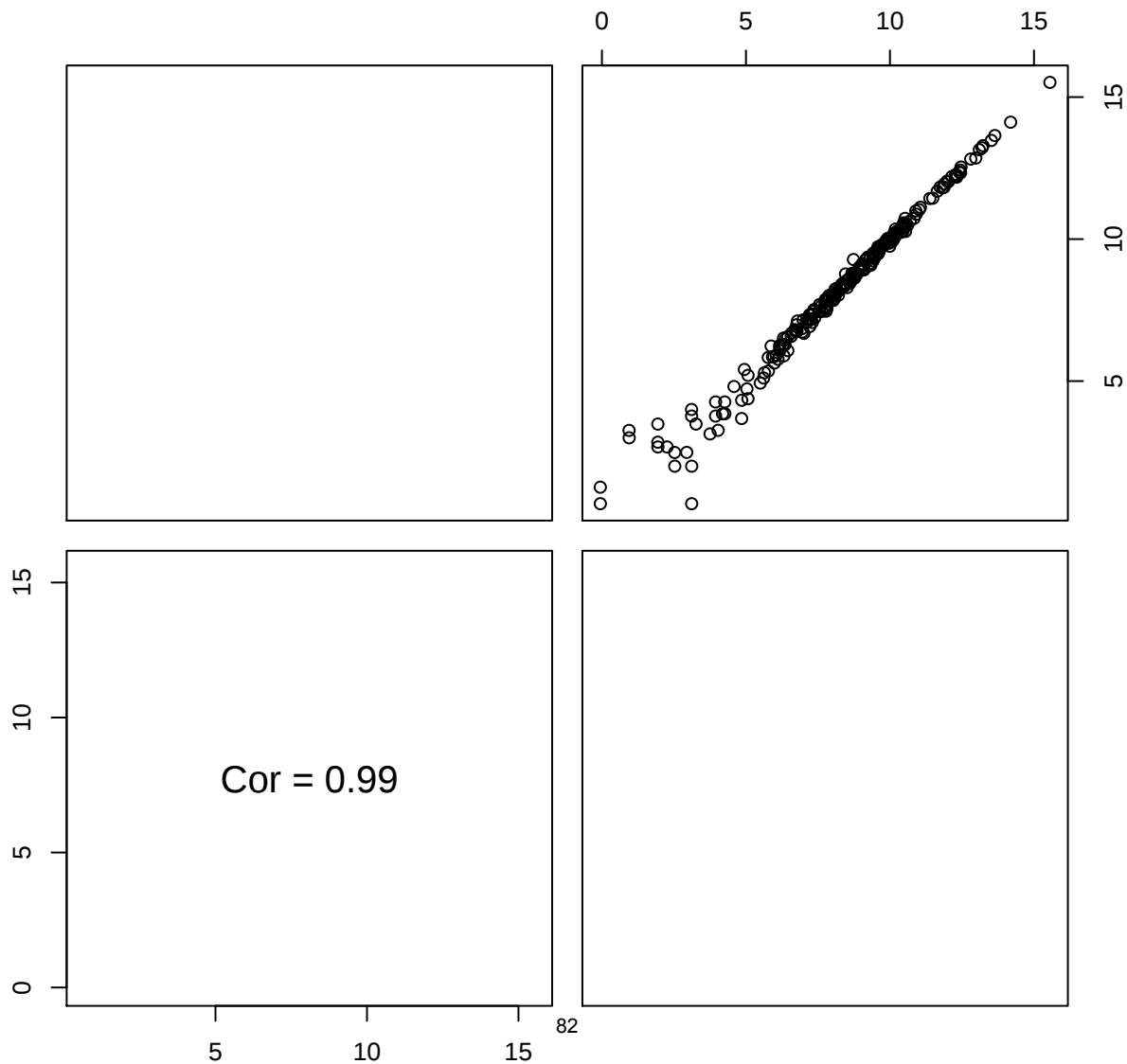
Nanostring -- Ovarian samples. Case: G366; block age: 6



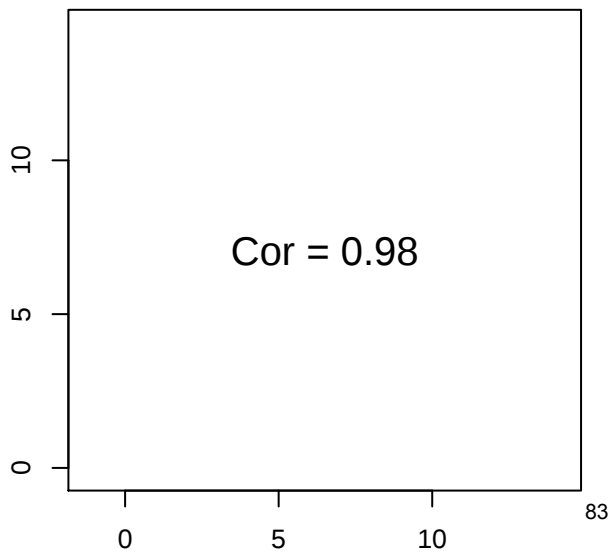
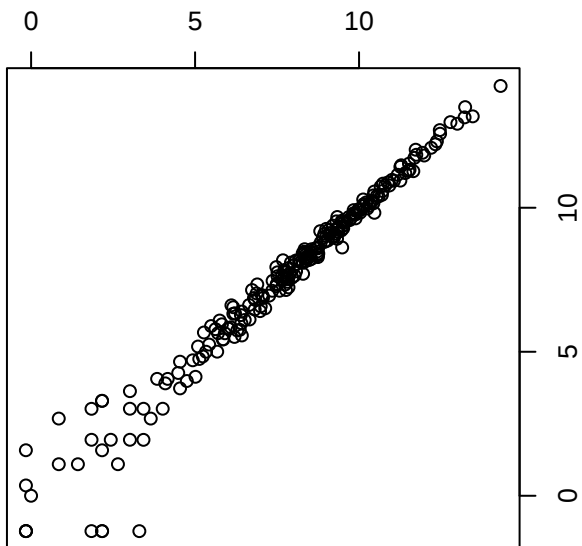
Nanostring -- Ovarian samples. Case: G794; block age: 4



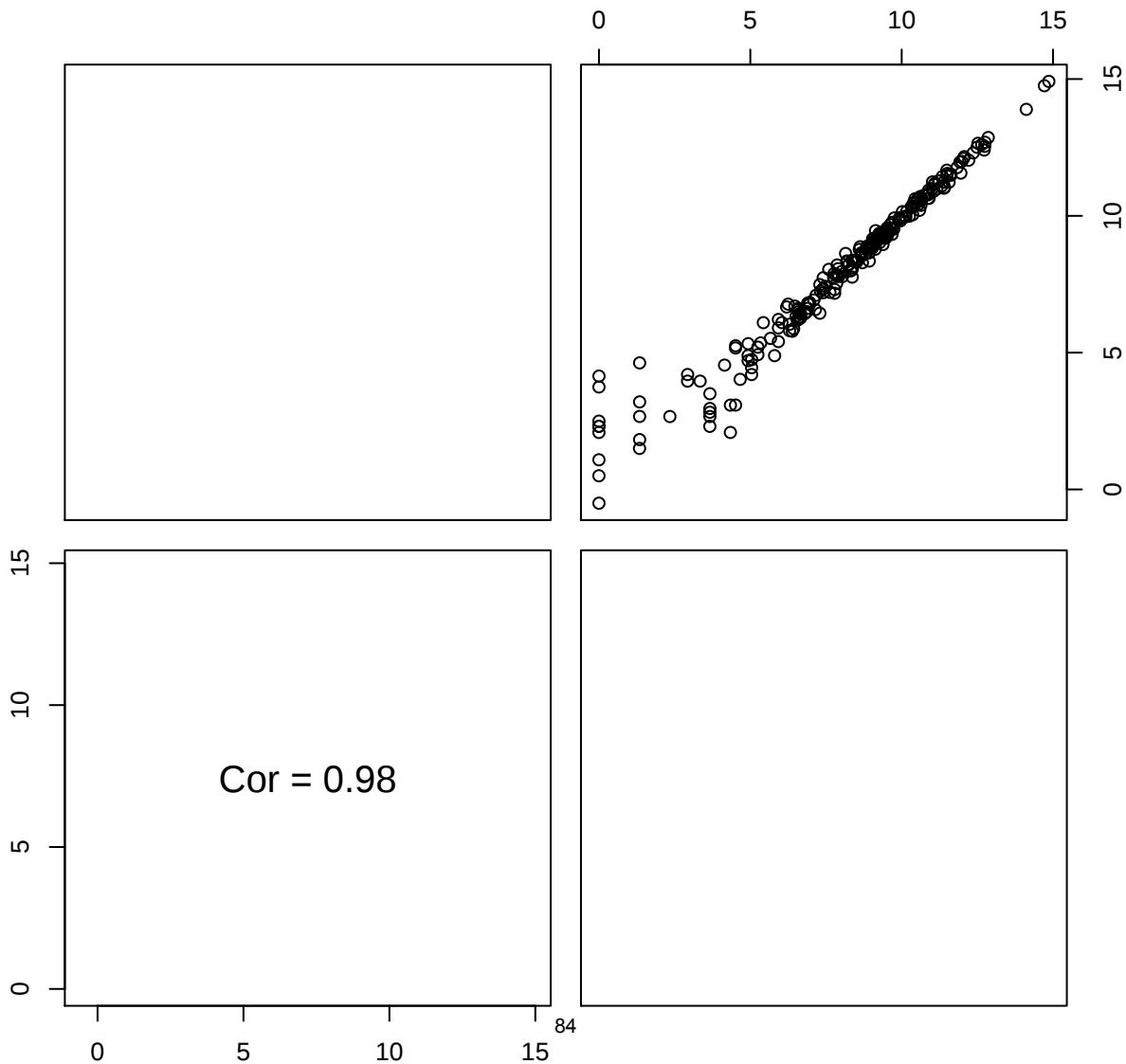
Nanostring -- Ovarian samples. Case: G678; block age: 5



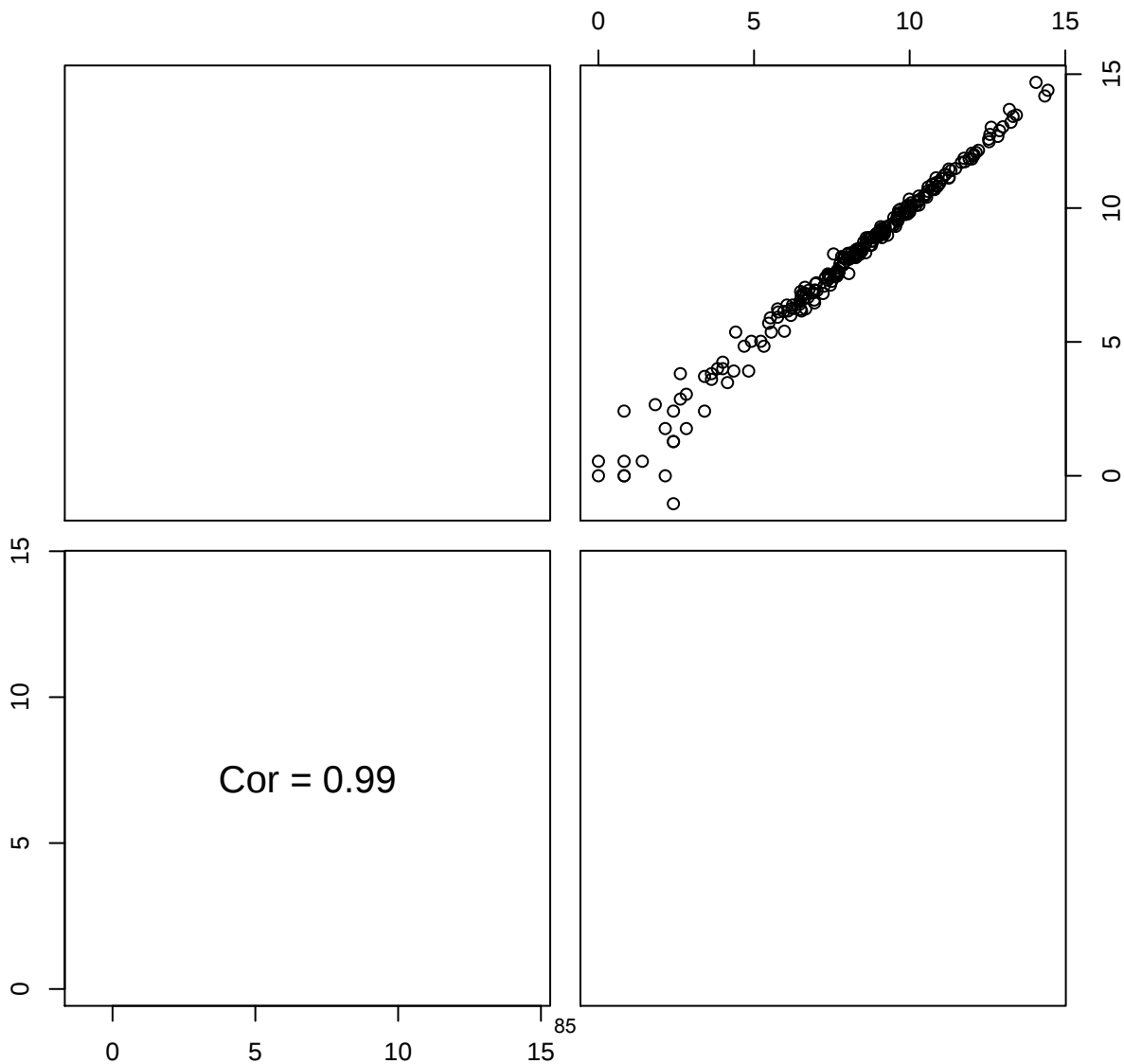
Nanostring -- Ovarian samples. Case: G776; block age: 4



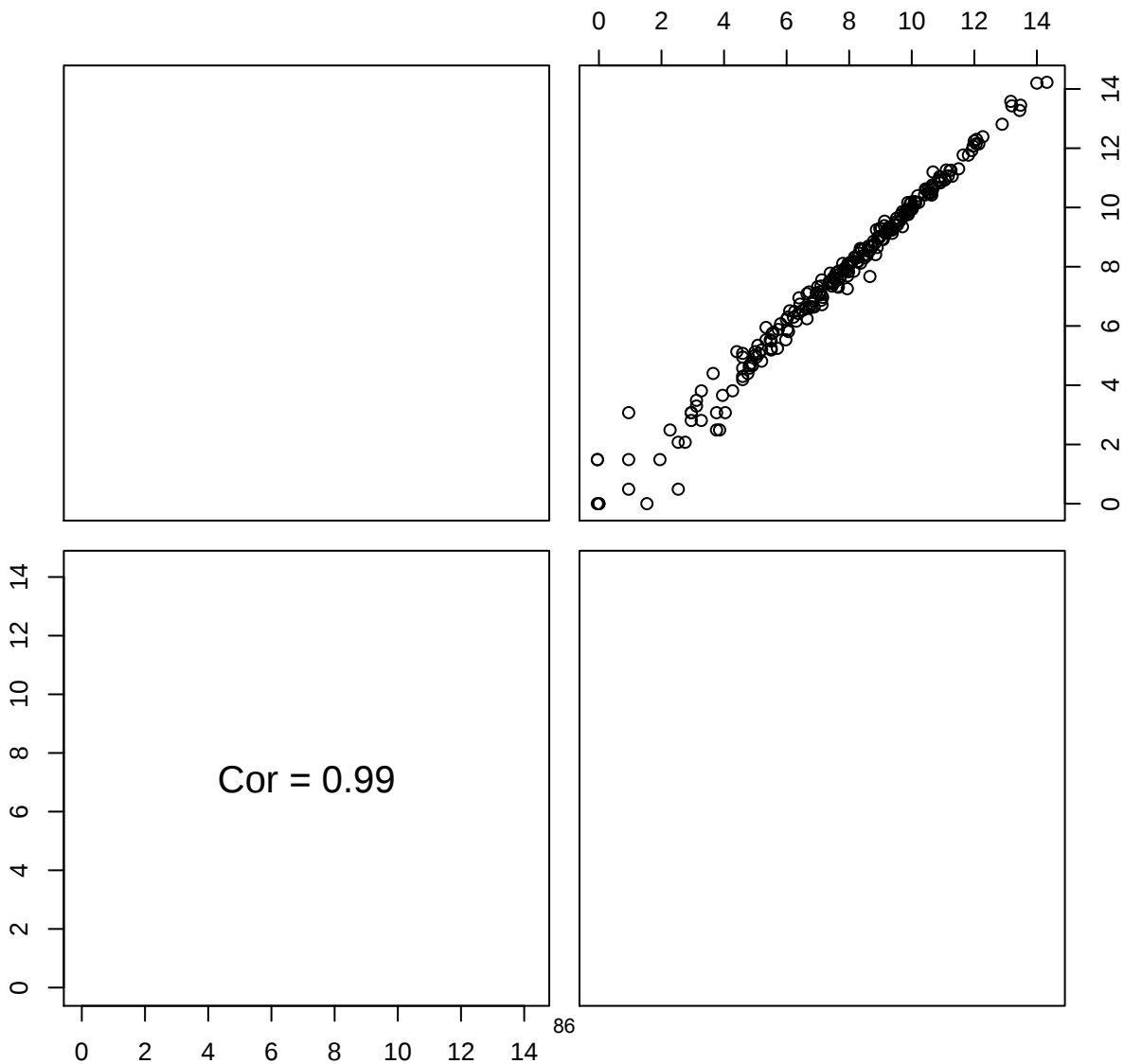
Nanostring -- Ovarian samples. Case: G653; block age: 7



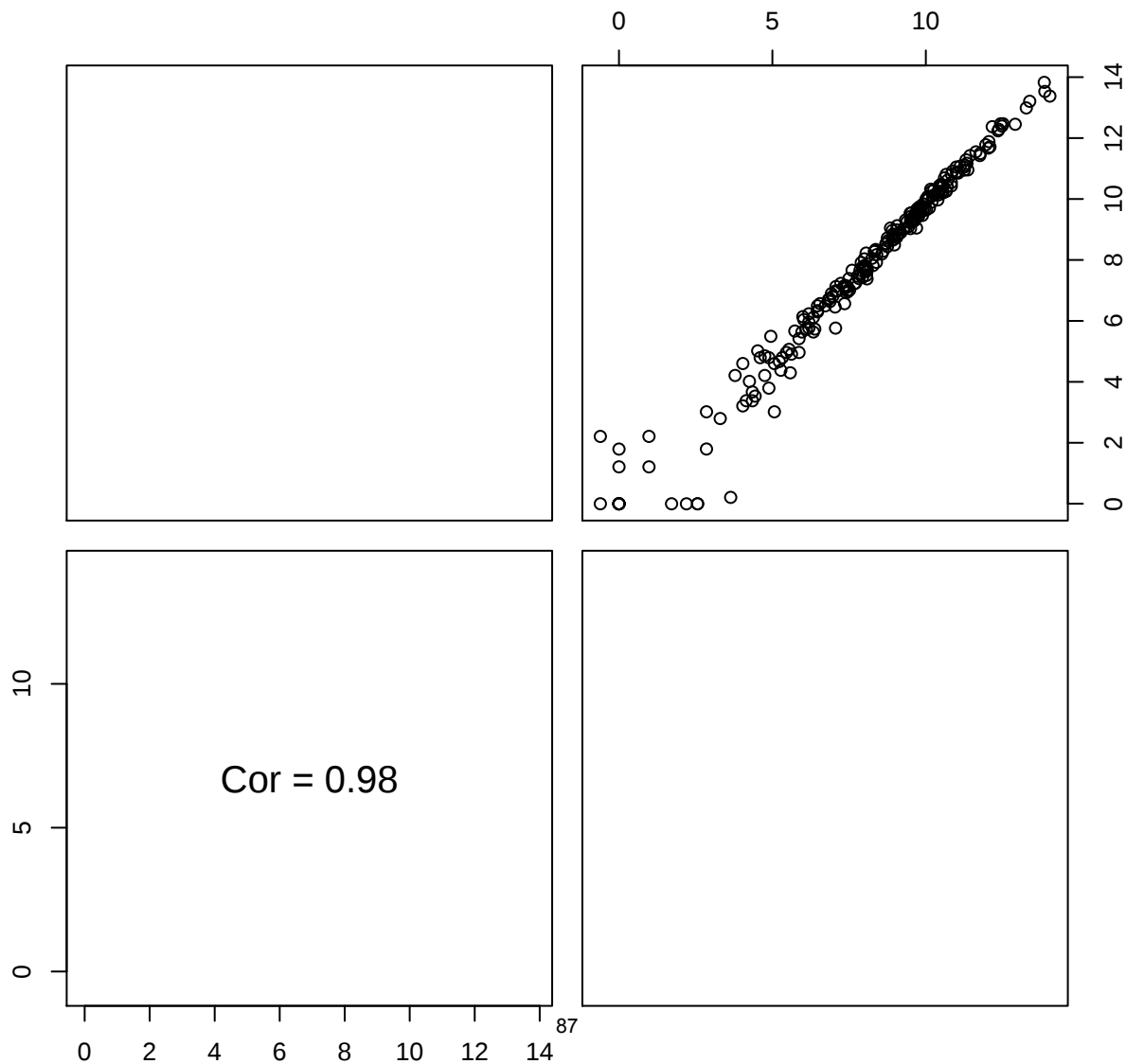
Nanostring -- Ovarian samples. Case: G403; block age: 5



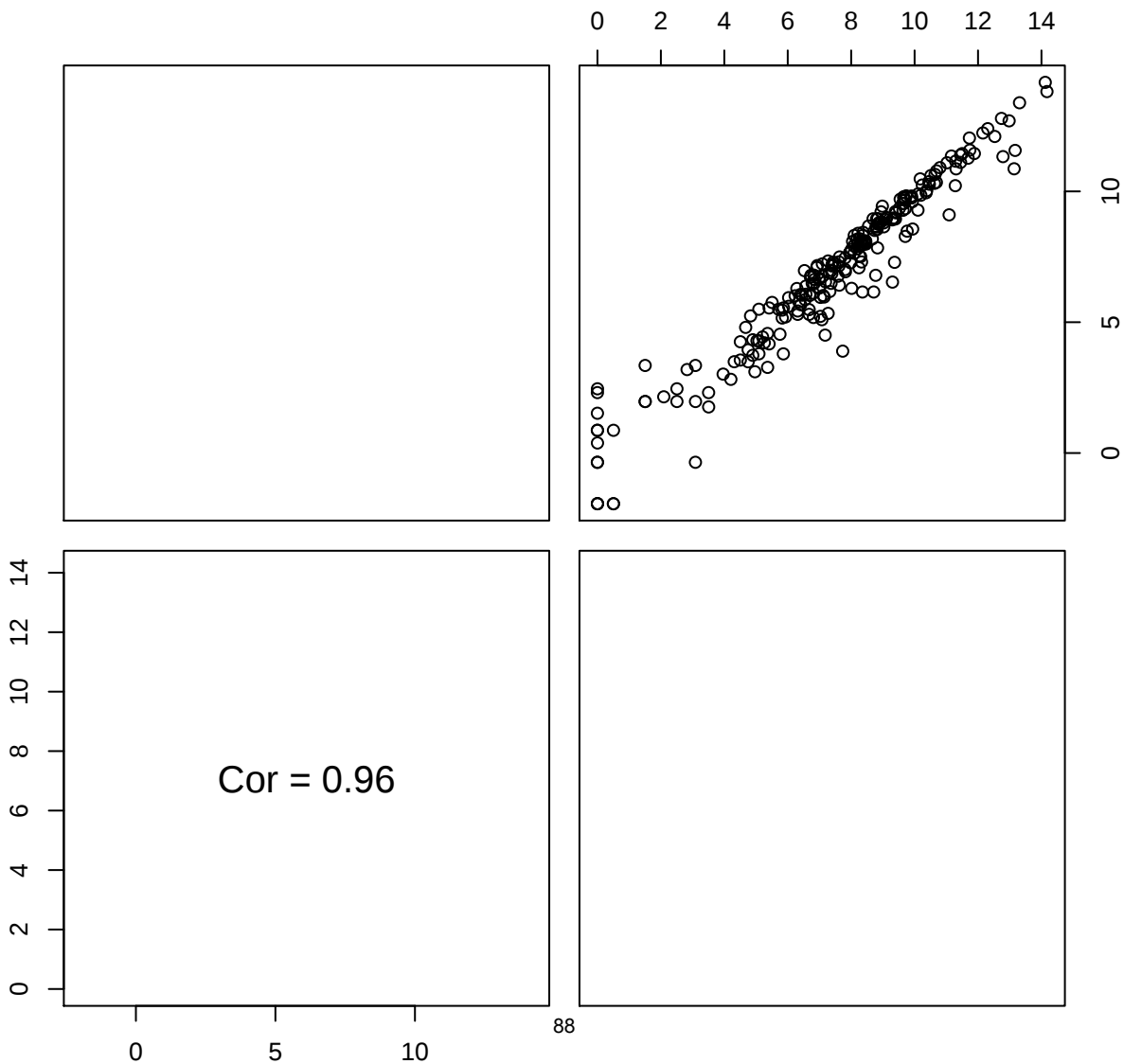
Nanostring -- Ovarian samples. Case: G765; block age: 4



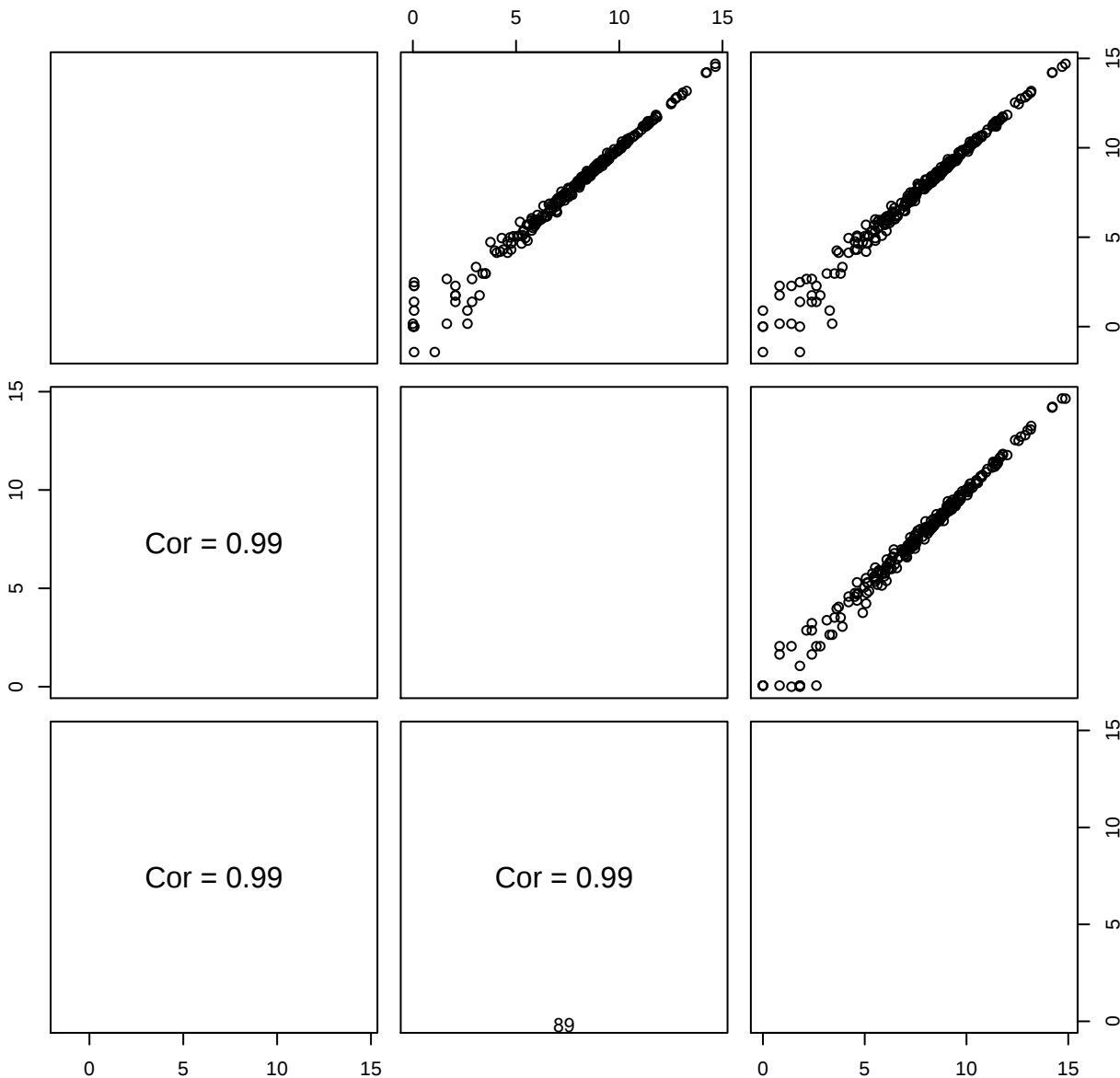
Nanostring -- Ovarian samples. Case: G969; block age: 6



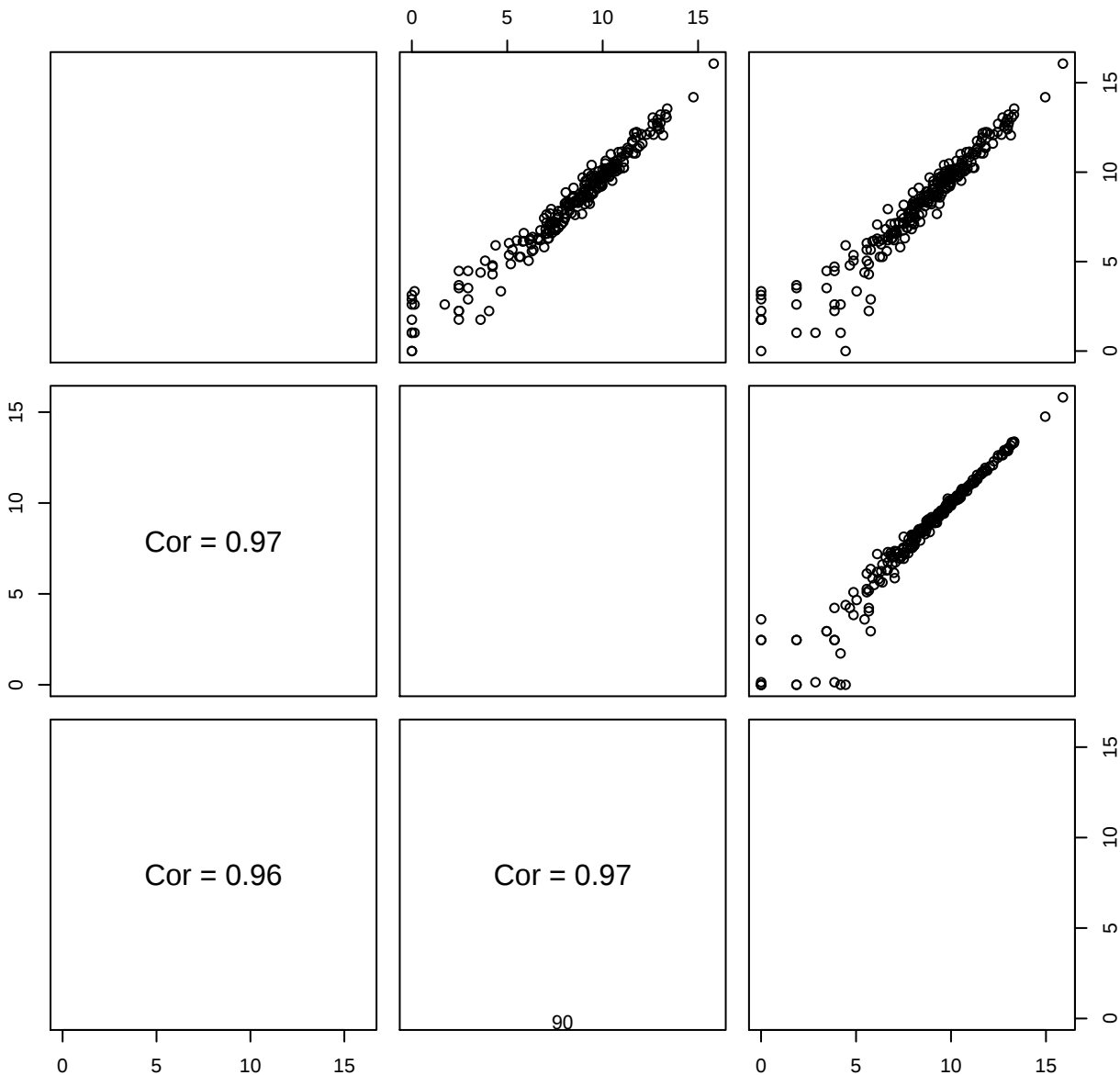
Nanostring -- Ovarian samples. Case: G901; block age: 6



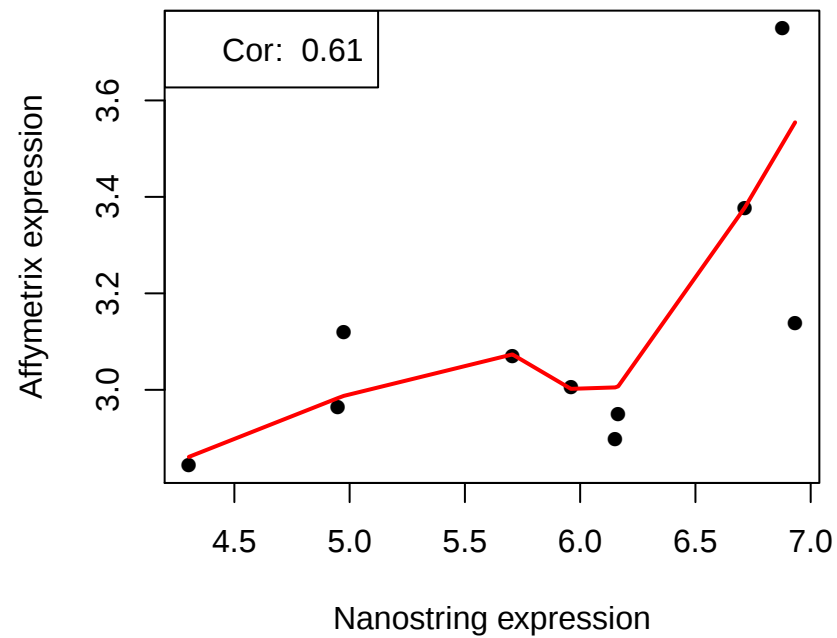
Nanostring -- Ovarian samples. Case: G800; block age: 5



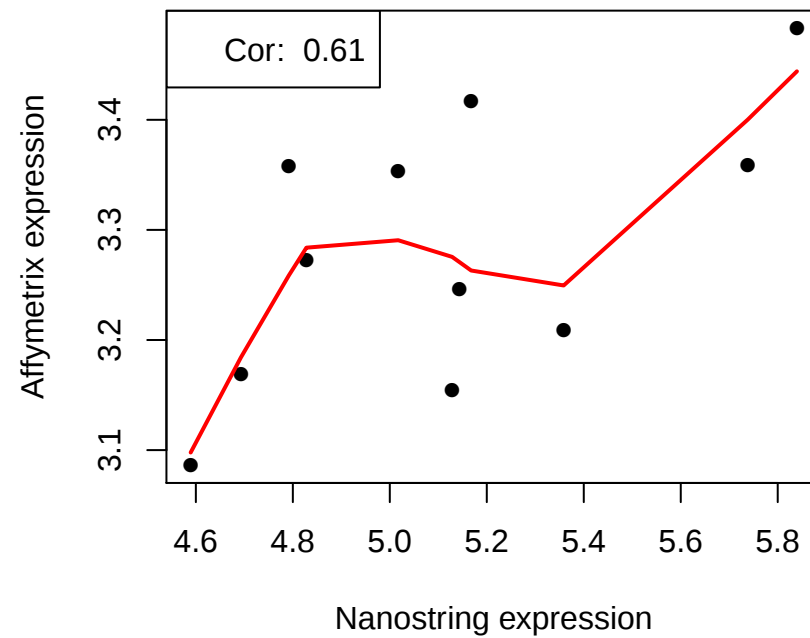
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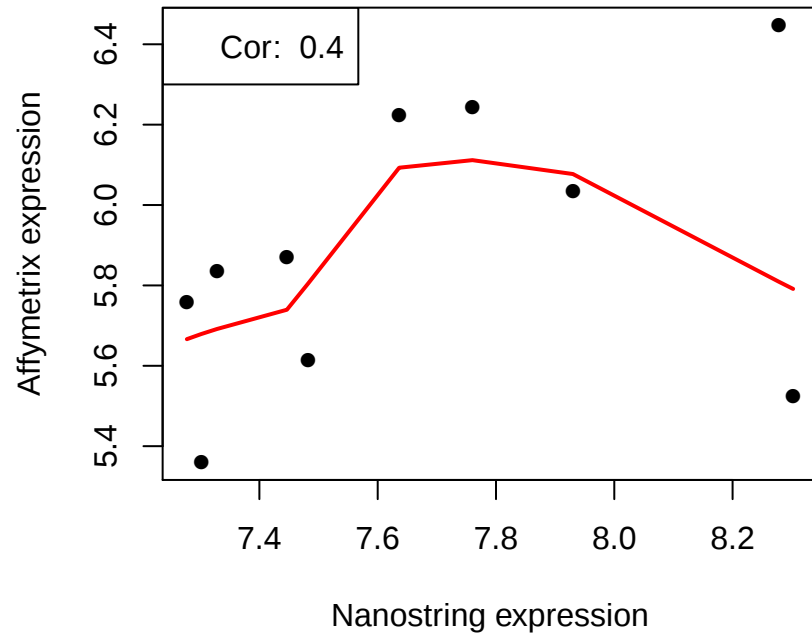
Prostate data



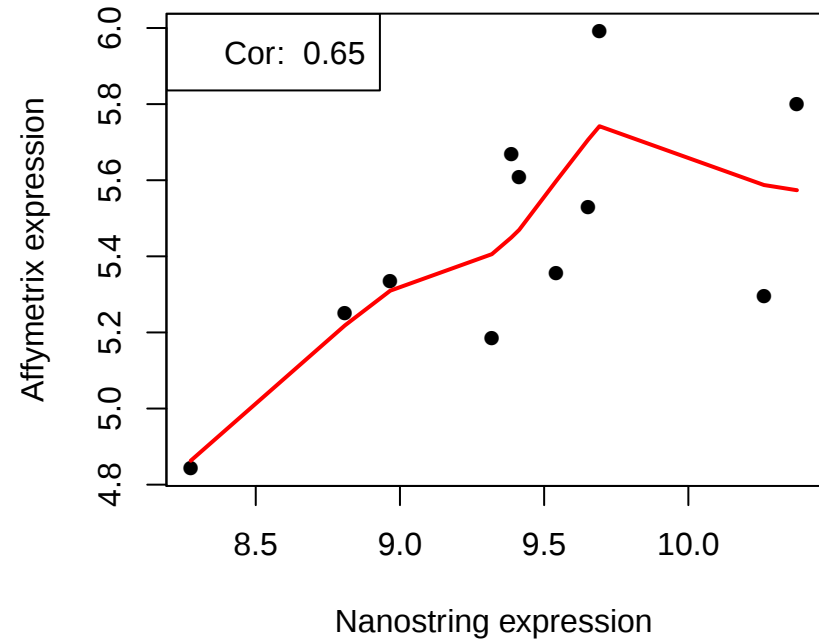
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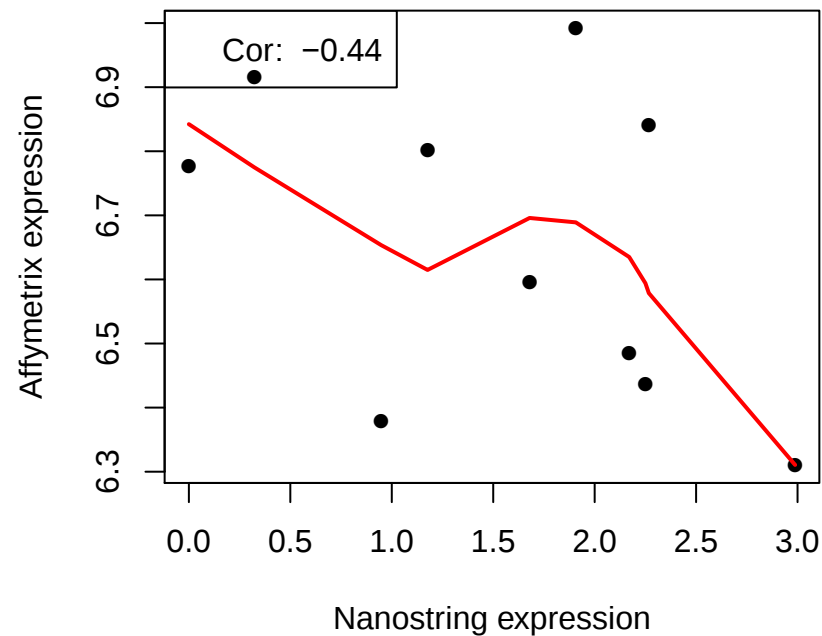
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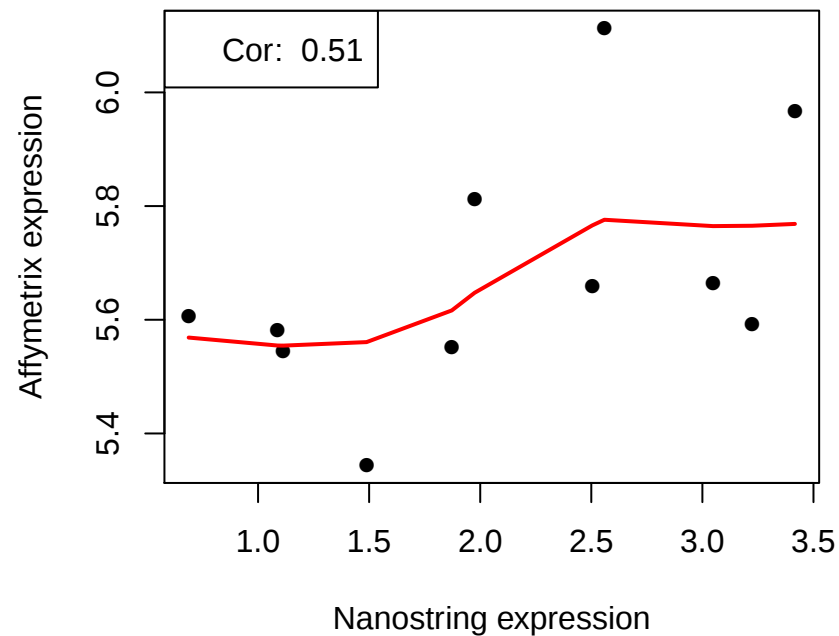
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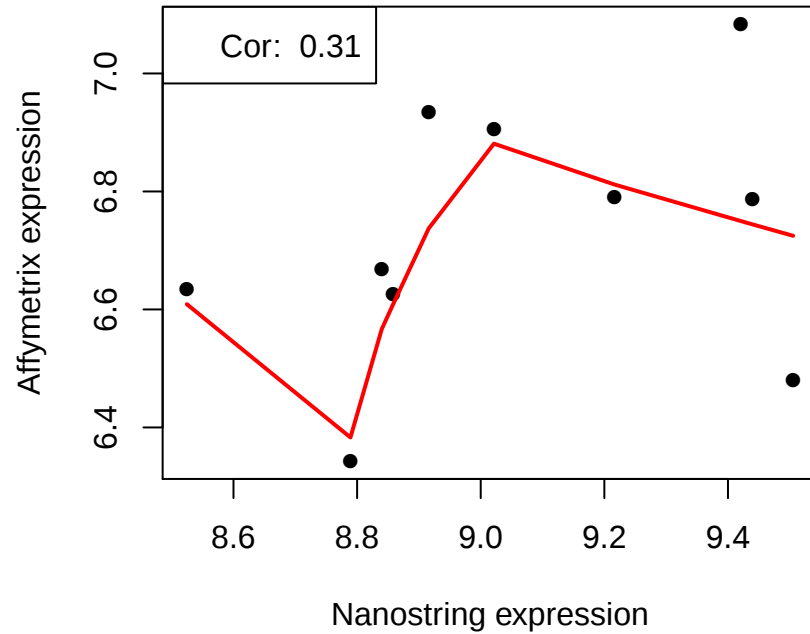
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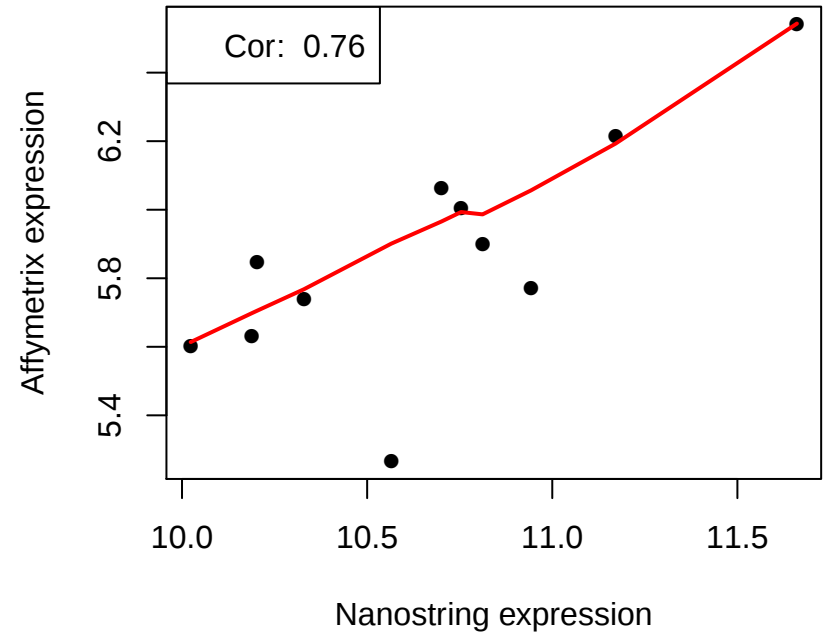
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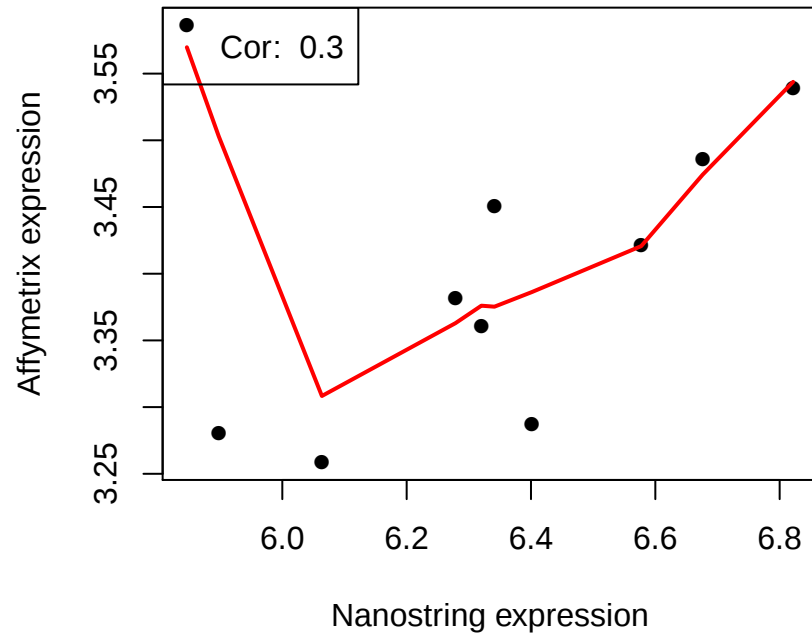
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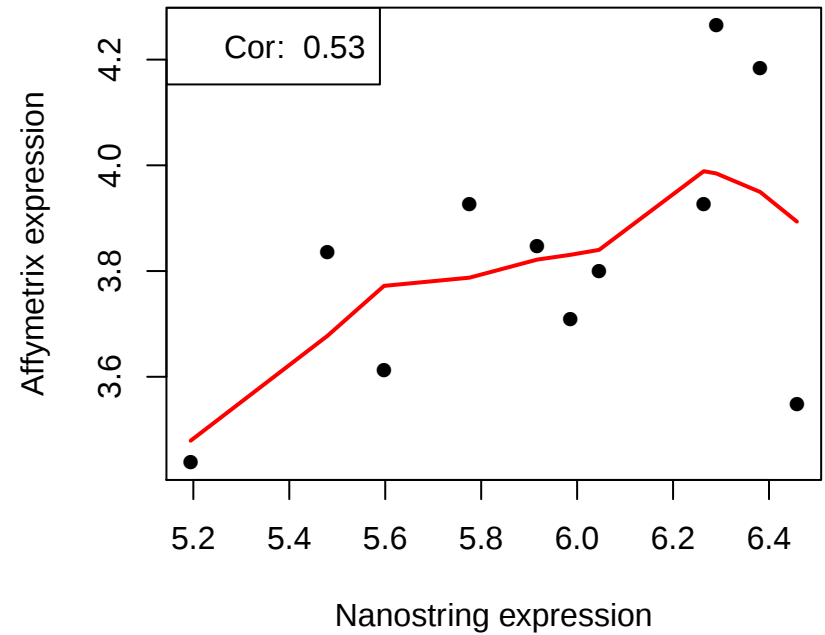
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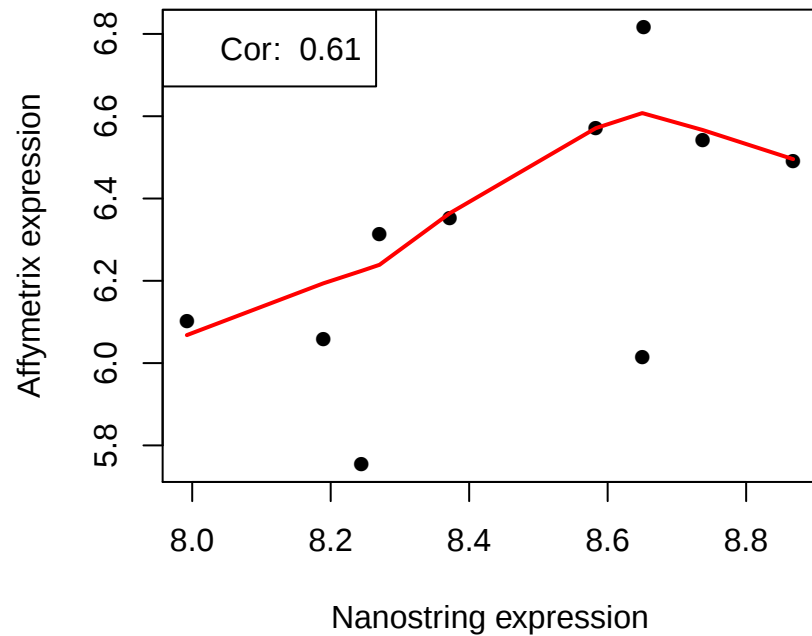
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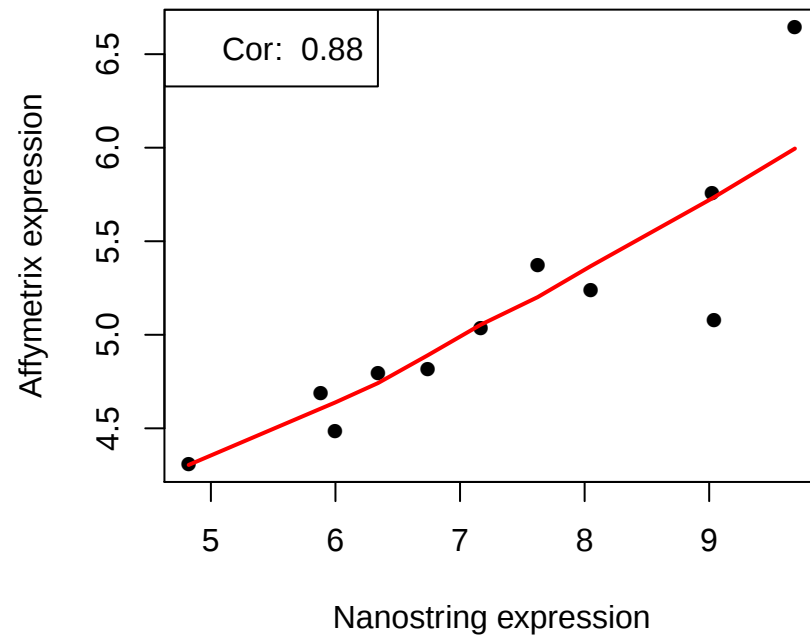
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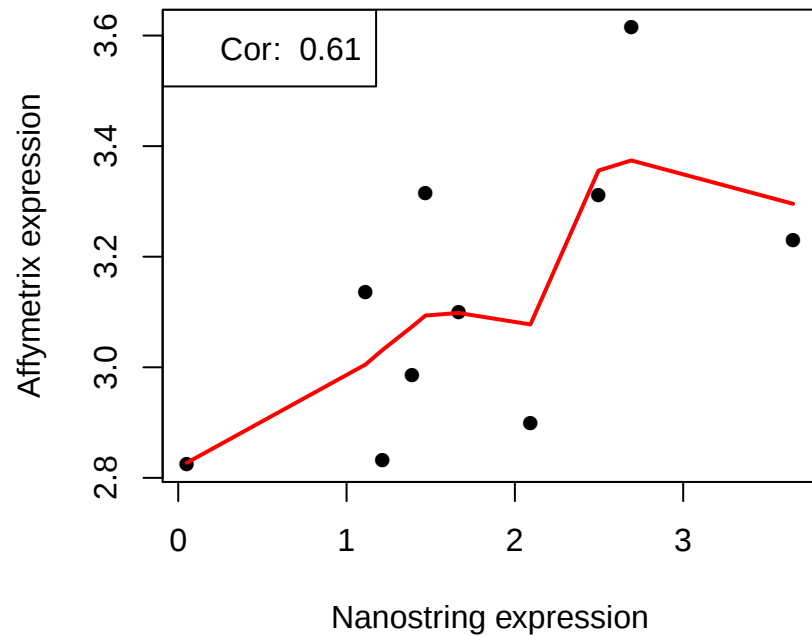
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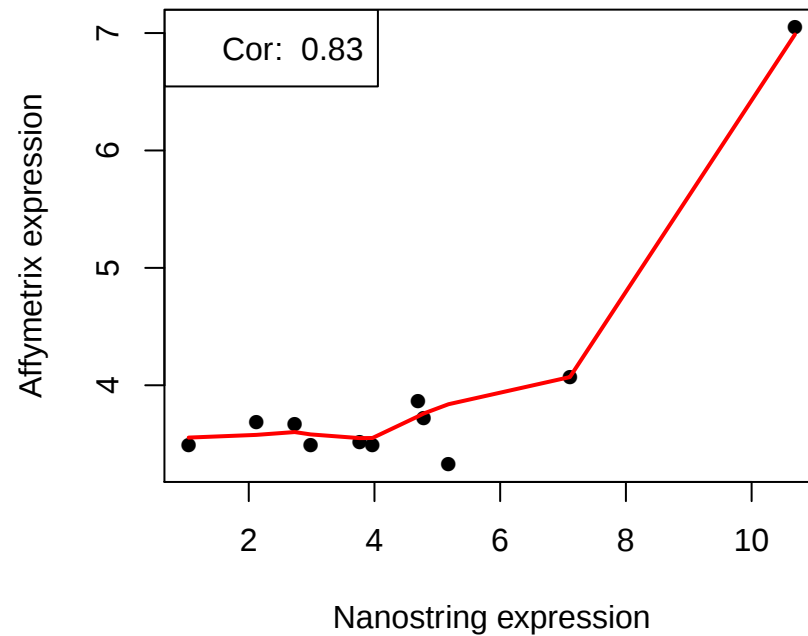
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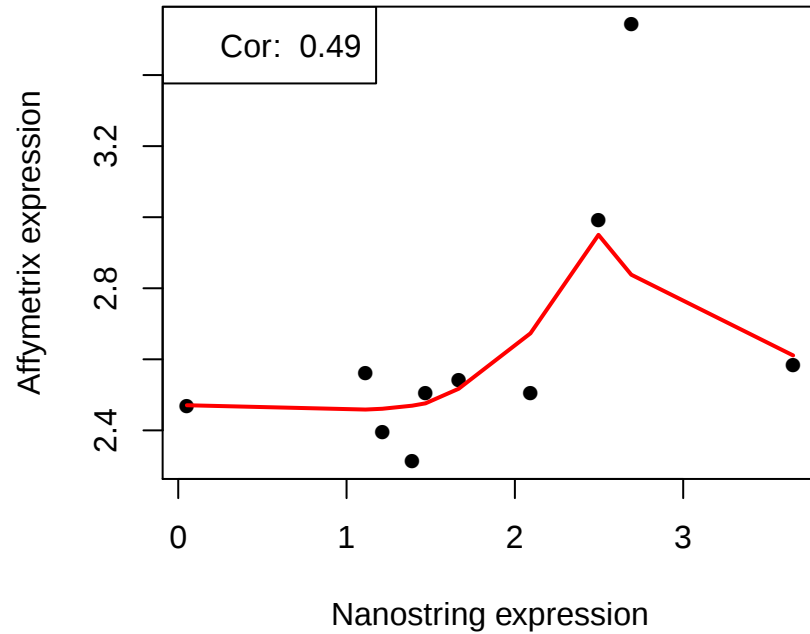
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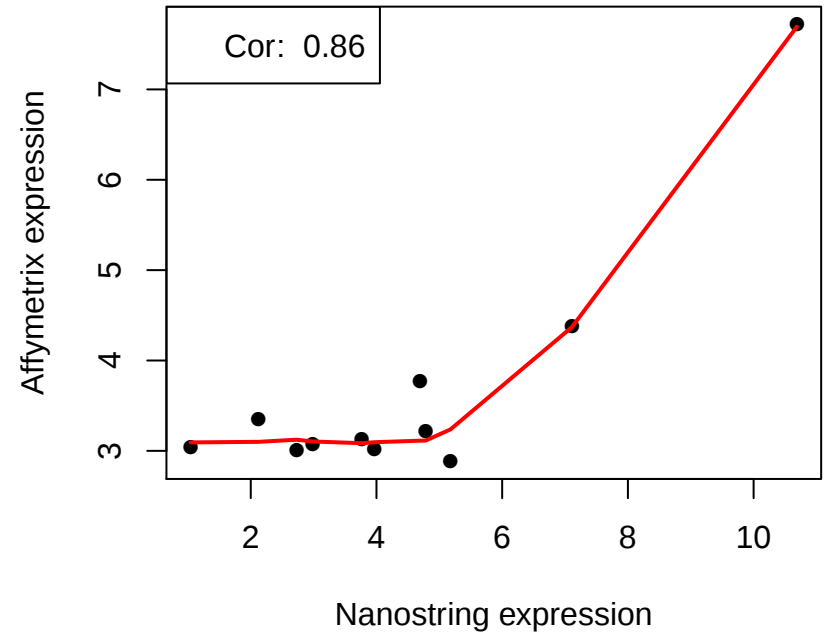
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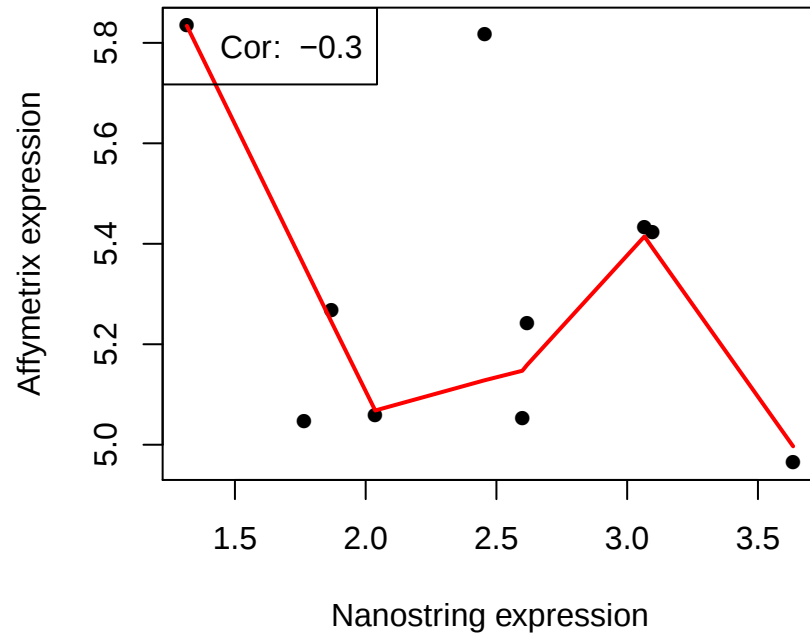
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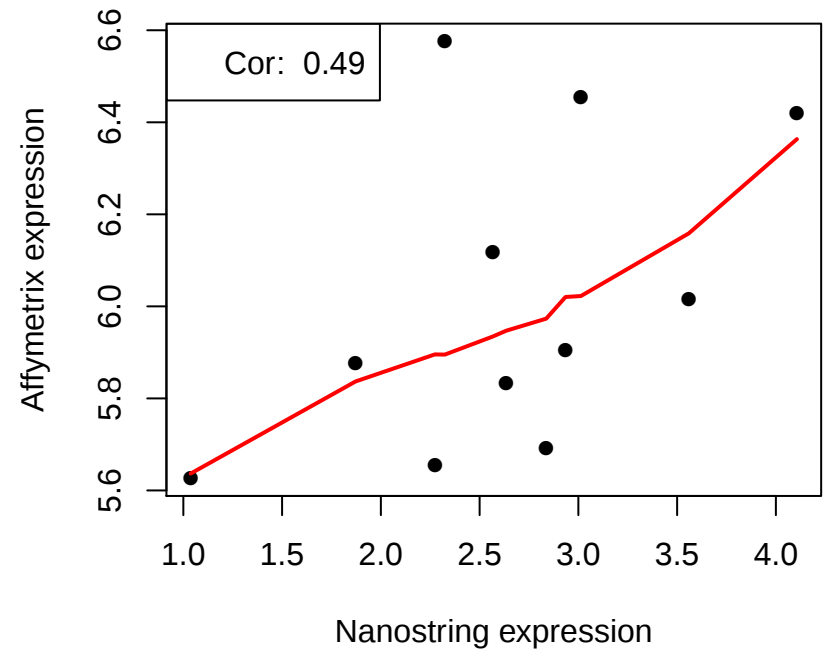
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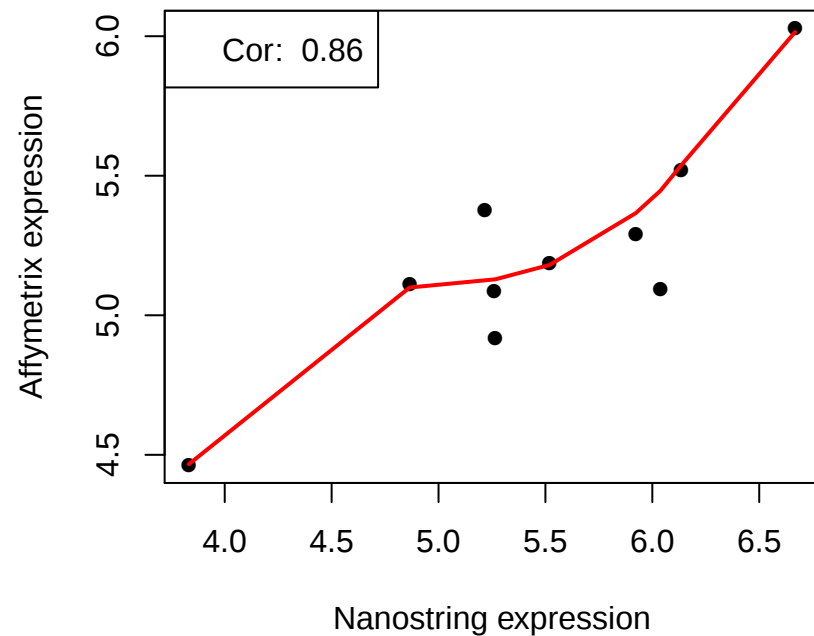
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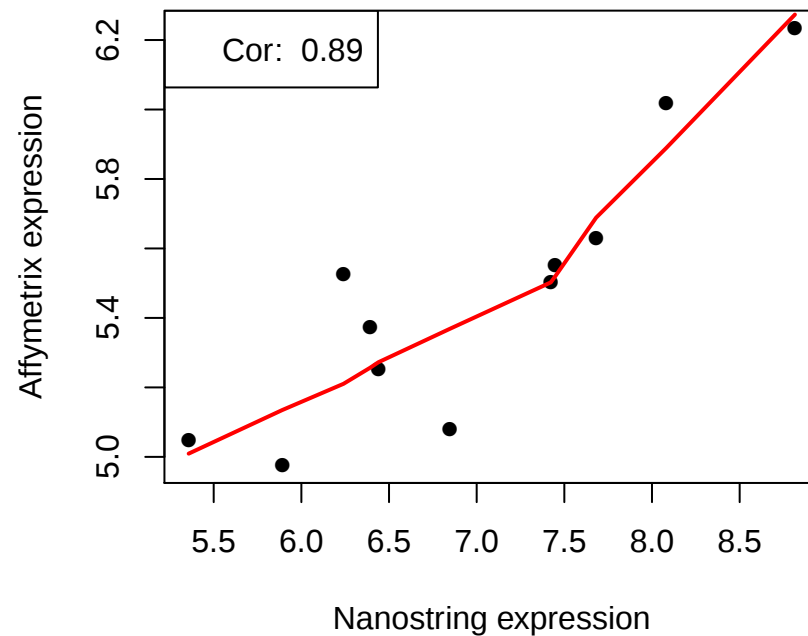
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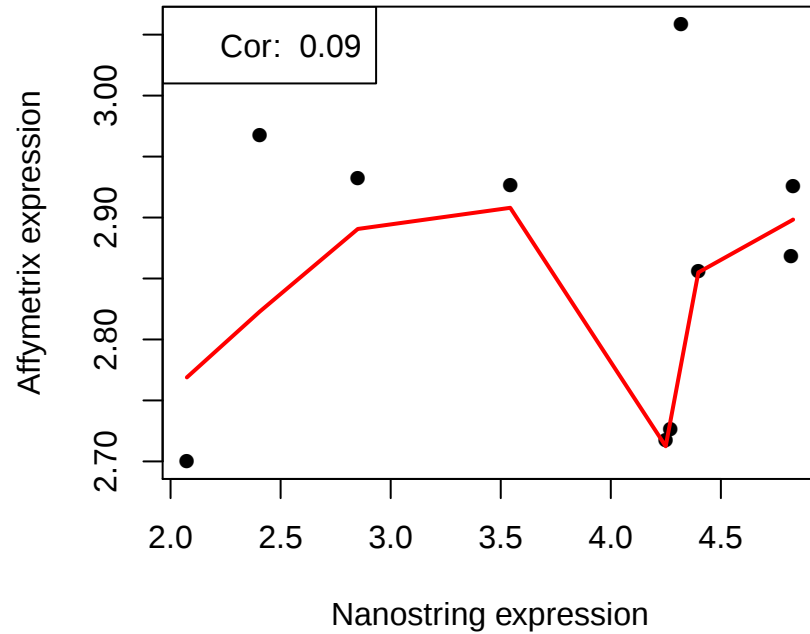
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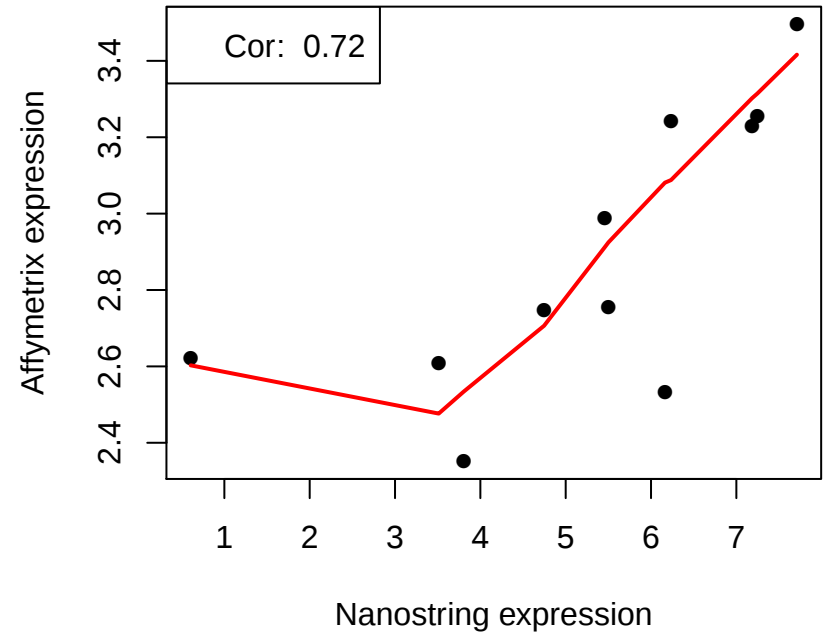
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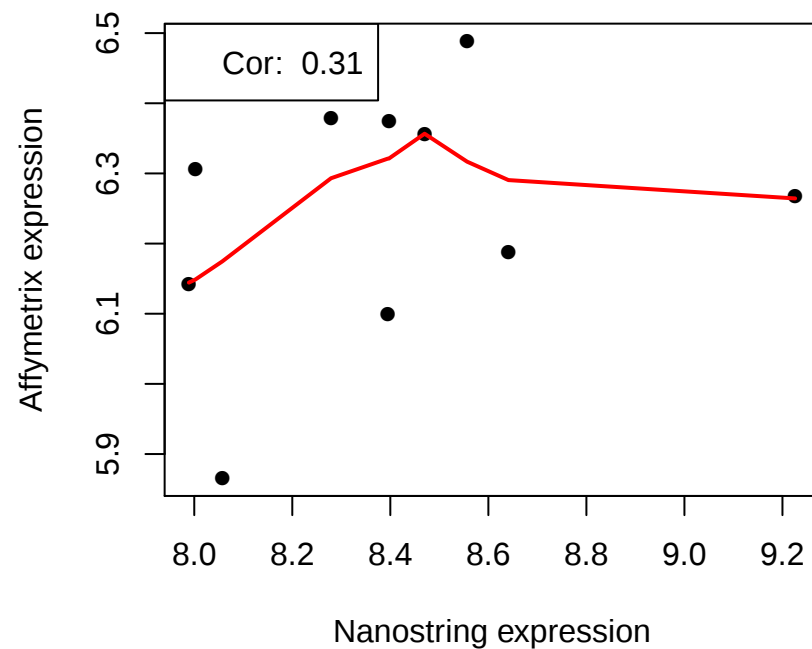
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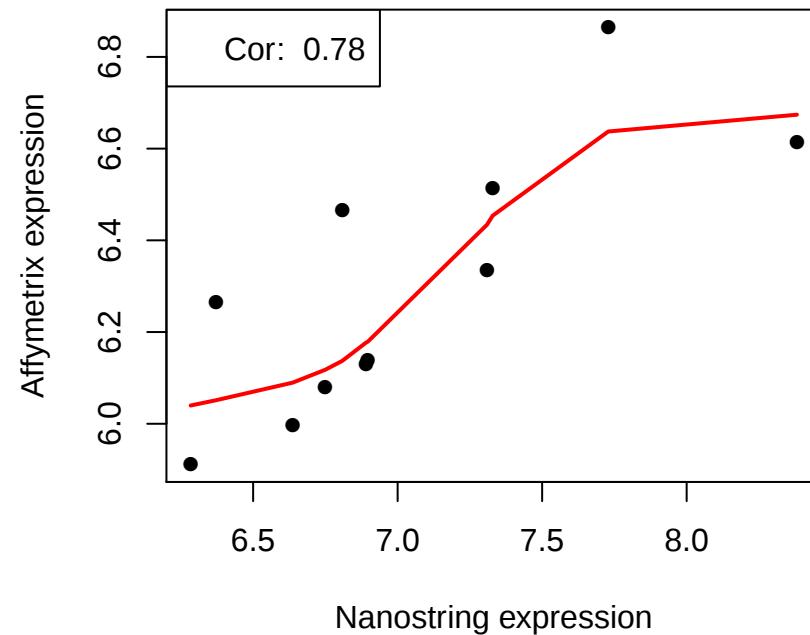
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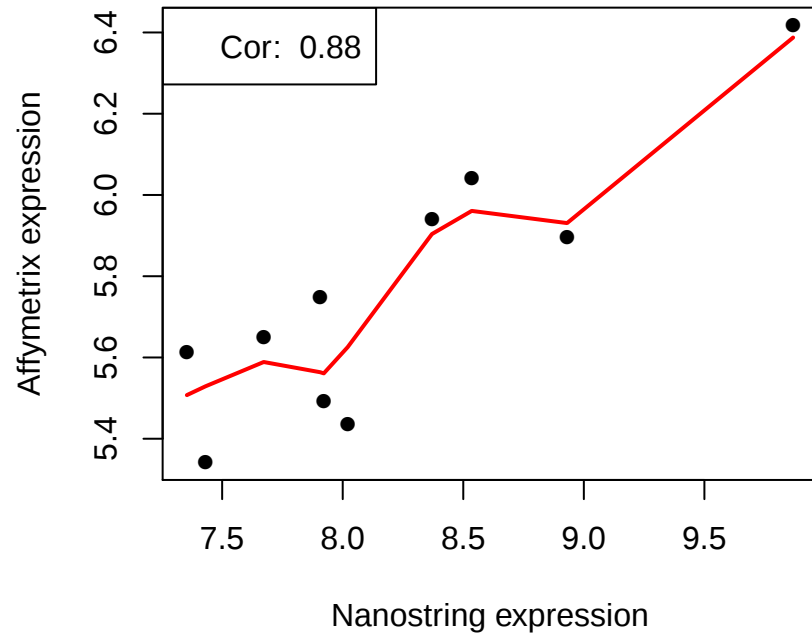
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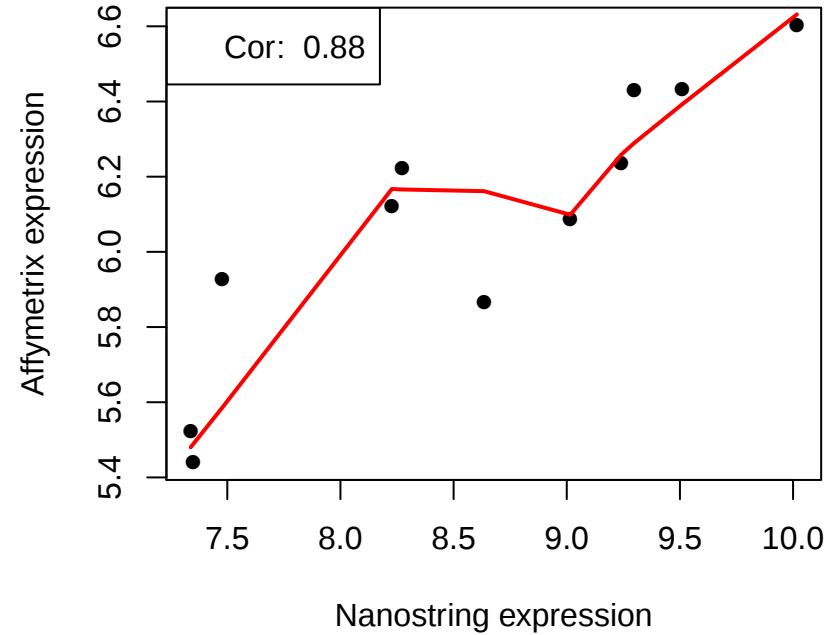
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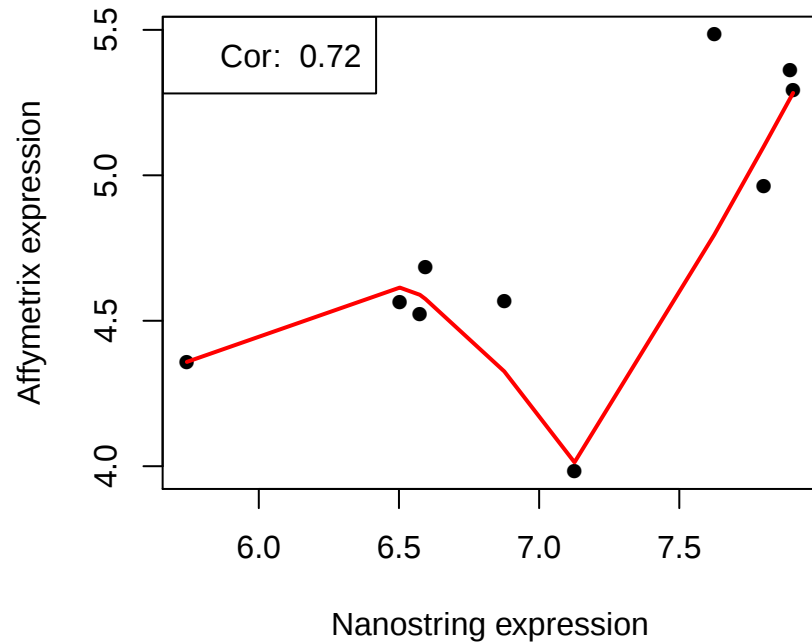
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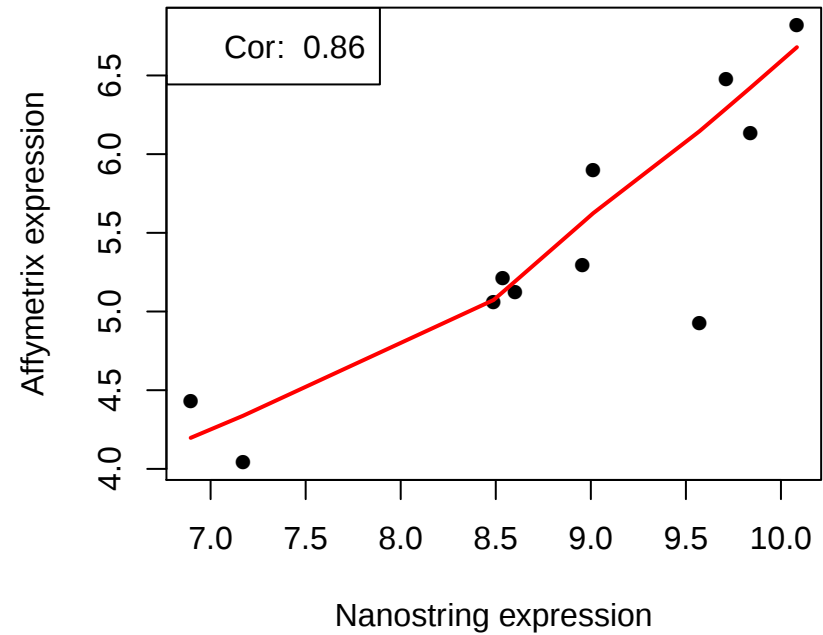
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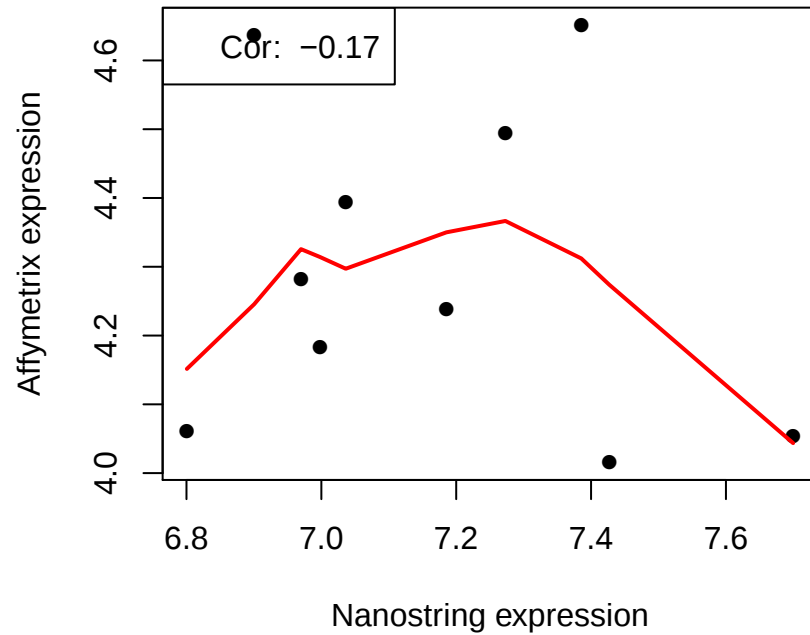
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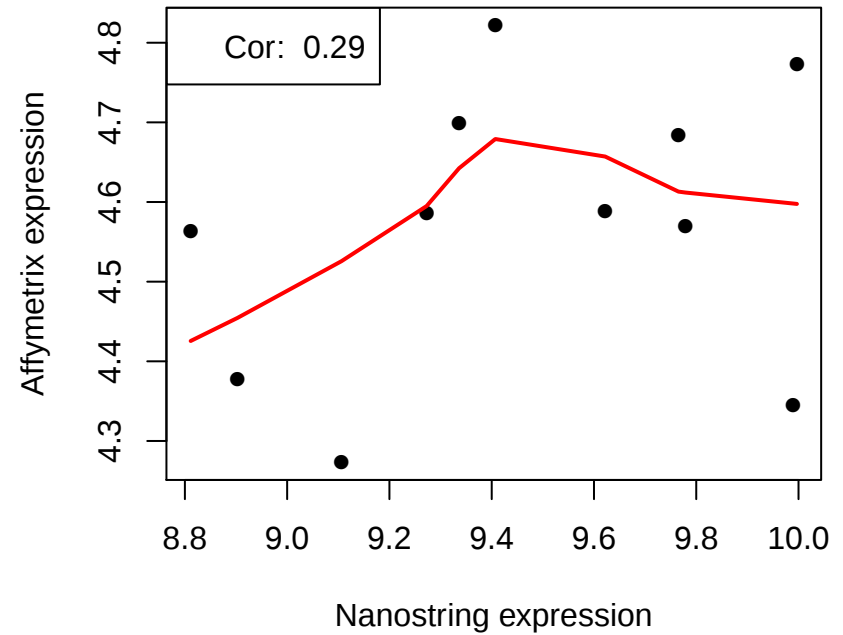
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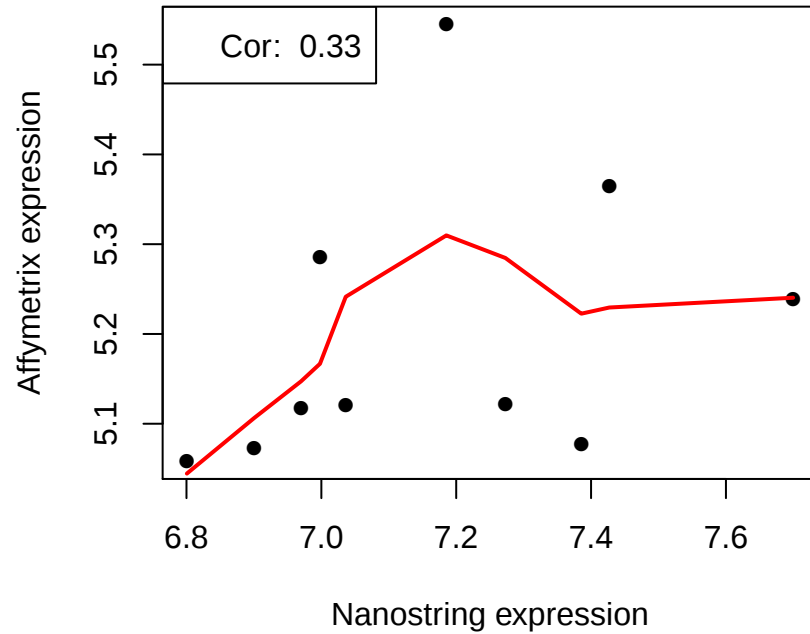
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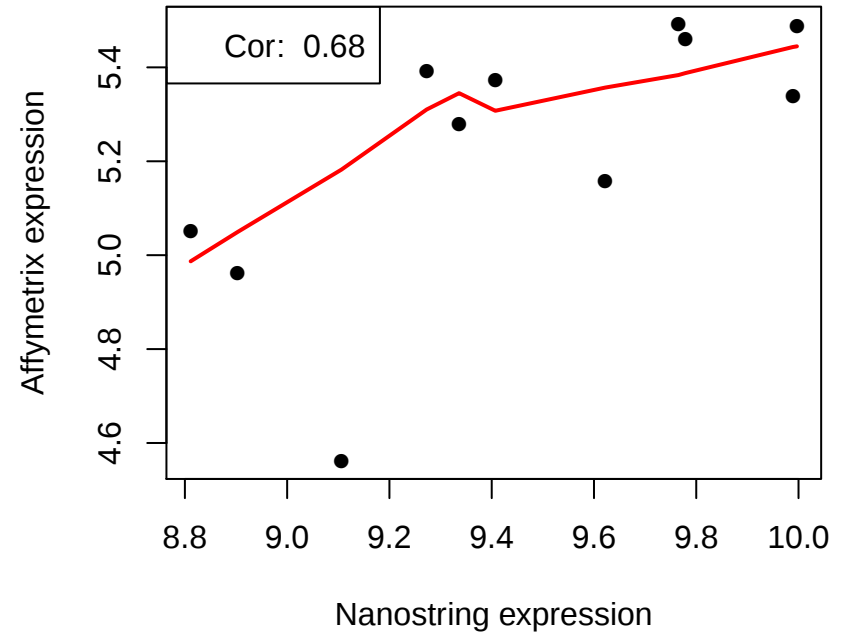
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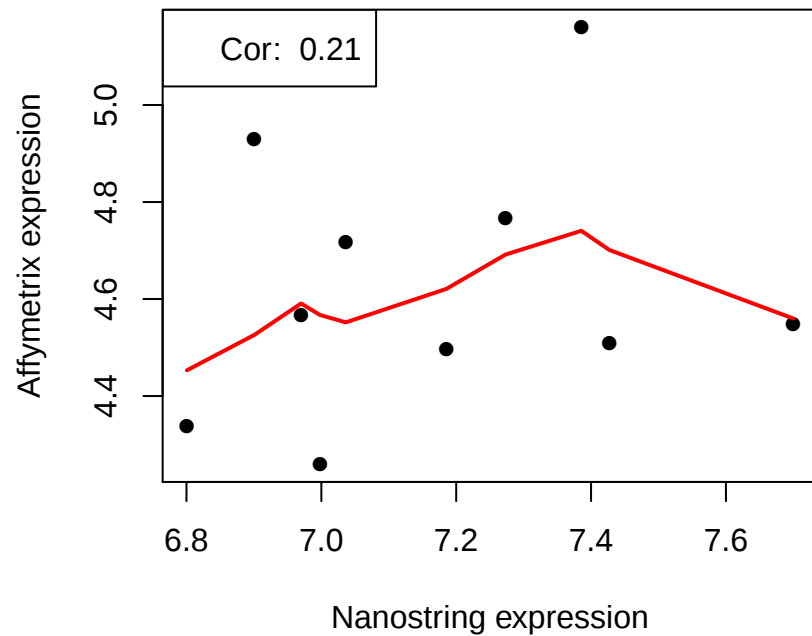
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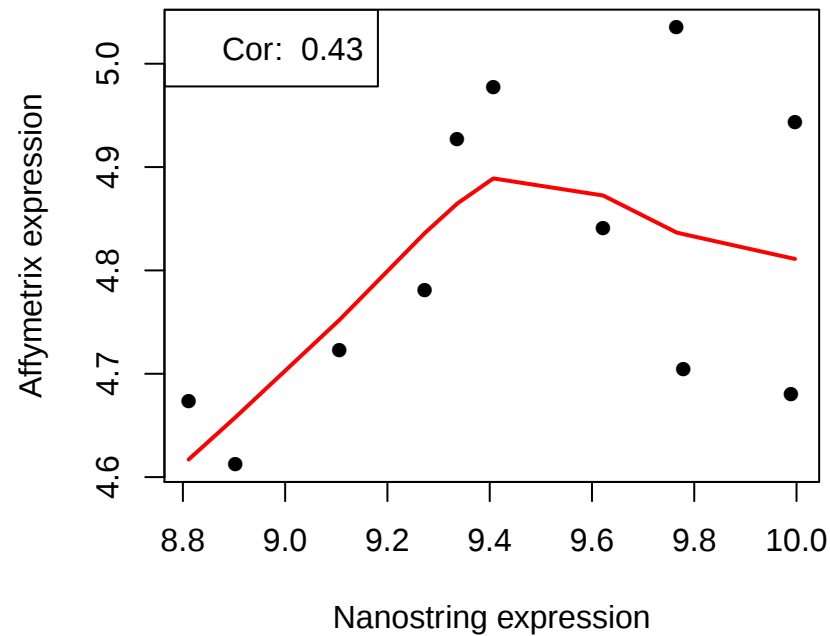
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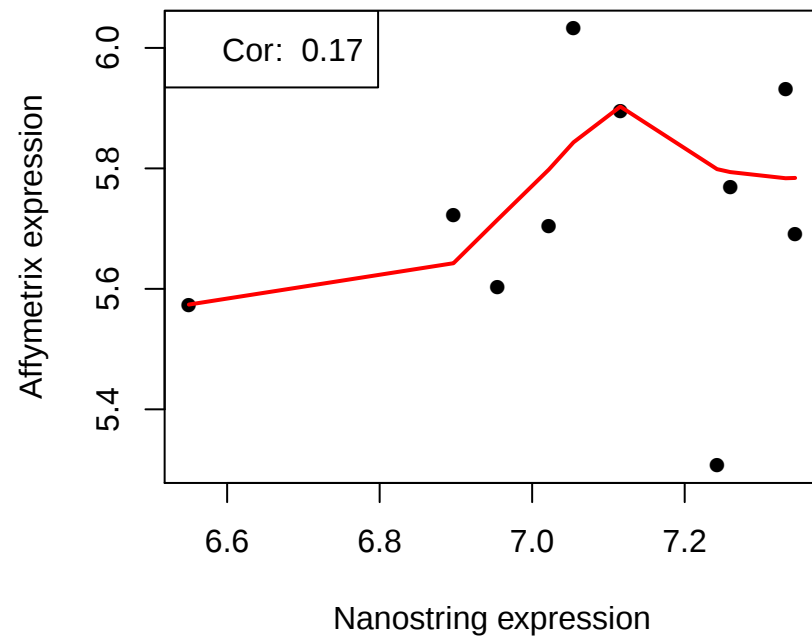
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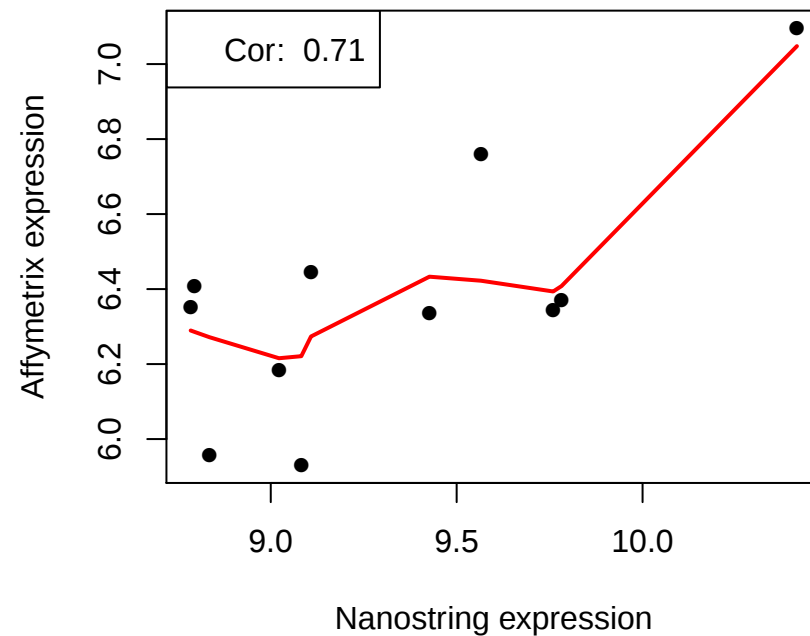
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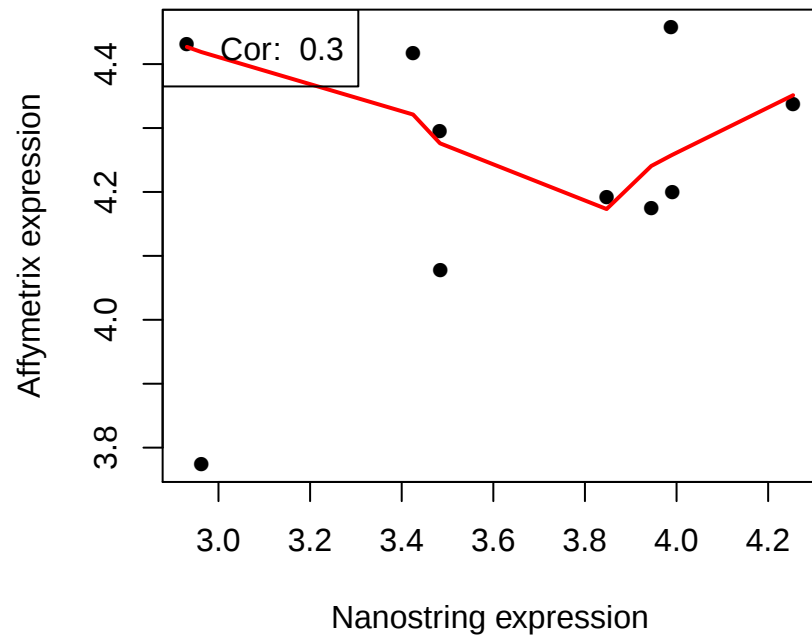
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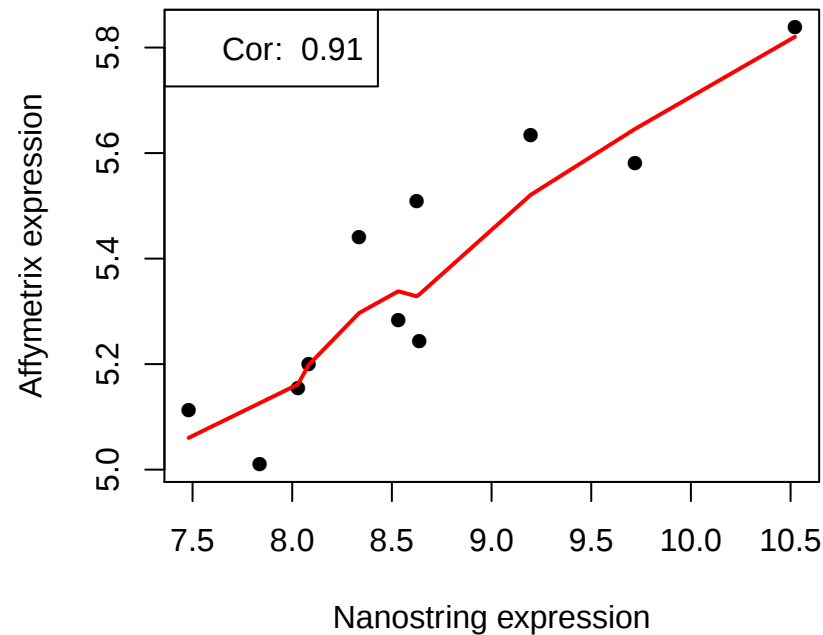
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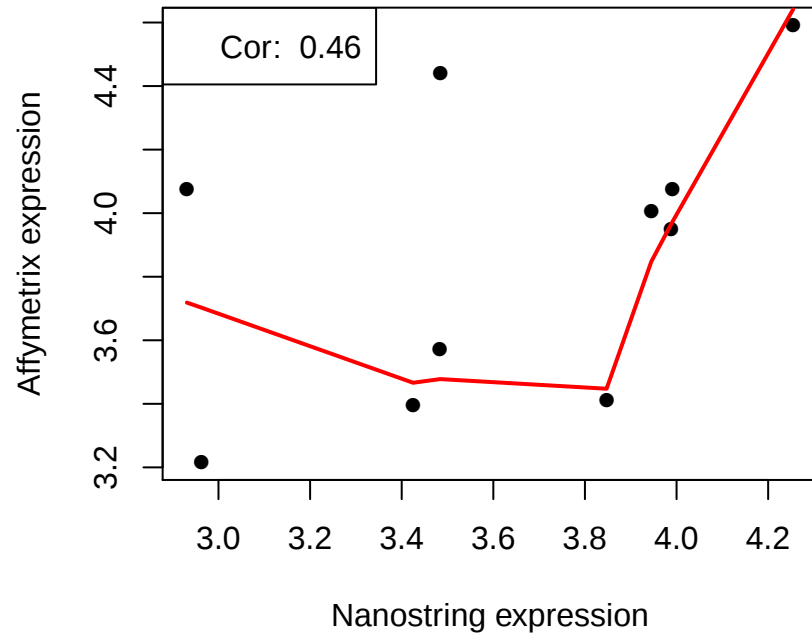
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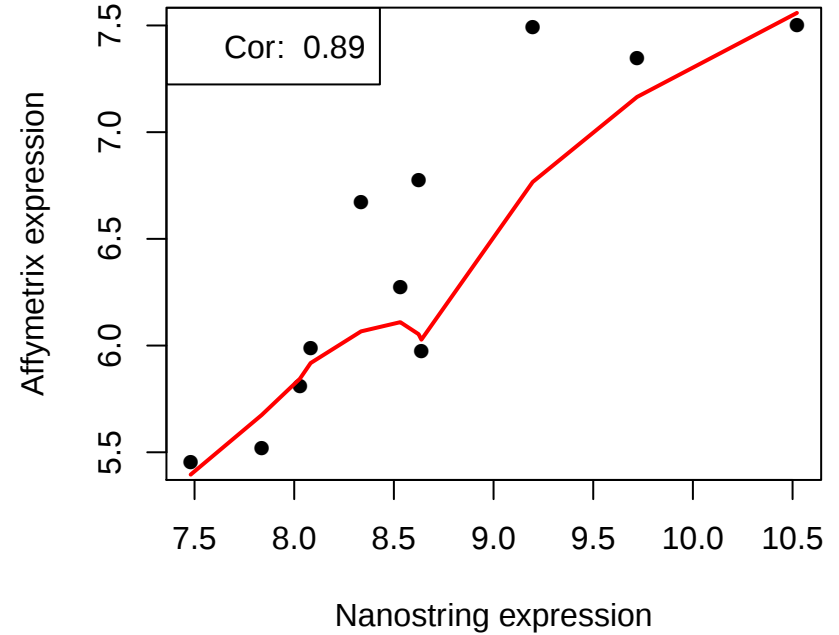
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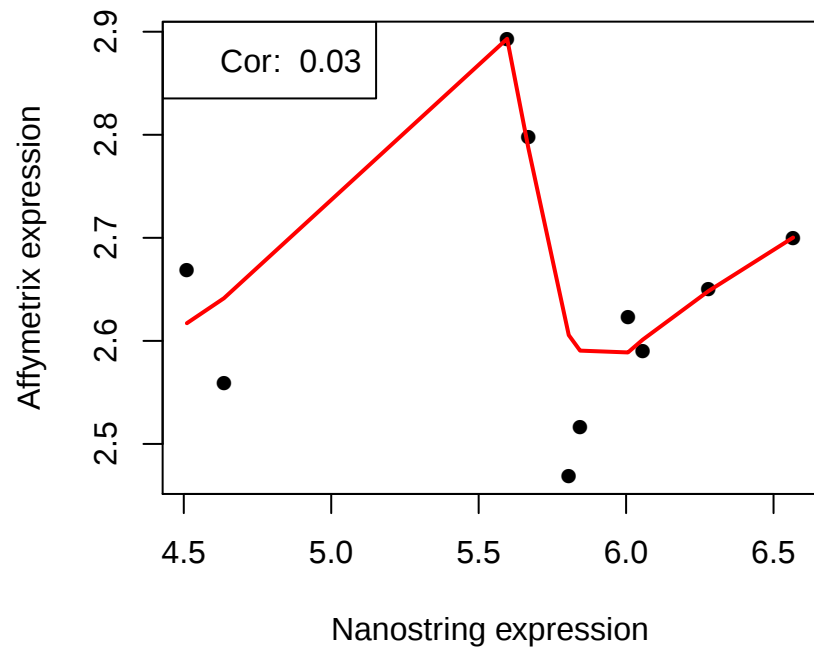
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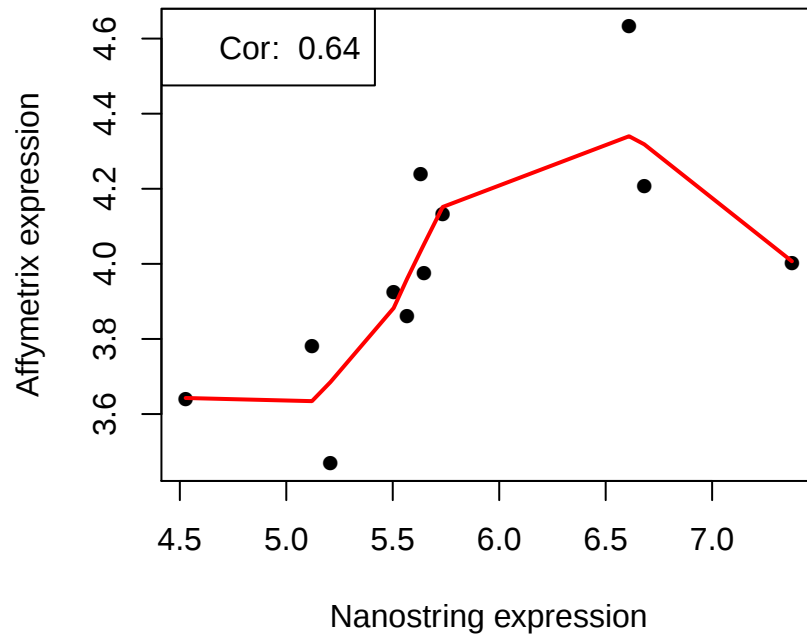
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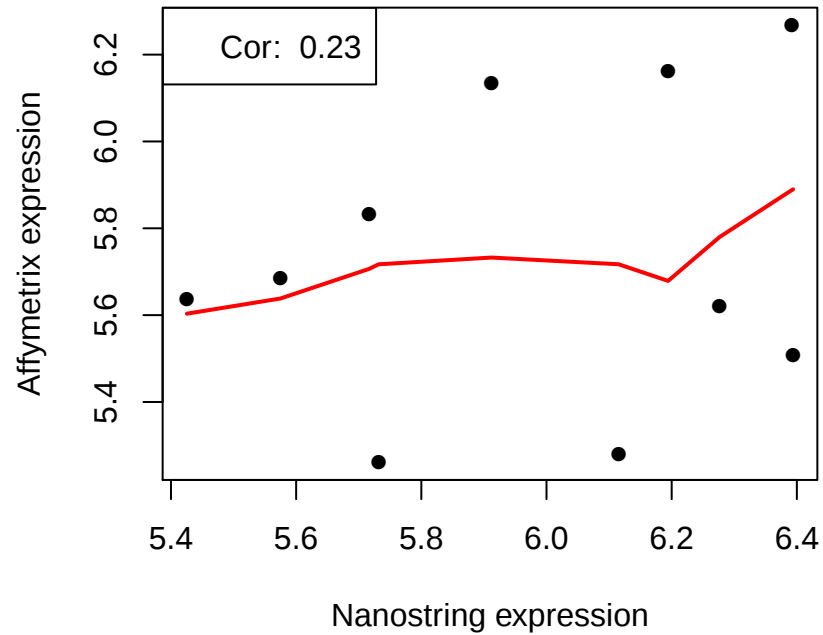
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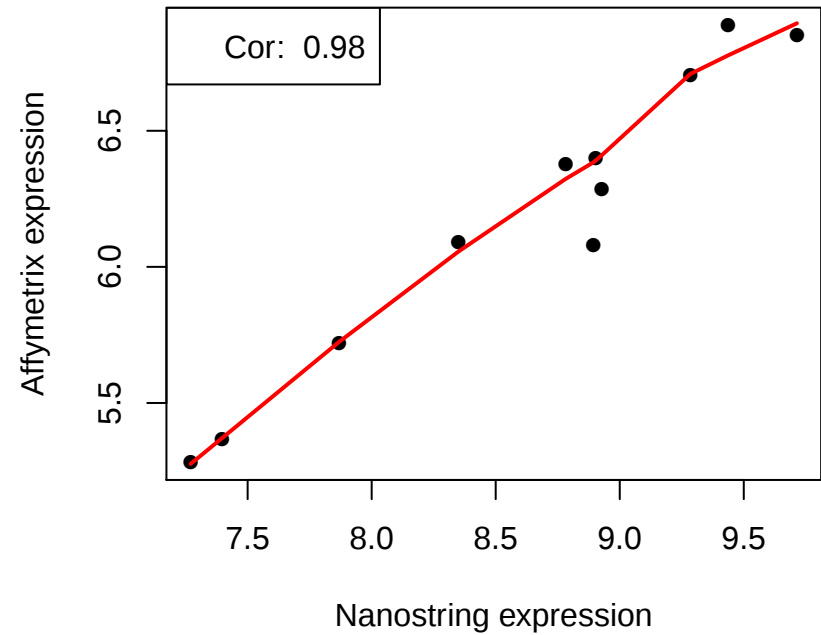
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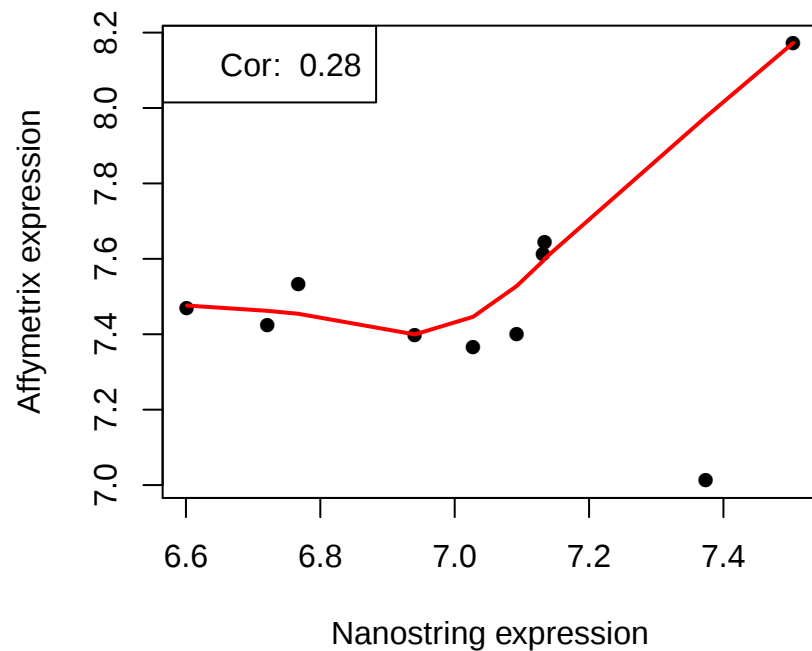
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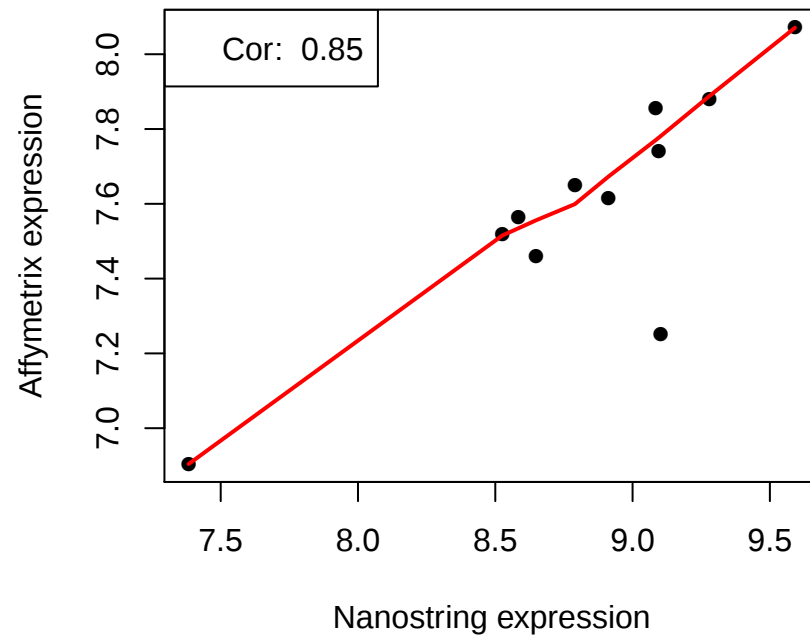
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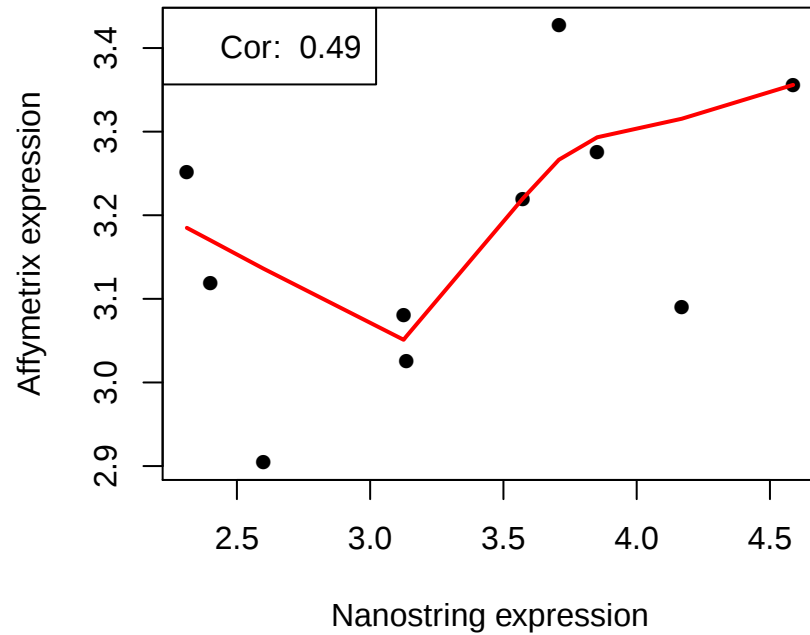
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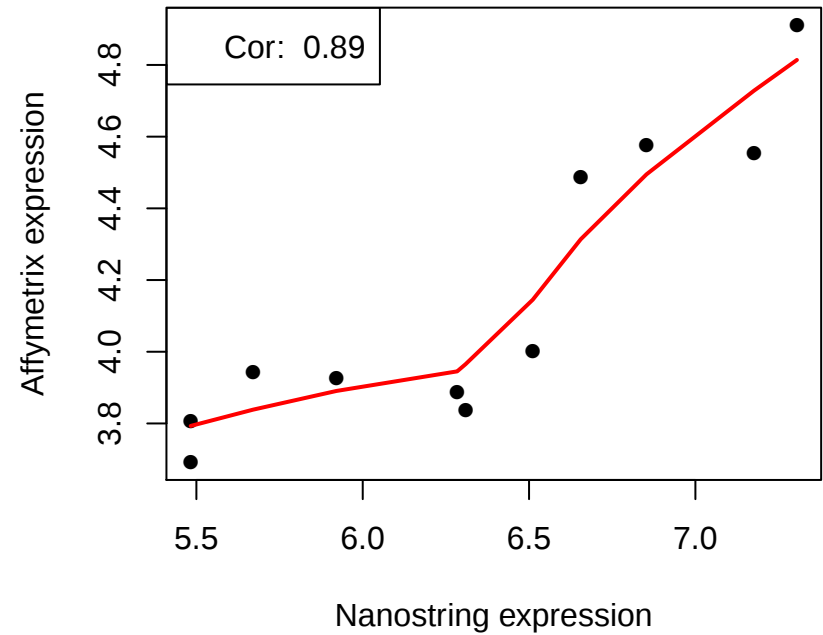
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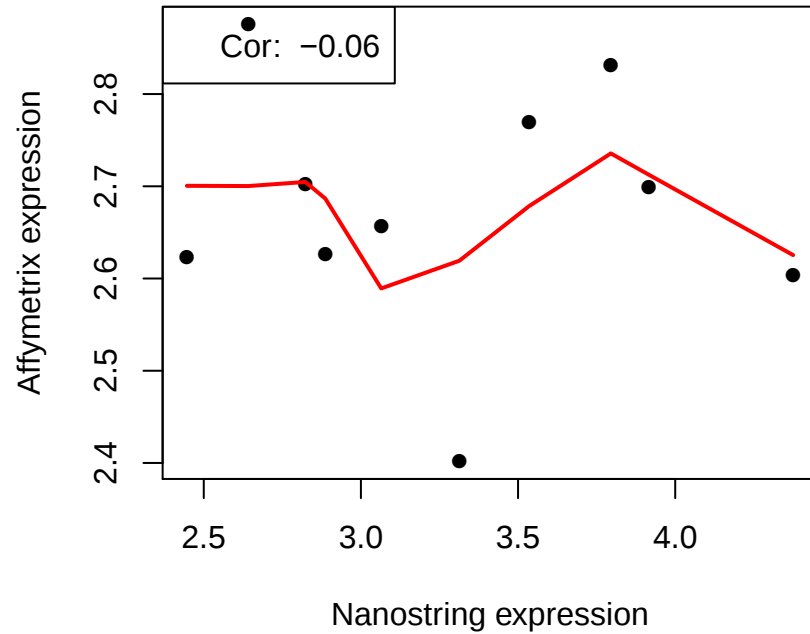
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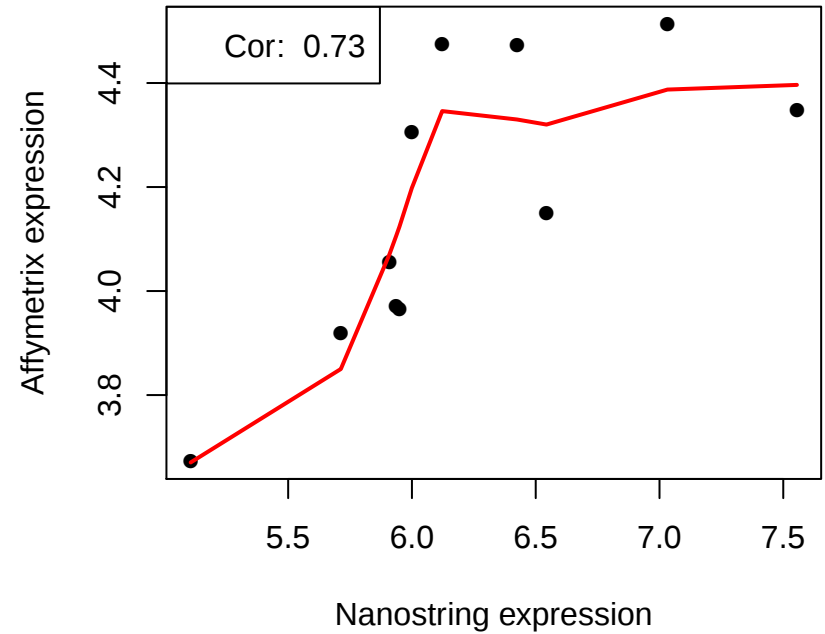
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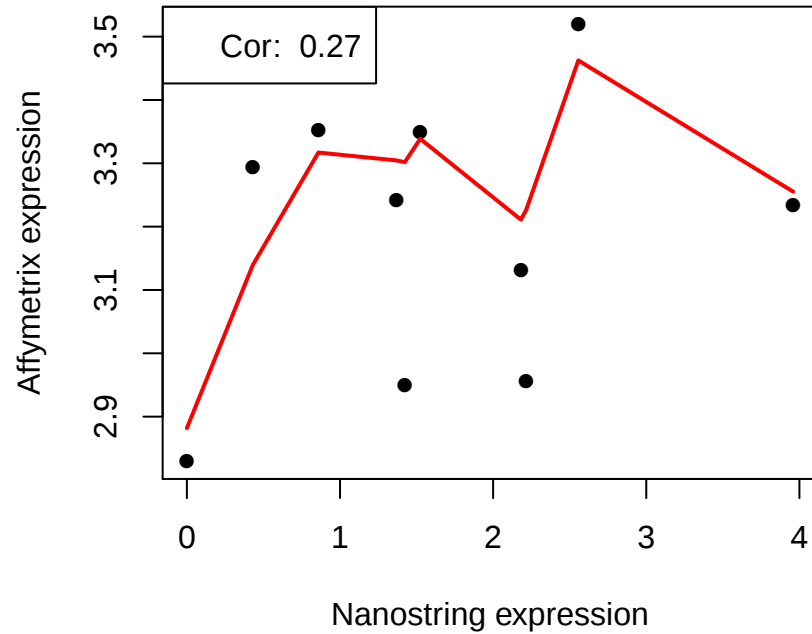
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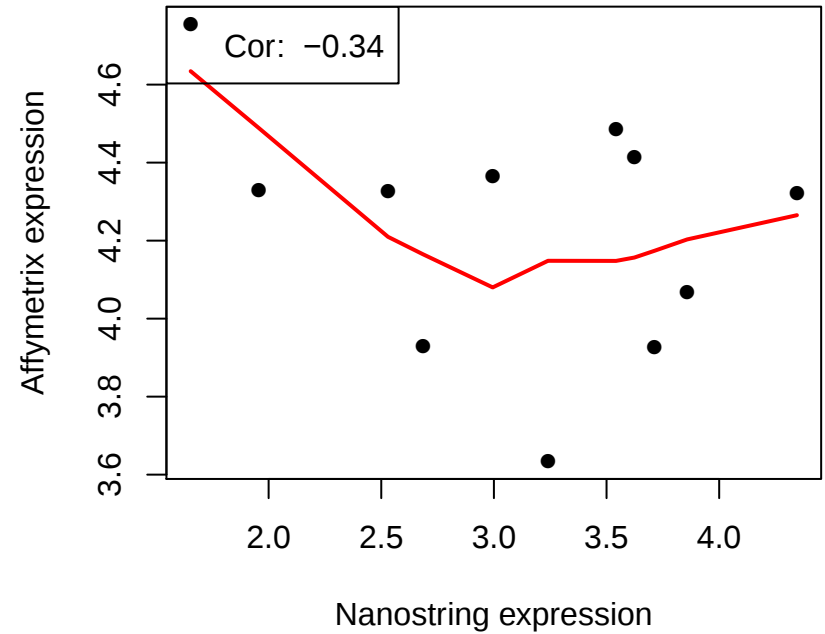
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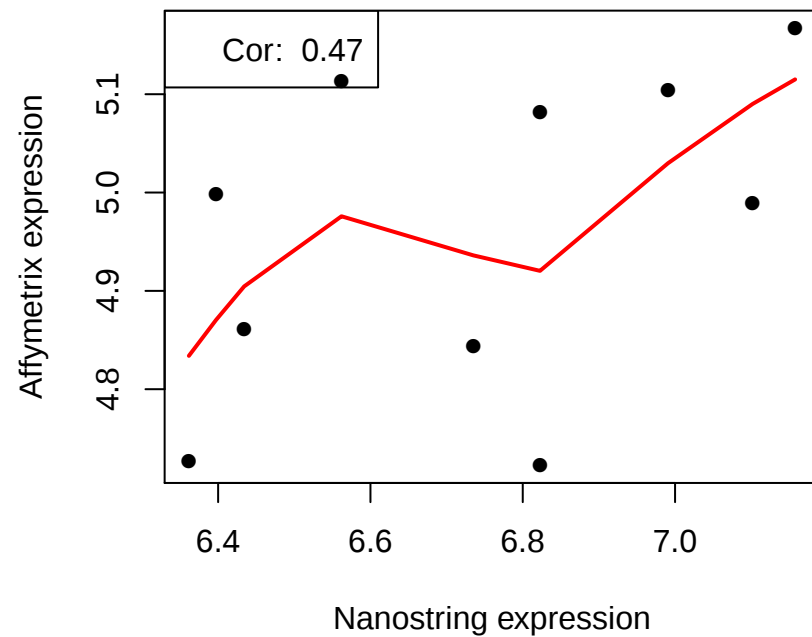
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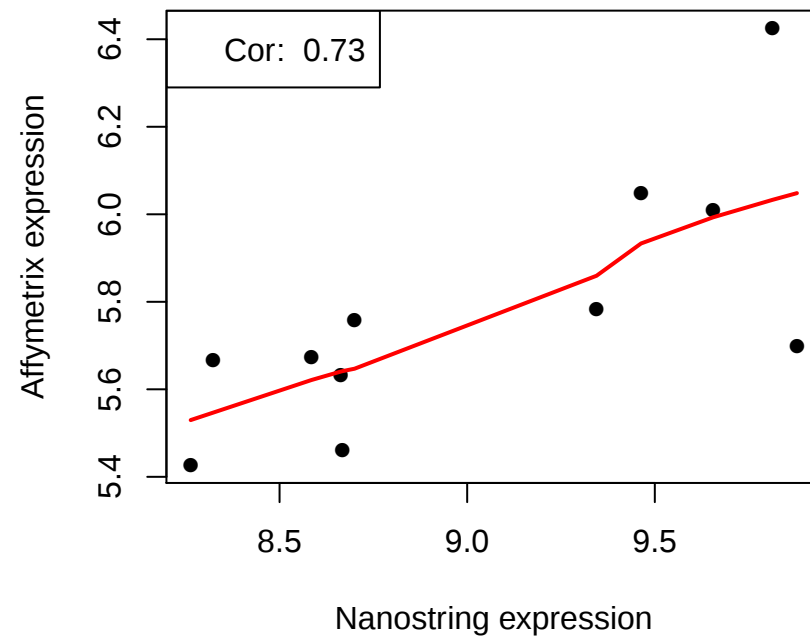
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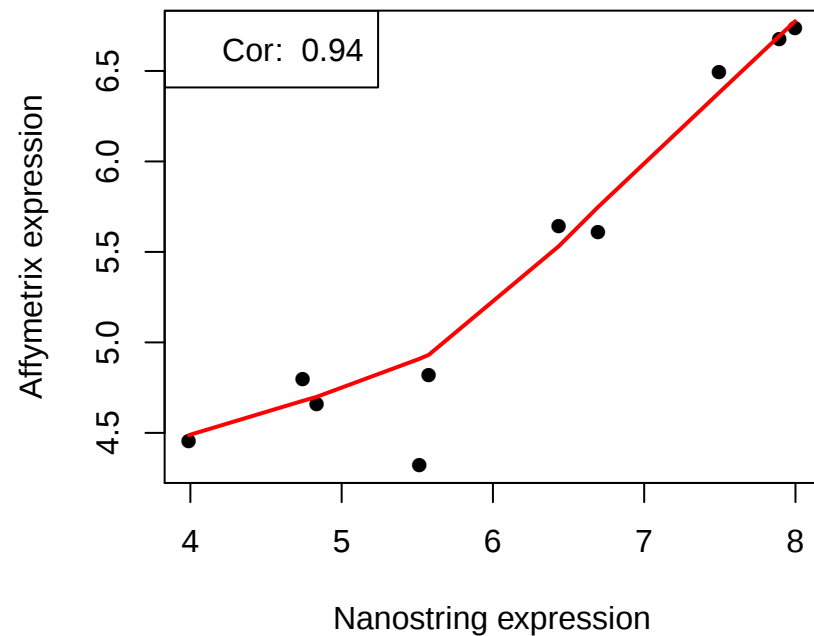
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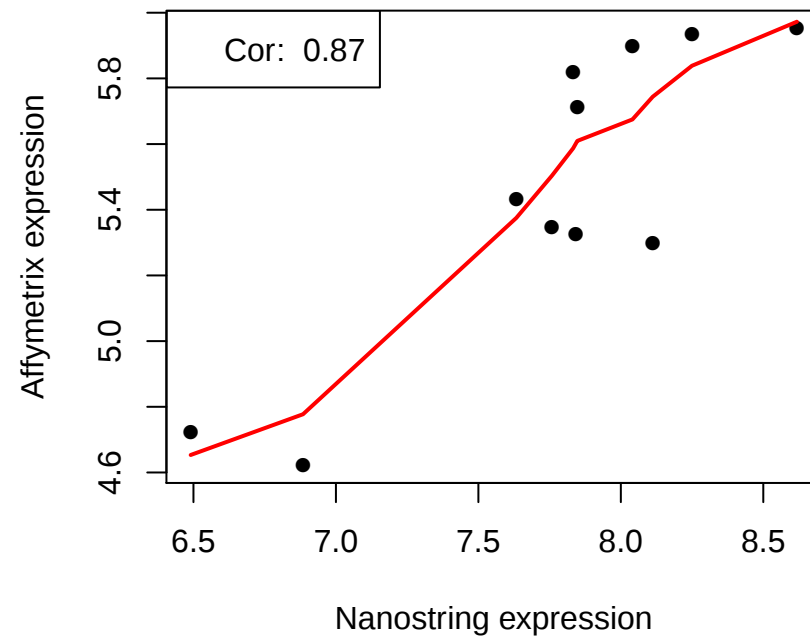
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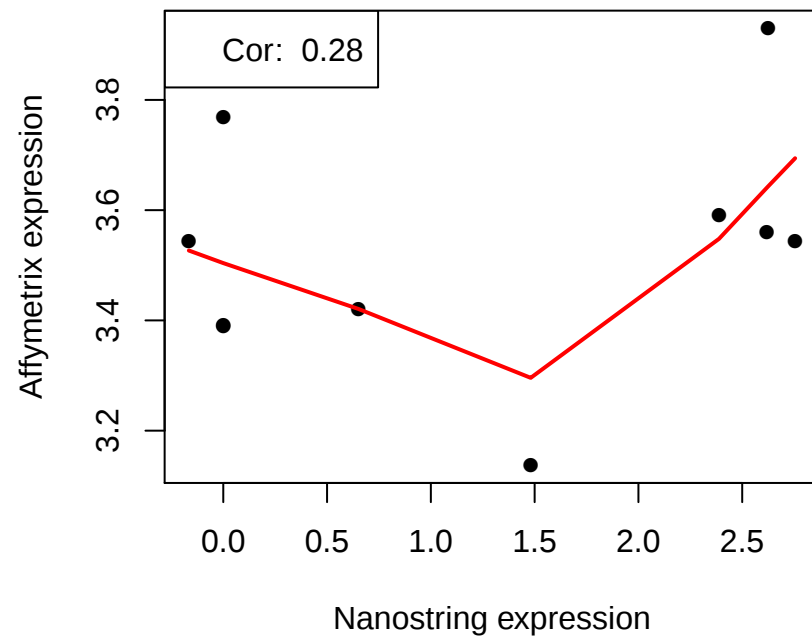
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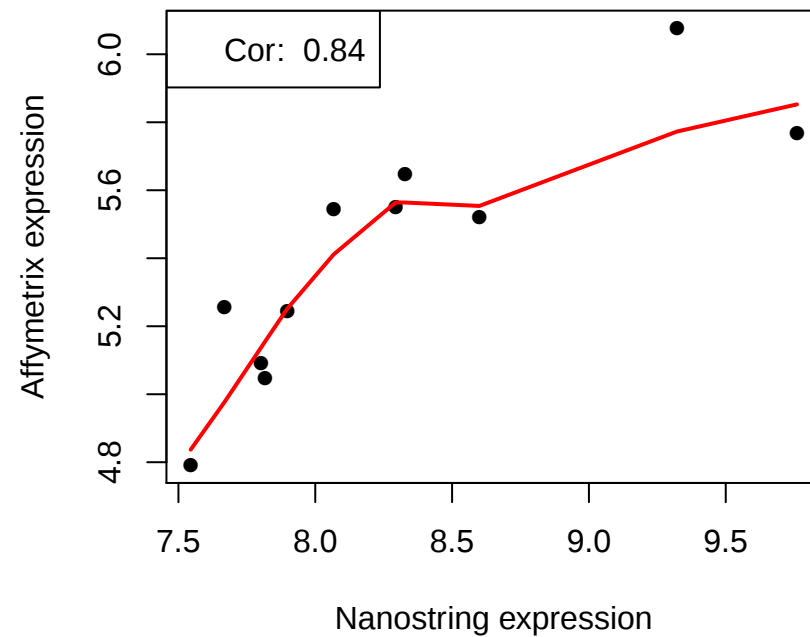
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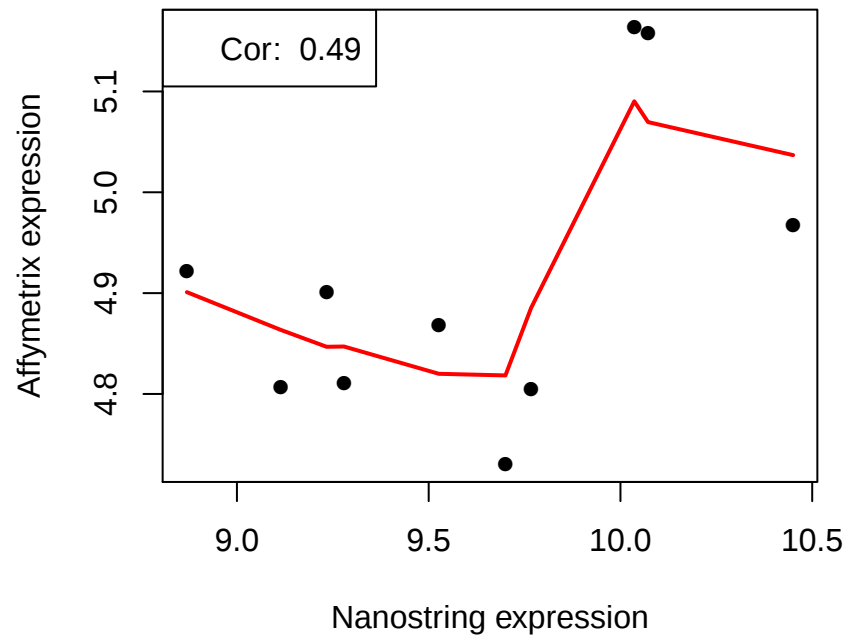
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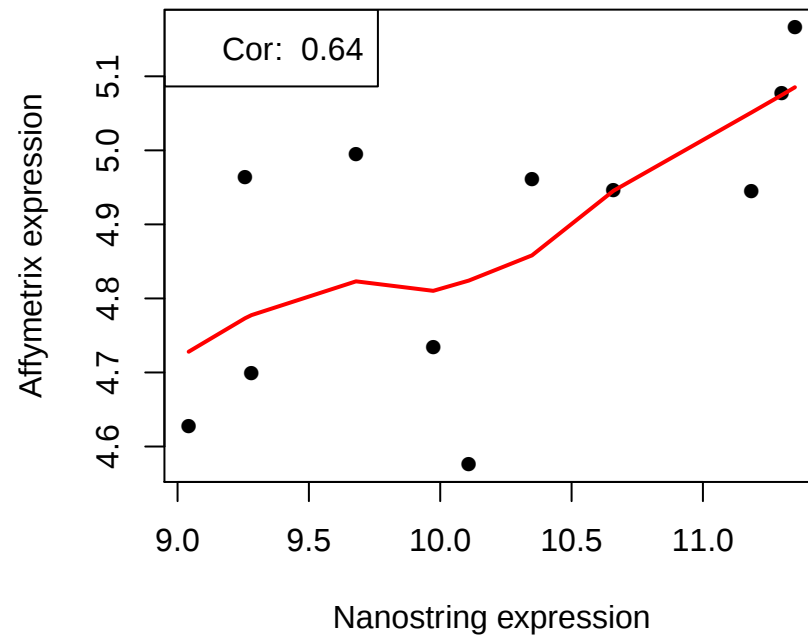
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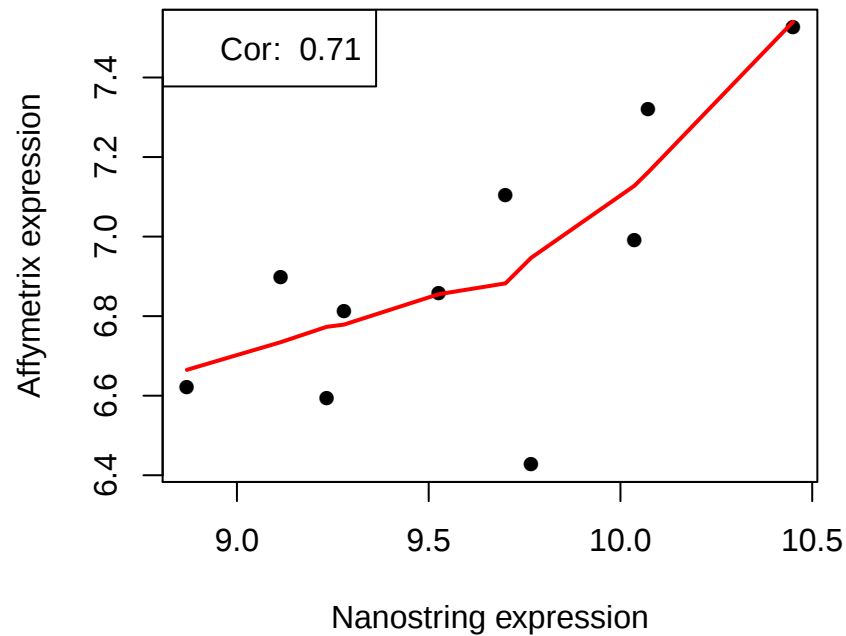
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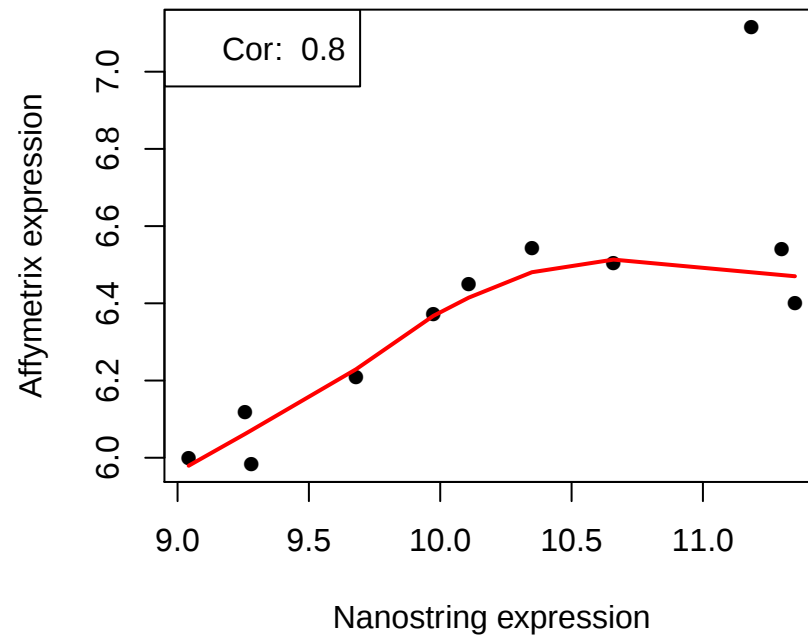
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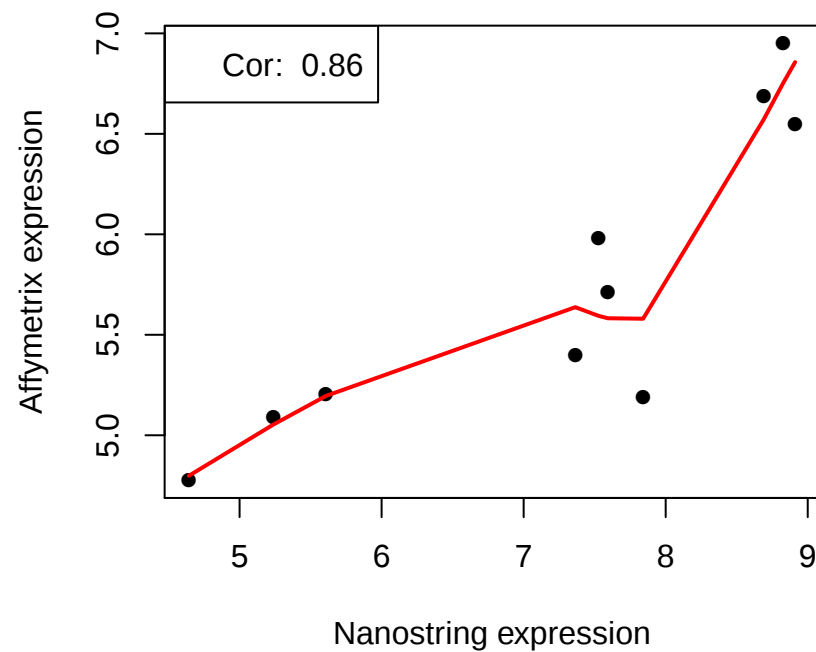
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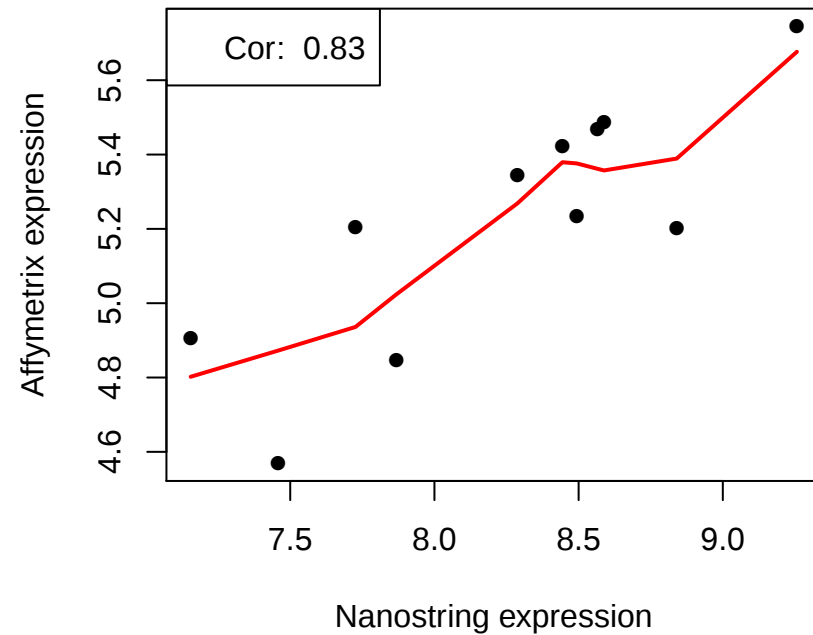
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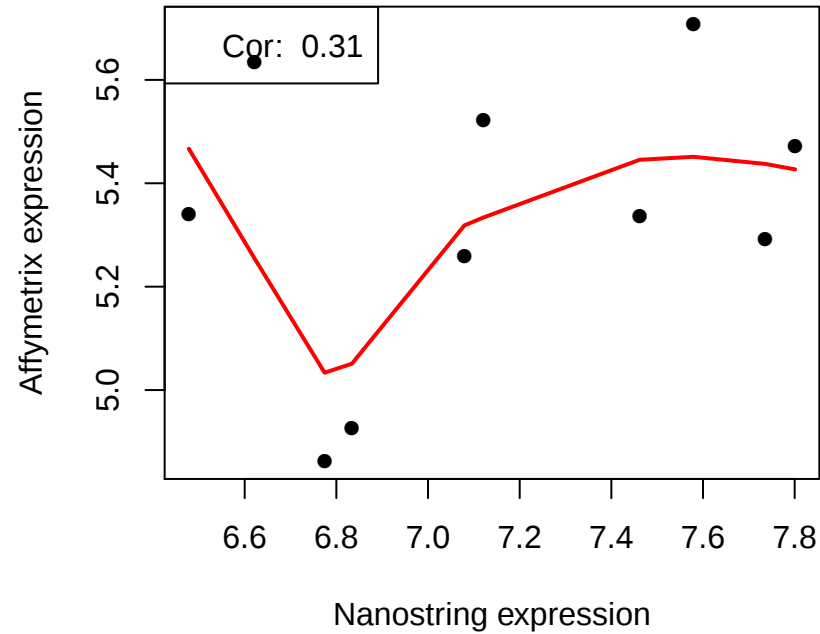
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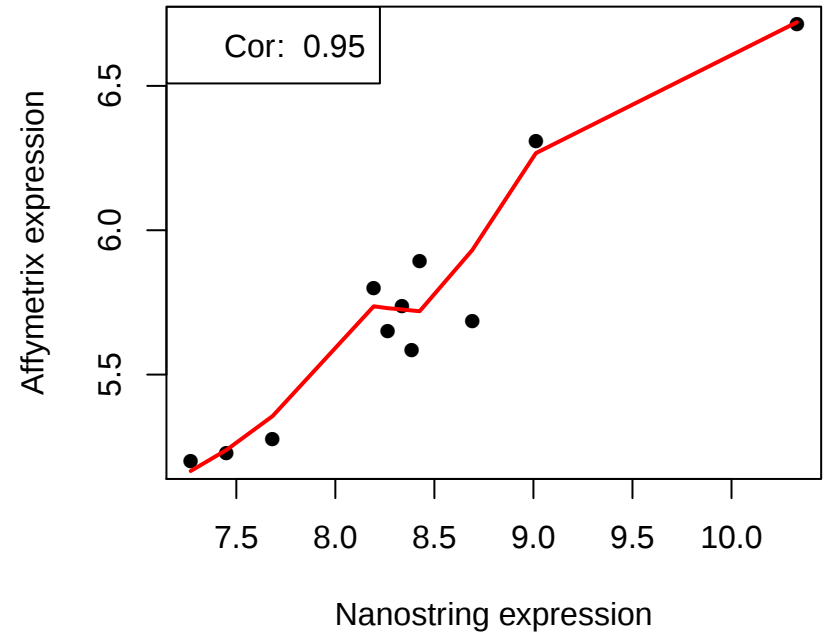
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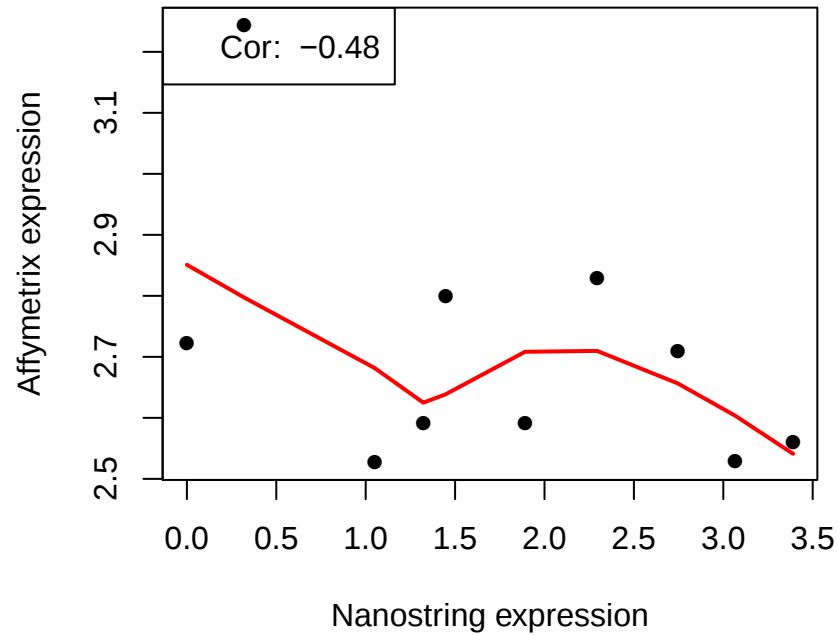
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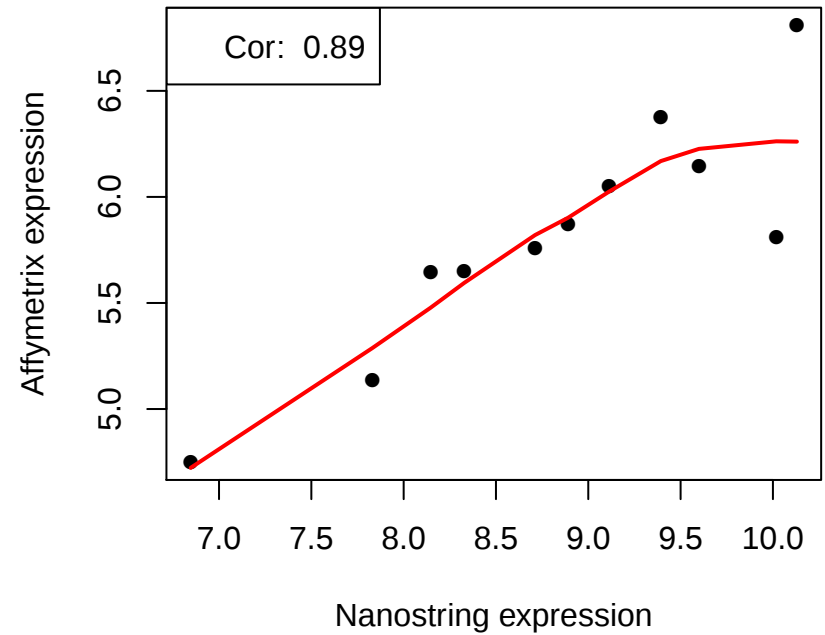
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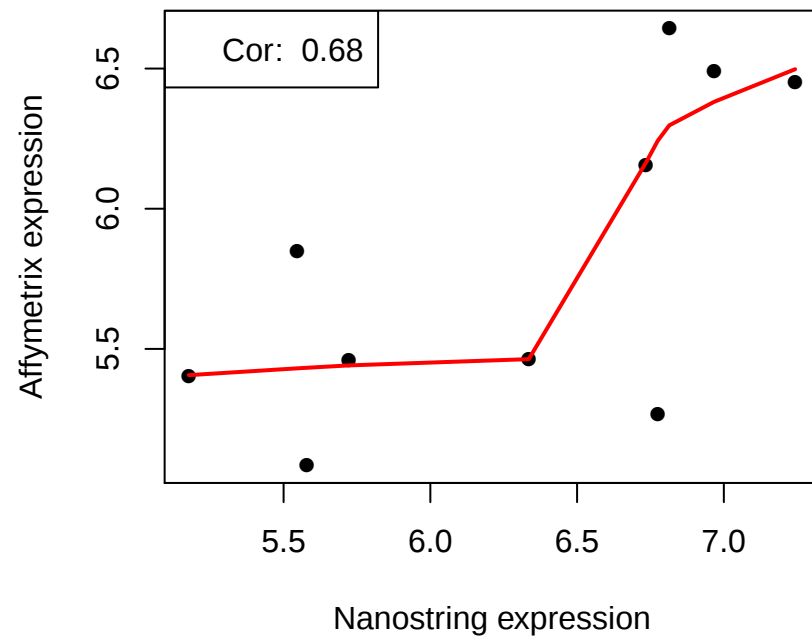
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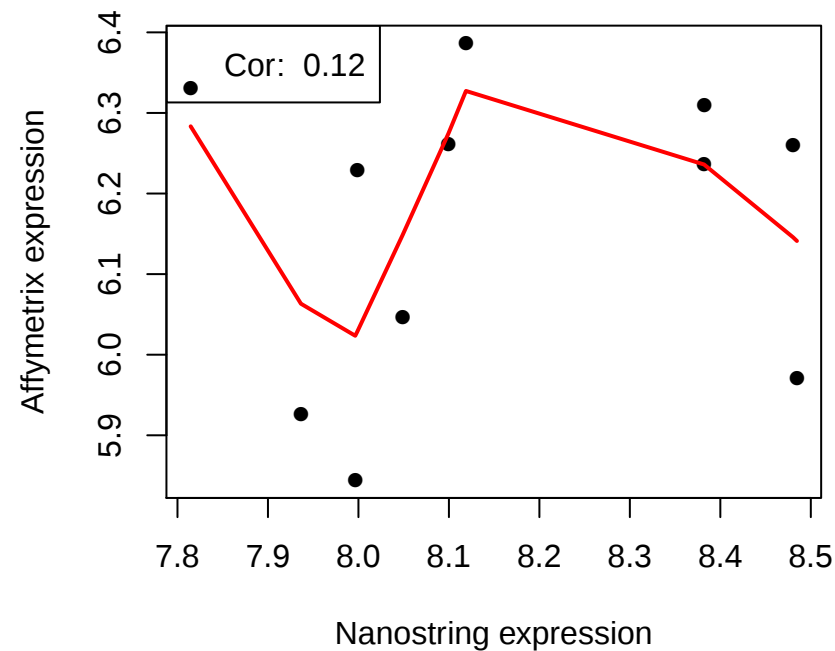
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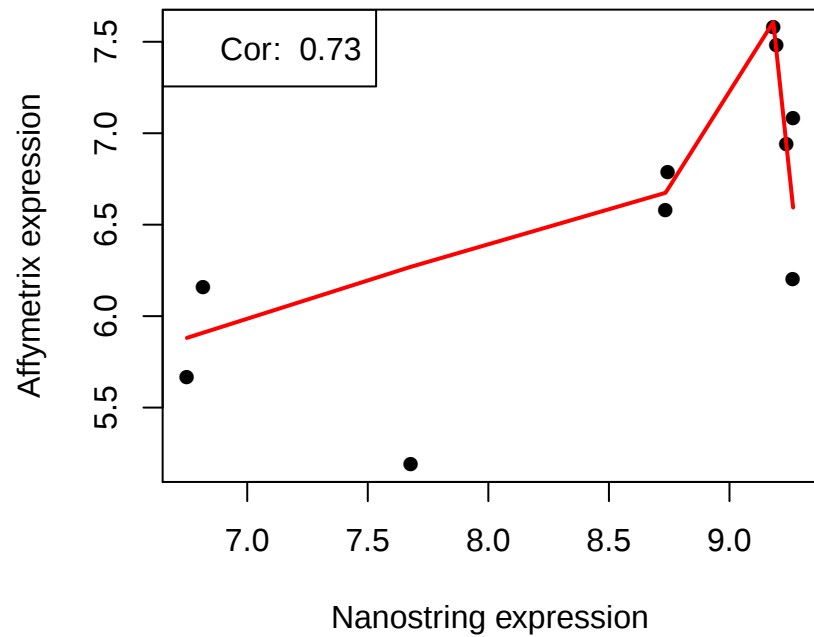
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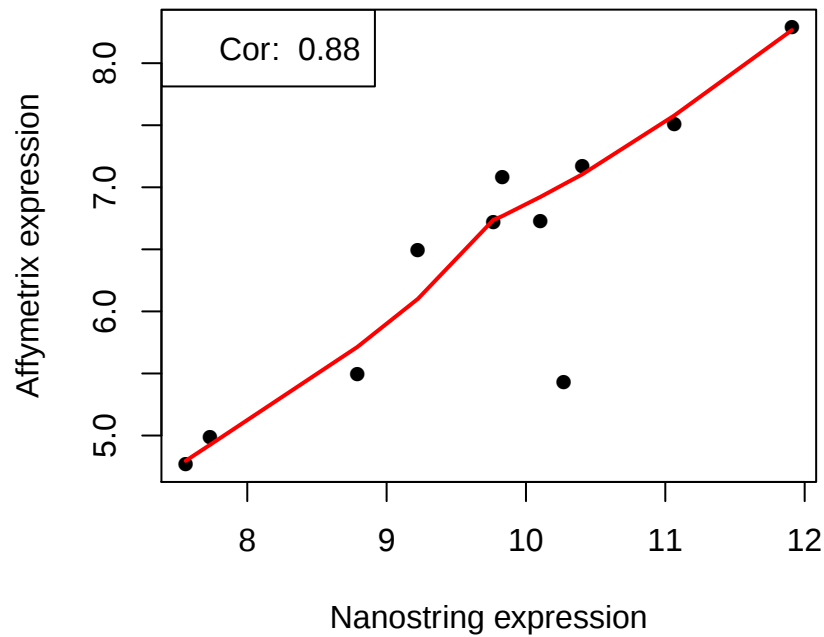
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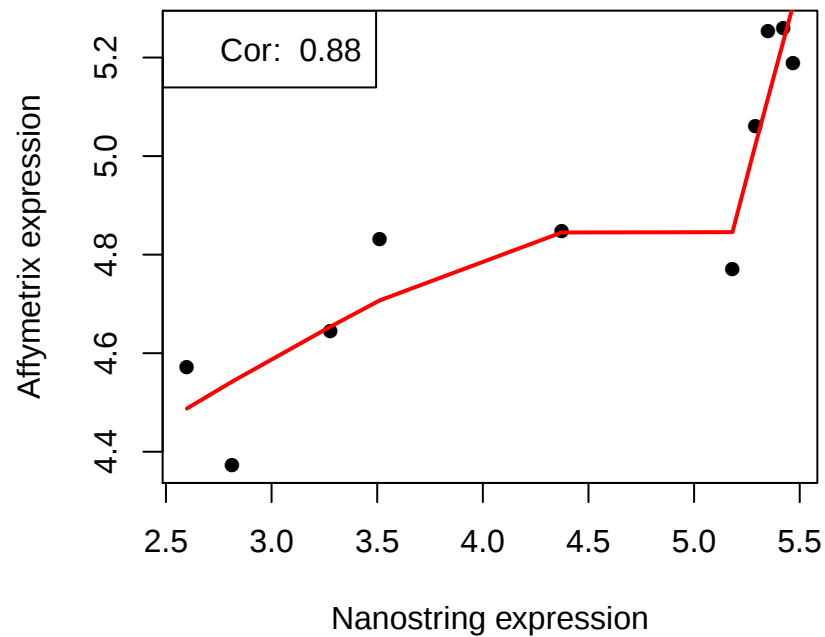
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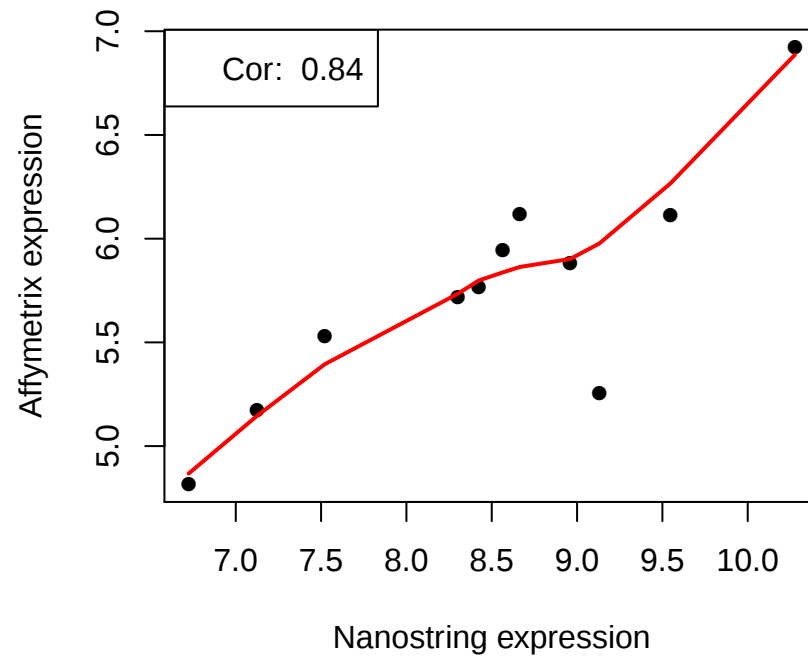
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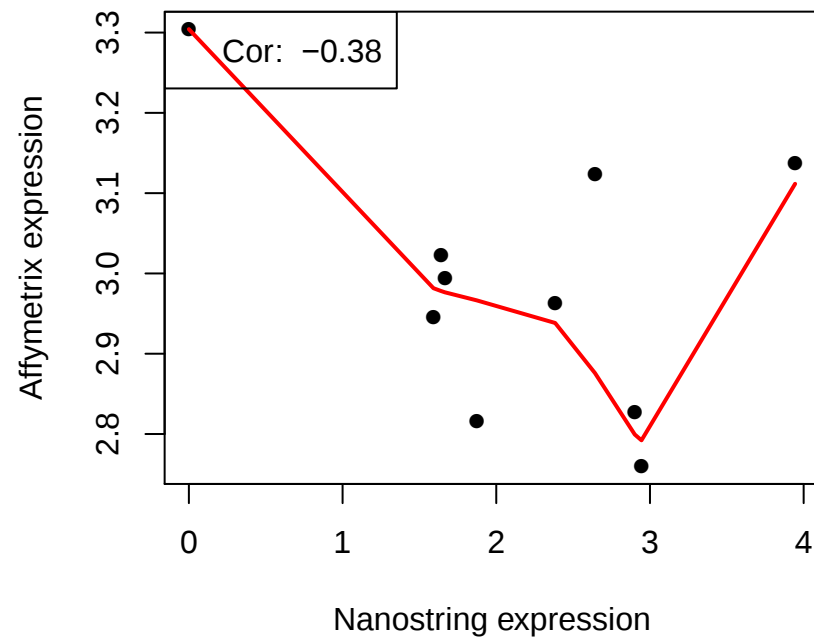
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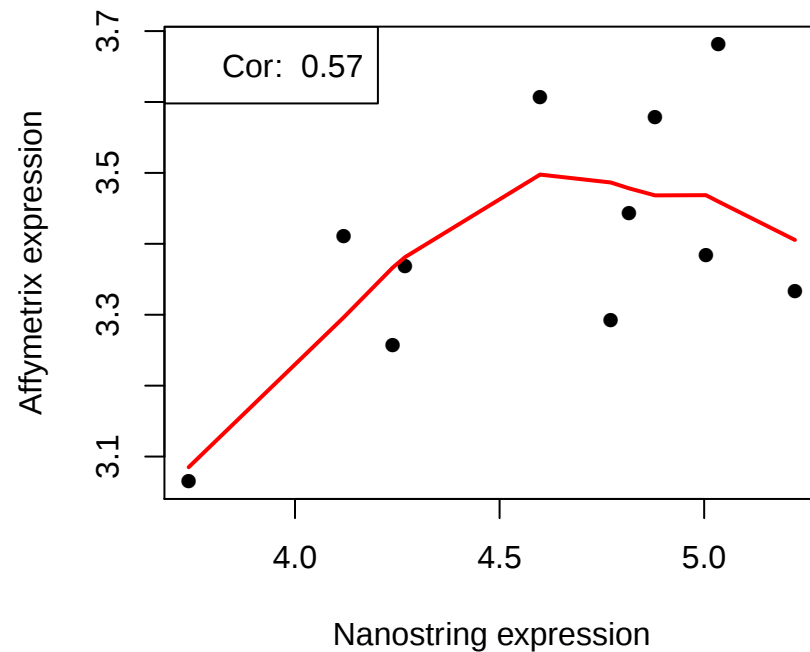
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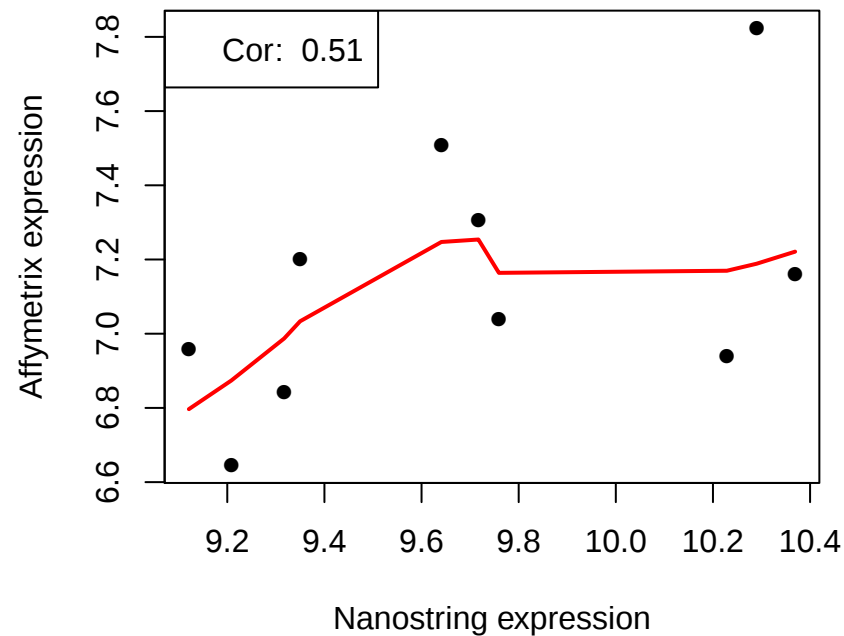
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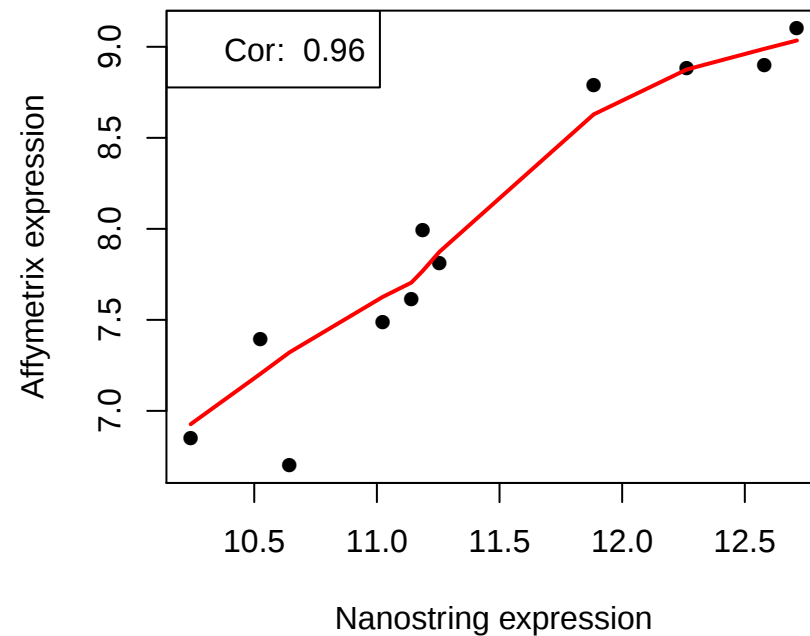
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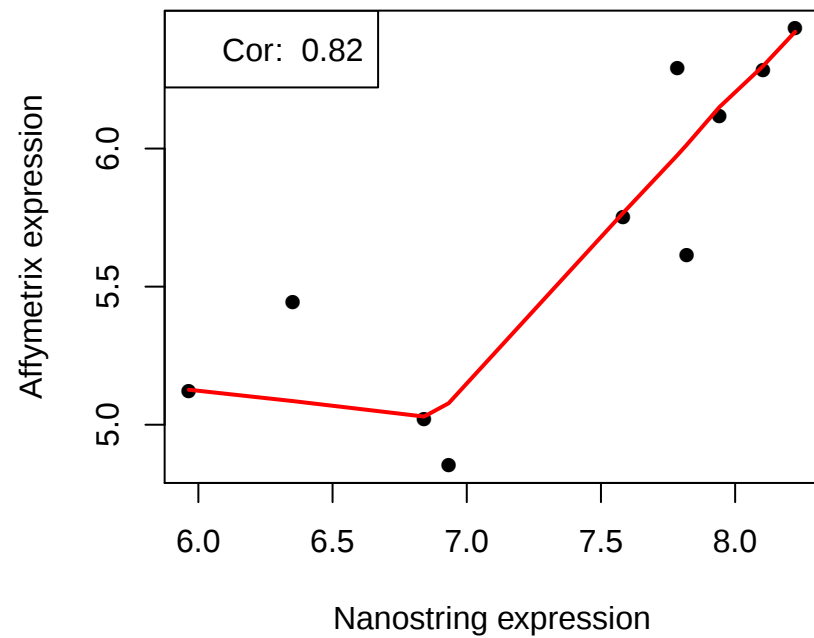
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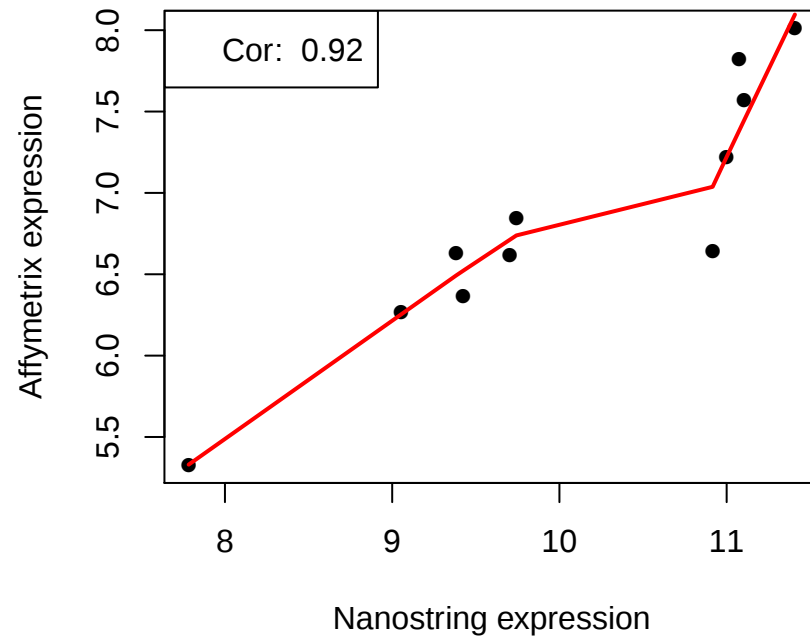
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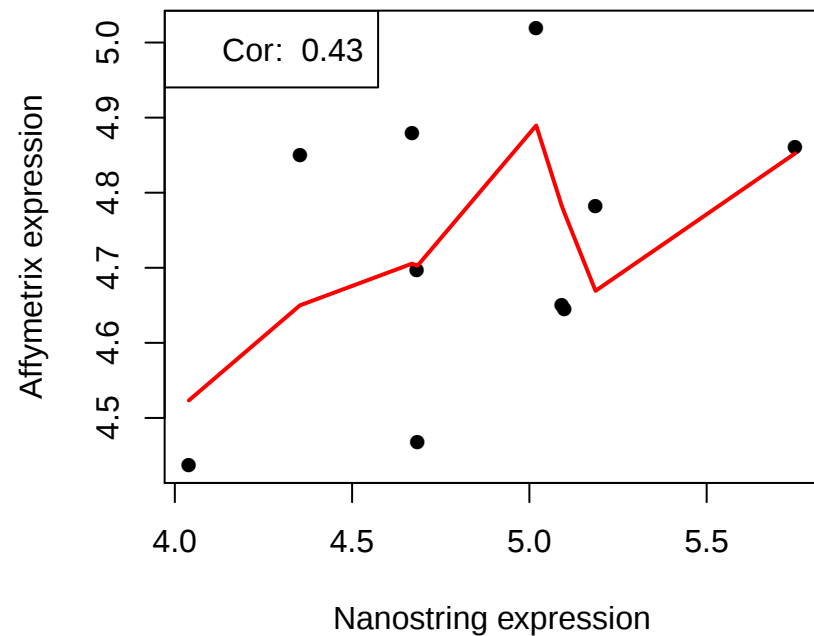
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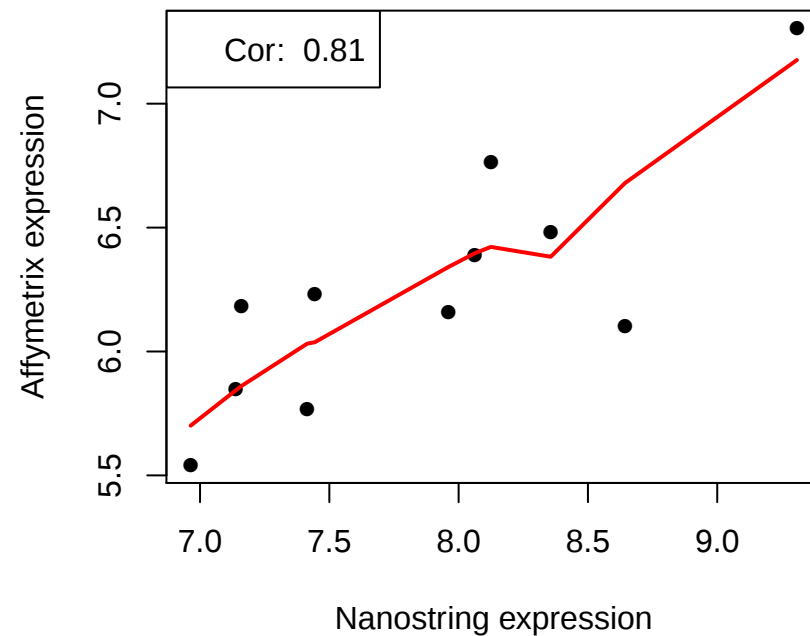
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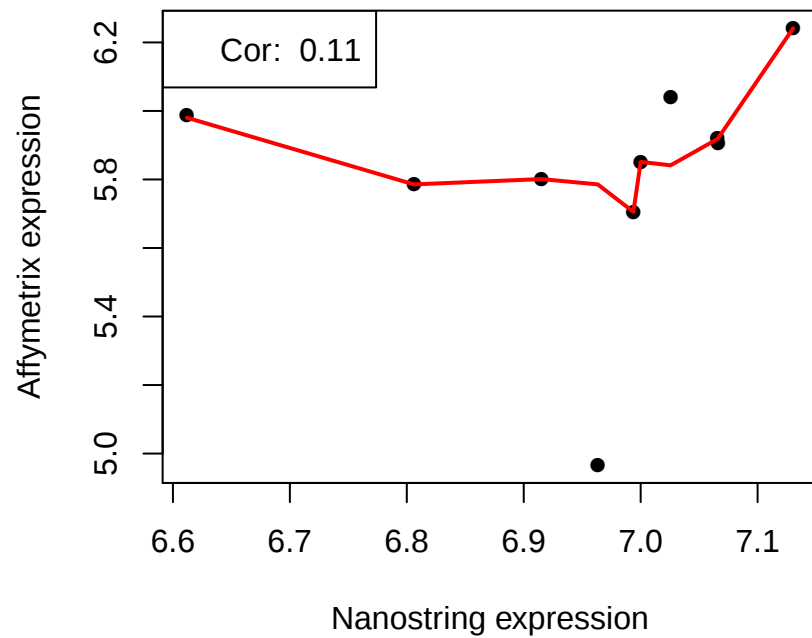
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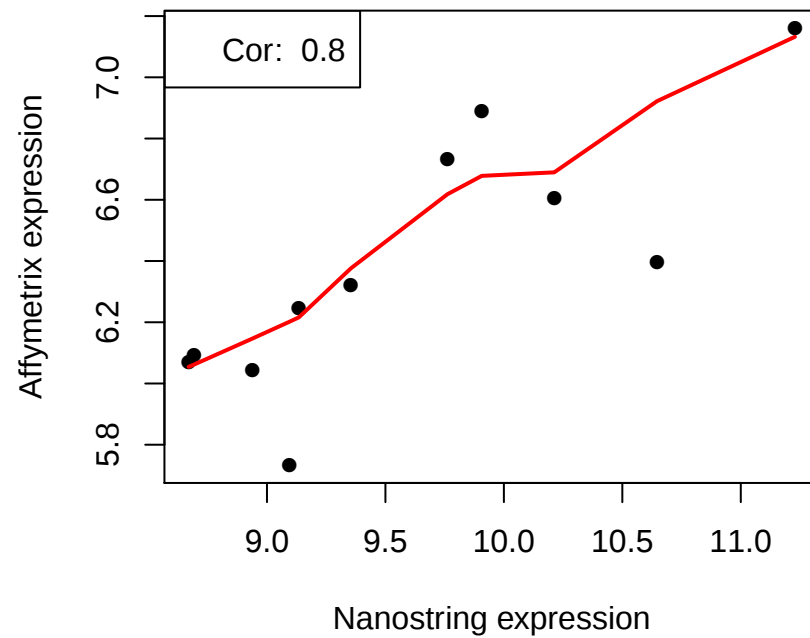
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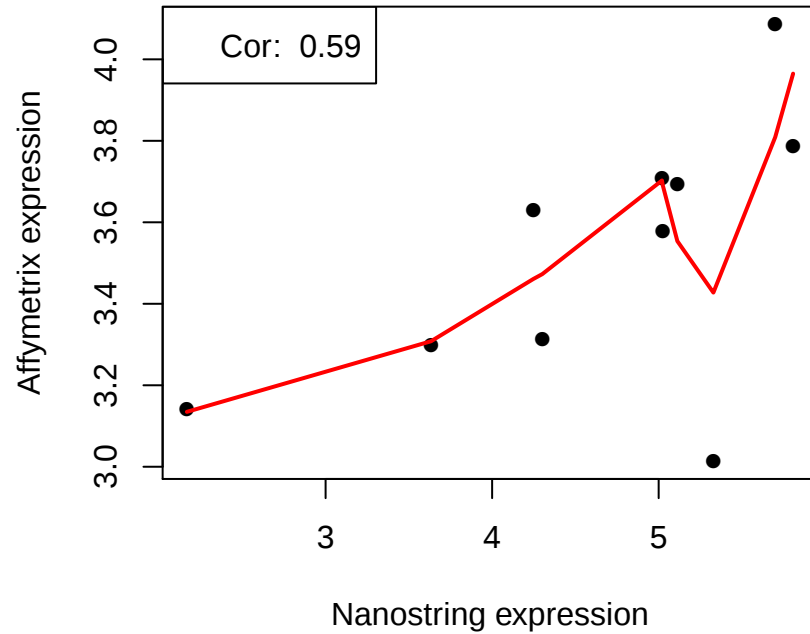
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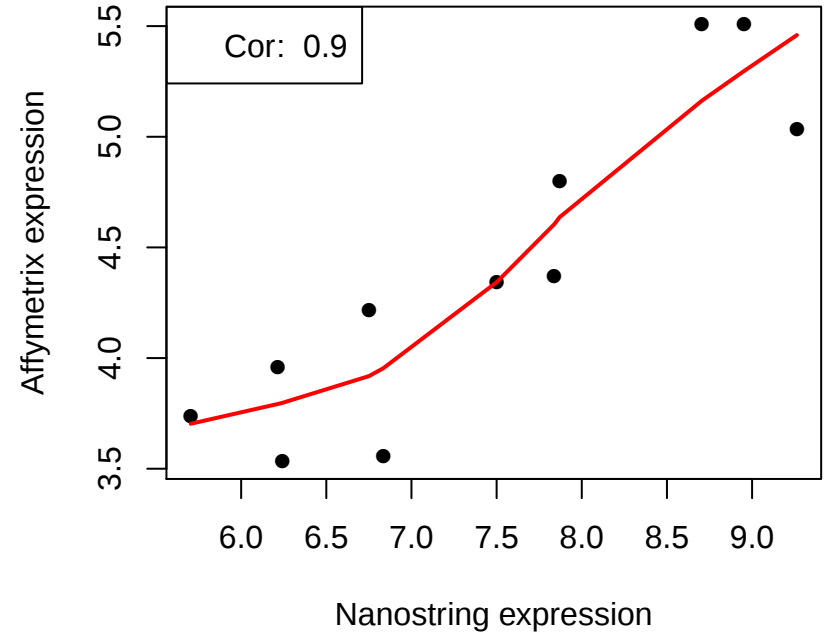
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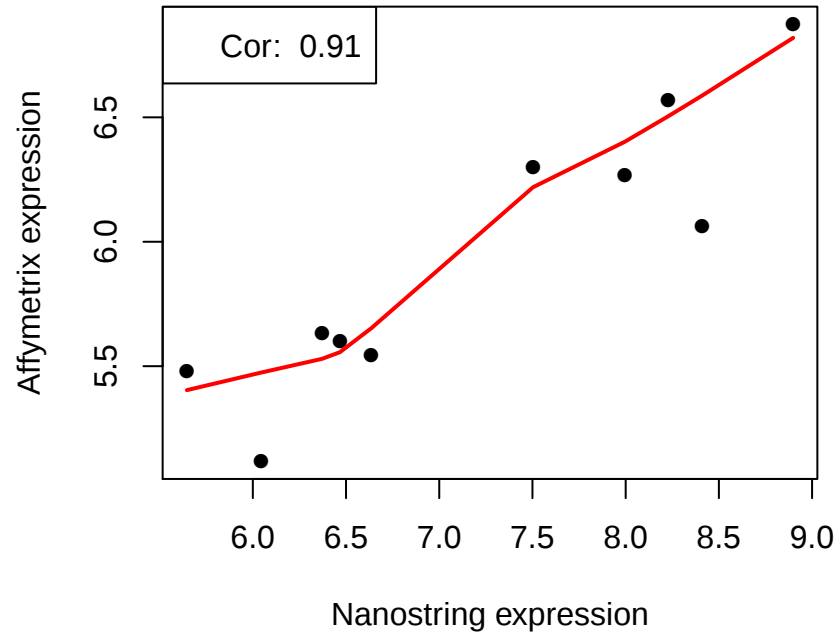
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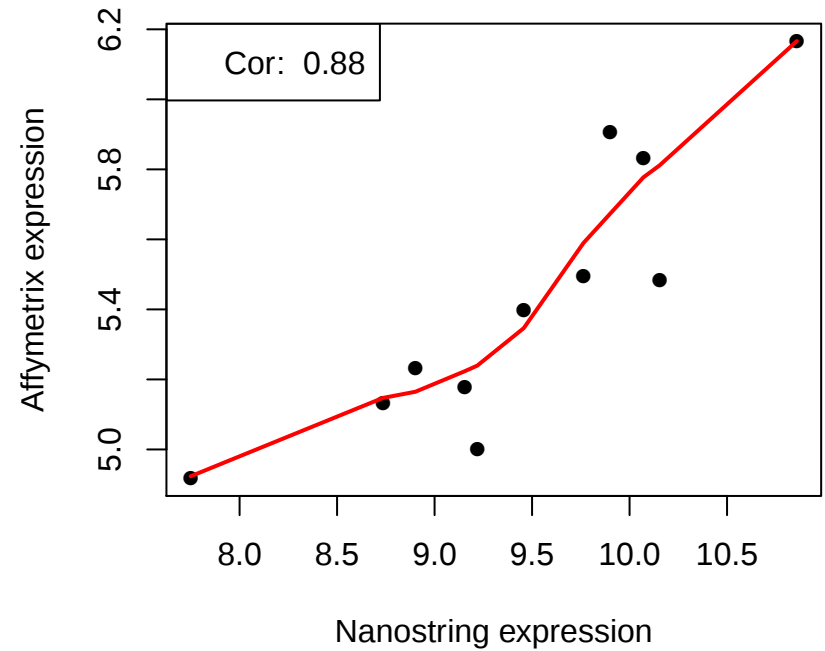
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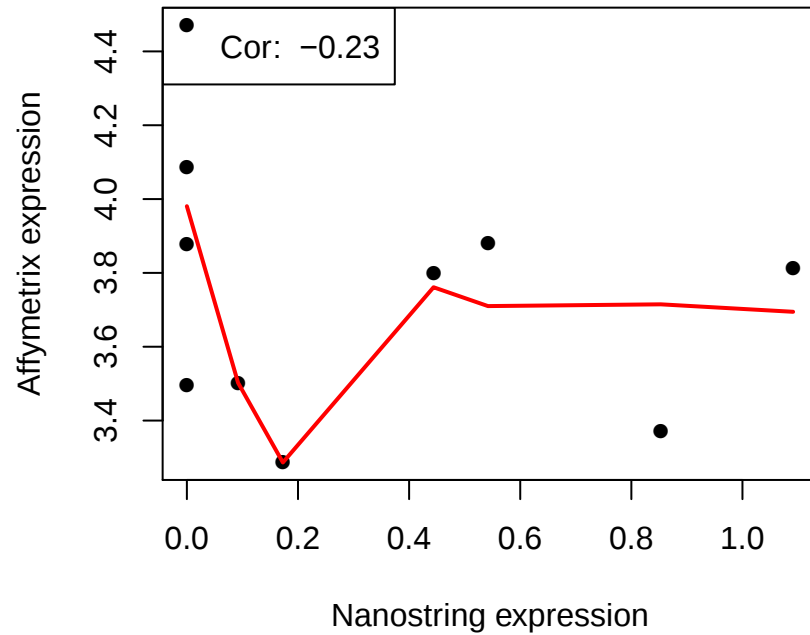
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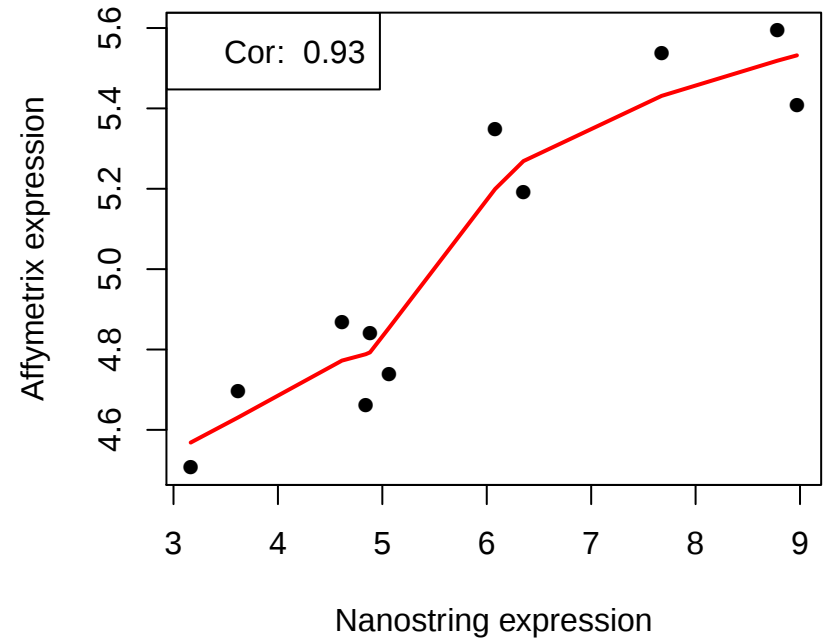
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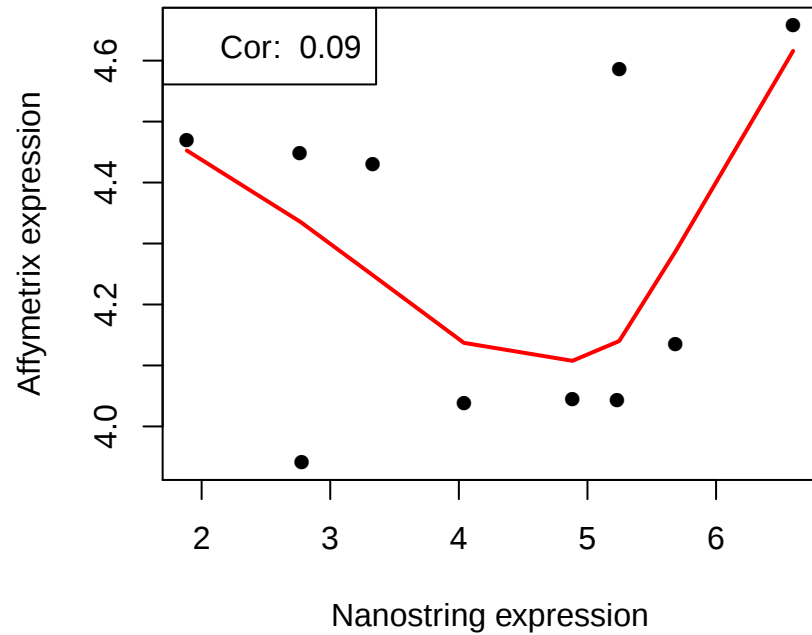
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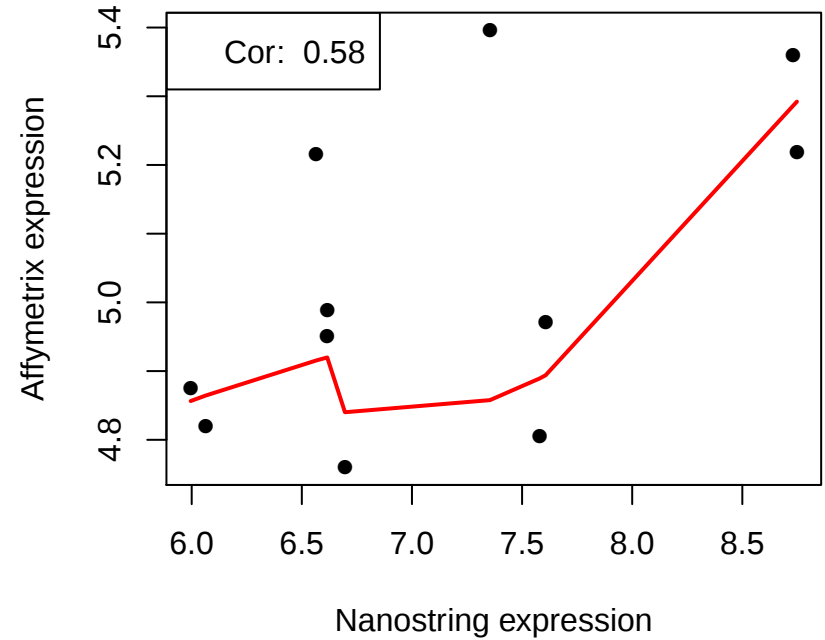
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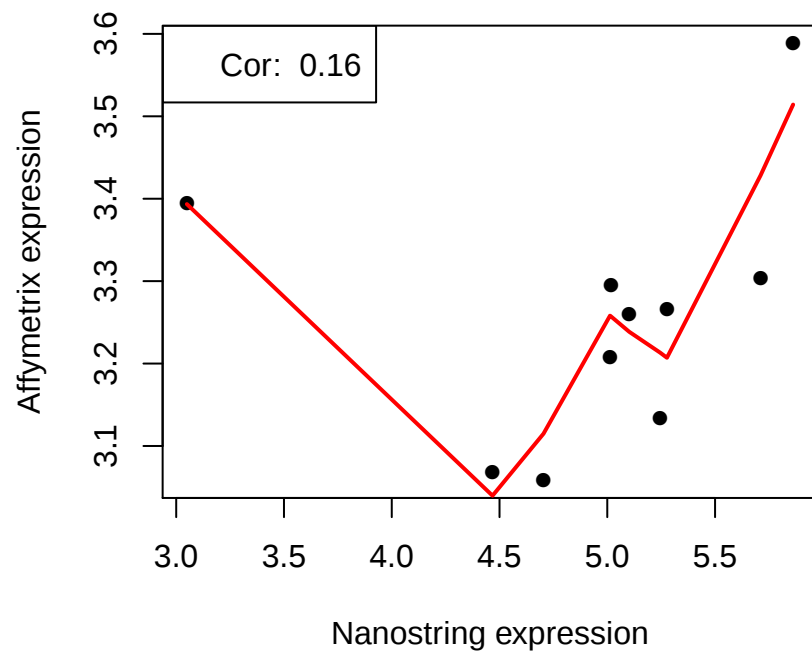
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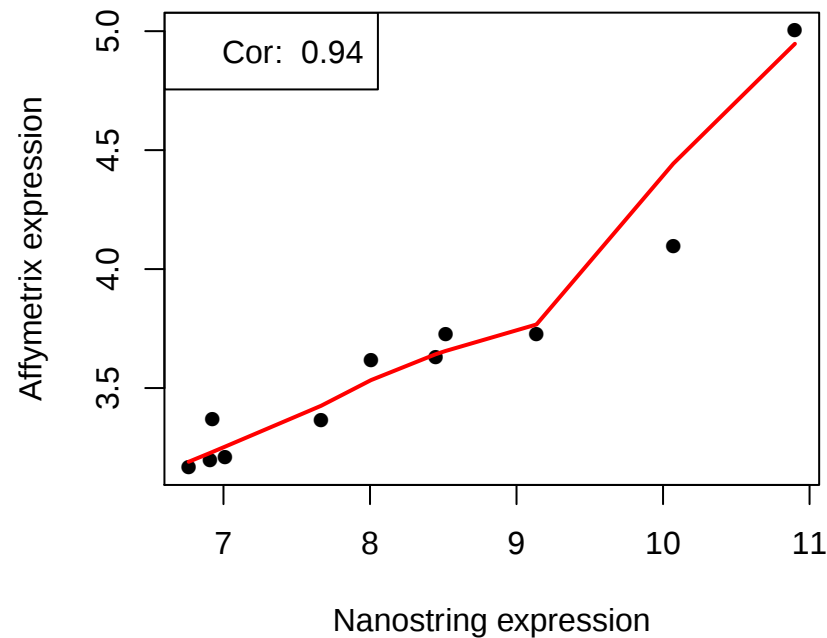
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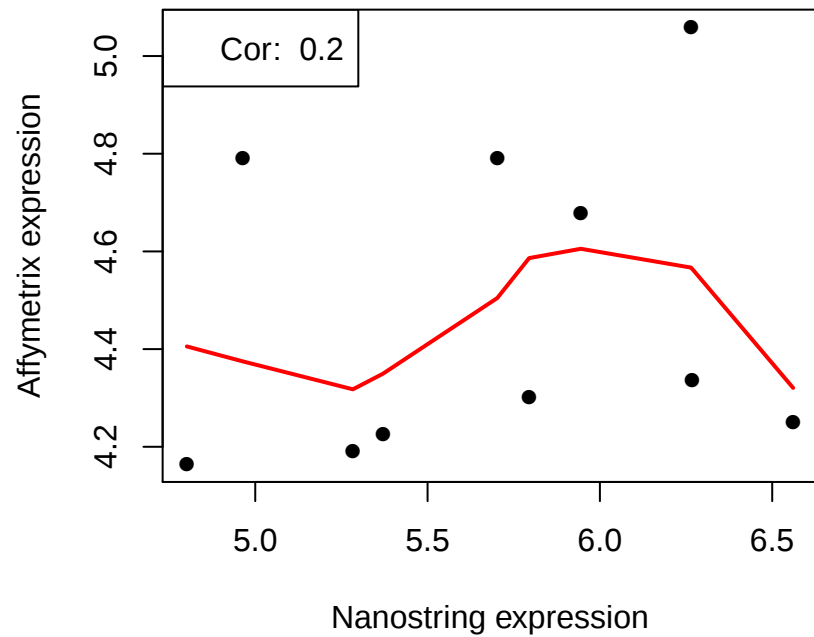
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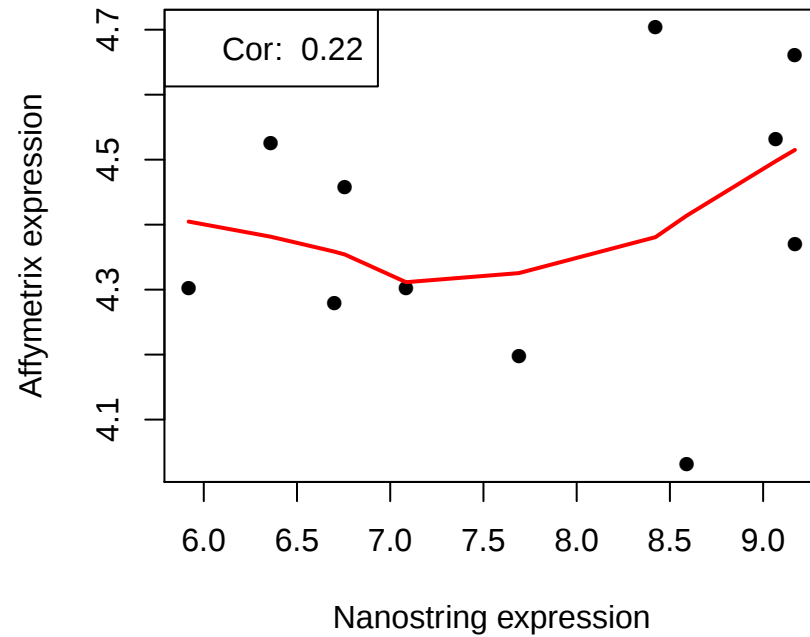
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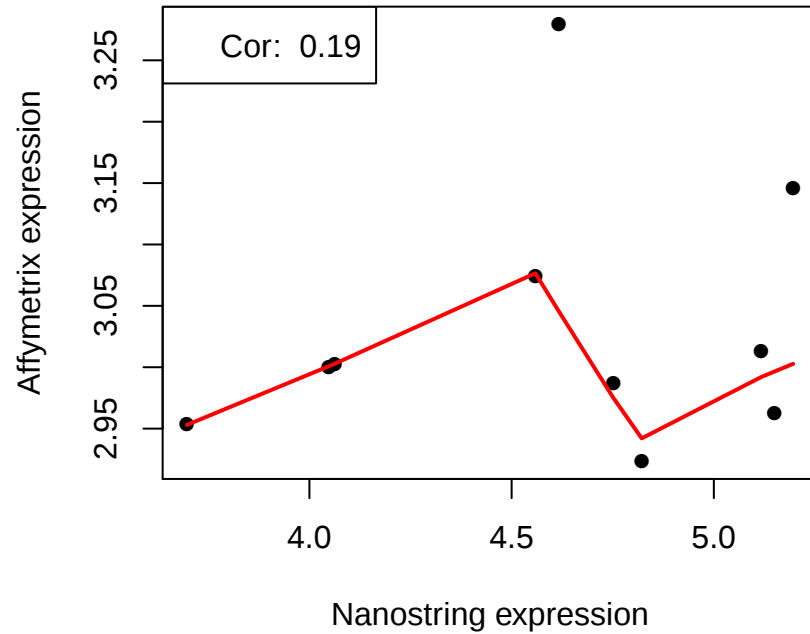
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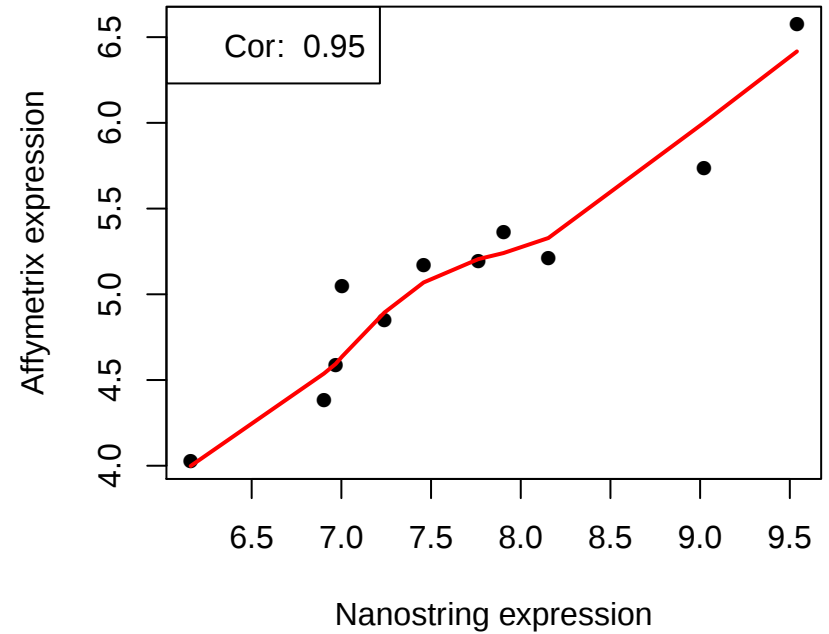
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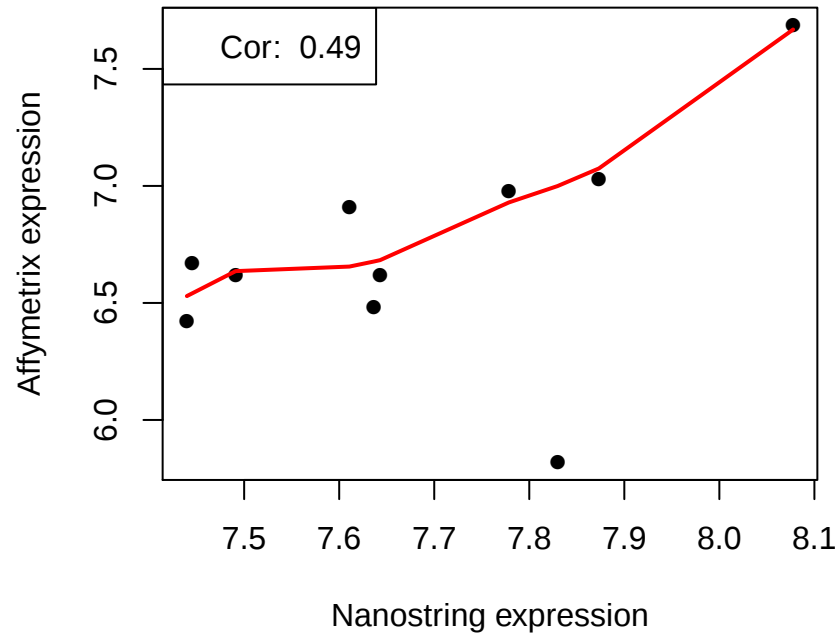
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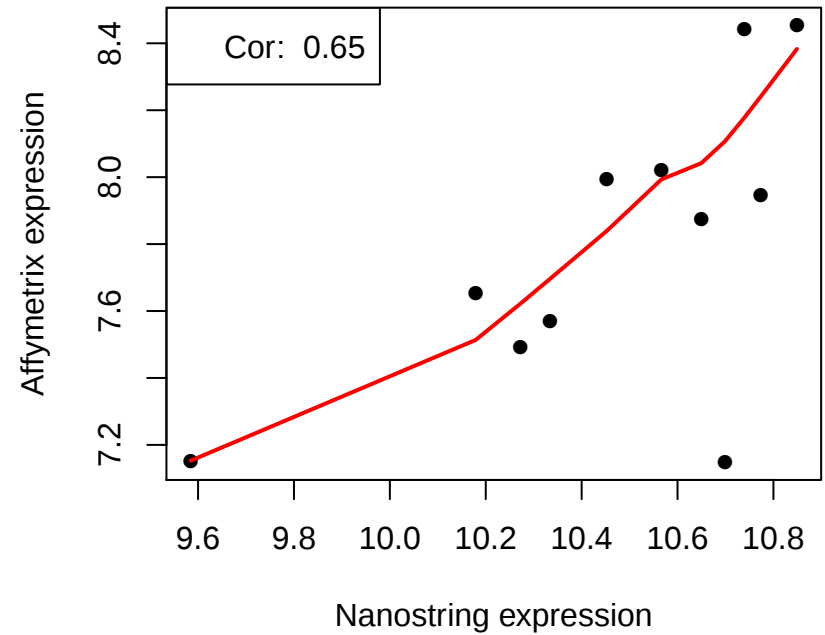
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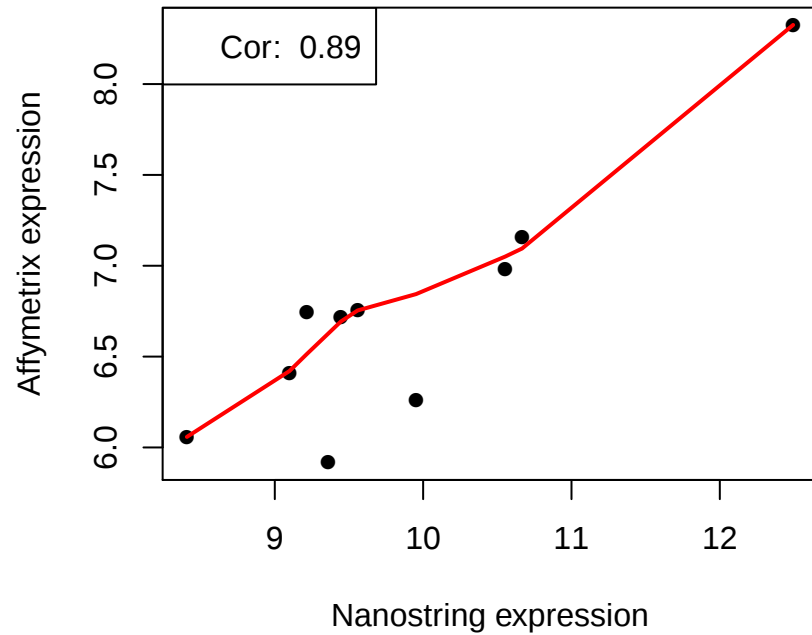
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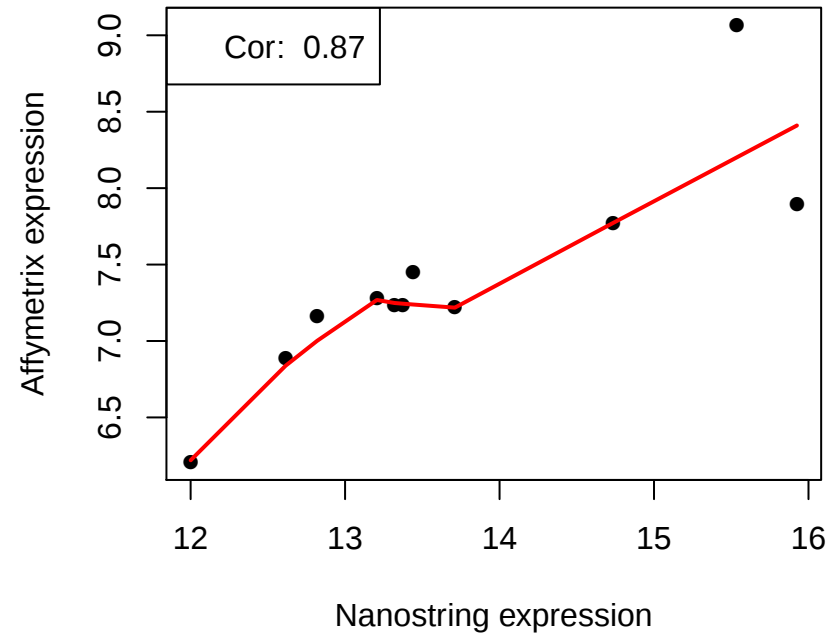
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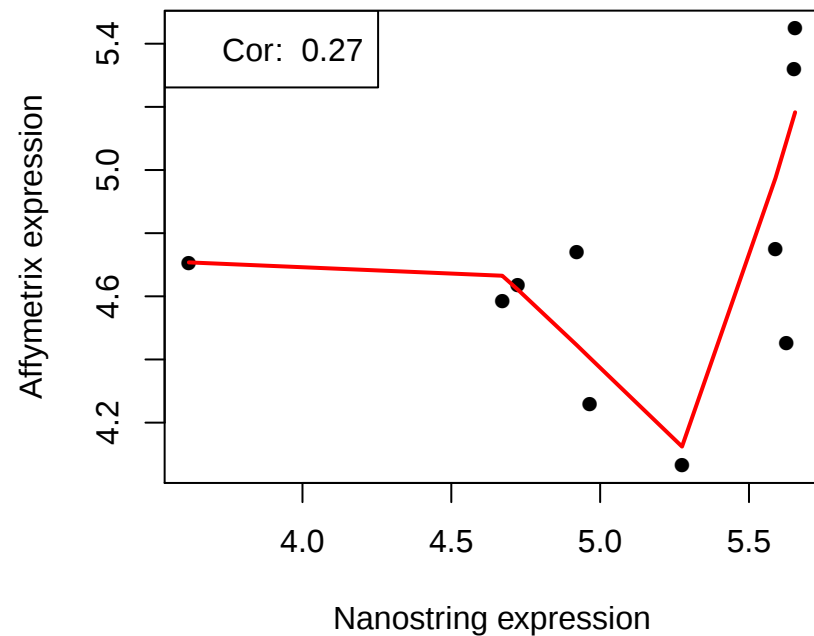
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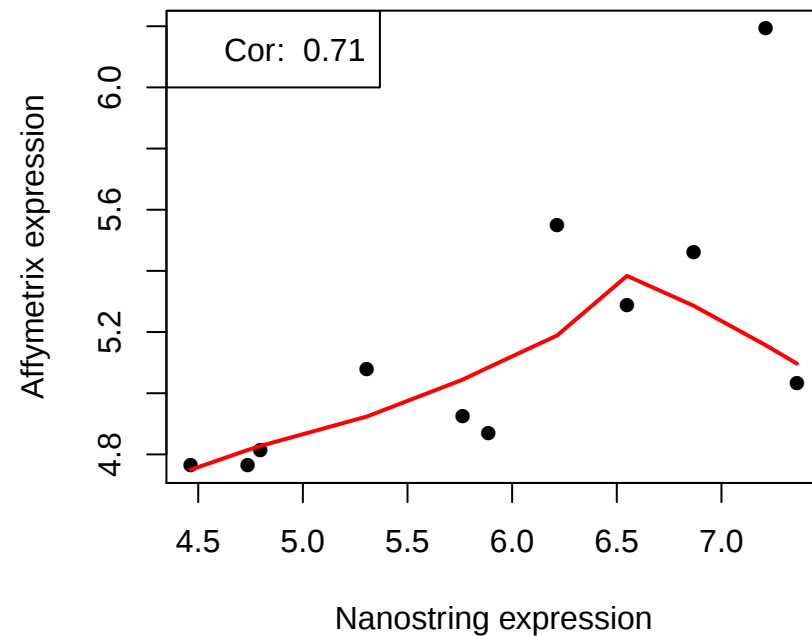
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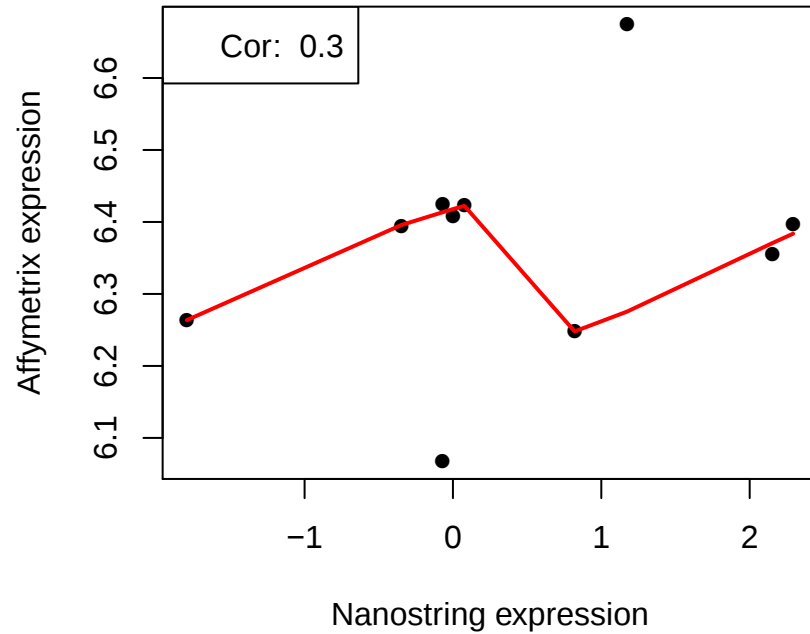
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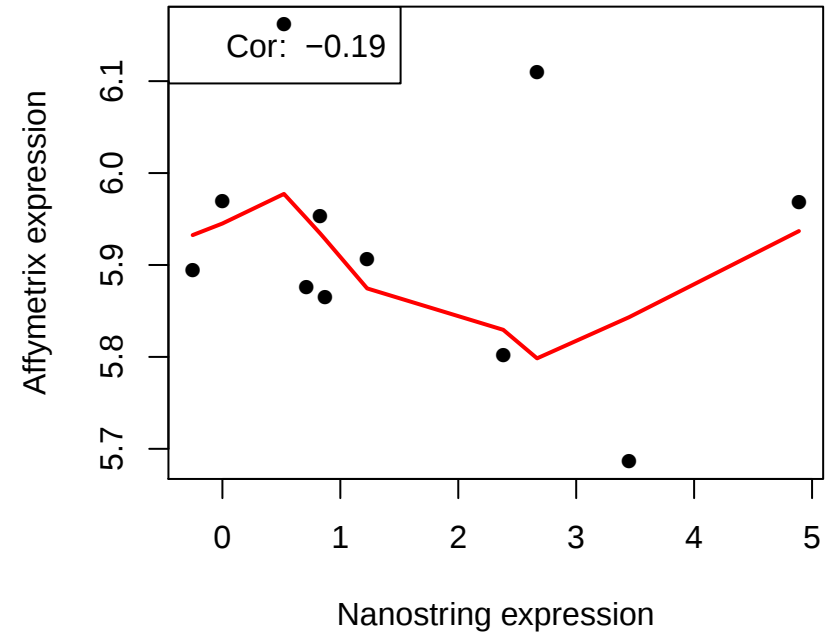
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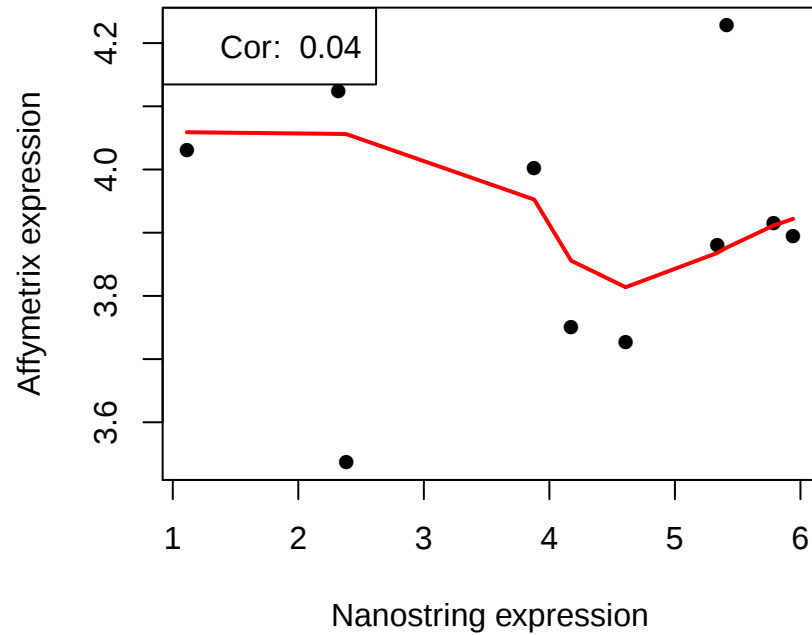
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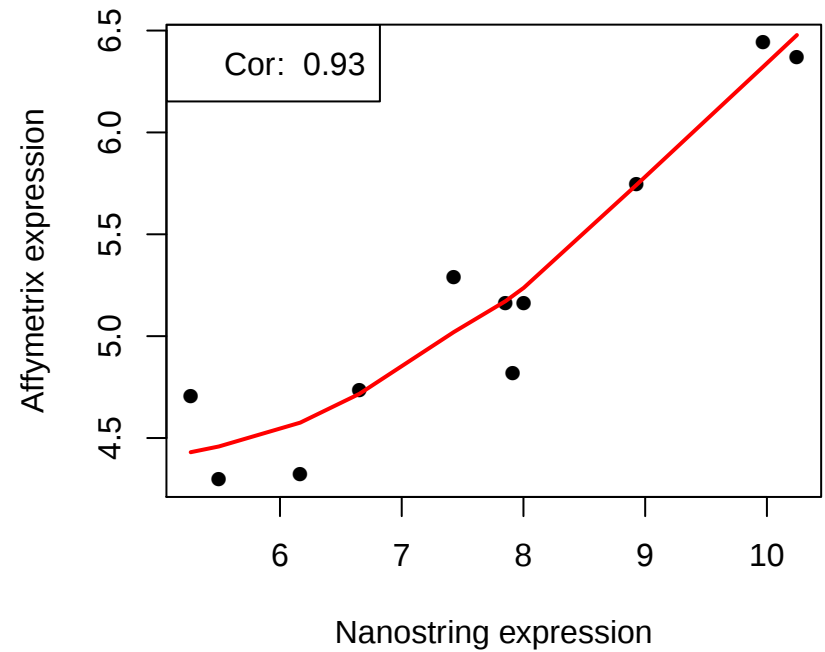
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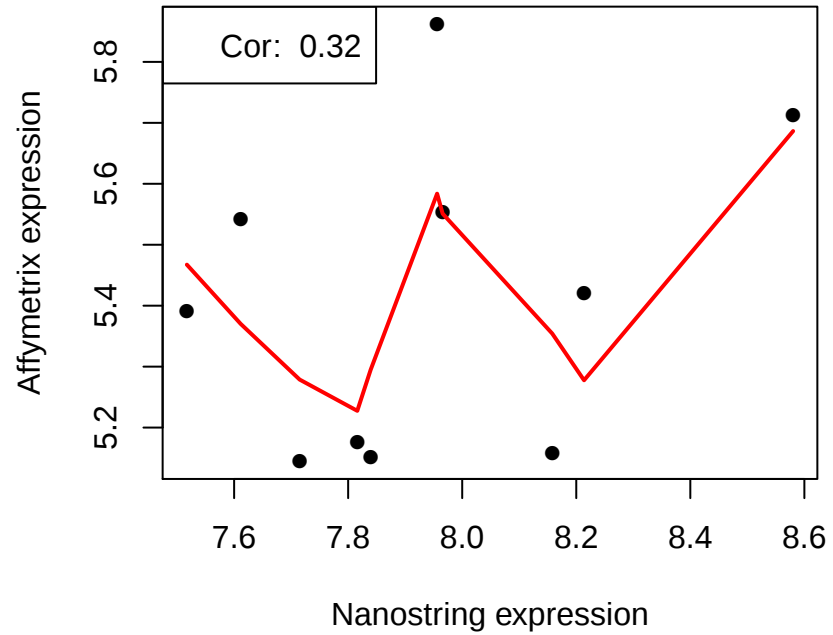
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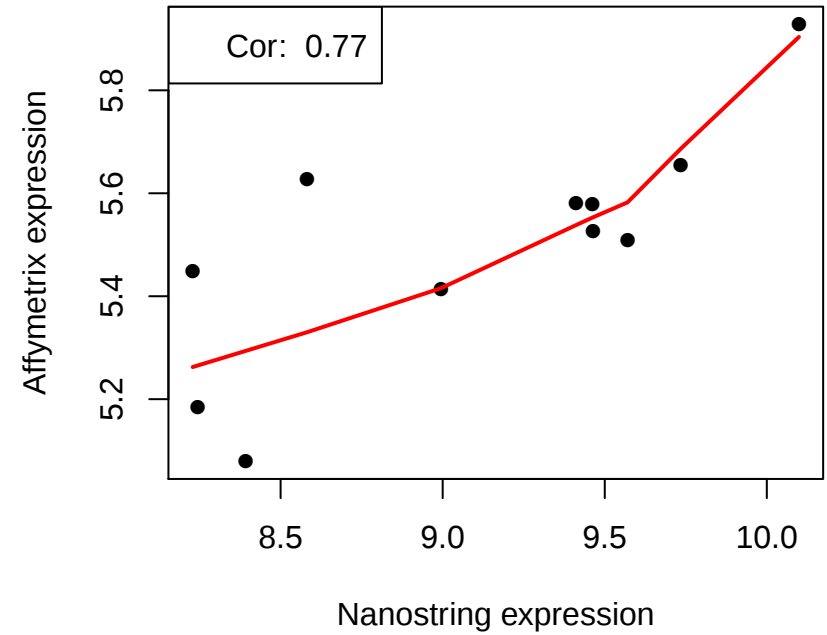
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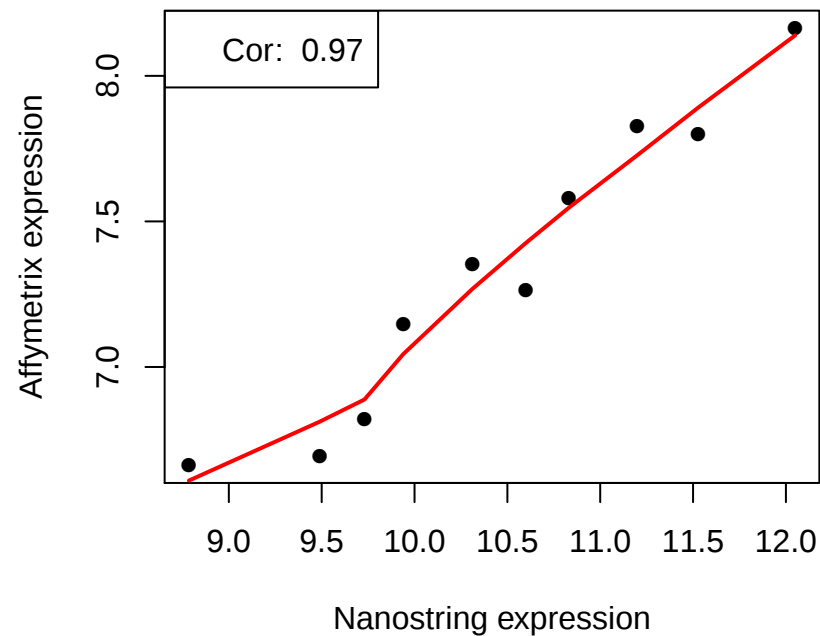
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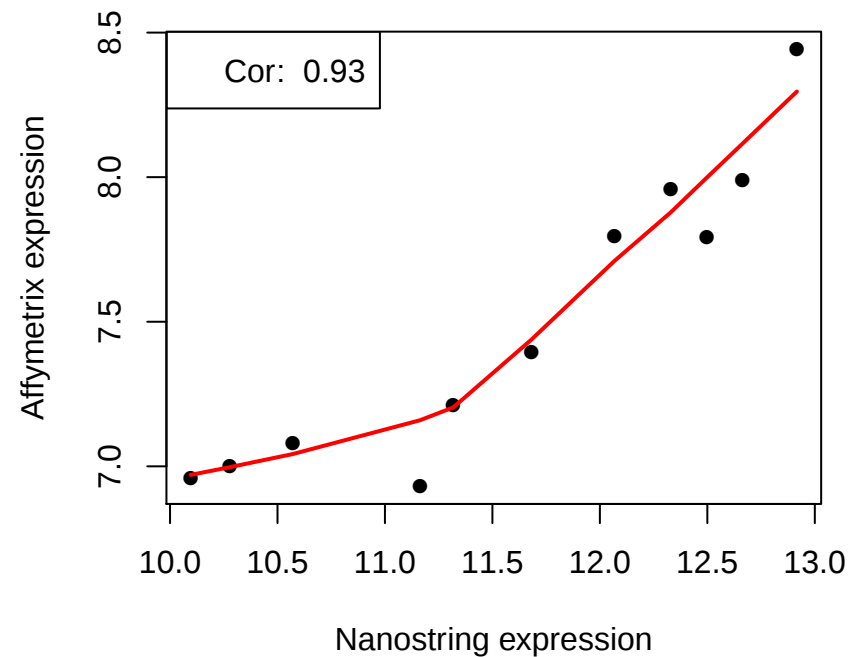
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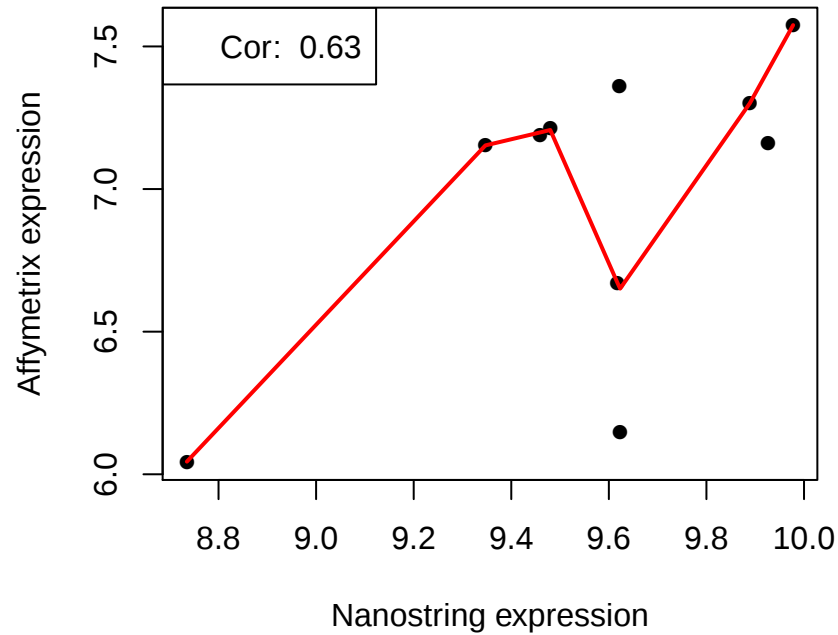
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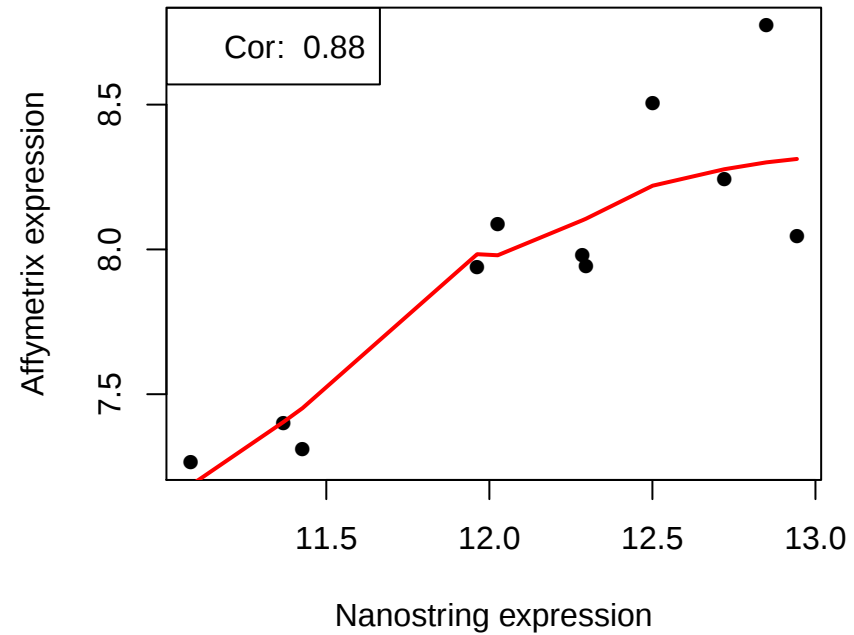
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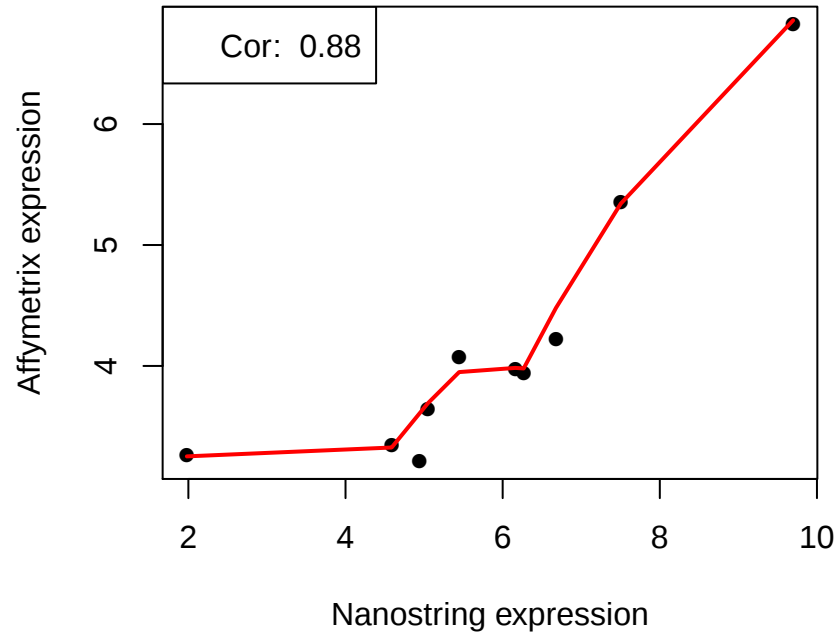
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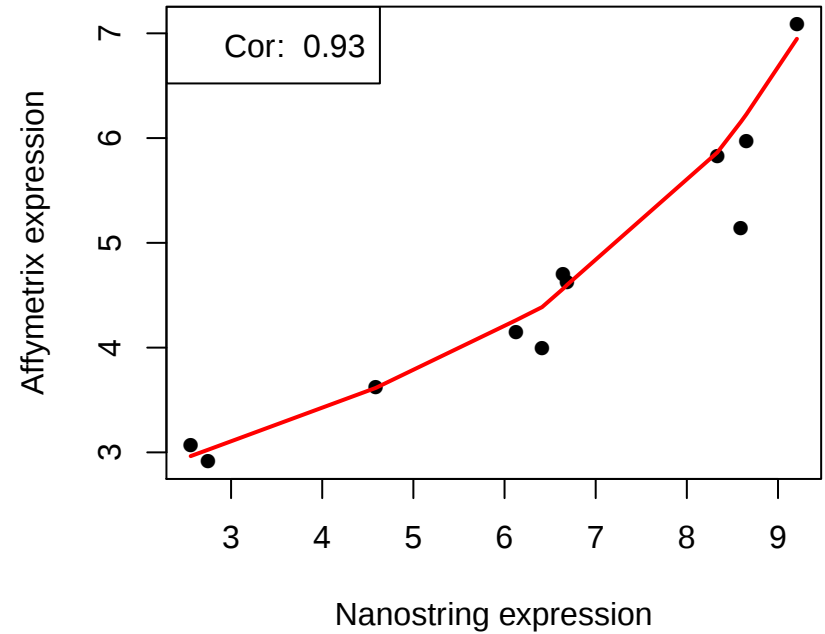
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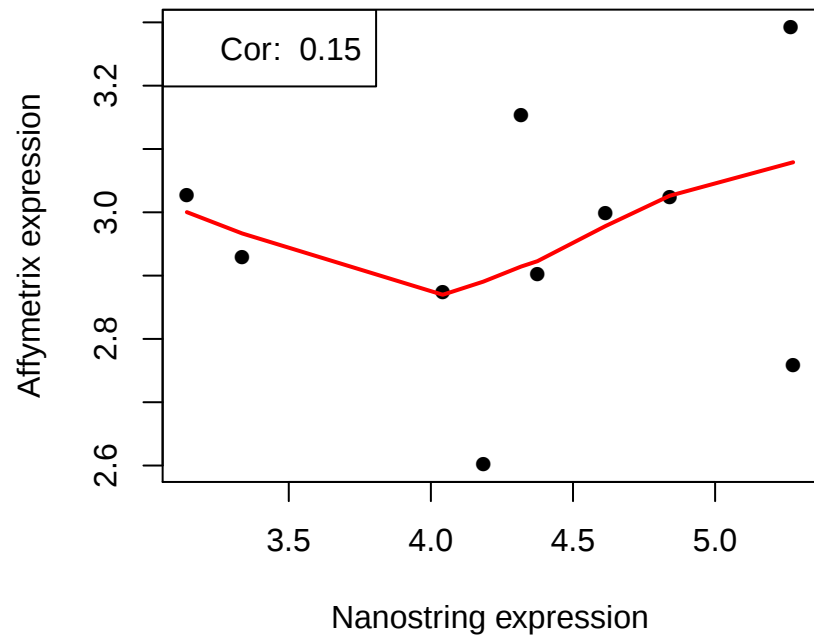
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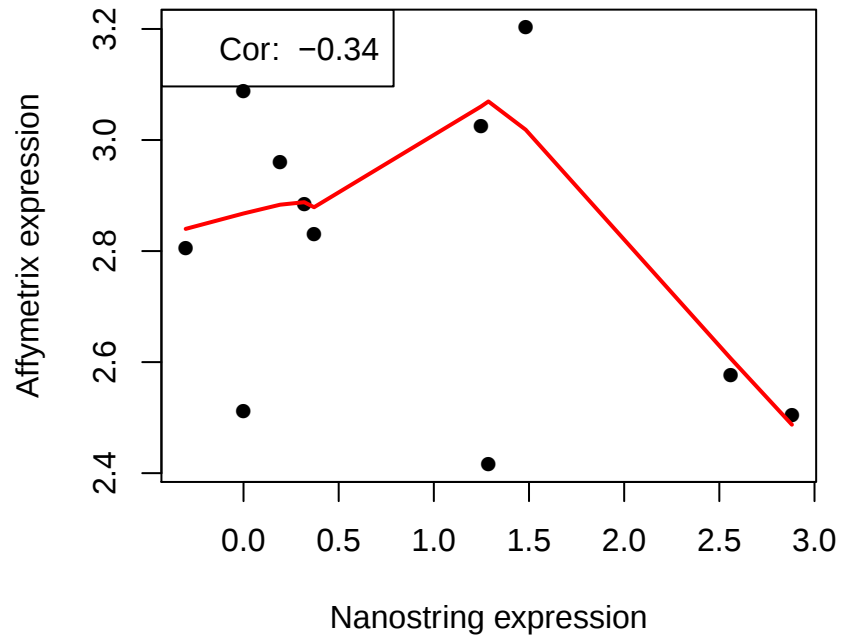
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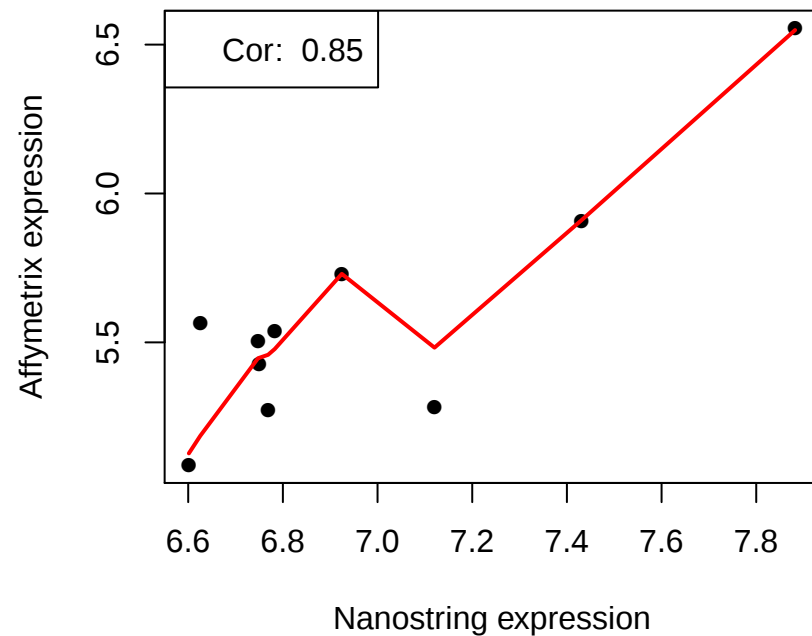
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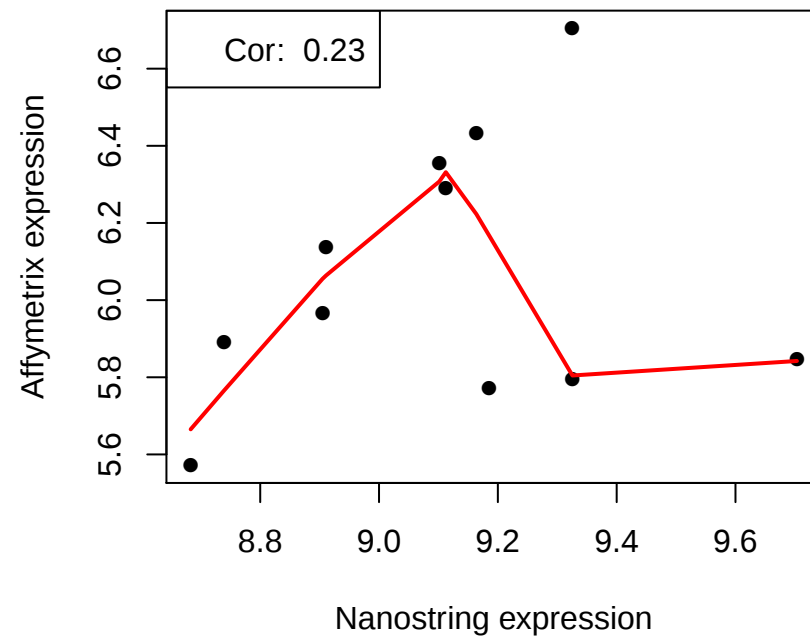
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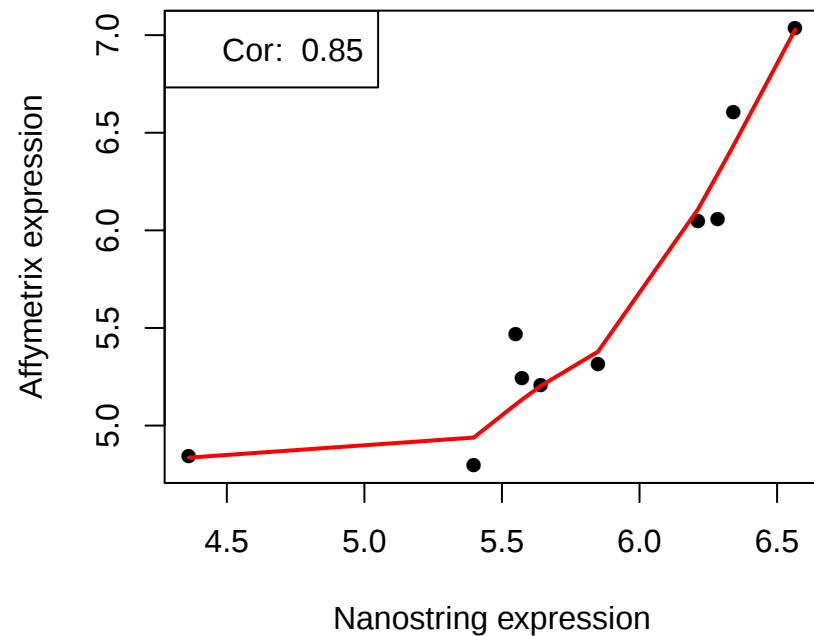
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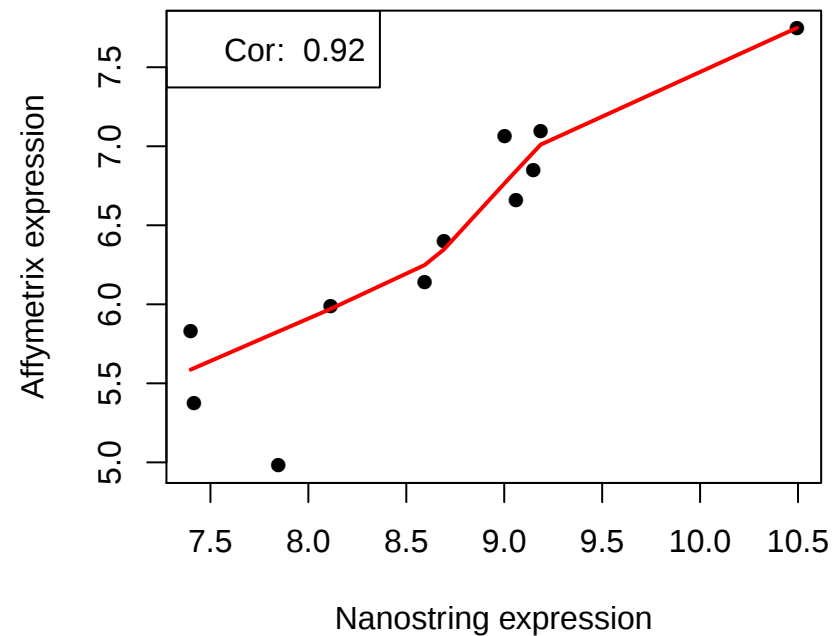
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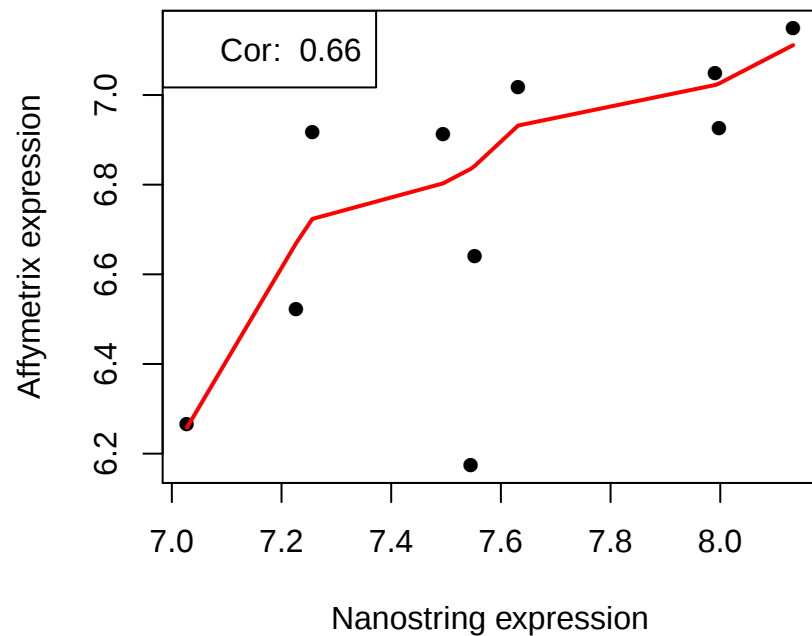
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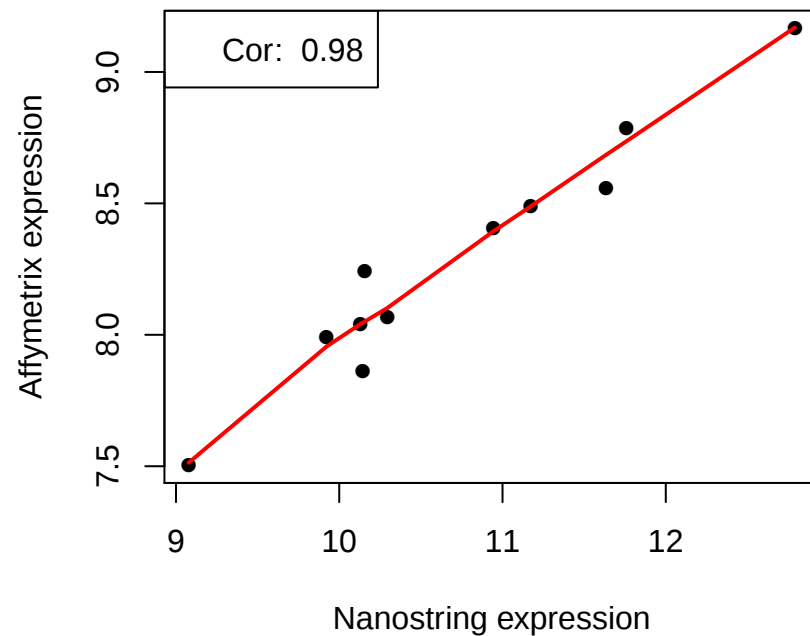
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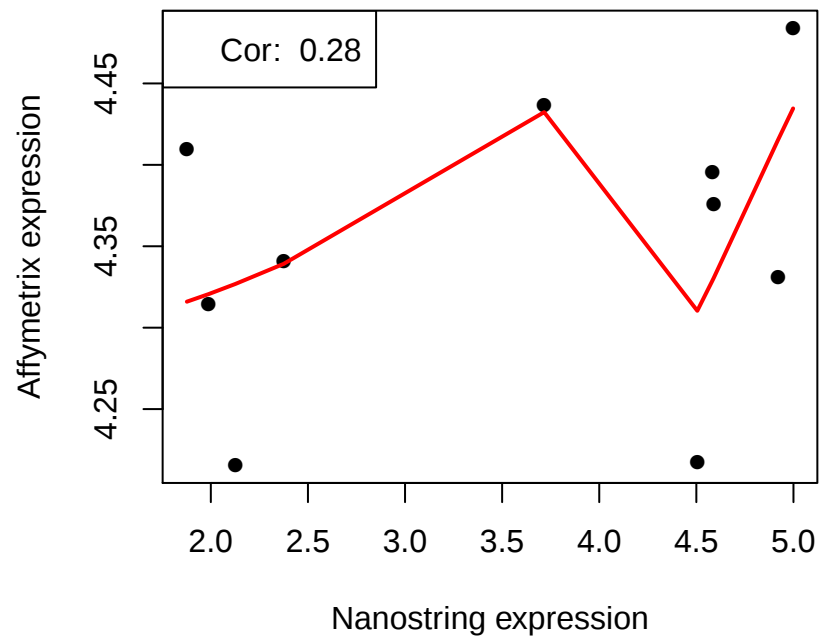
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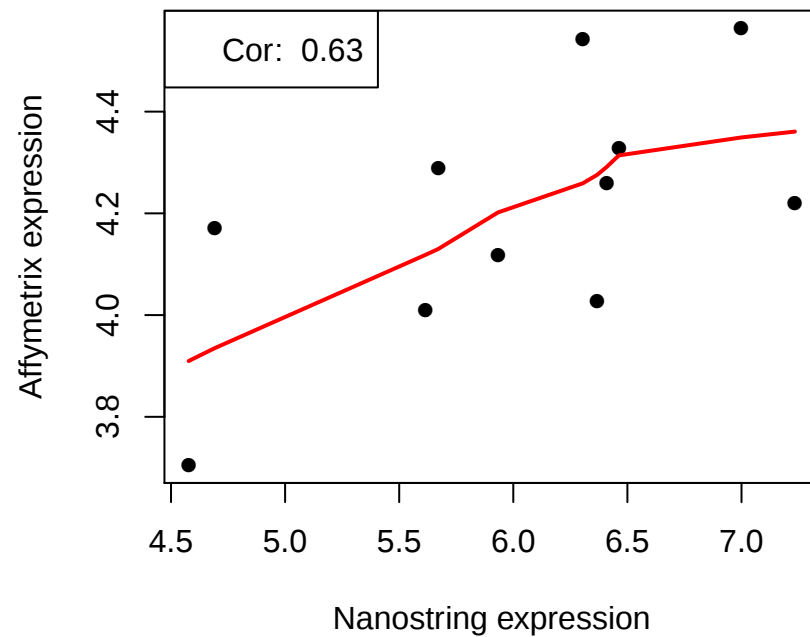
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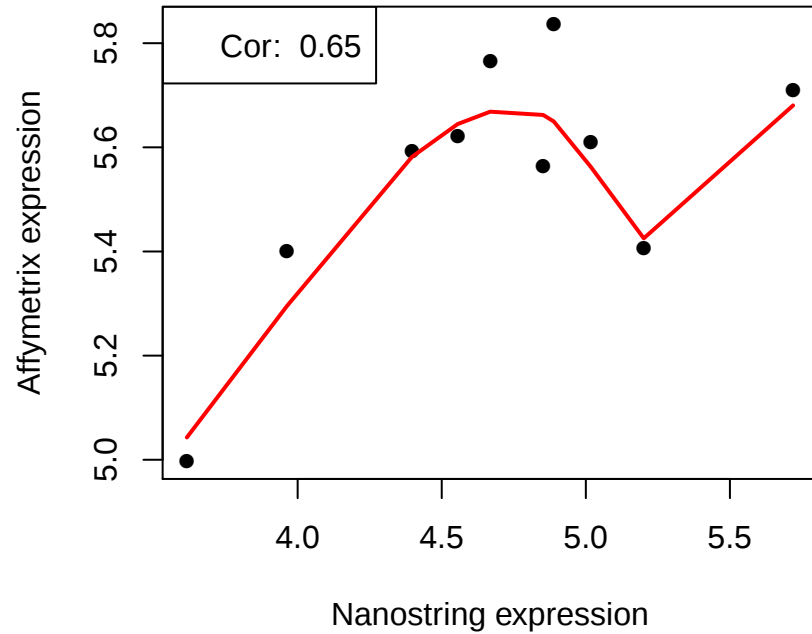
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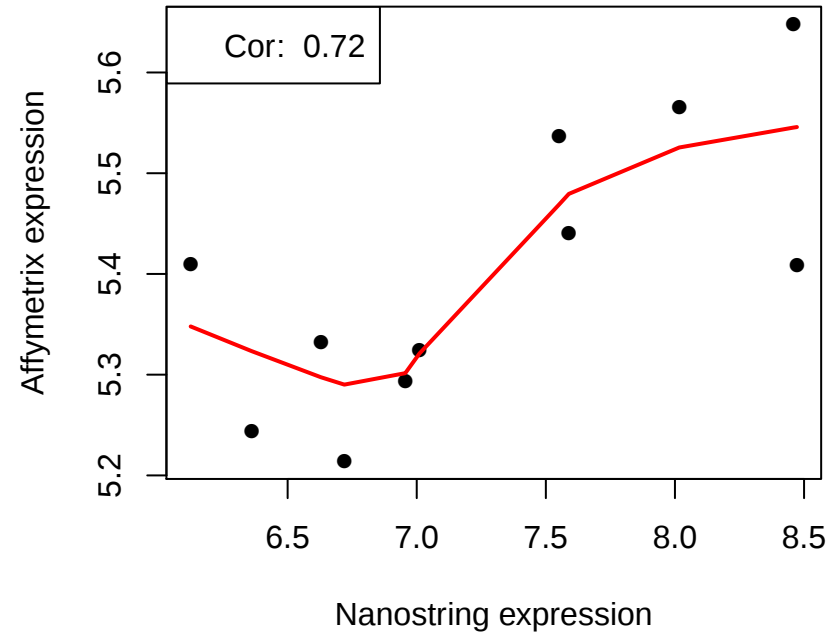
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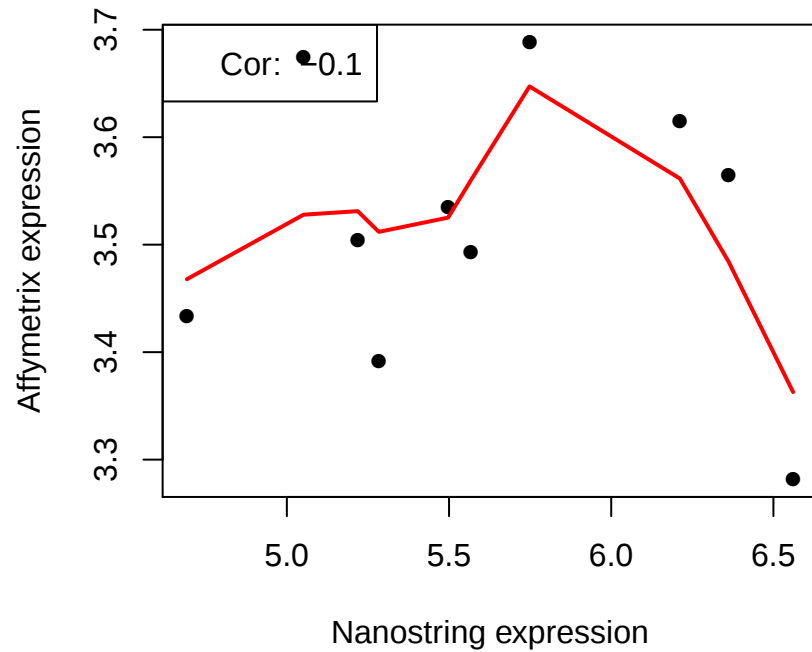
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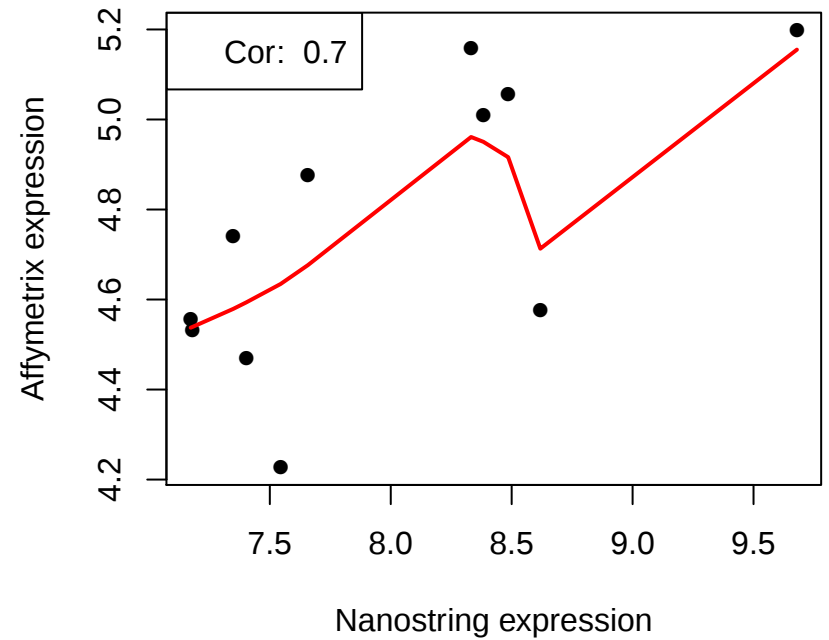
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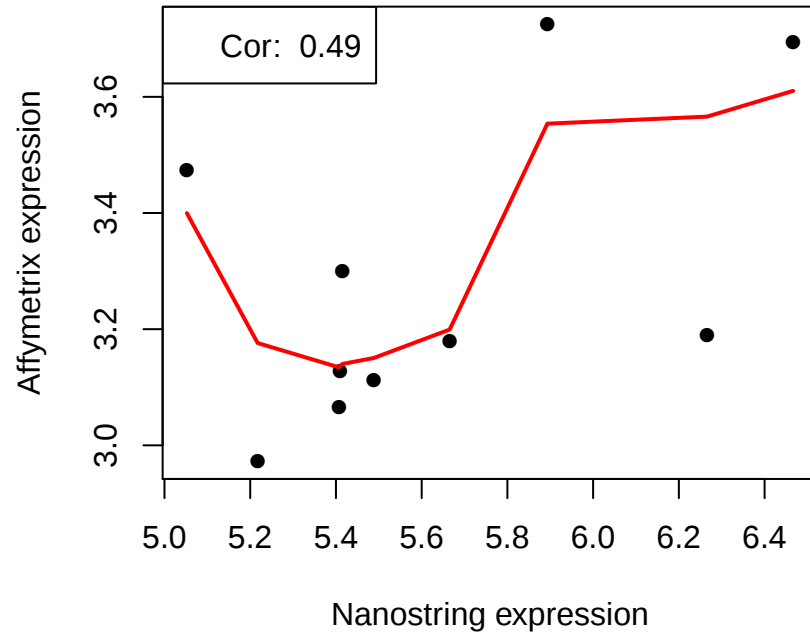
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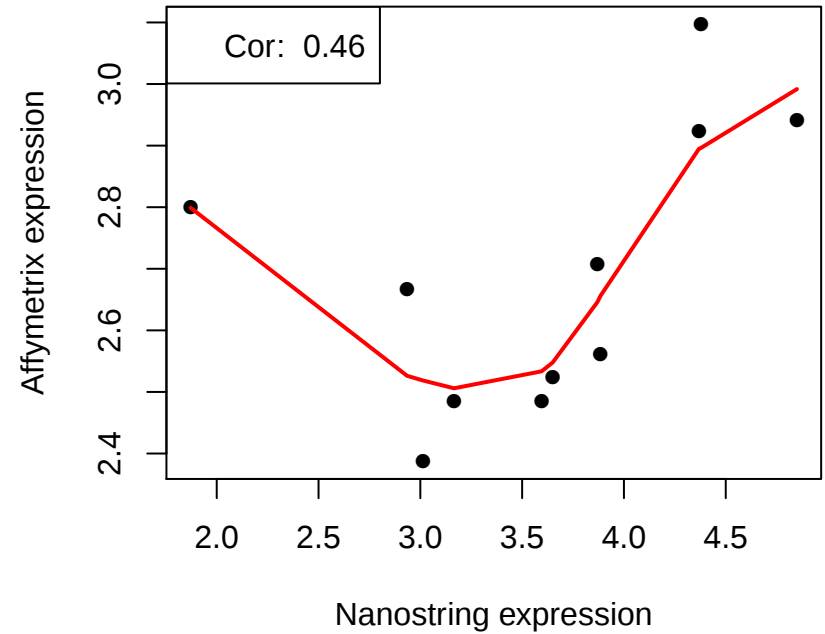
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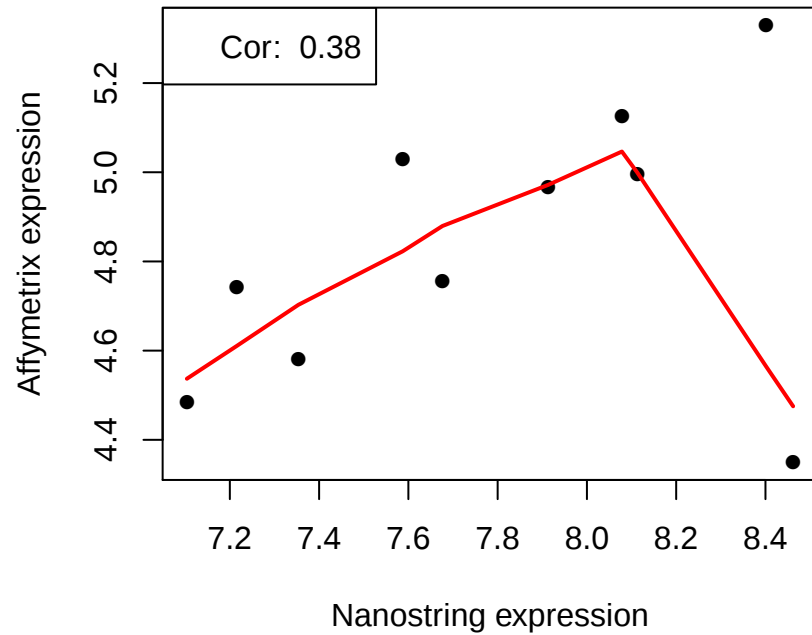
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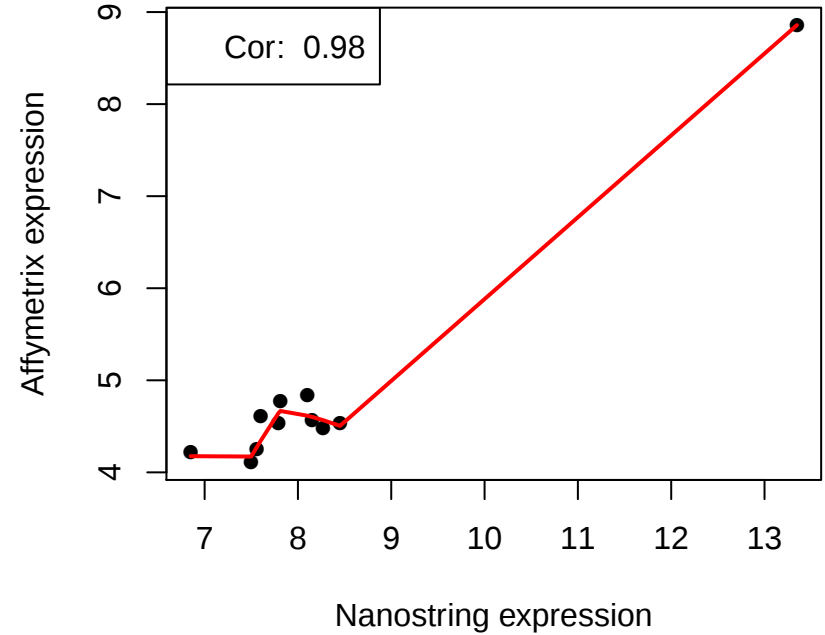
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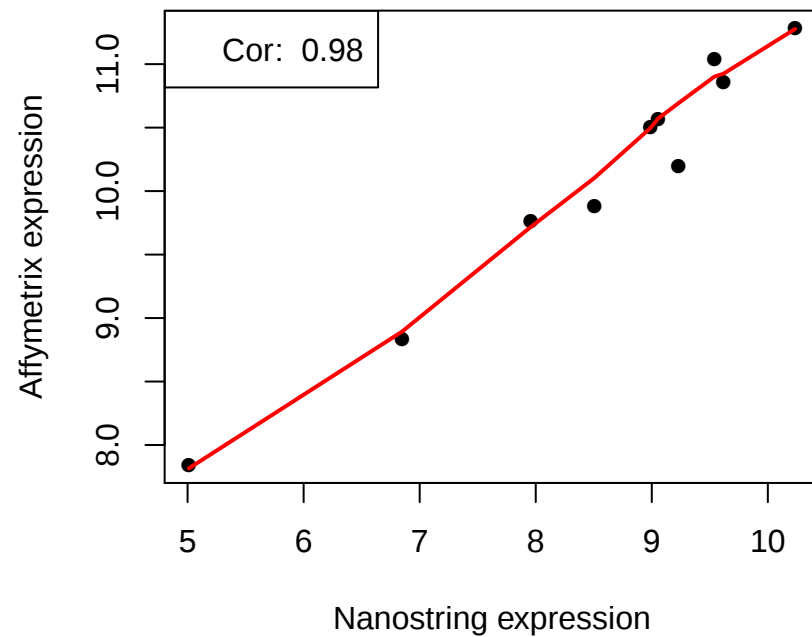
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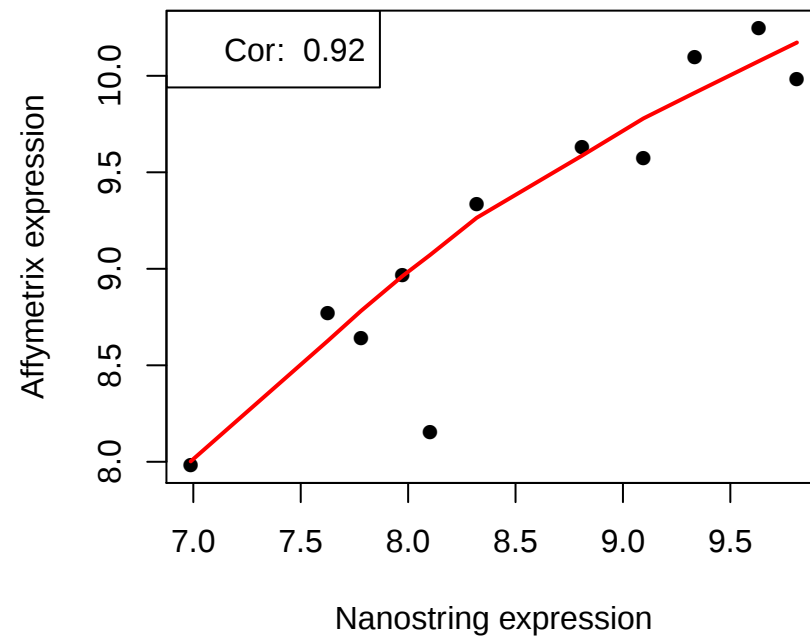
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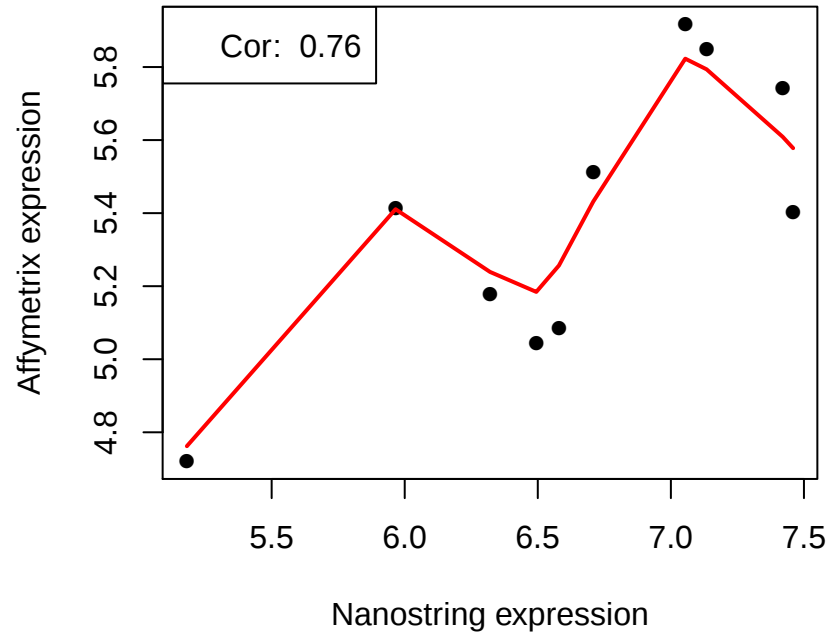
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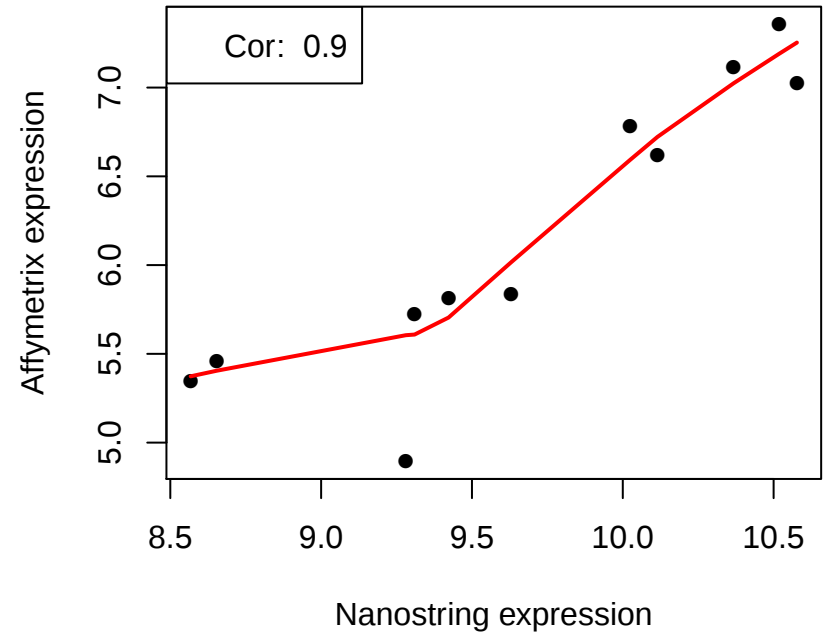
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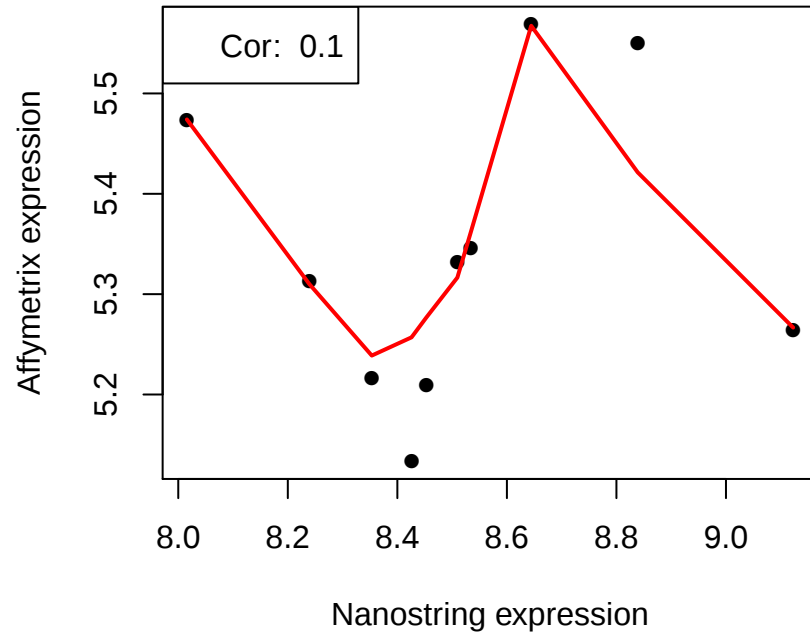
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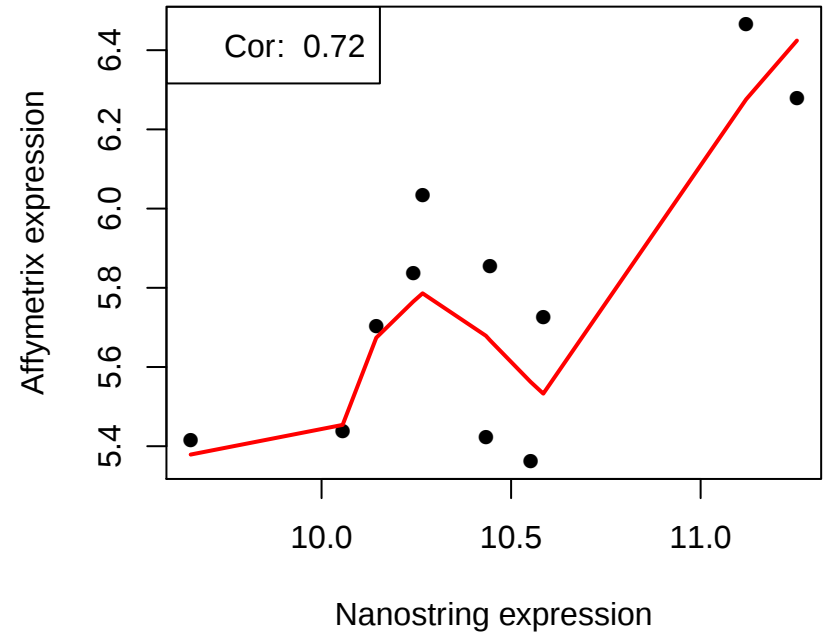
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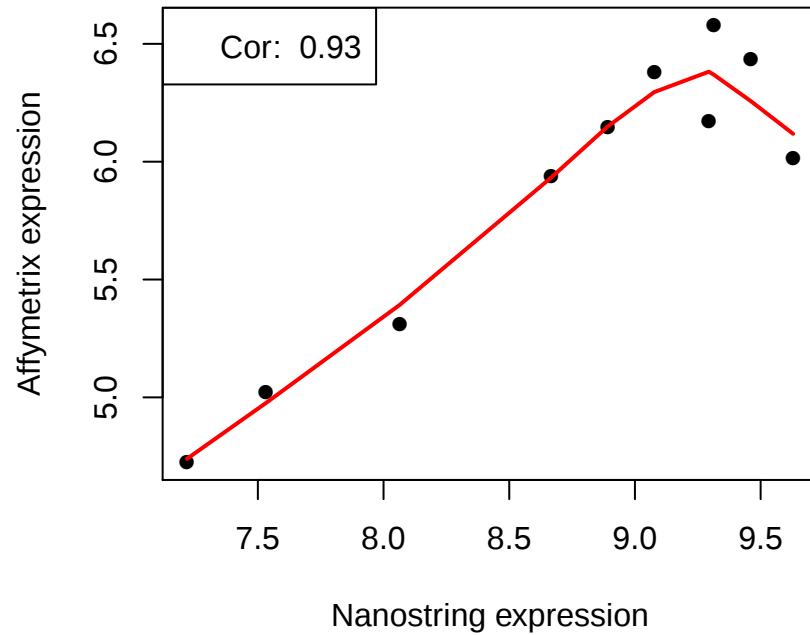
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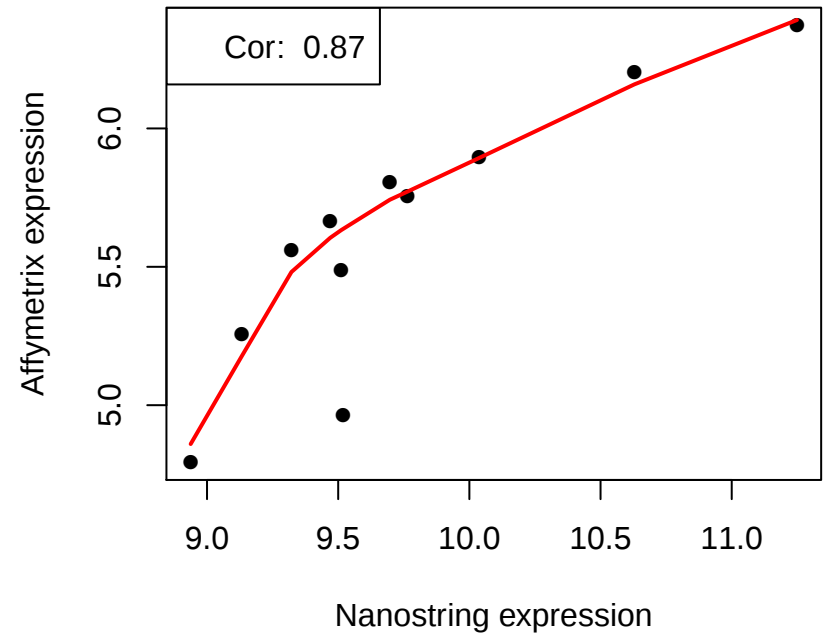
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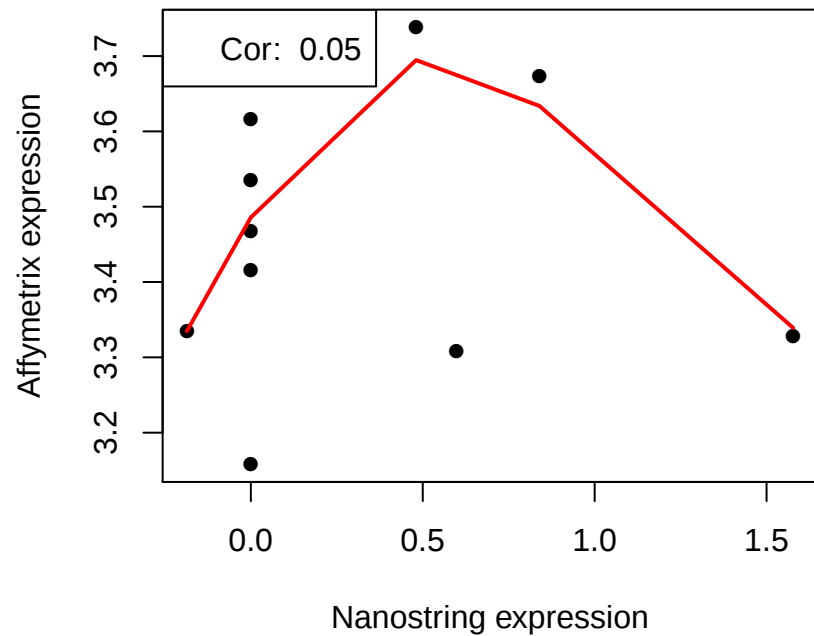
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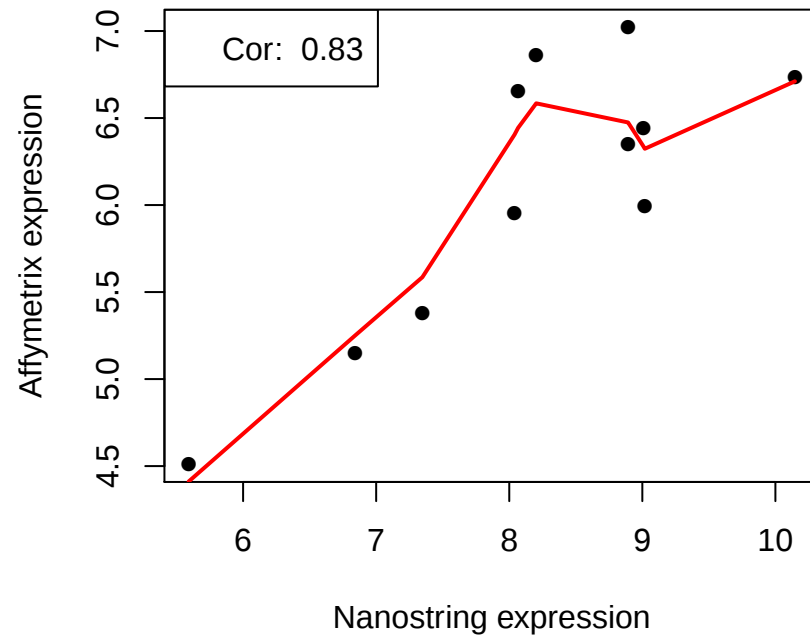
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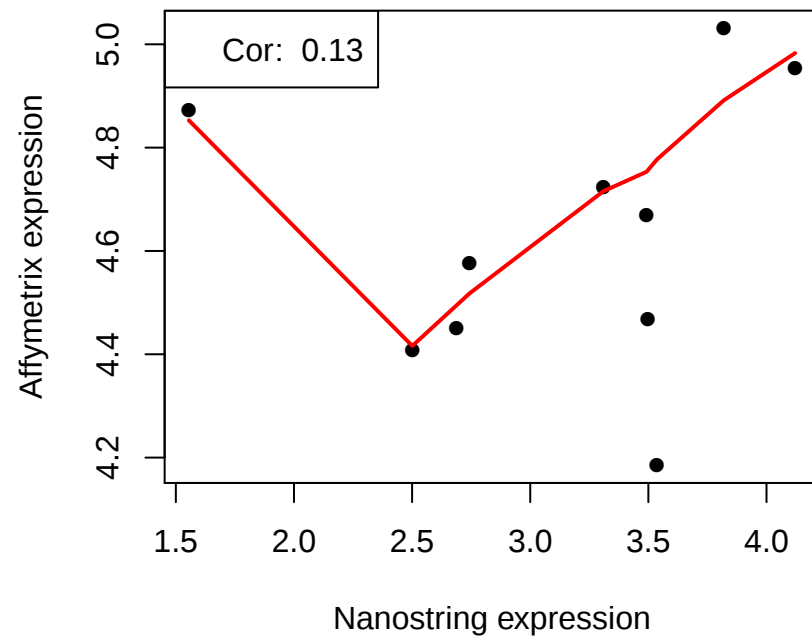
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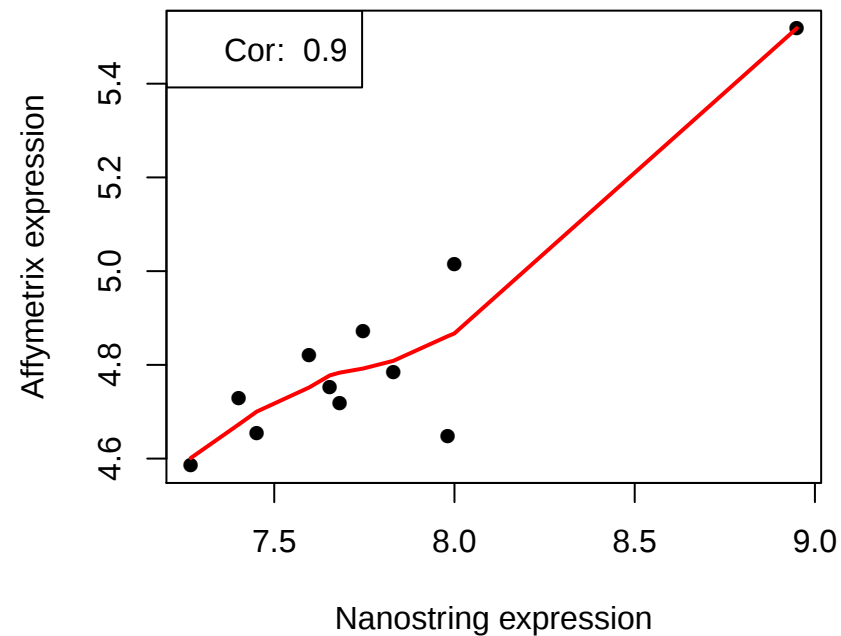
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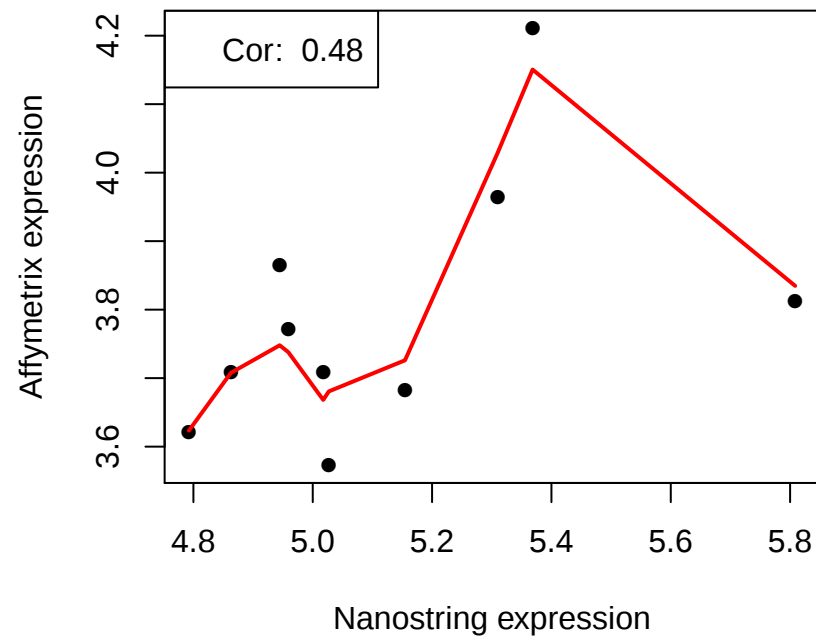
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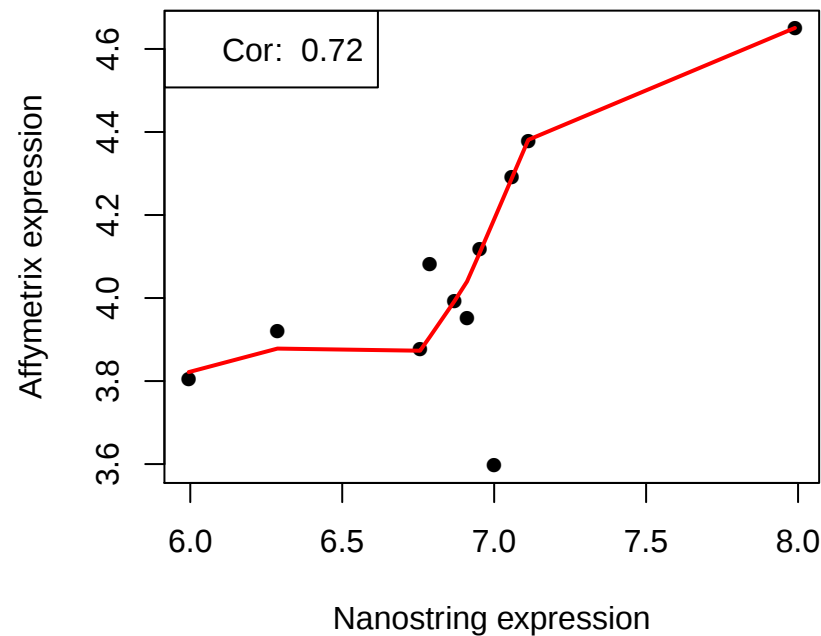
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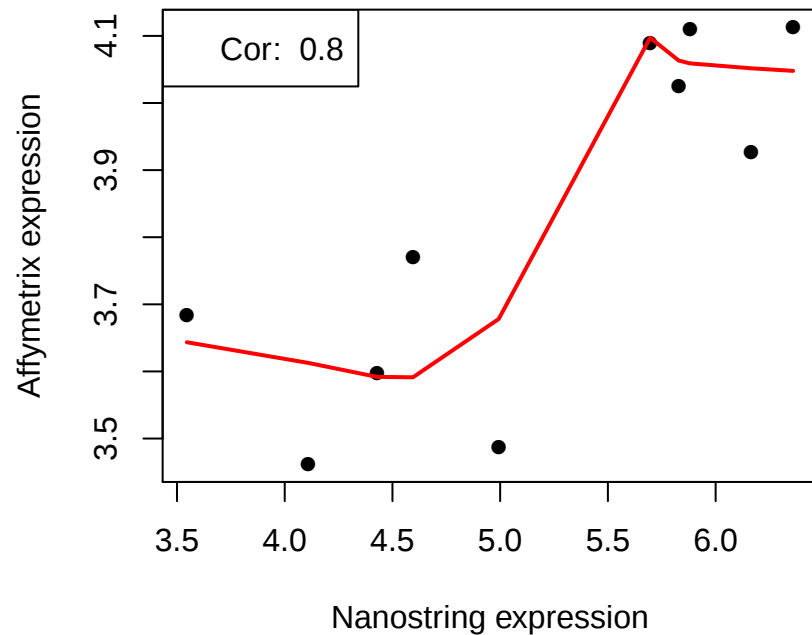
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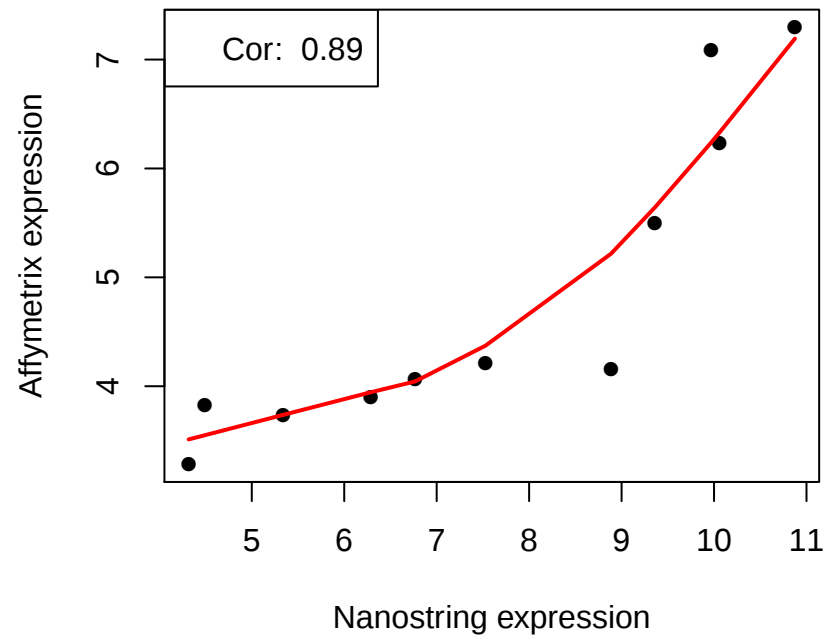
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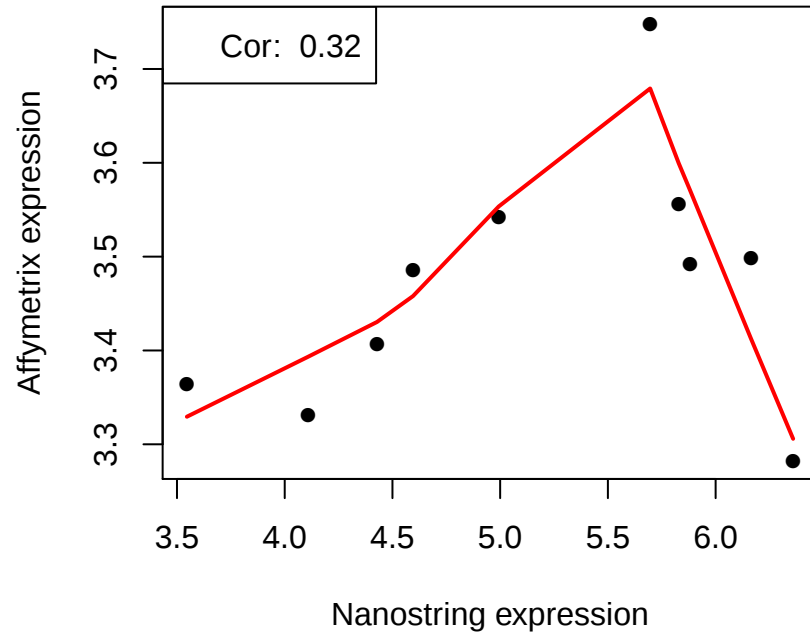
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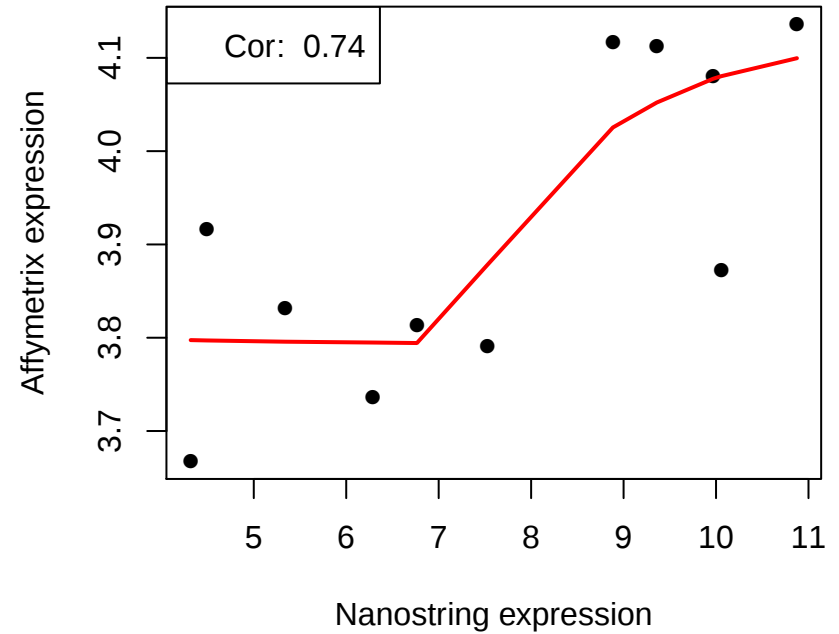
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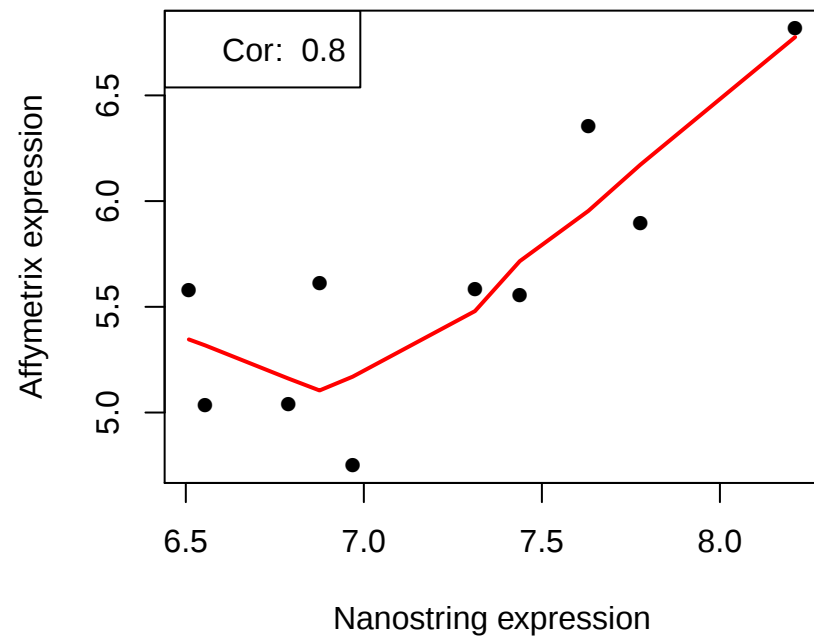
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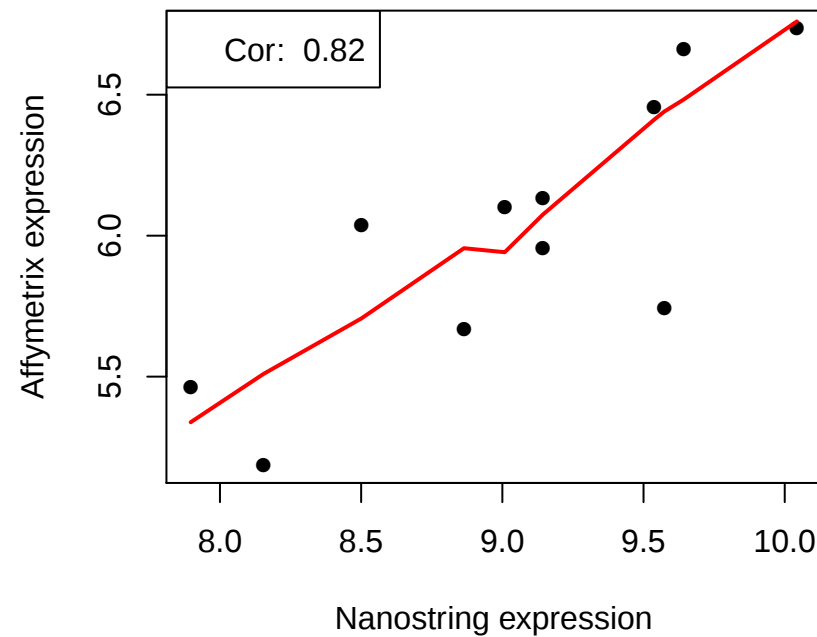
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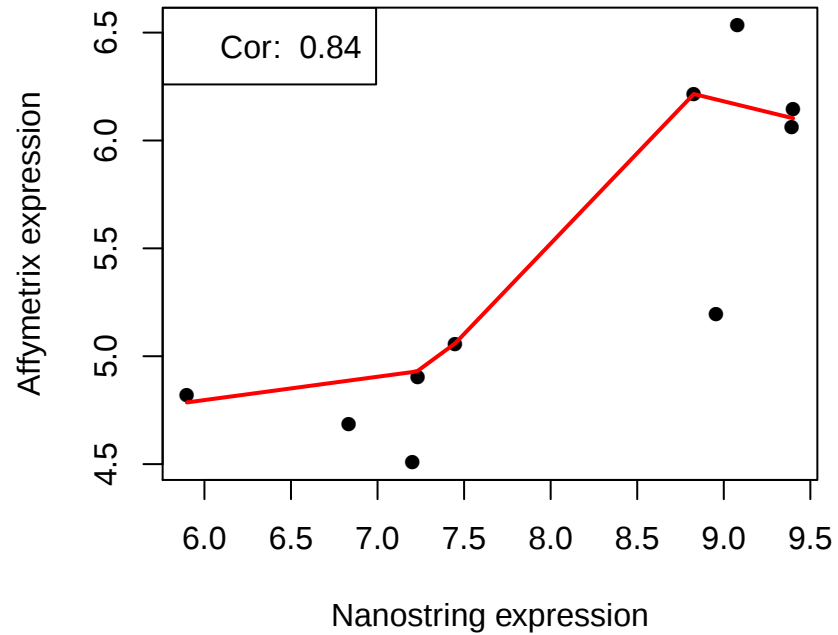
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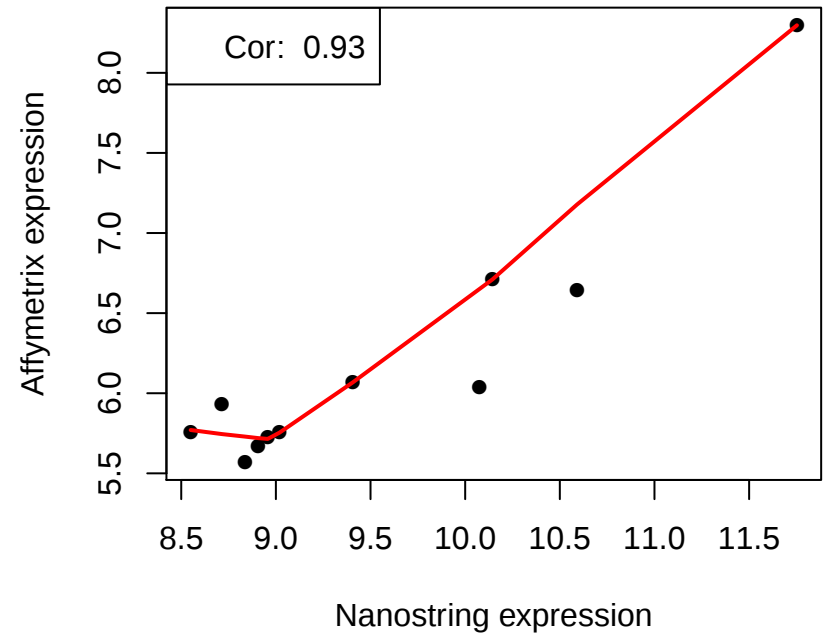
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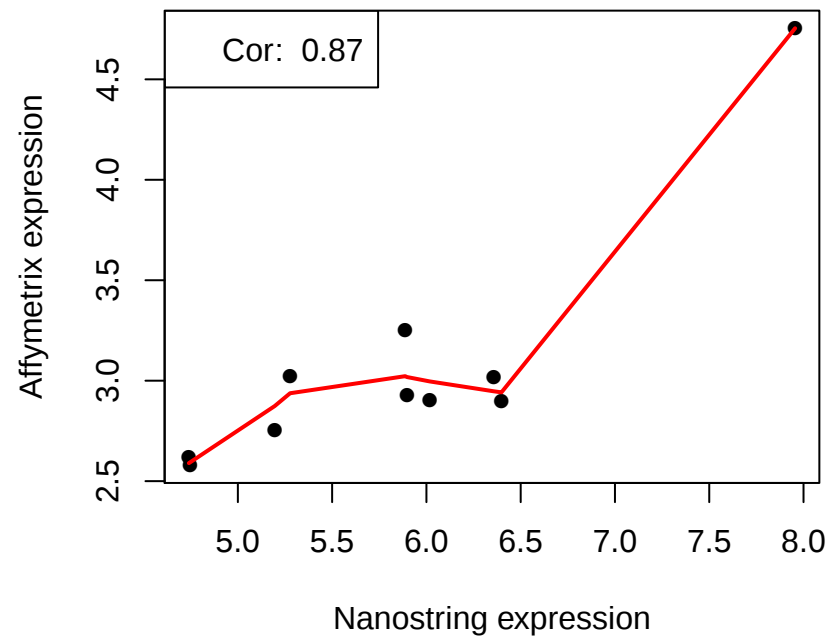
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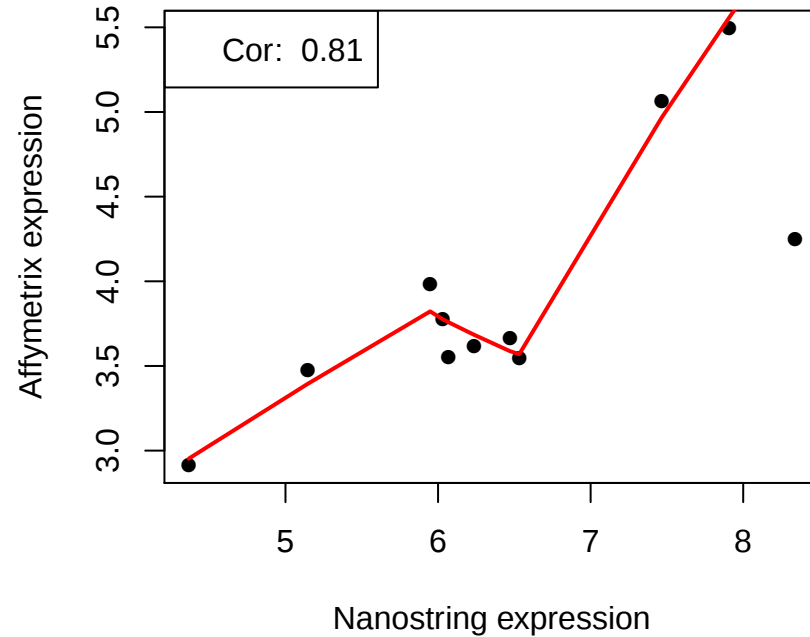
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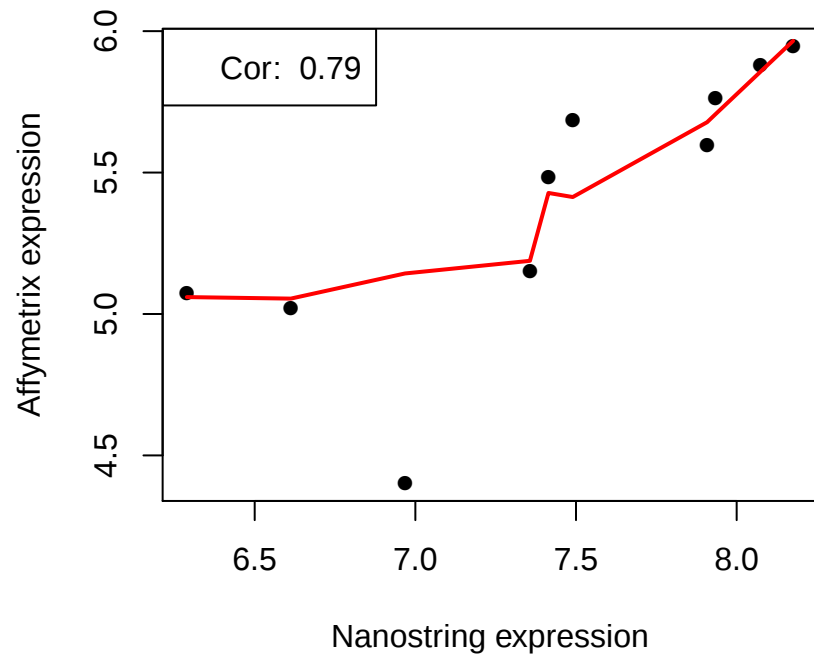
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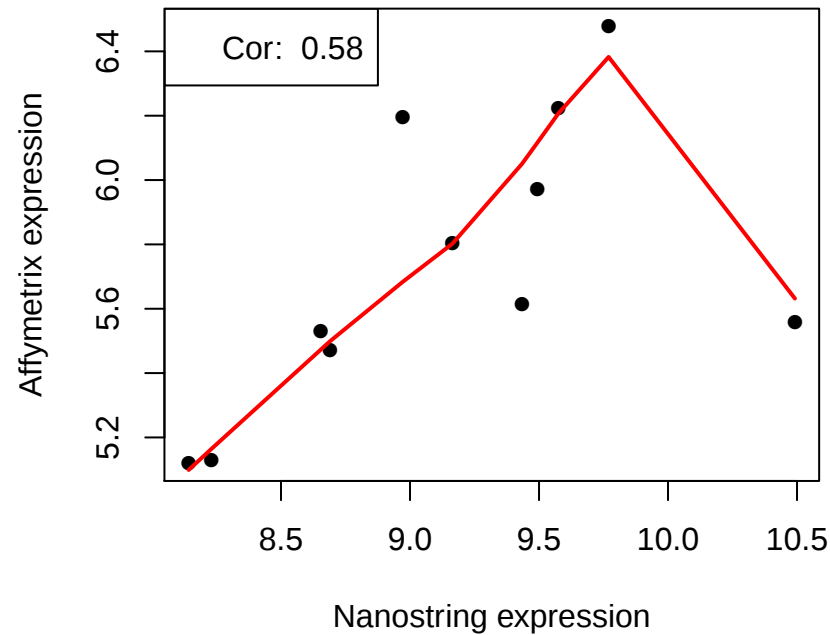
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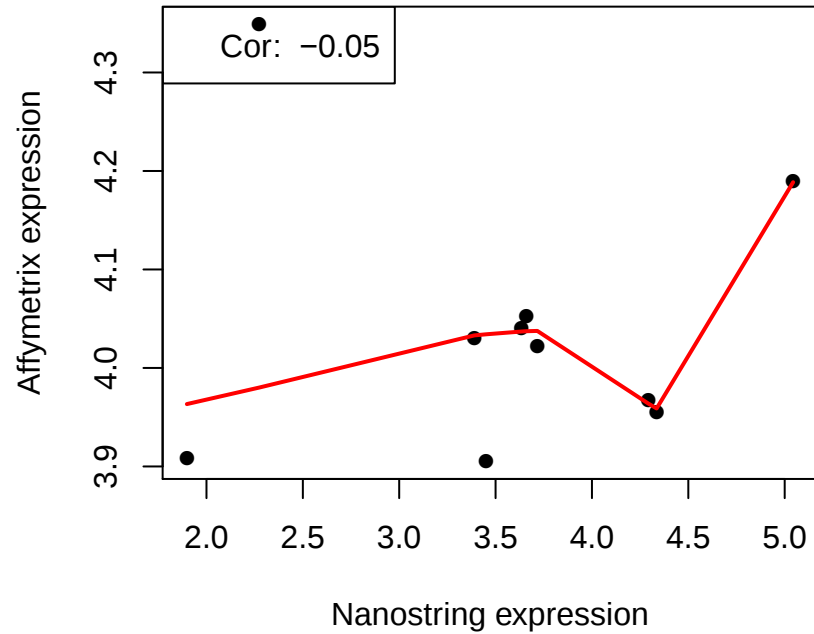
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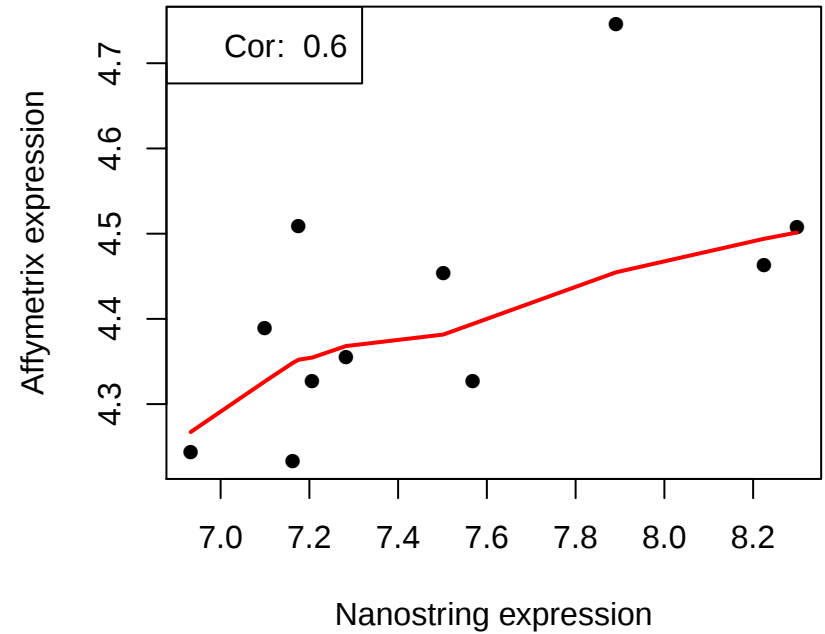
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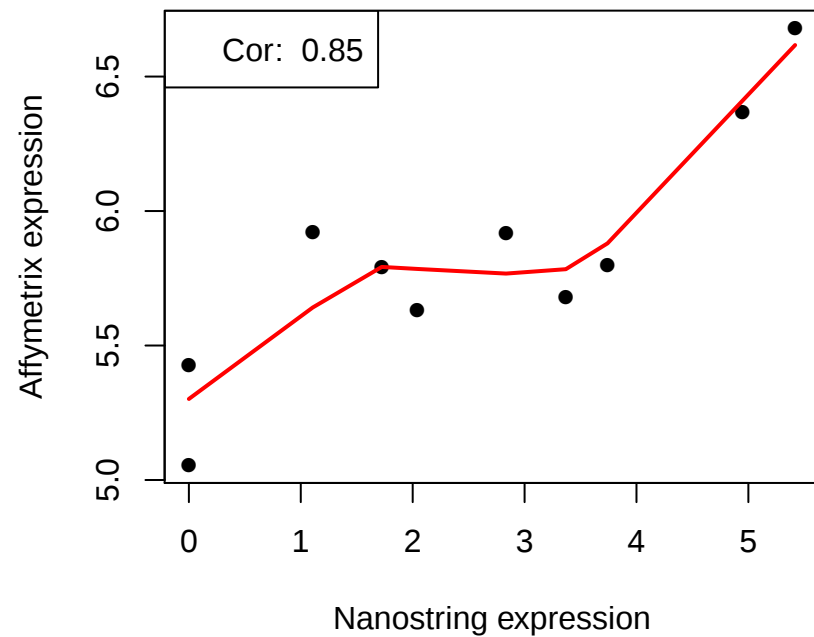
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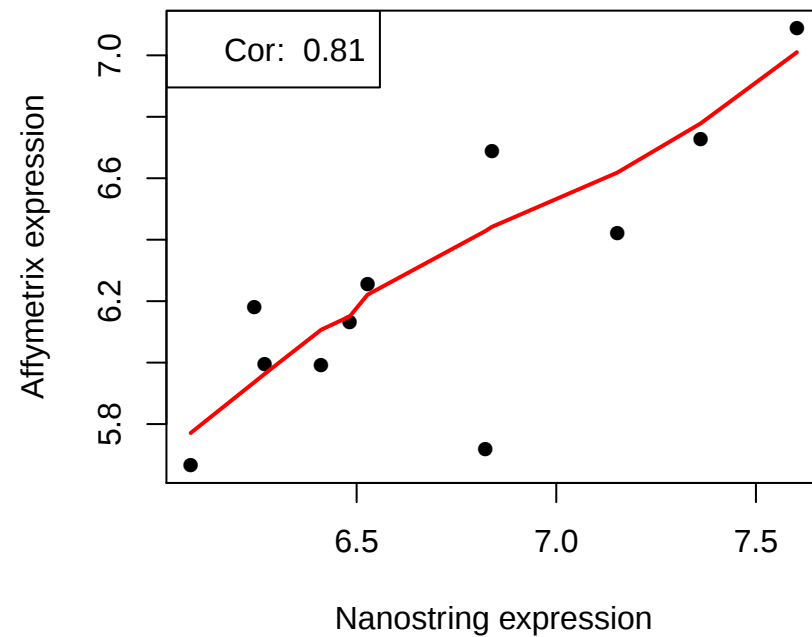
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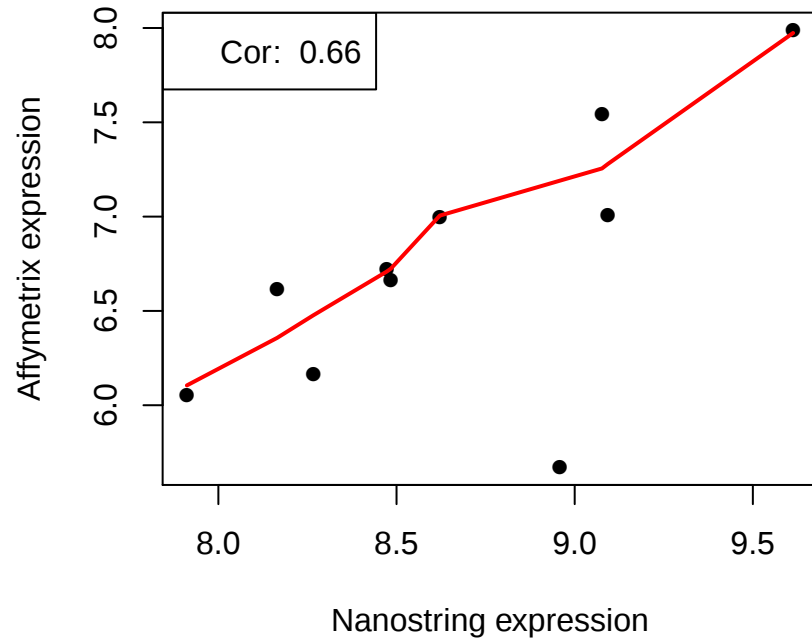
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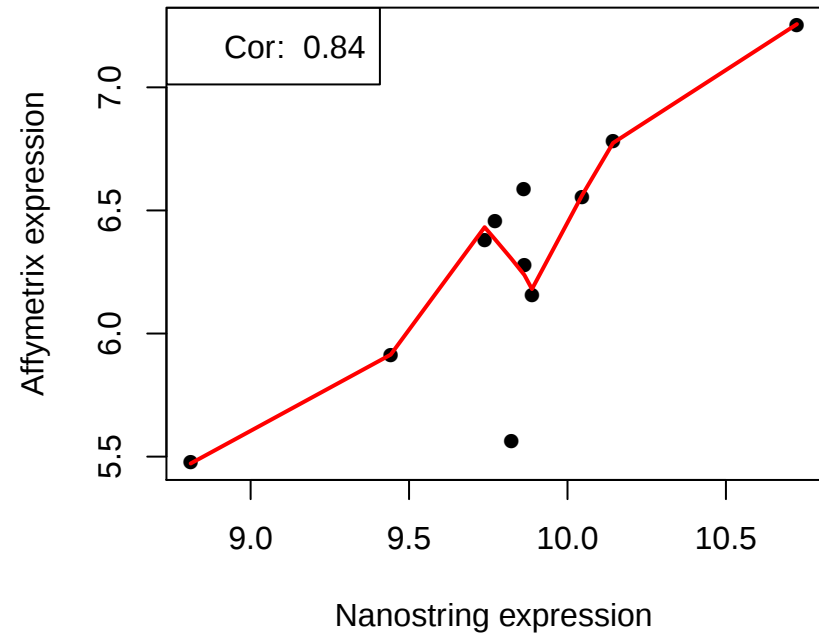
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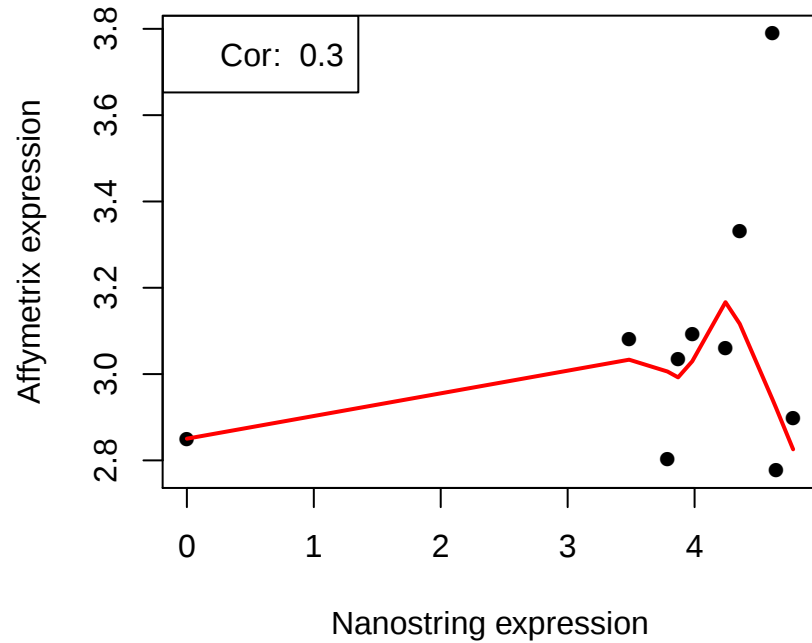
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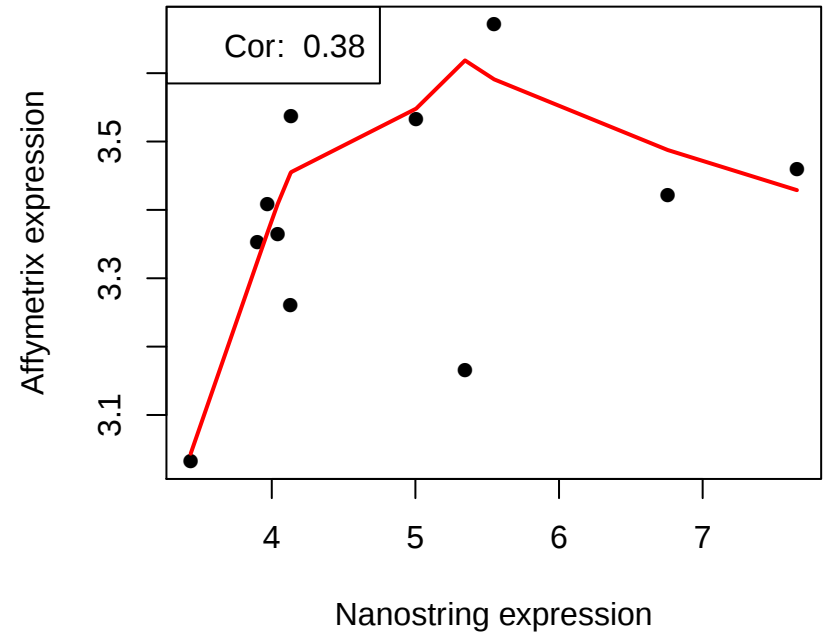
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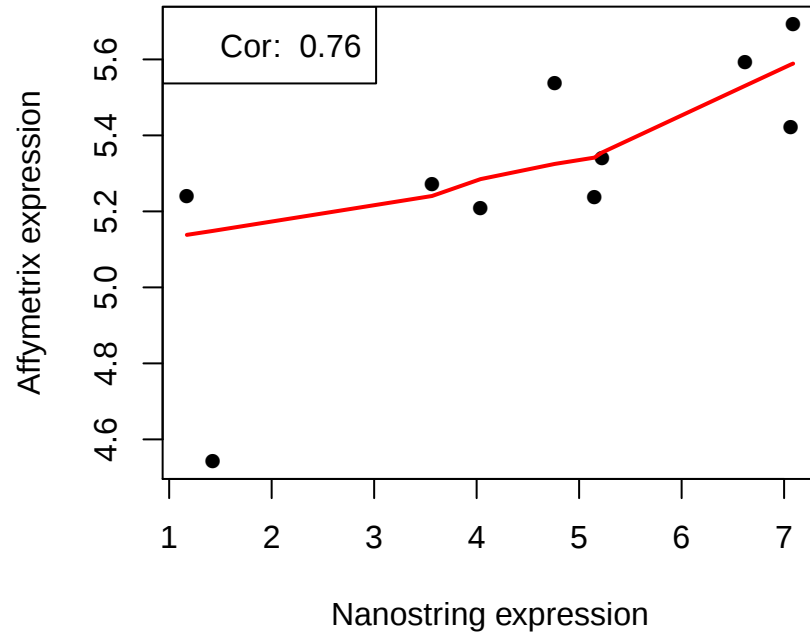
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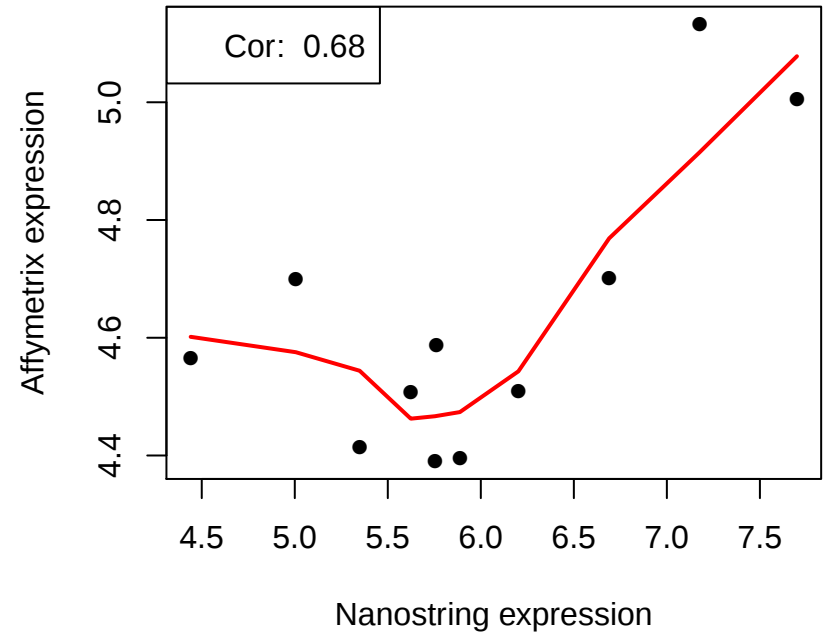
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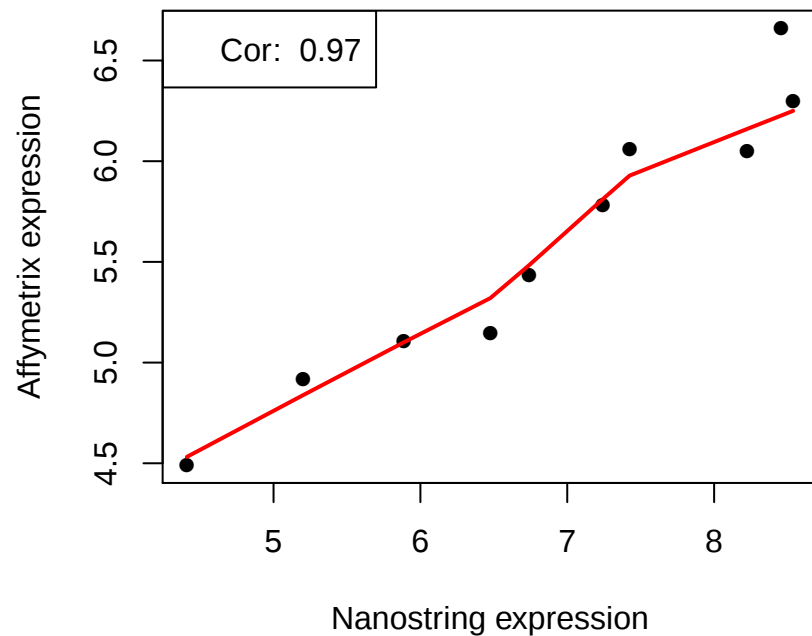
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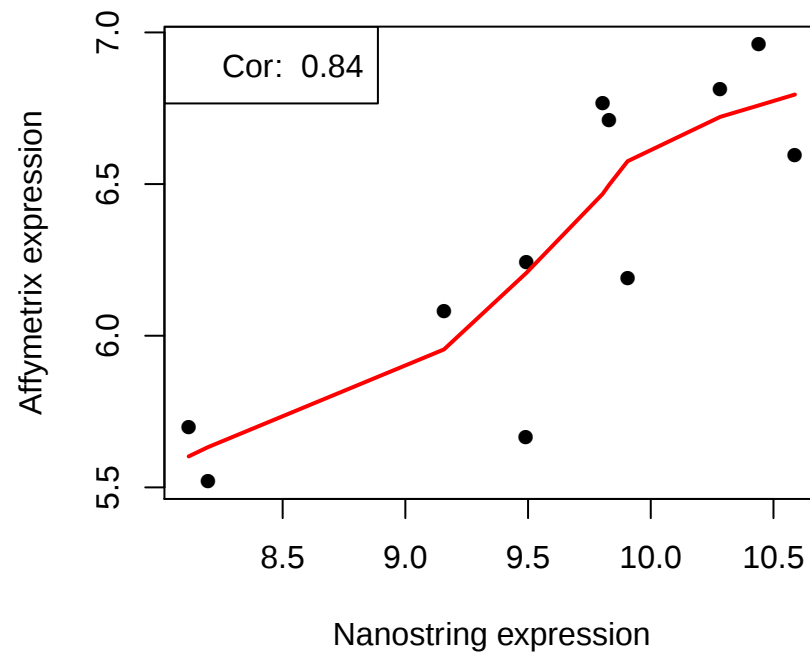
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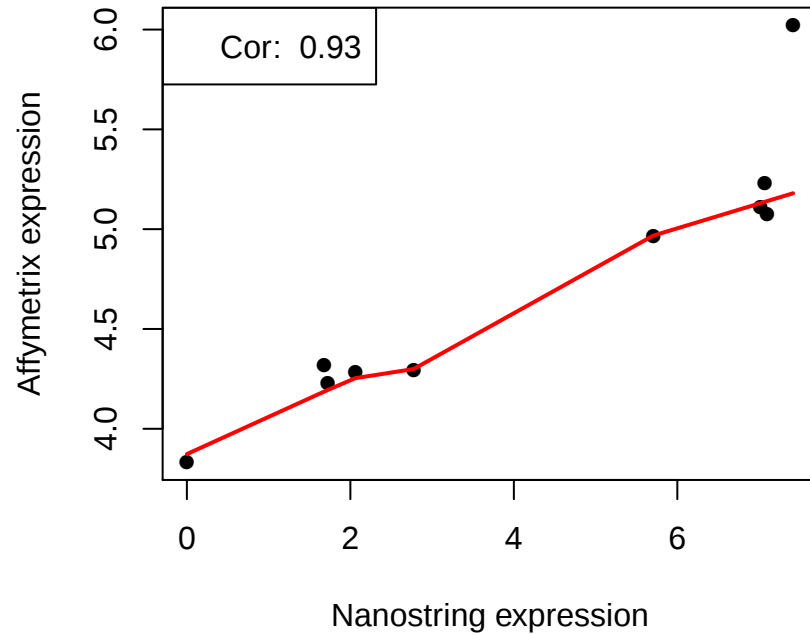
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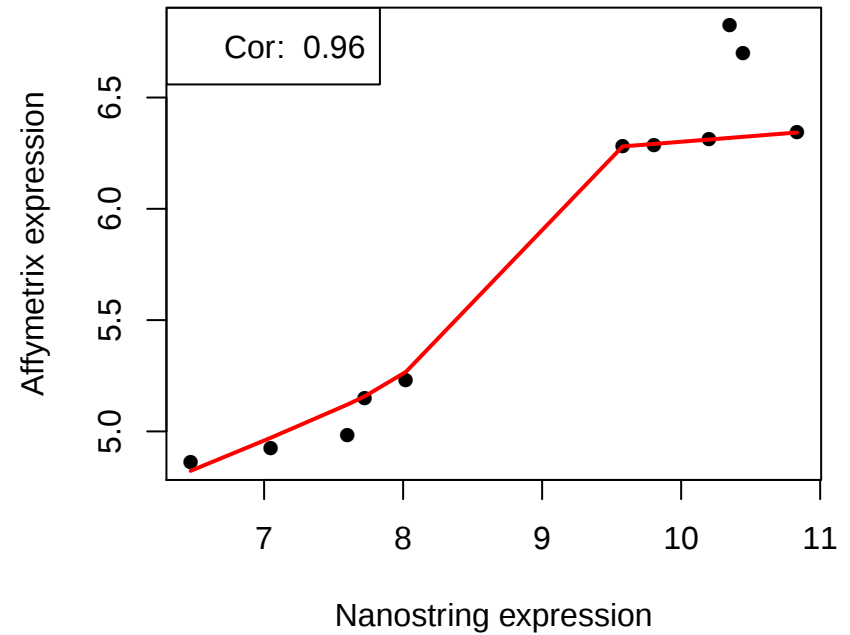
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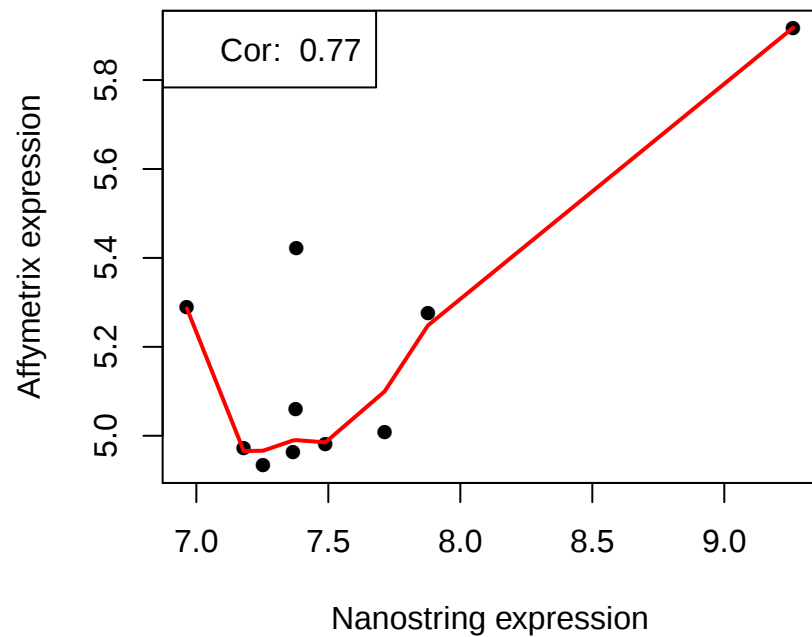
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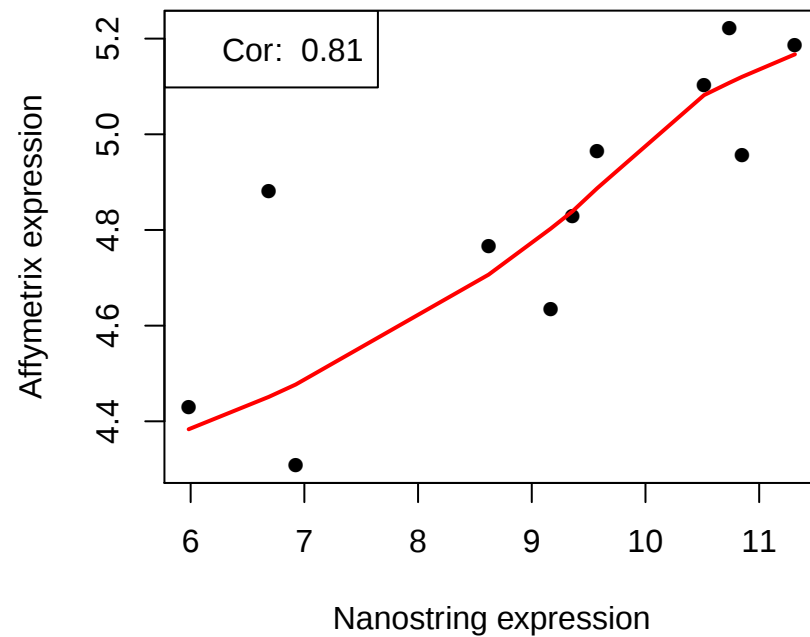
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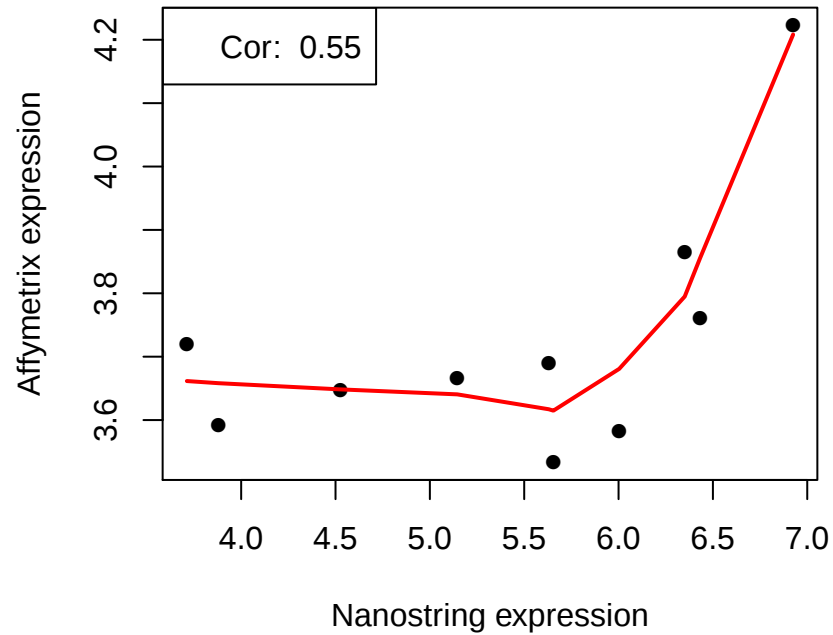
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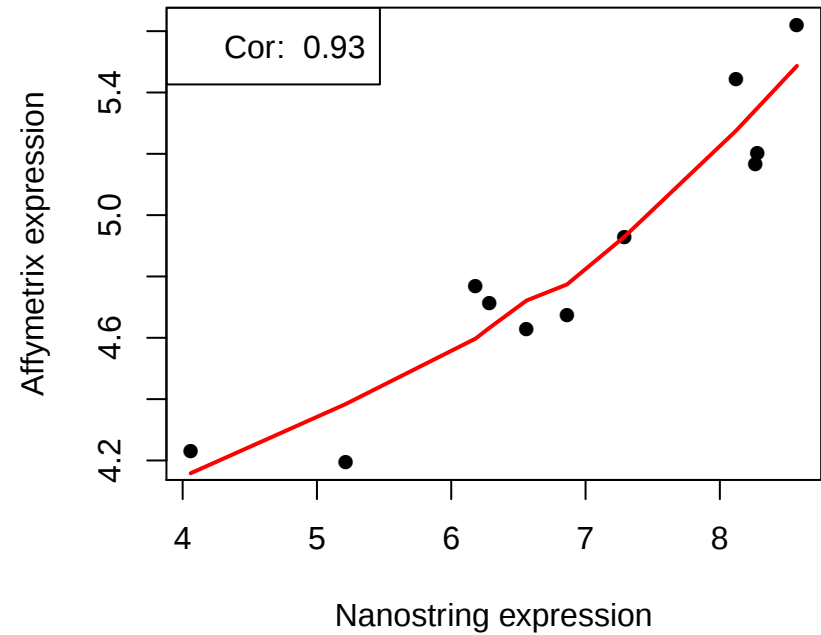
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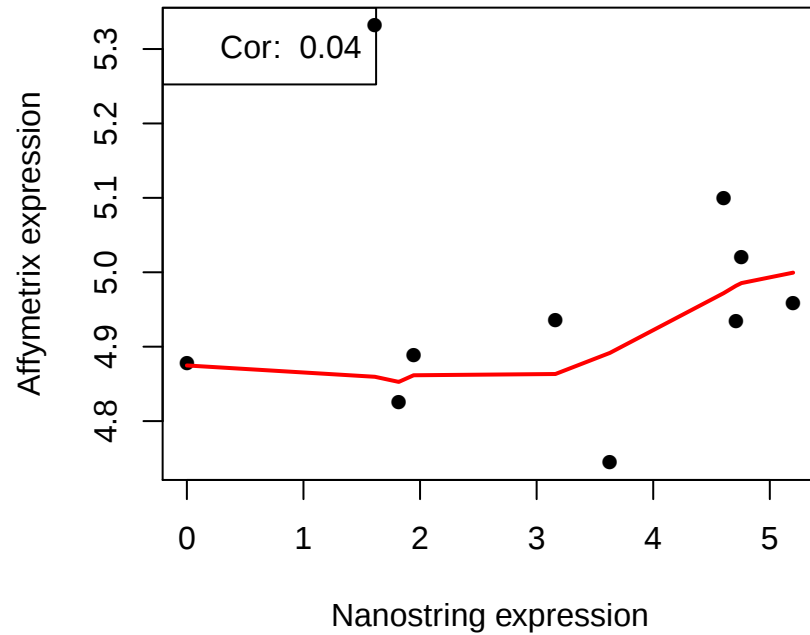
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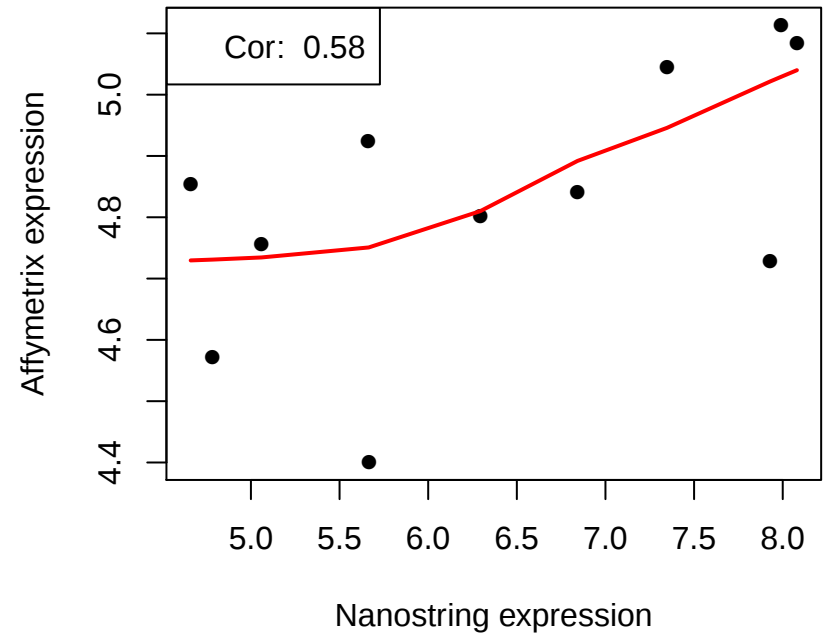
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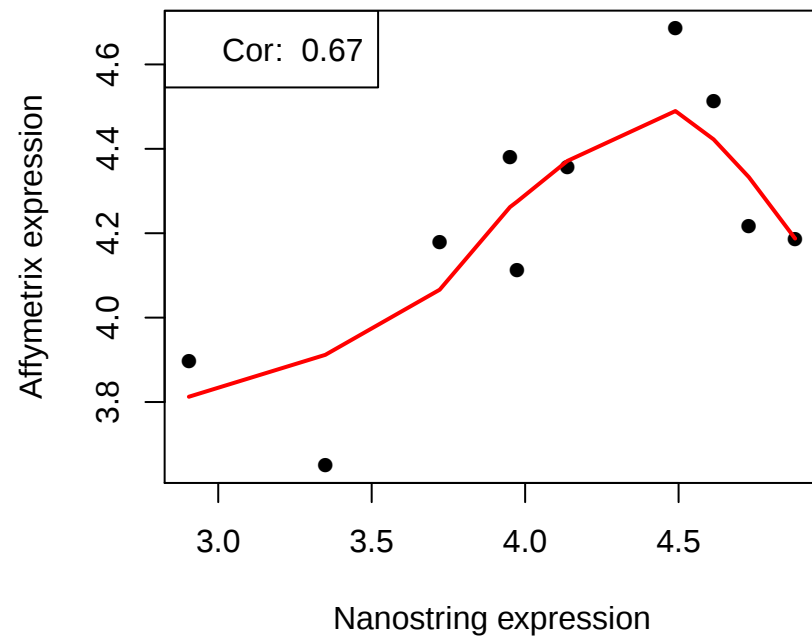
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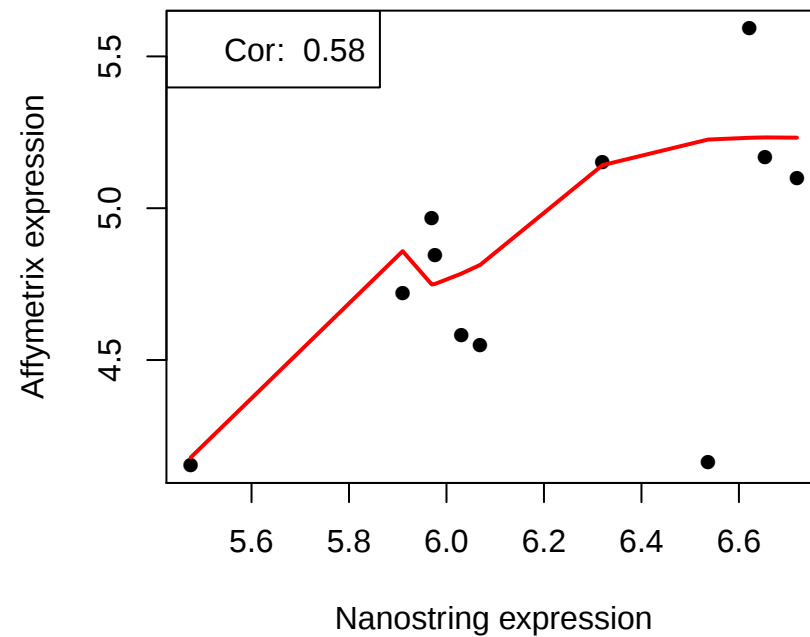
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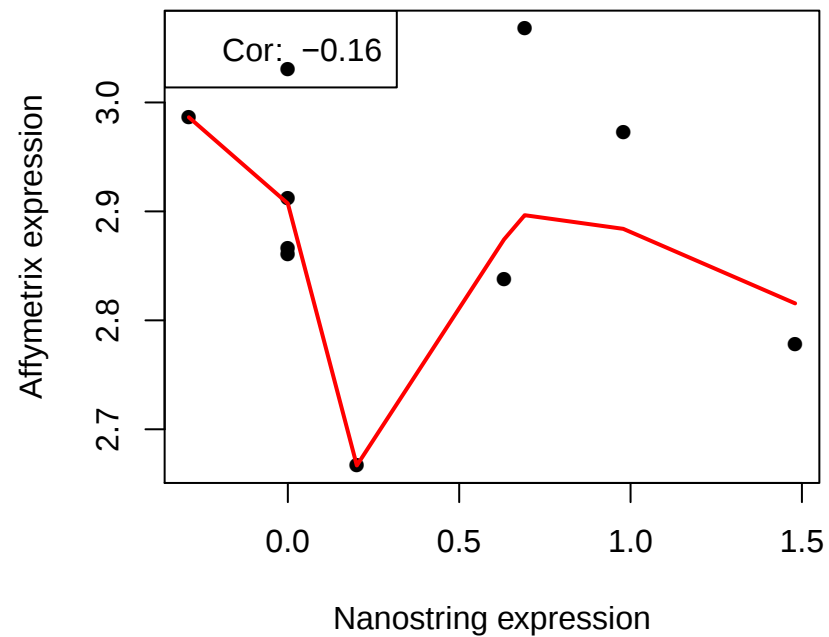
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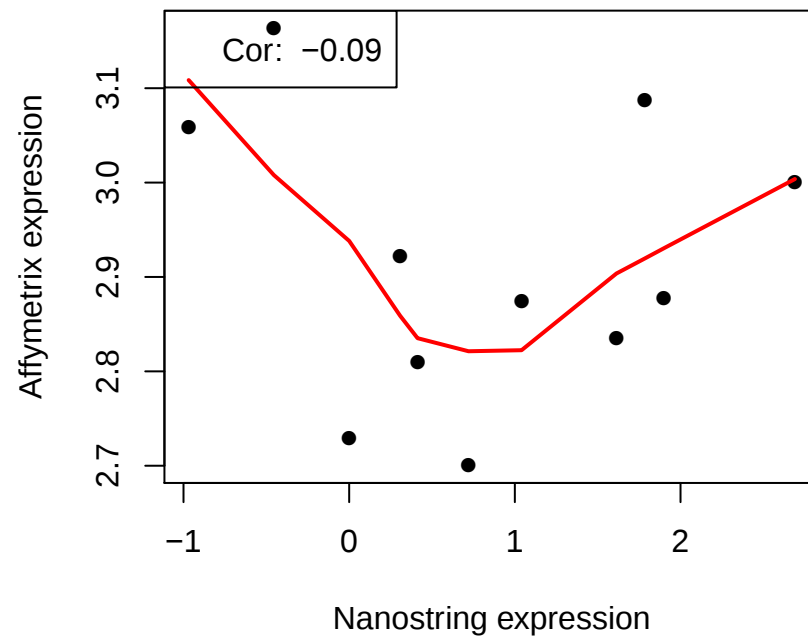
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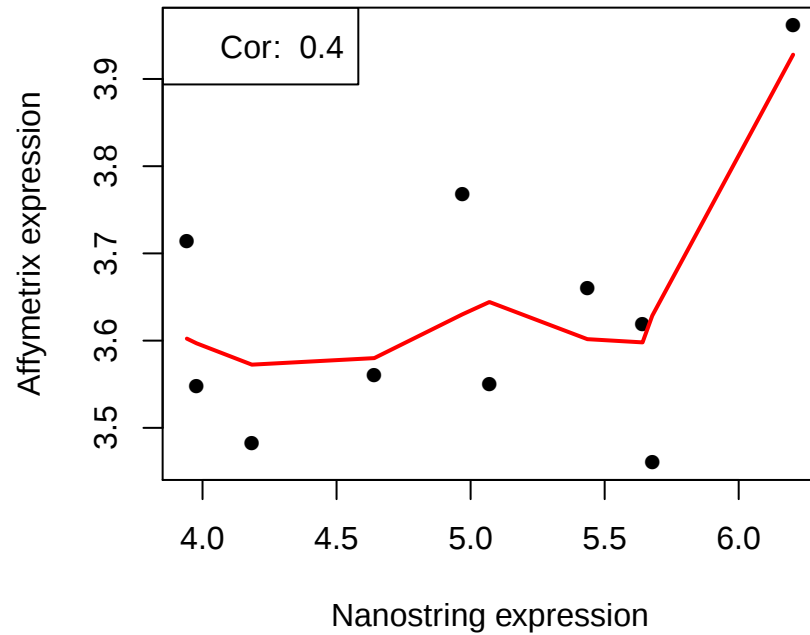
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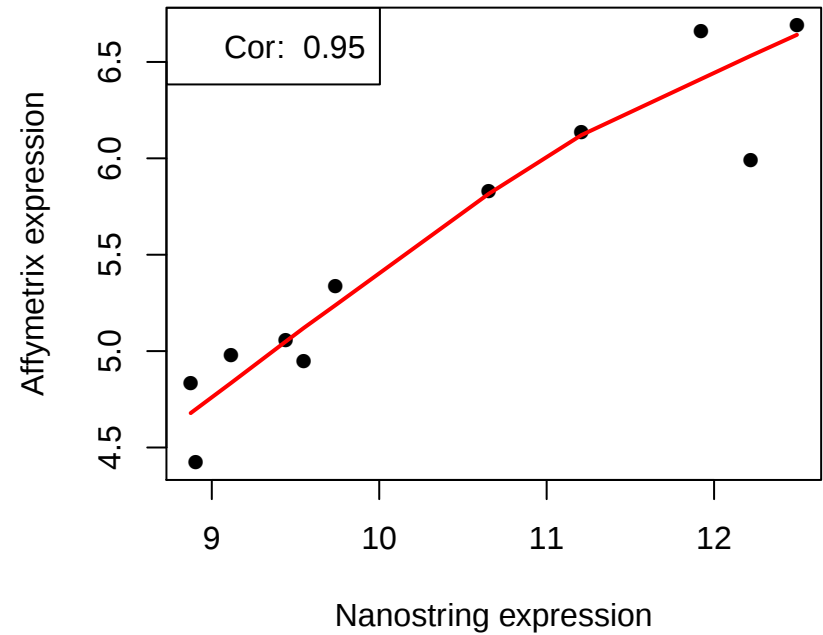
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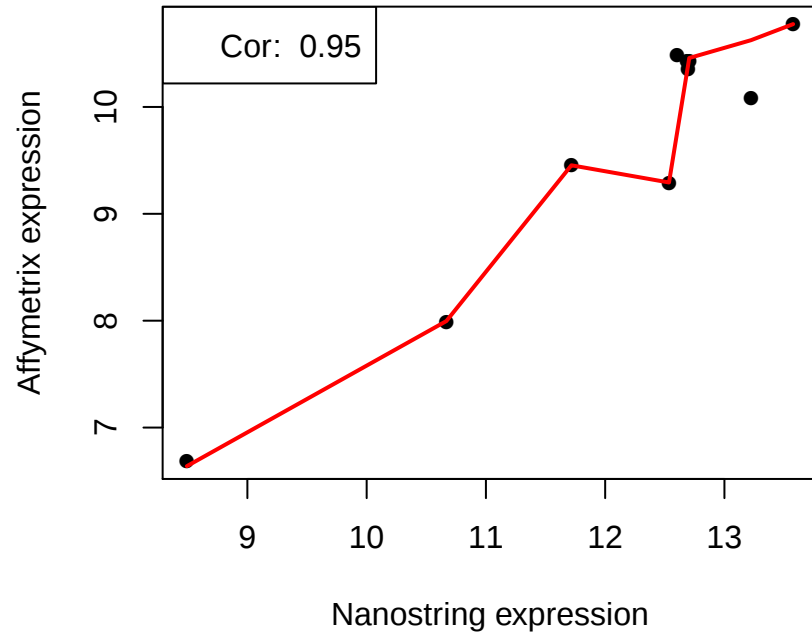
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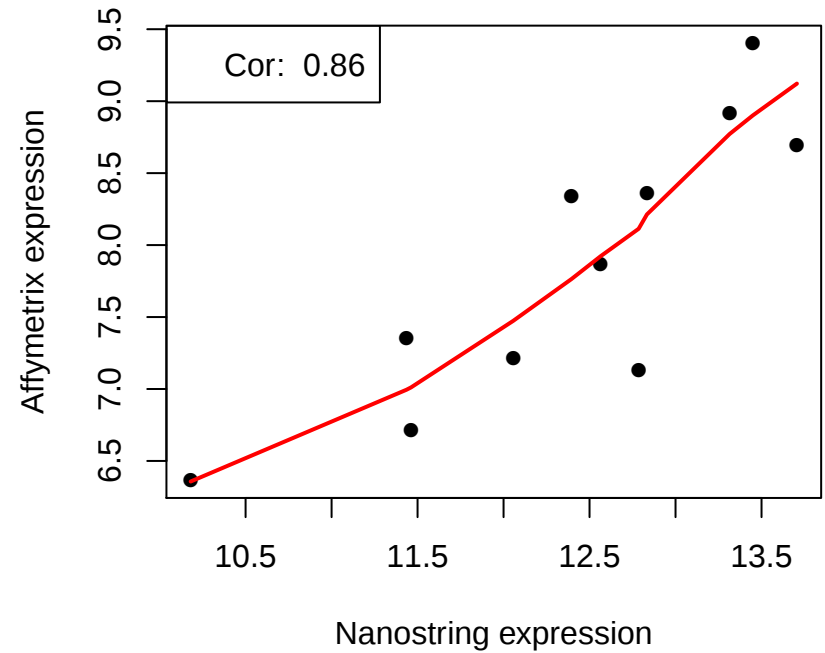
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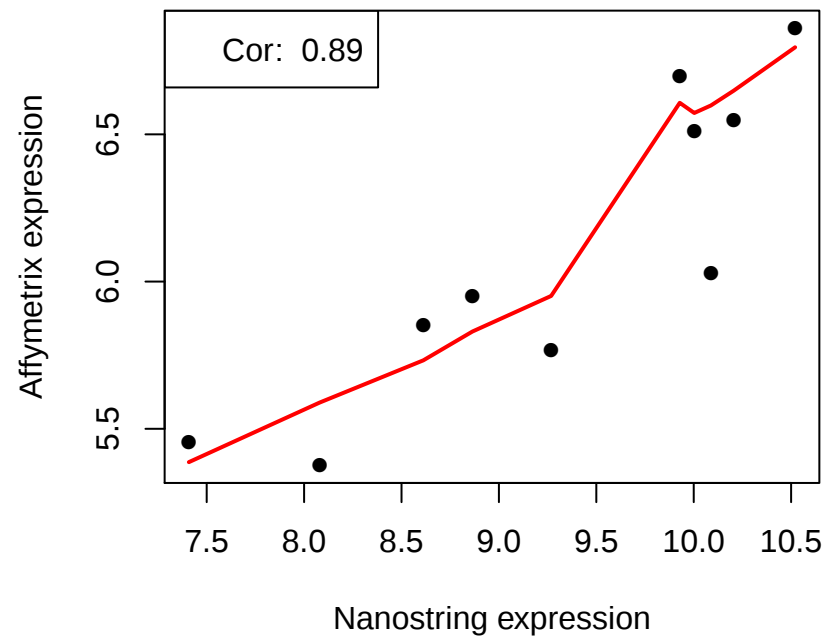
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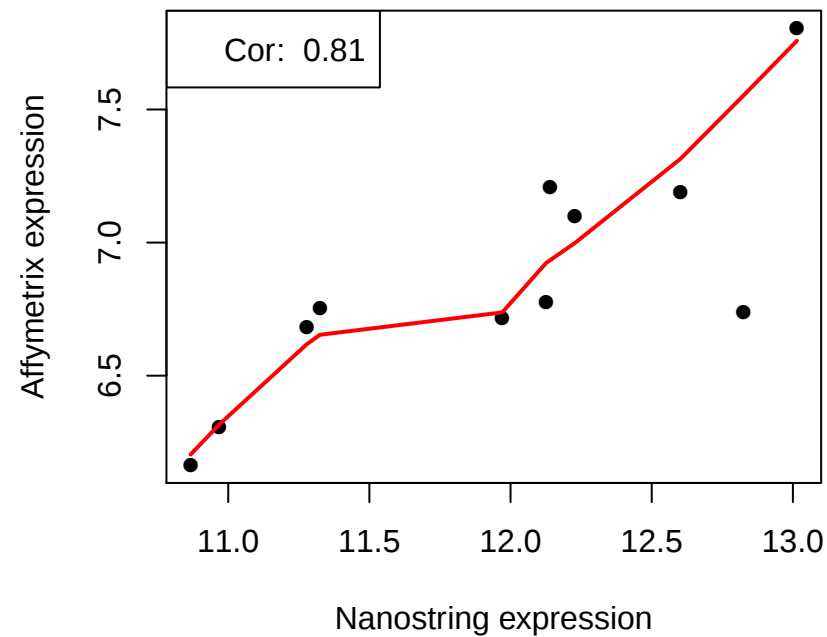
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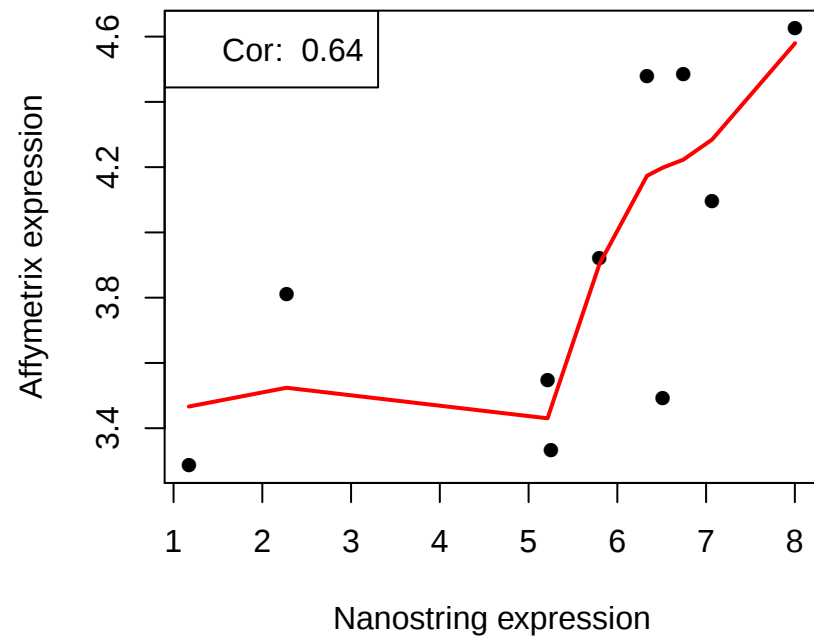
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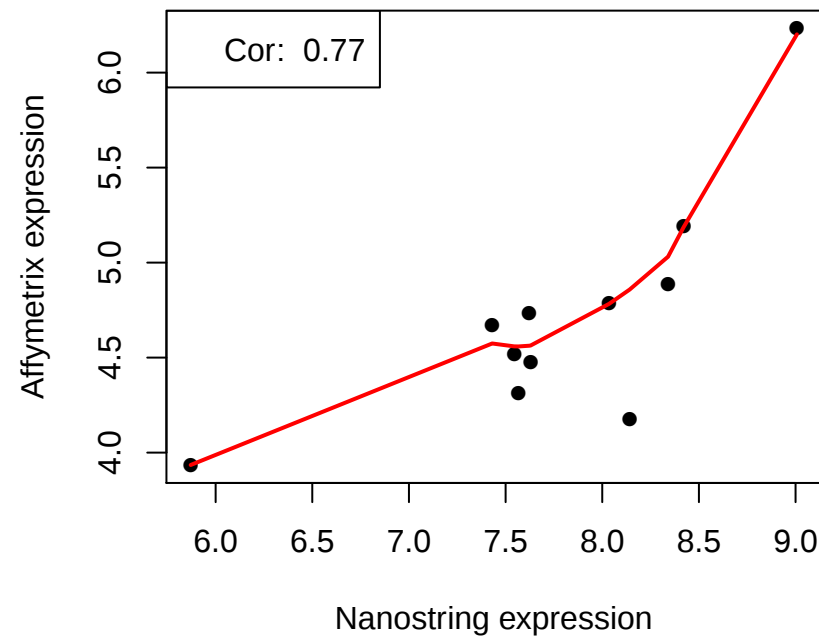
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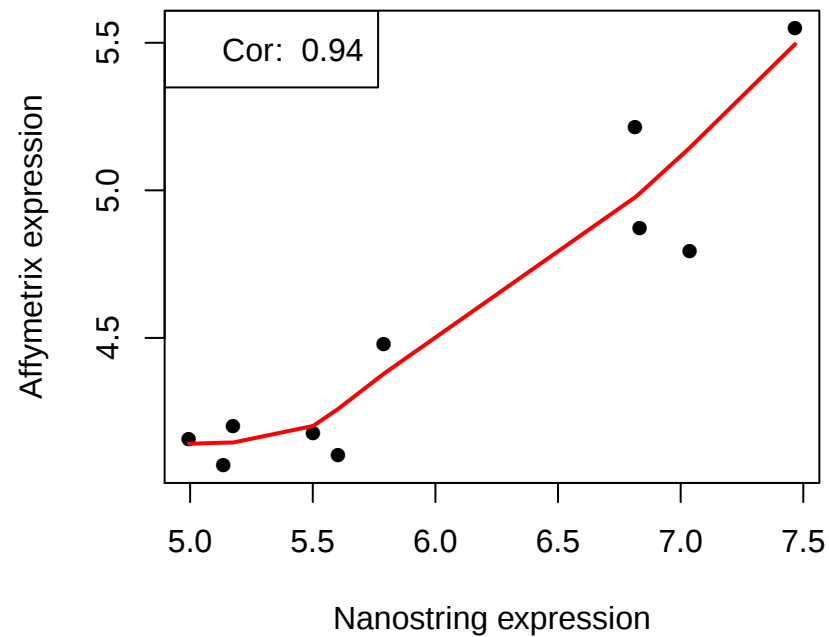
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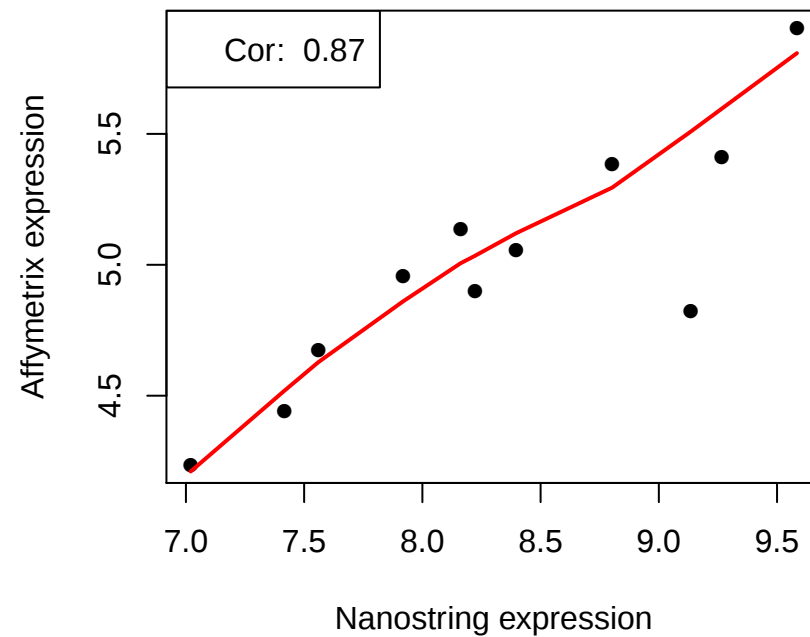
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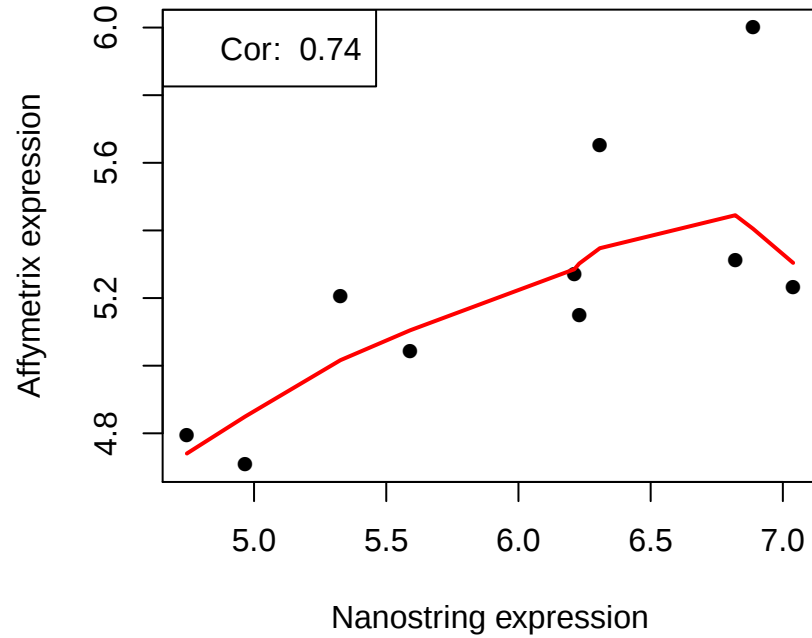
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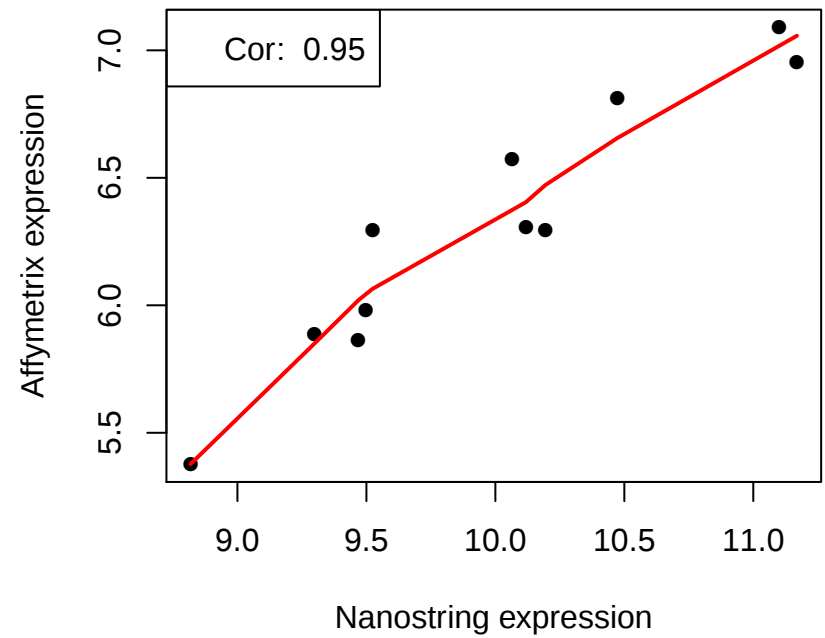
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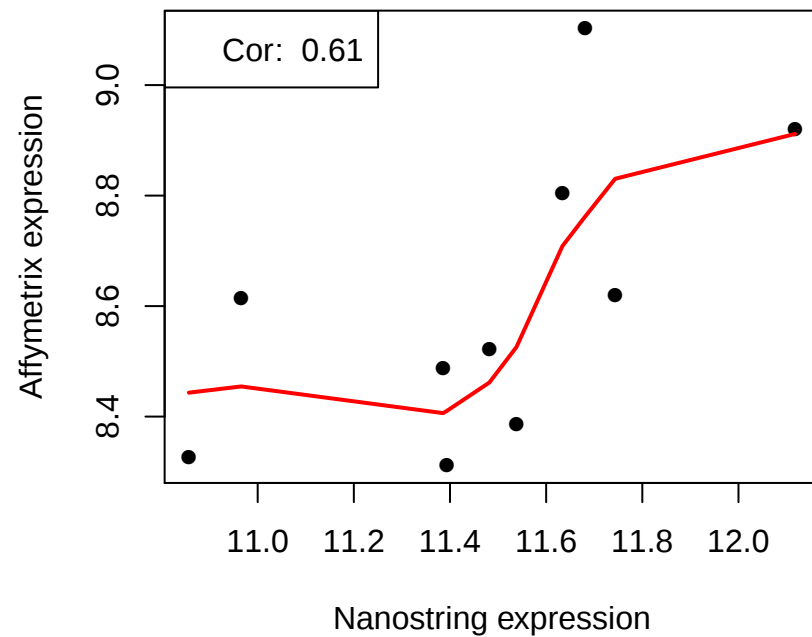
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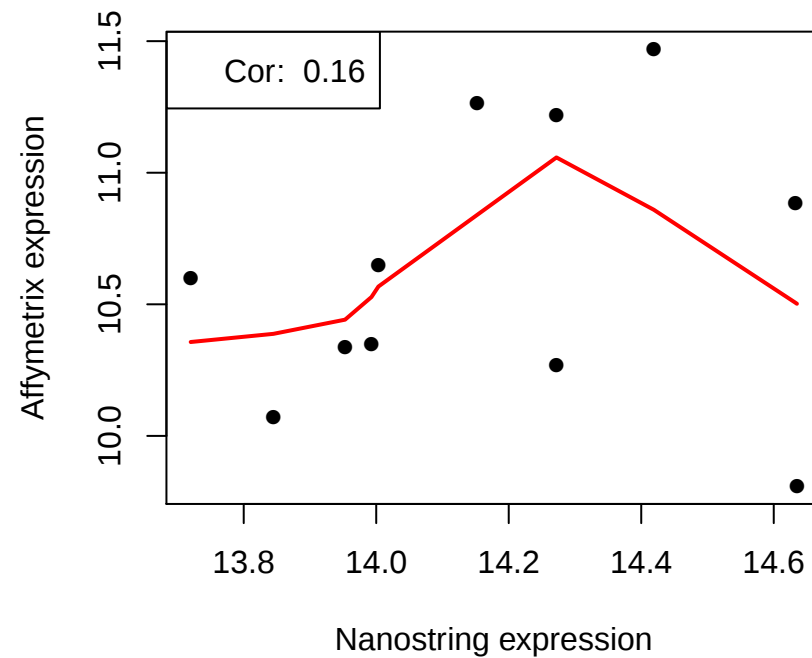
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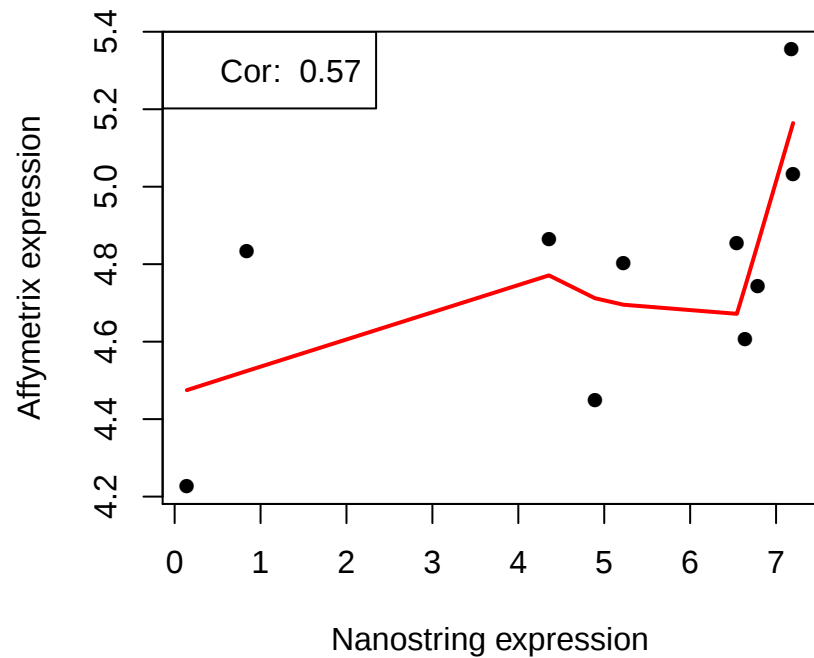
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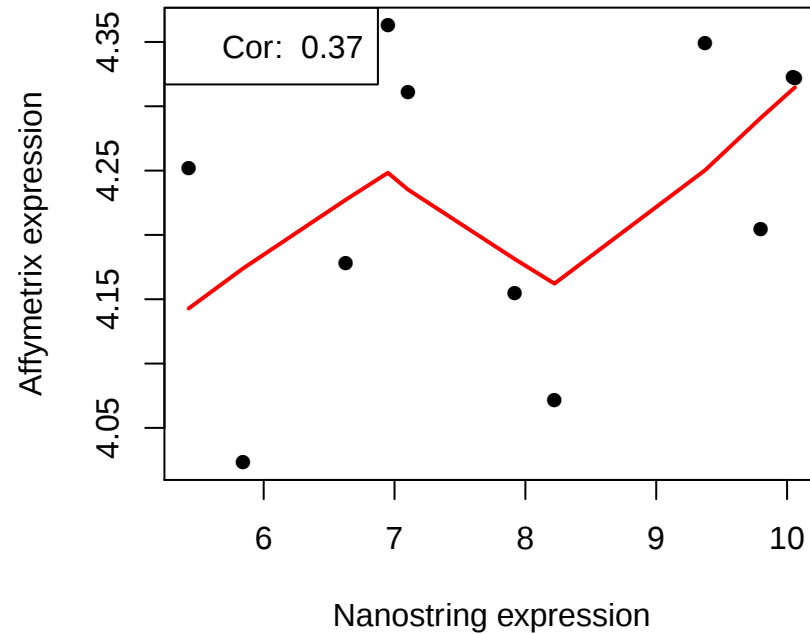
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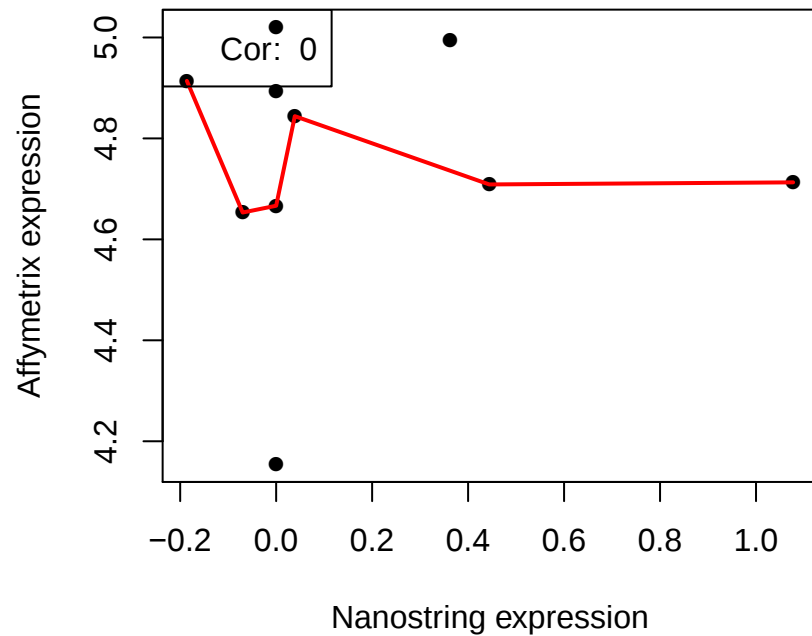
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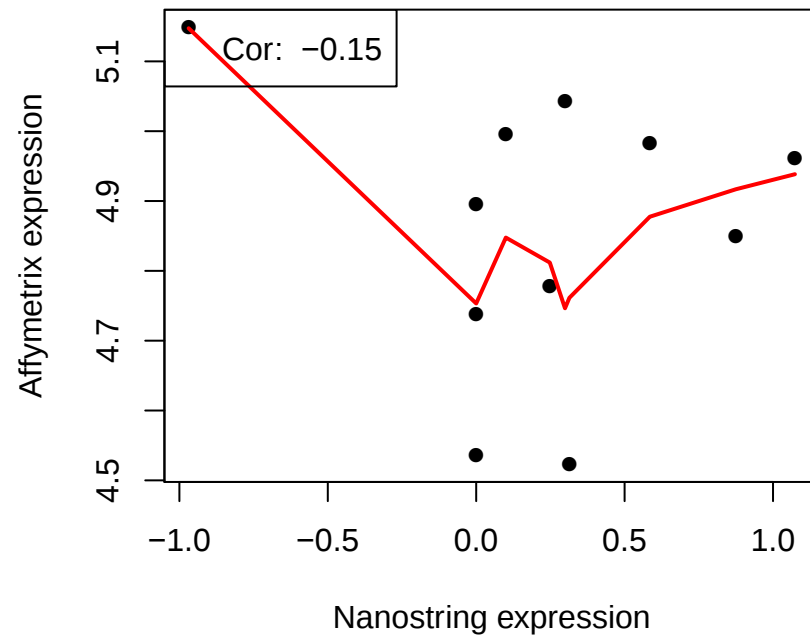
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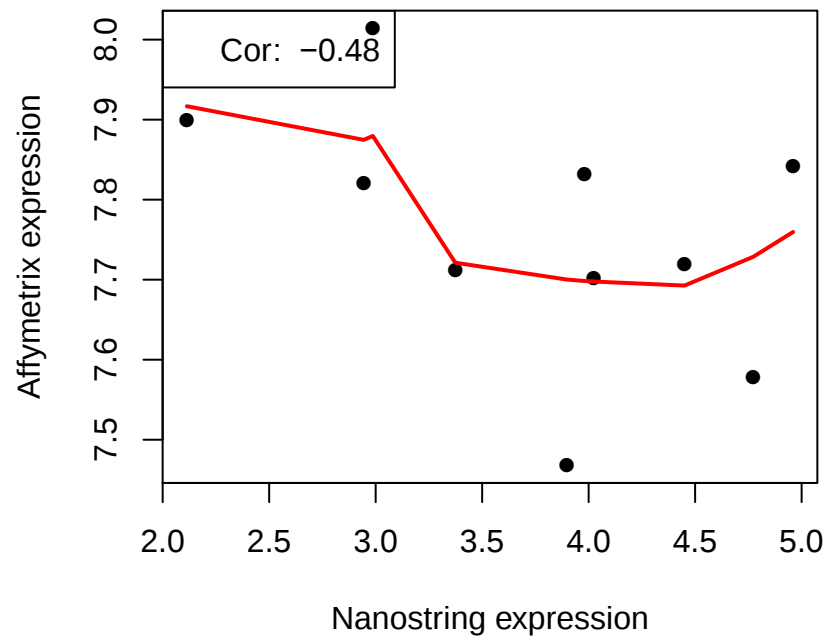
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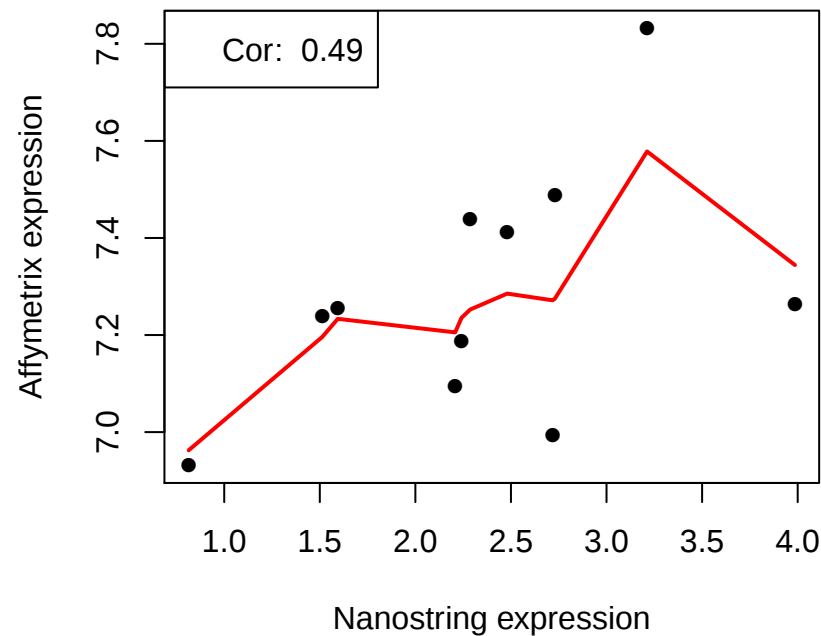
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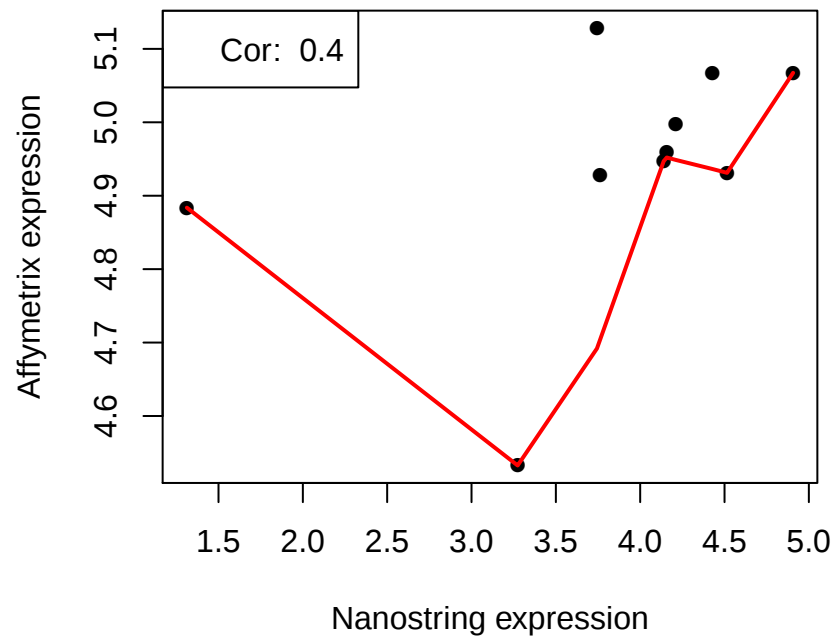
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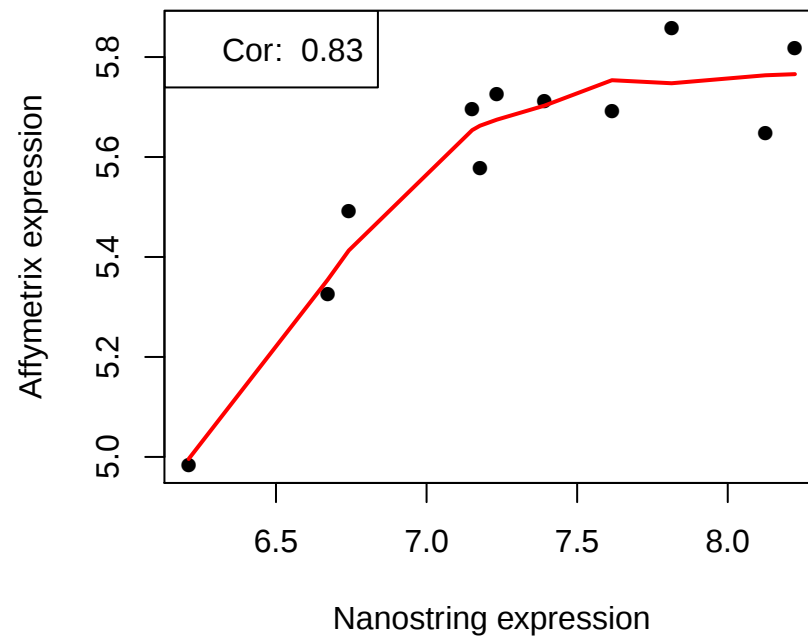
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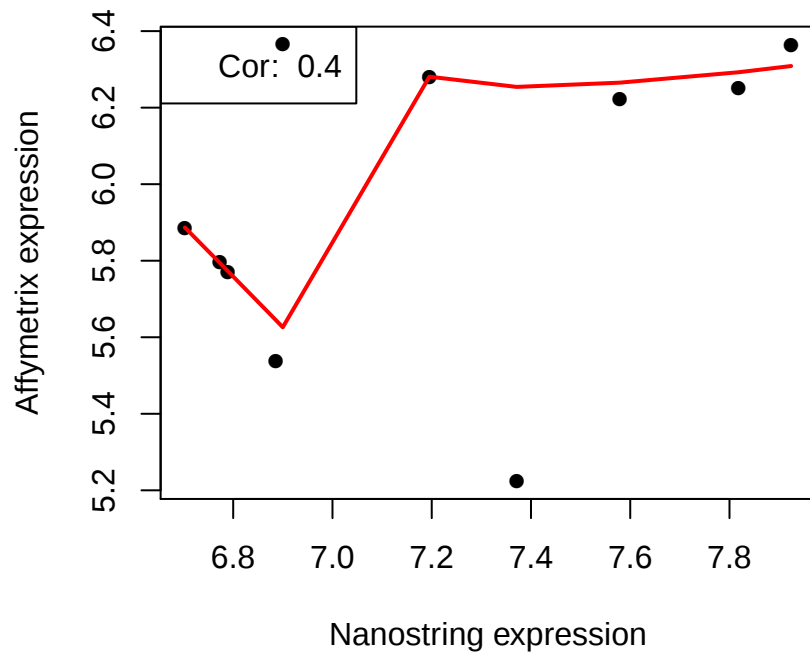
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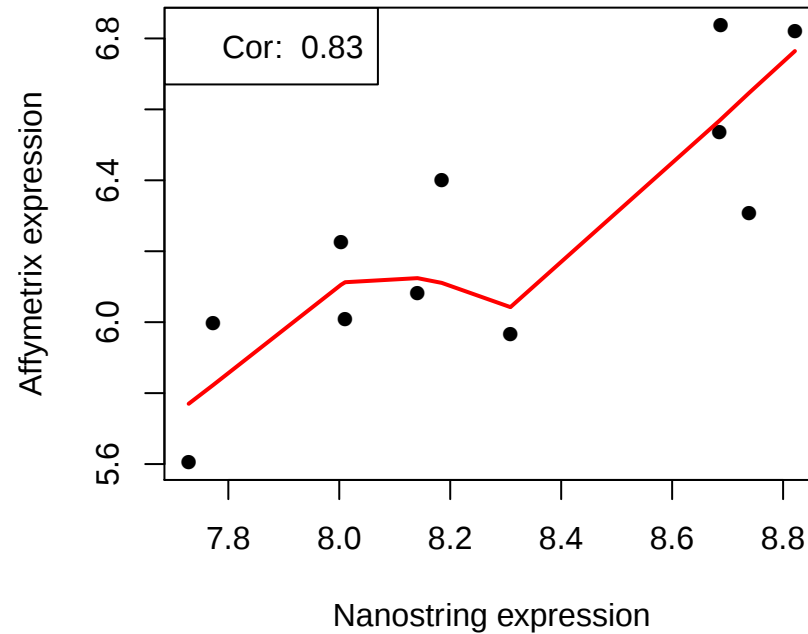
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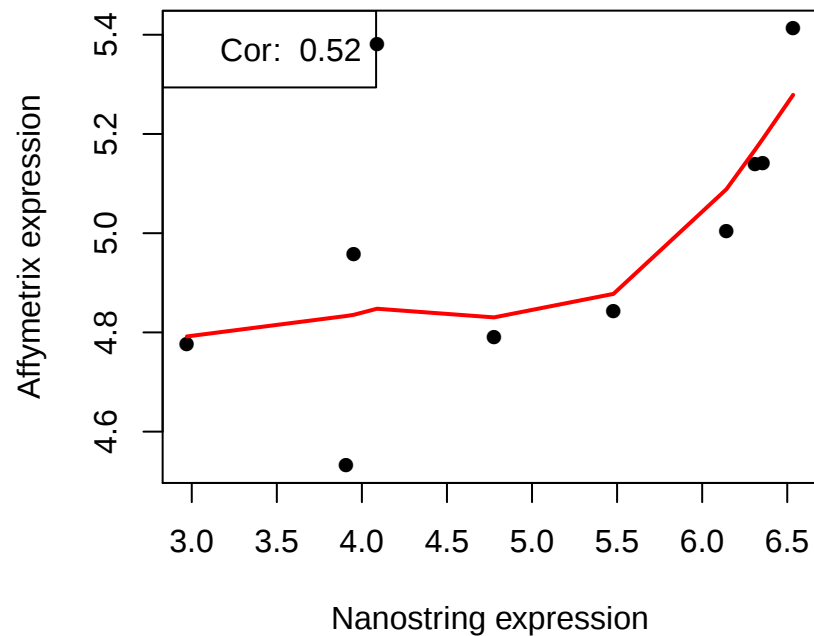
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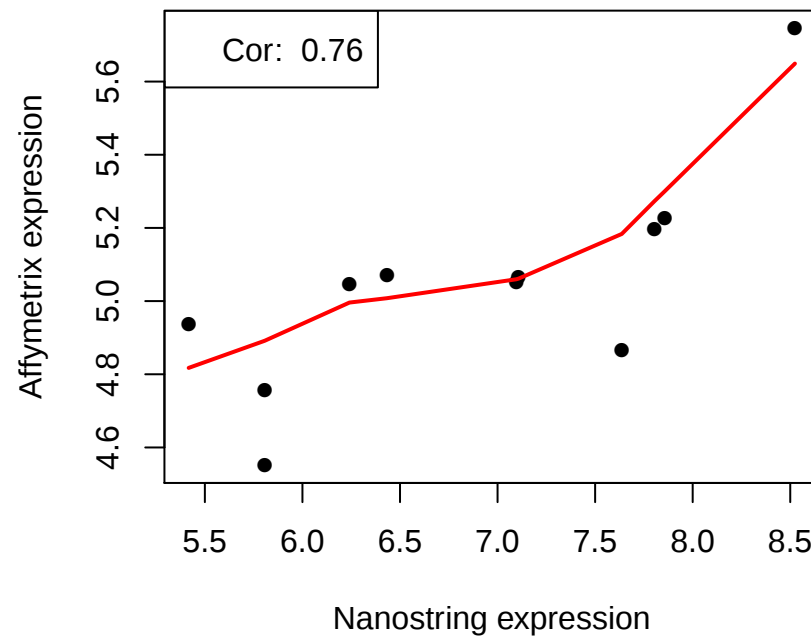
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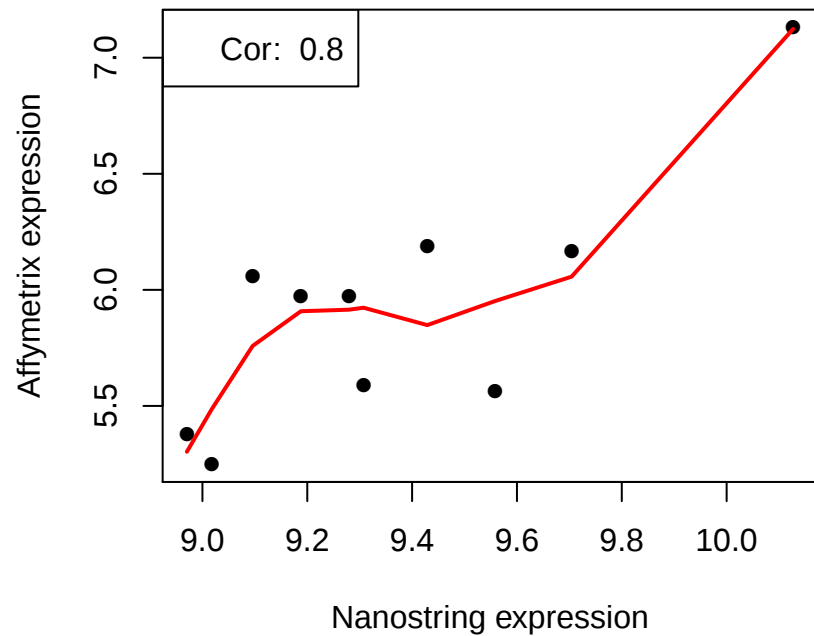
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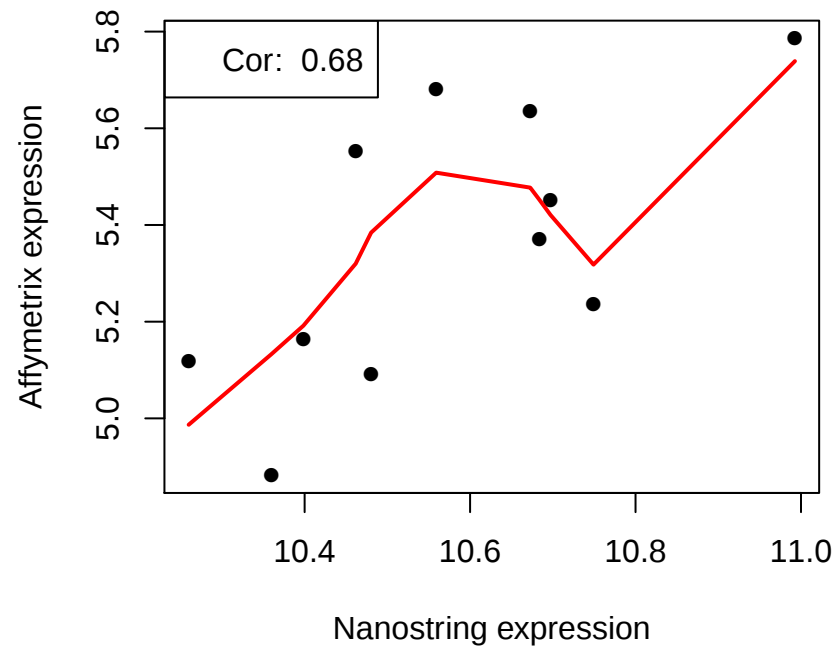
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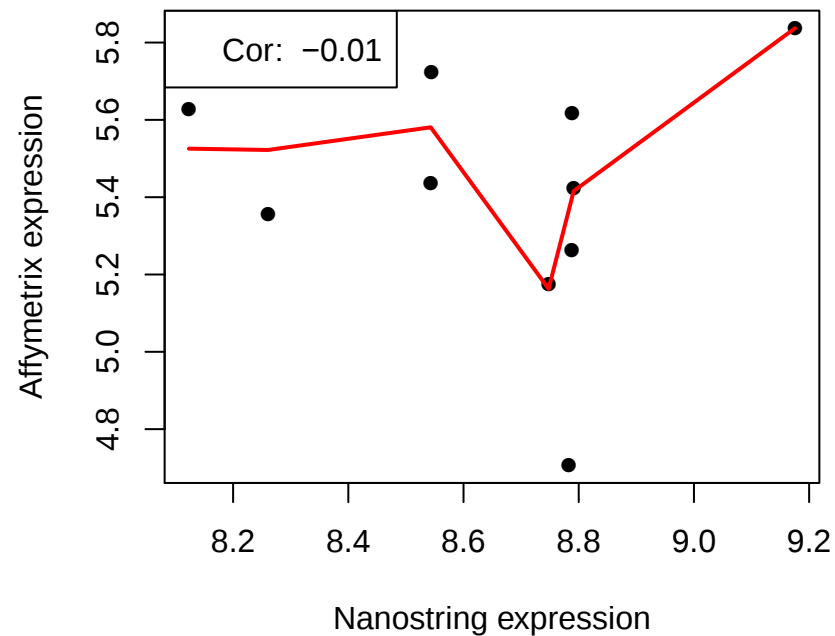
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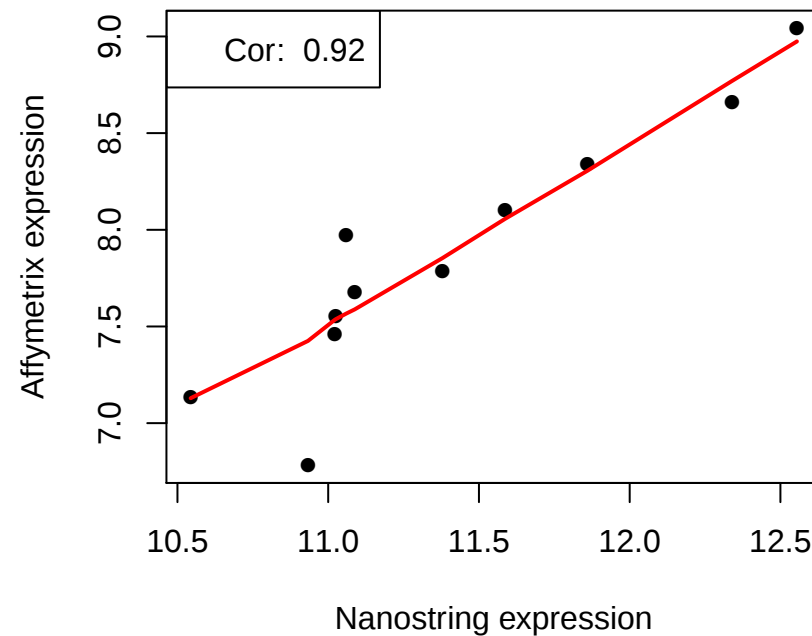
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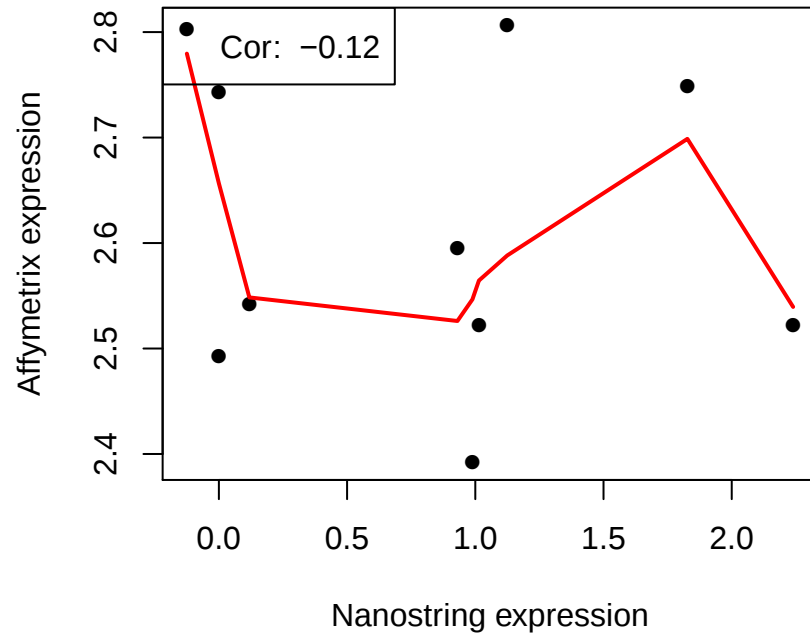
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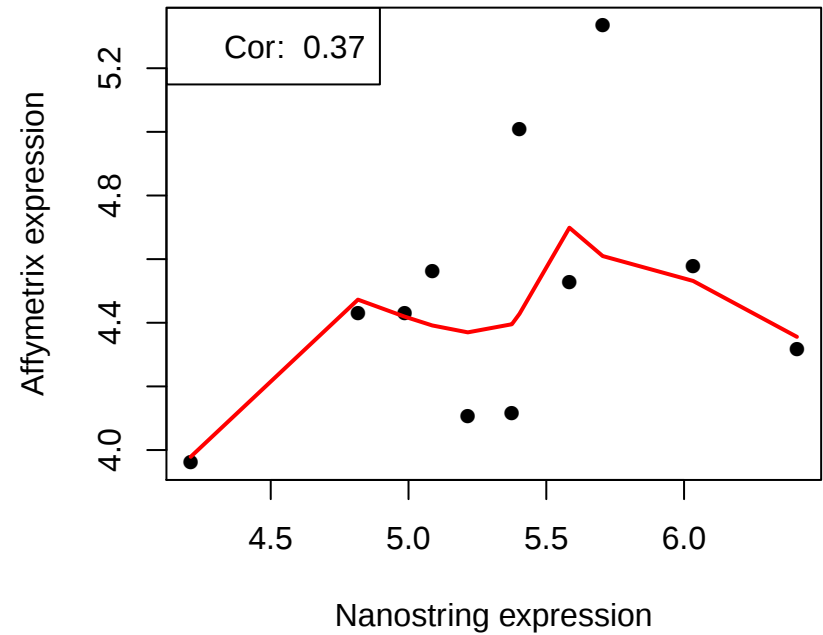
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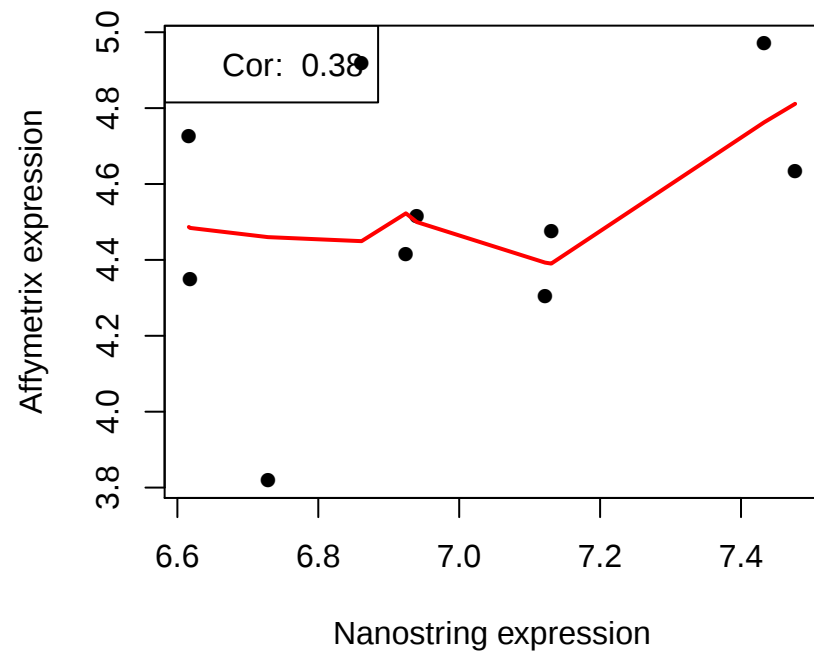
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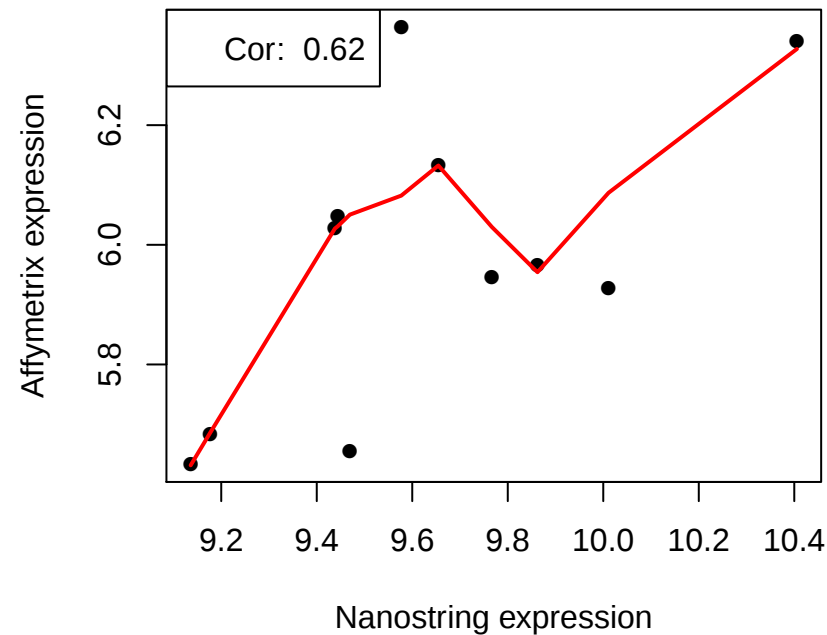
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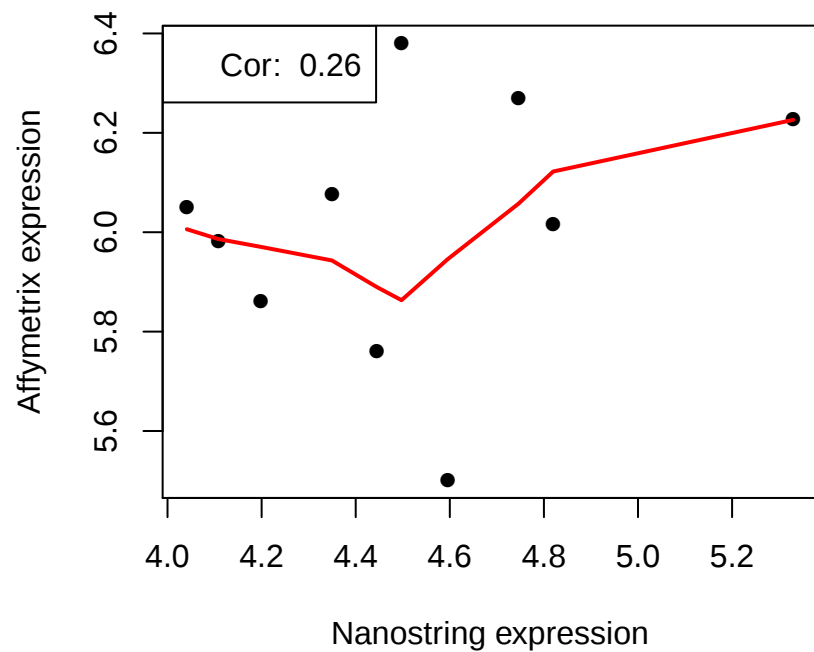
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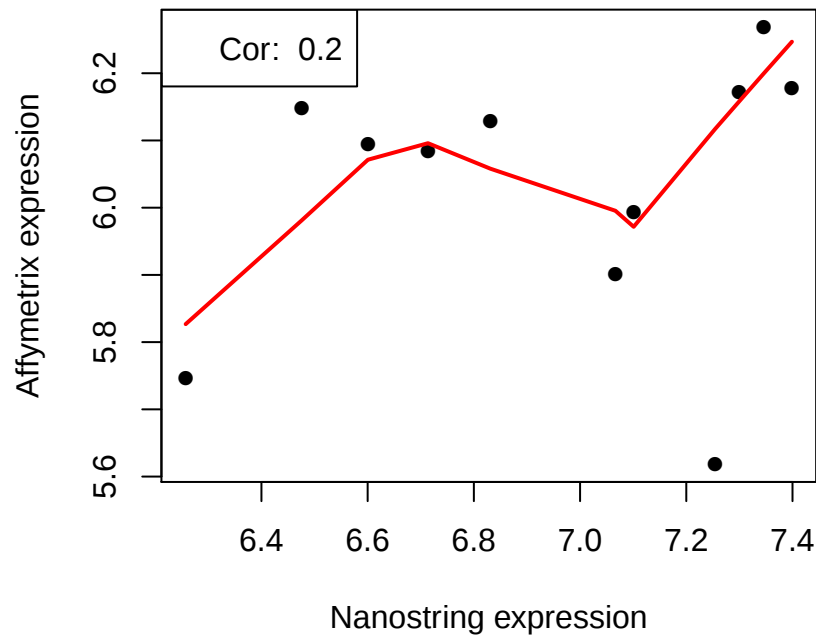
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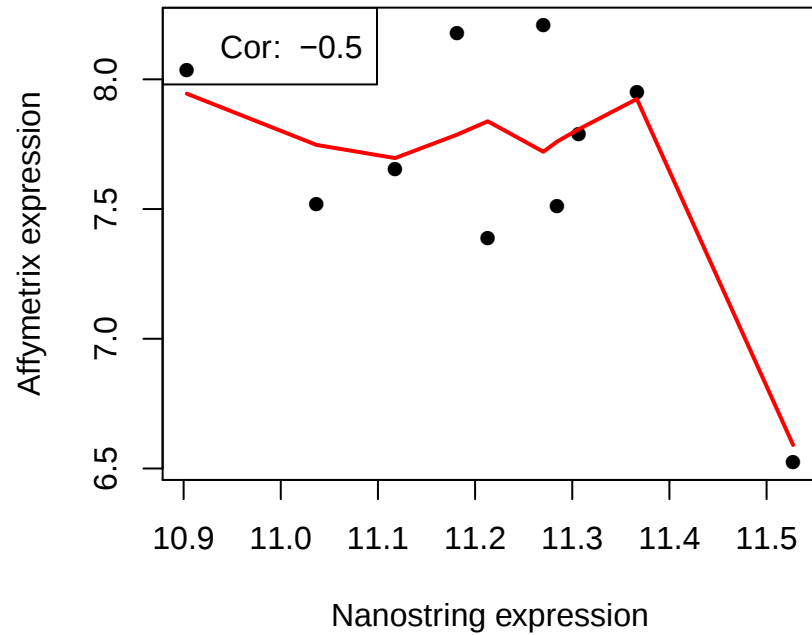
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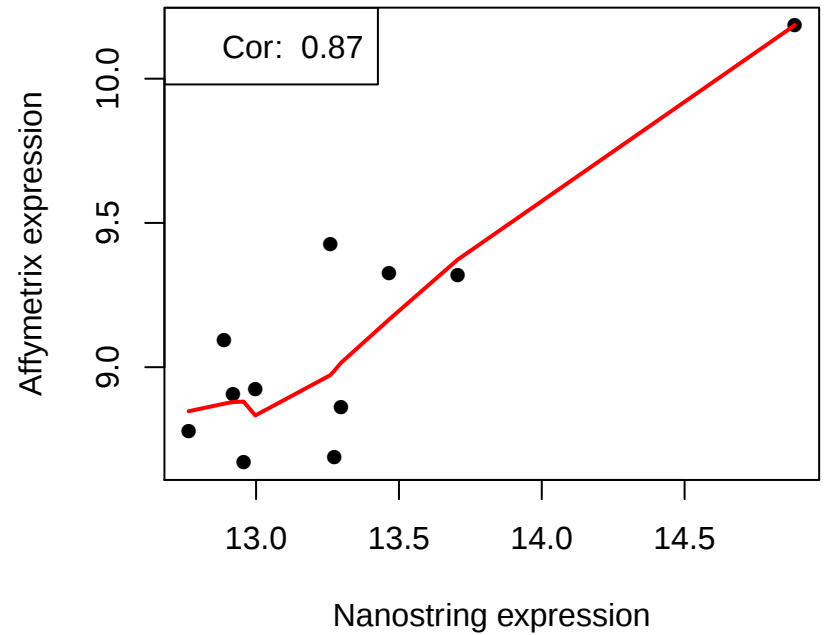
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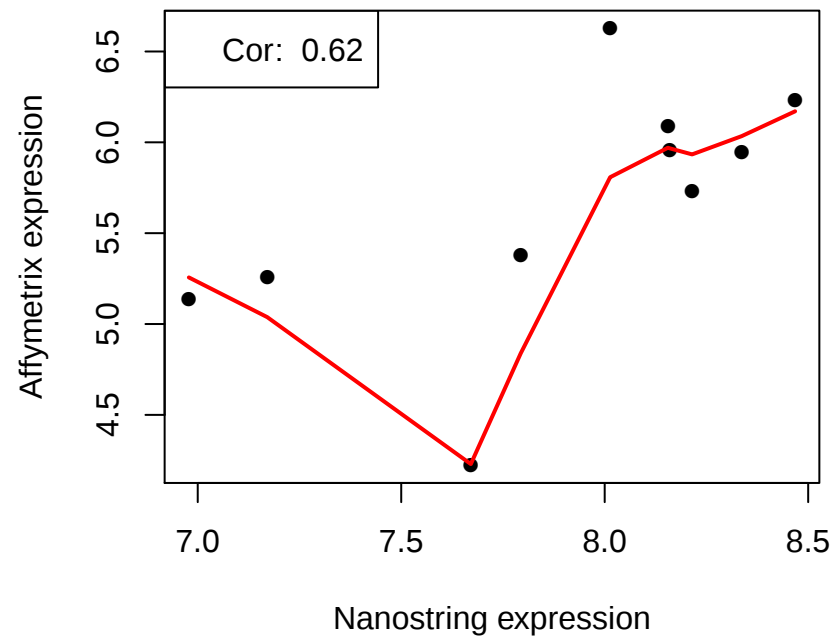
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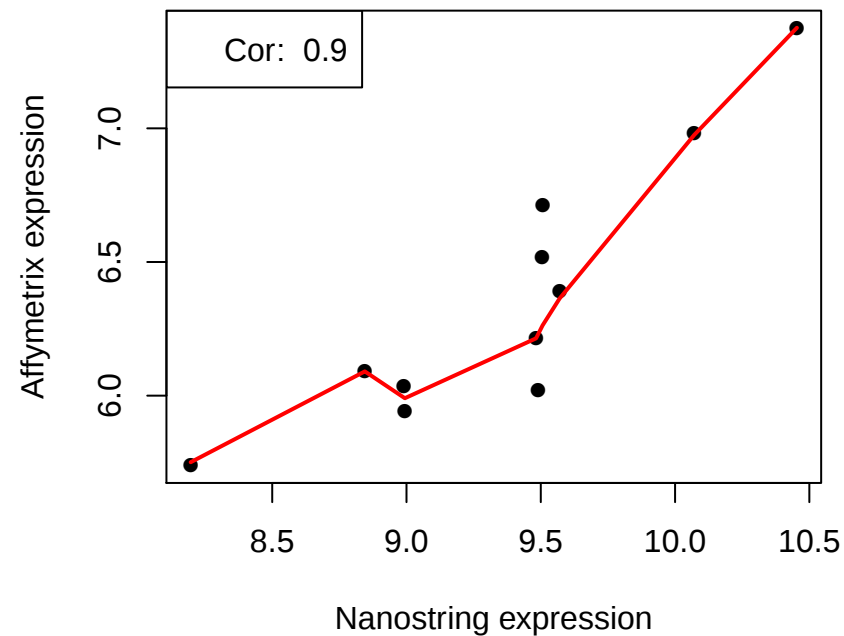
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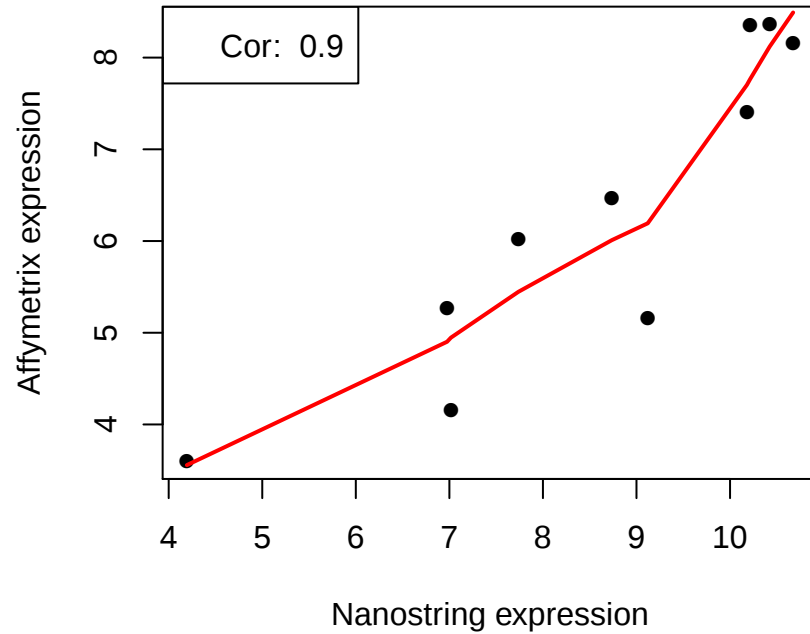
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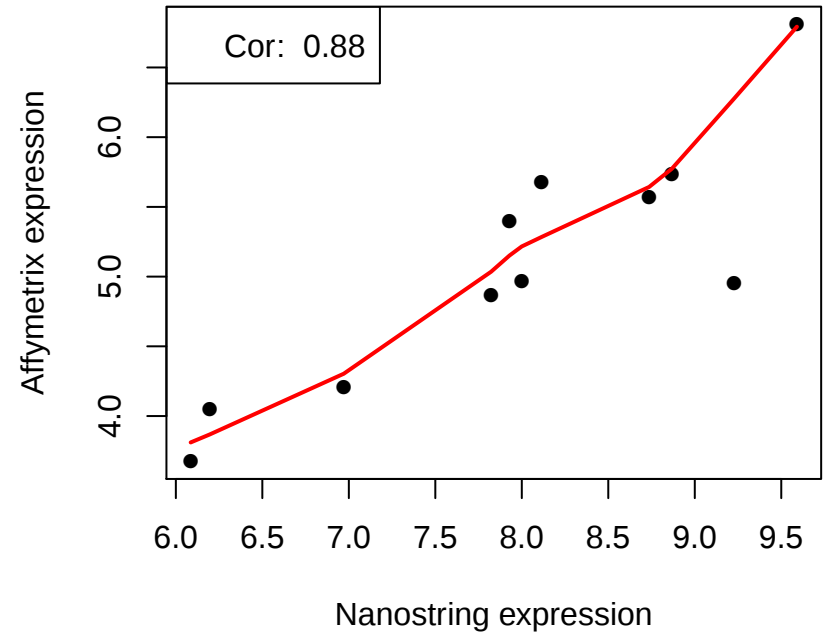
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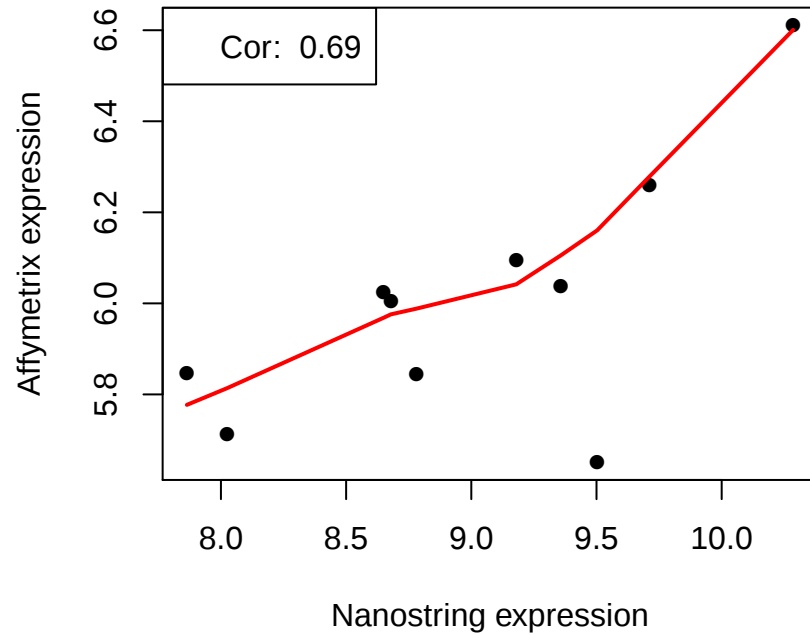
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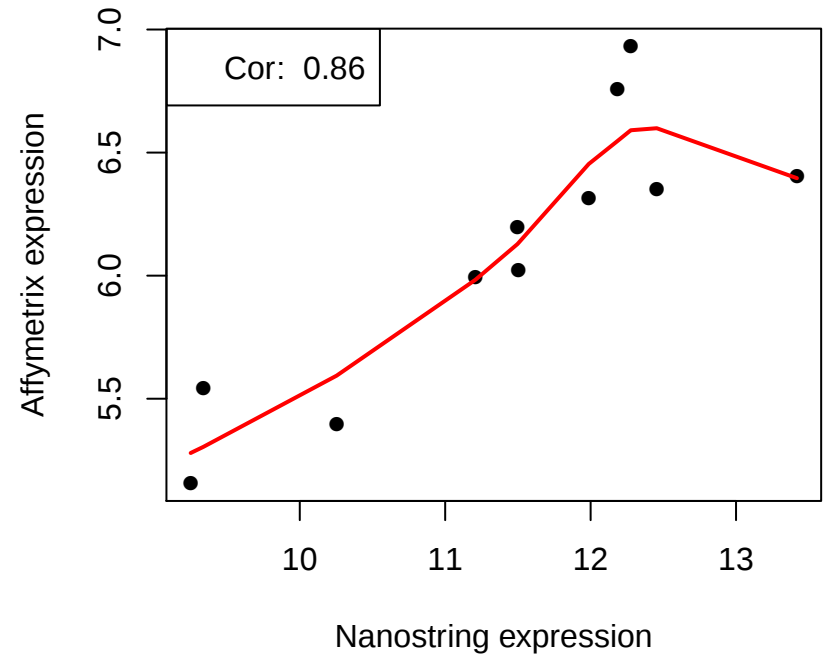
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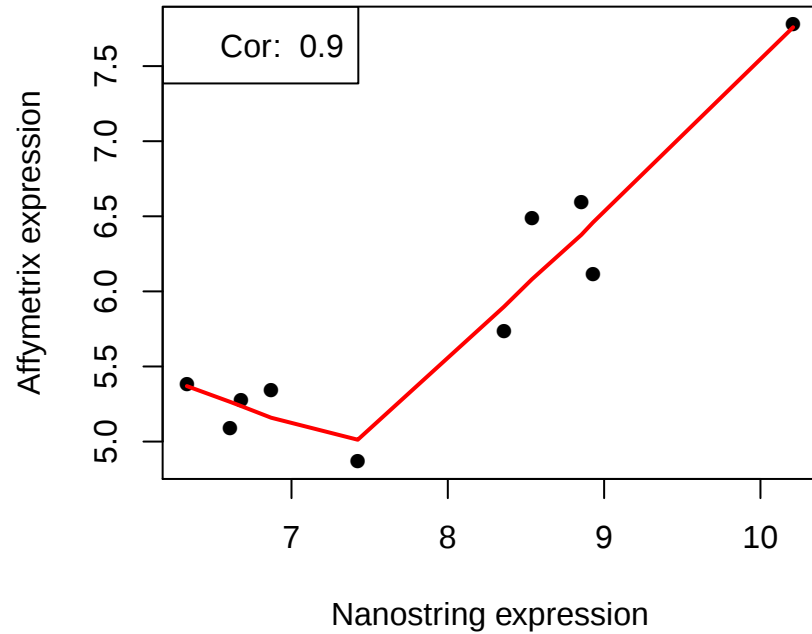
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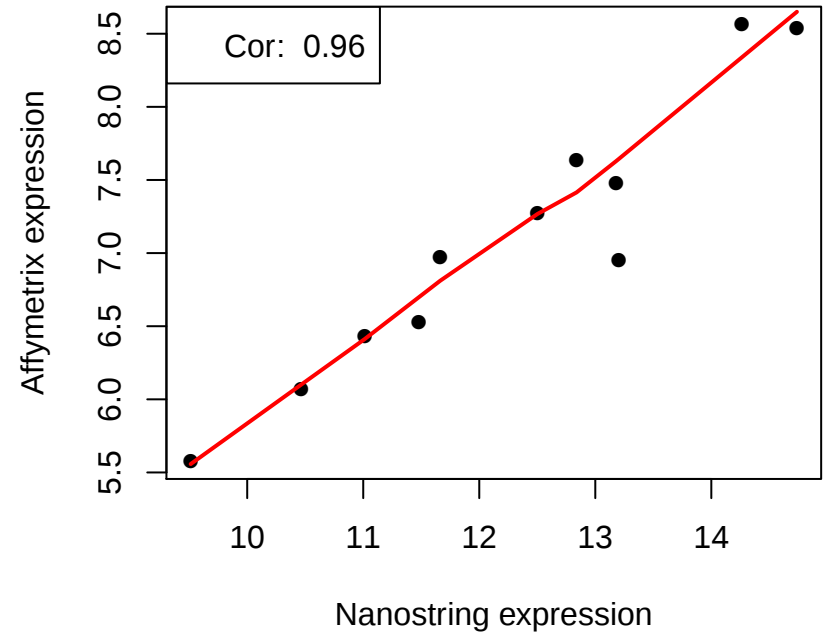
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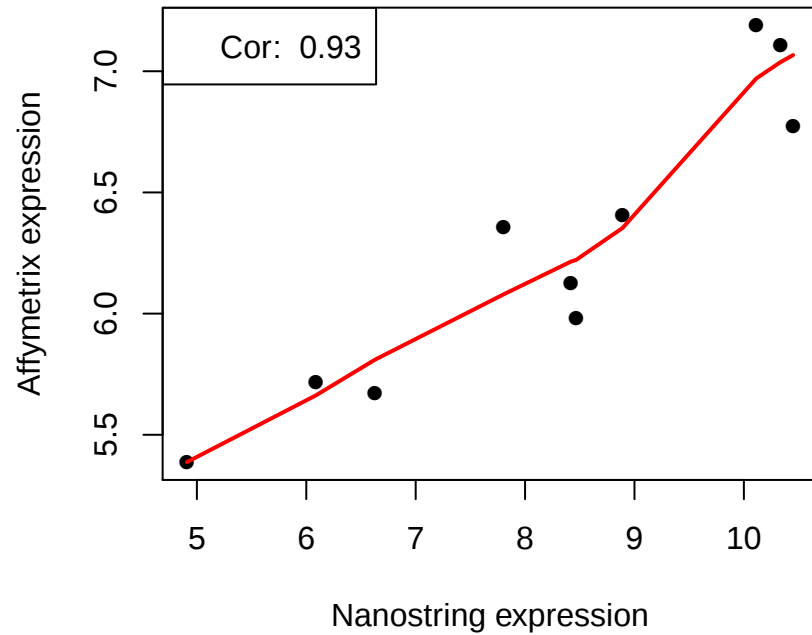
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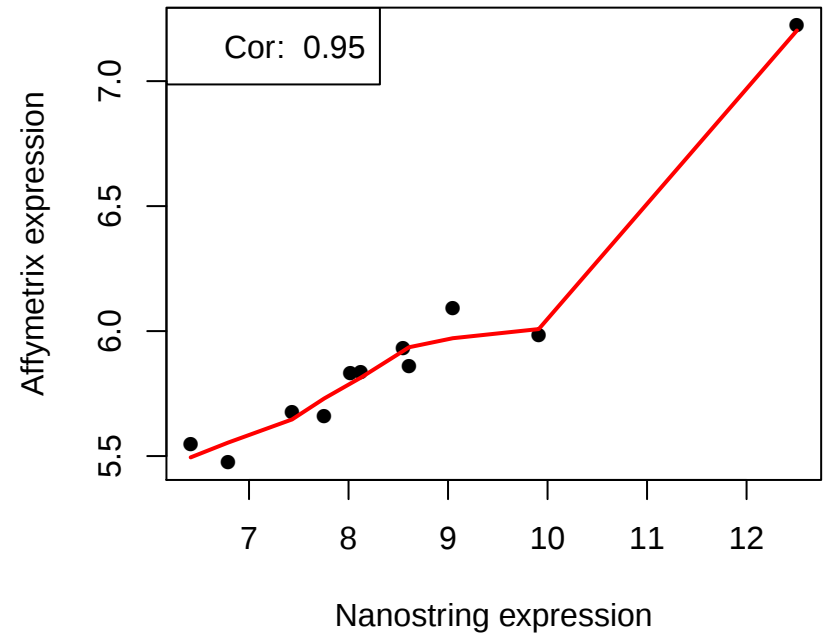
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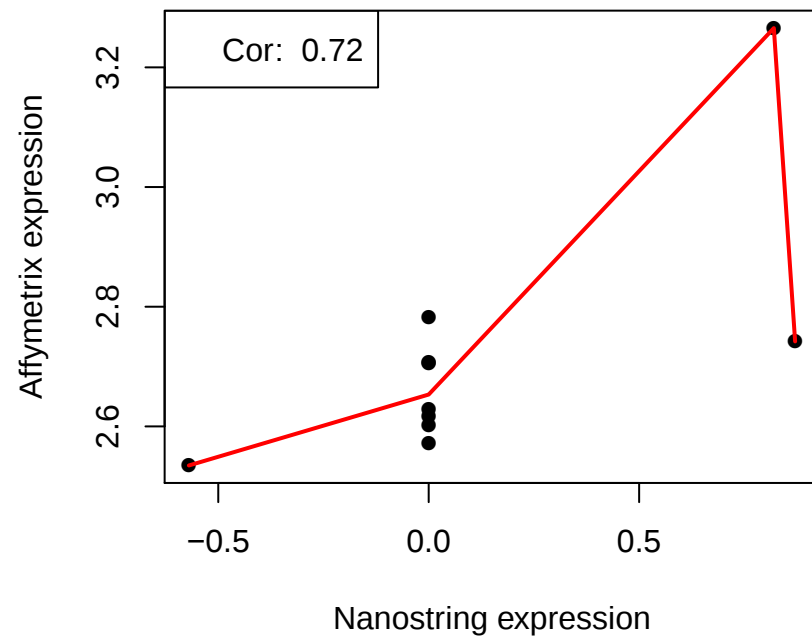
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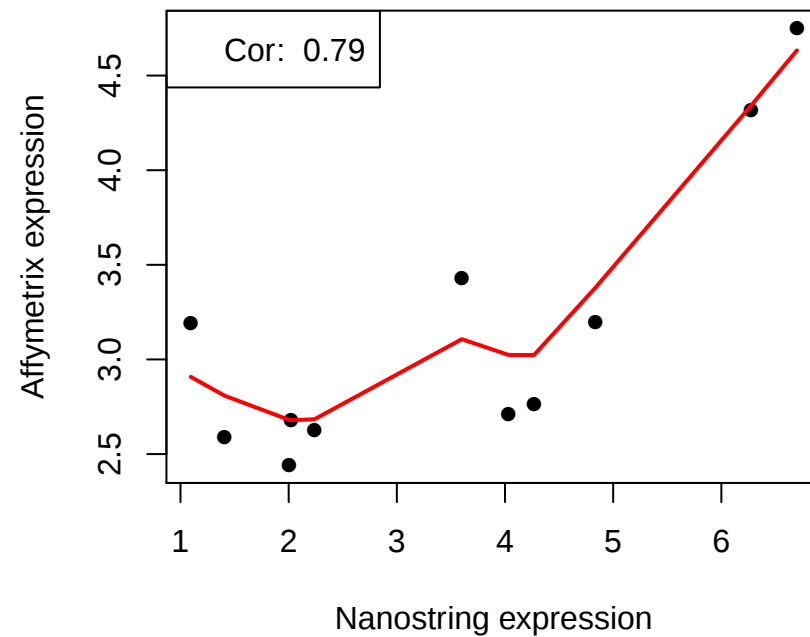
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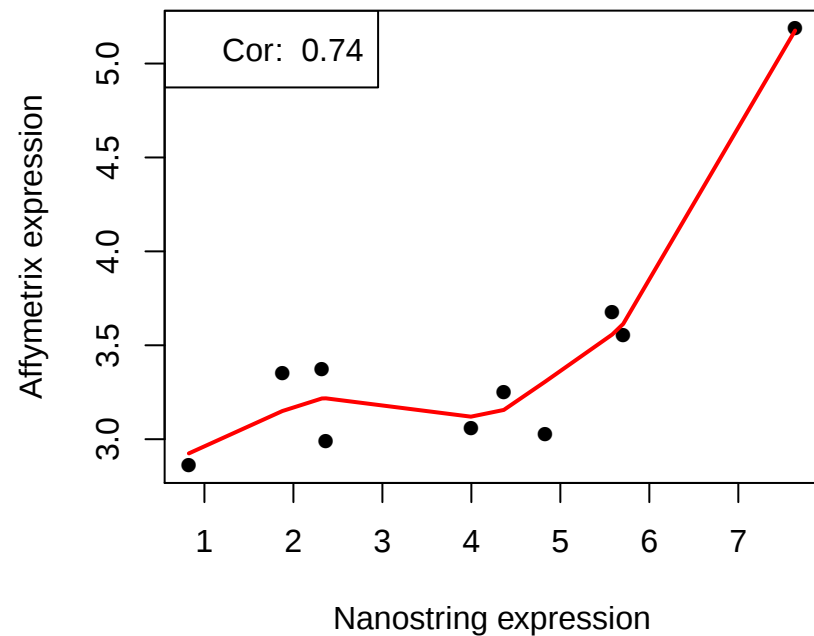
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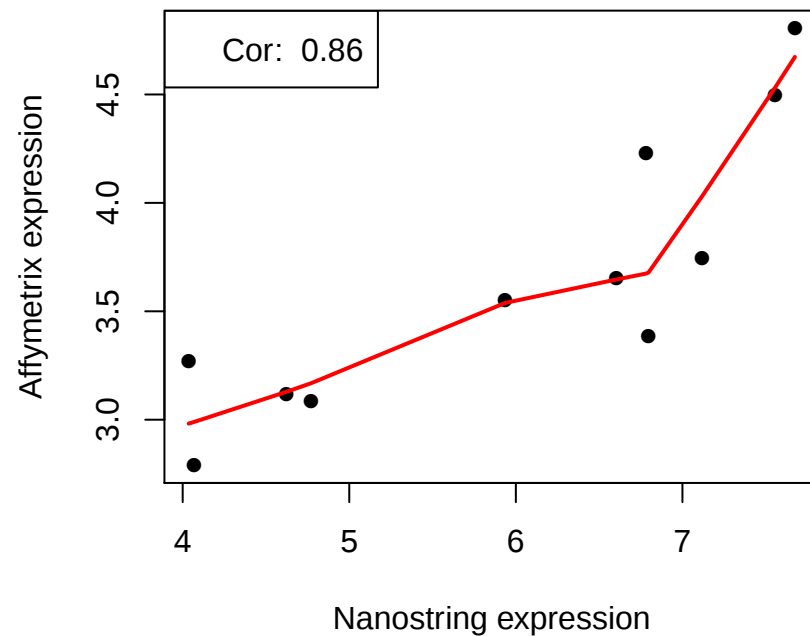
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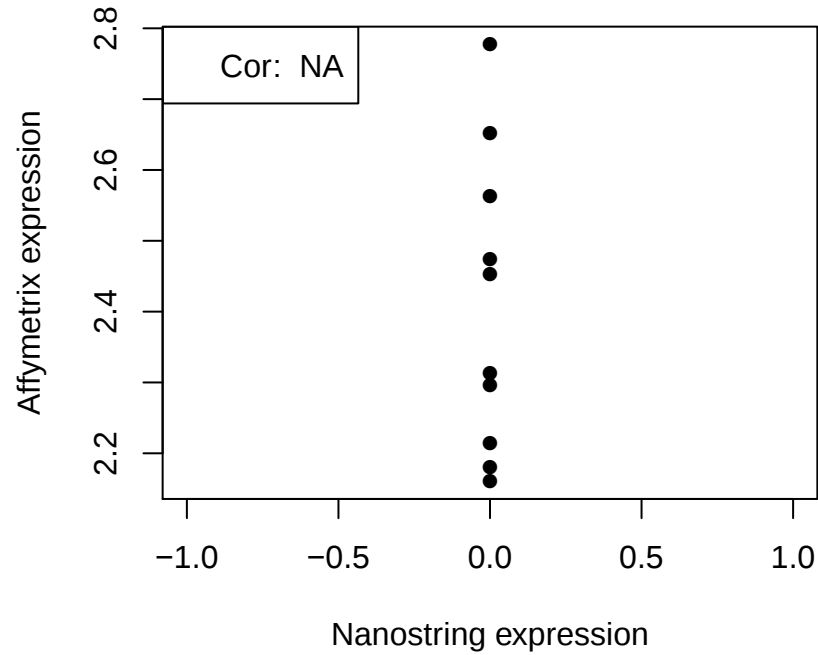
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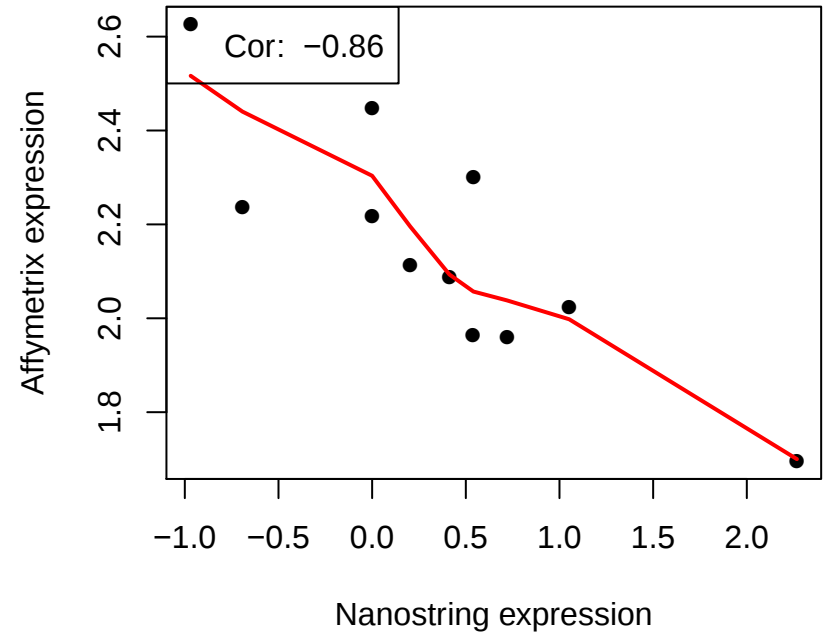
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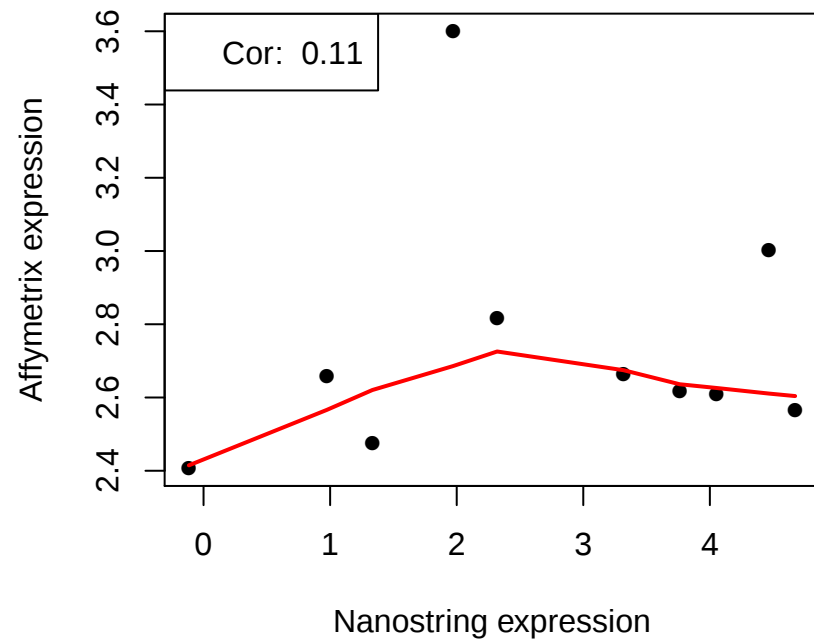
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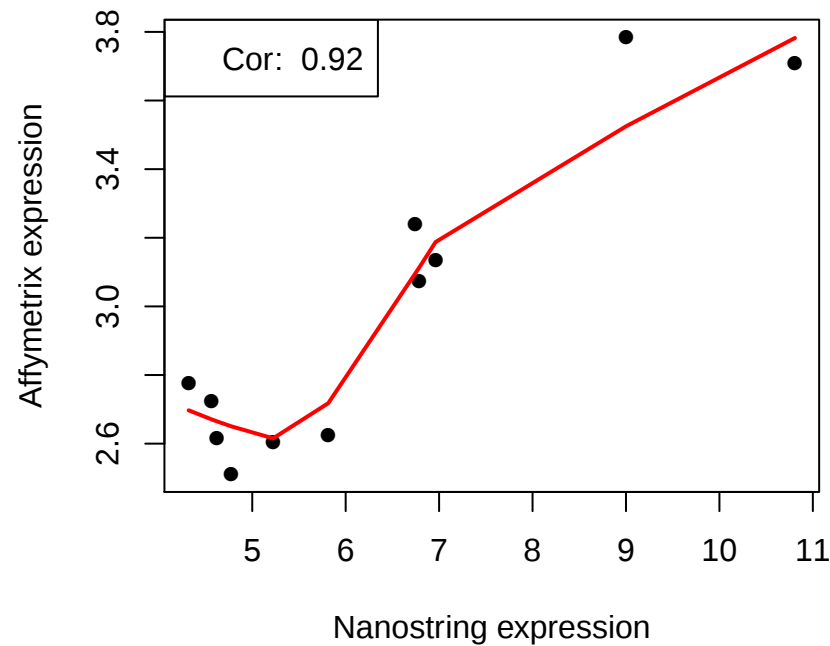
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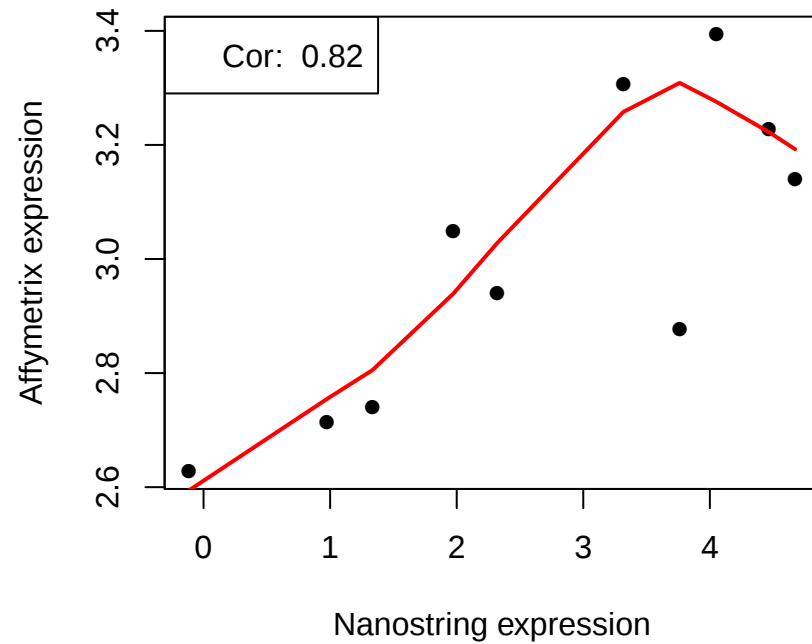
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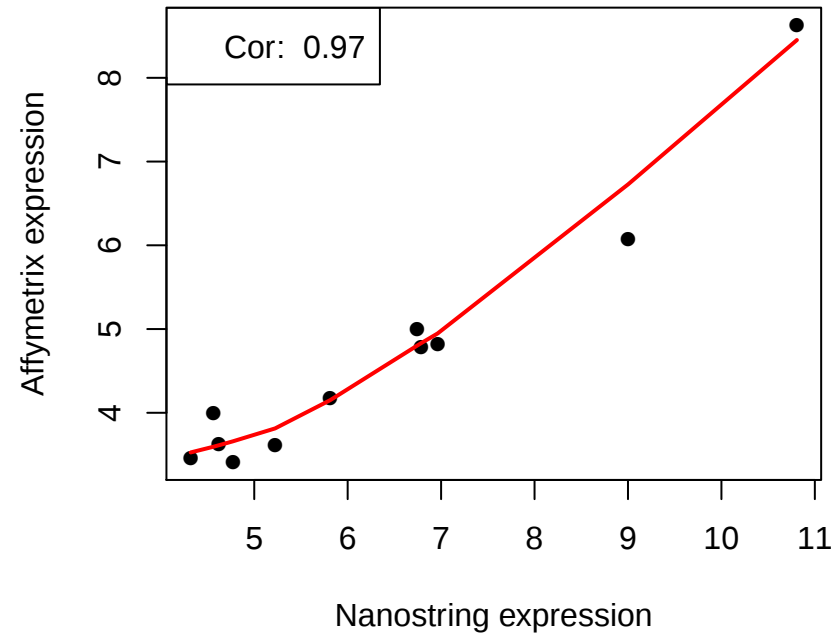
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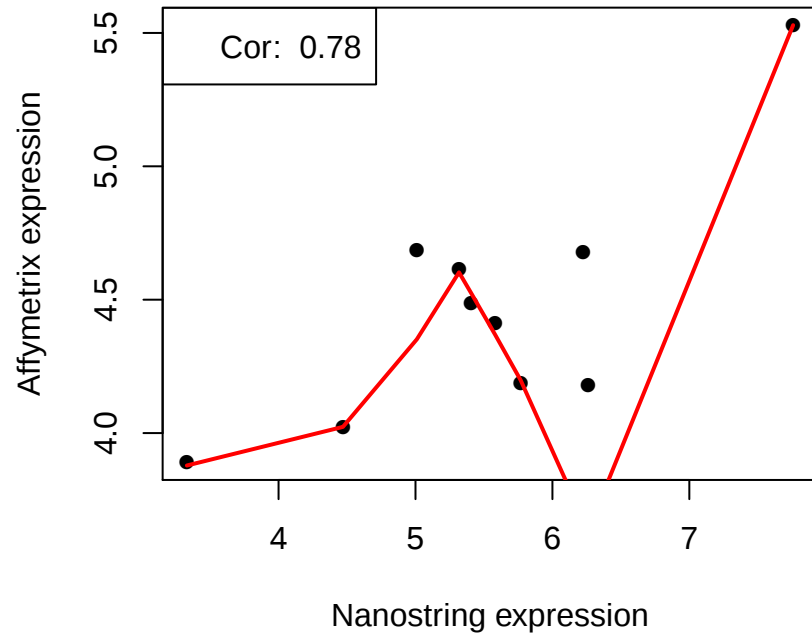
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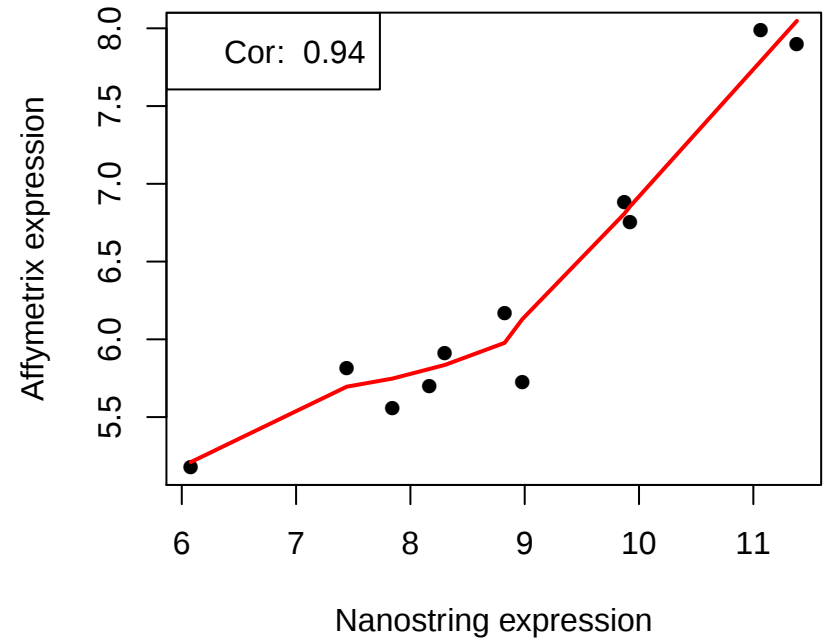
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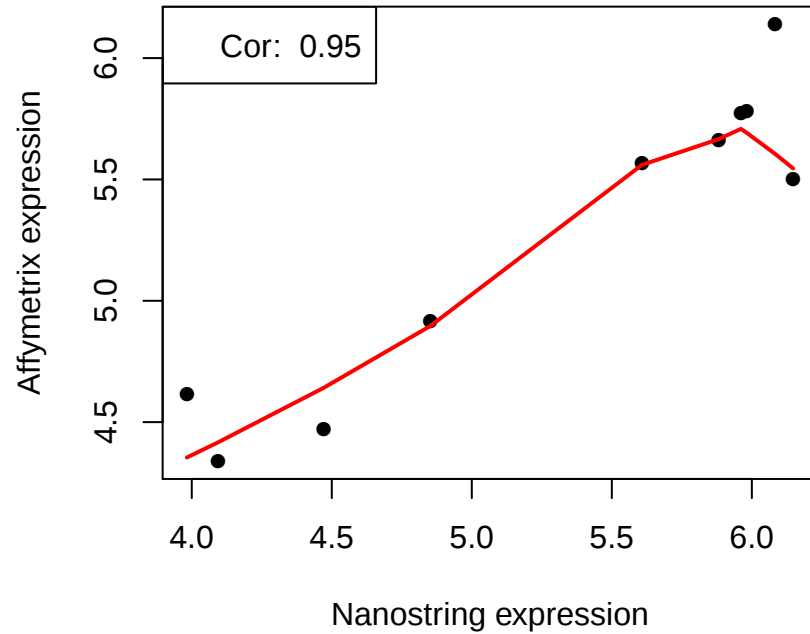
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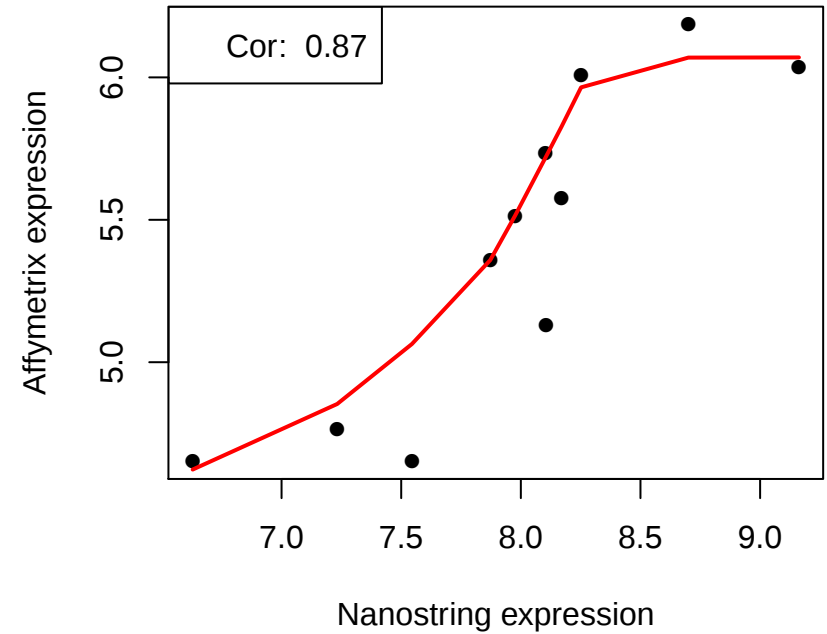
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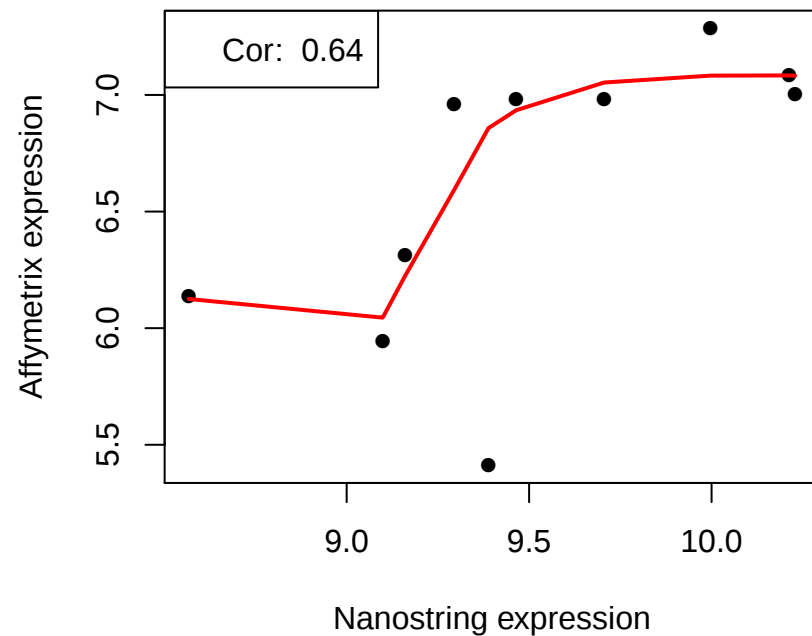
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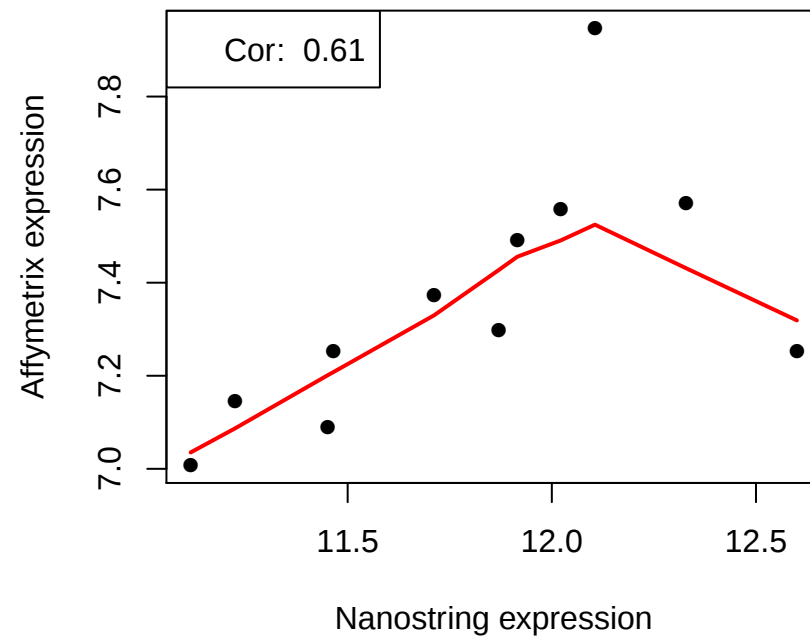
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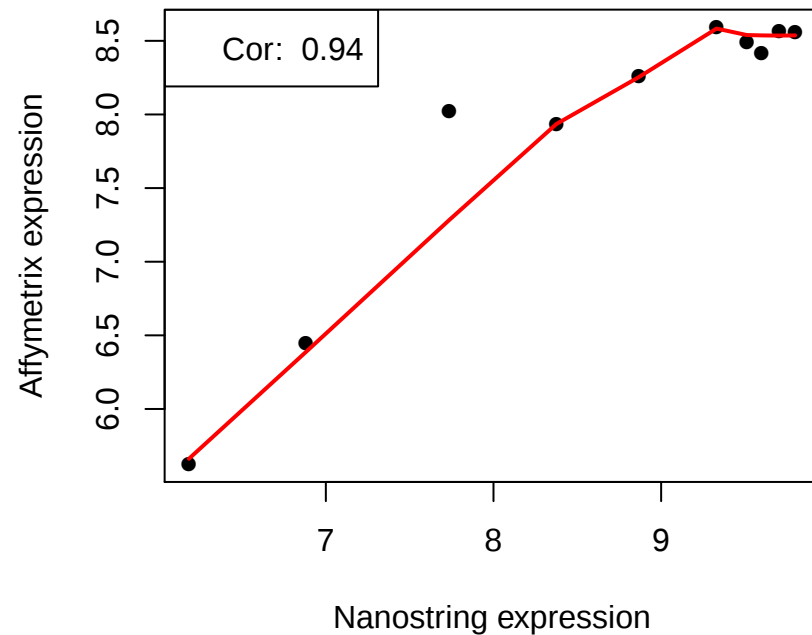
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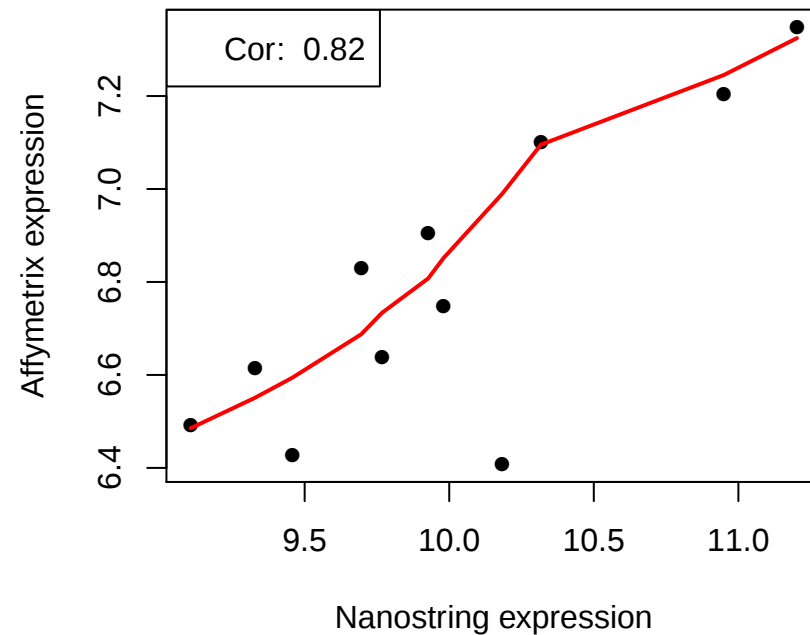
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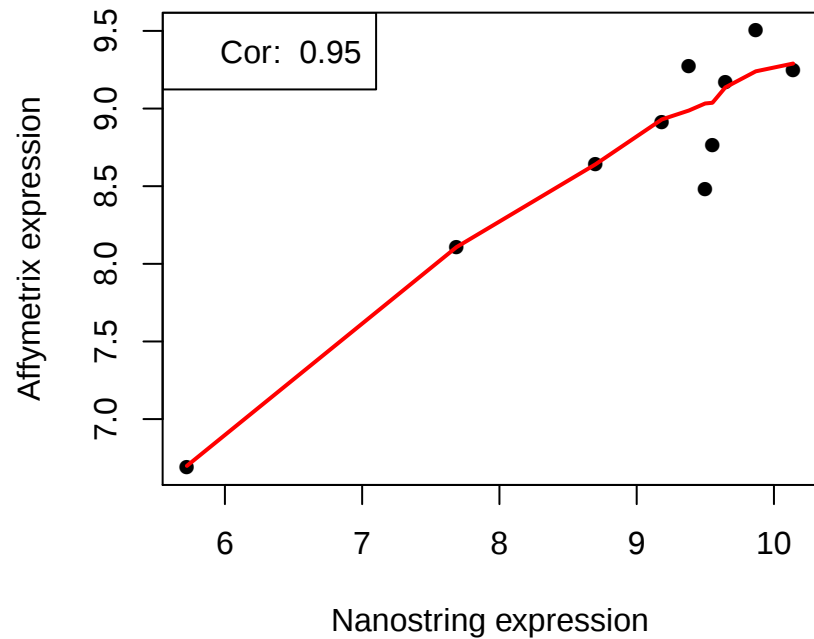
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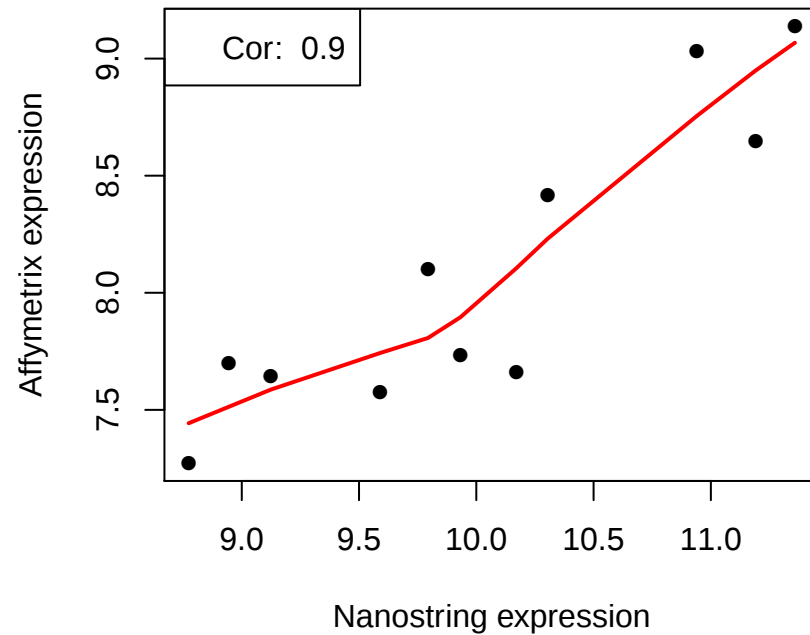
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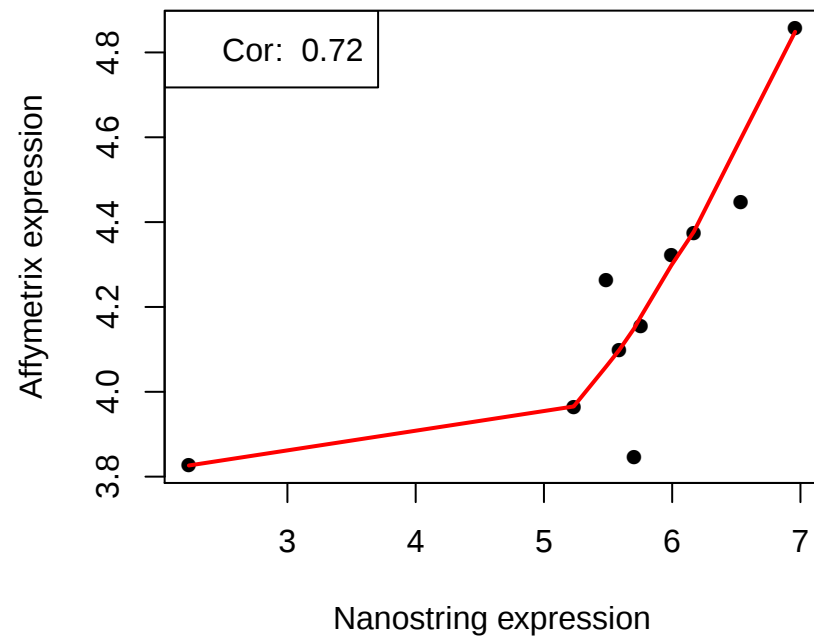
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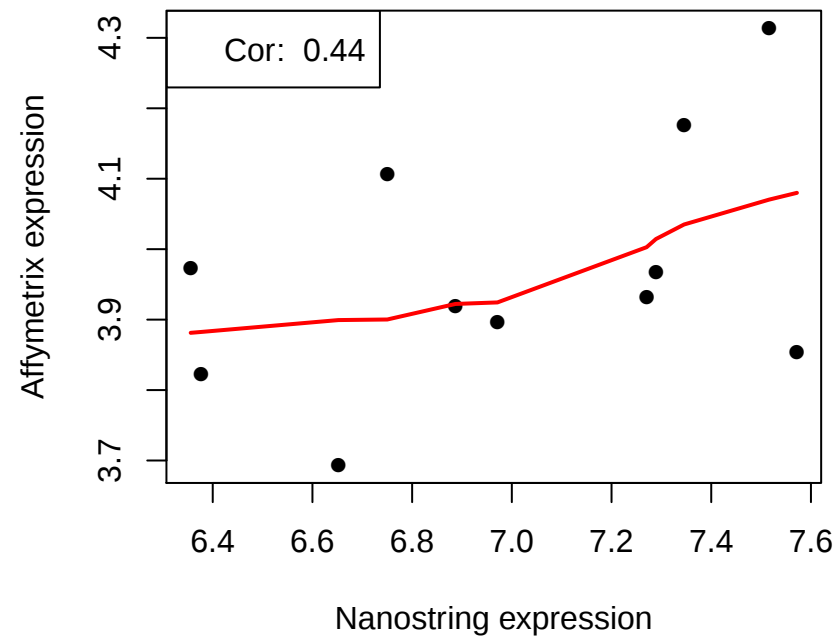
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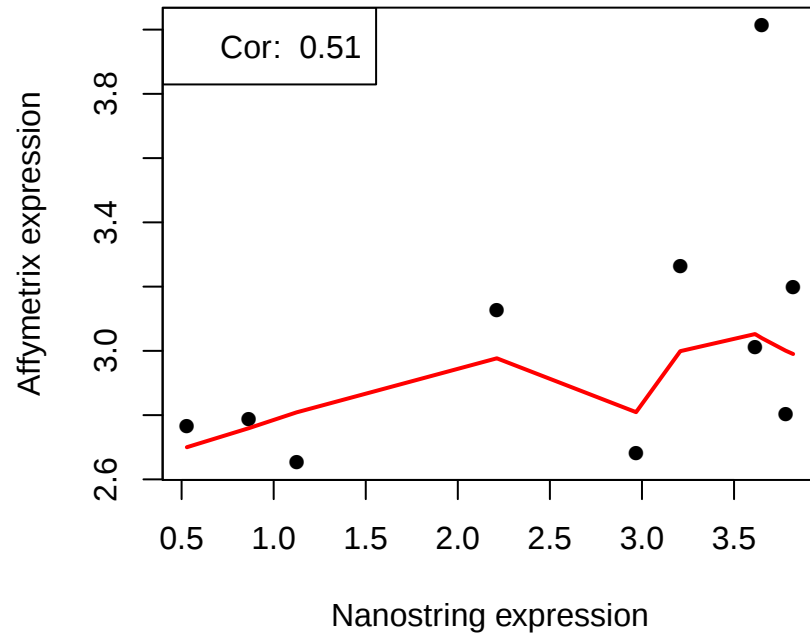
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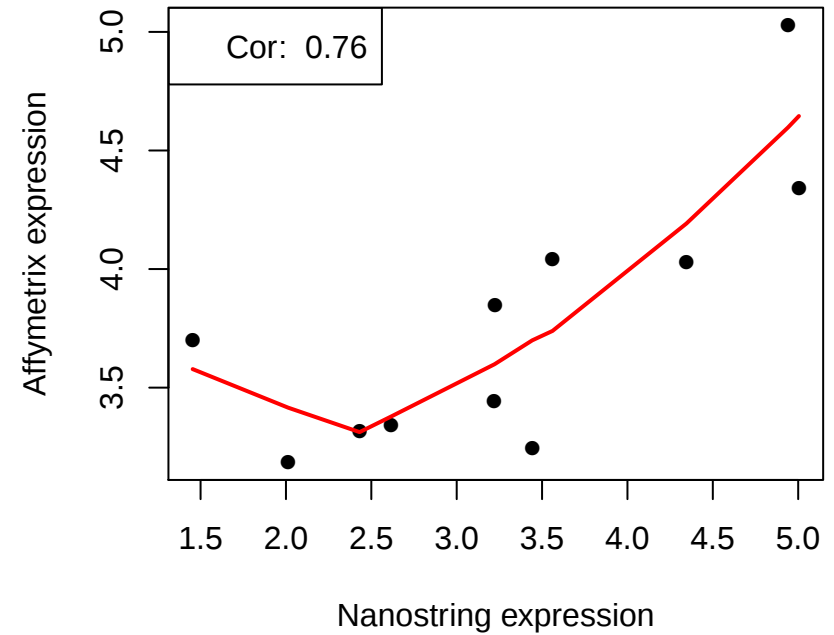
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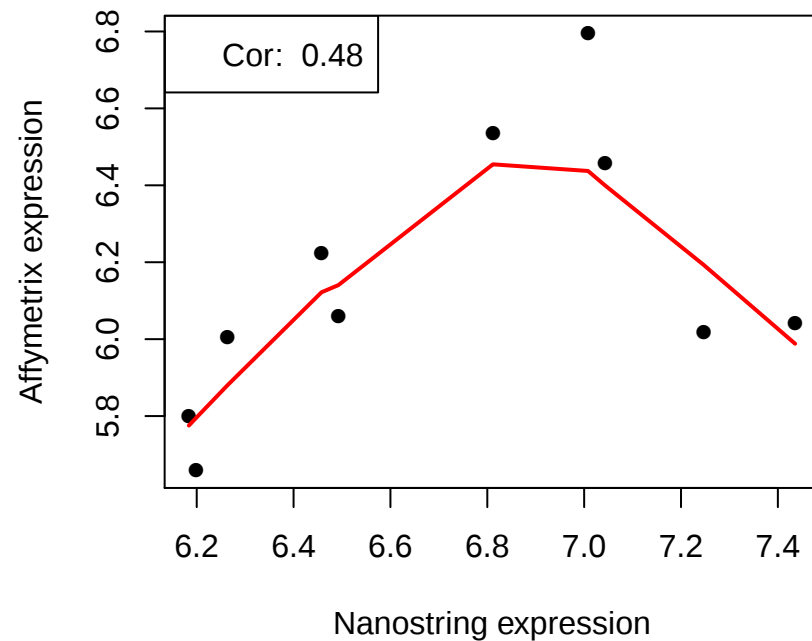
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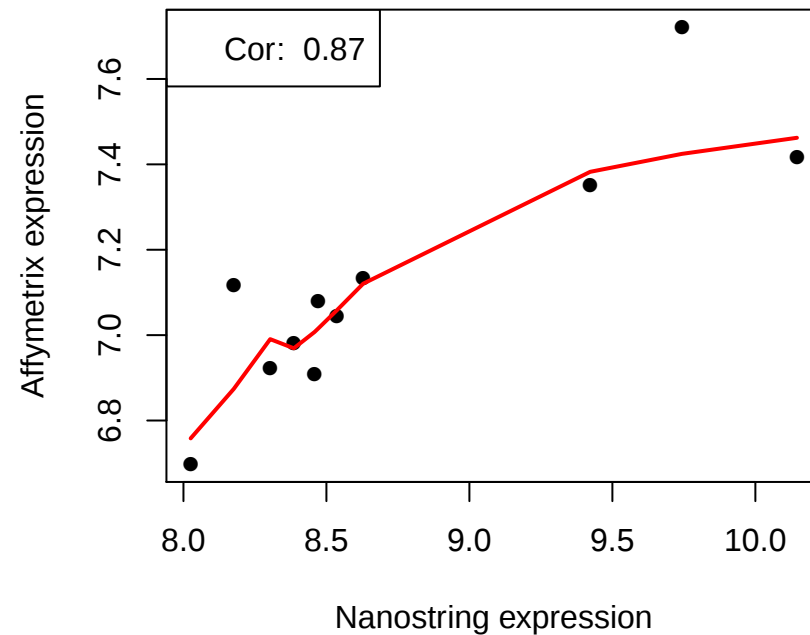
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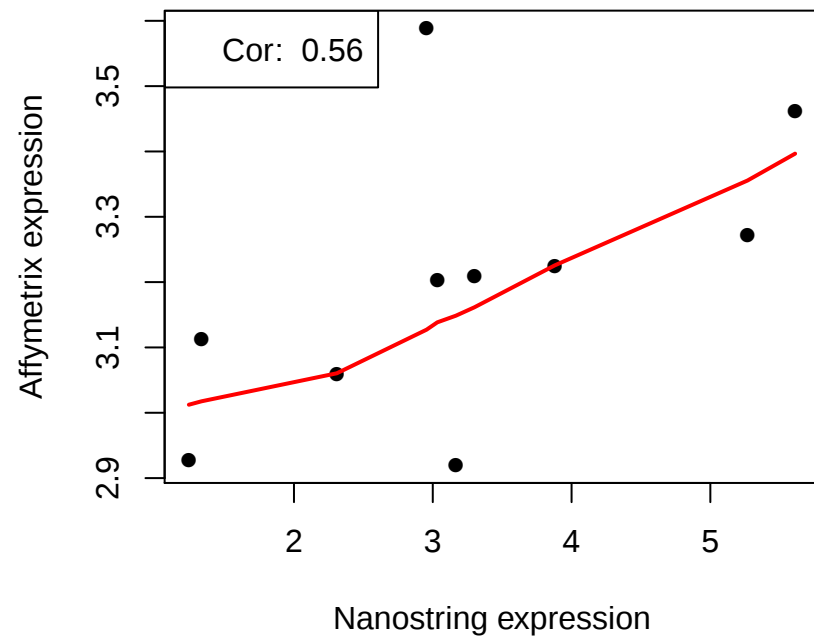
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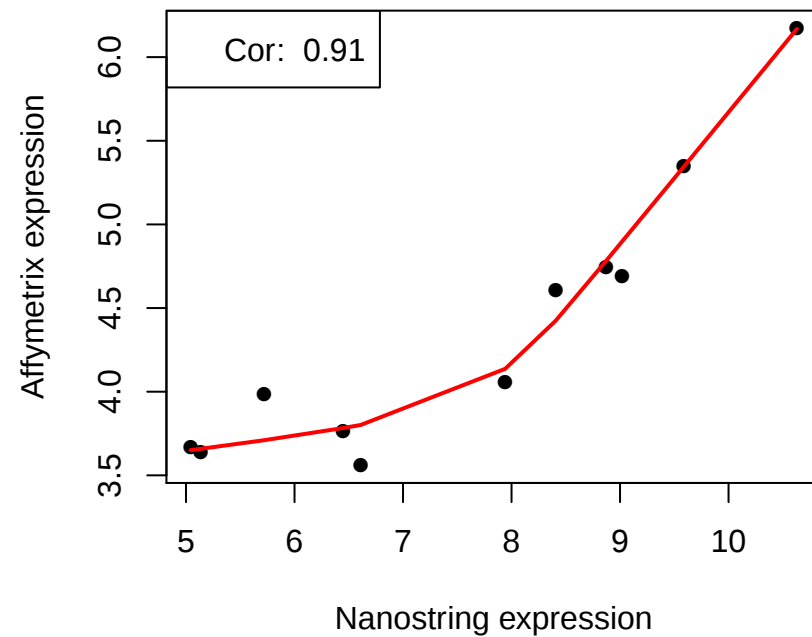
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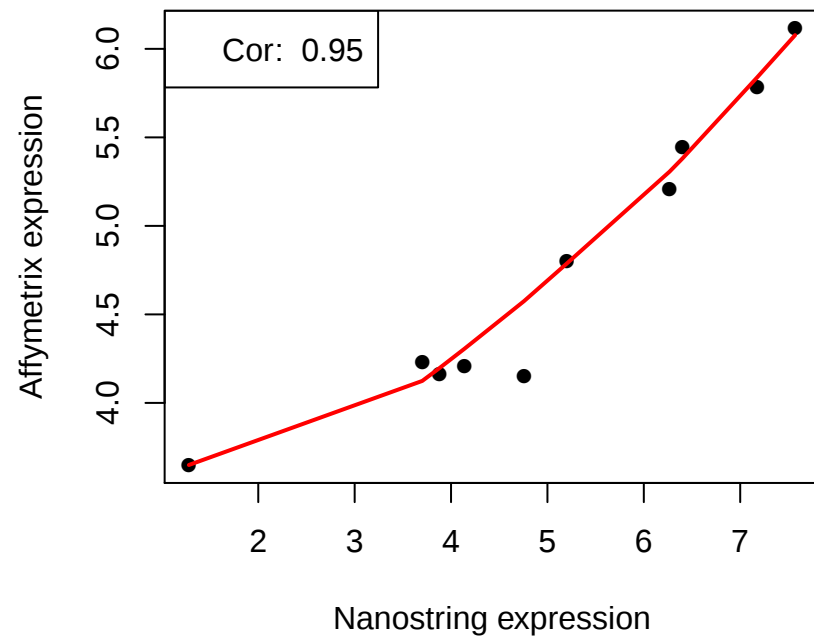
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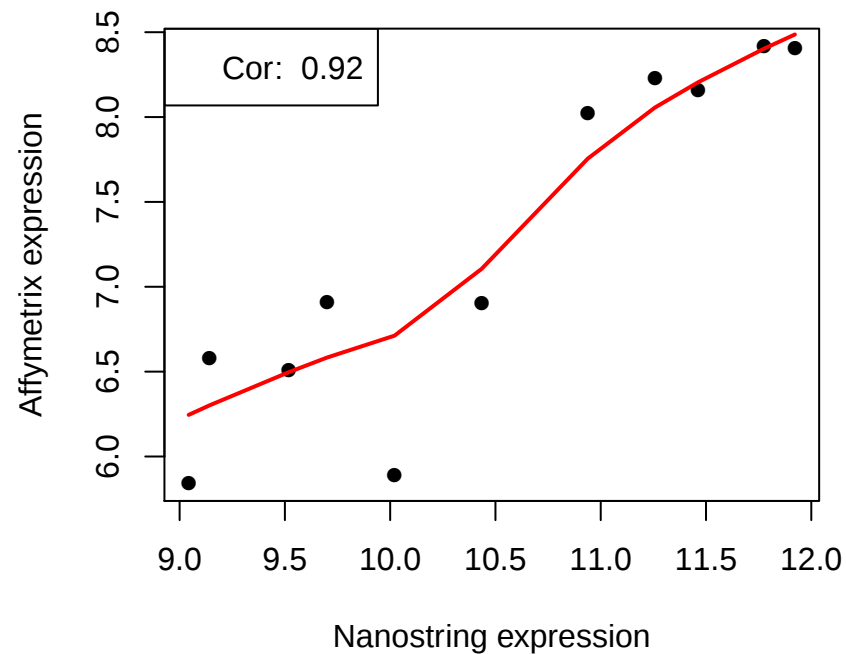
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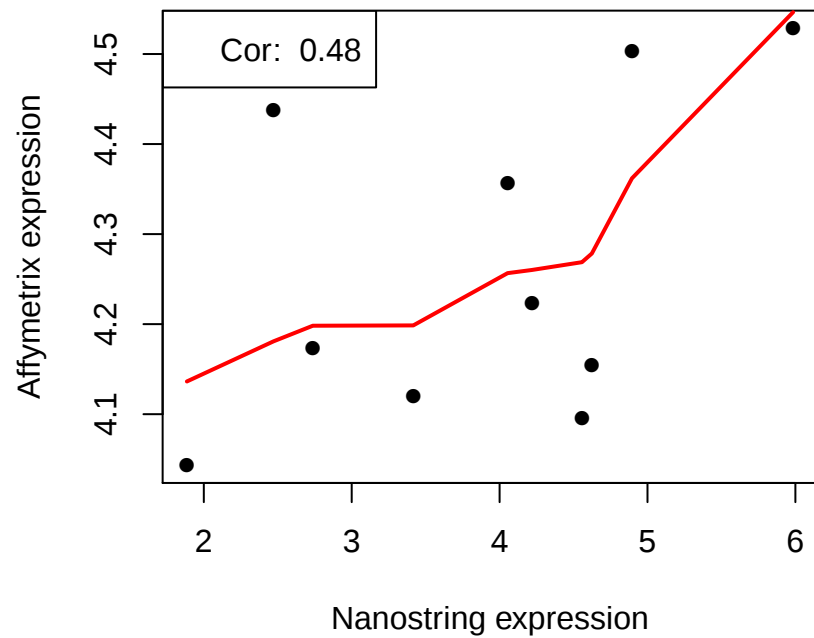
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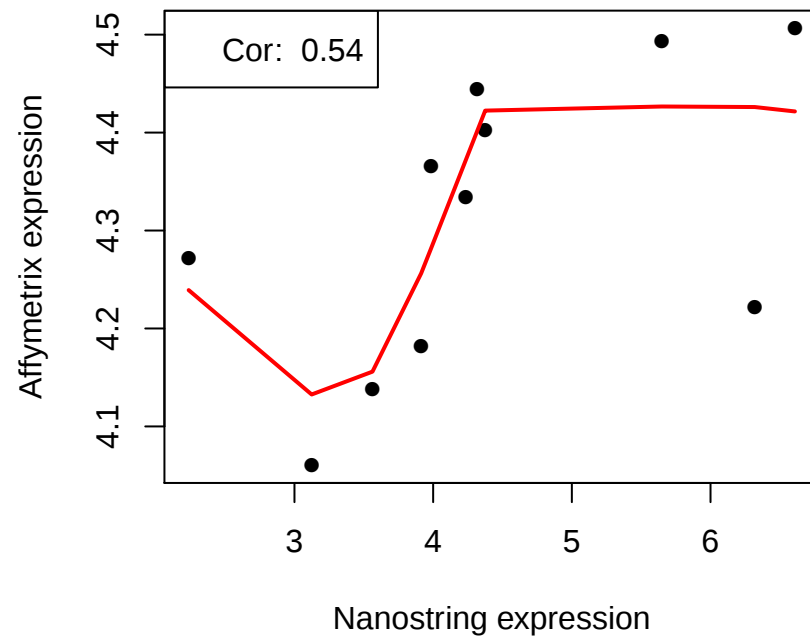
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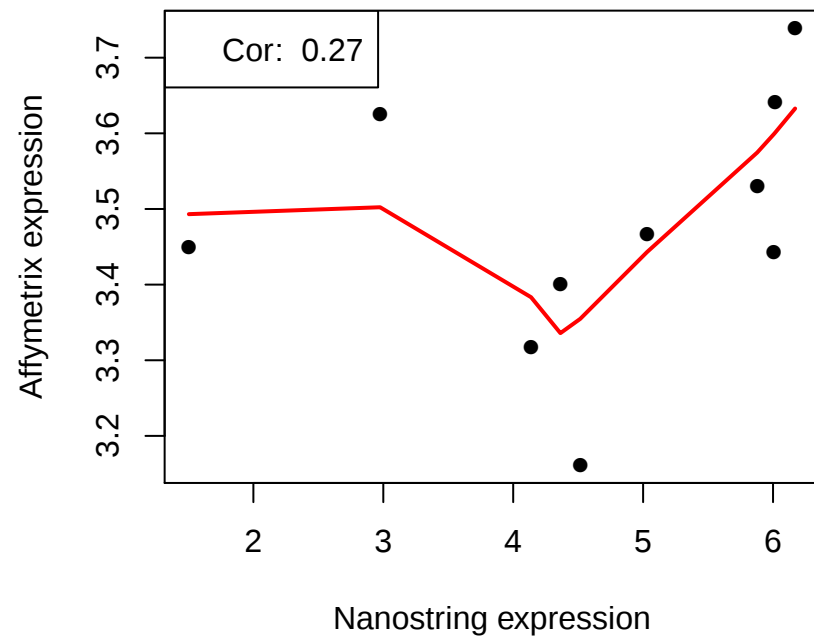
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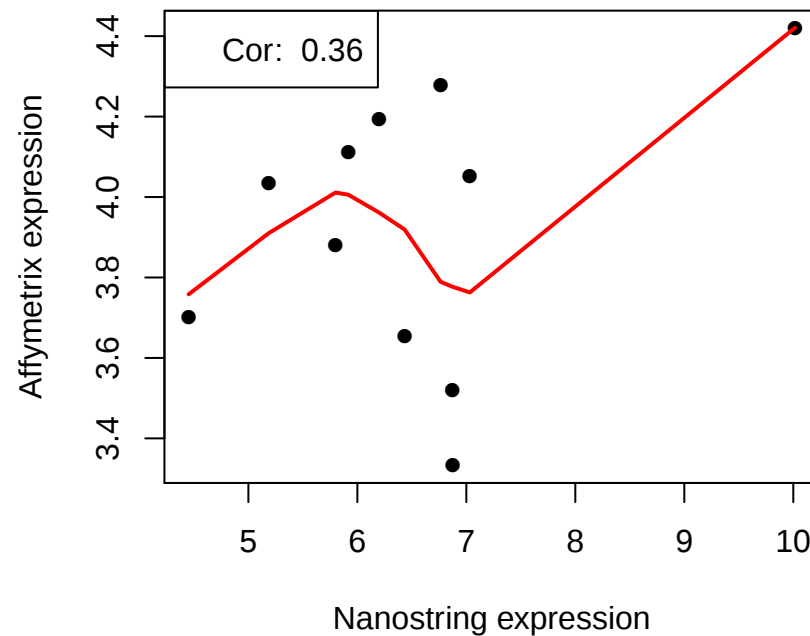
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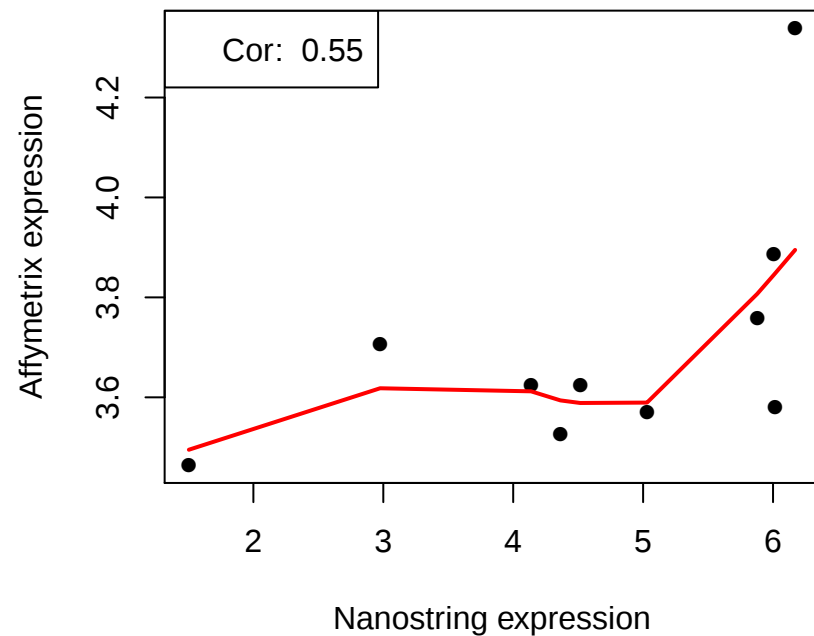
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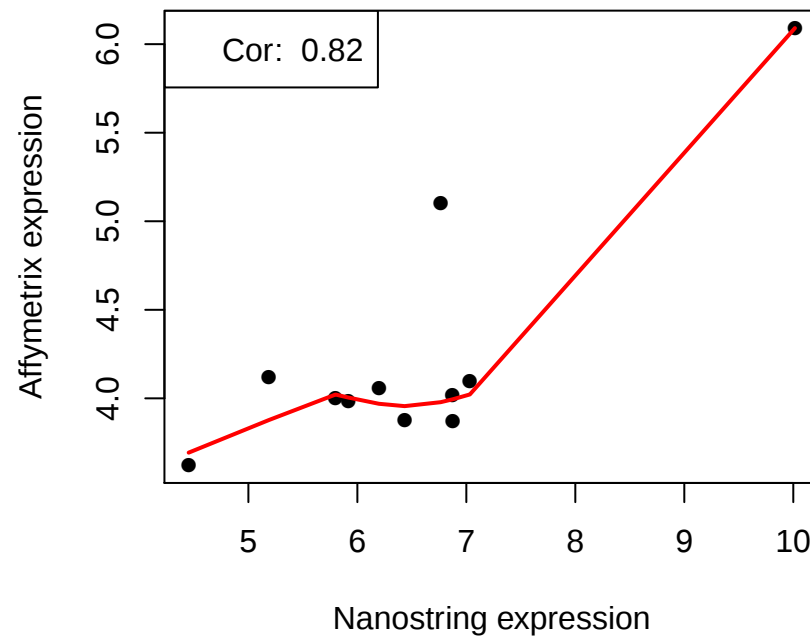
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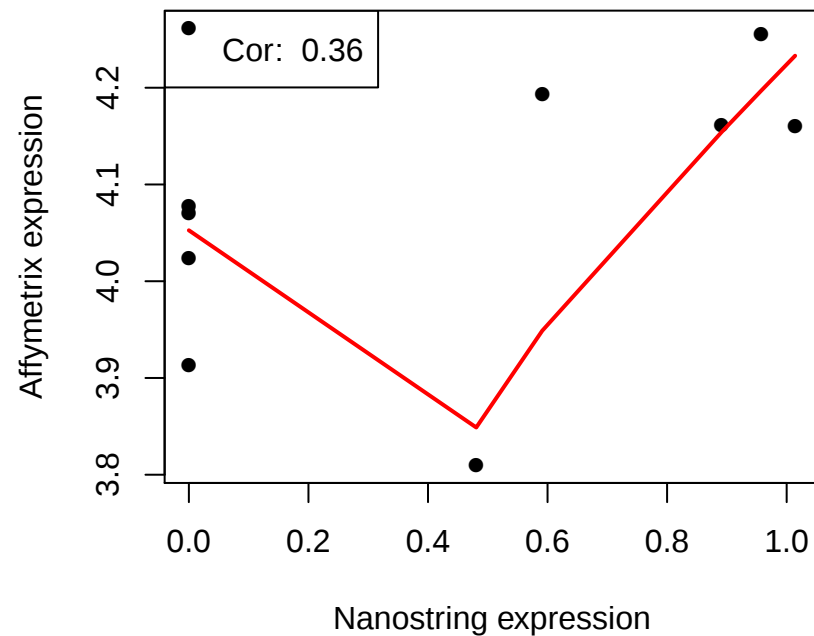
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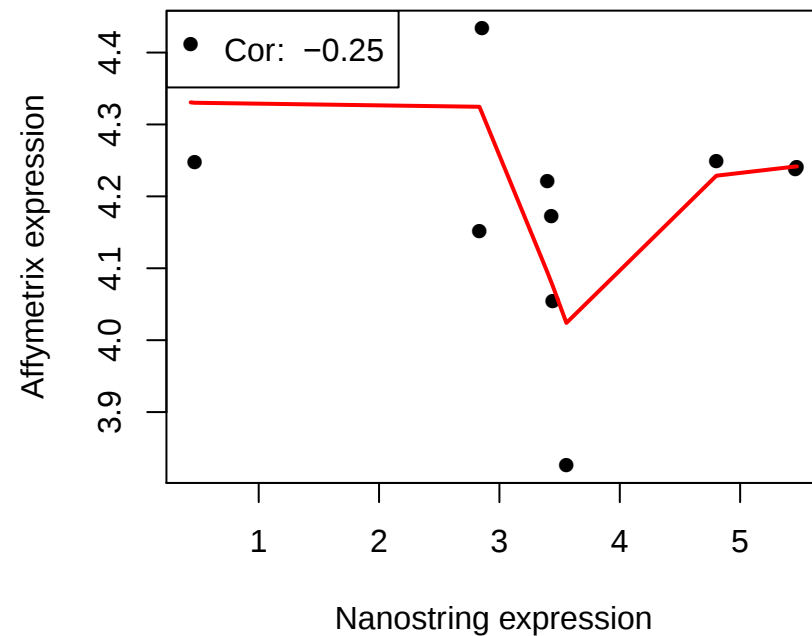
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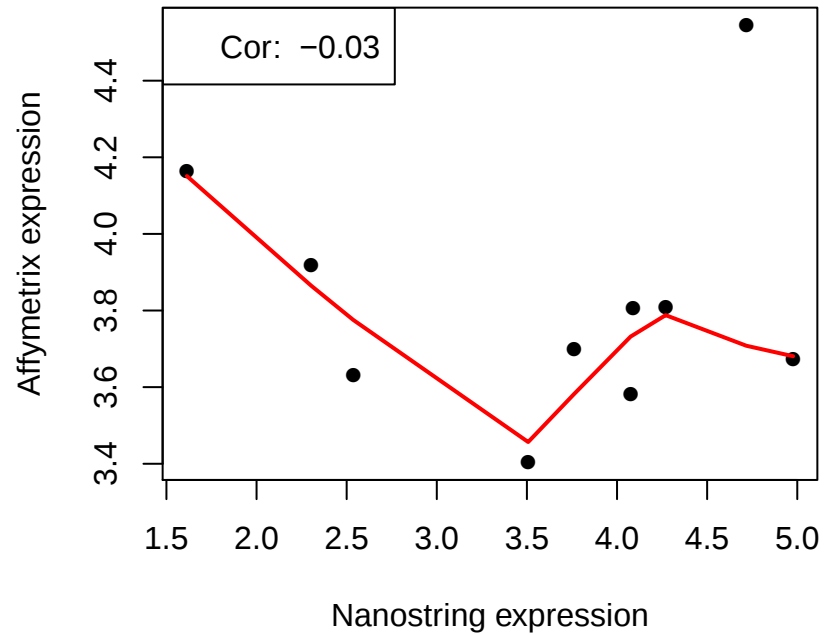
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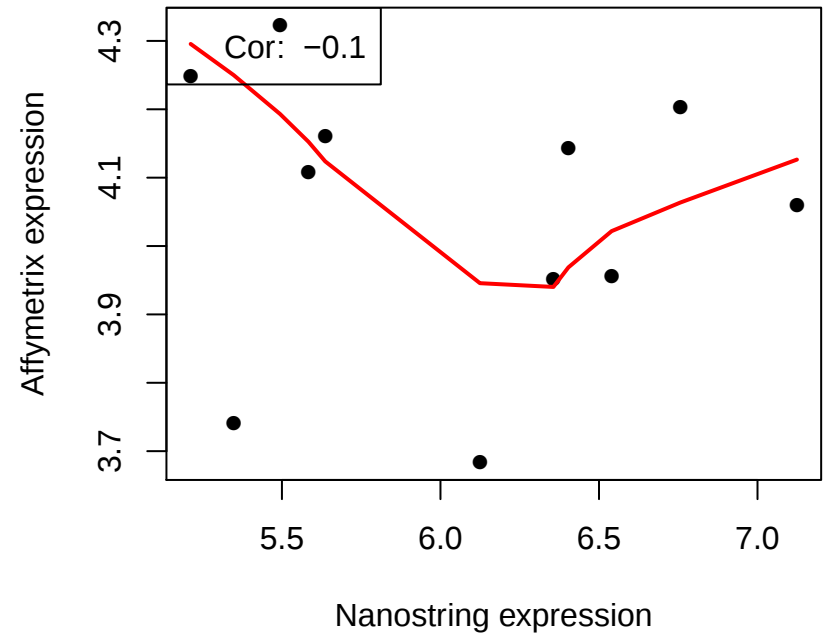
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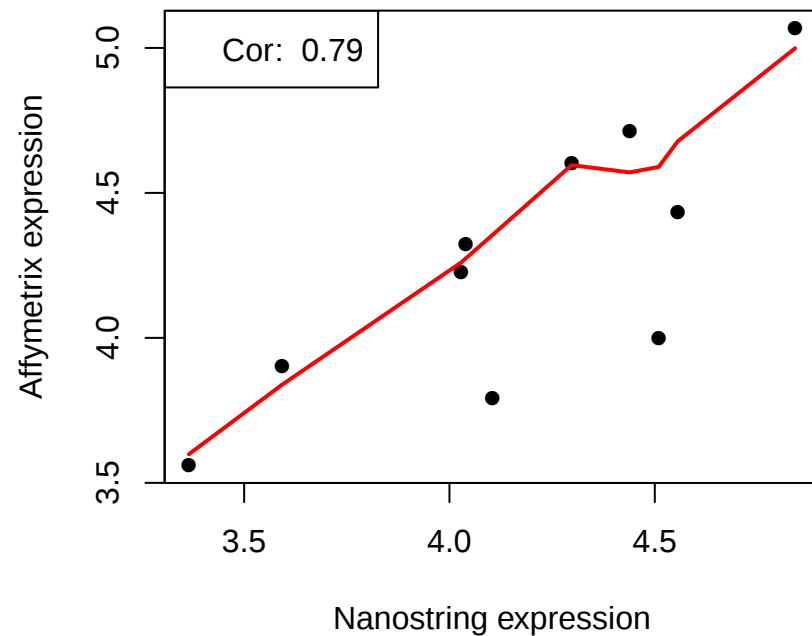
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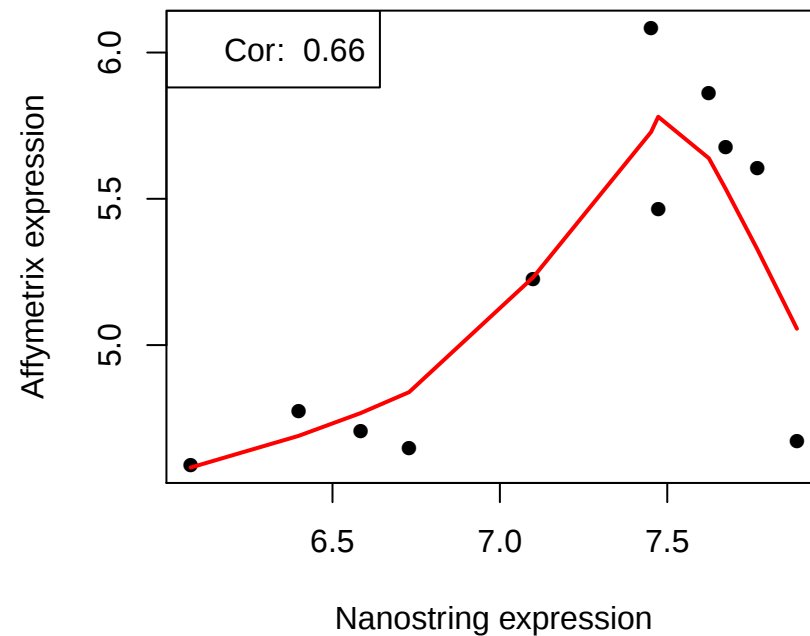
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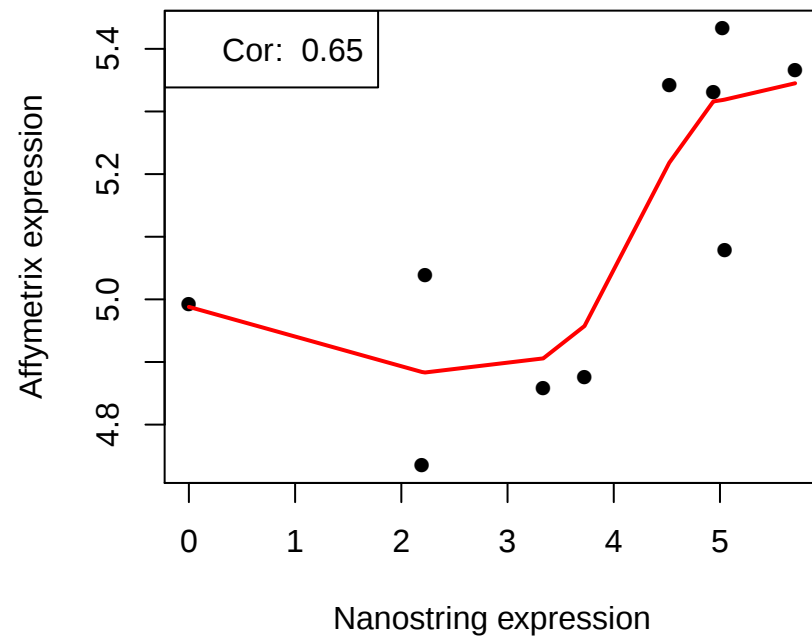
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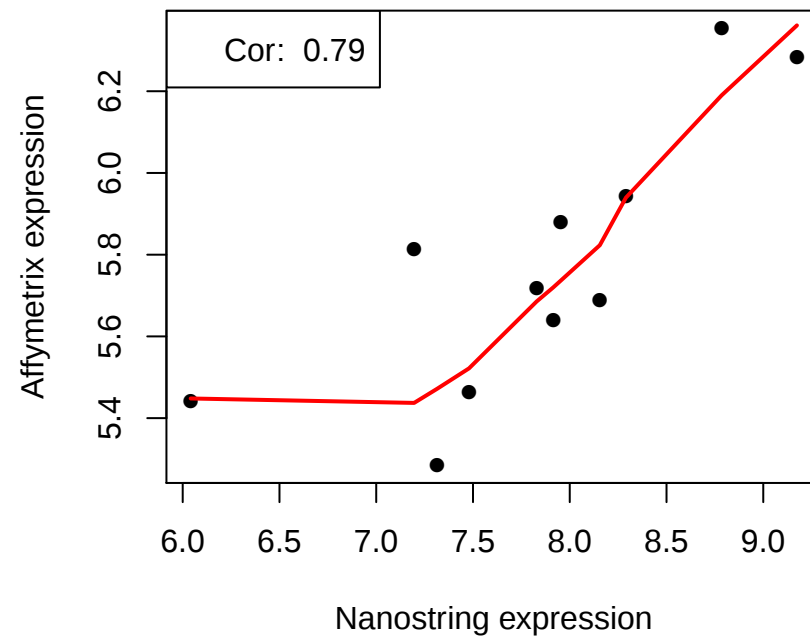
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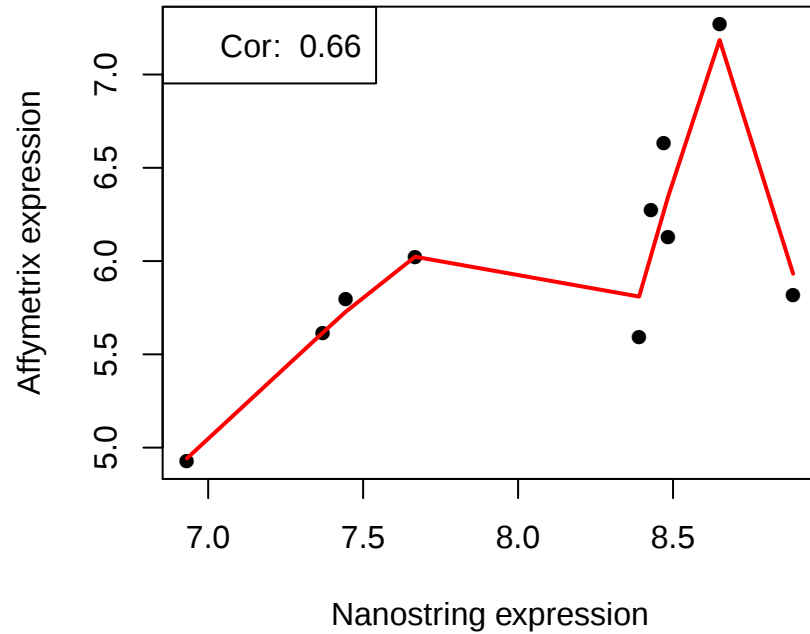
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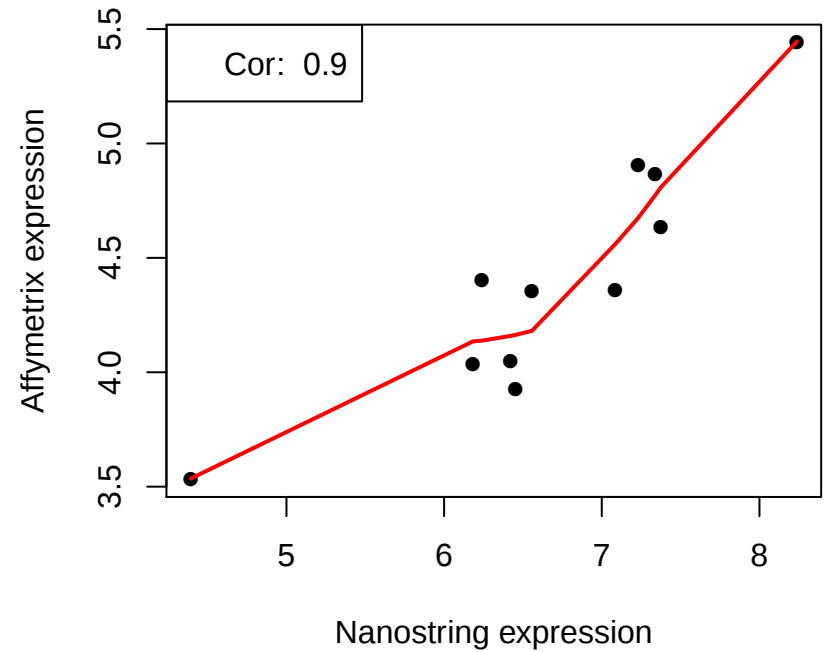
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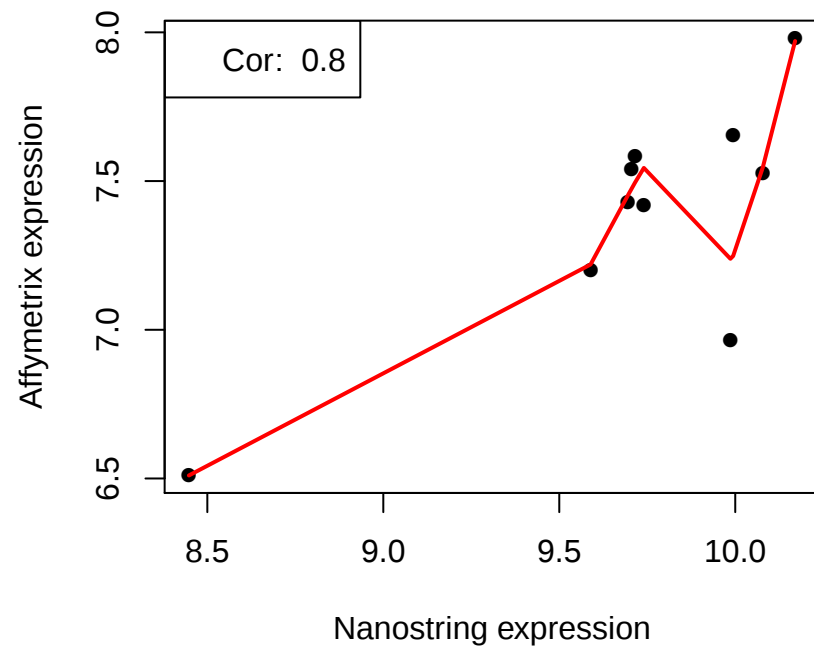
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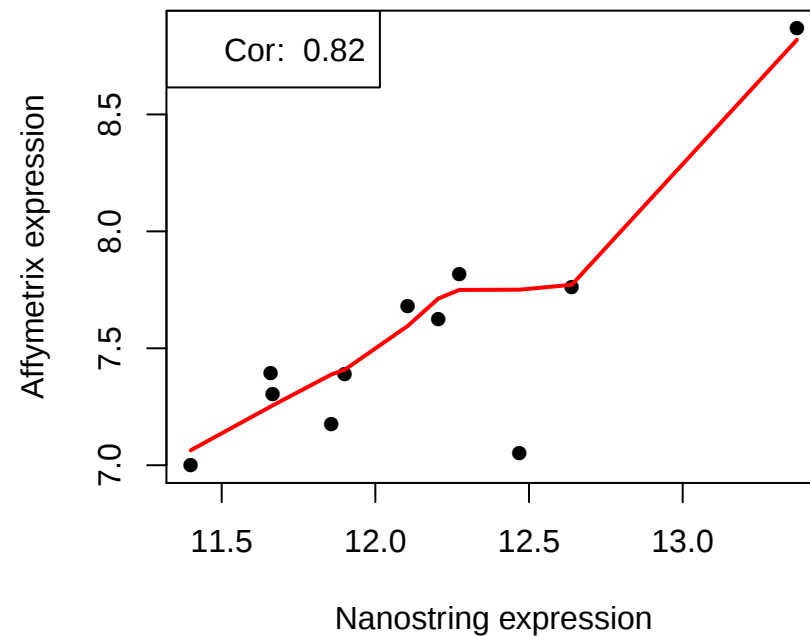
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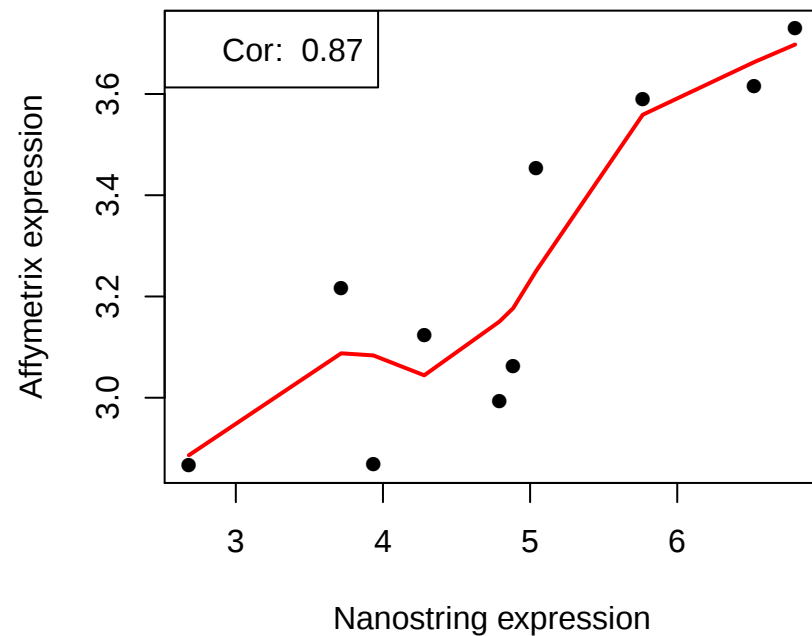
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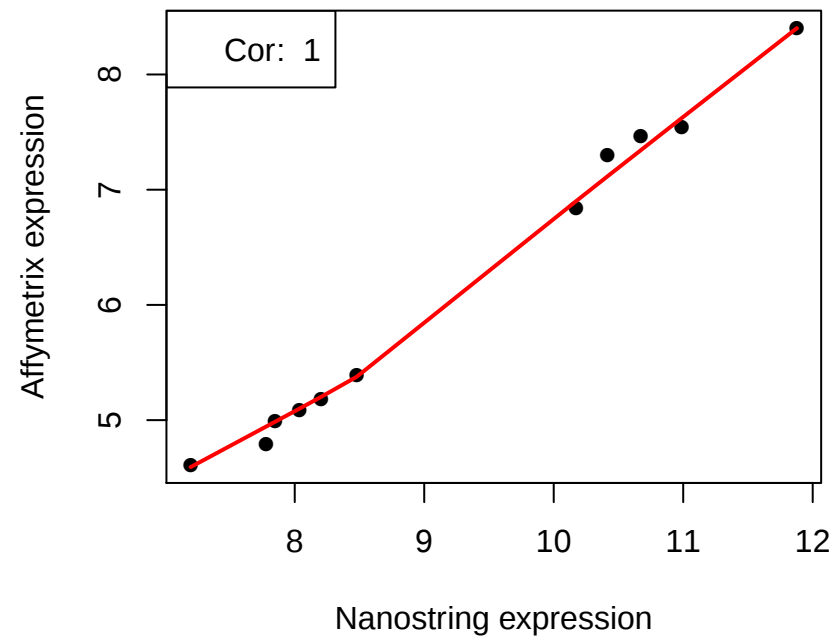
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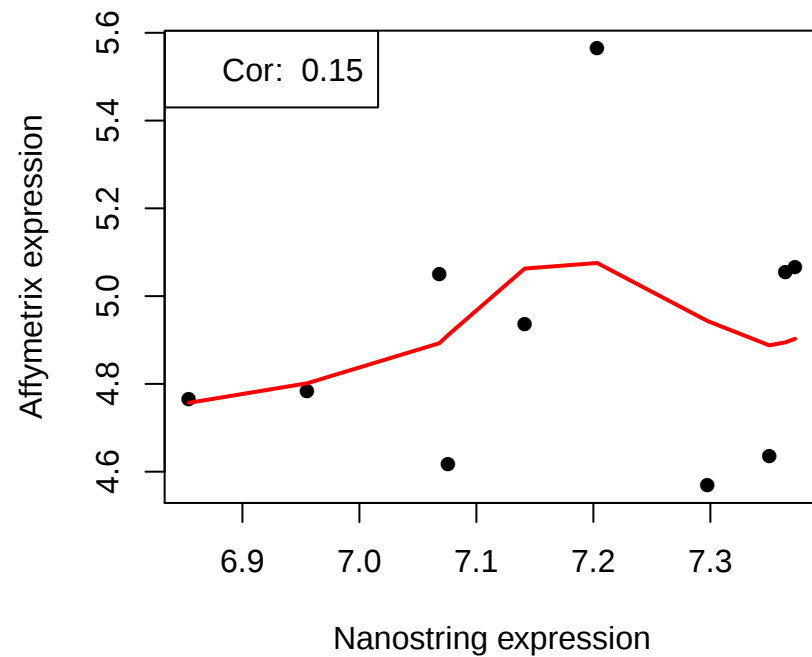
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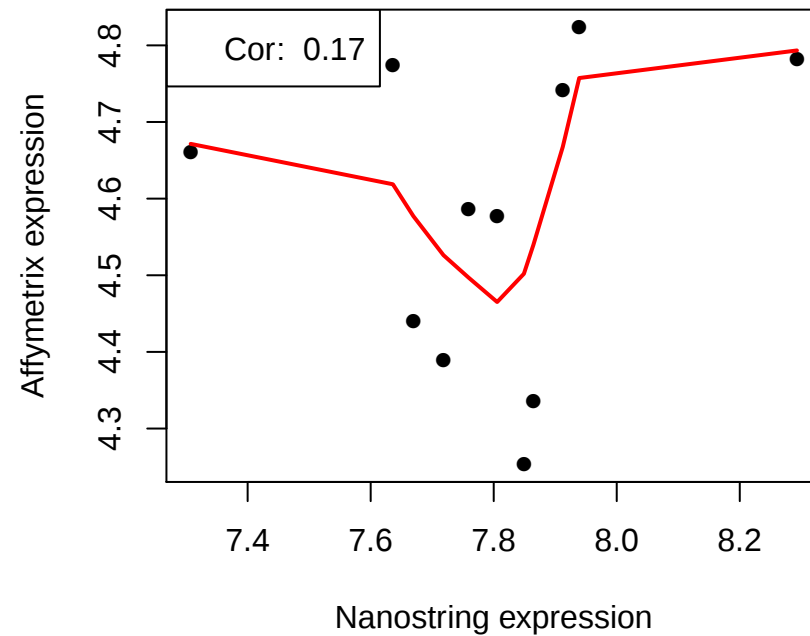
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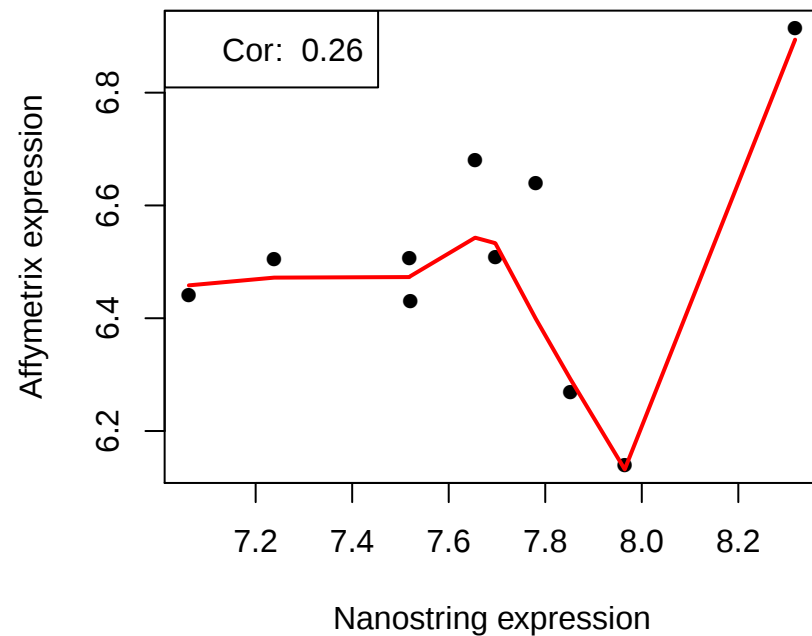
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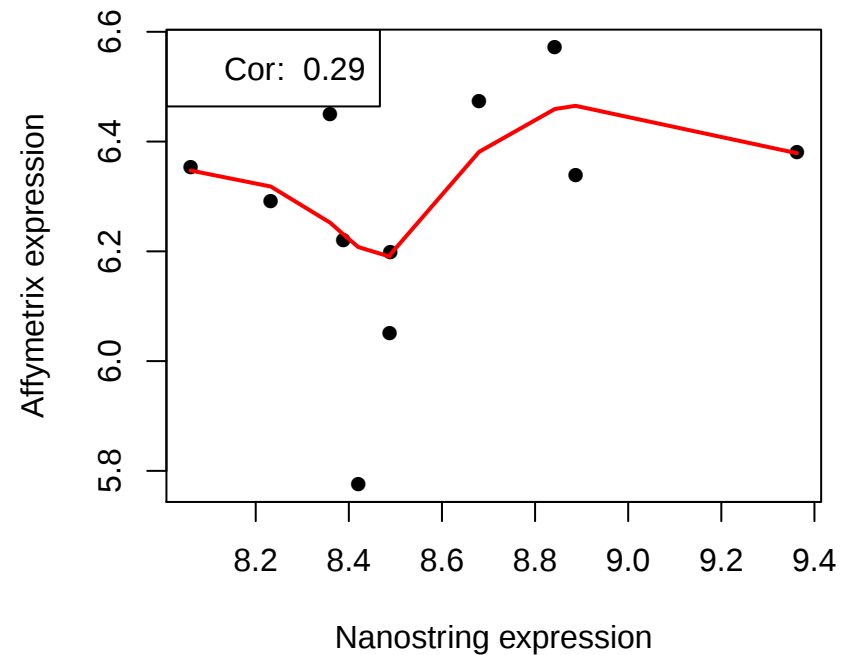
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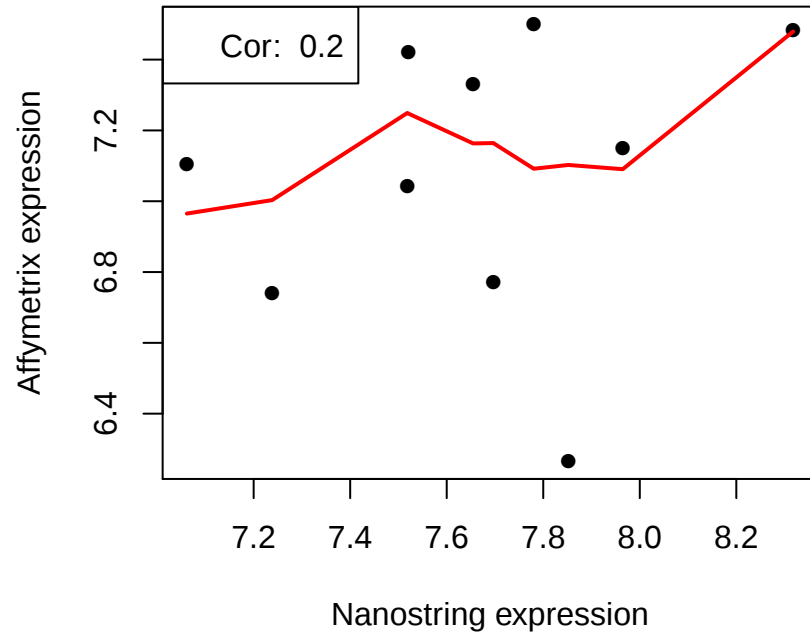
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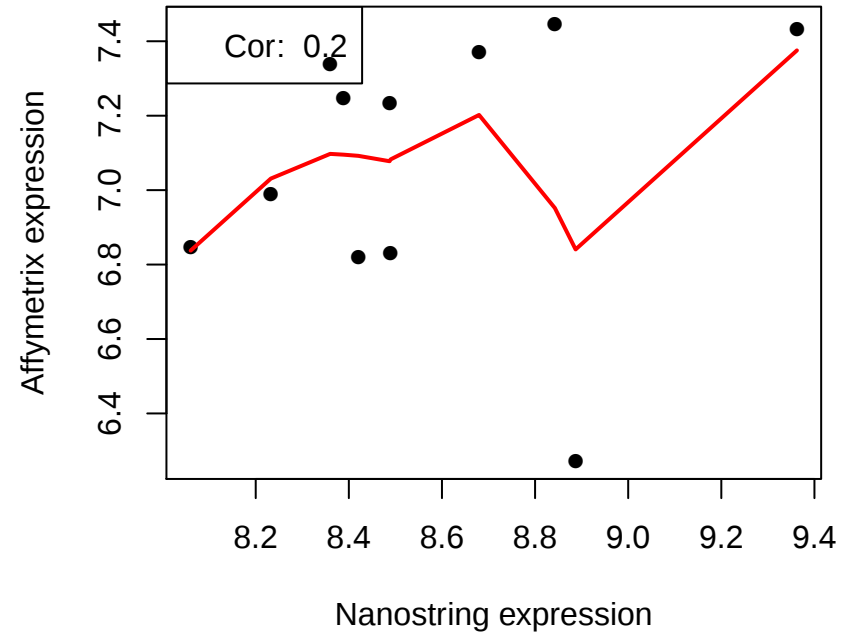
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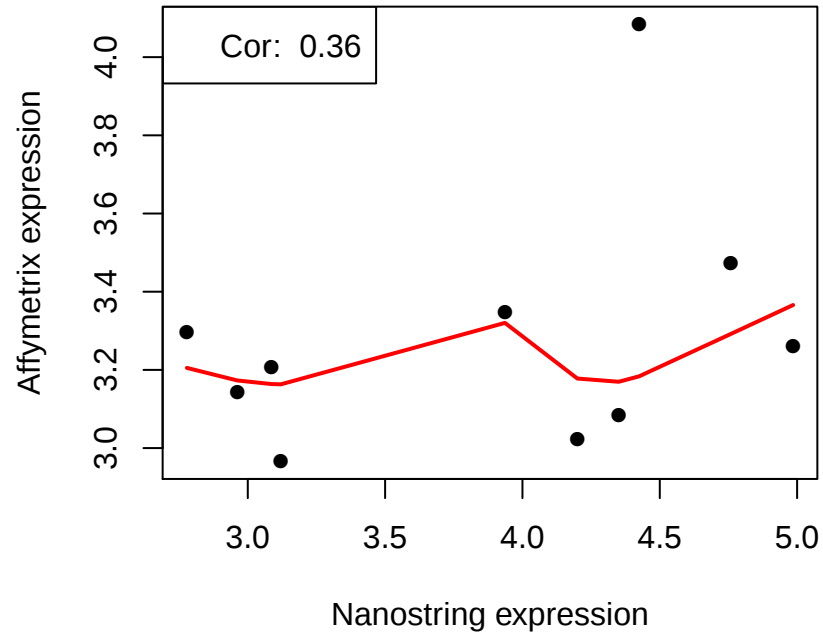
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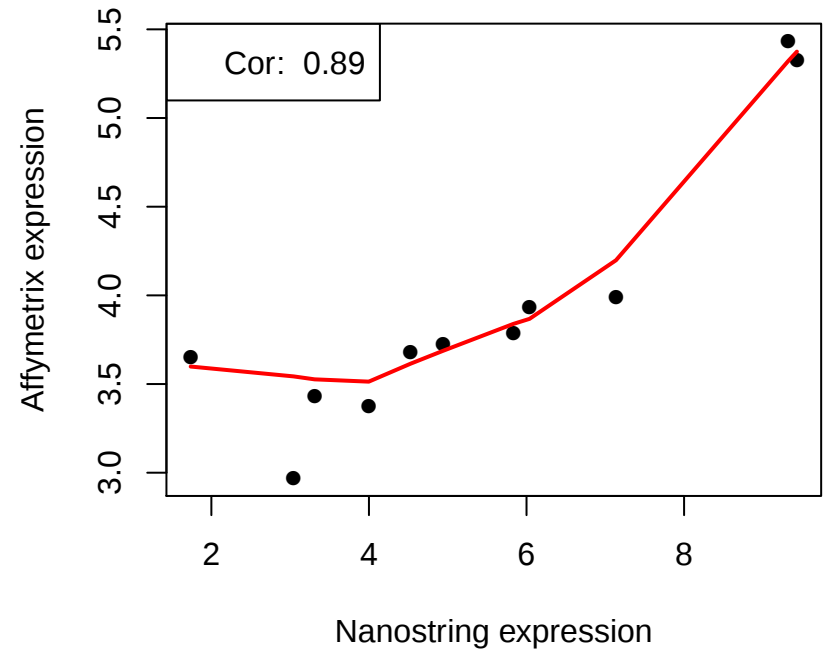
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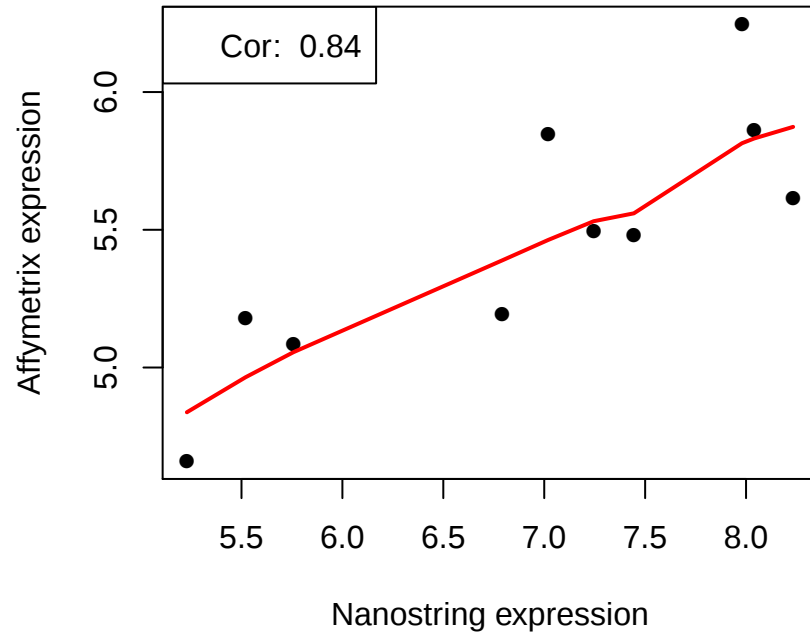
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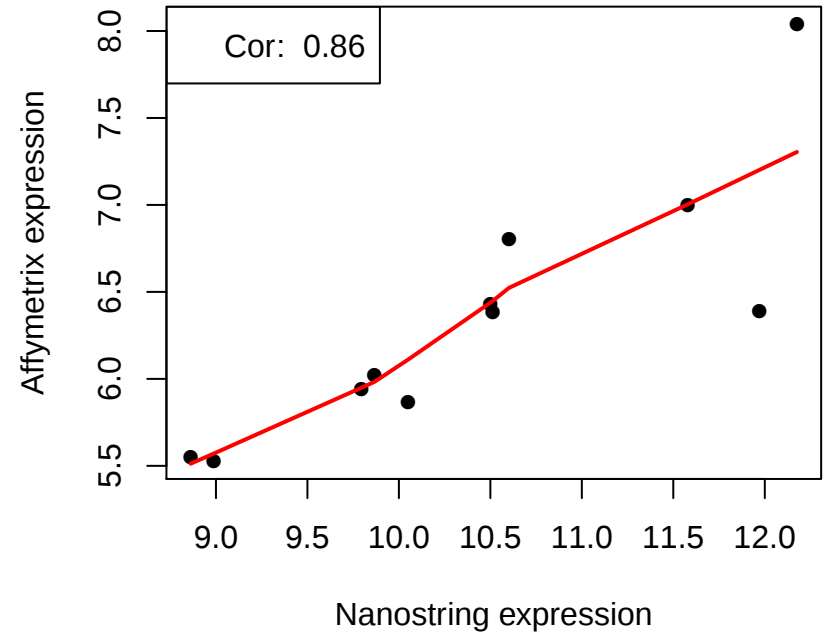
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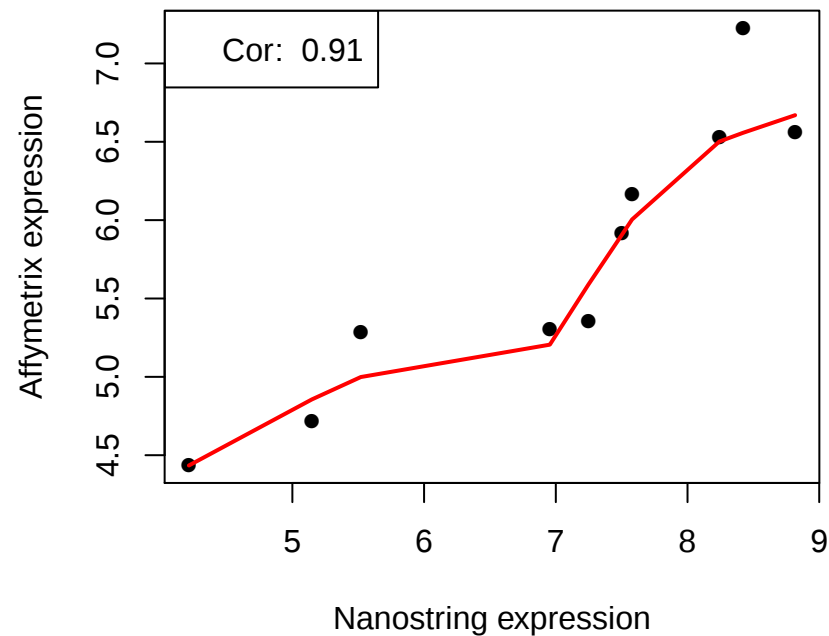
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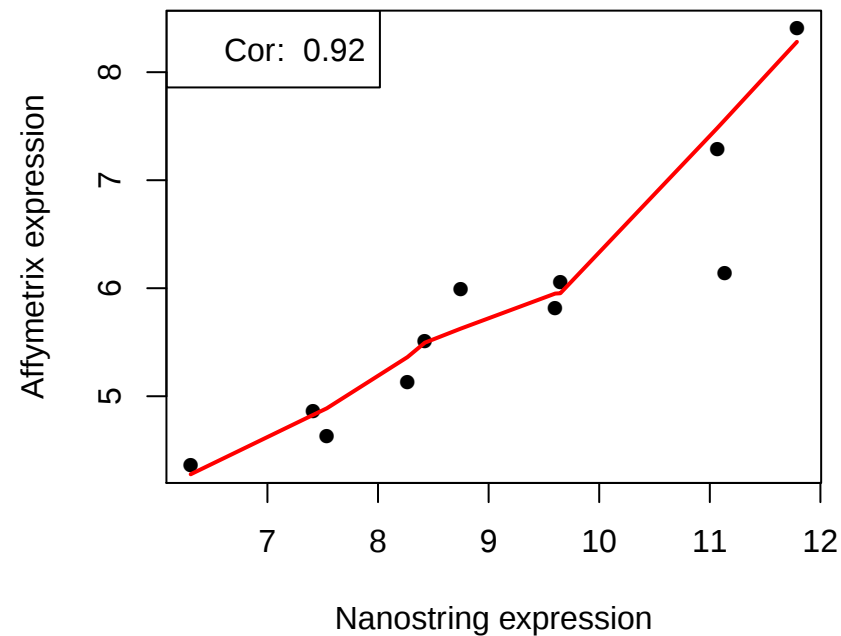
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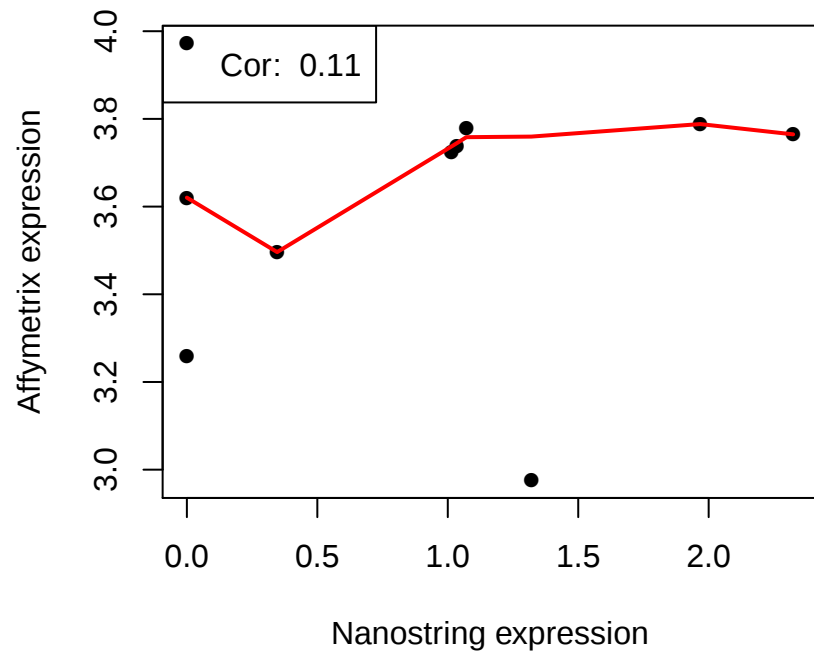
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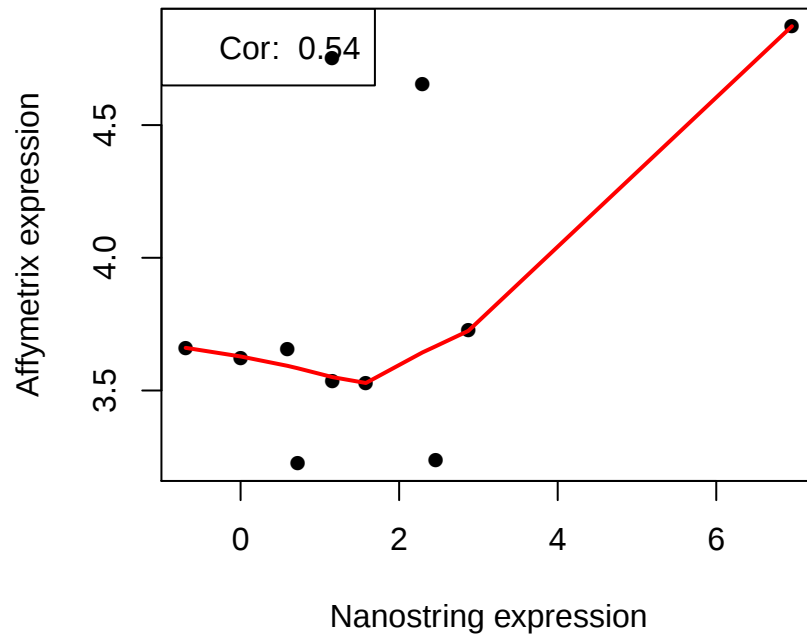
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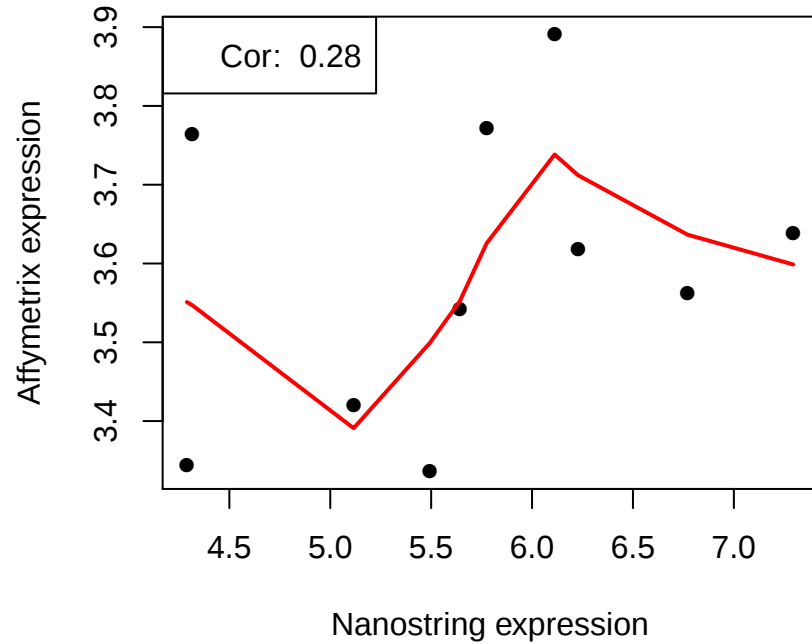
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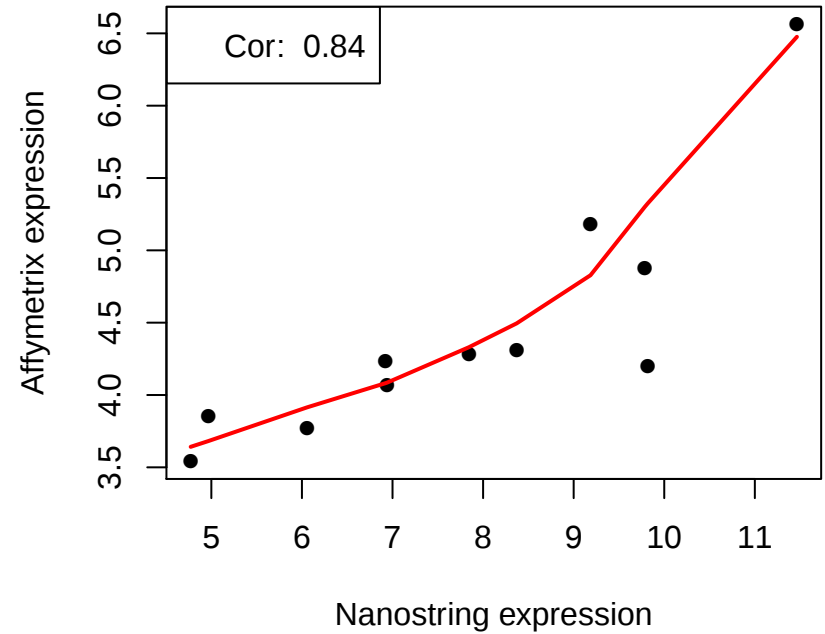
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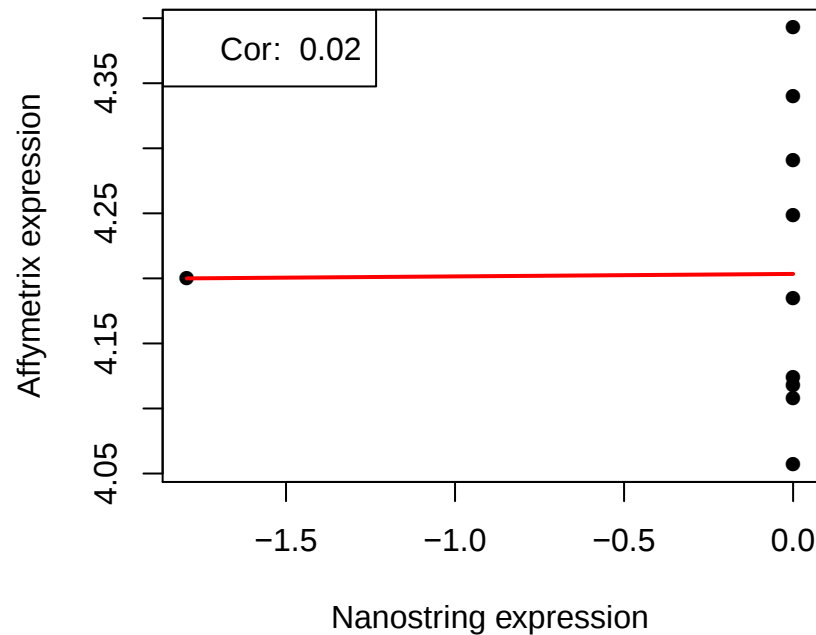
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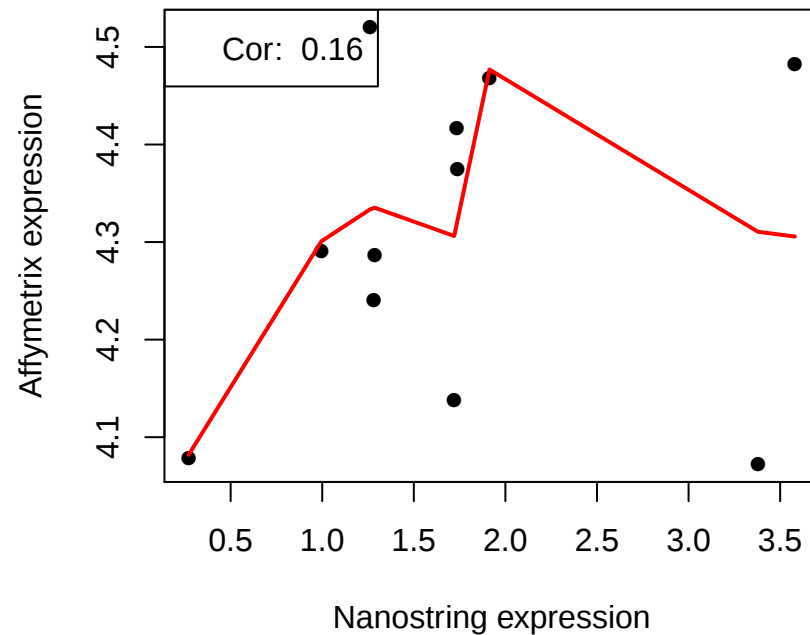
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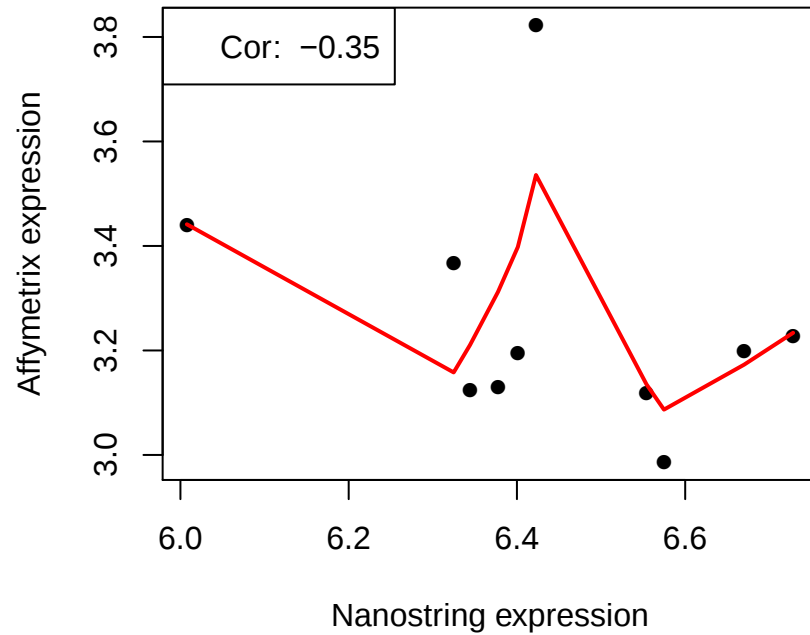
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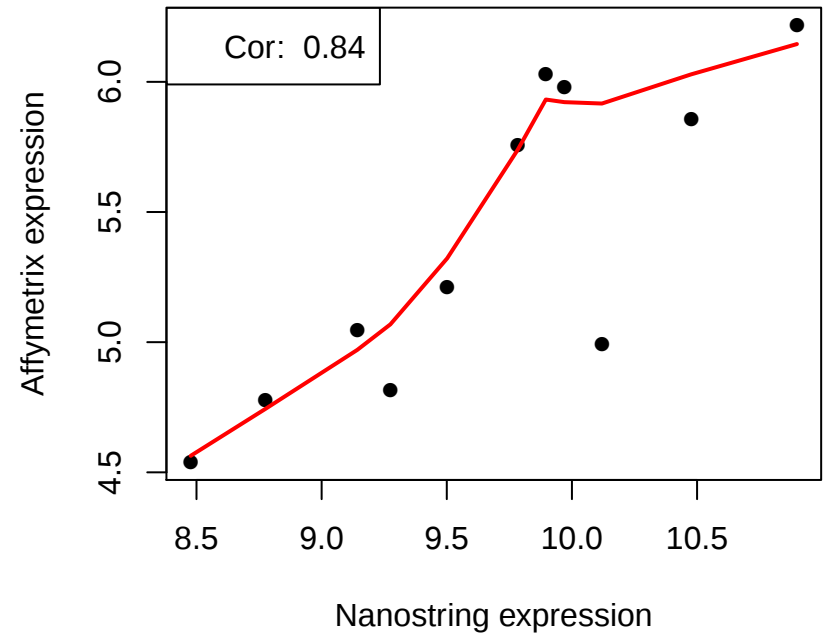
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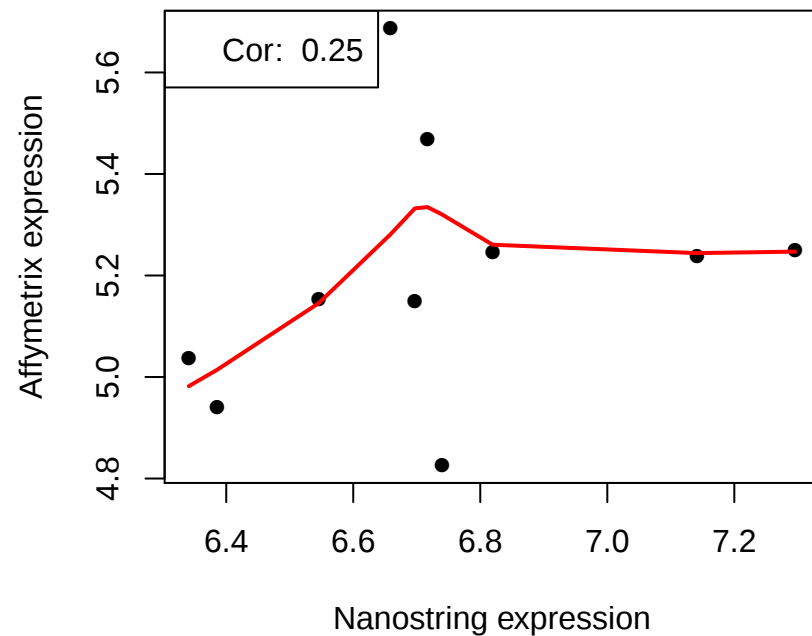
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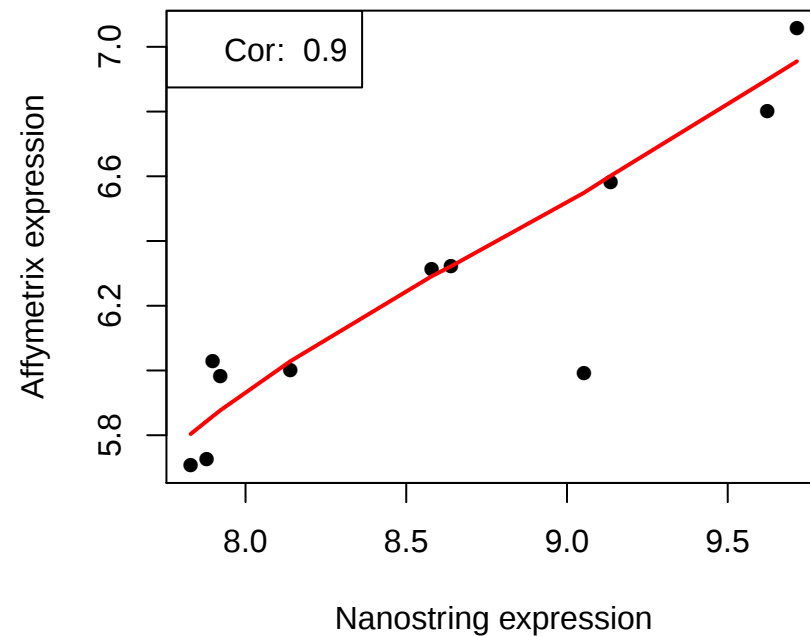
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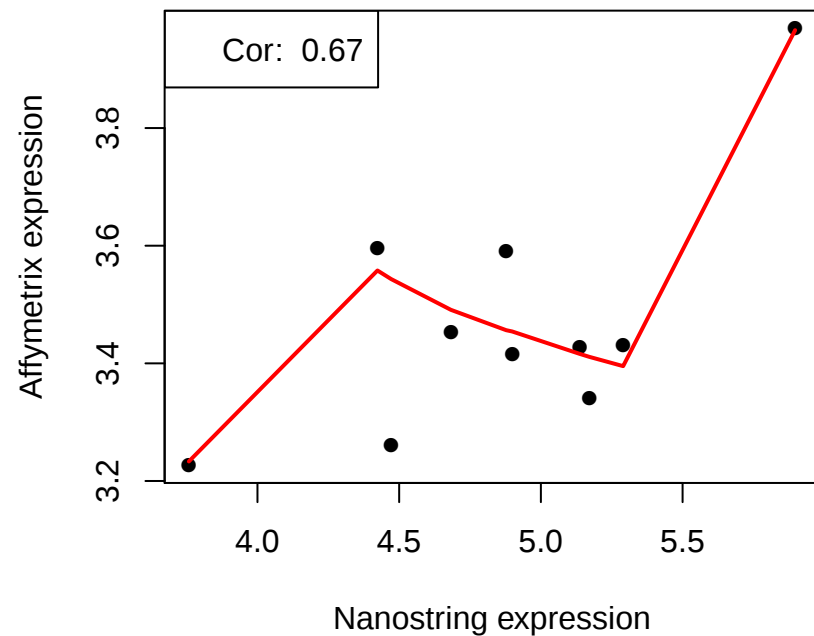
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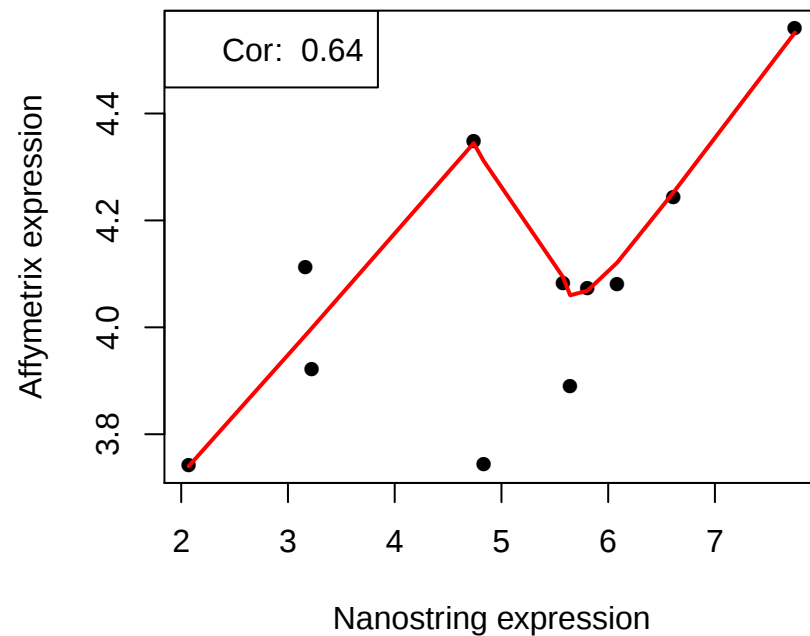
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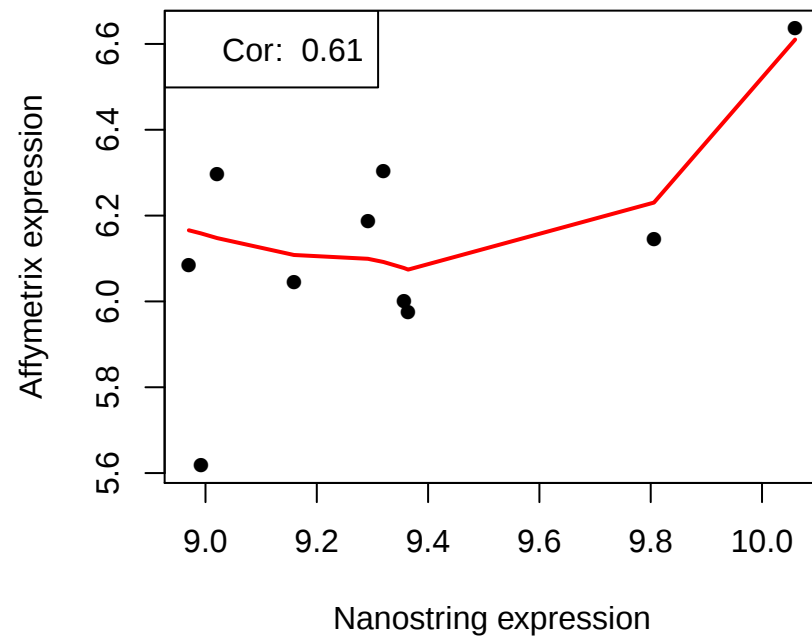
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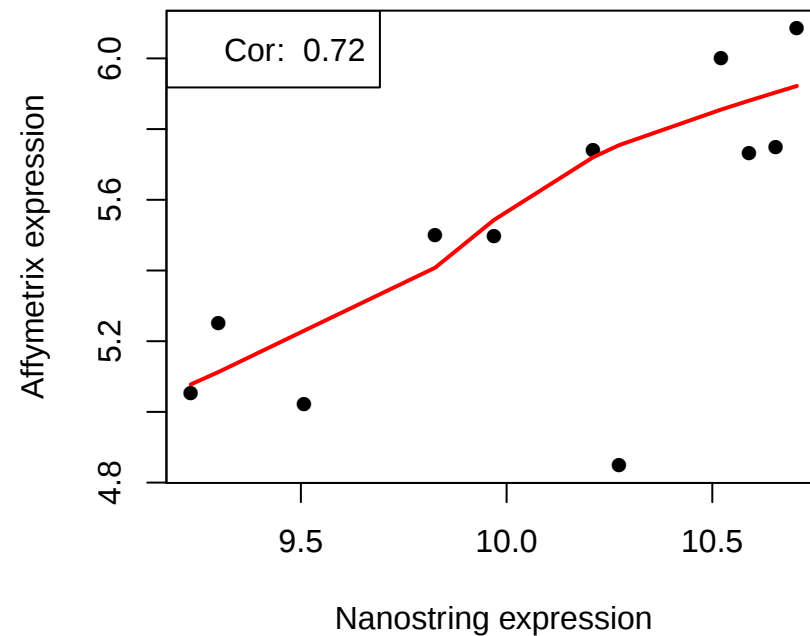
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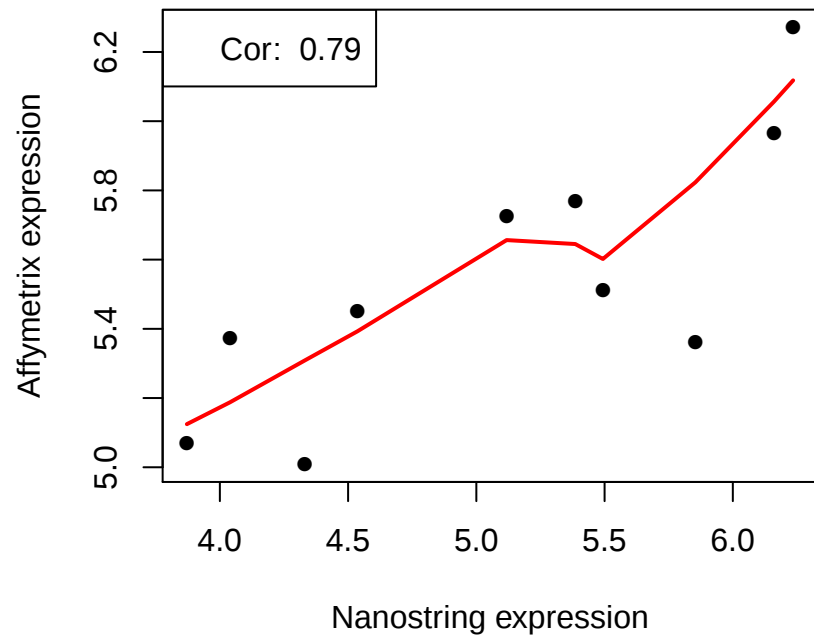
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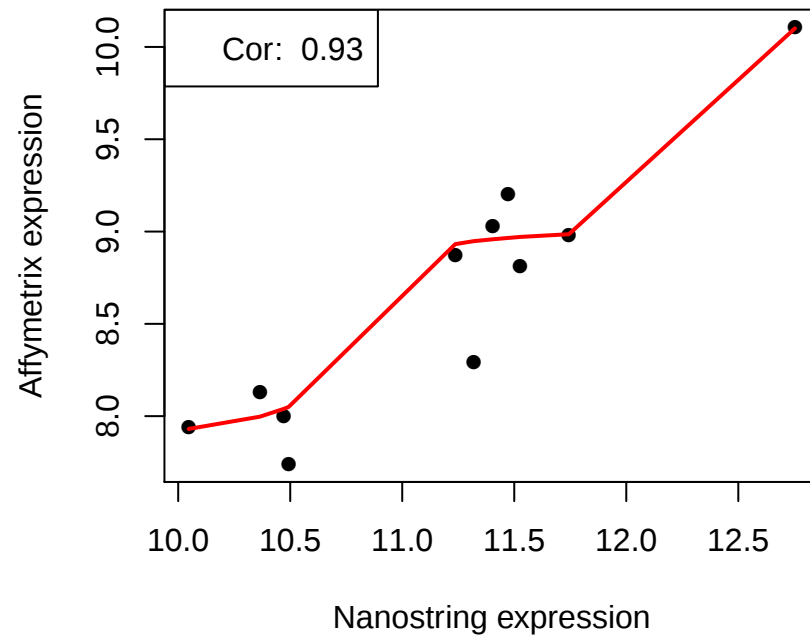
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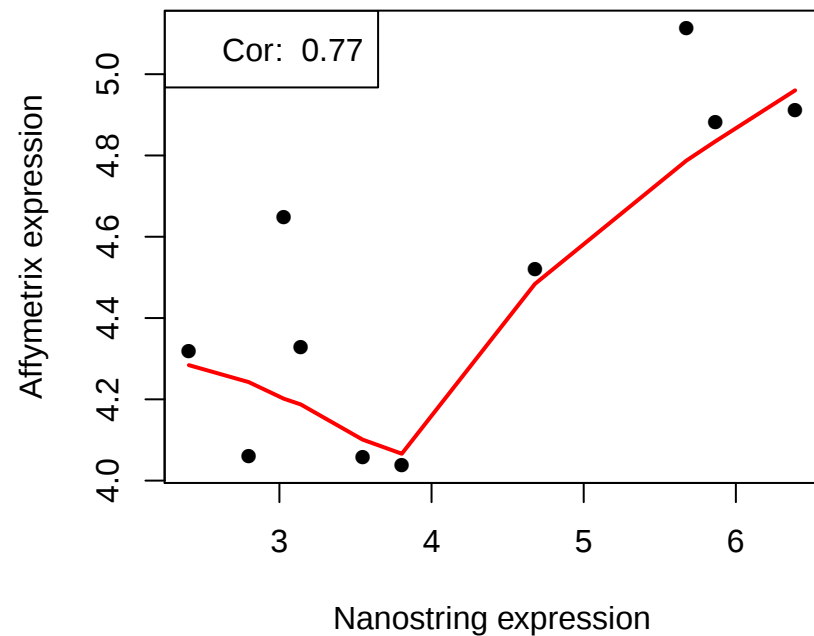
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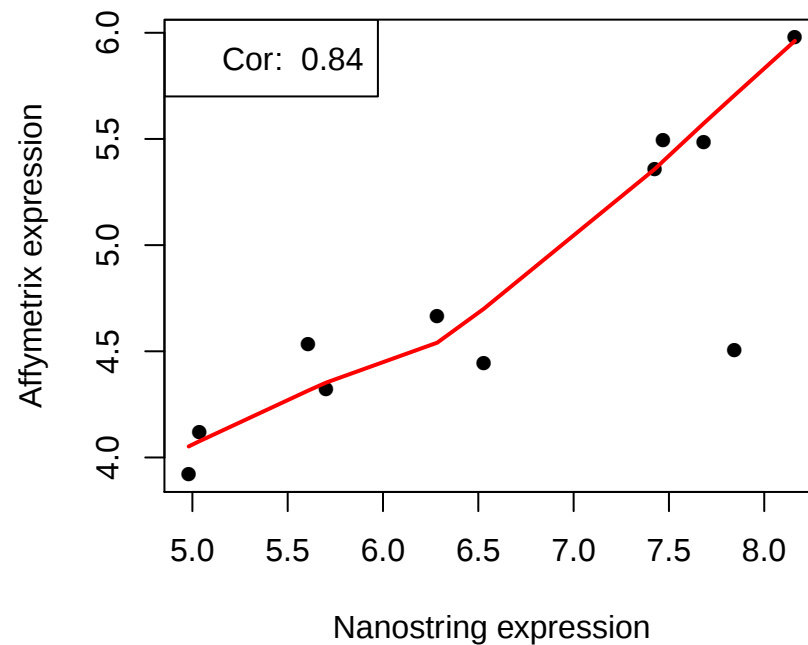
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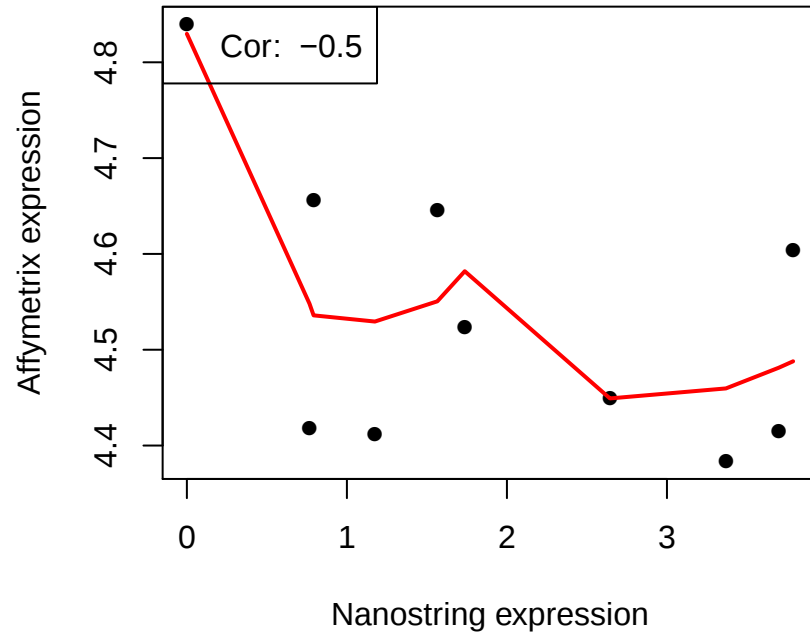
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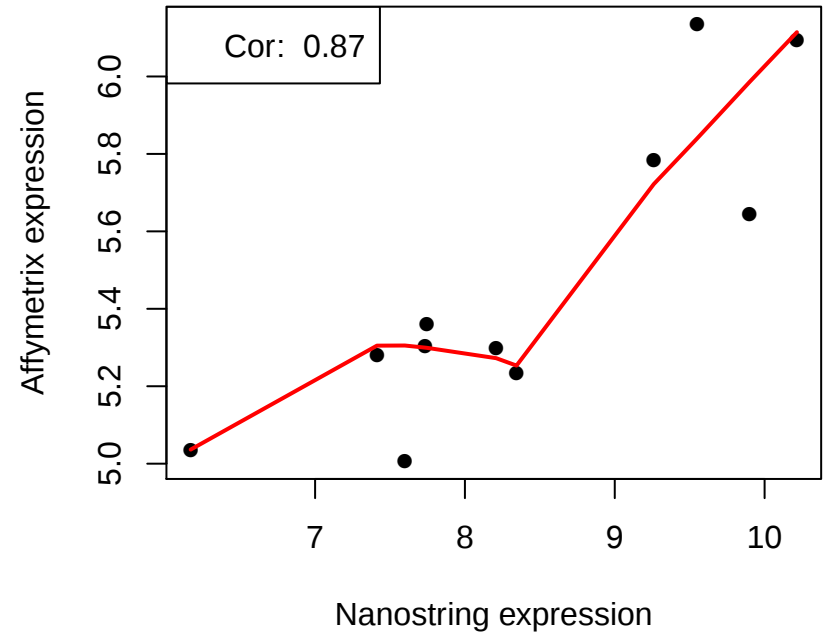
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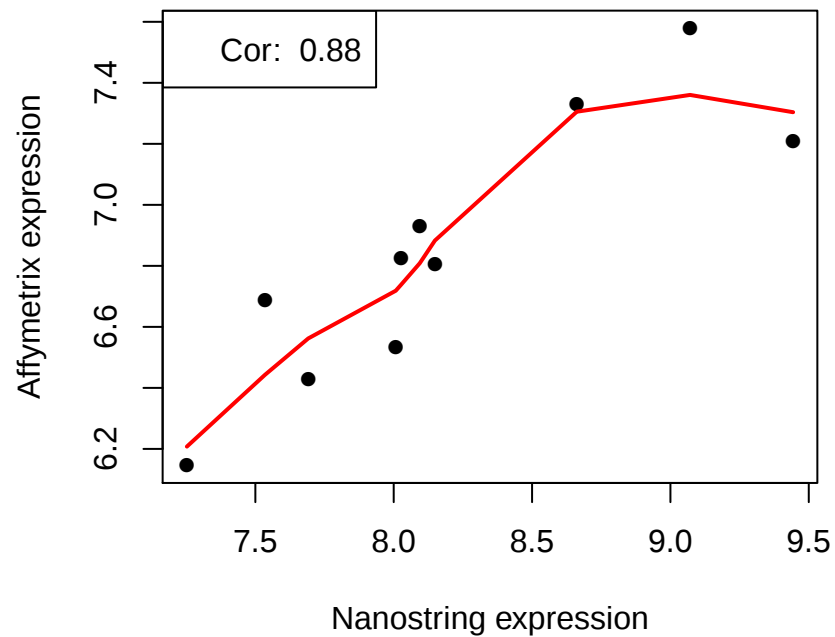
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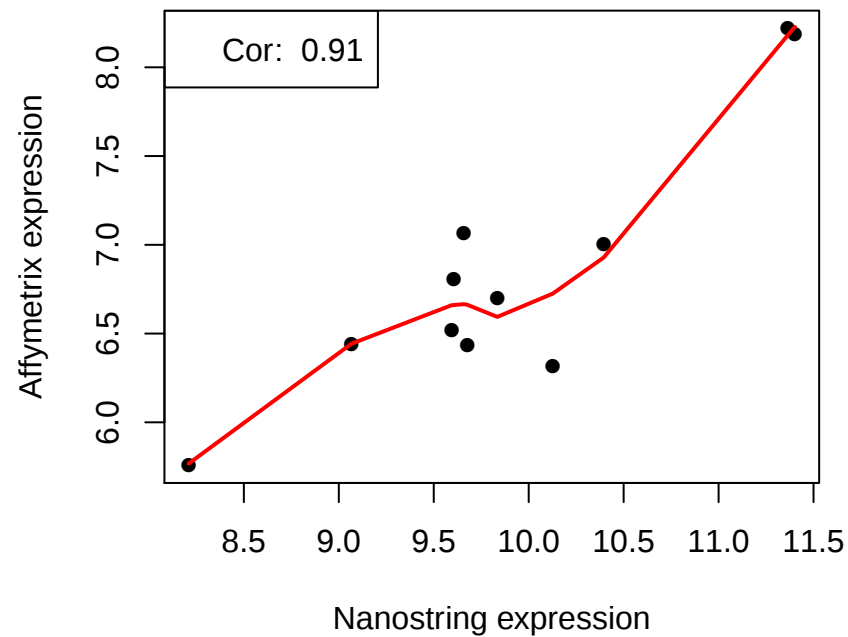
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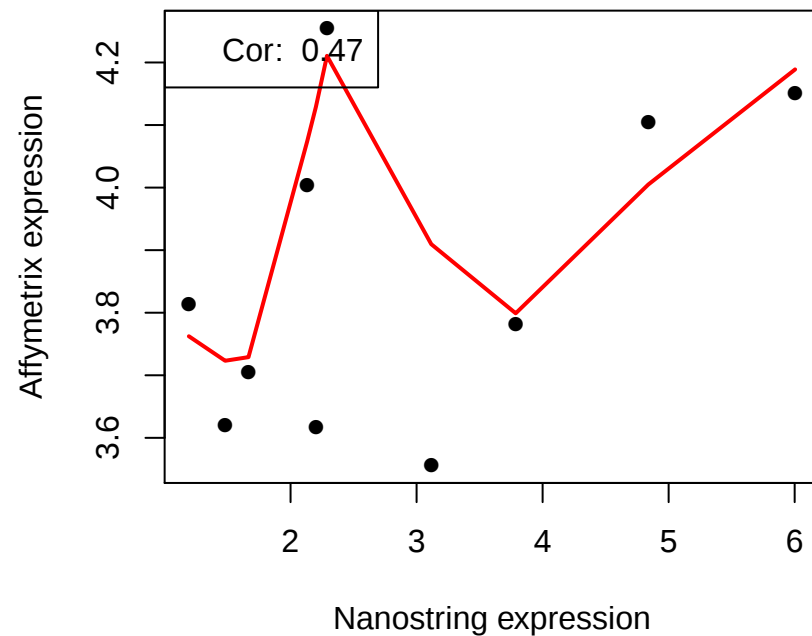
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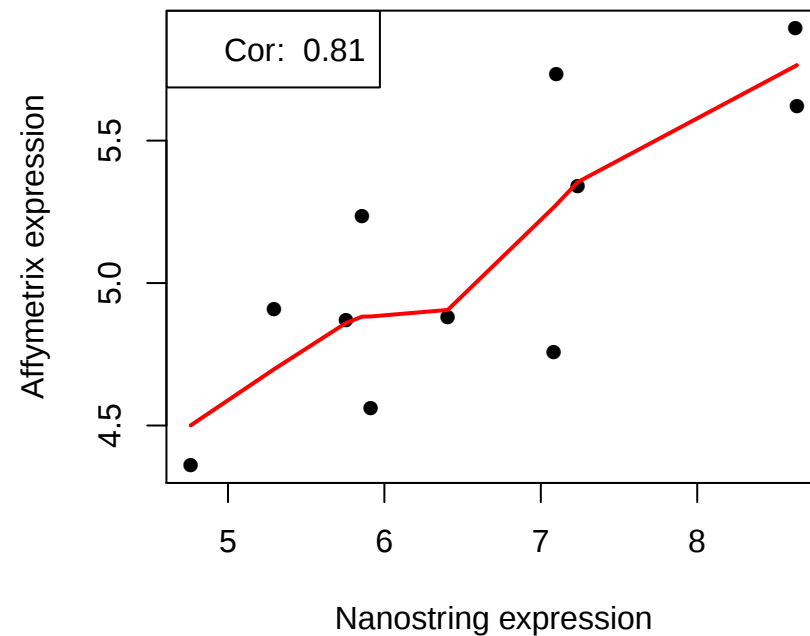
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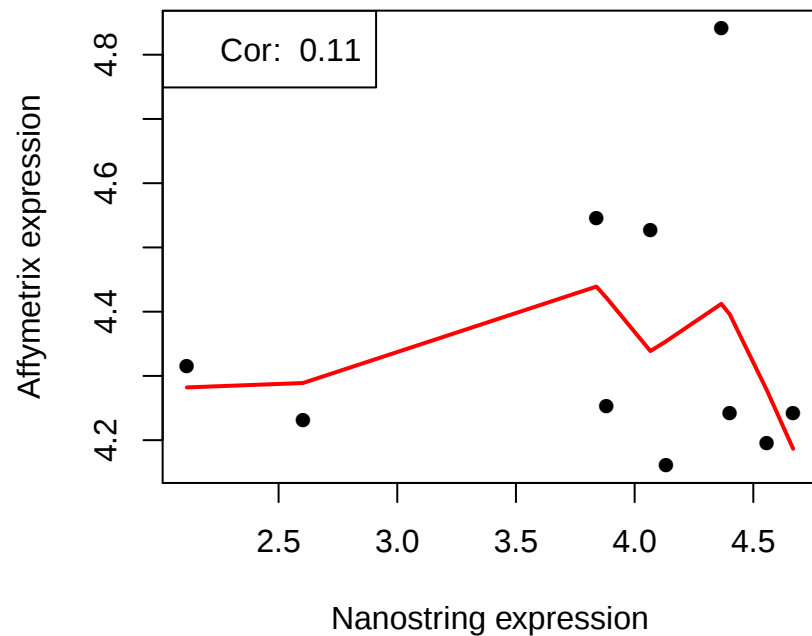
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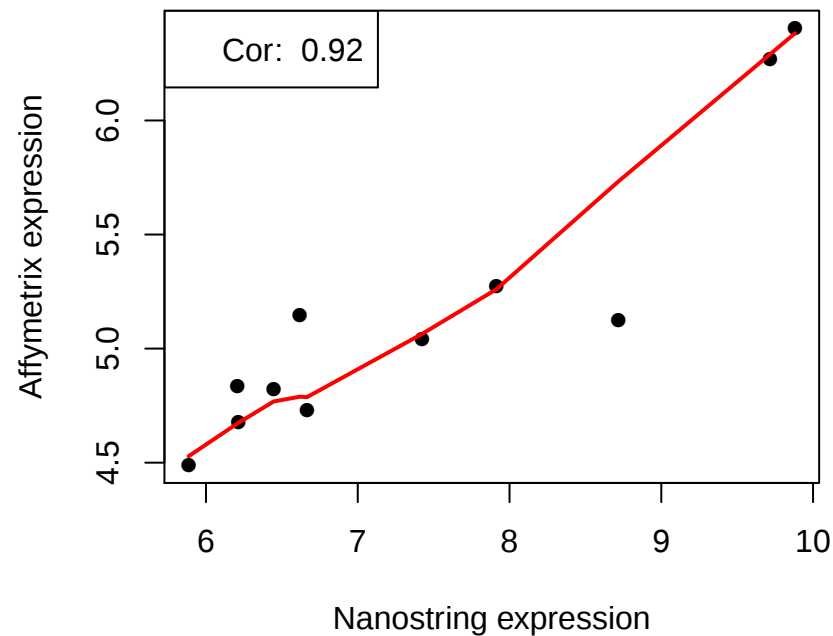
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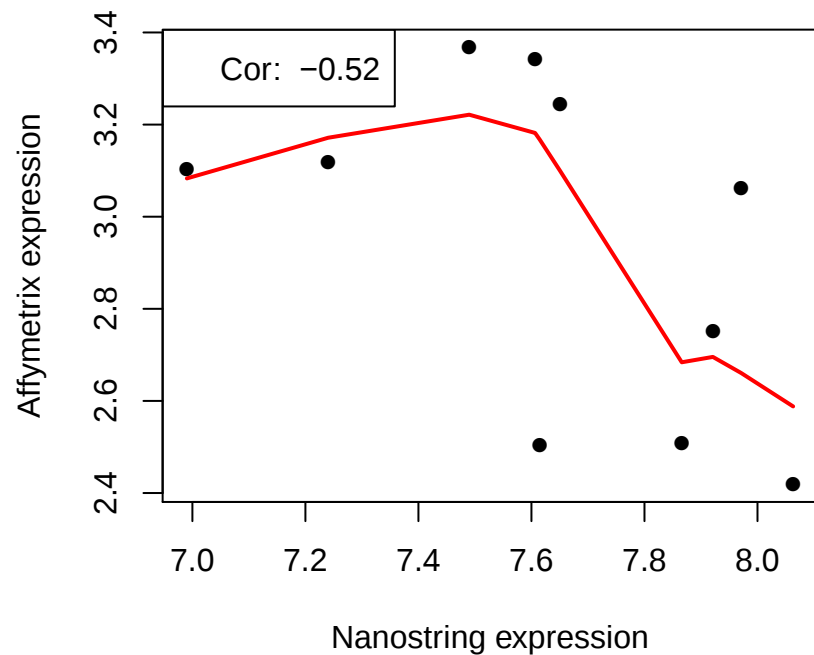
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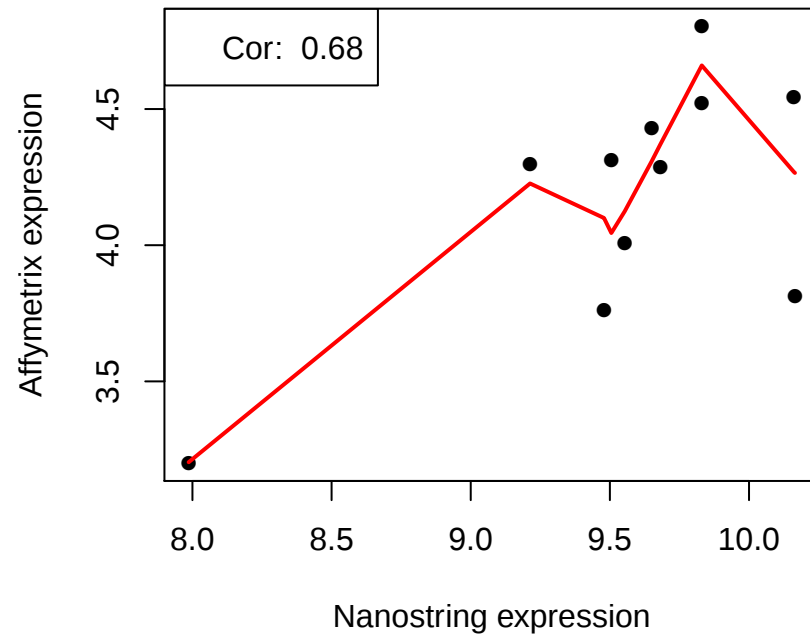
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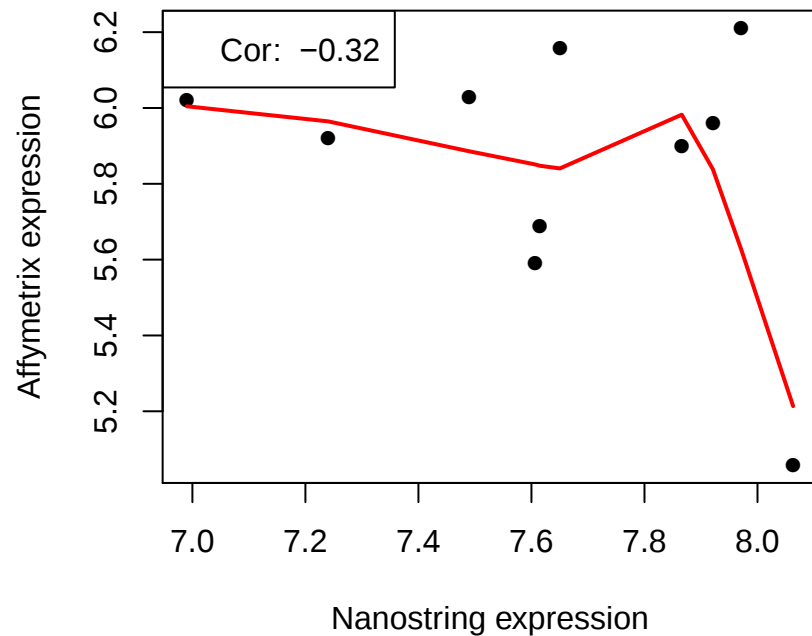
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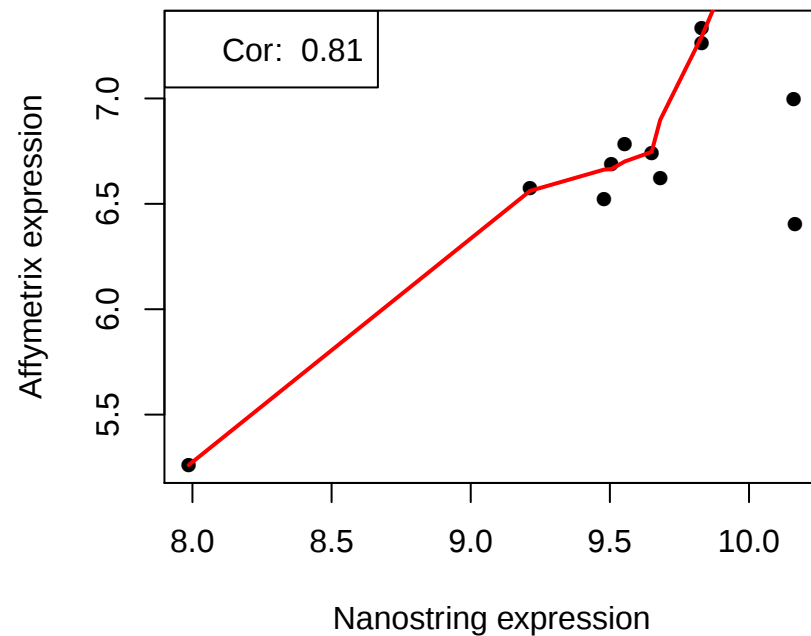
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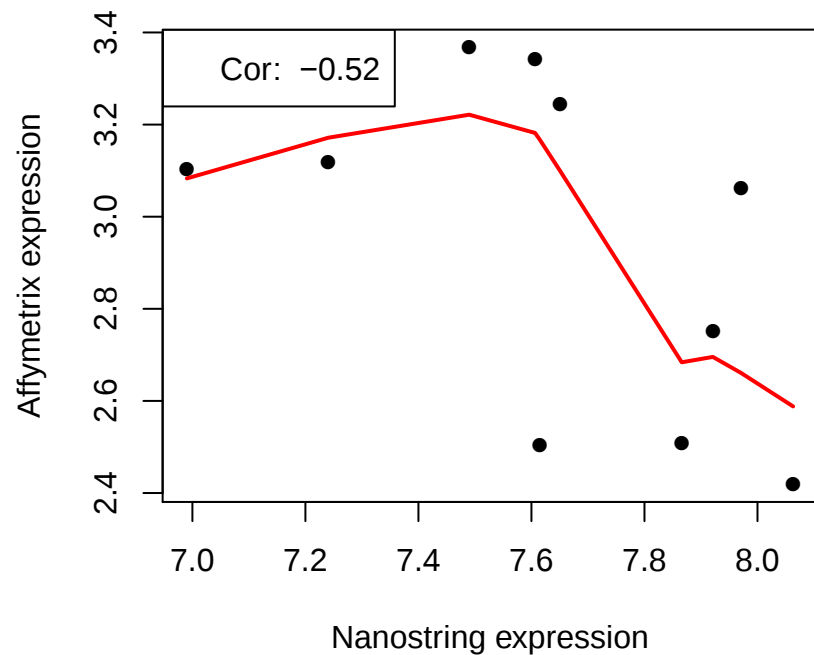
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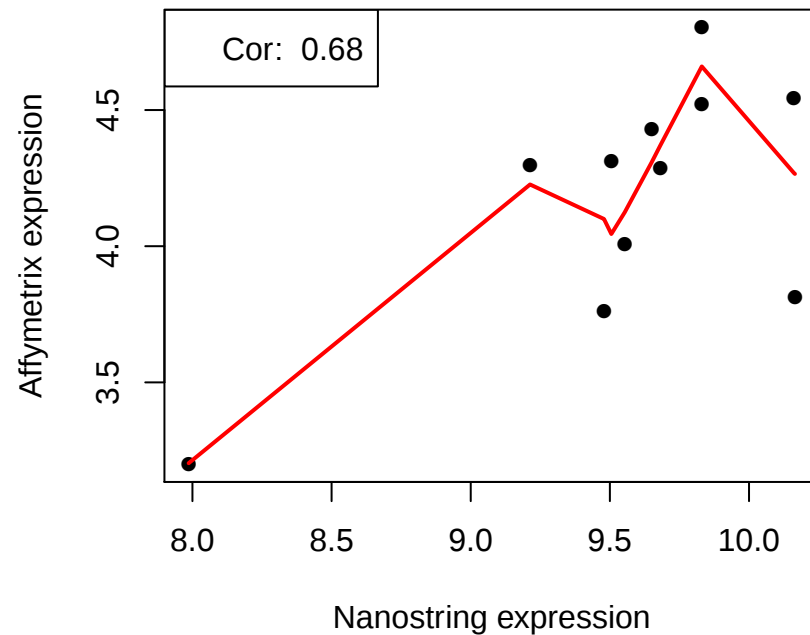
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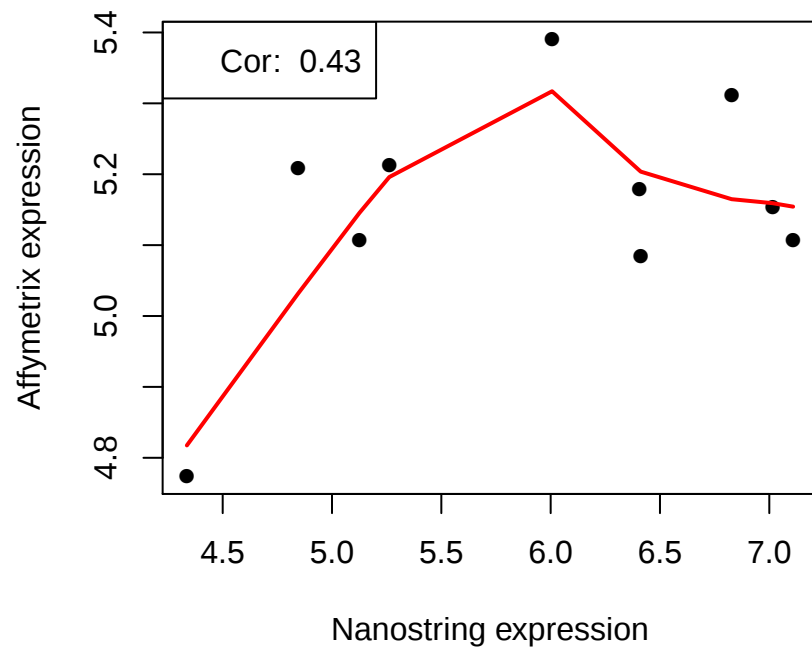
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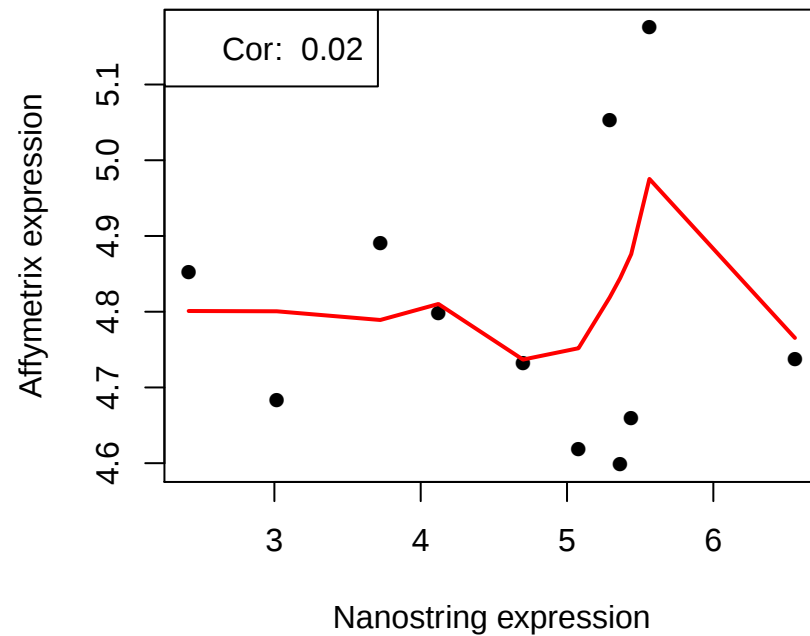
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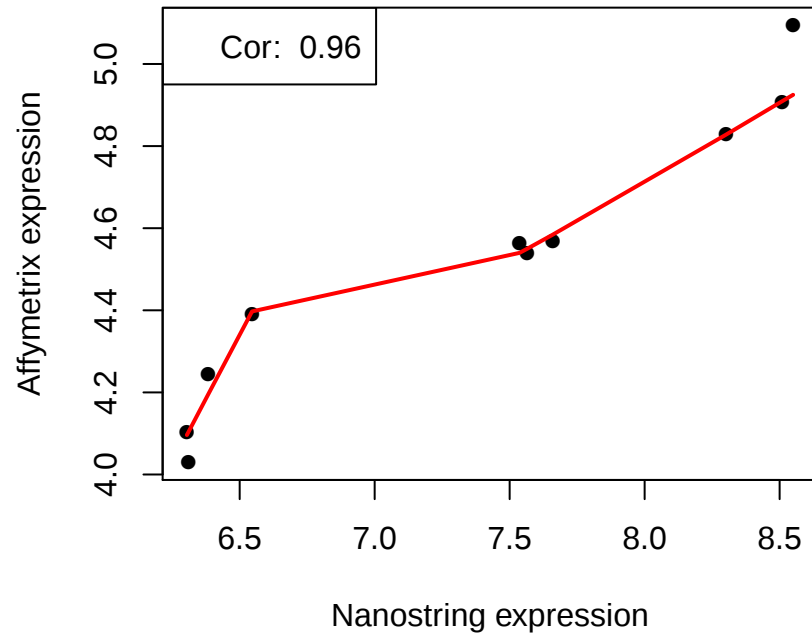
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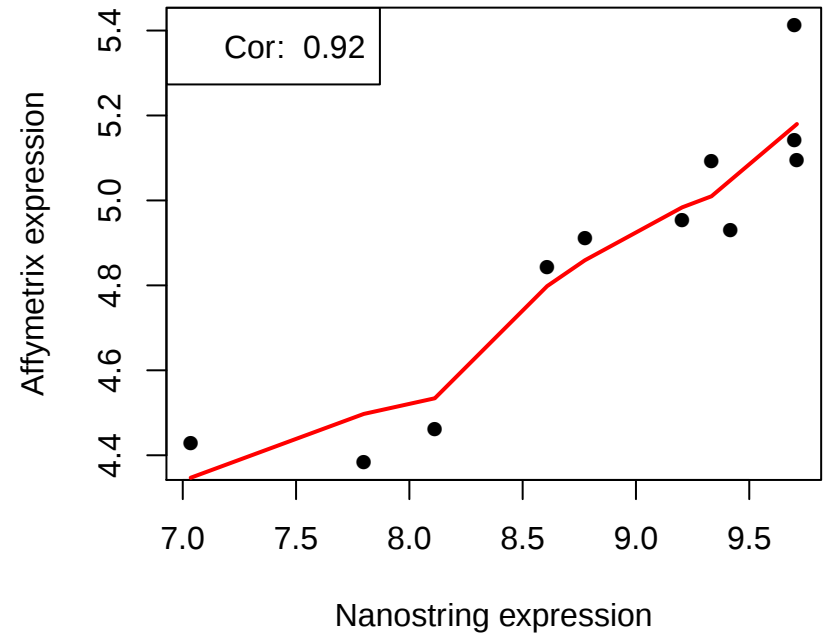
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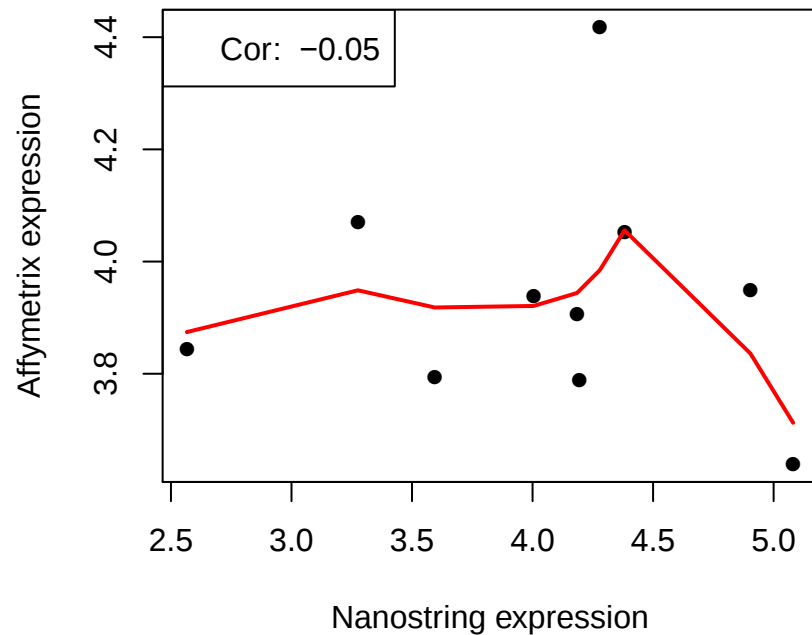
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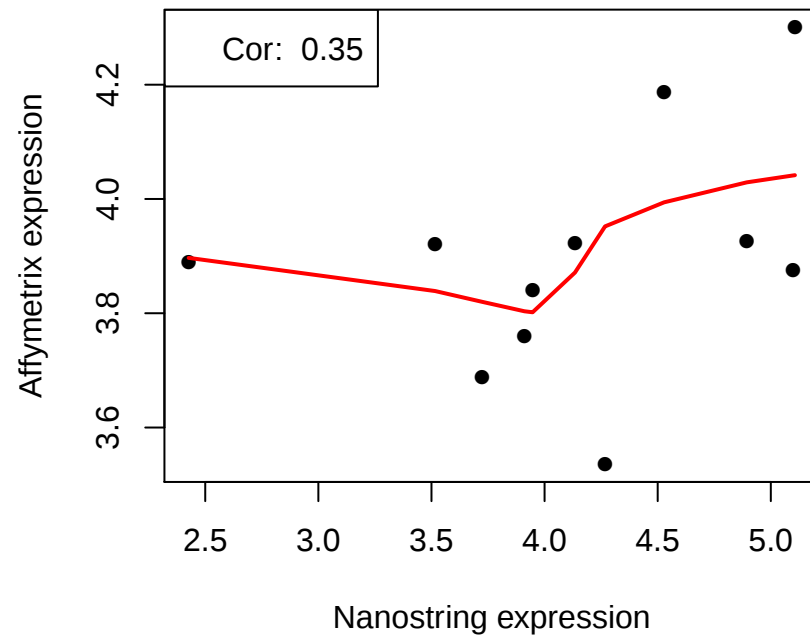
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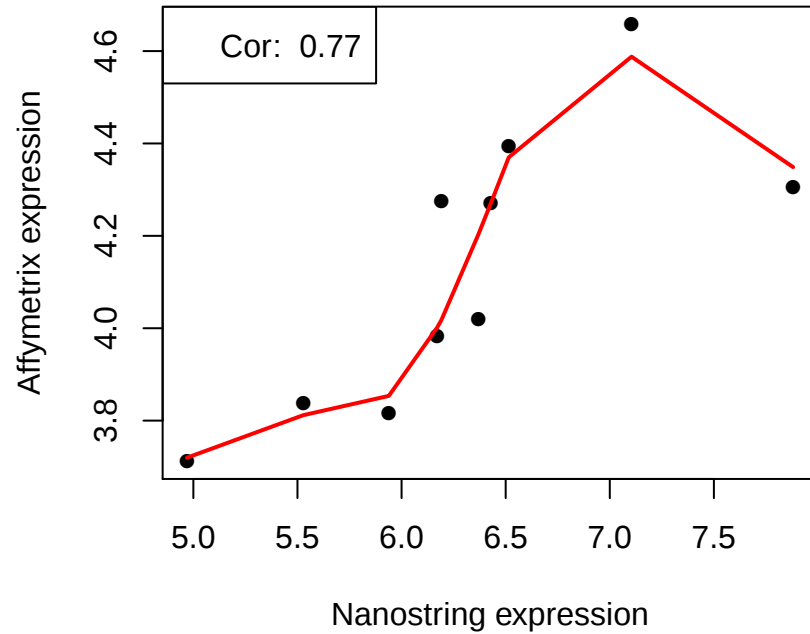
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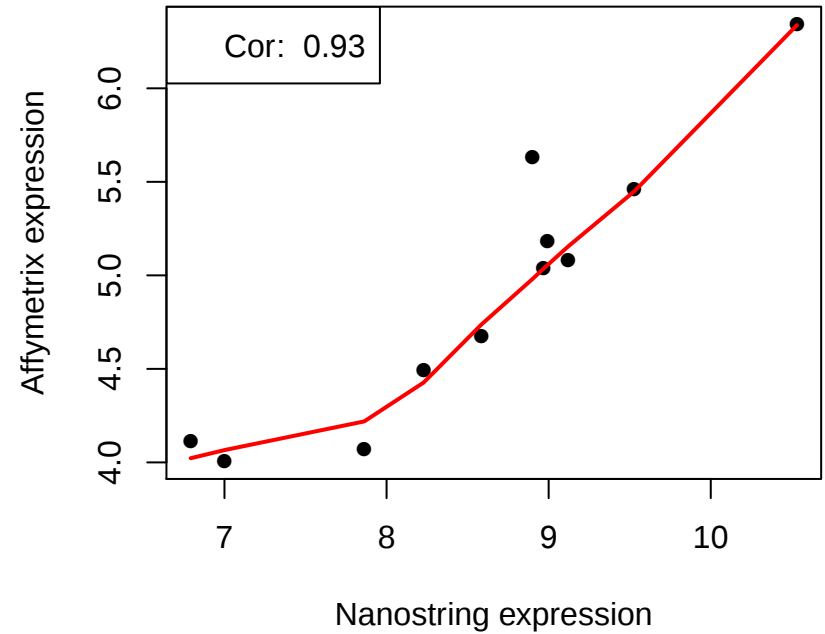
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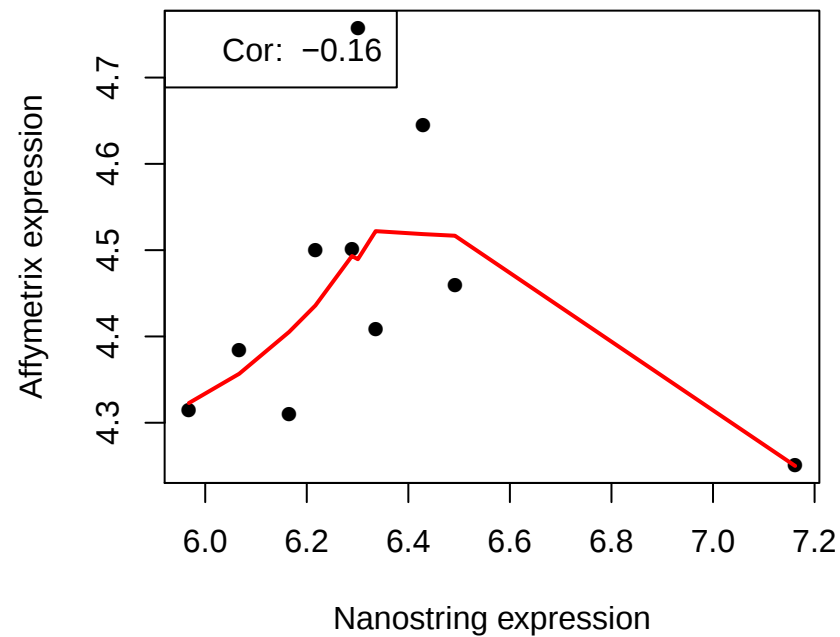
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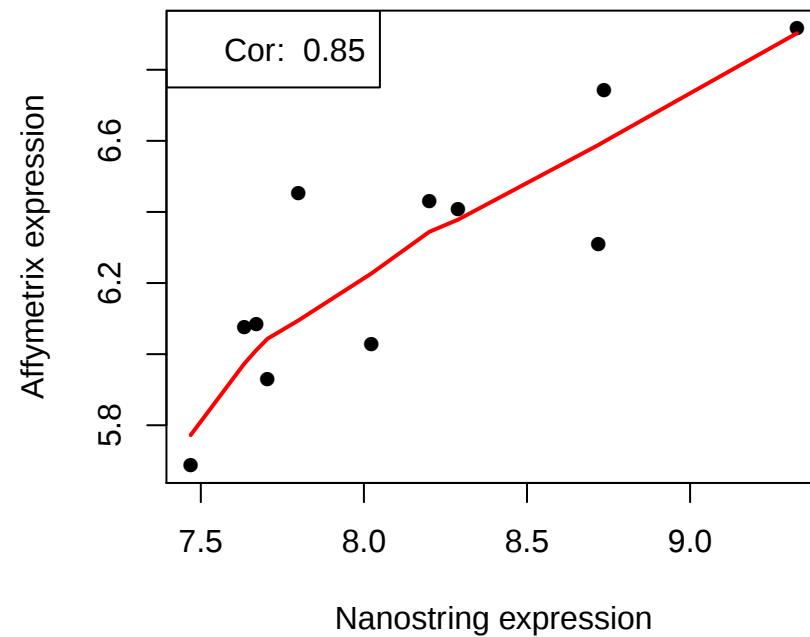
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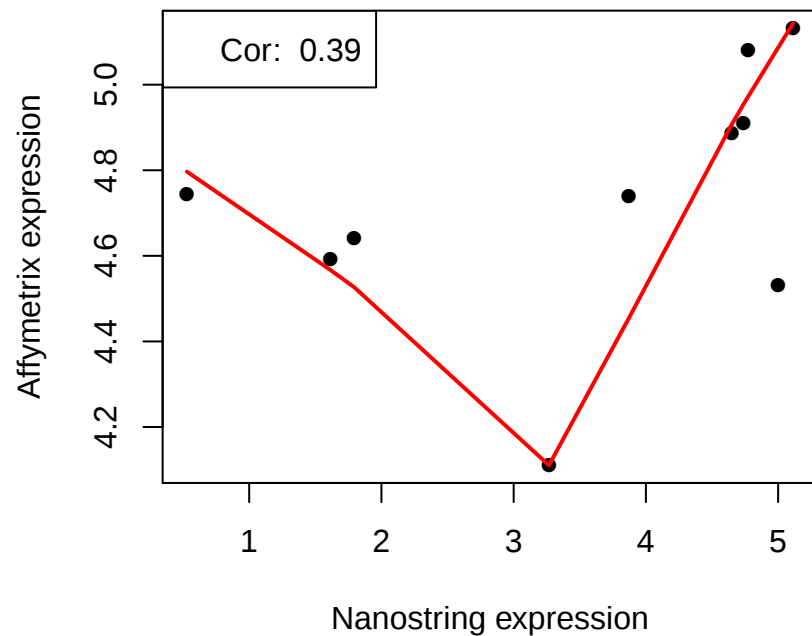
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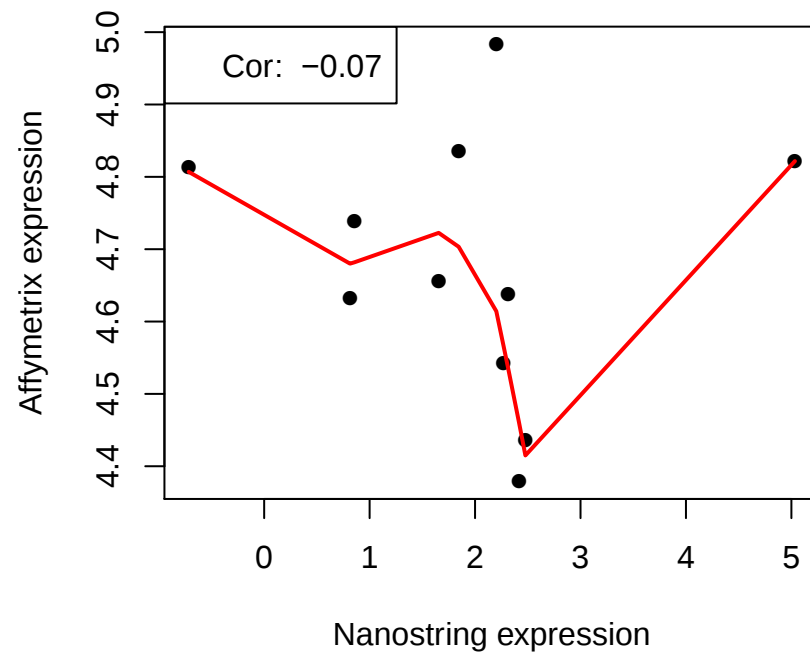
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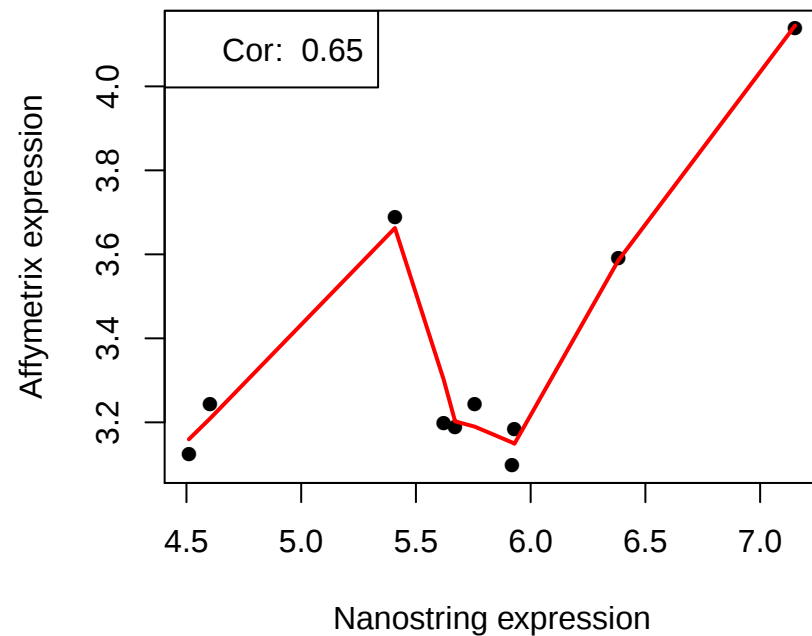
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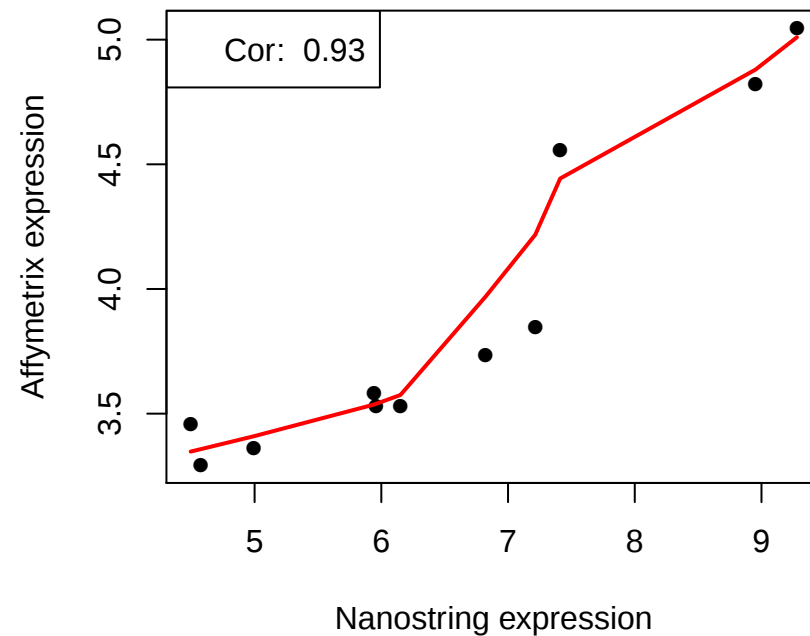
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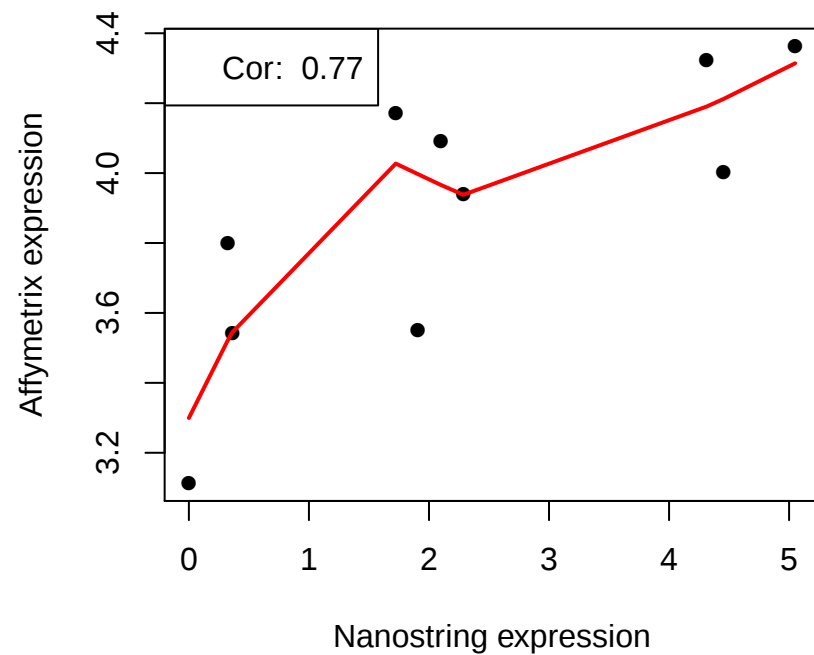
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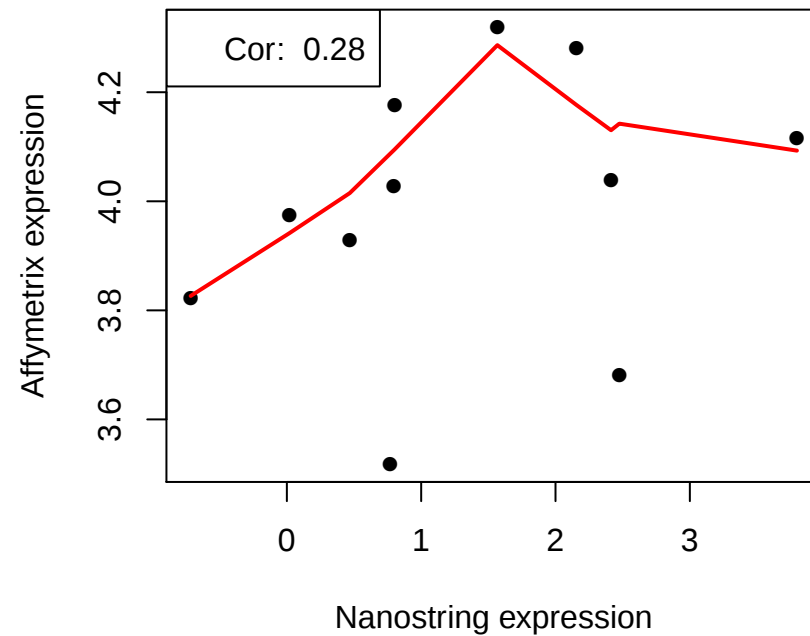
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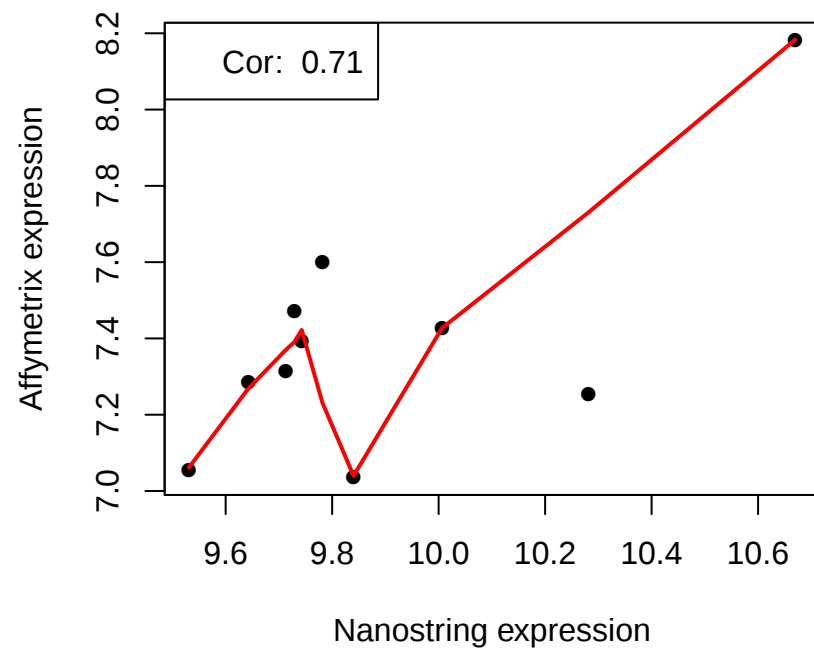
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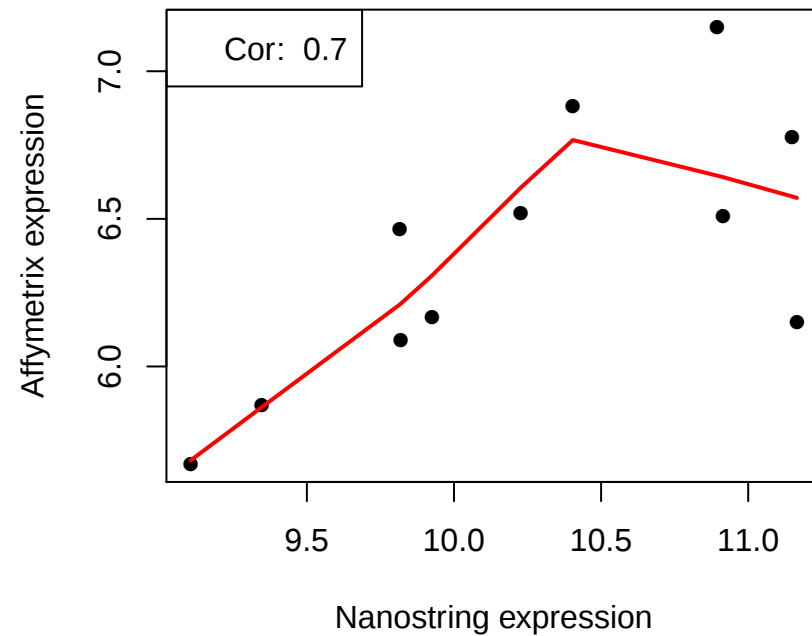
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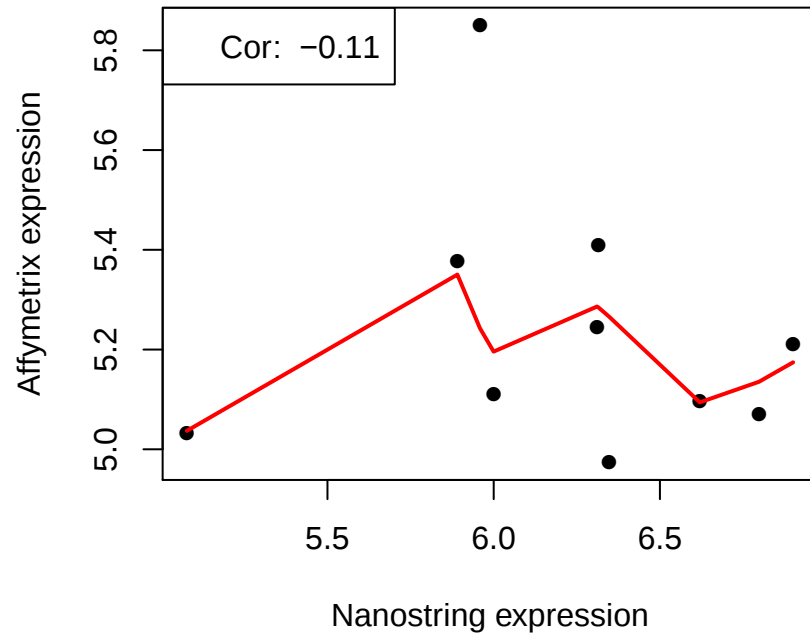
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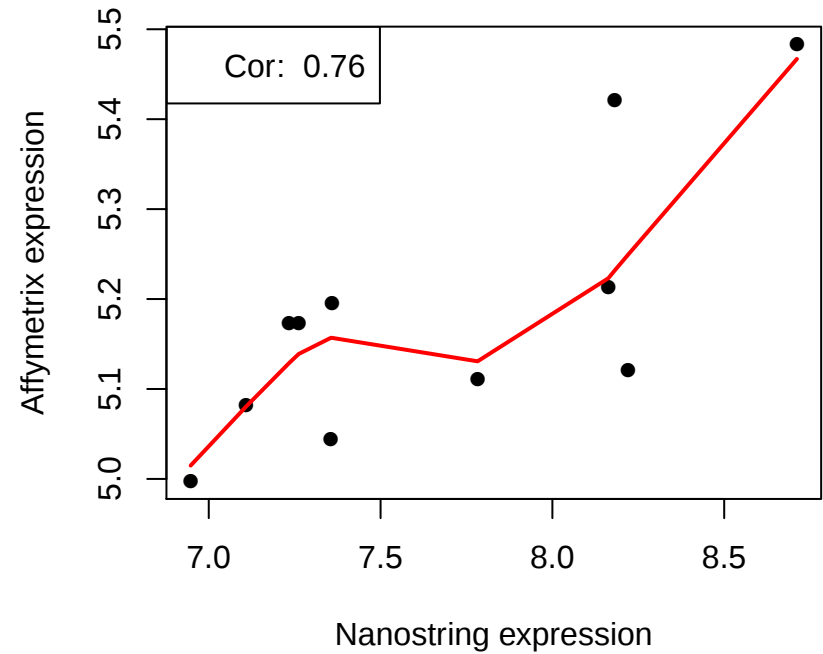
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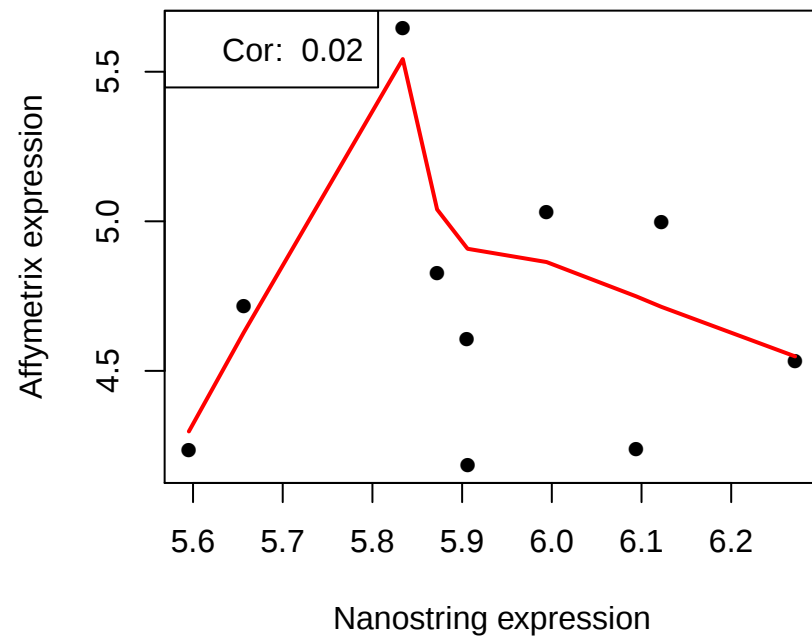
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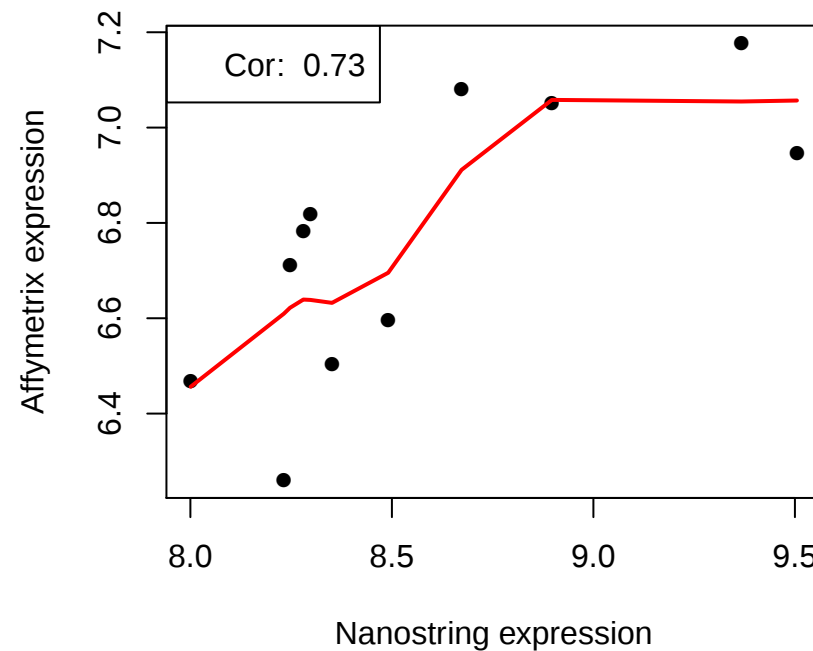
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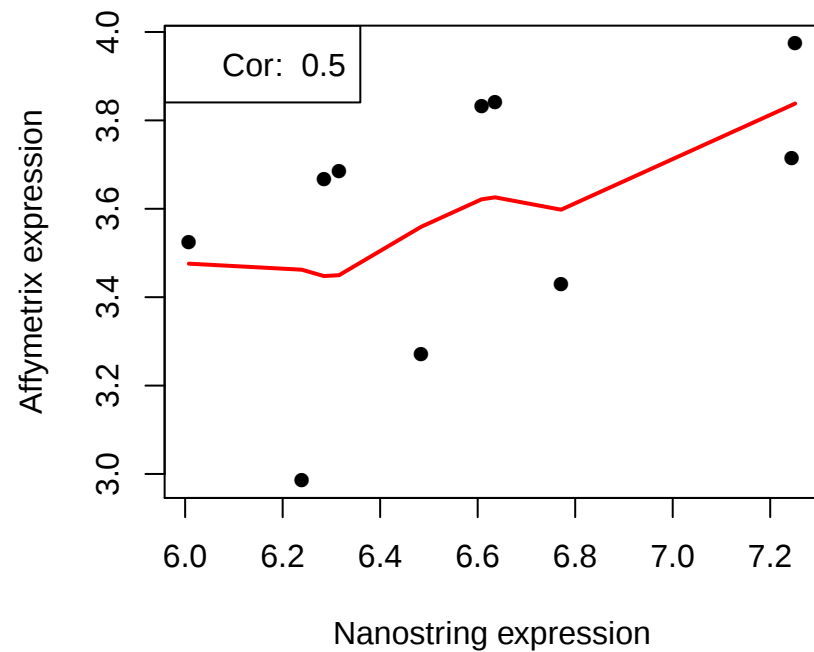
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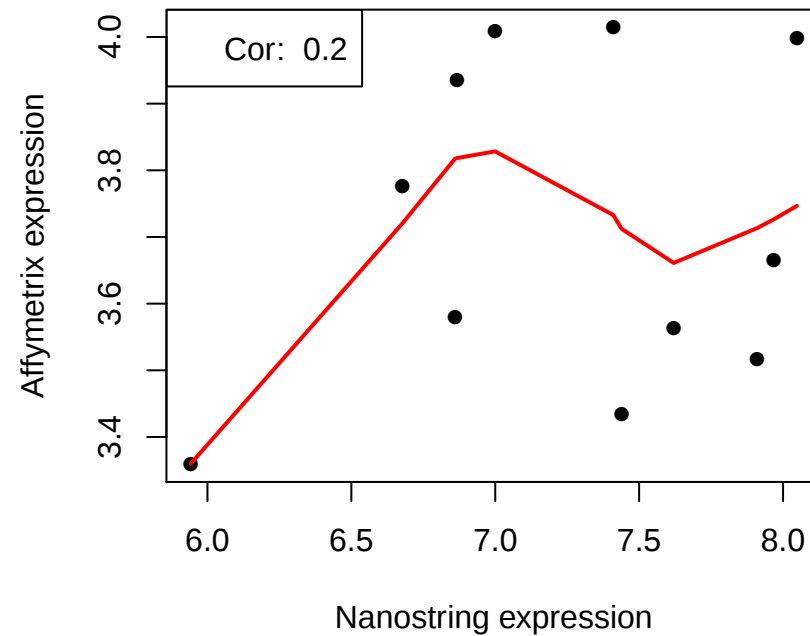
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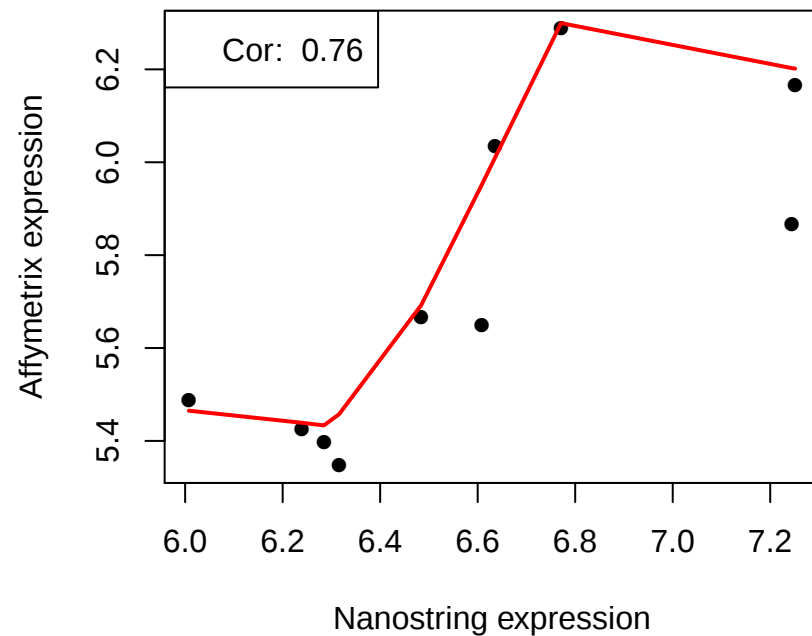
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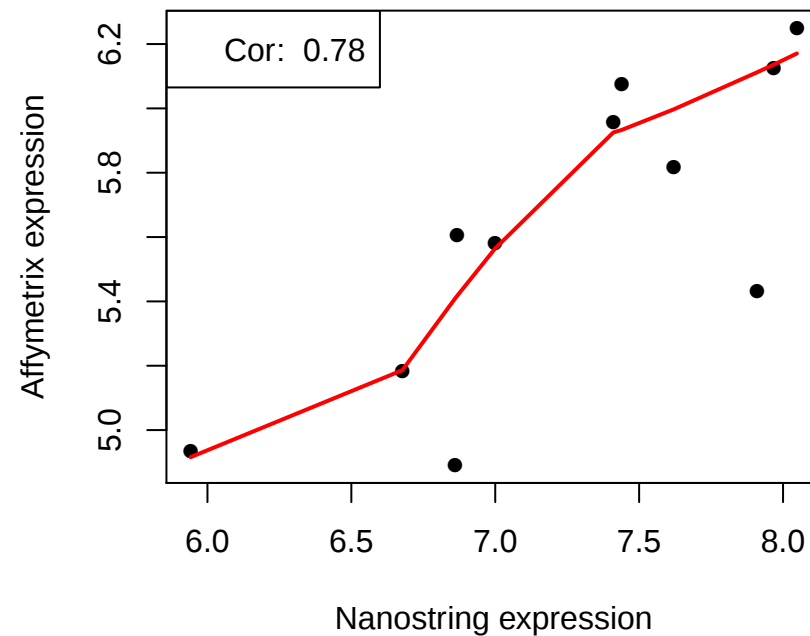
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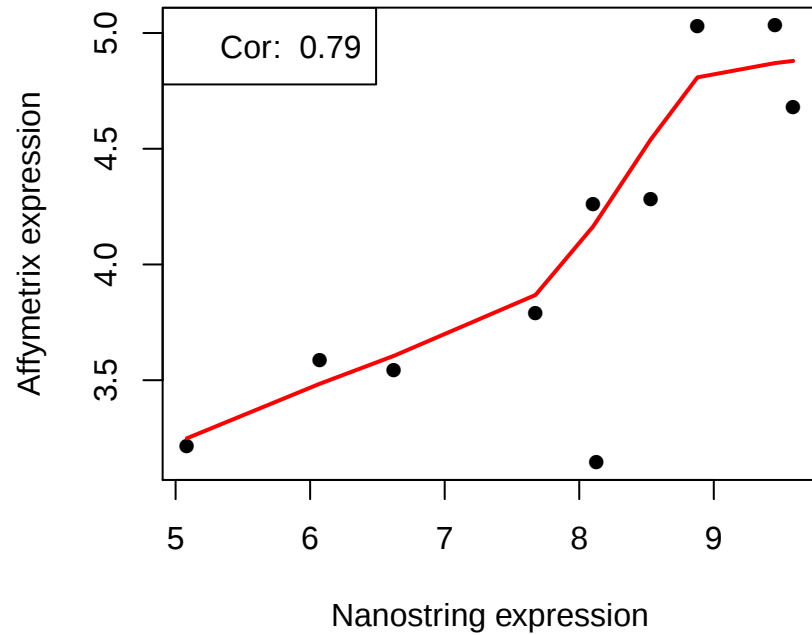
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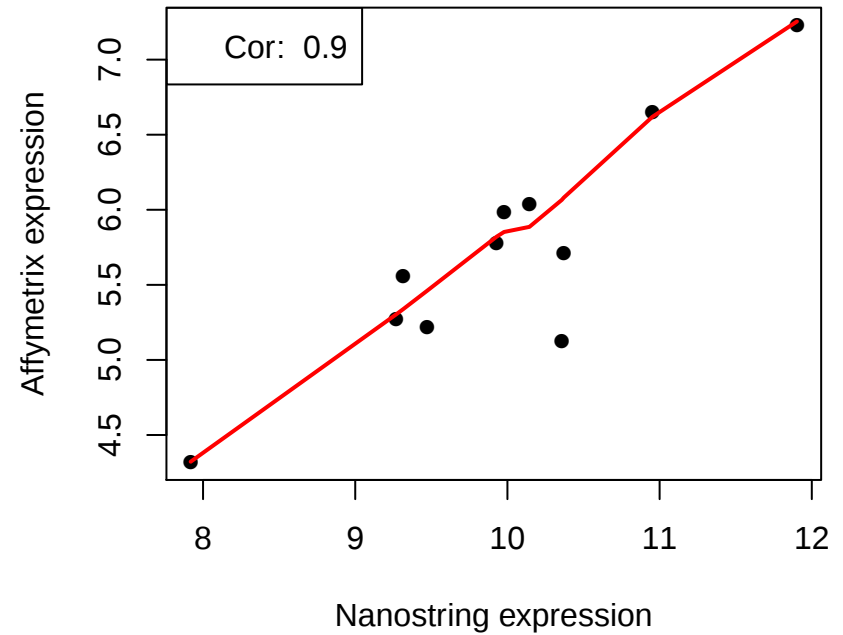
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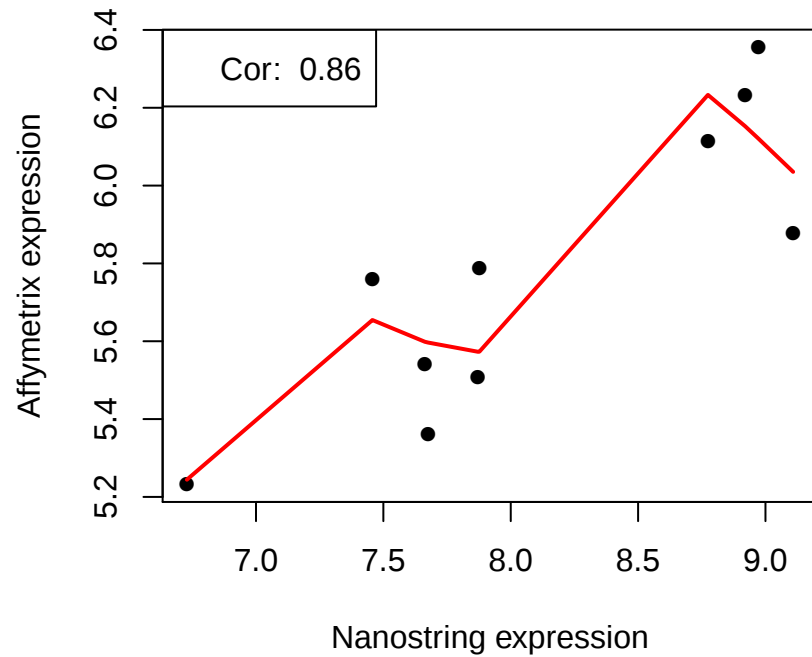
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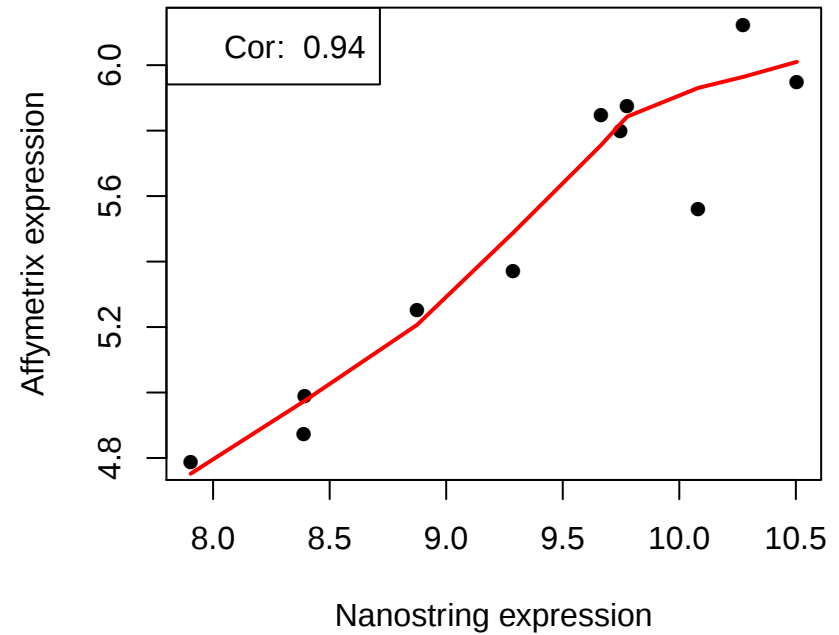
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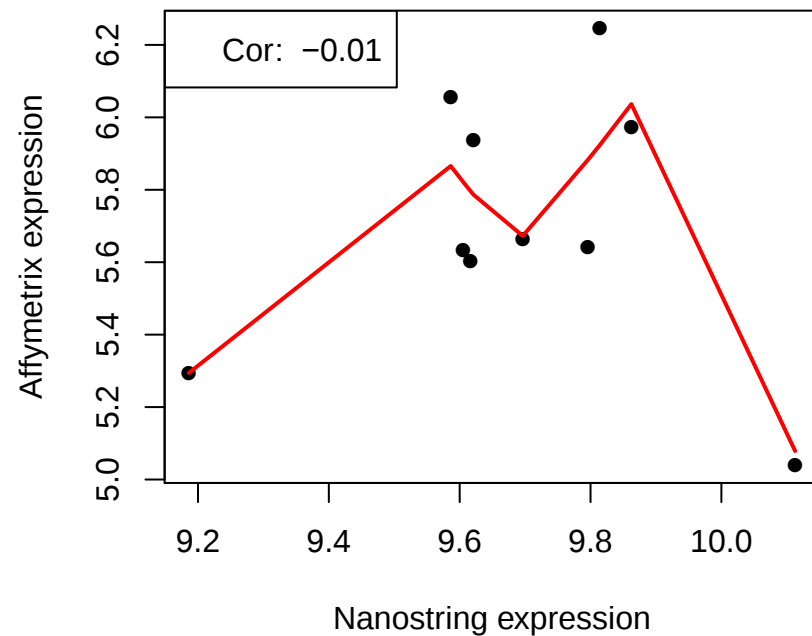
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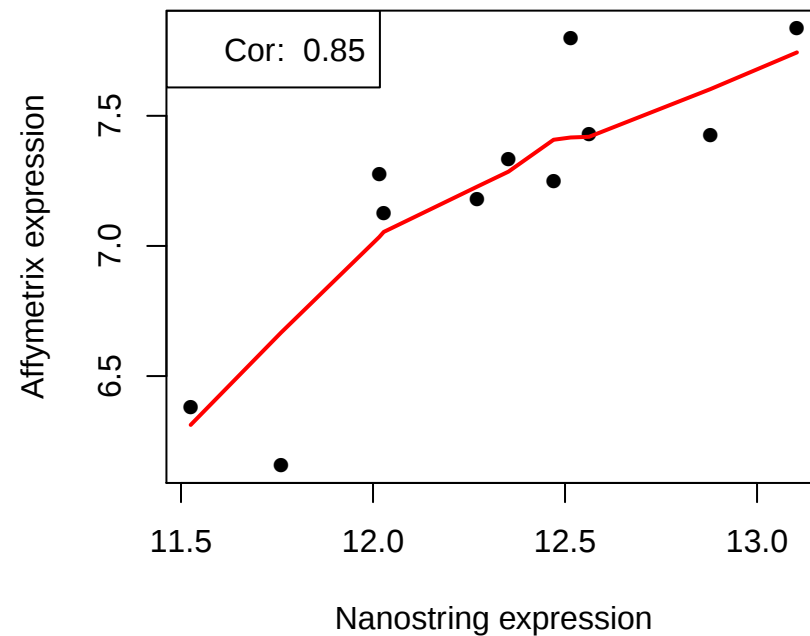
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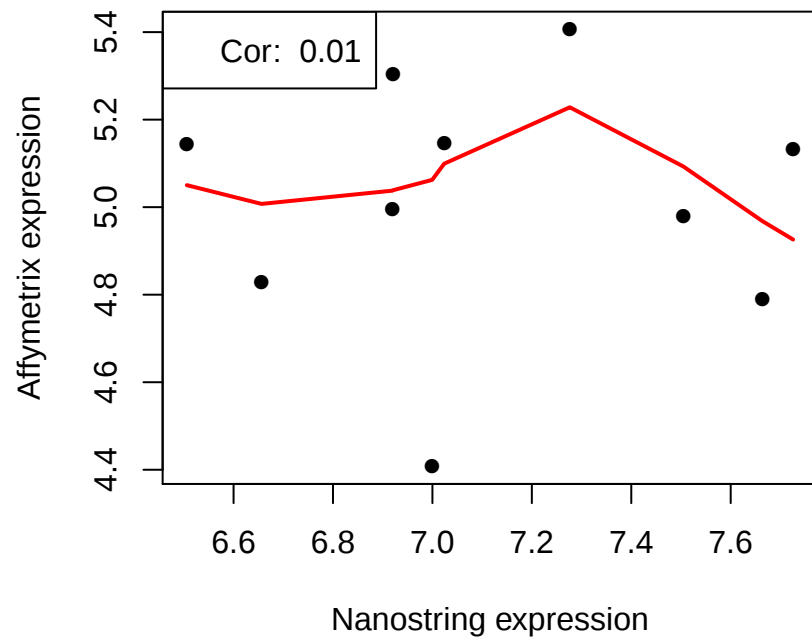
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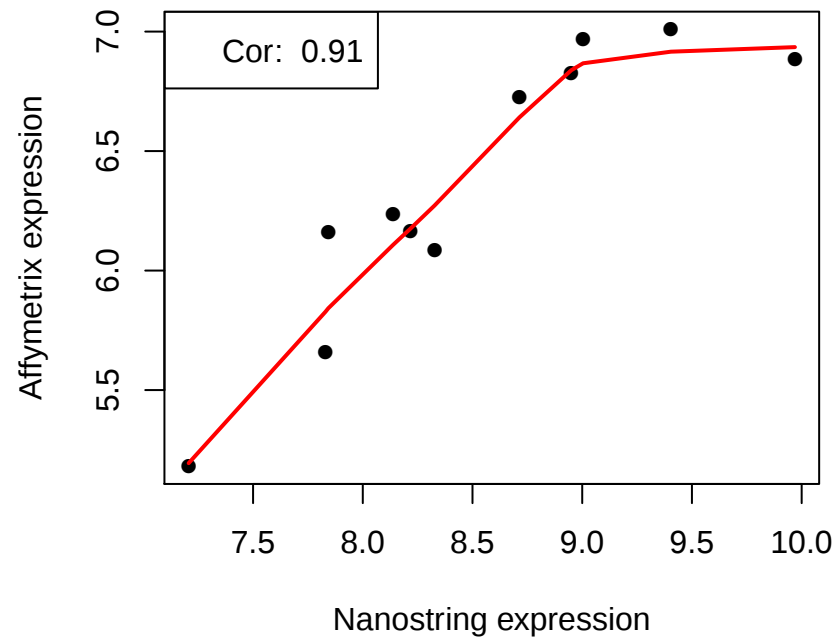
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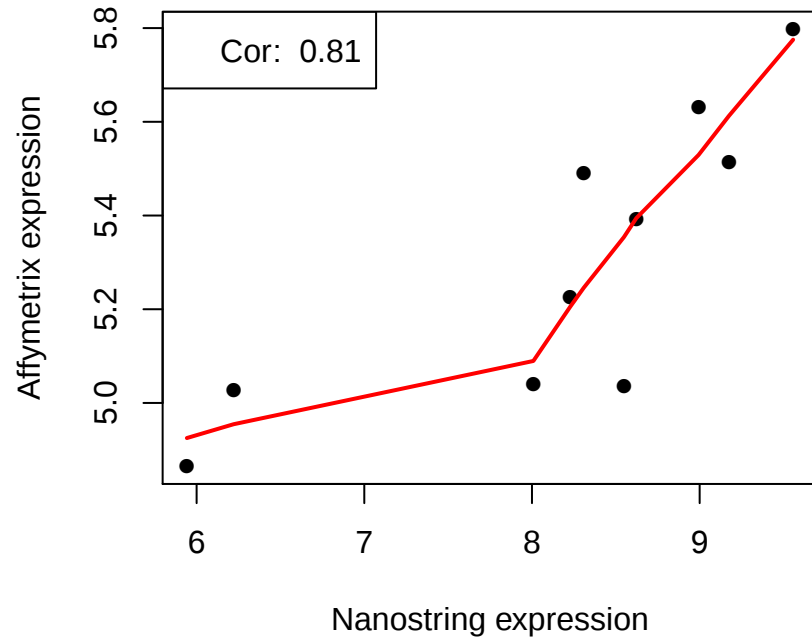
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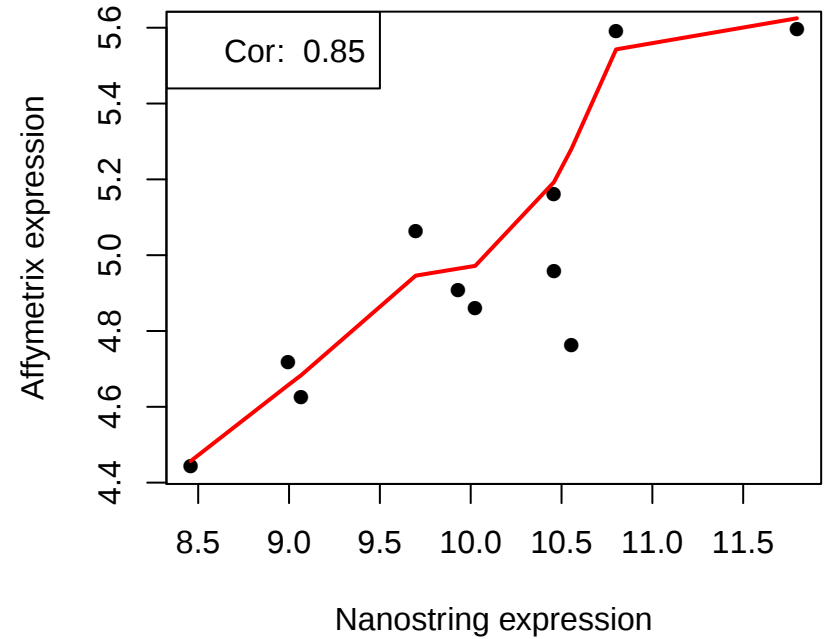
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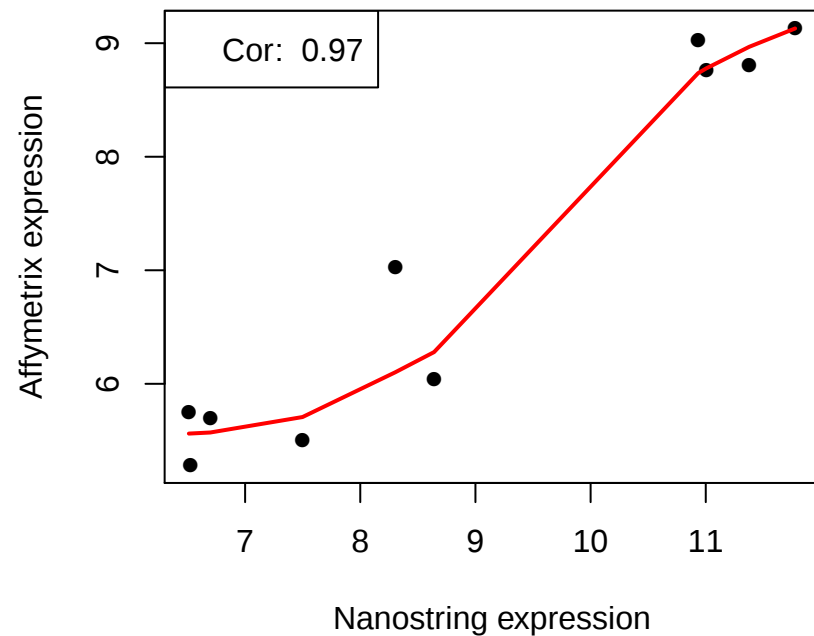
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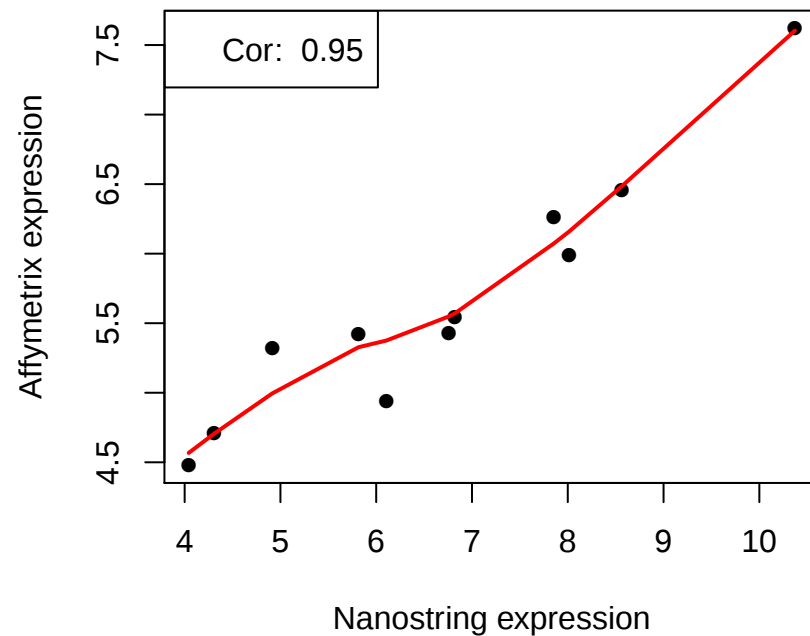
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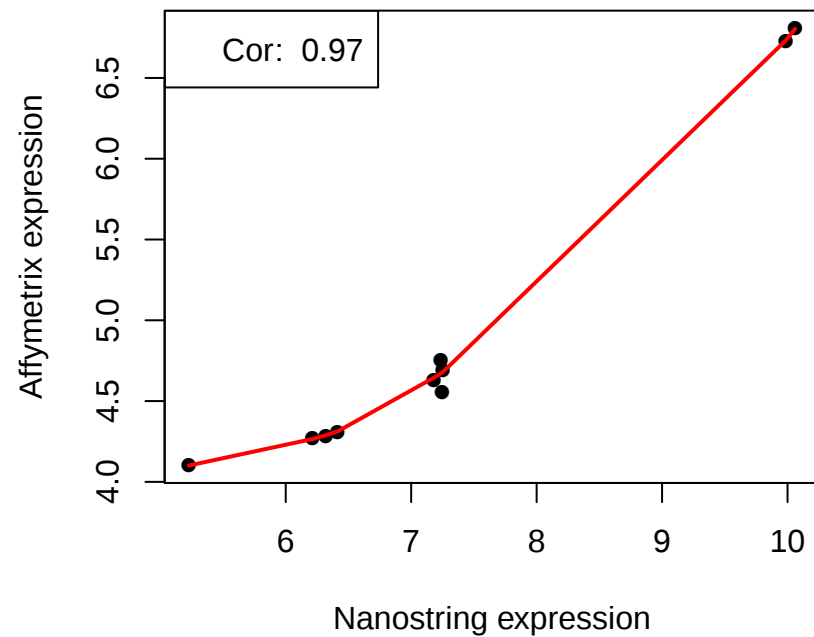
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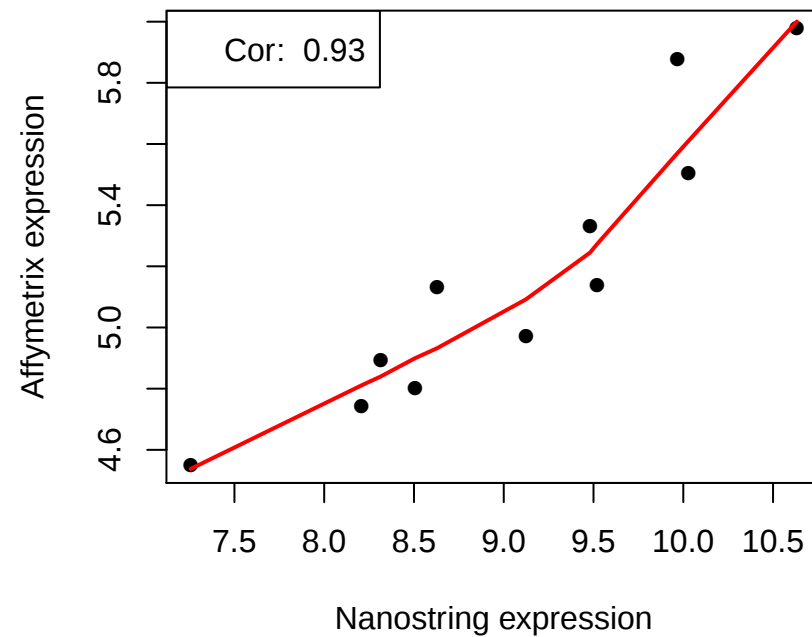
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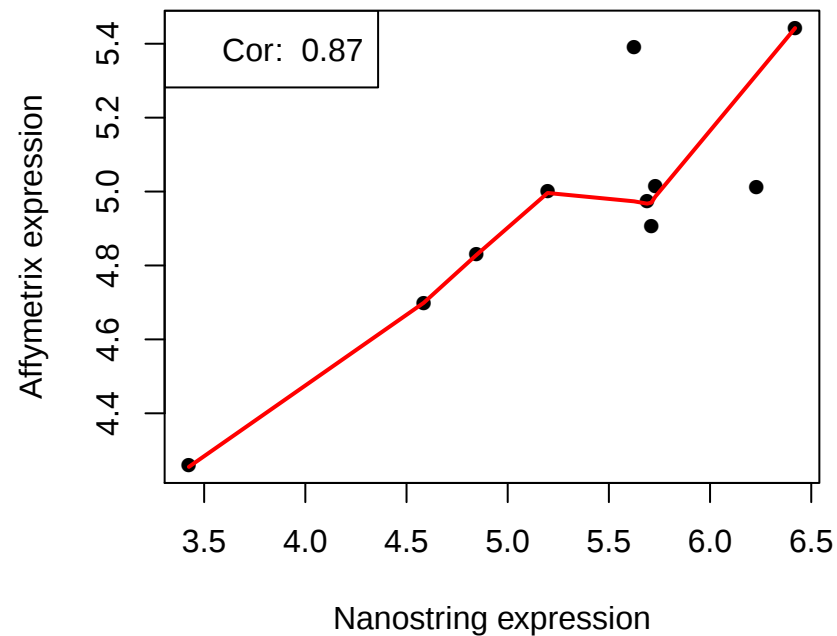
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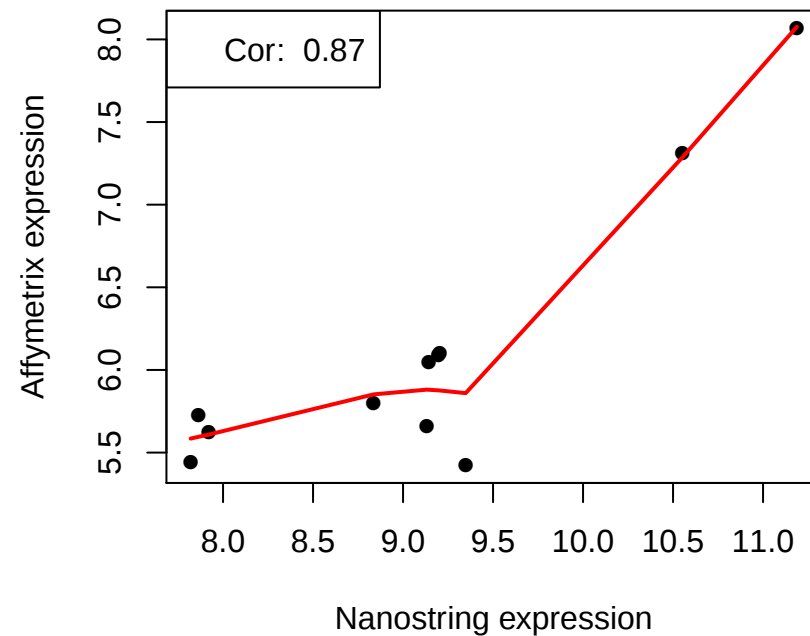
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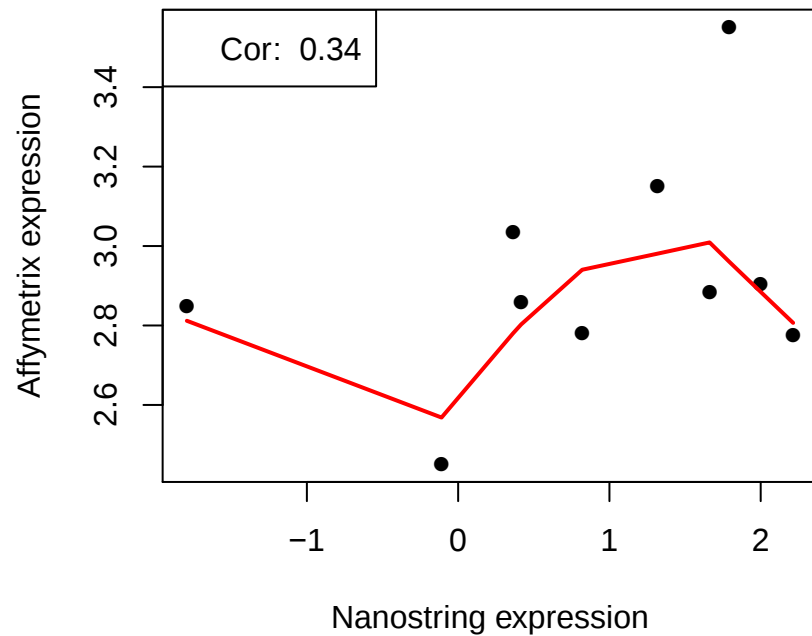
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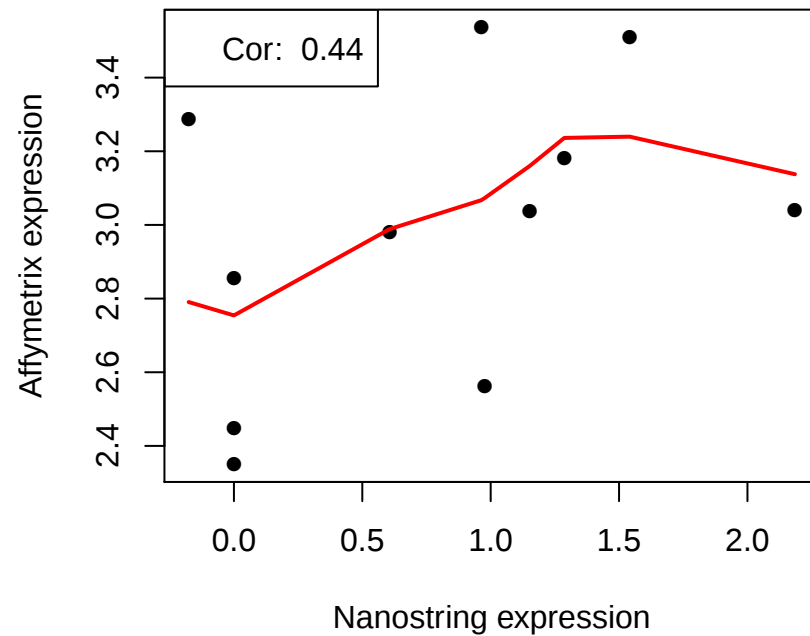
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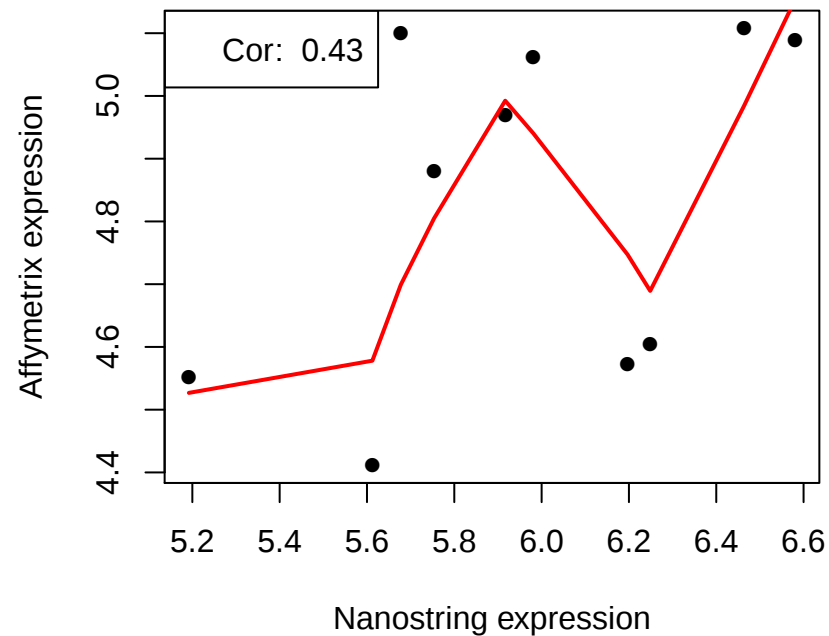
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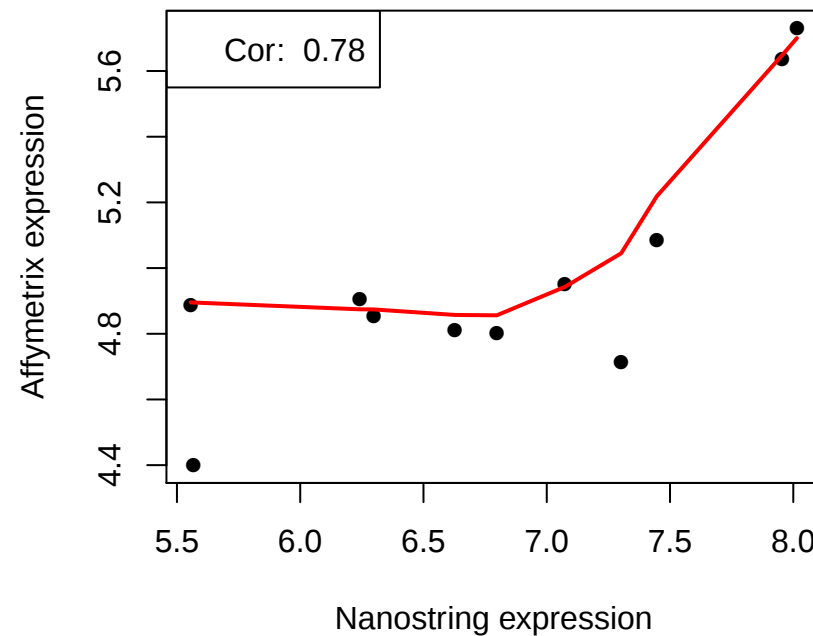
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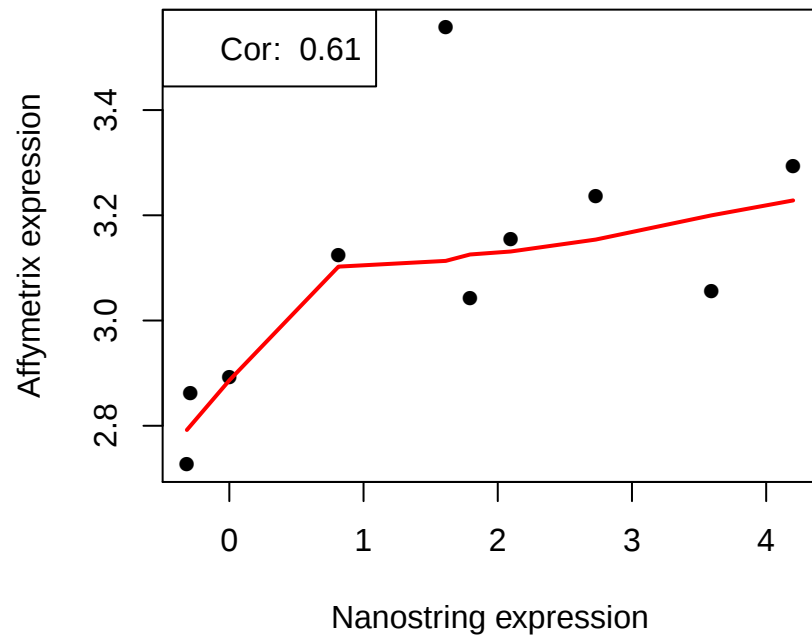
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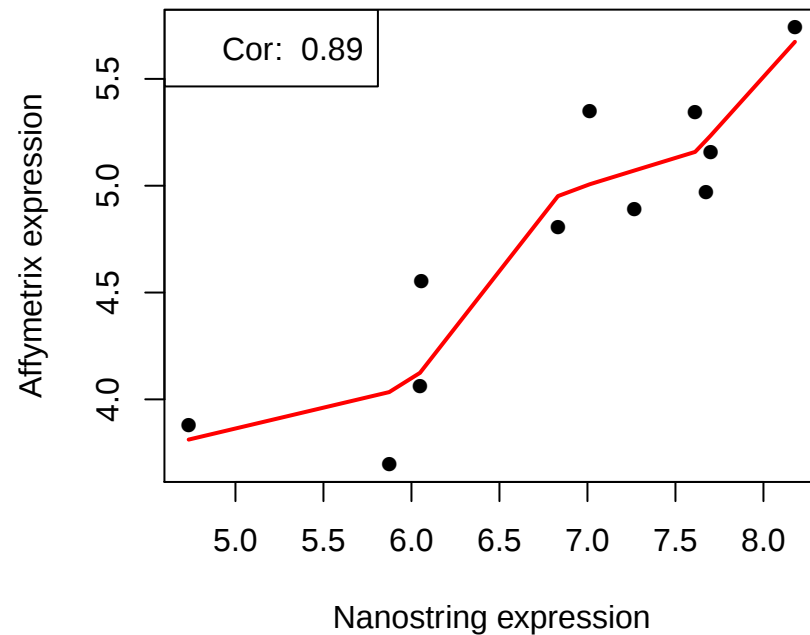
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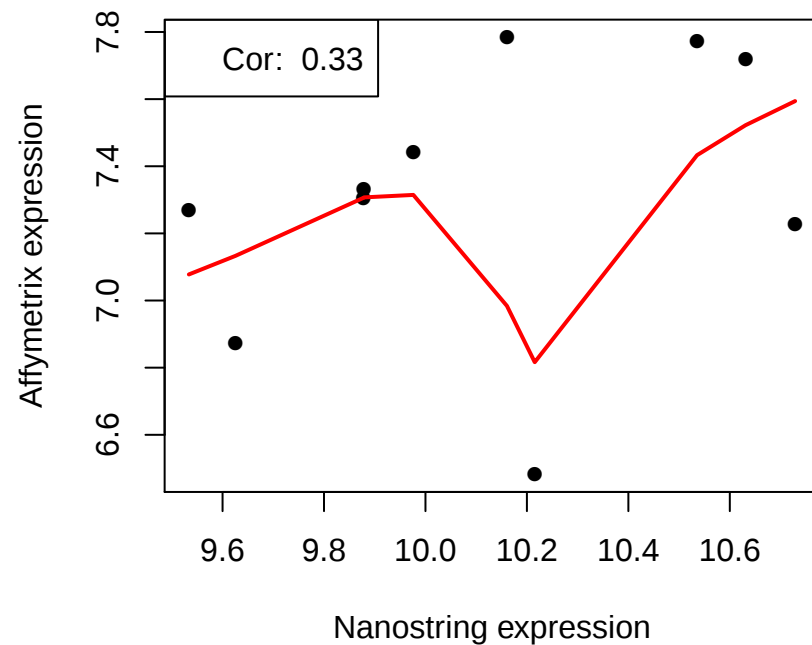
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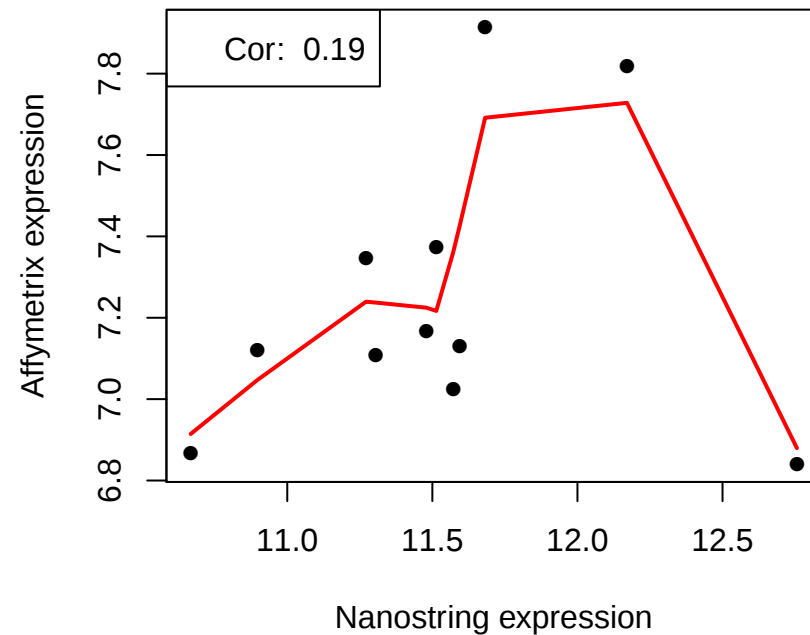
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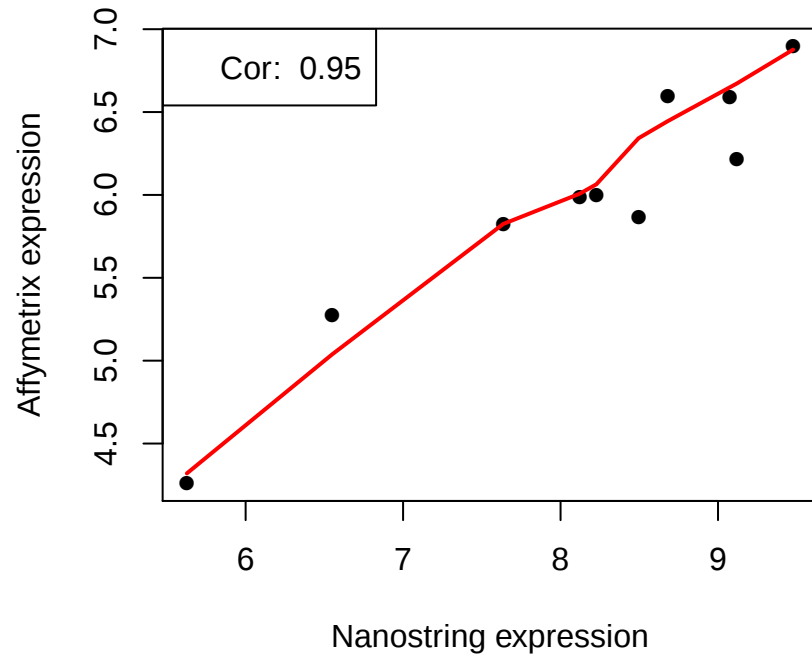
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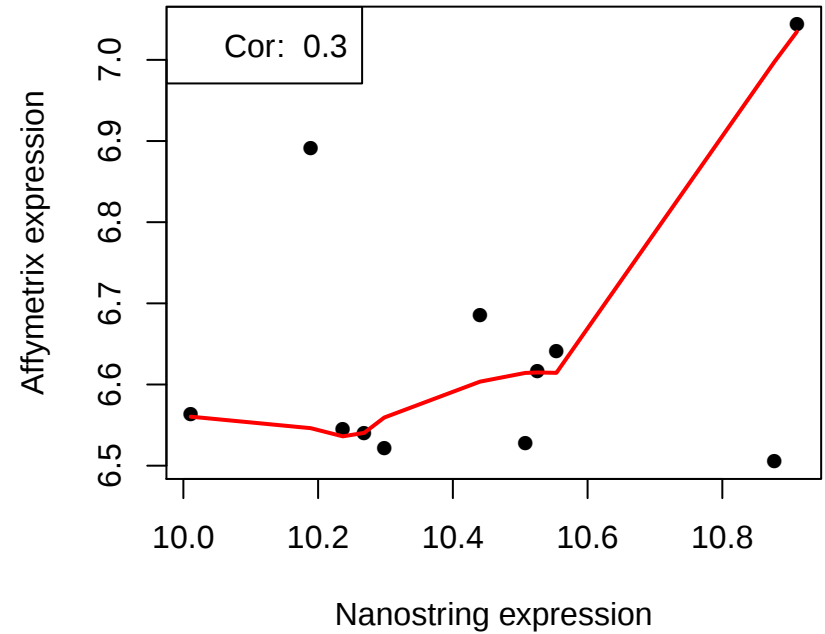
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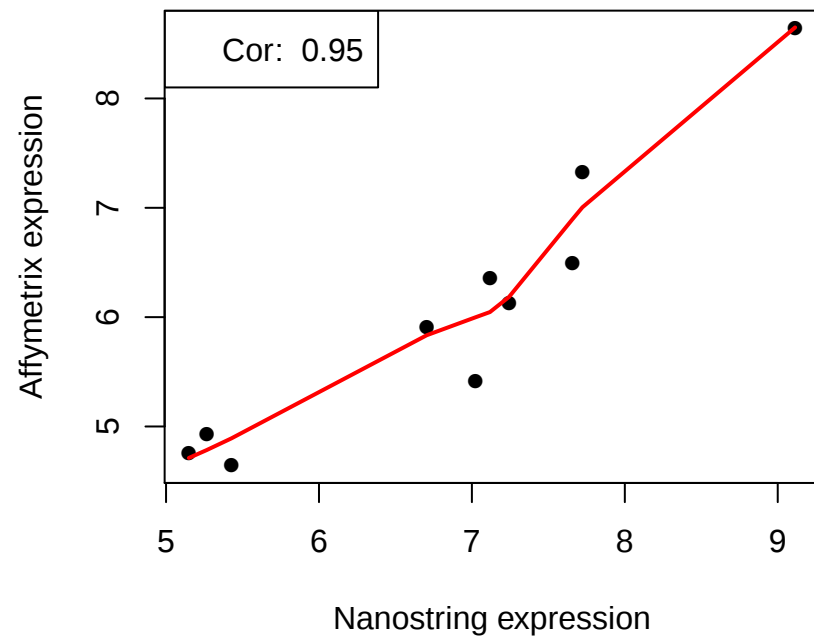
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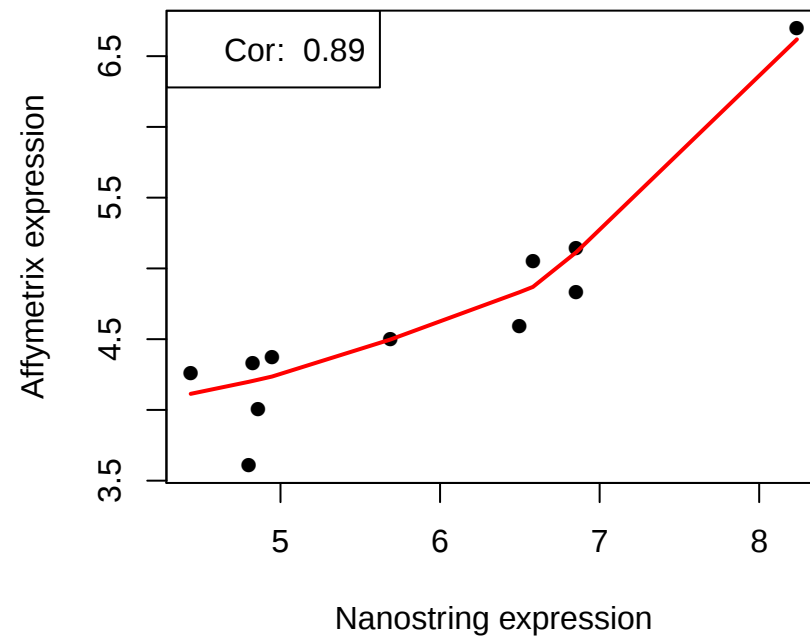
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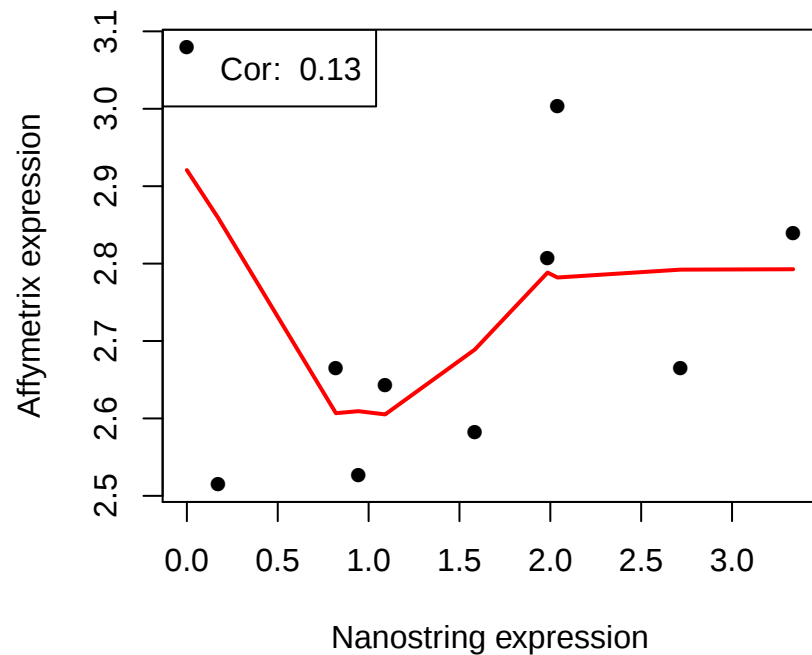
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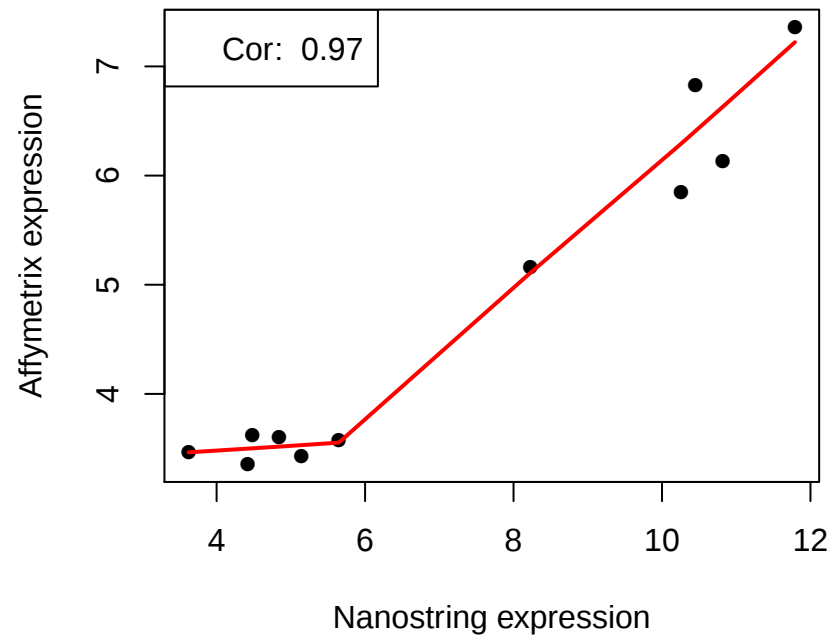
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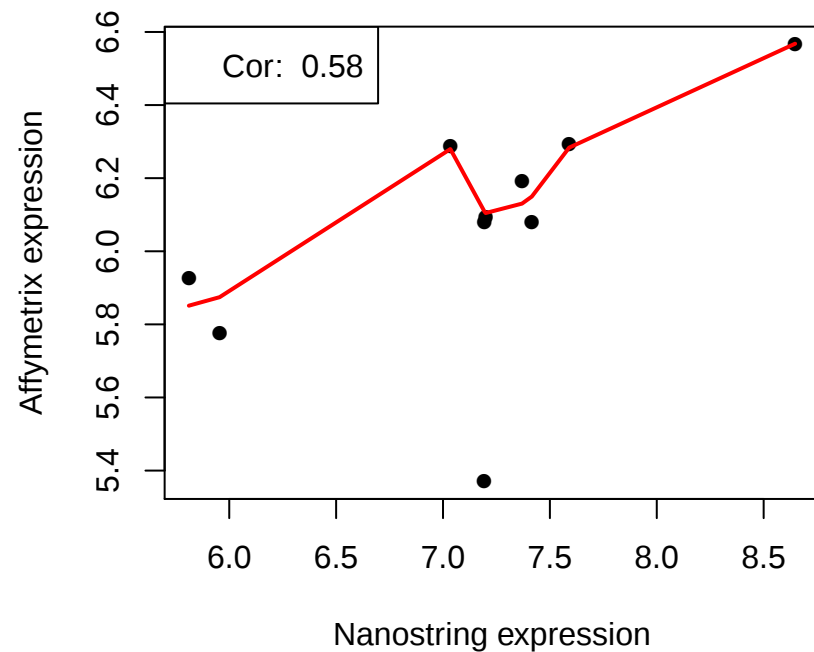
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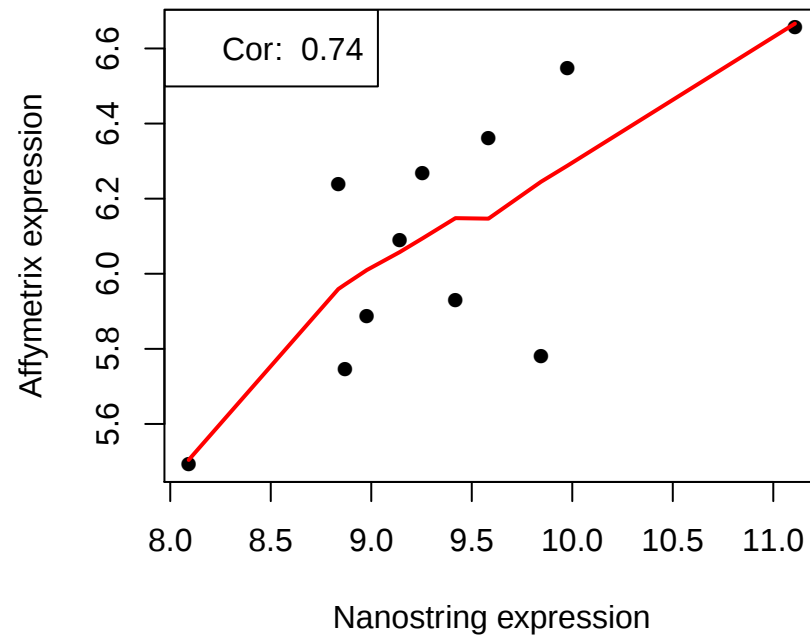
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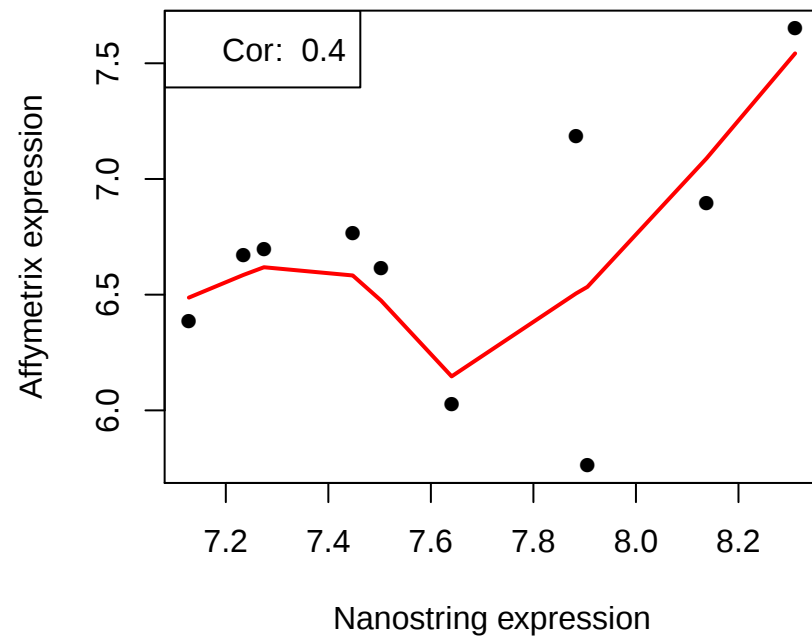
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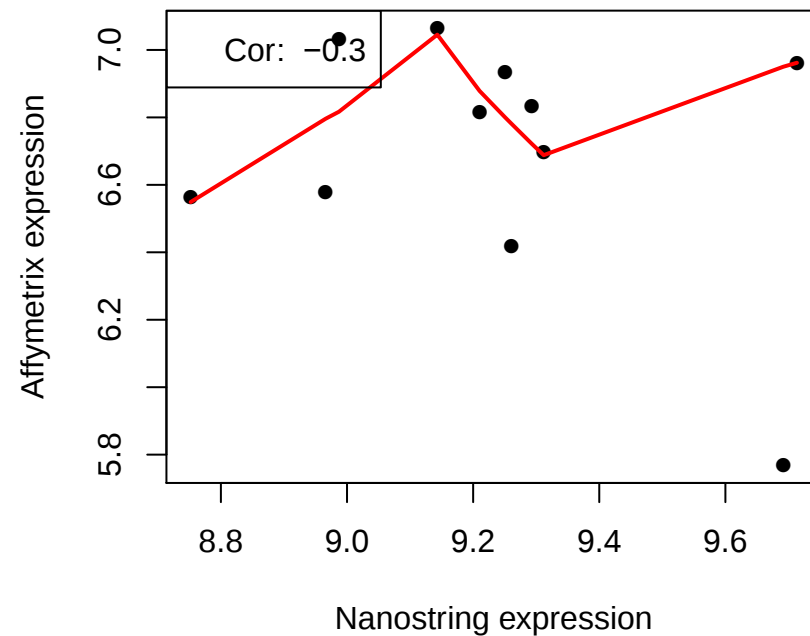
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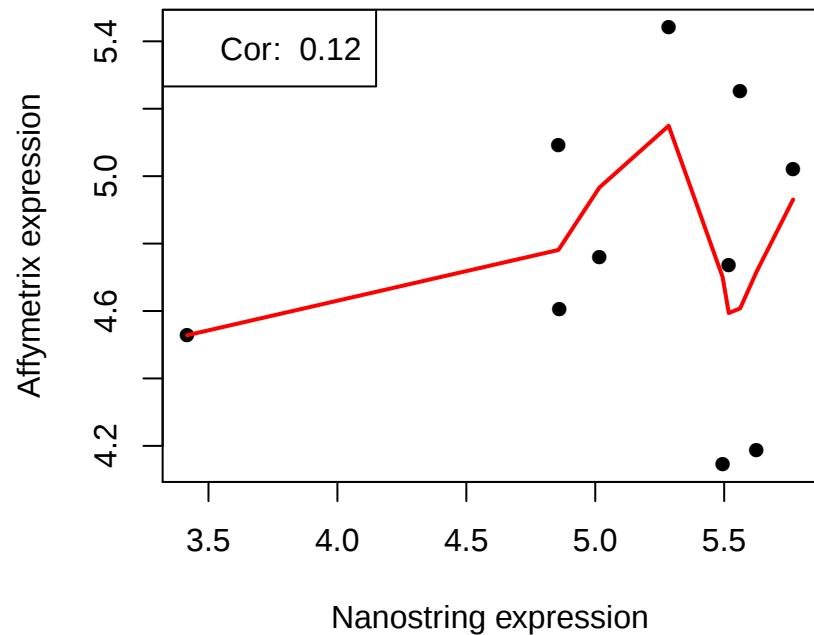
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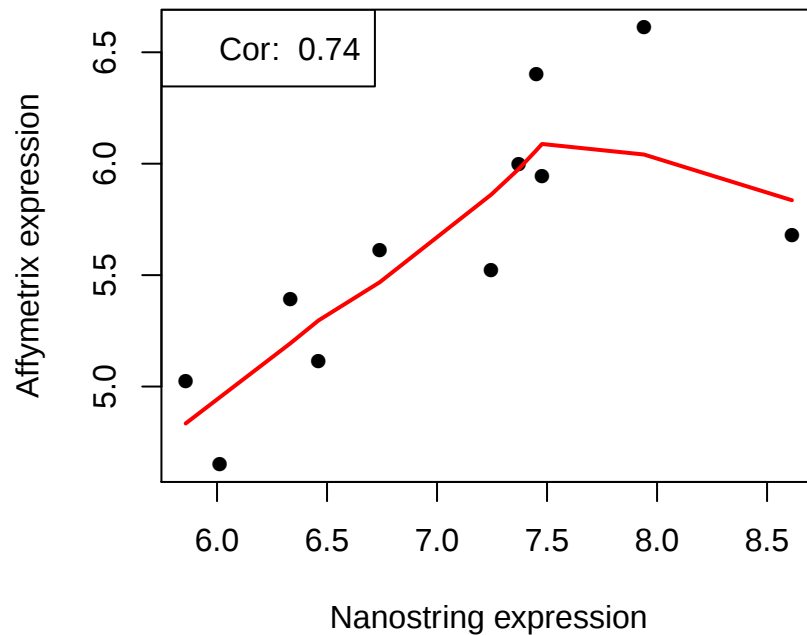
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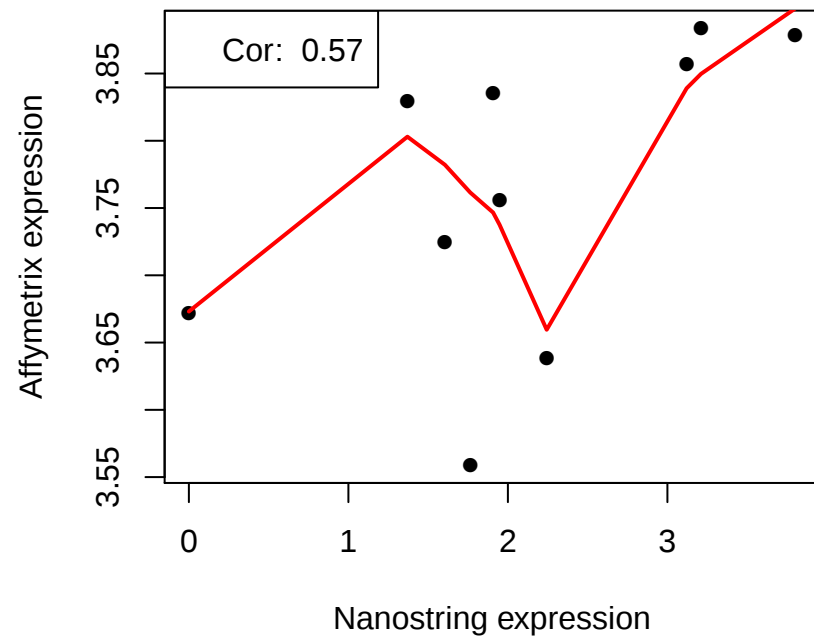
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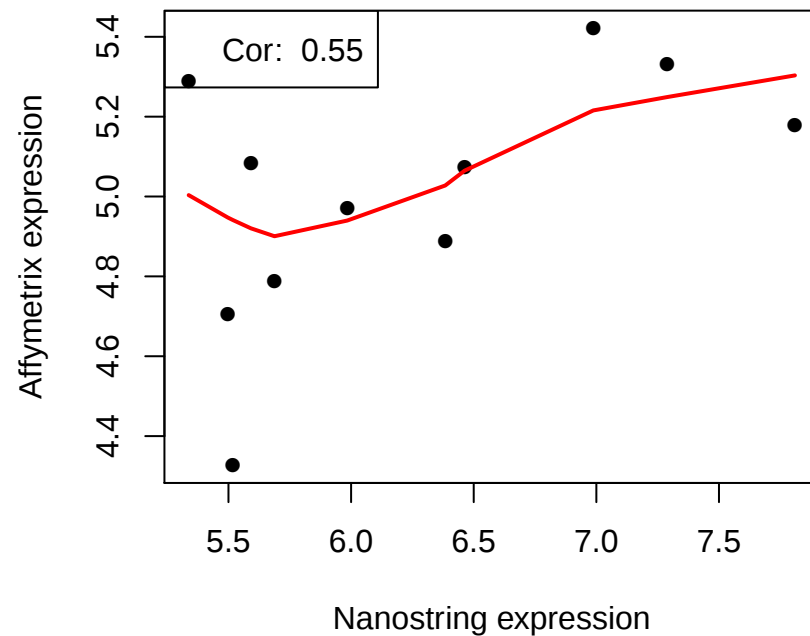
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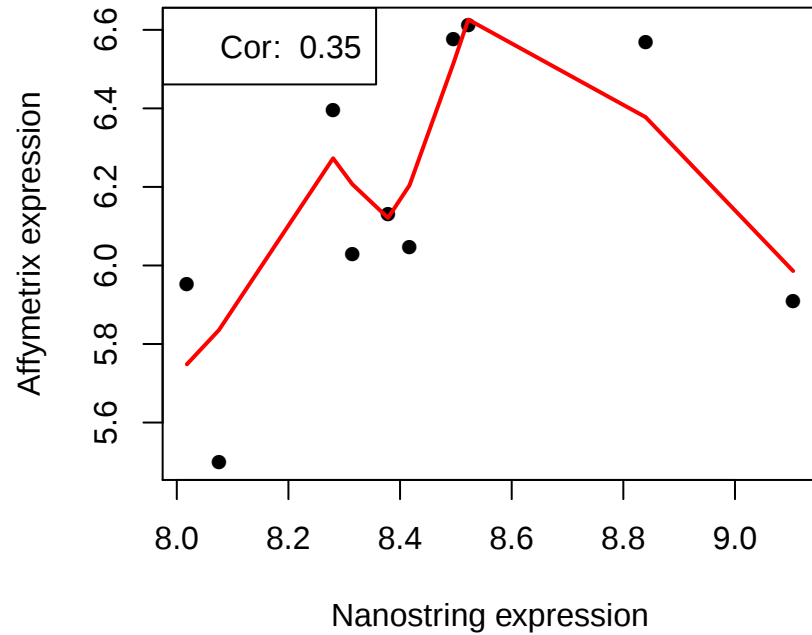
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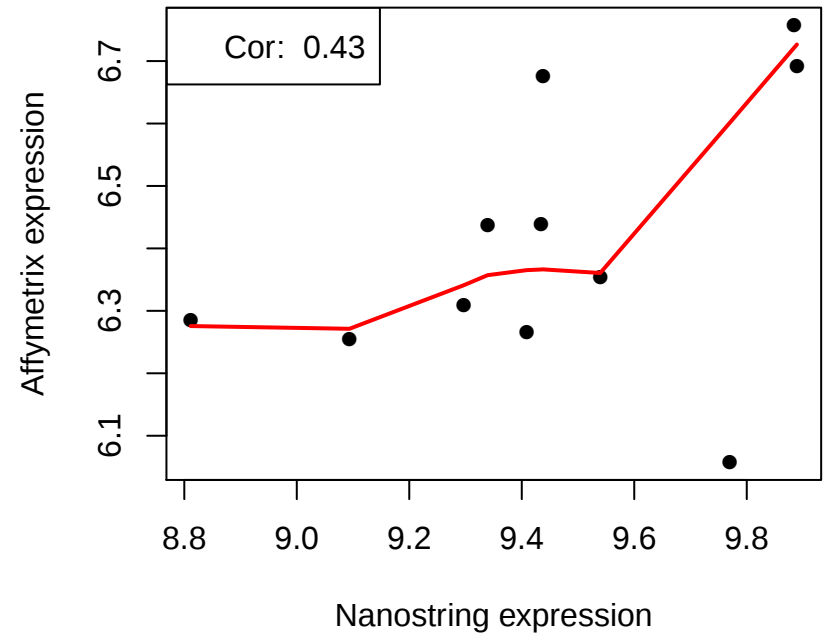
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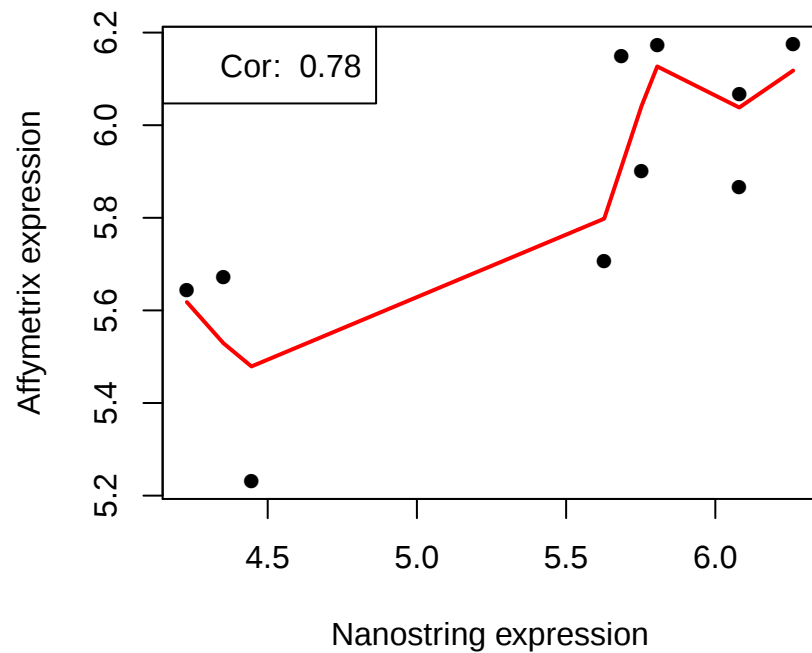
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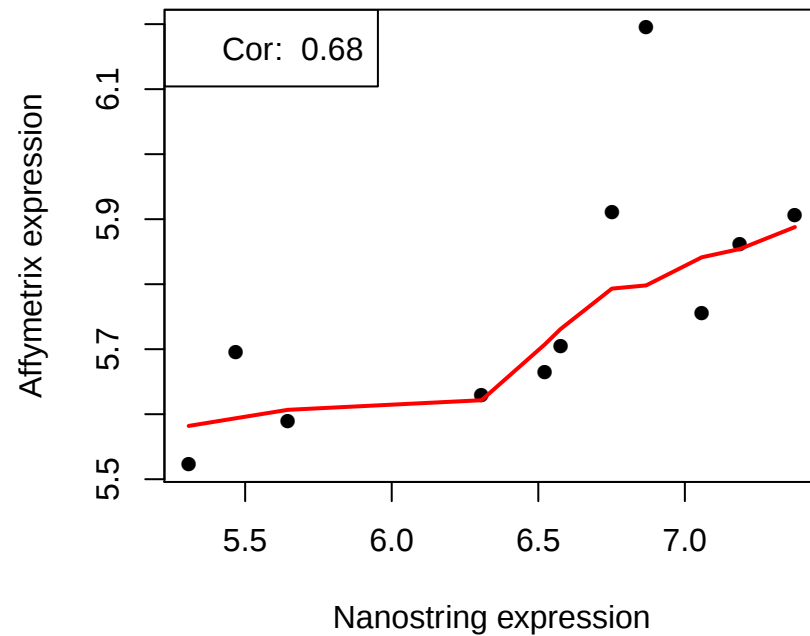
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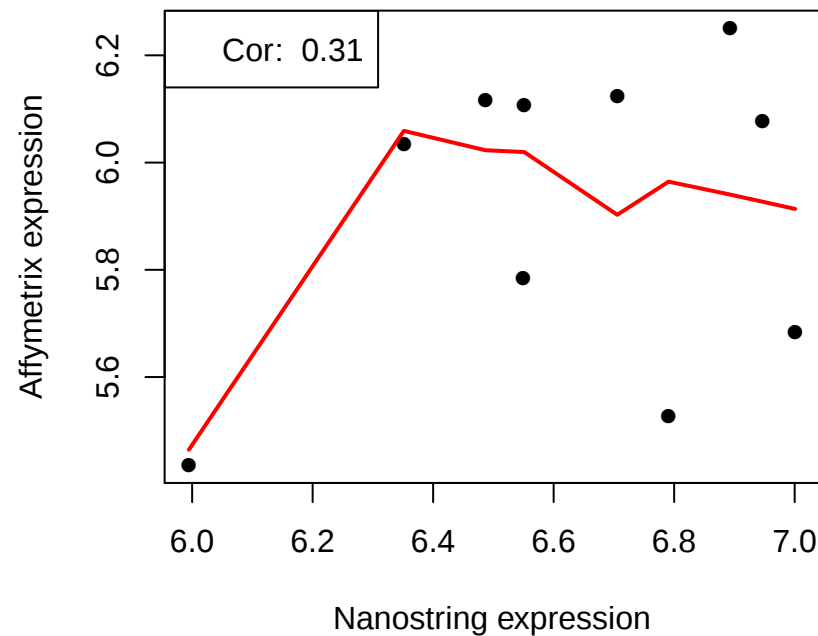
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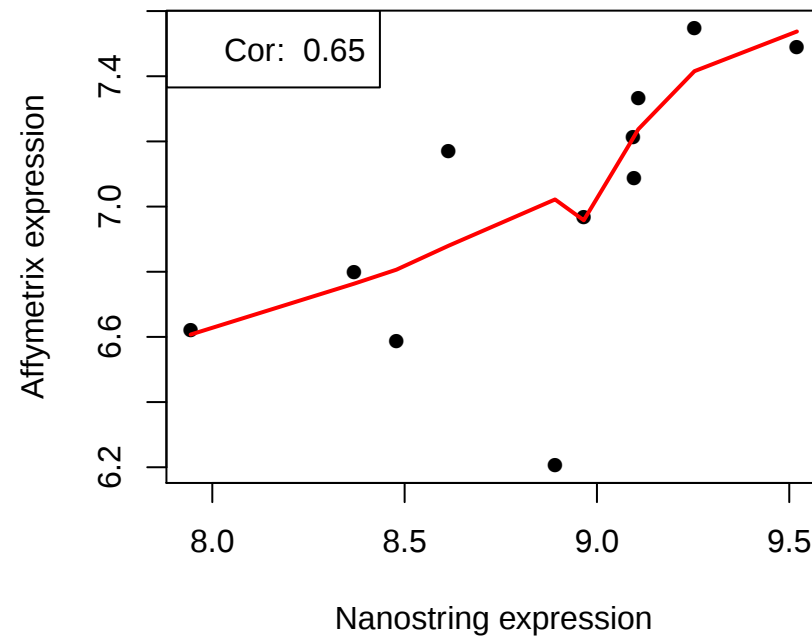
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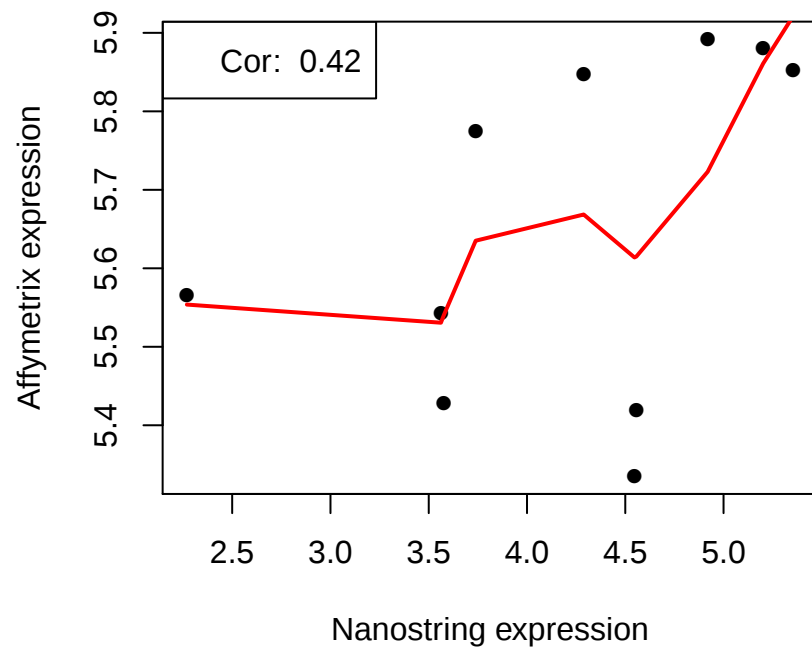
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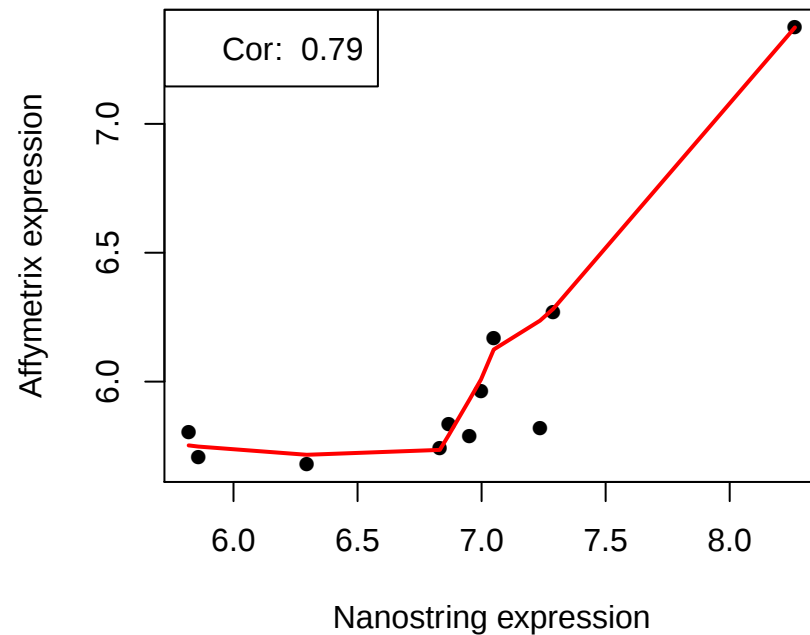
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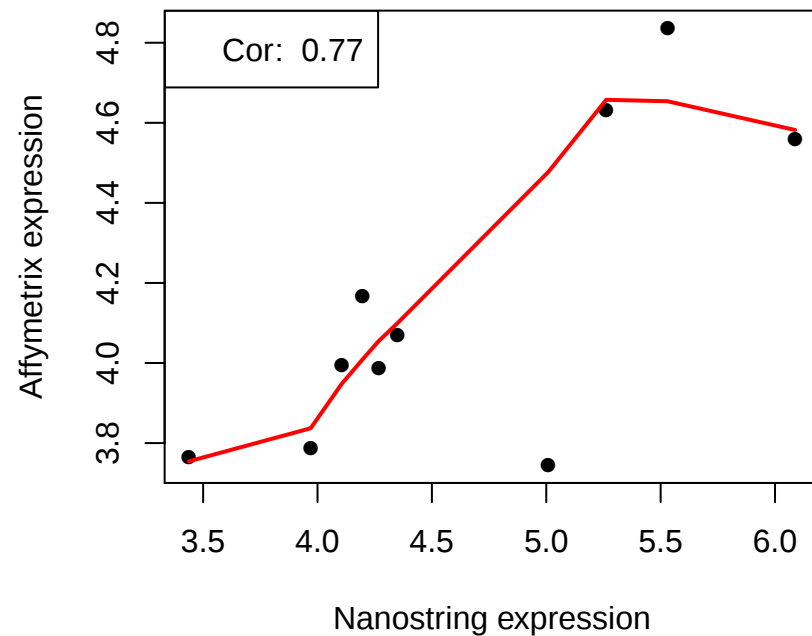
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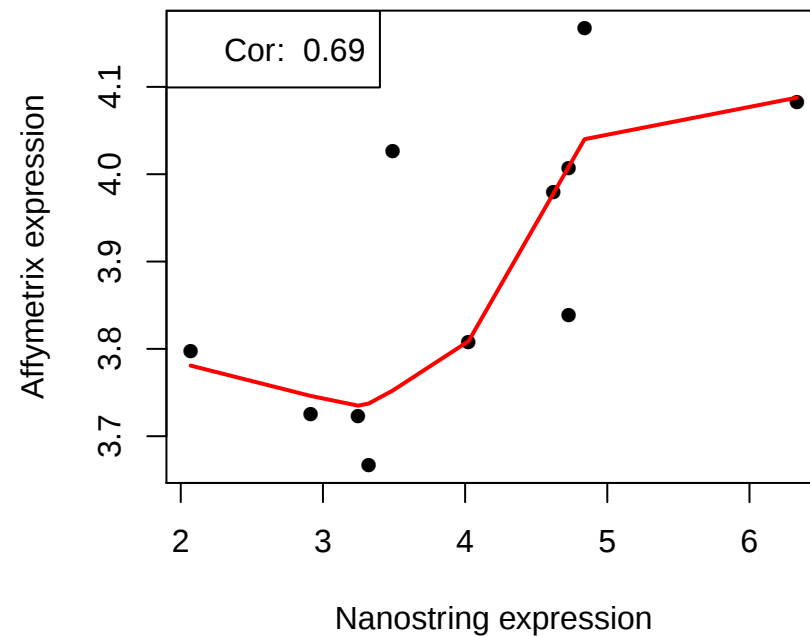
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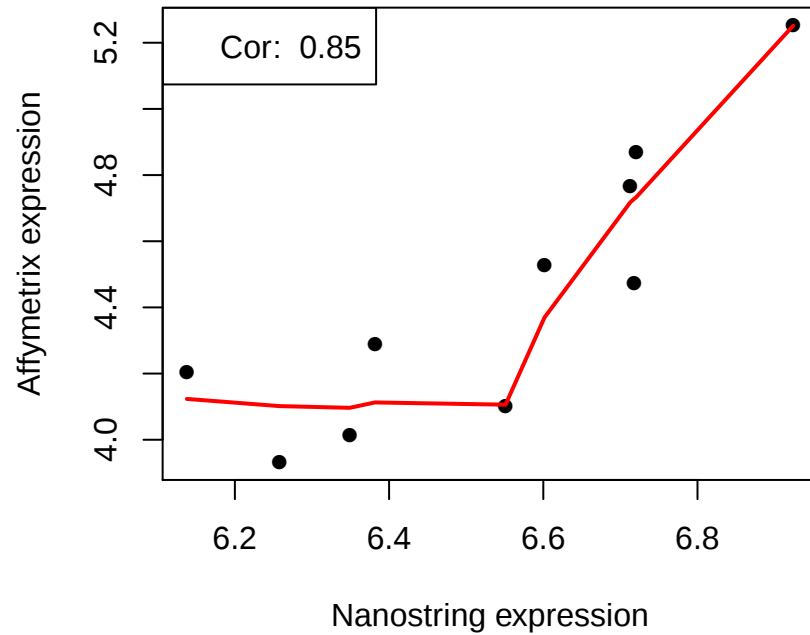
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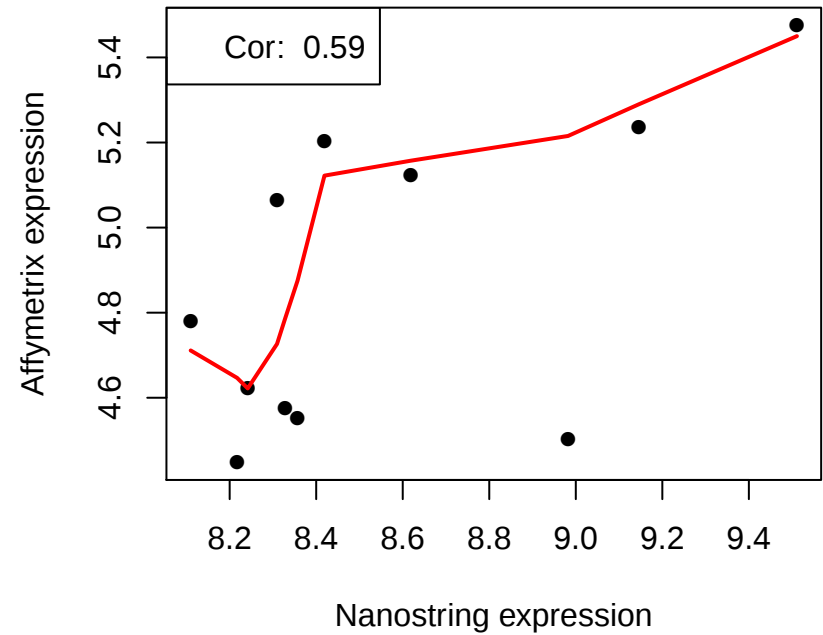
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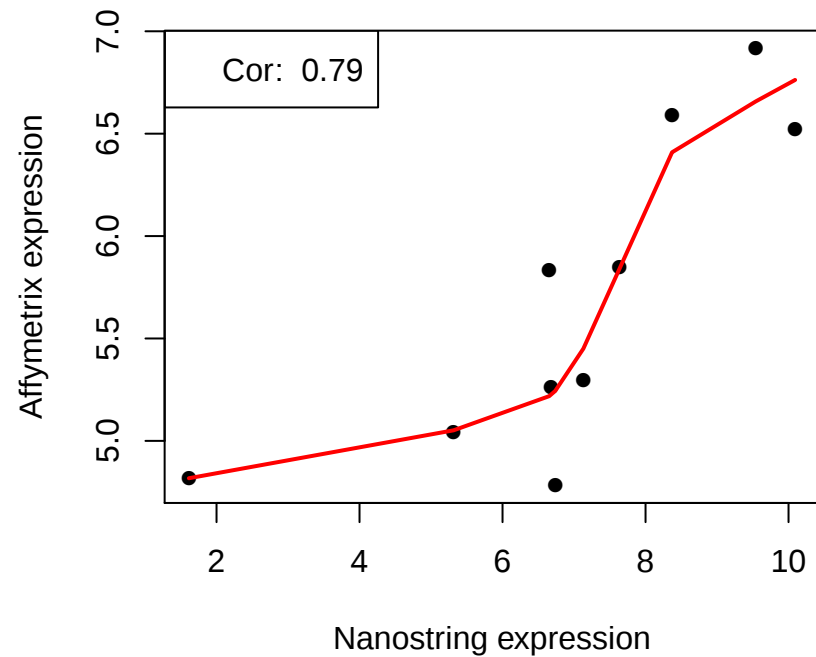
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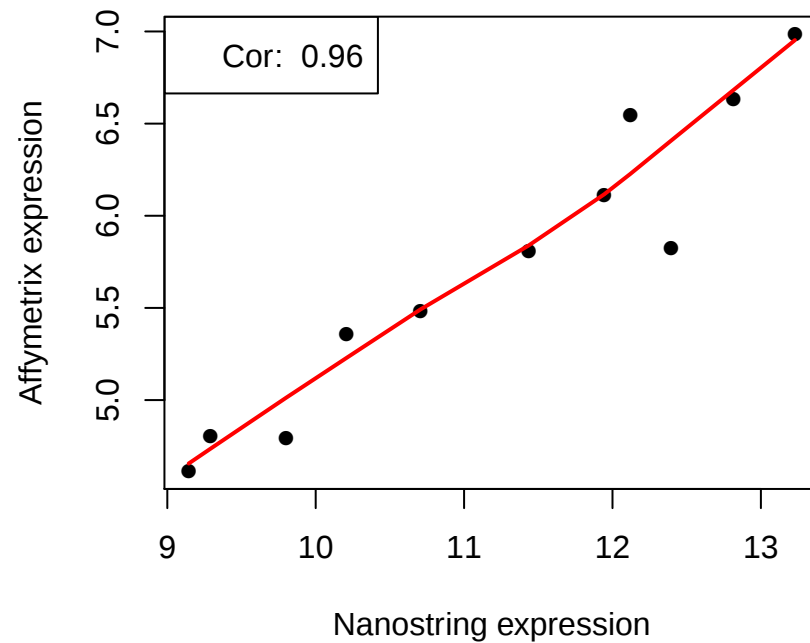
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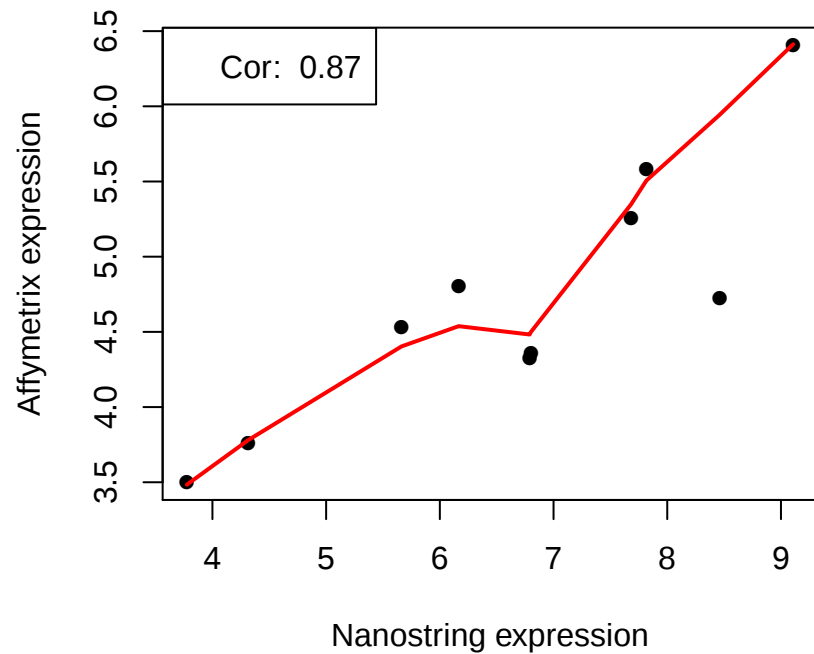
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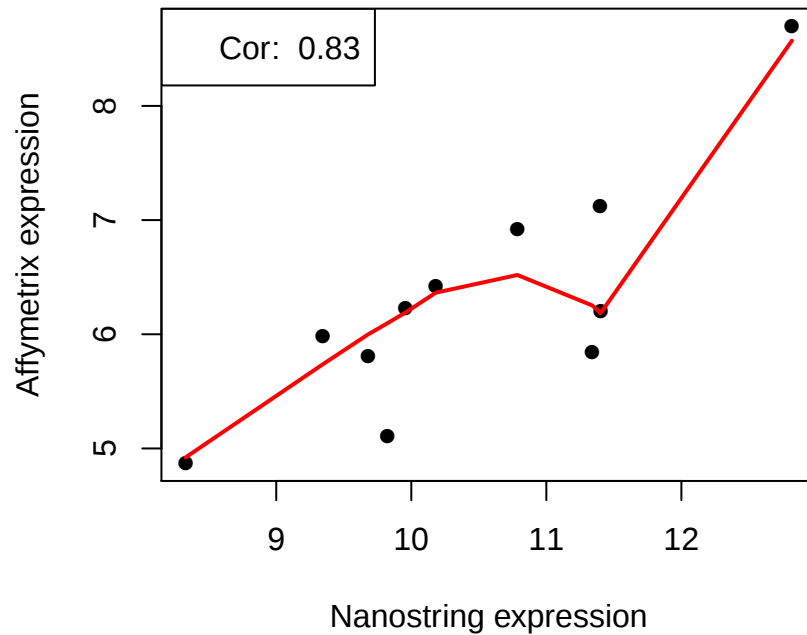
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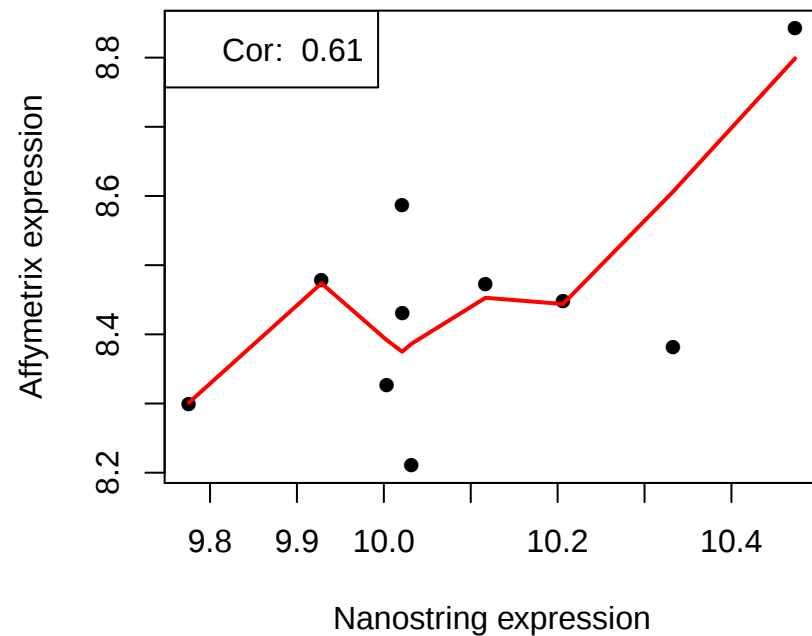
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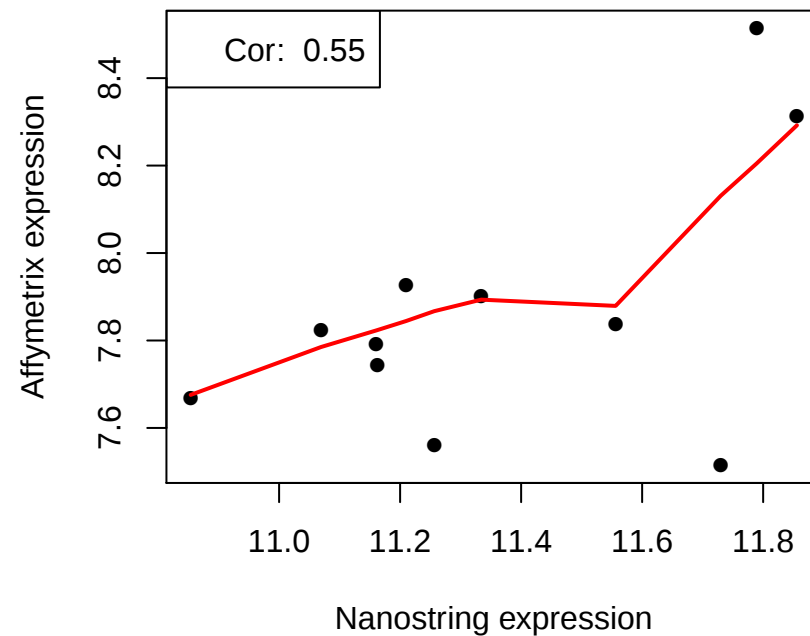
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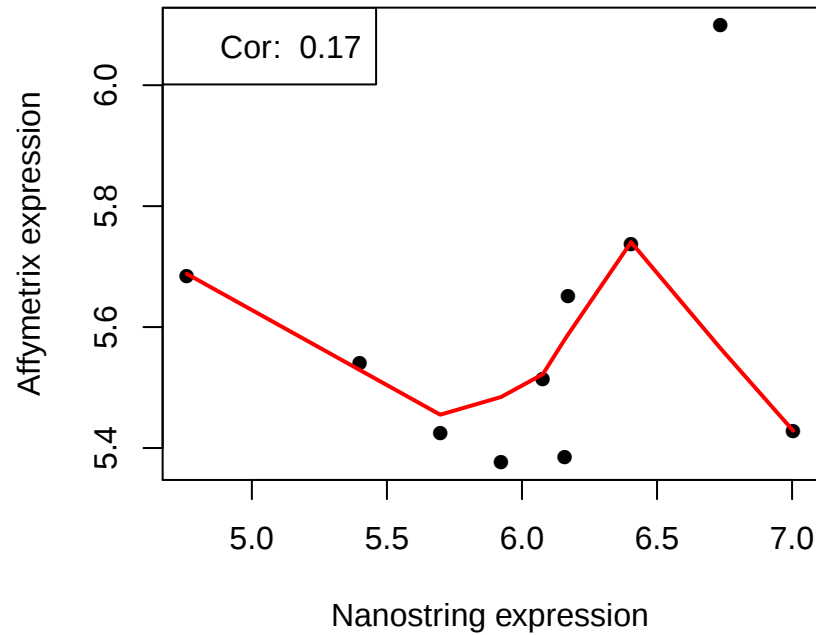
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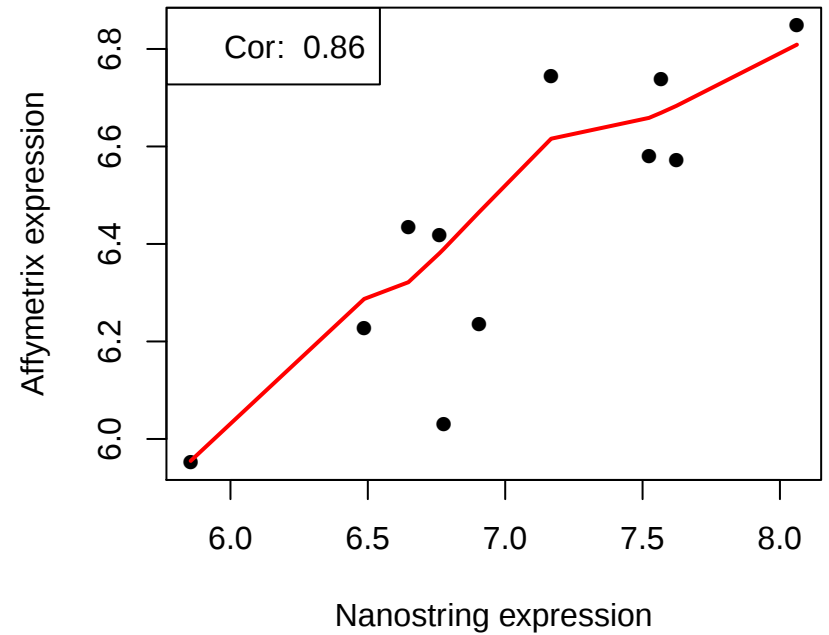
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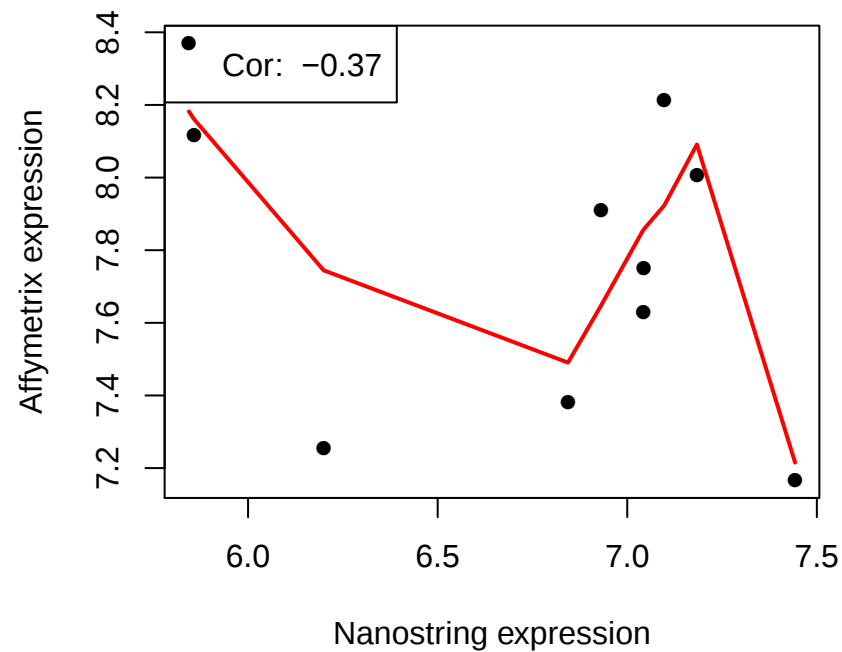
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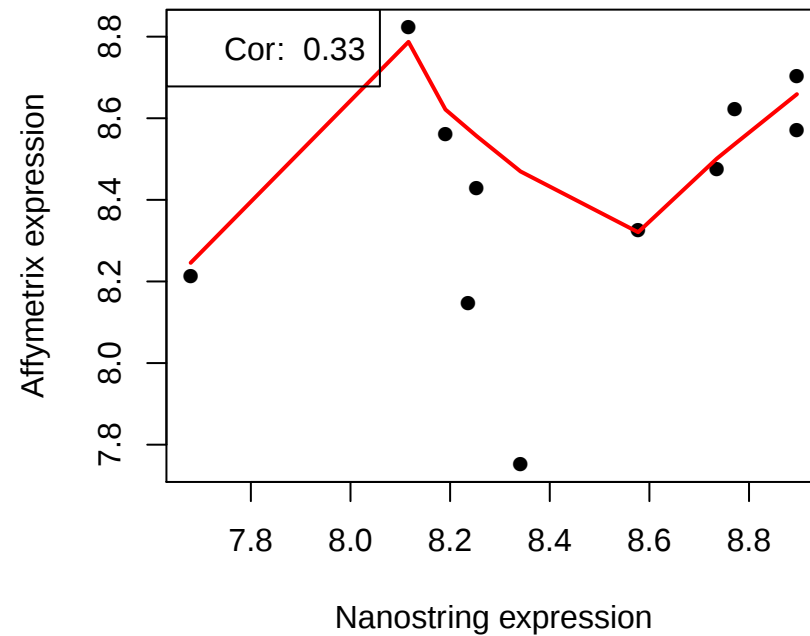
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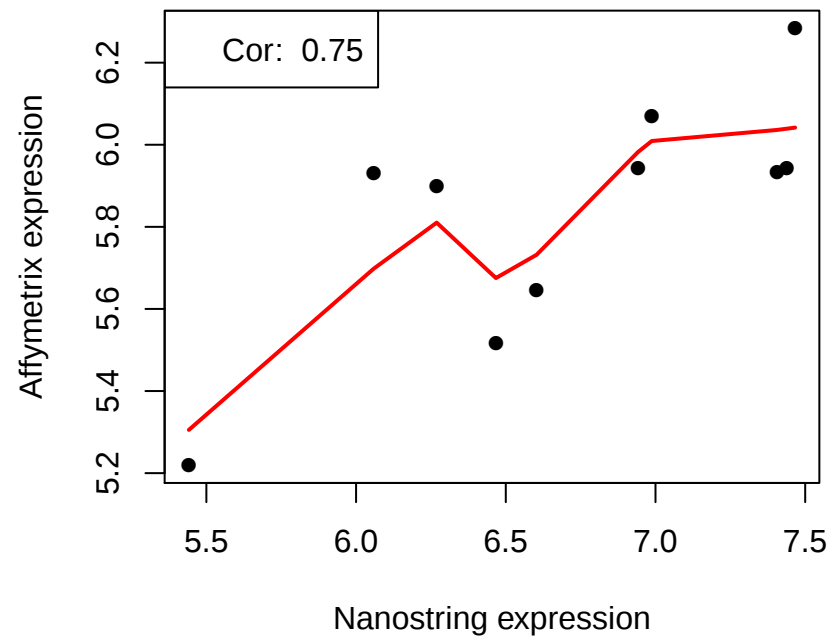
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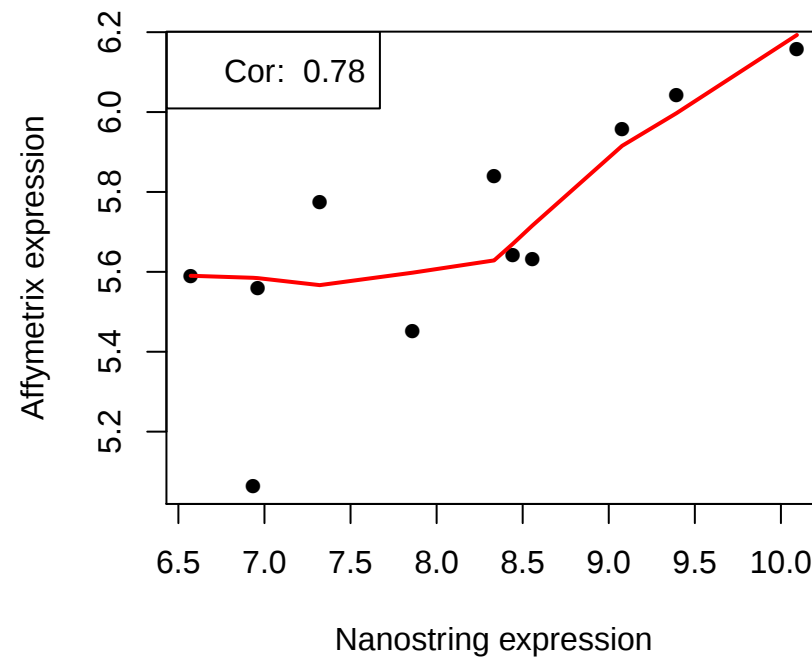
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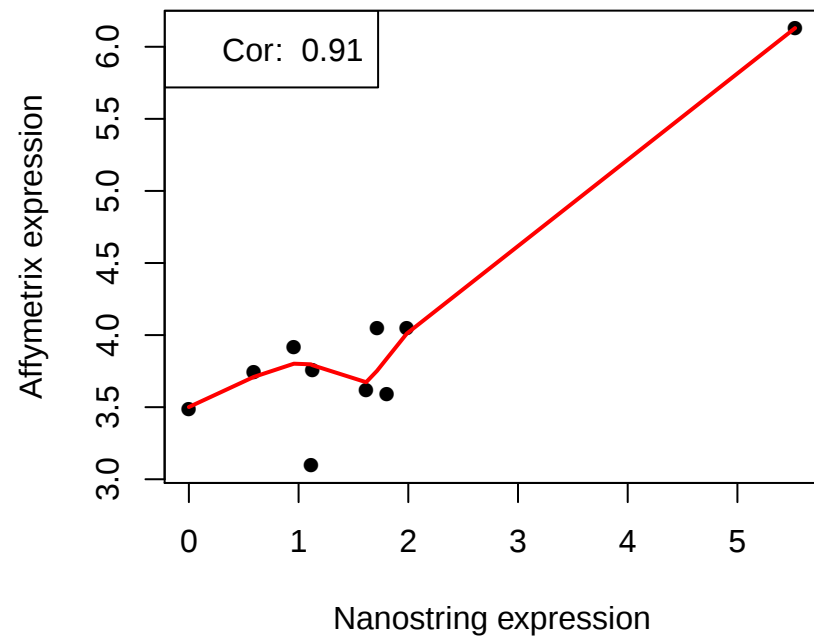
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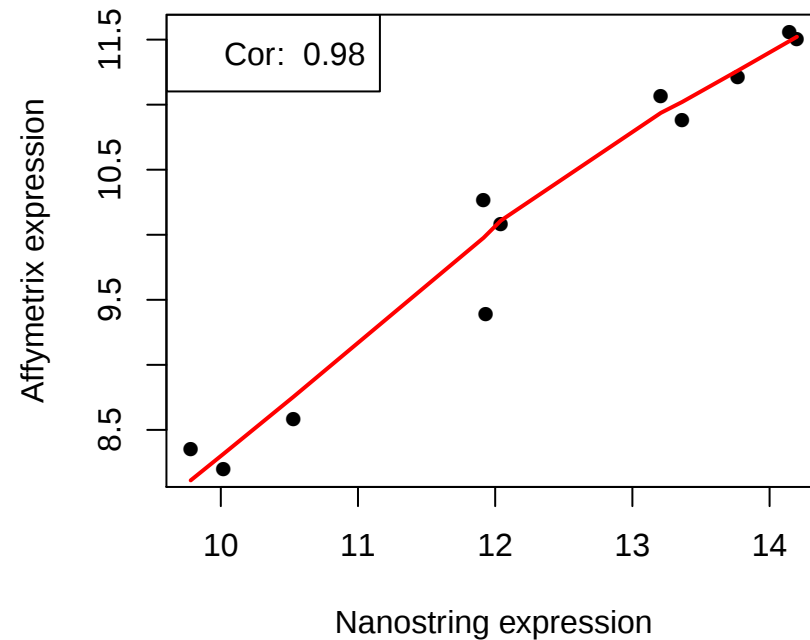
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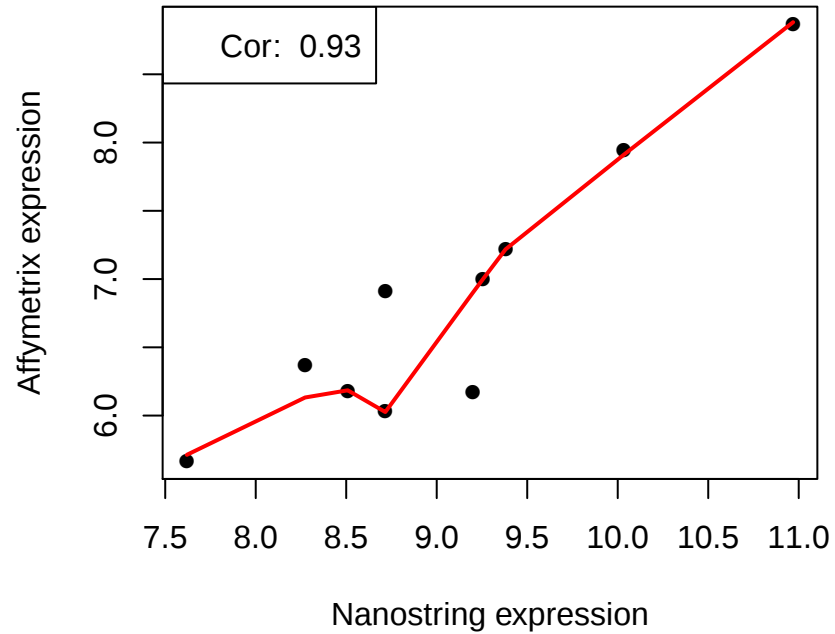
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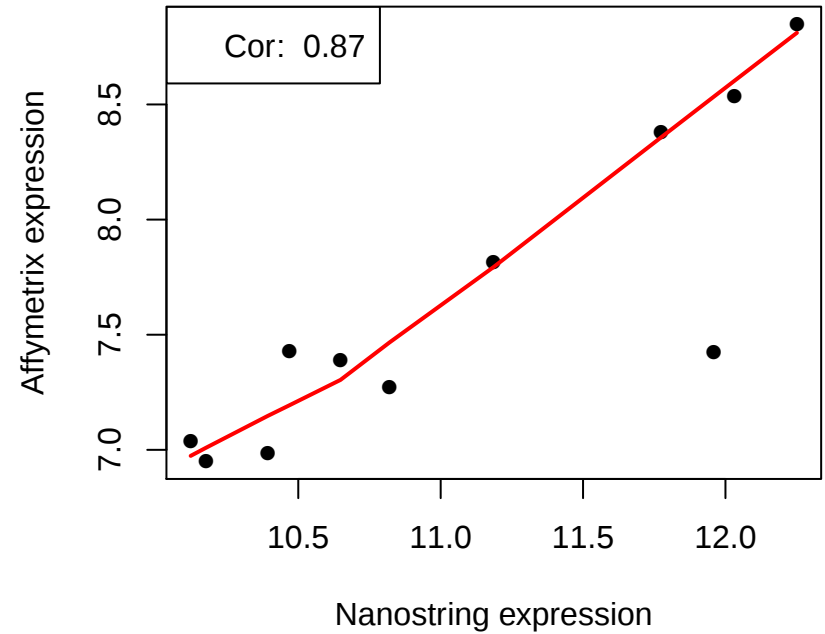
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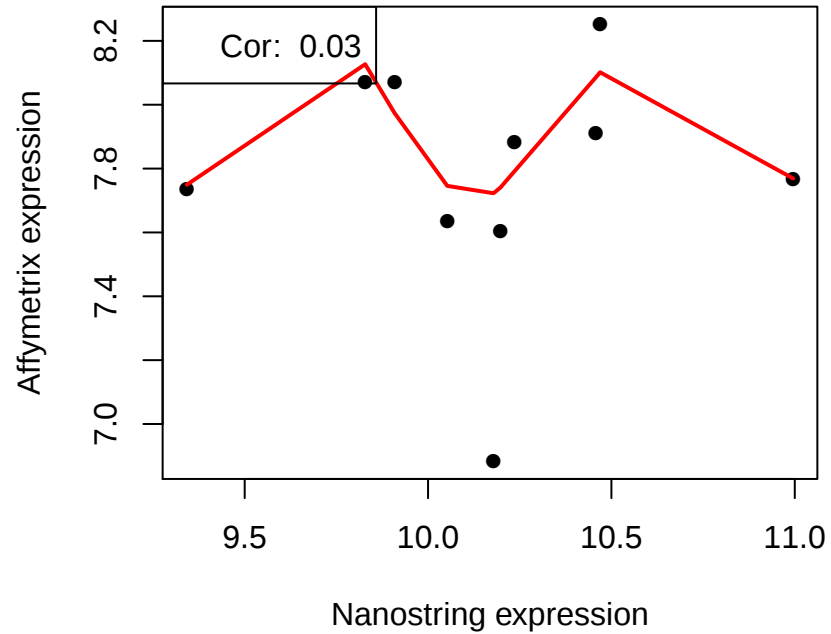
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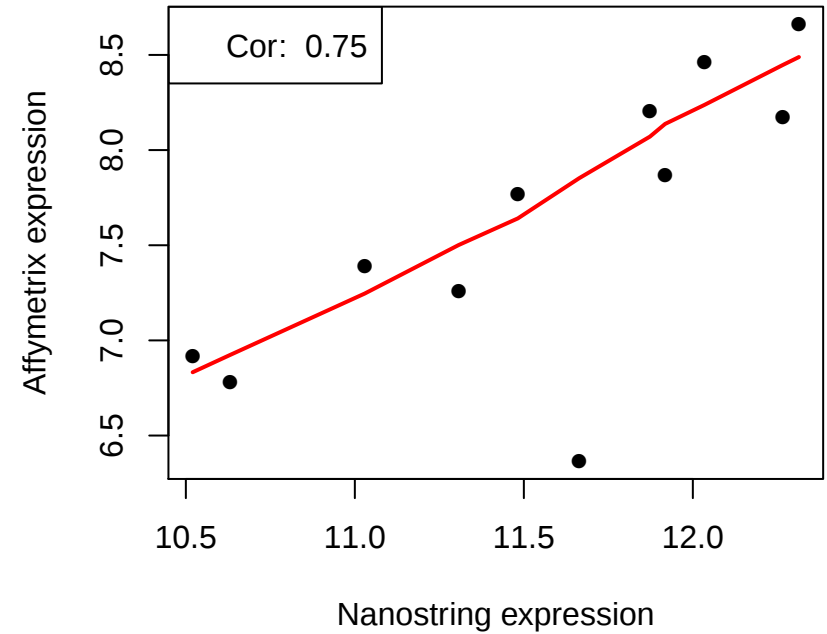
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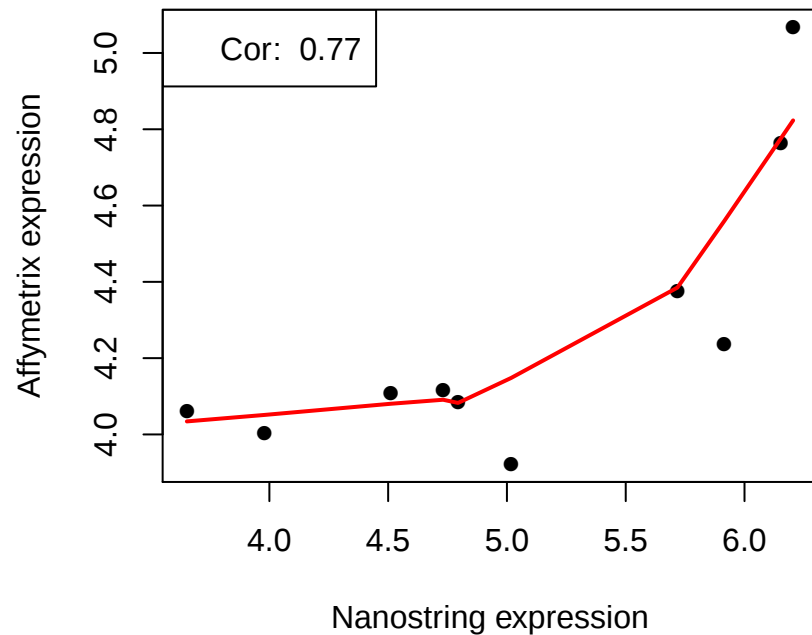
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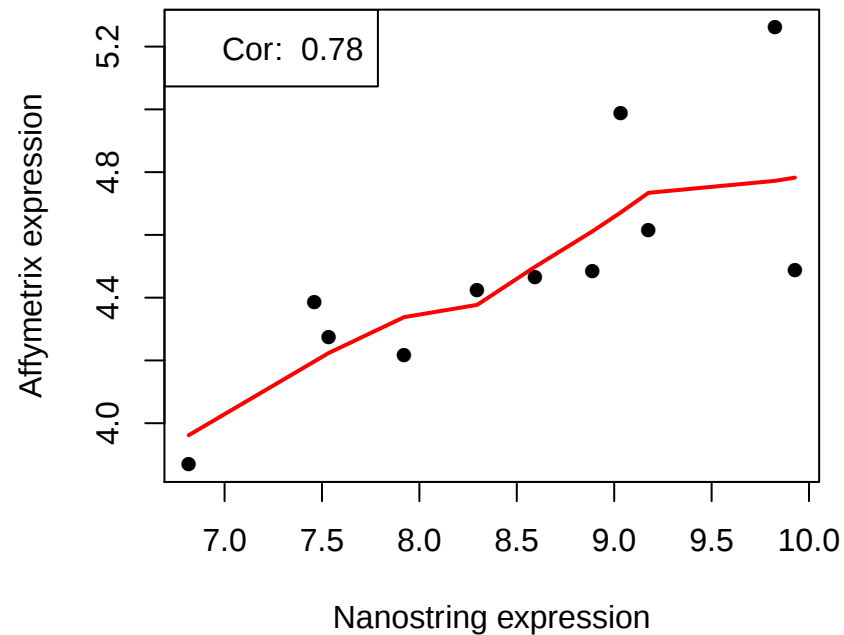
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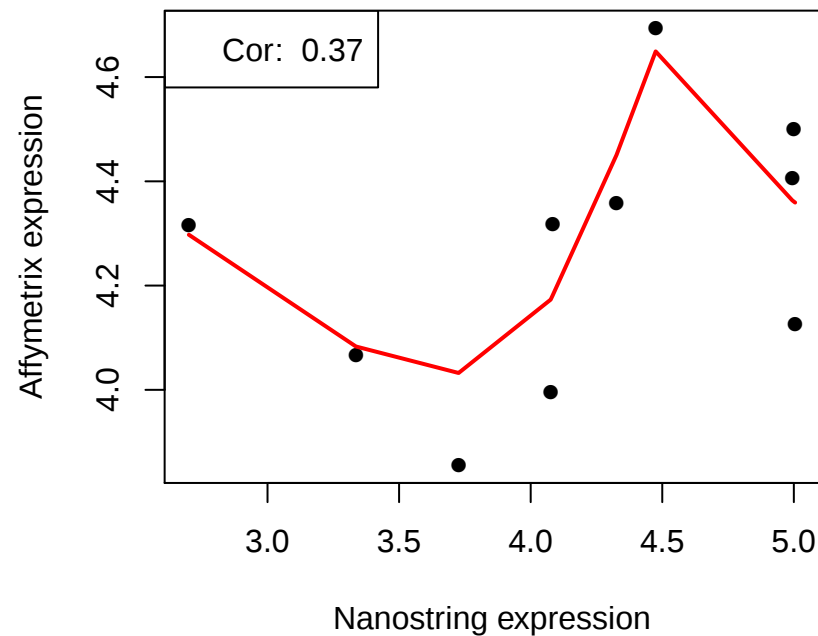
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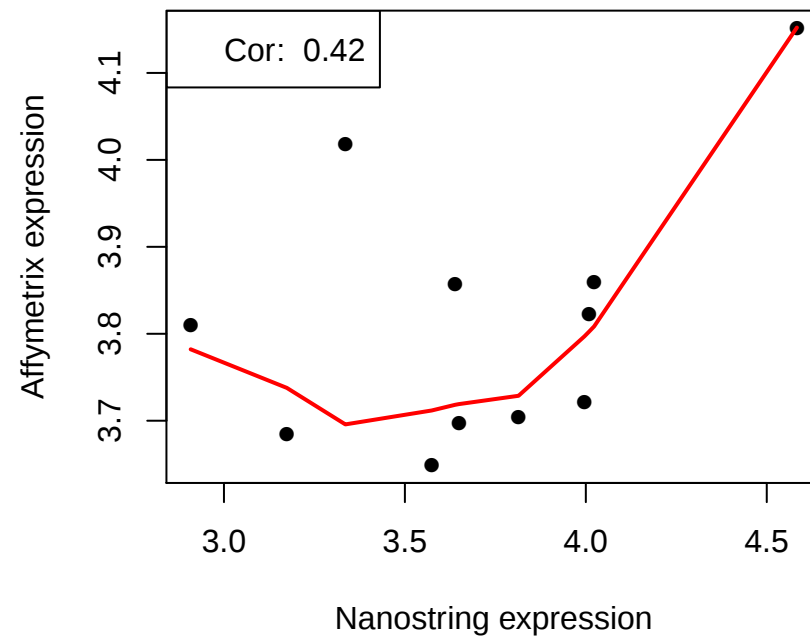
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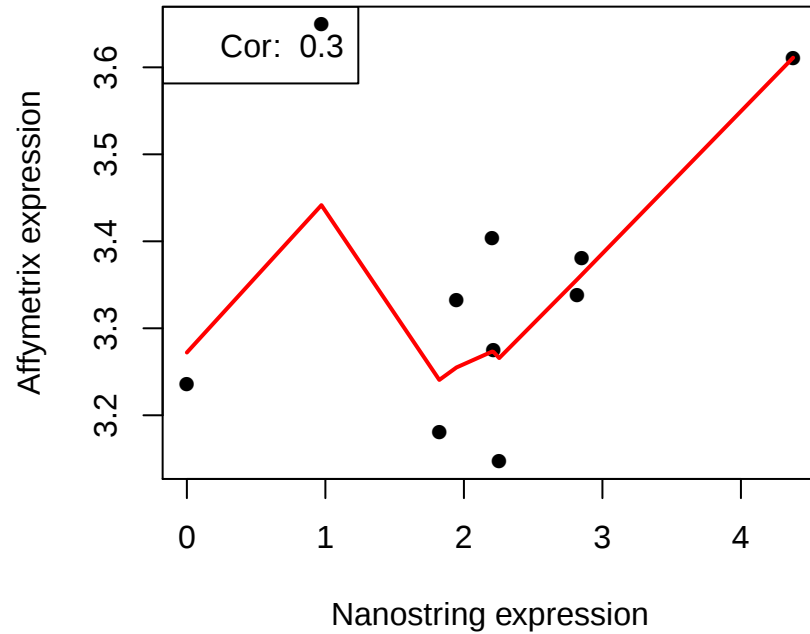
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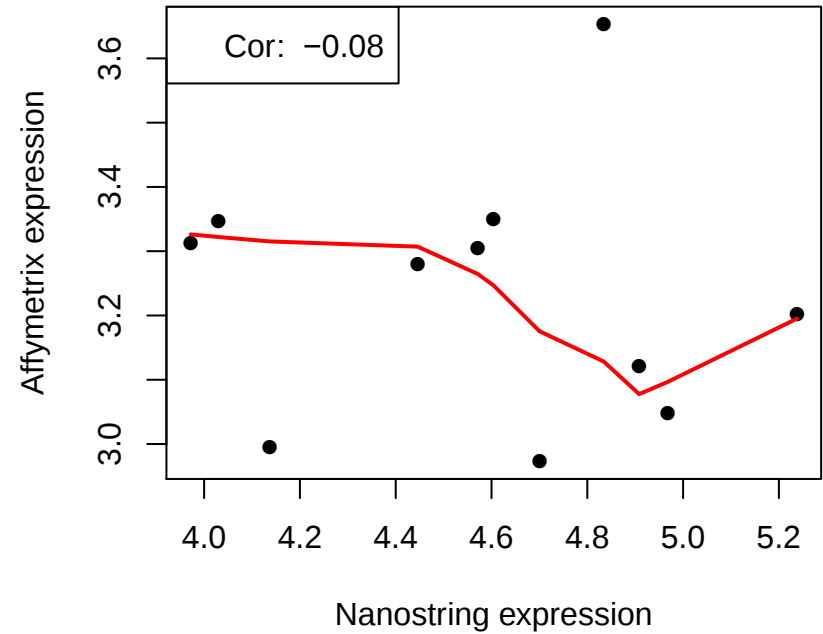
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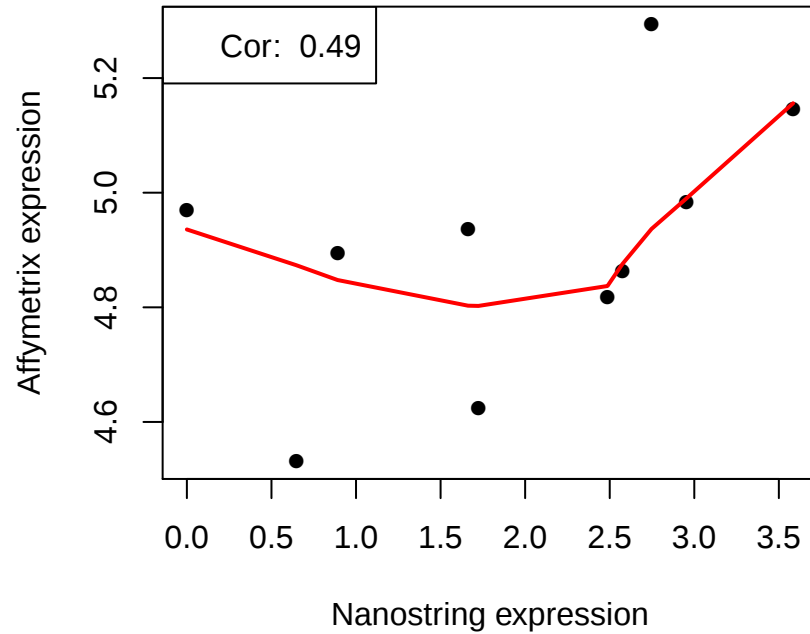
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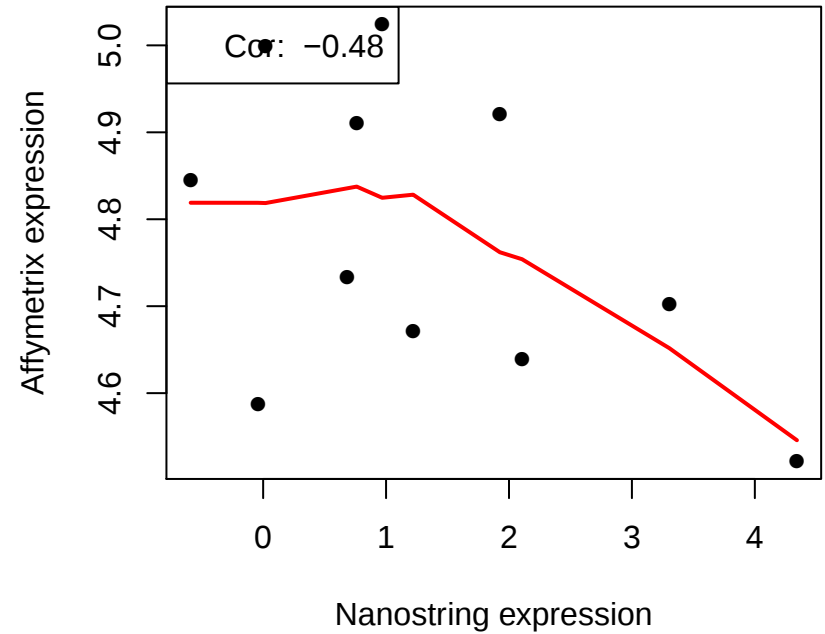
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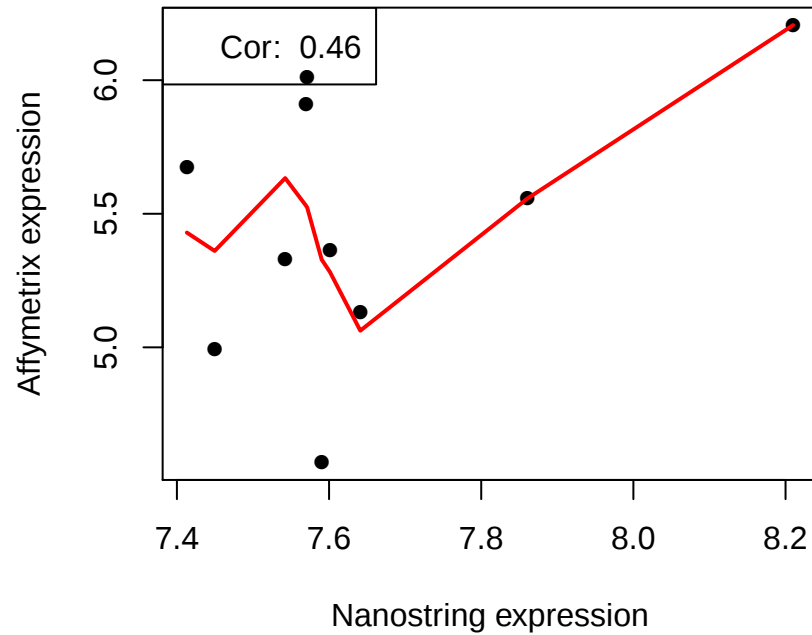
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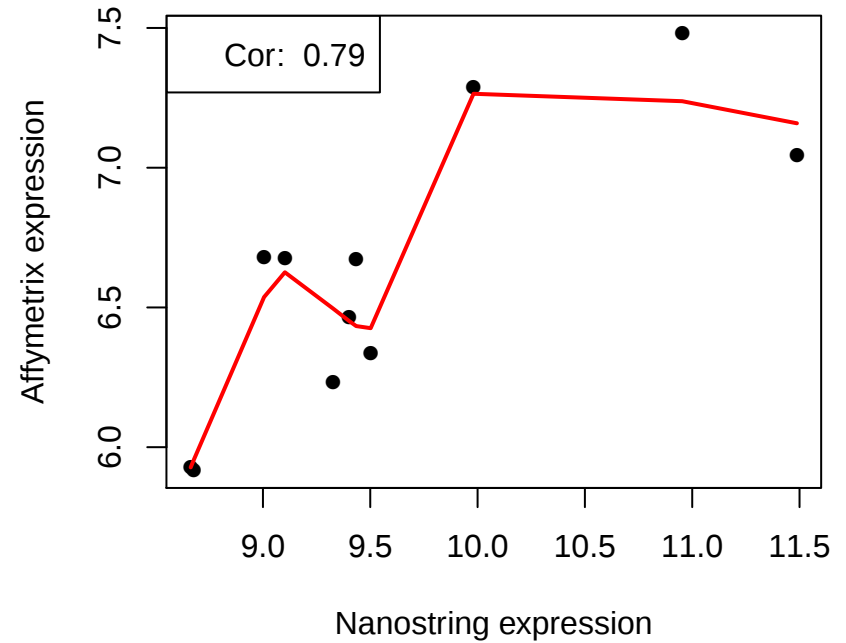
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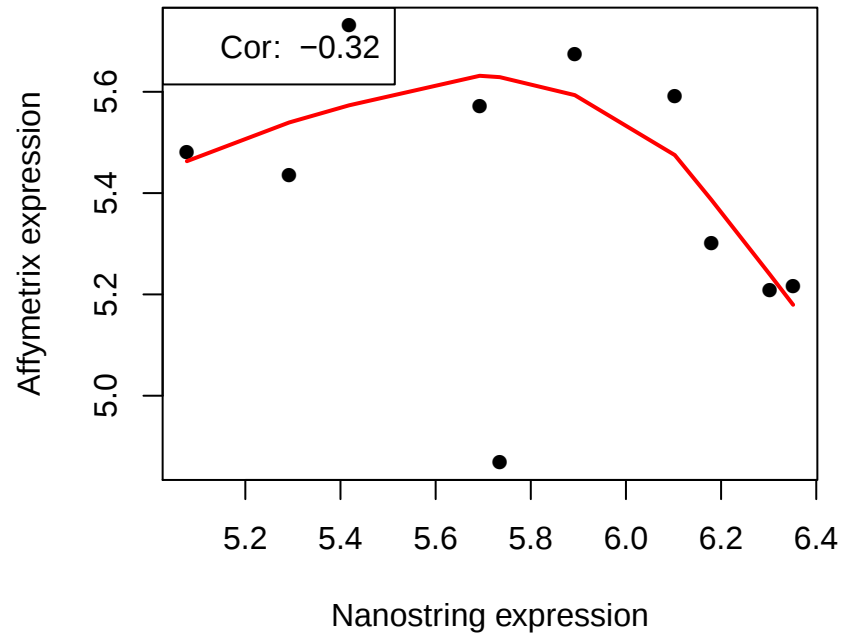
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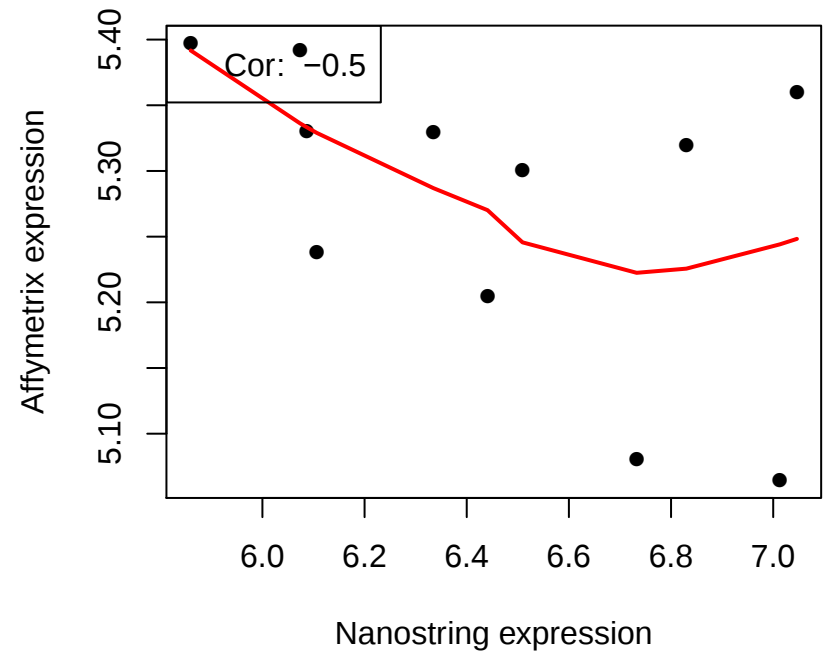
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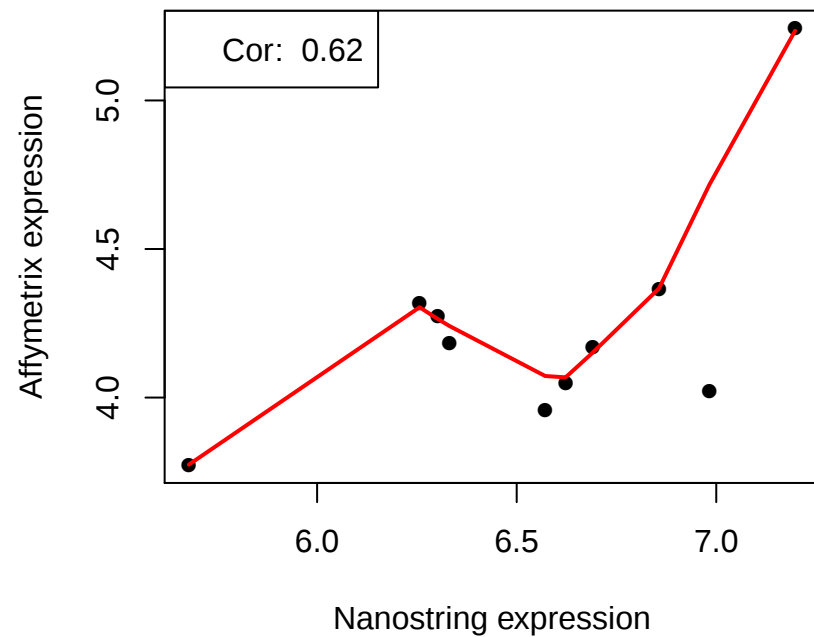
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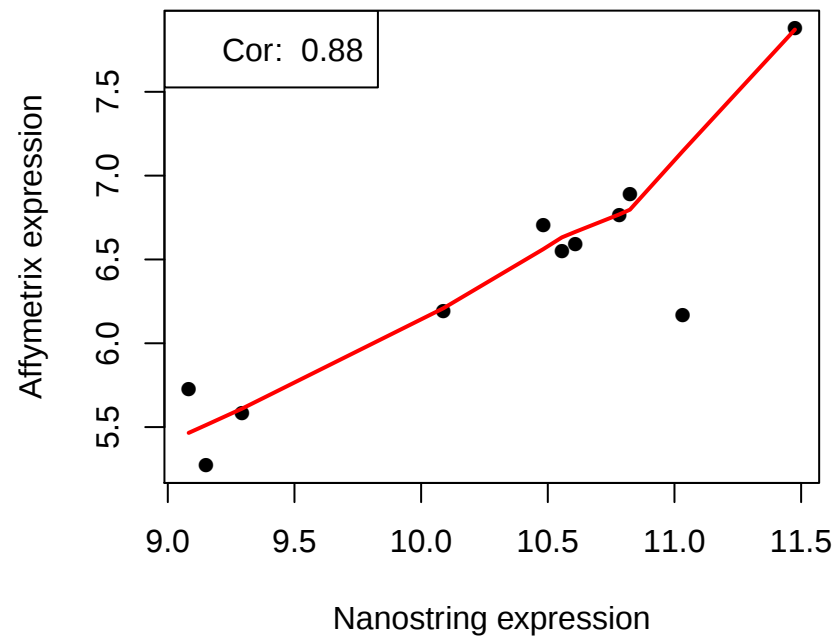
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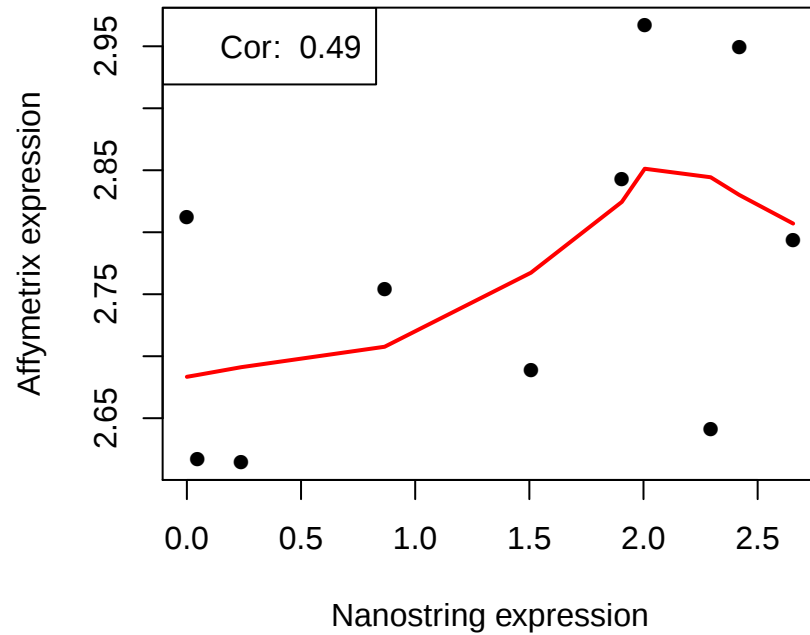
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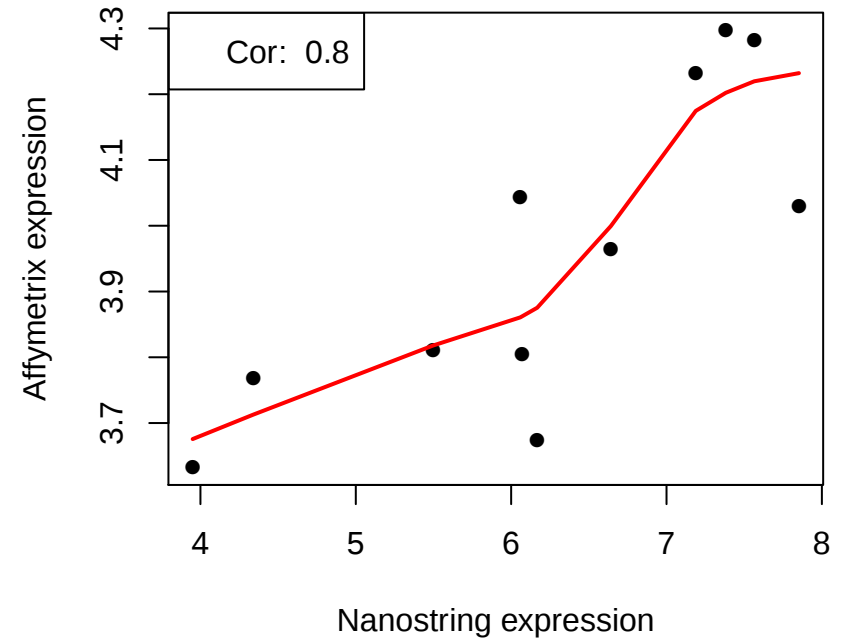
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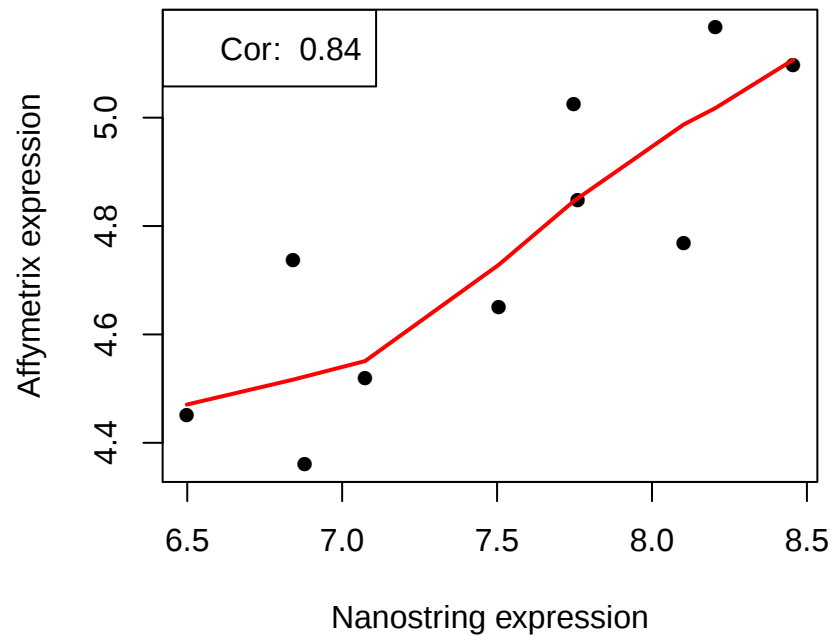
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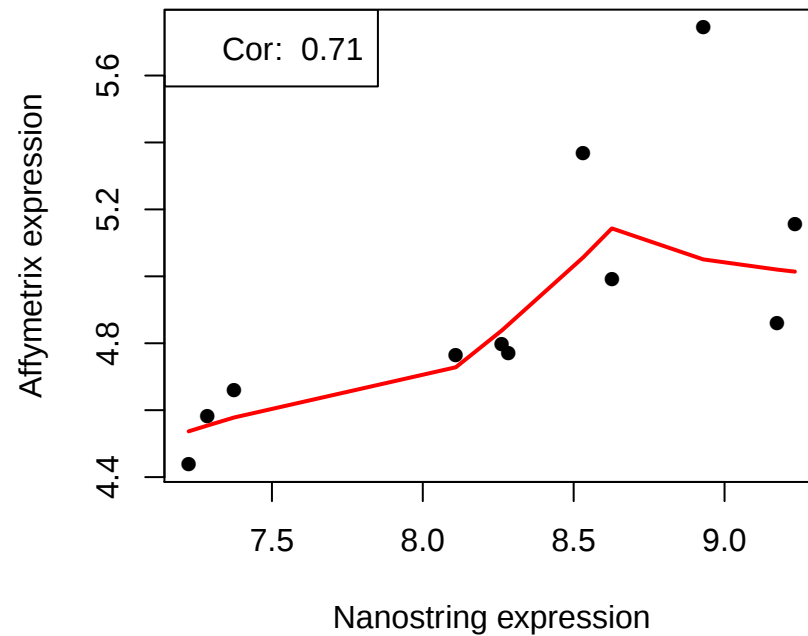
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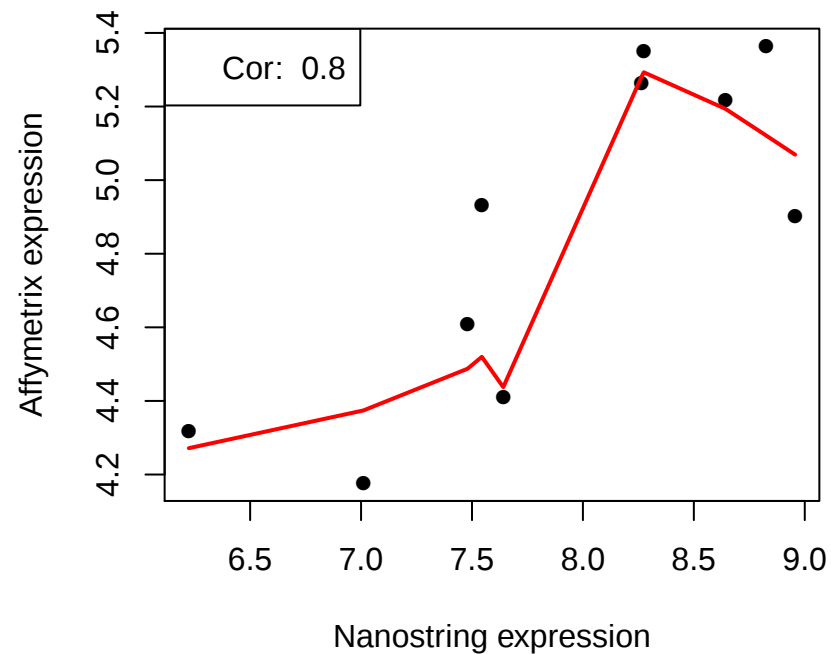
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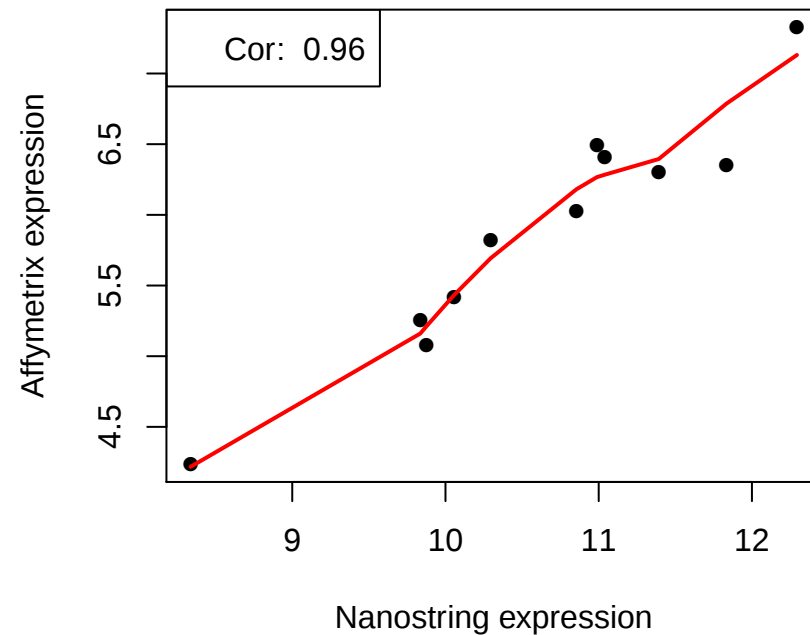
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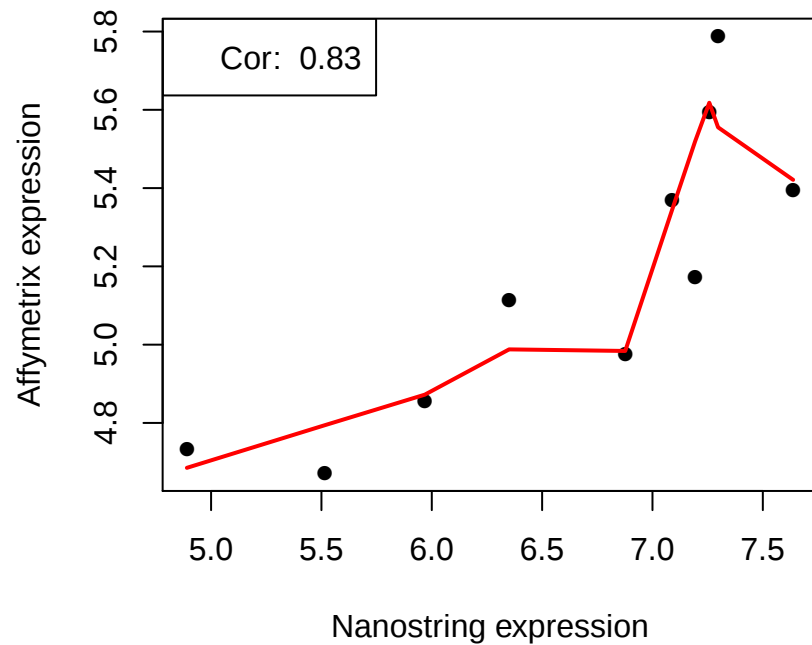
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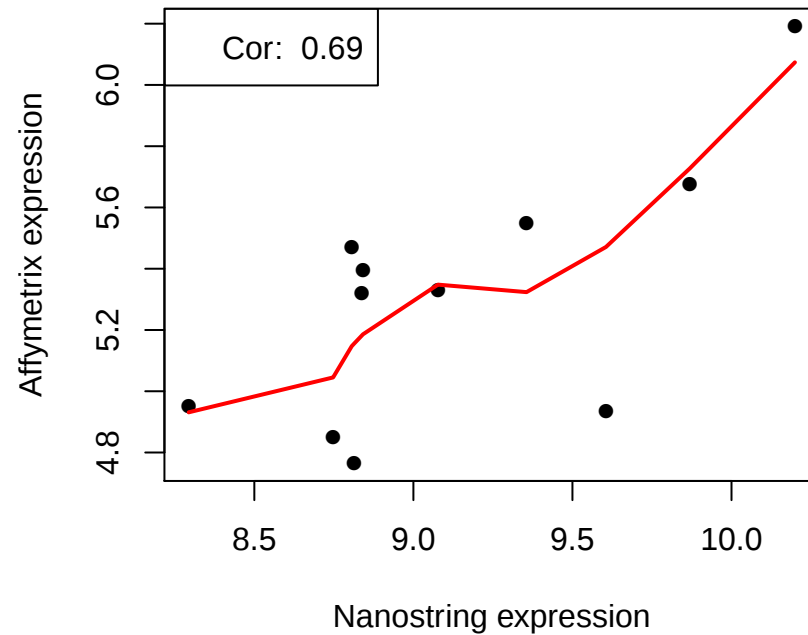
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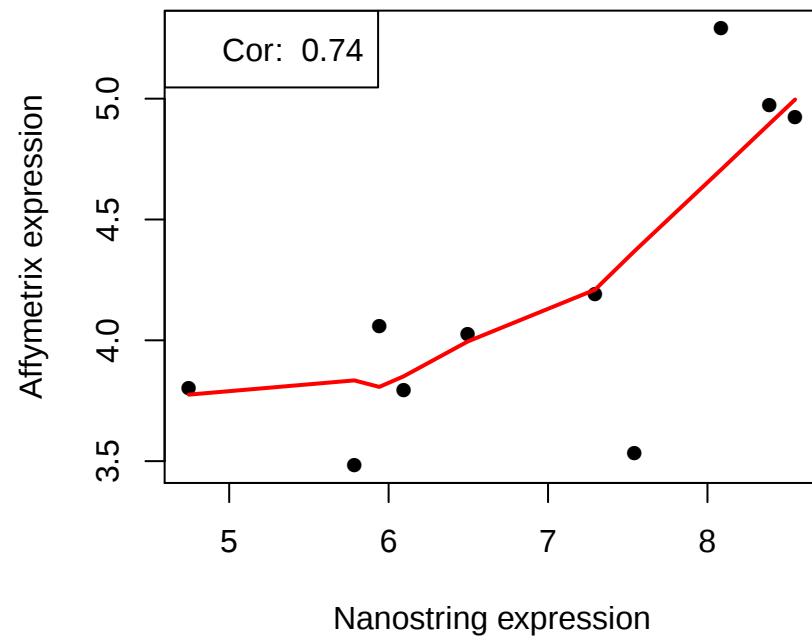
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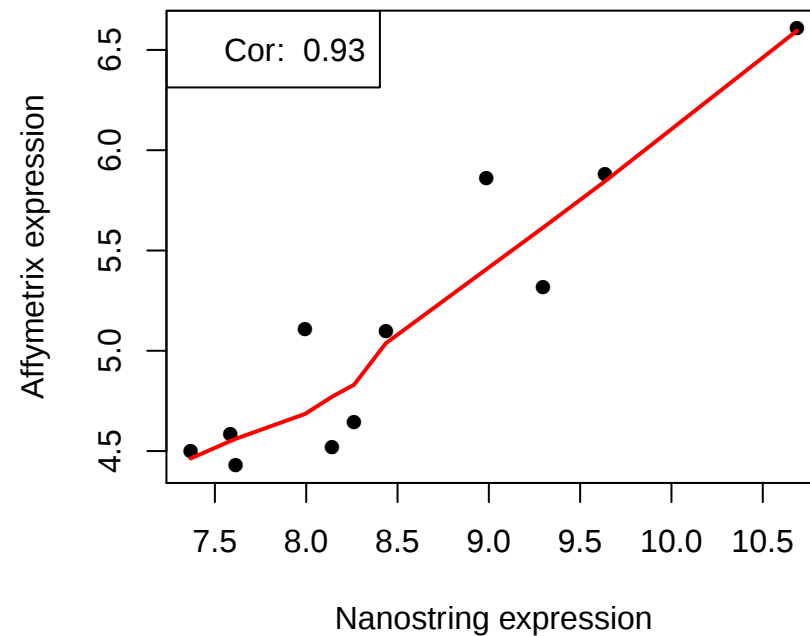
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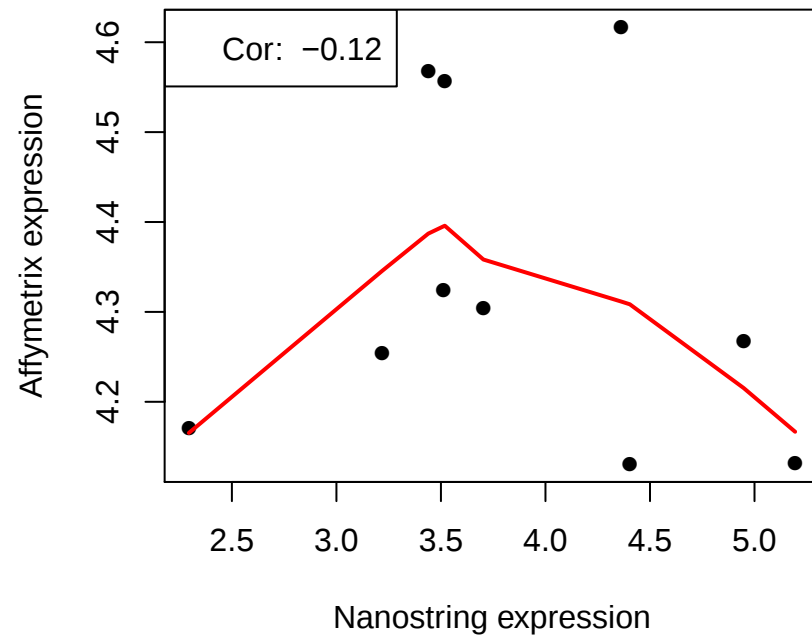
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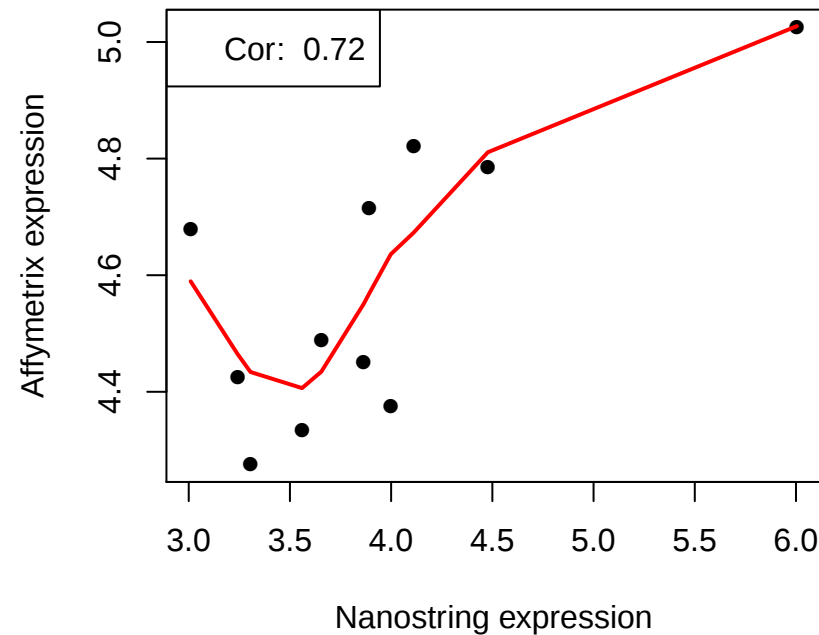
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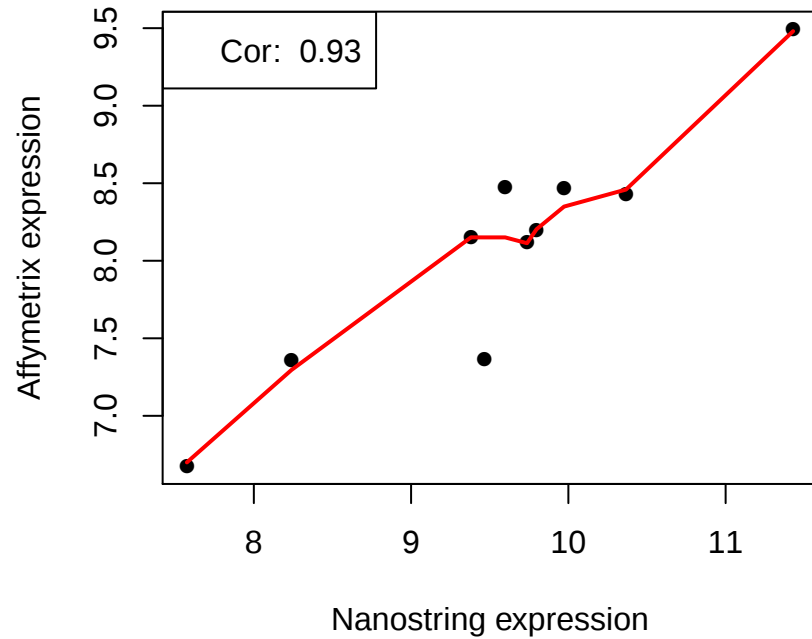
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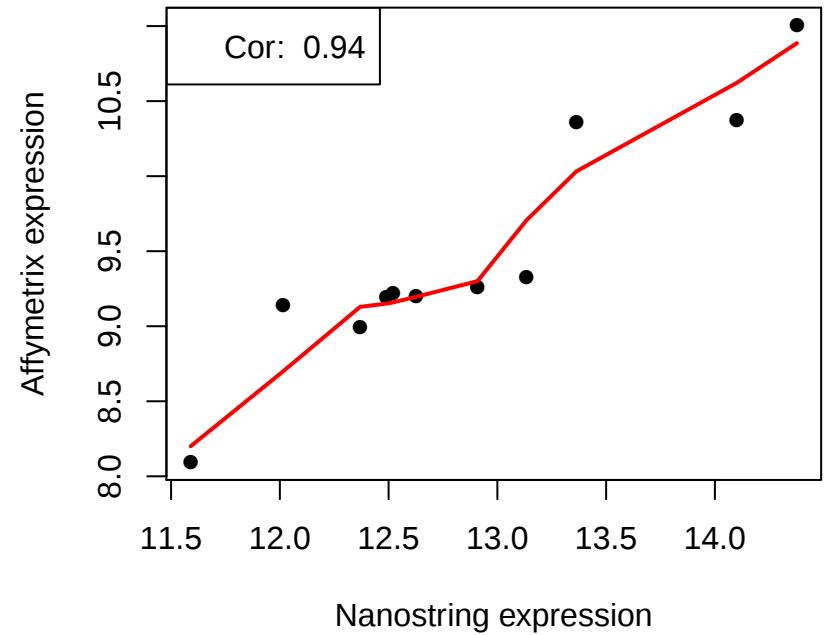
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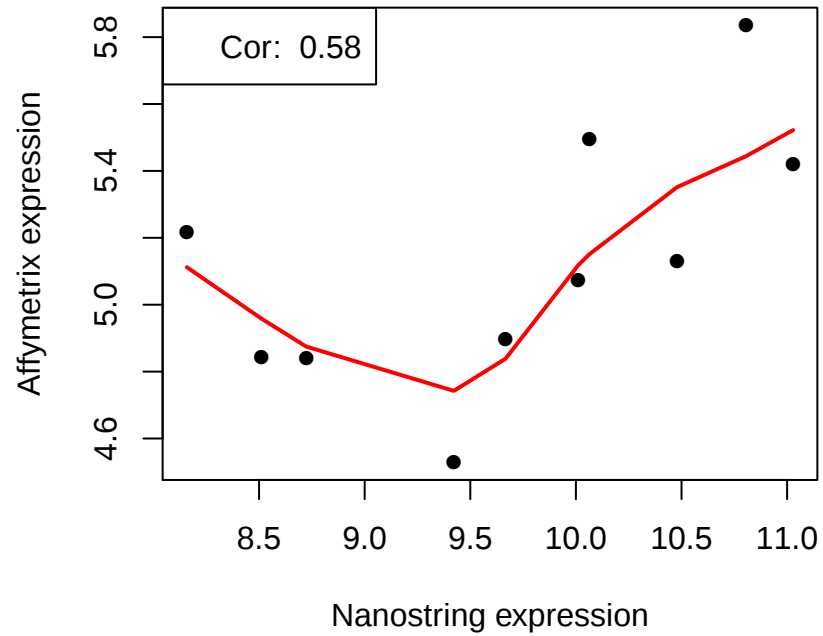
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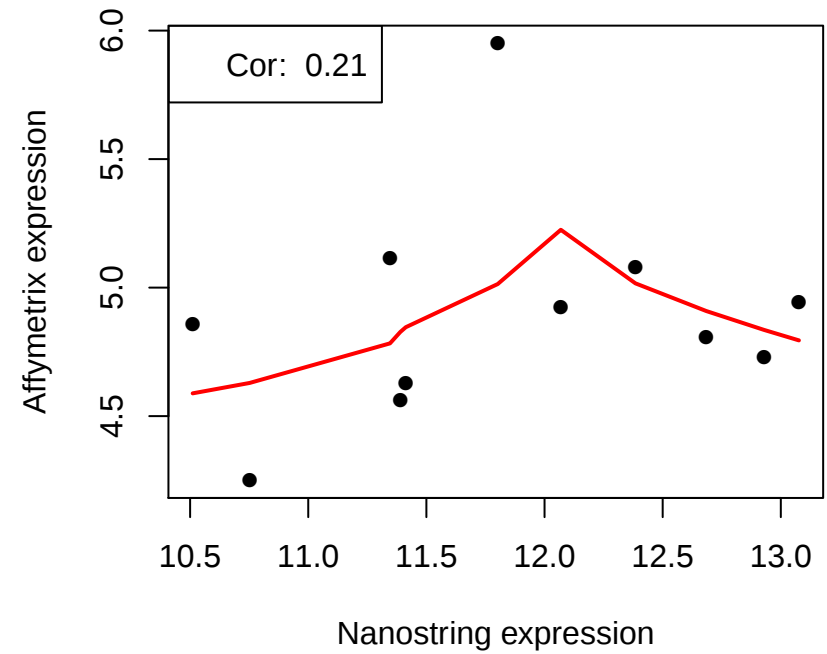
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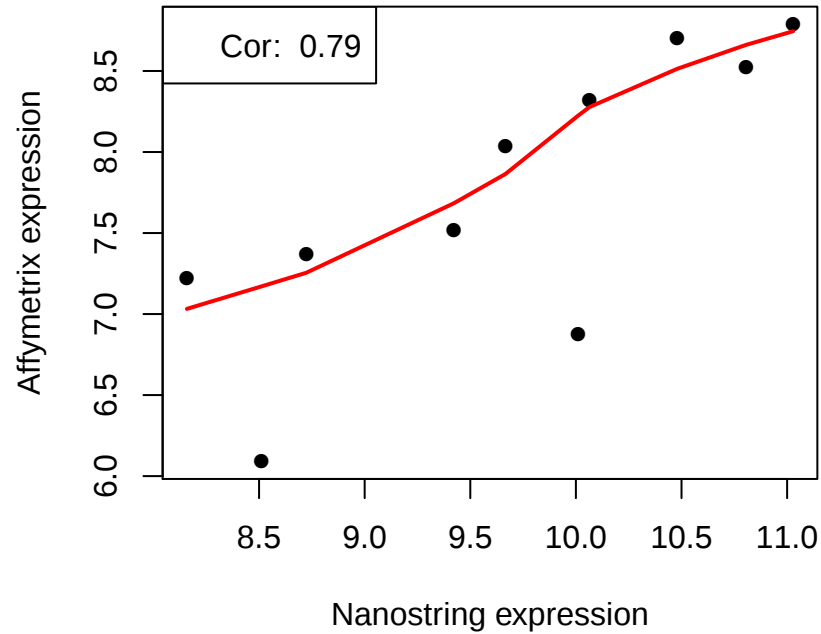
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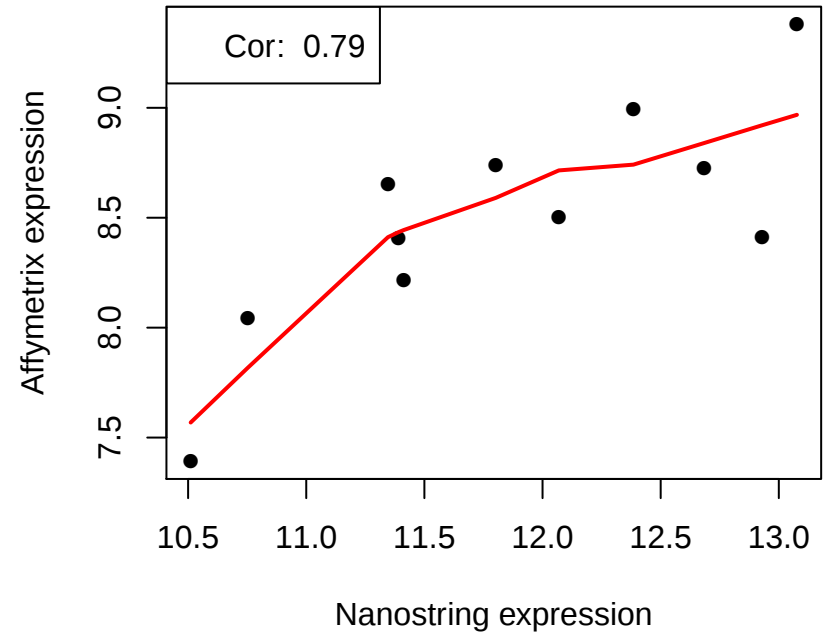
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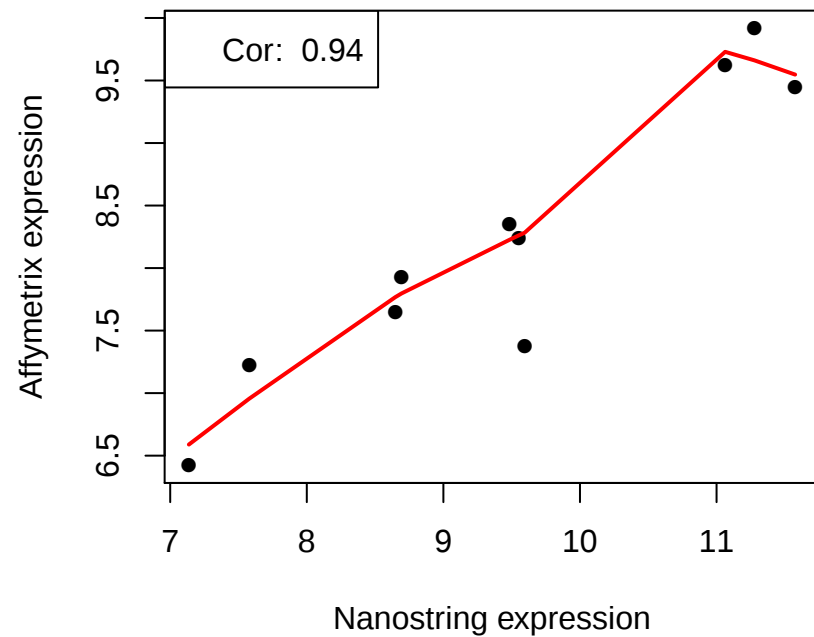
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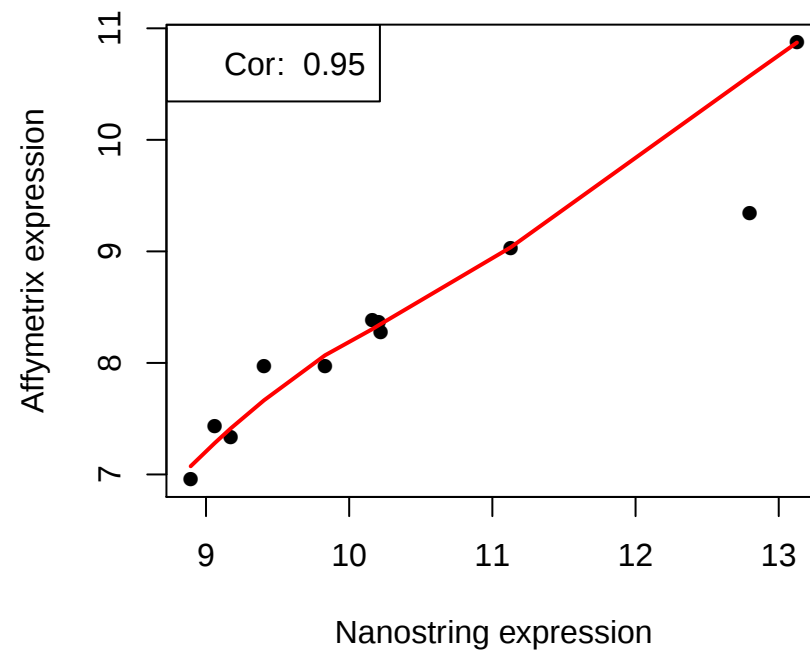
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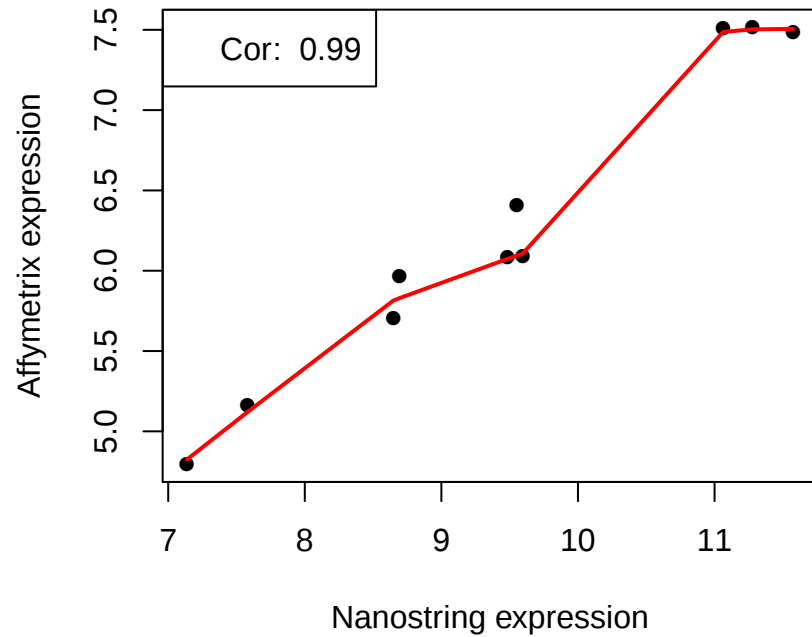
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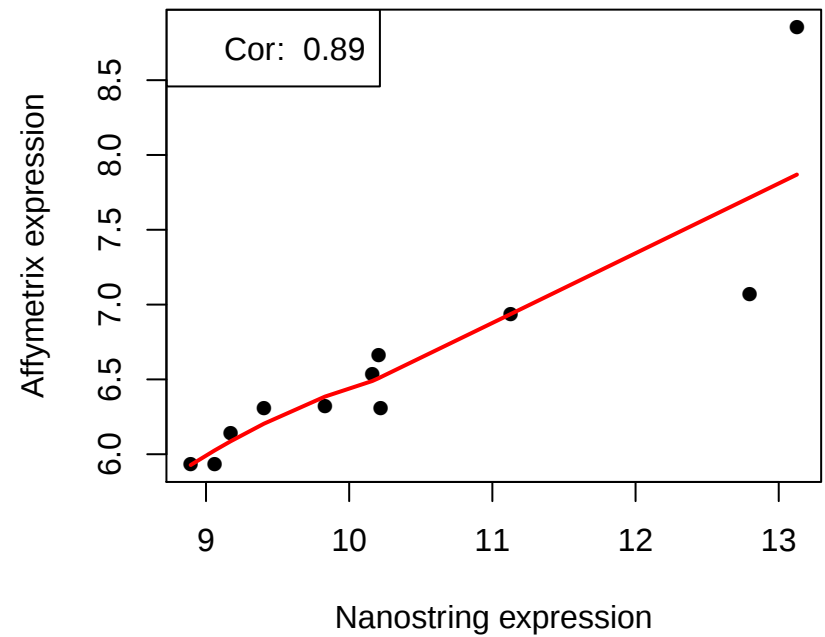
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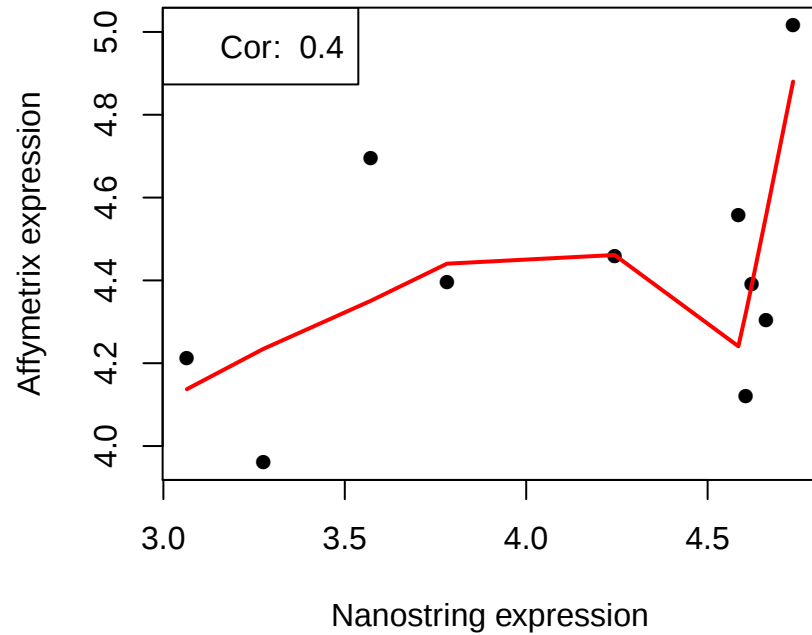
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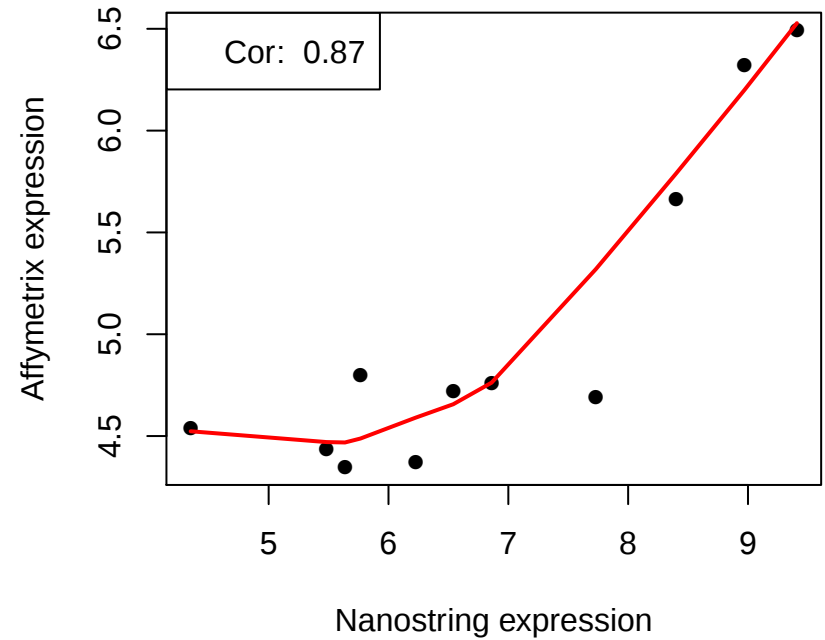
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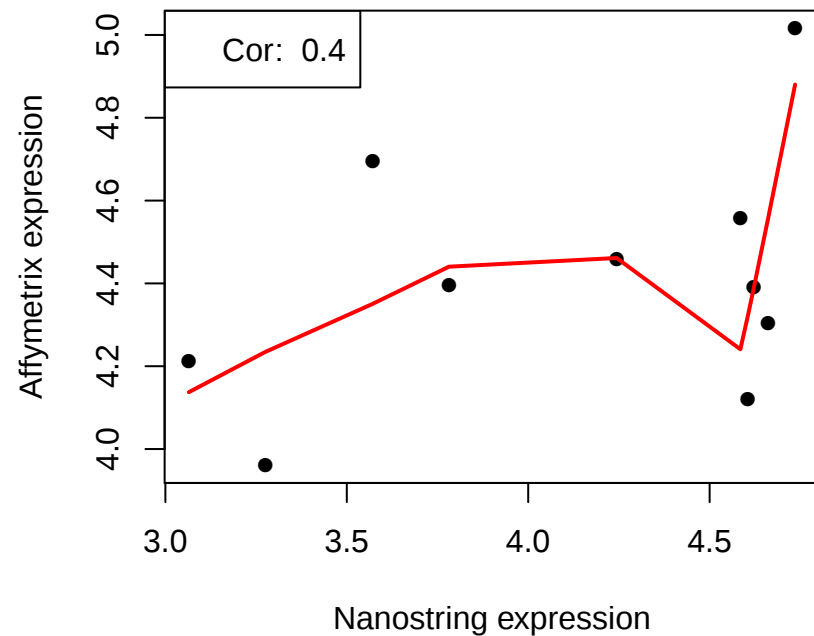
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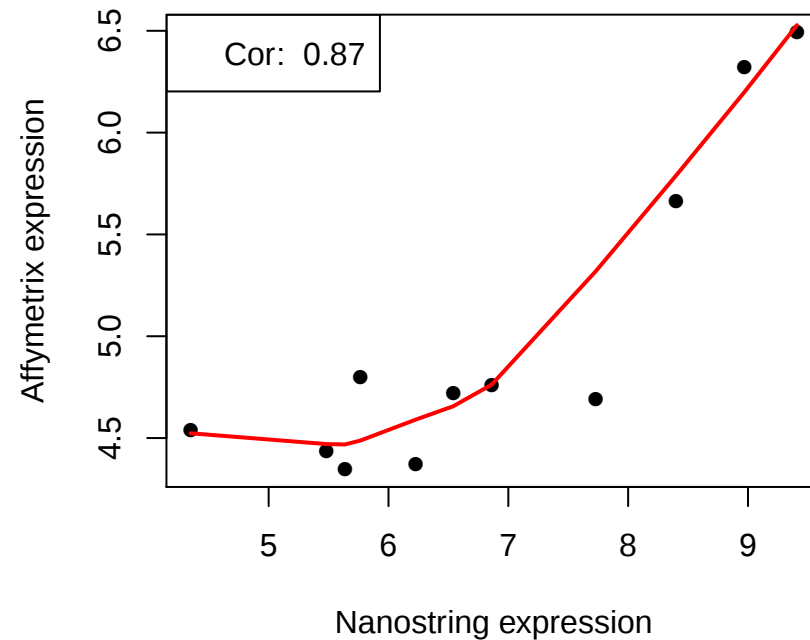
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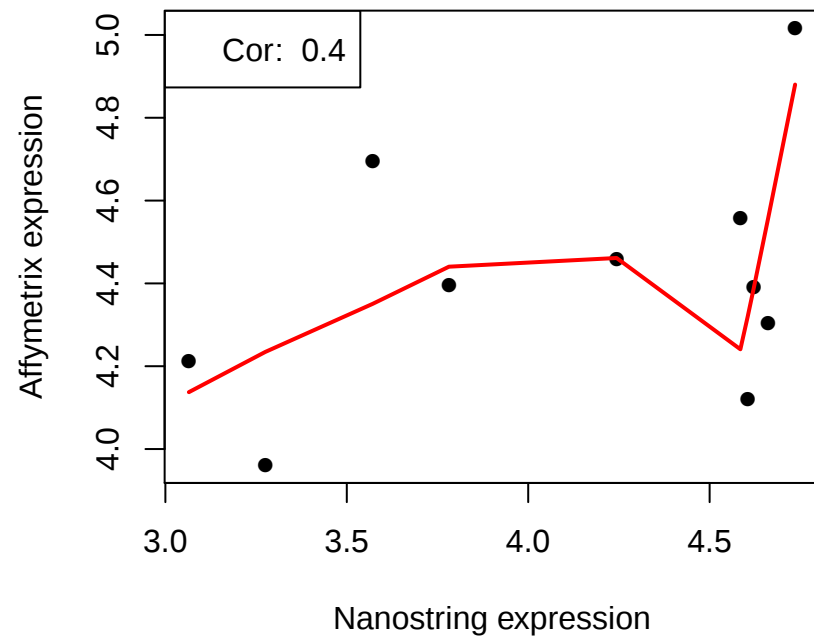
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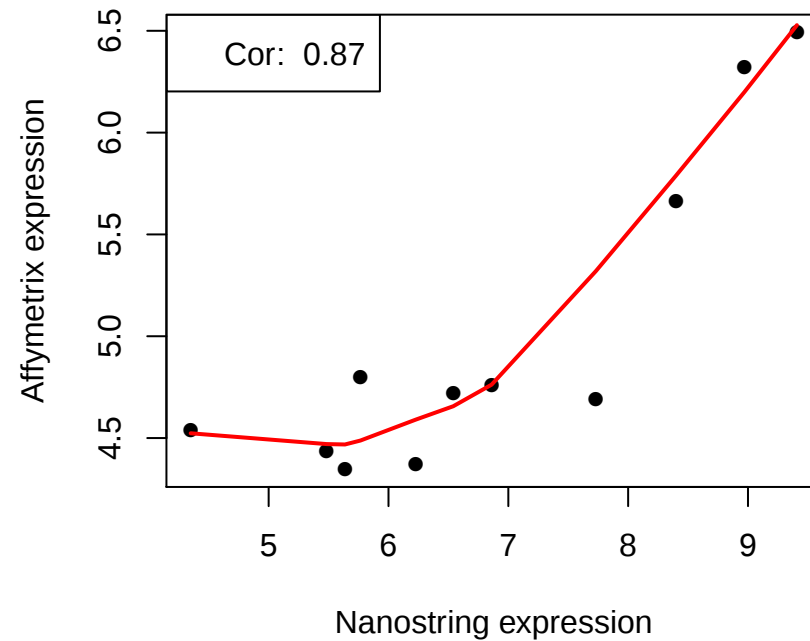
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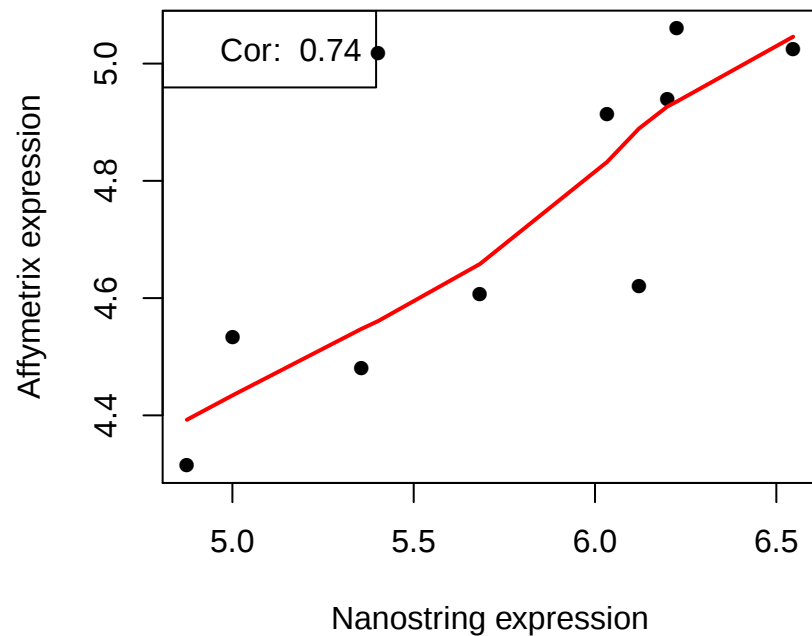
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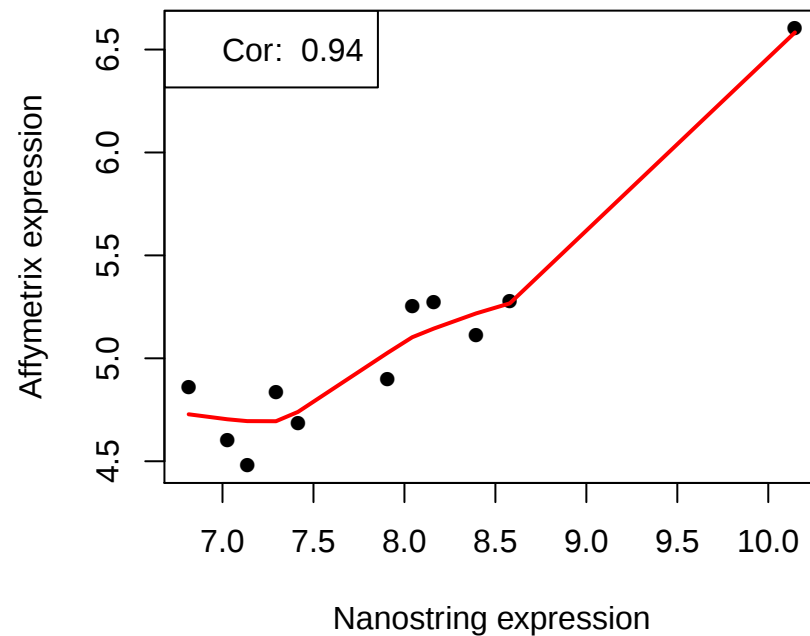
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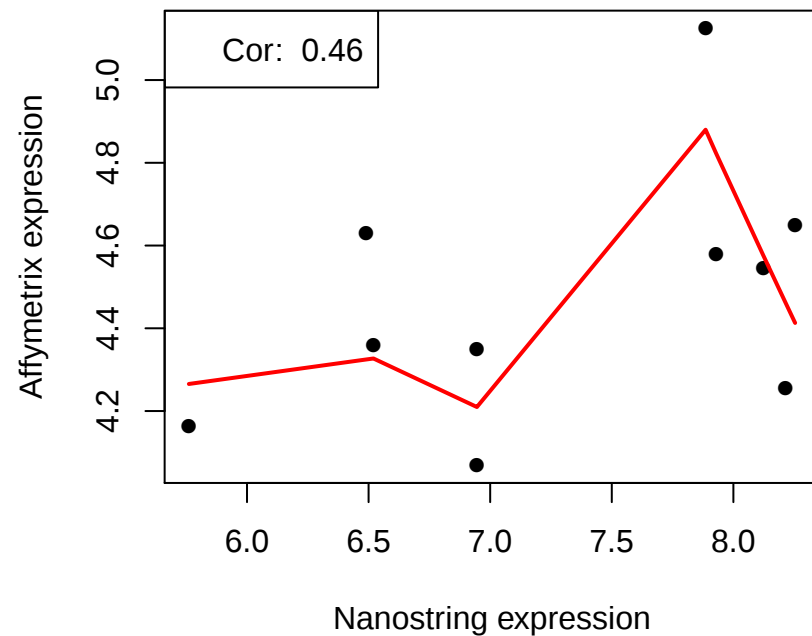
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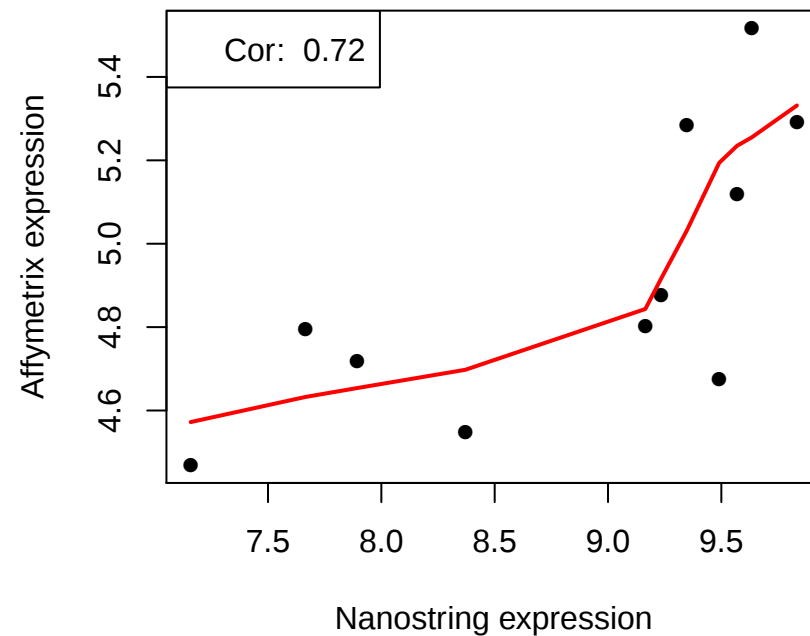
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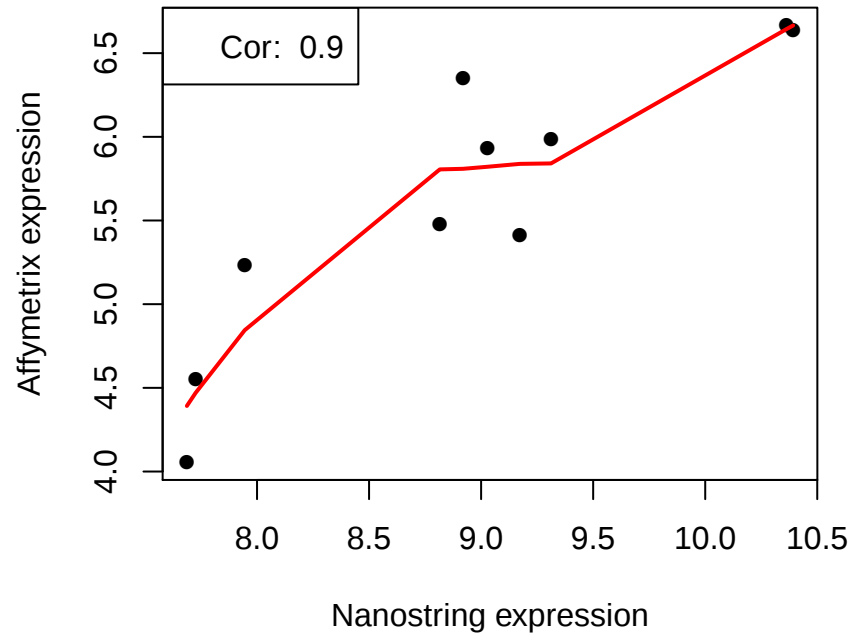
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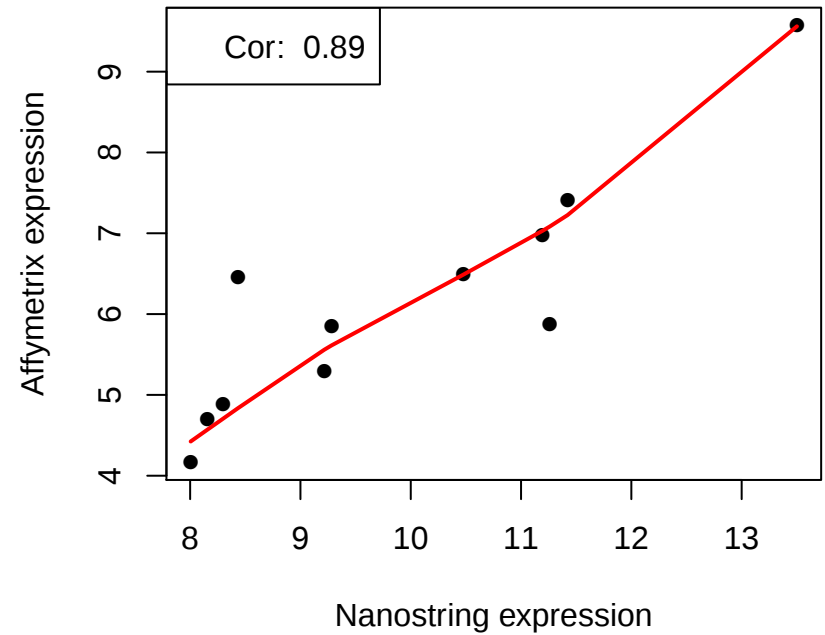
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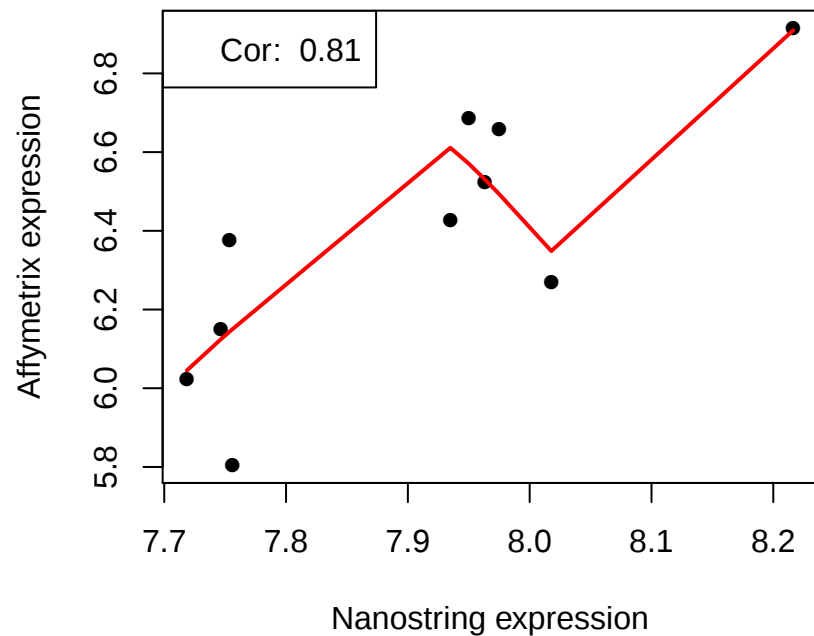
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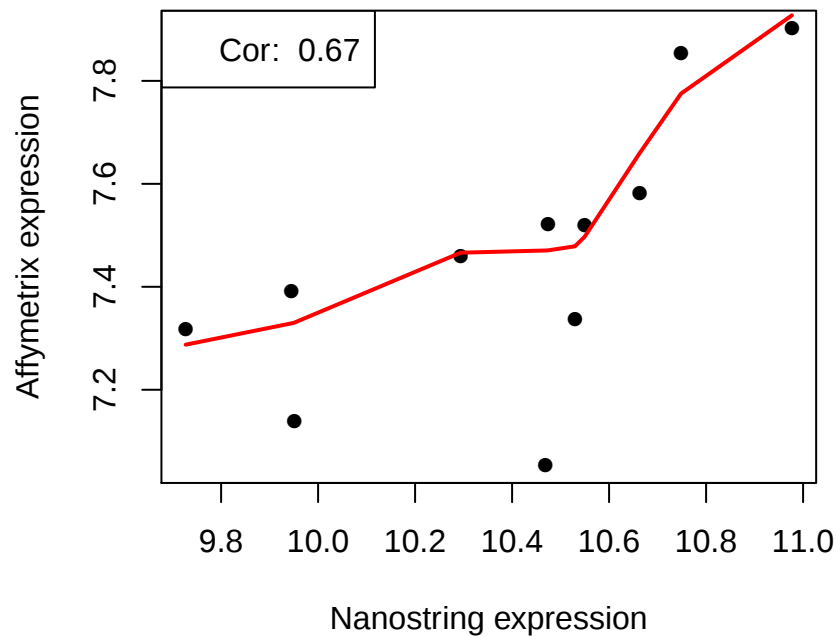
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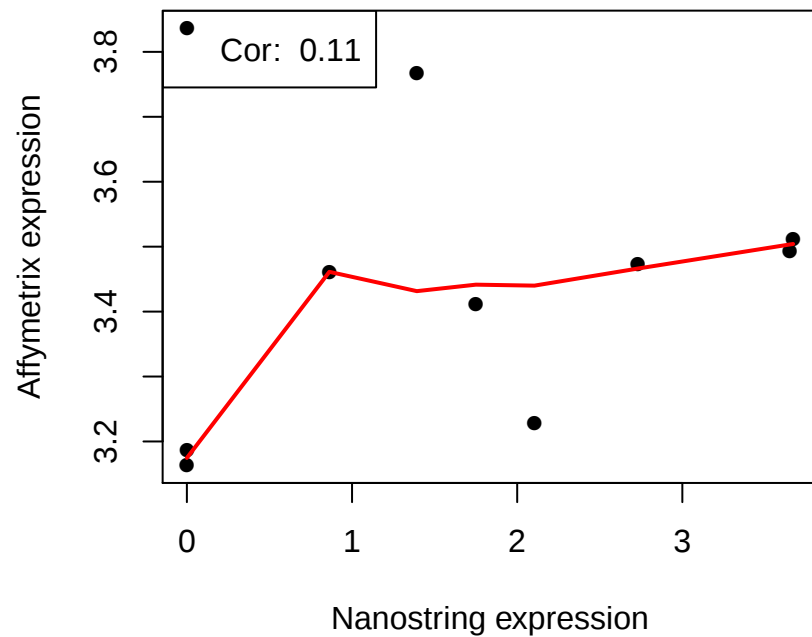
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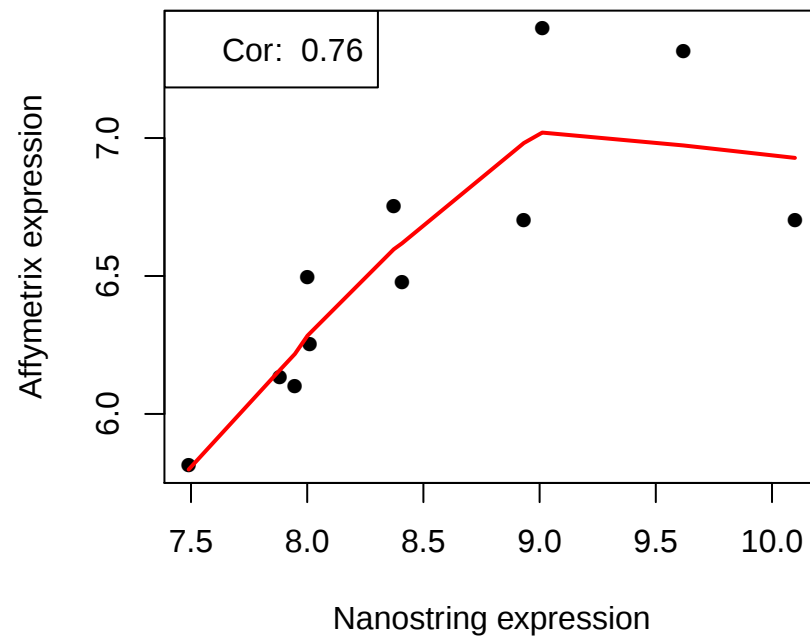
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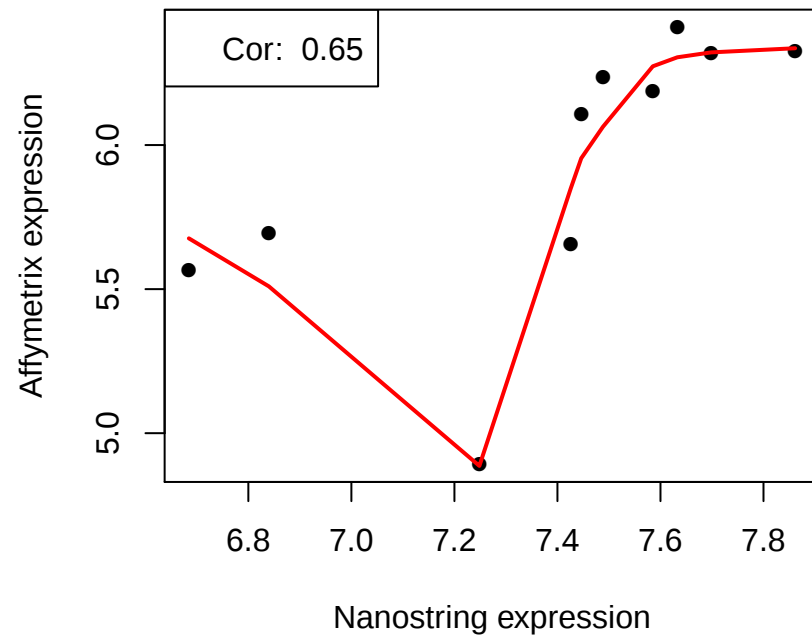
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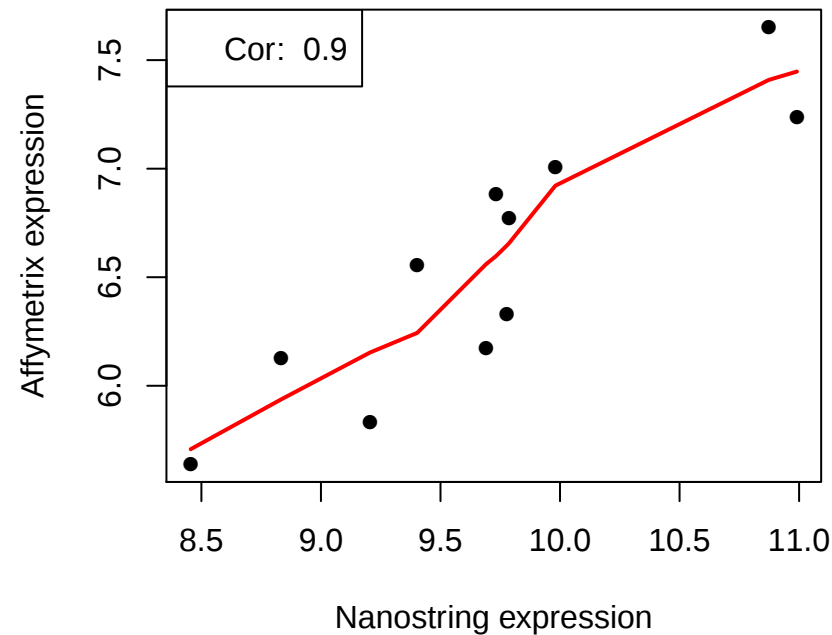
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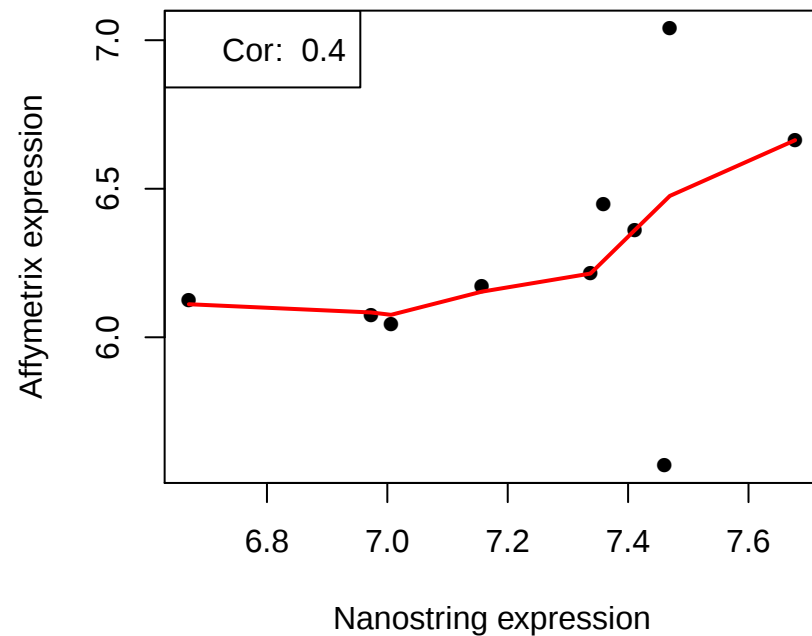
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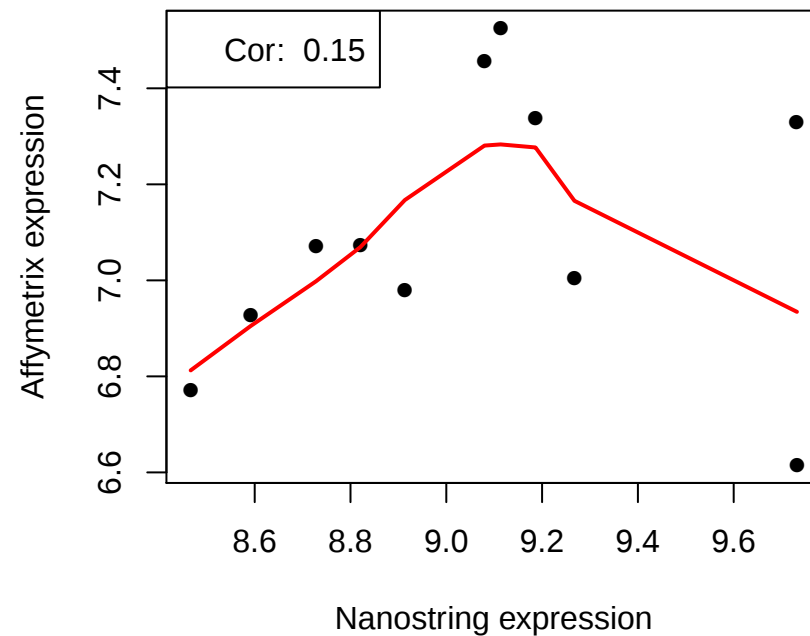
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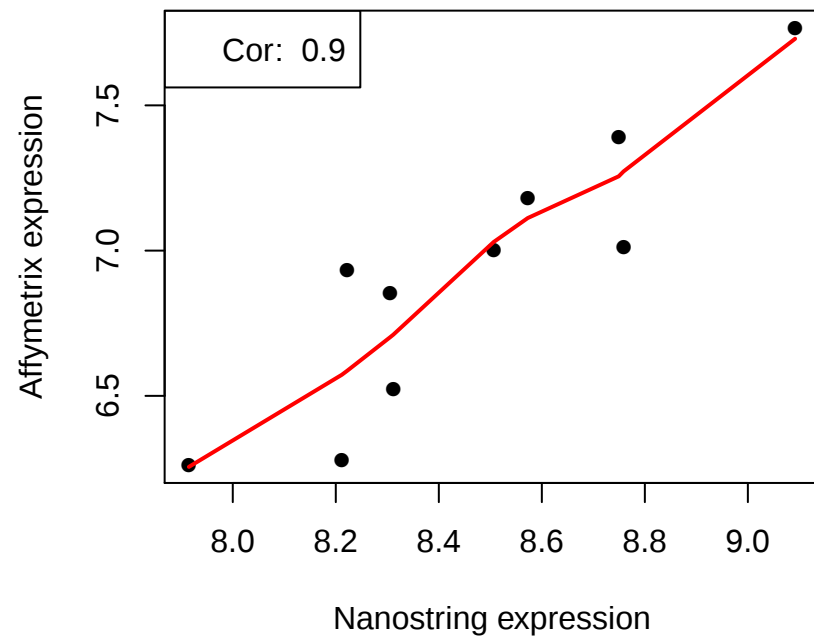
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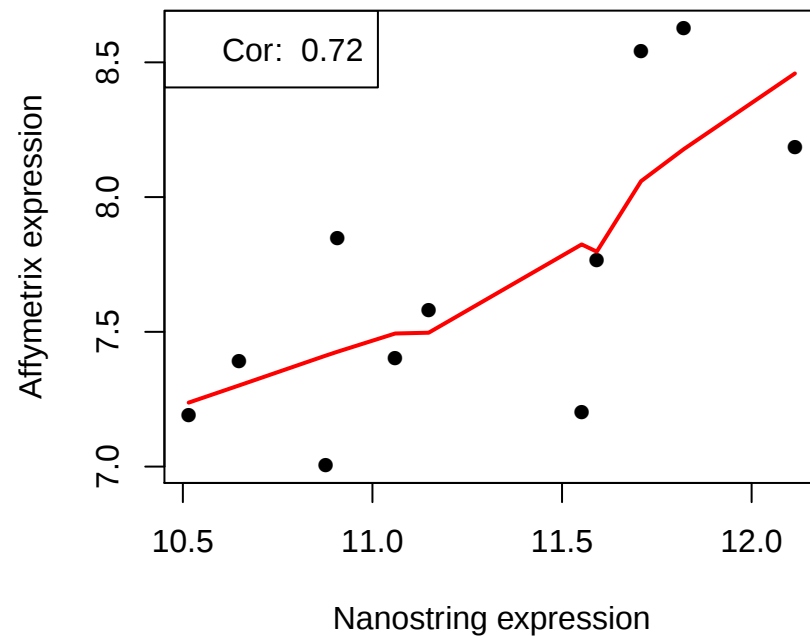
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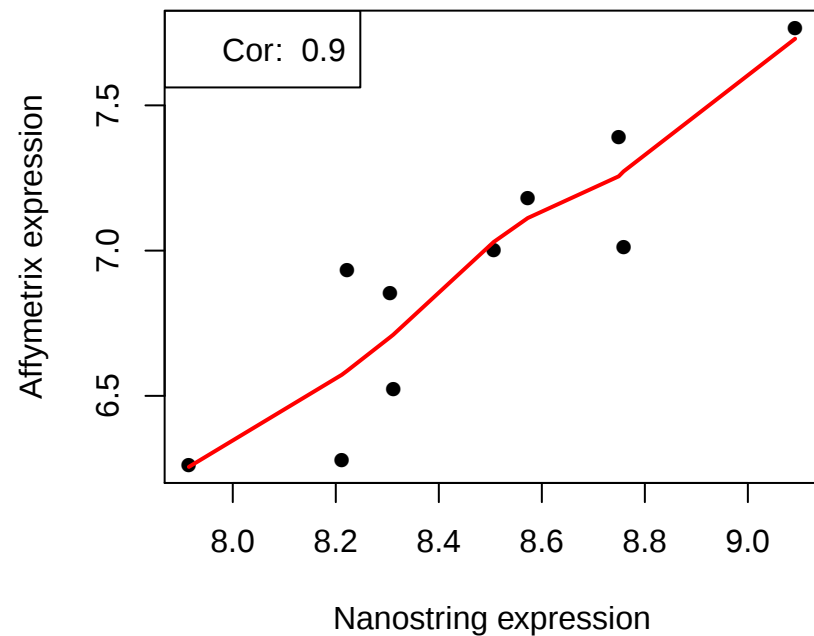
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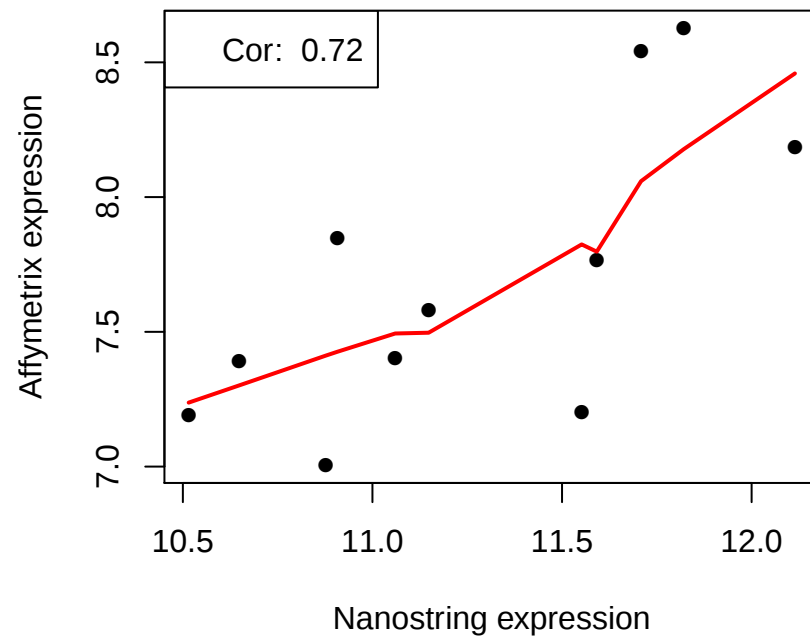
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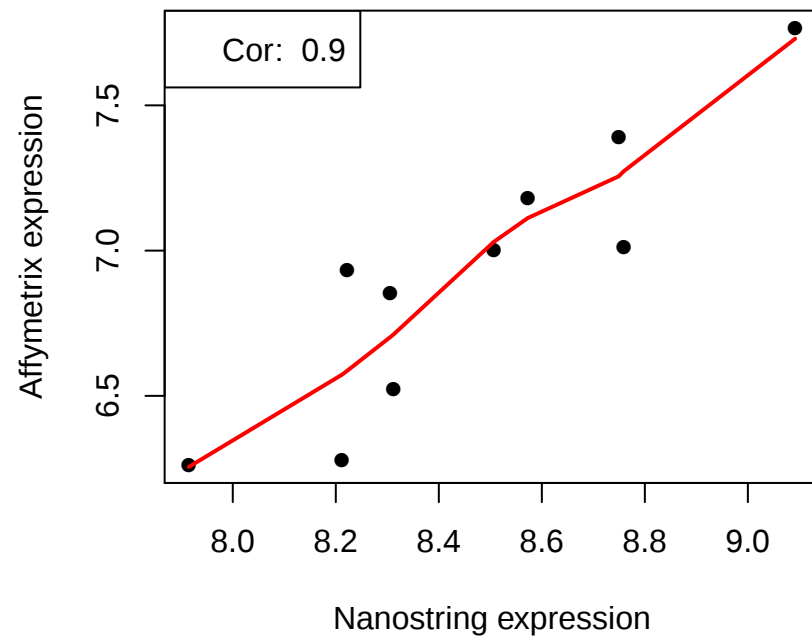
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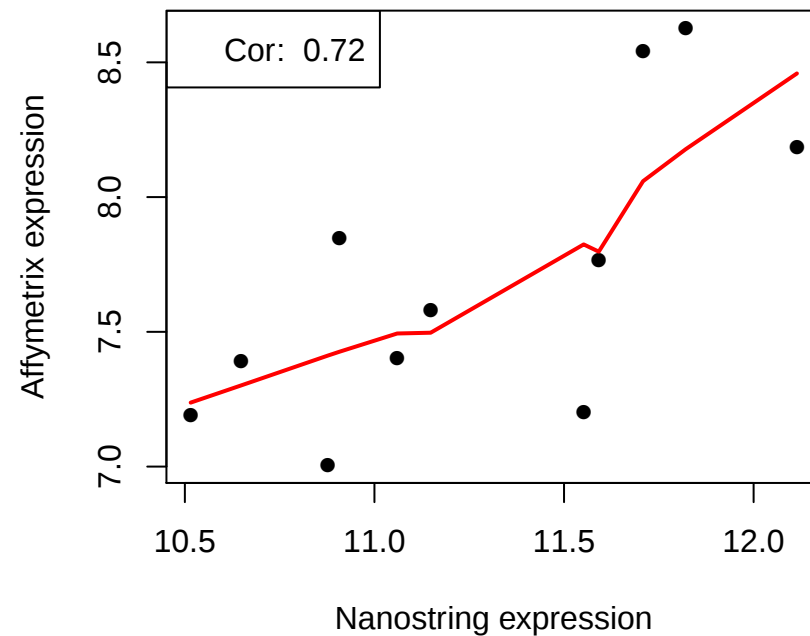
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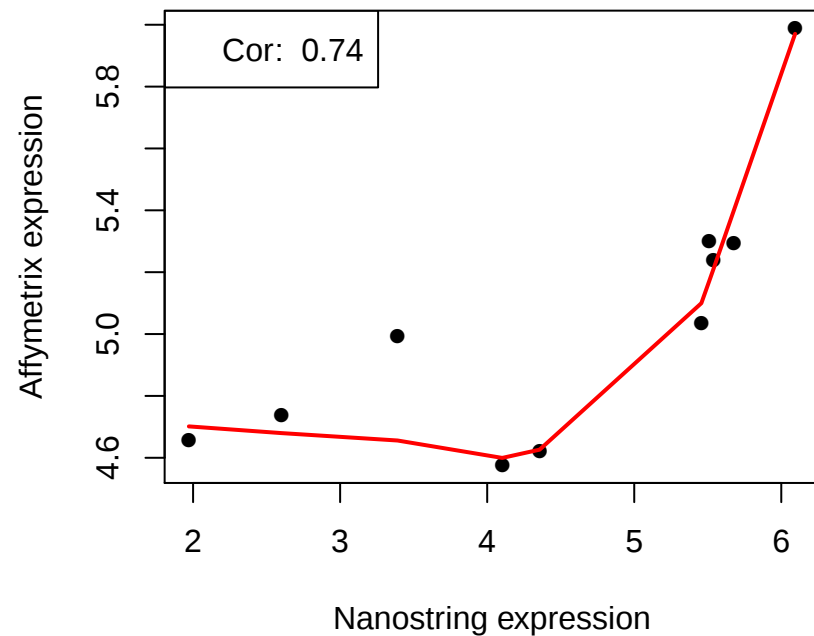
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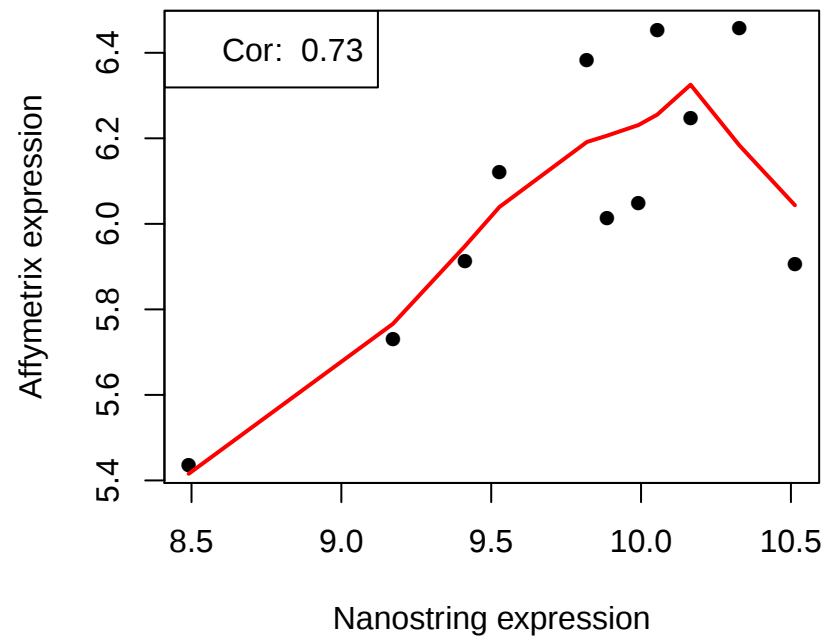
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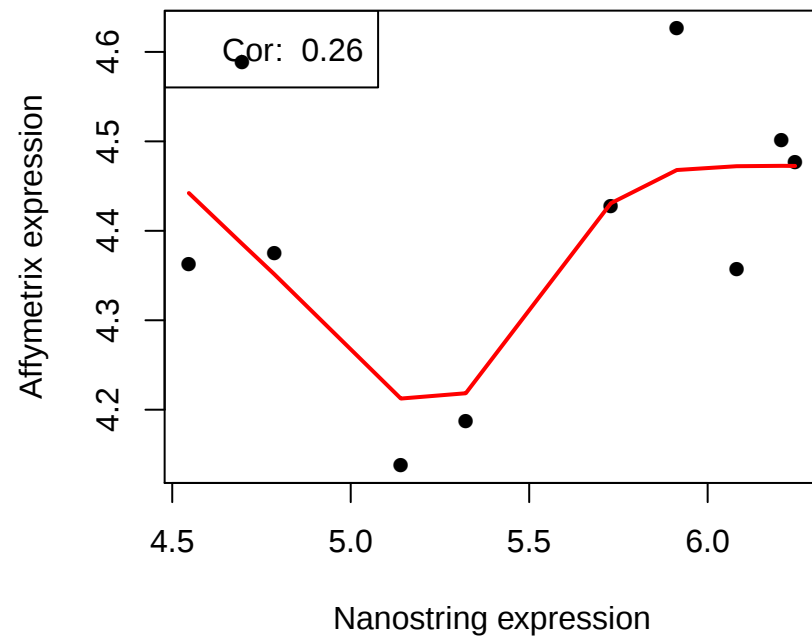
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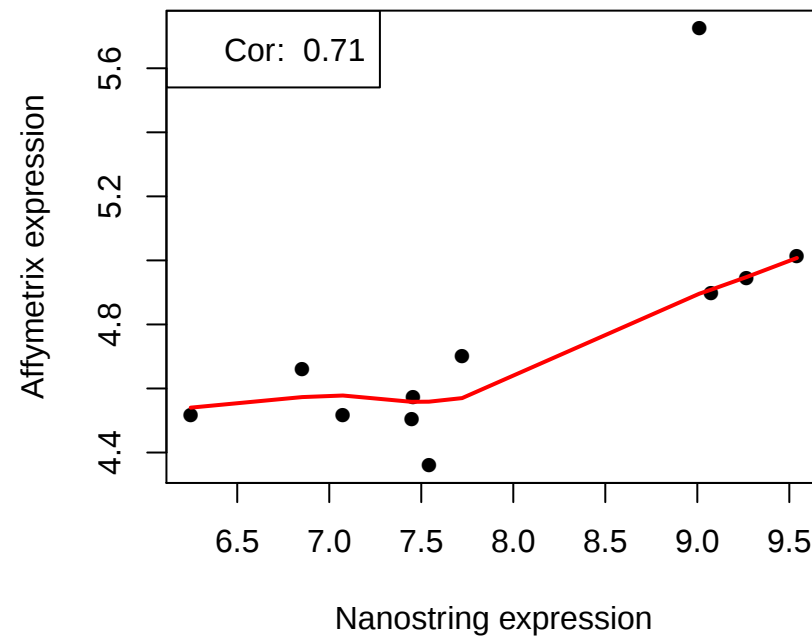
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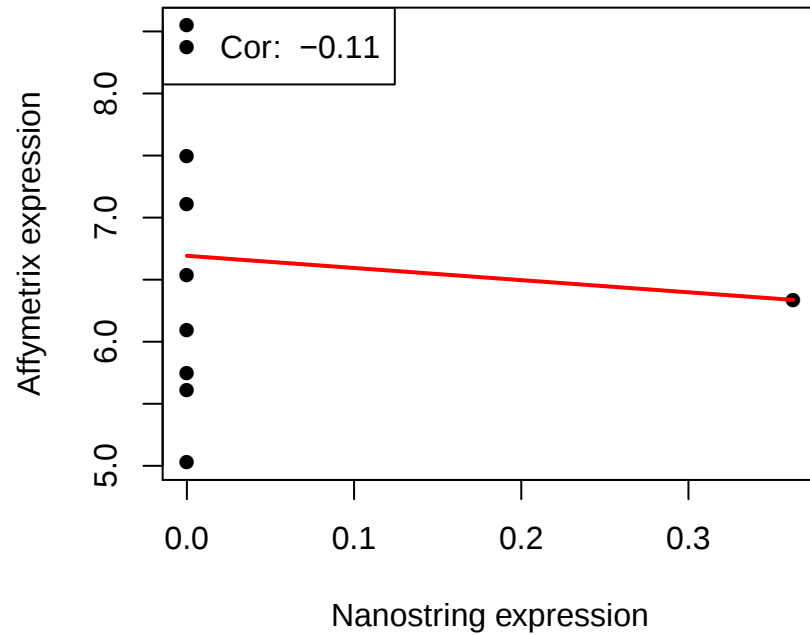
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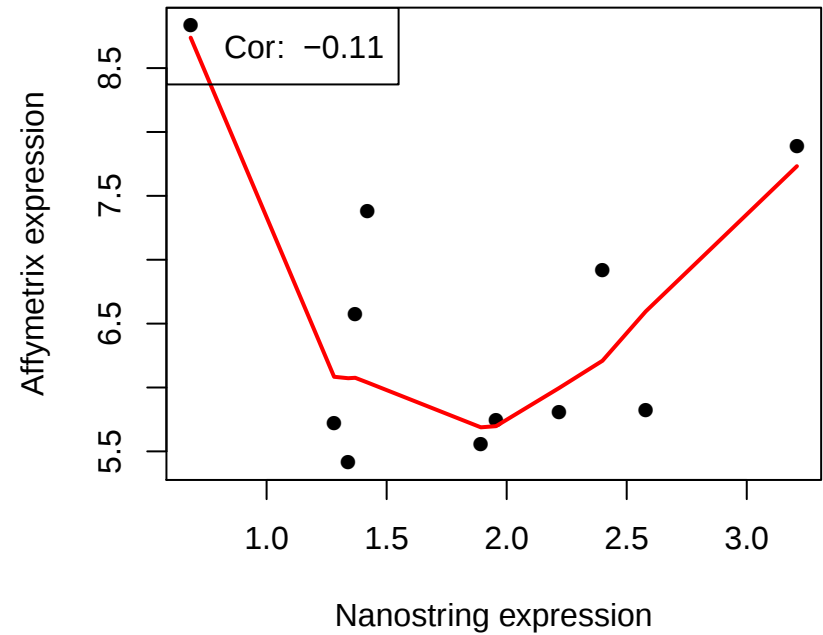
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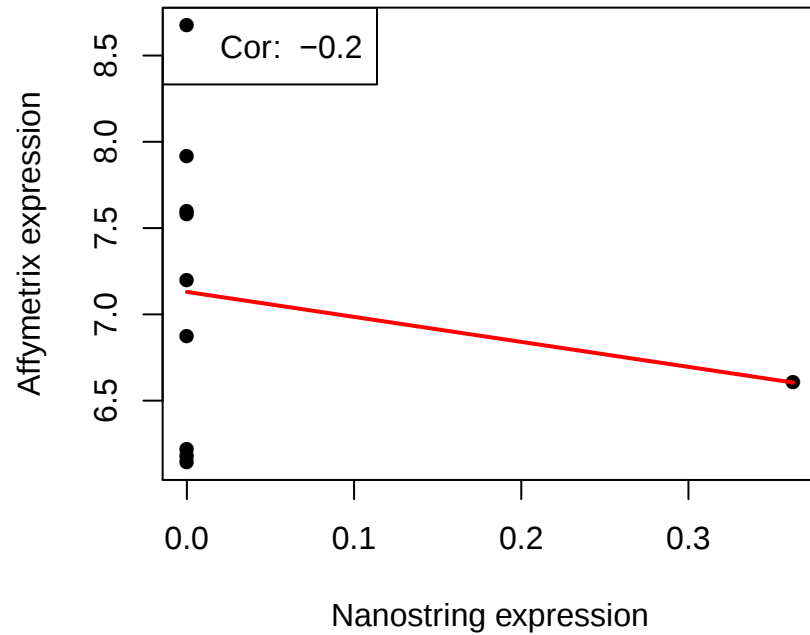
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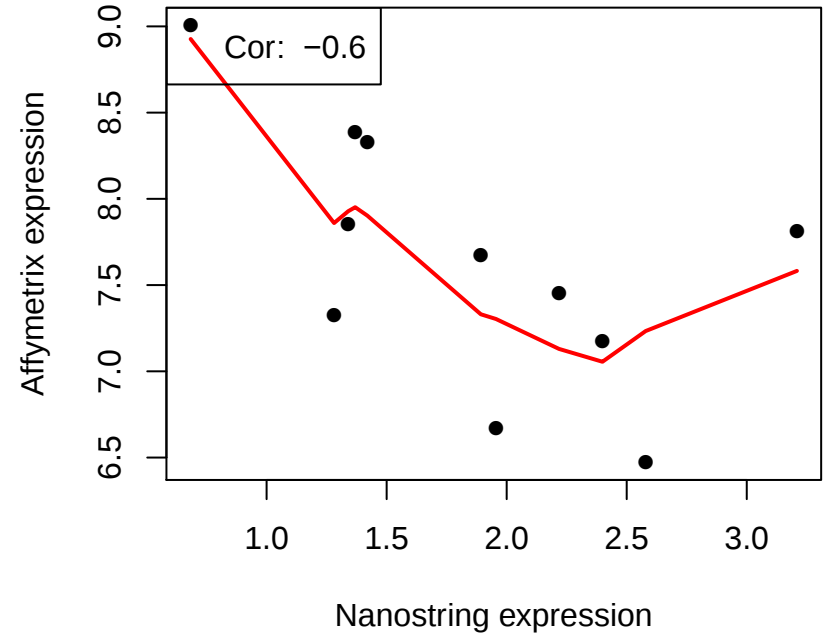
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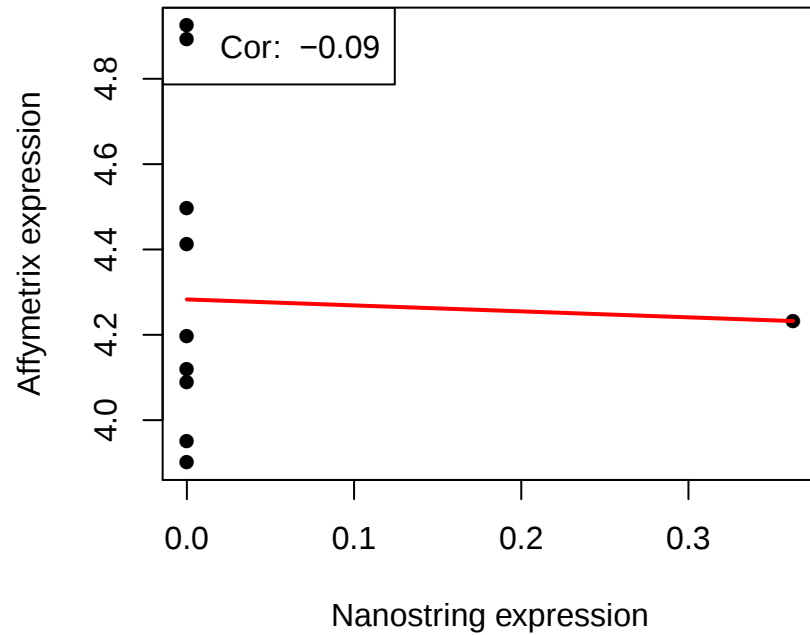
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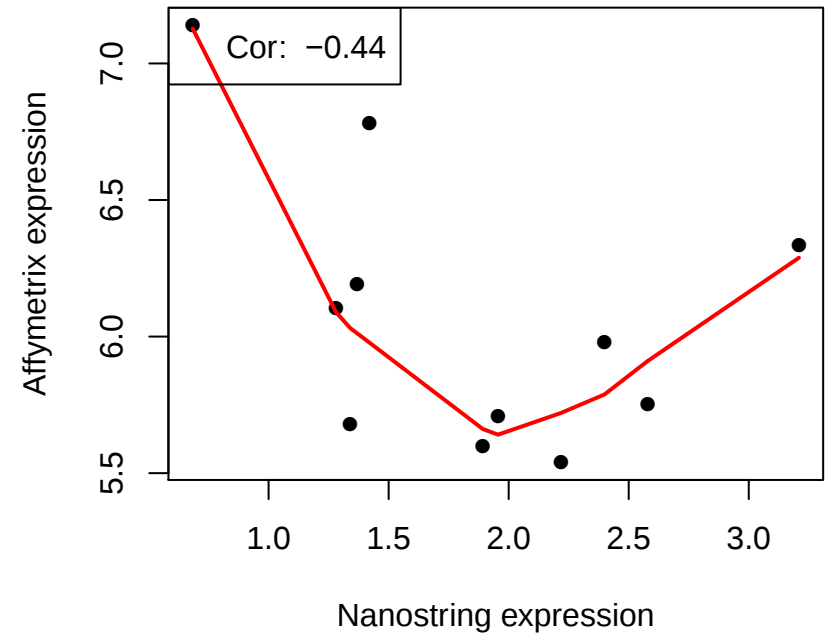
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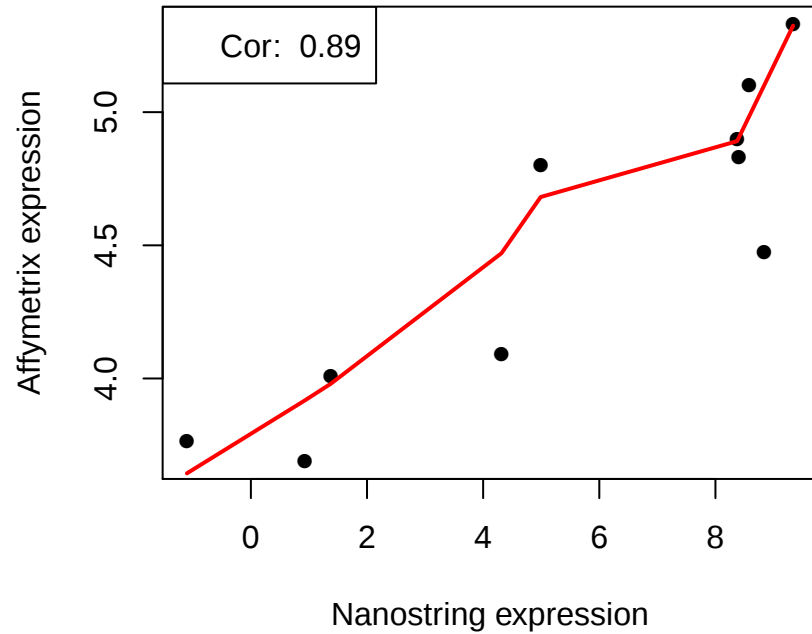
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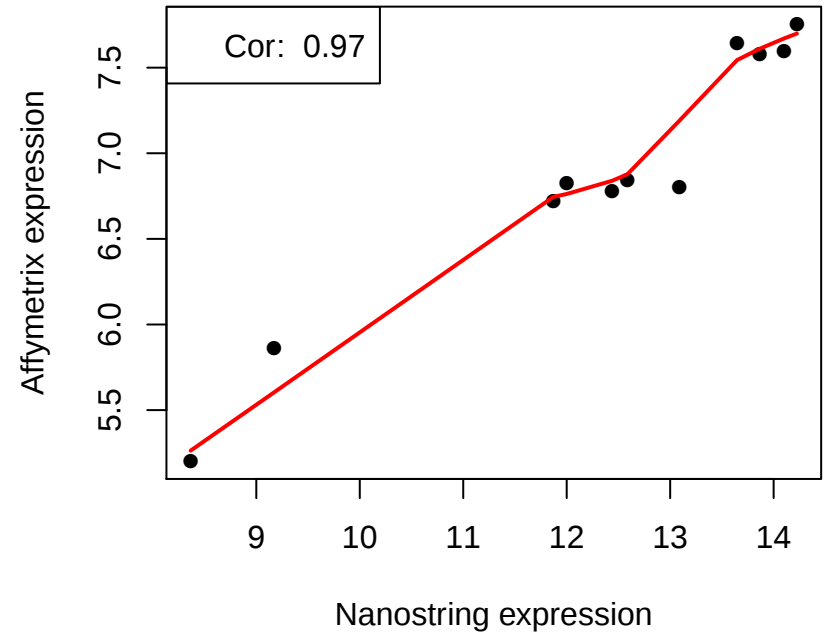
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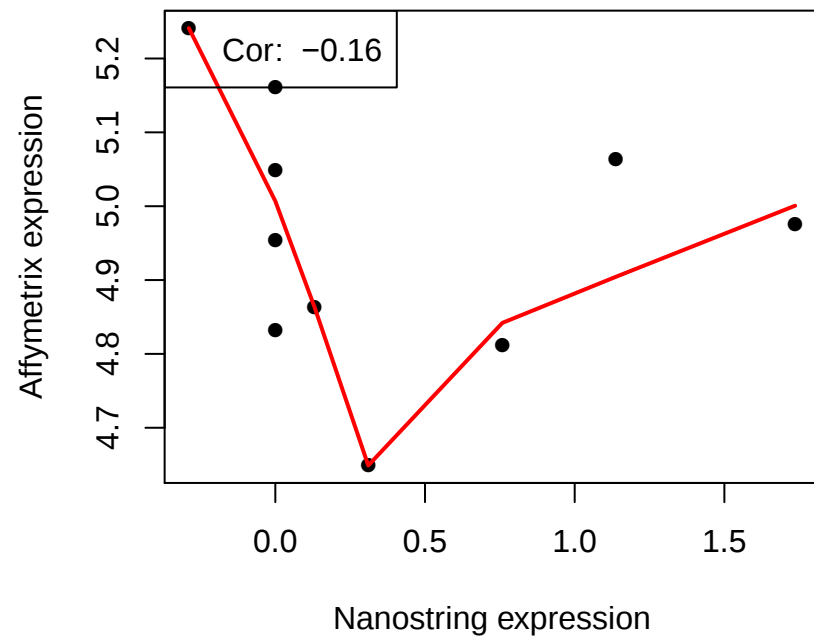
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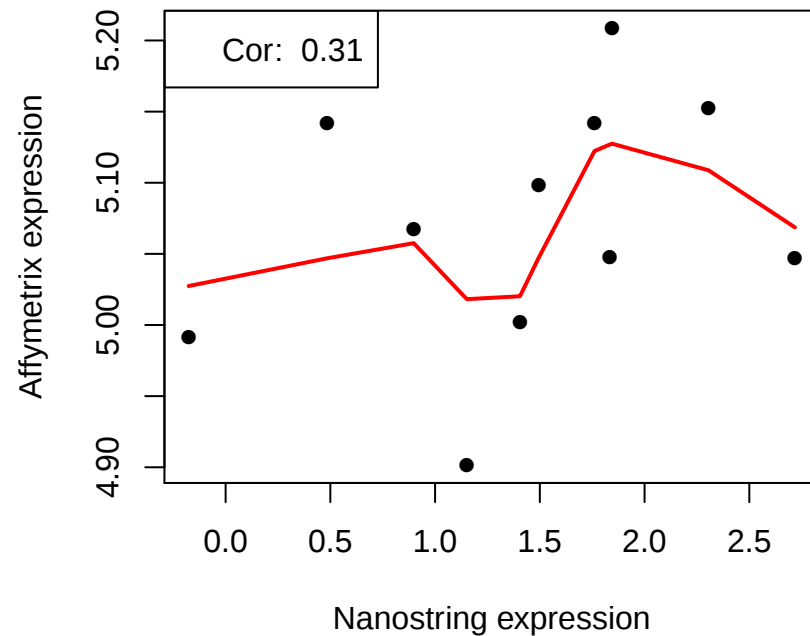
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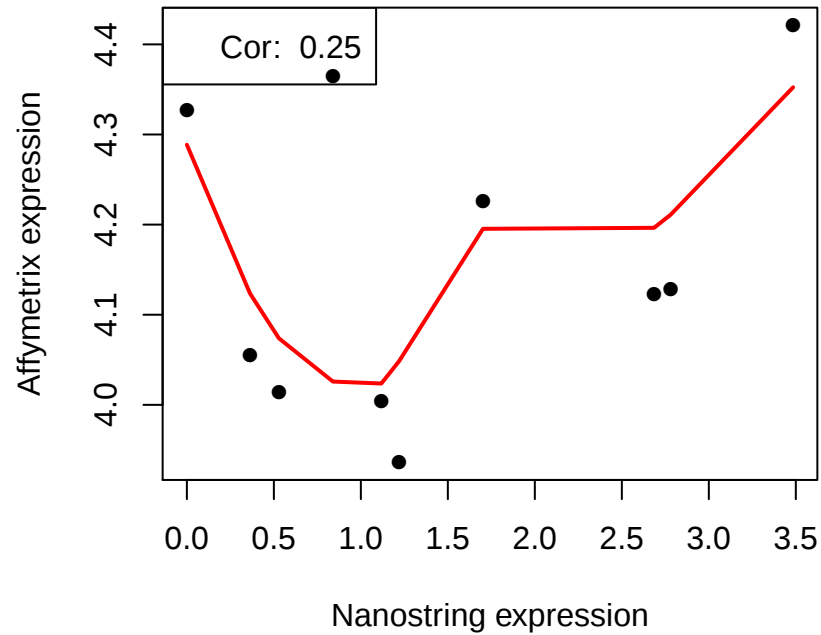
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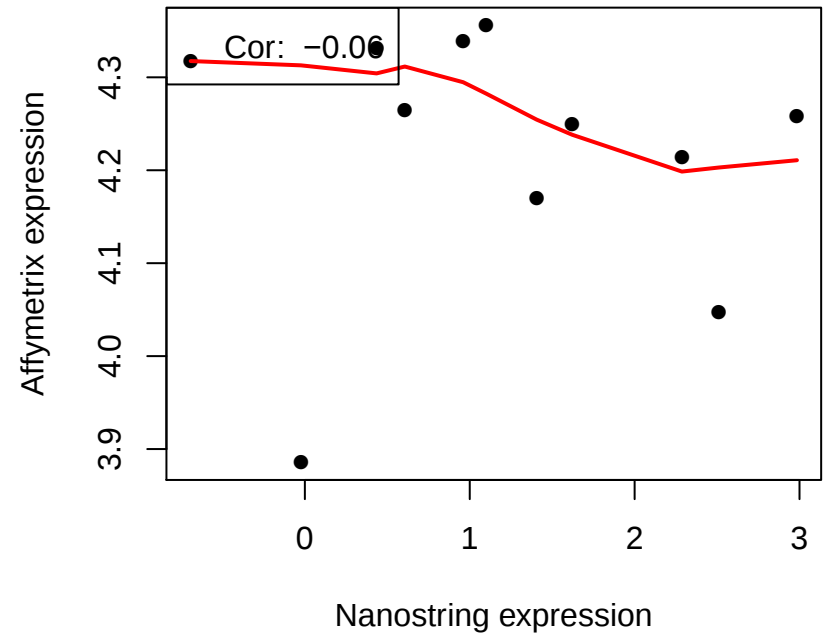
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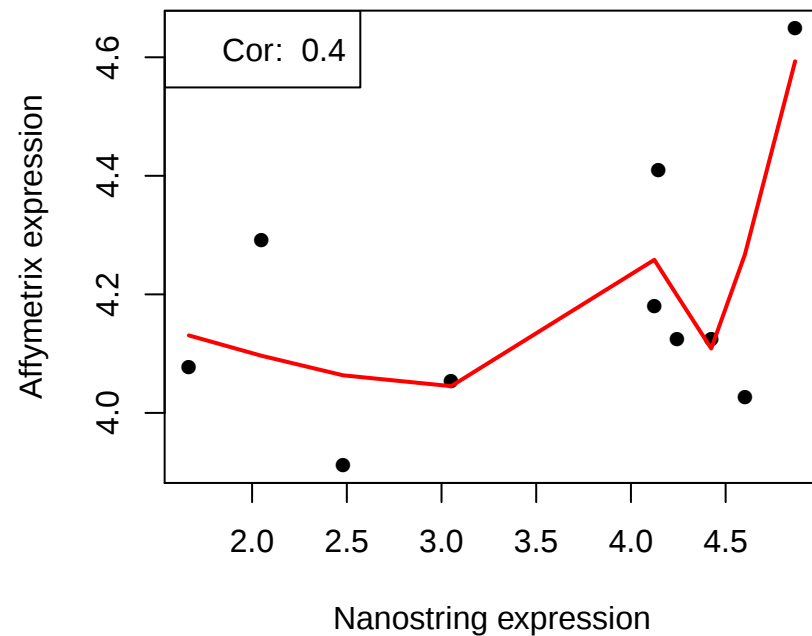
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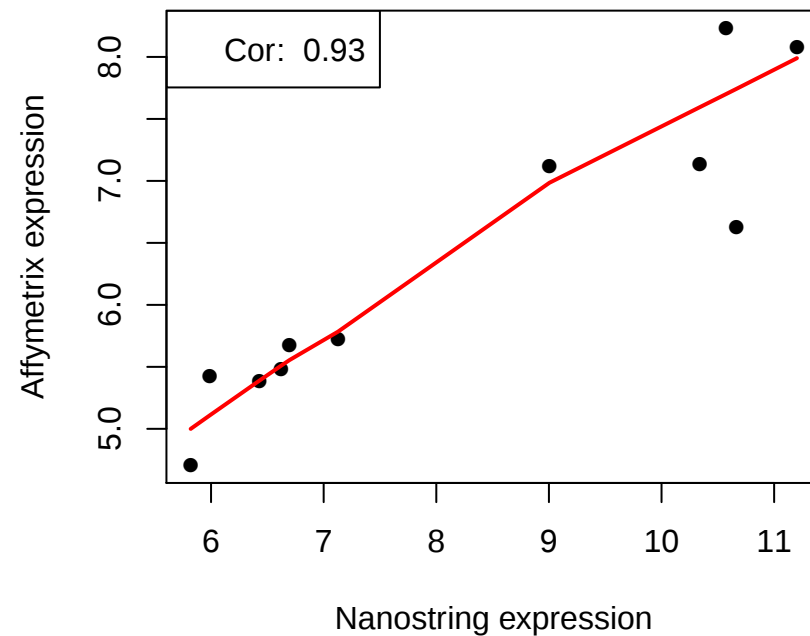
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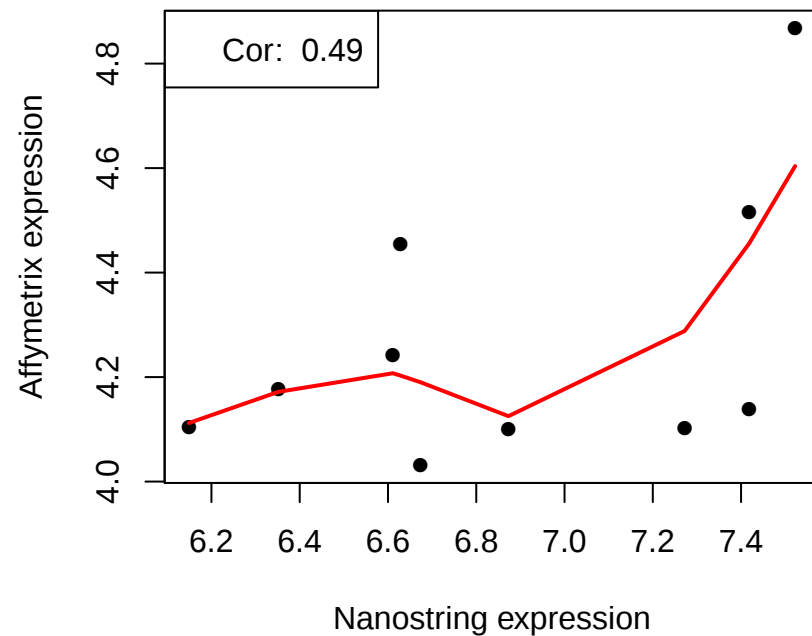
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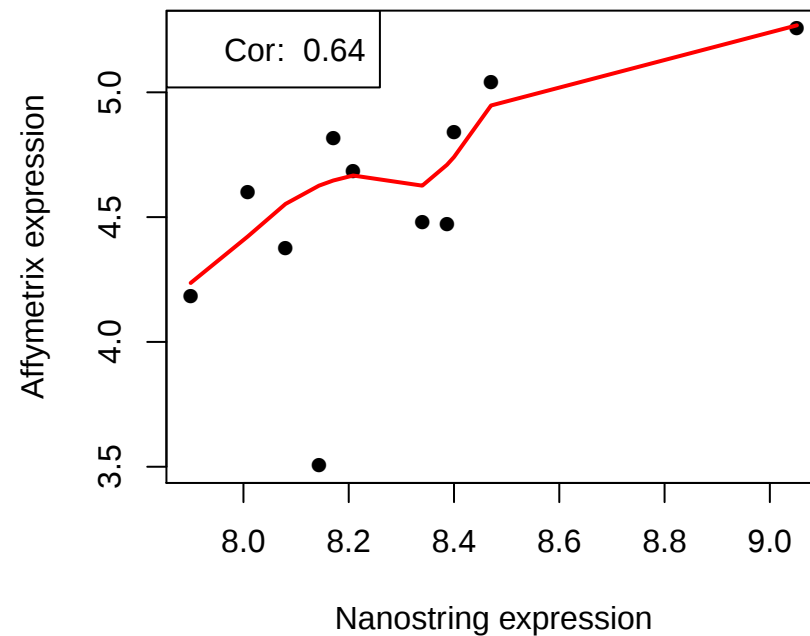
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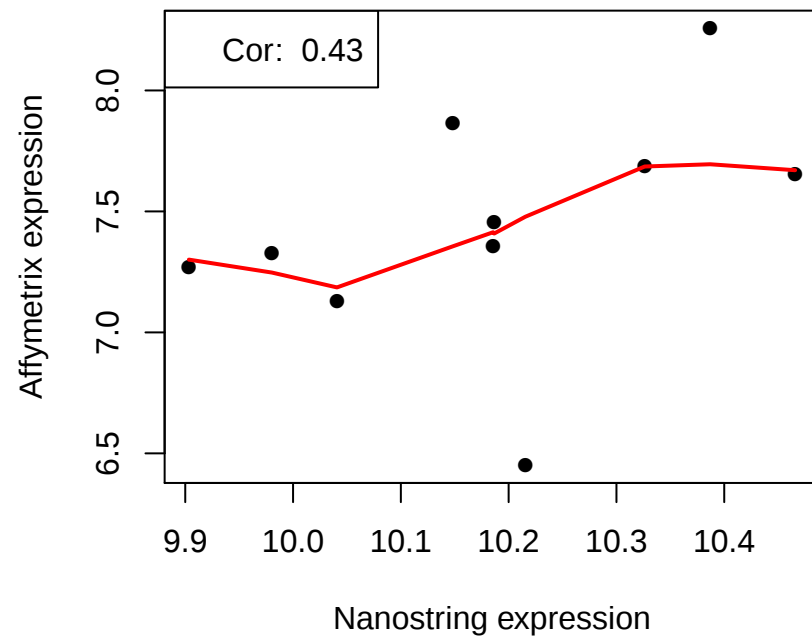
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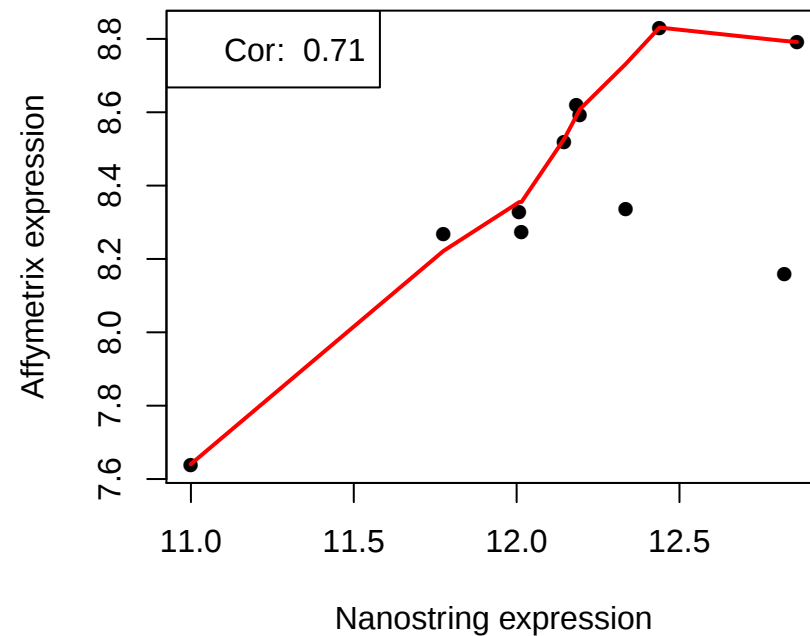
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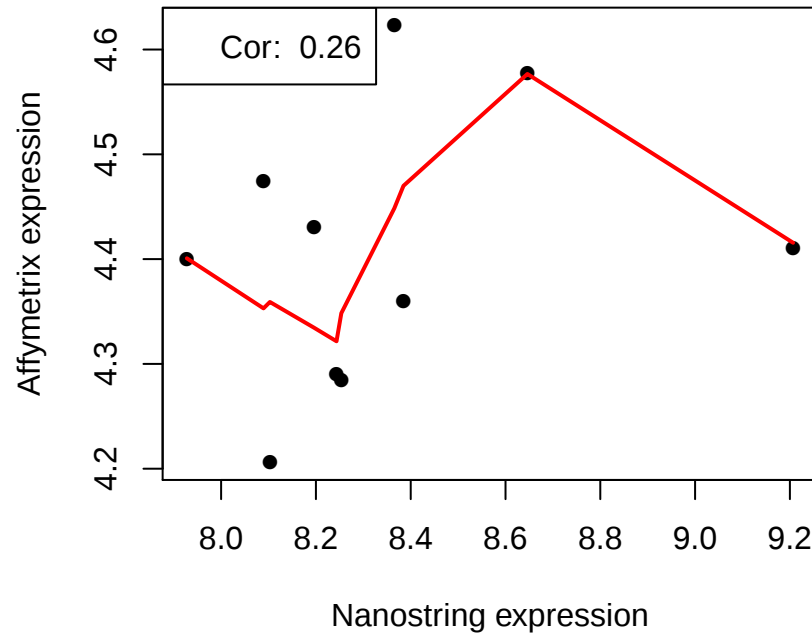
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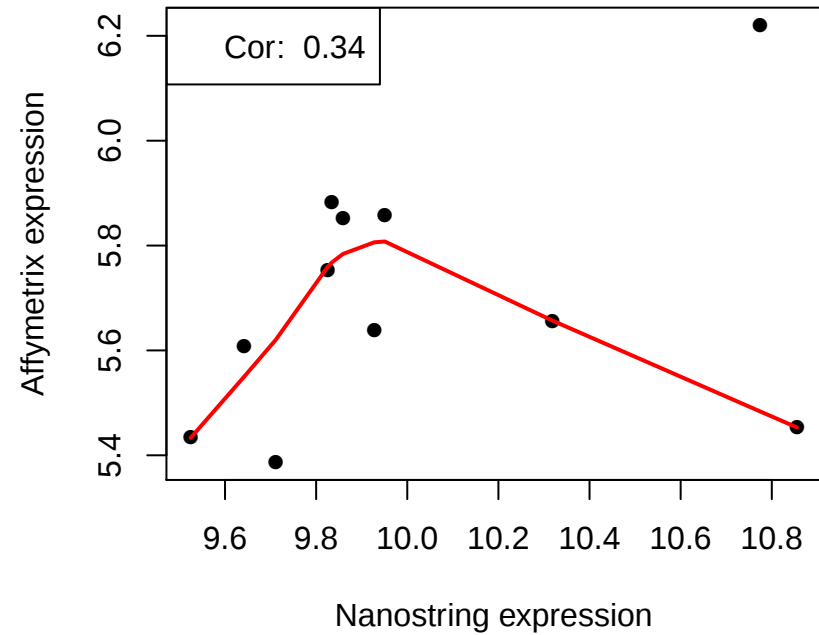
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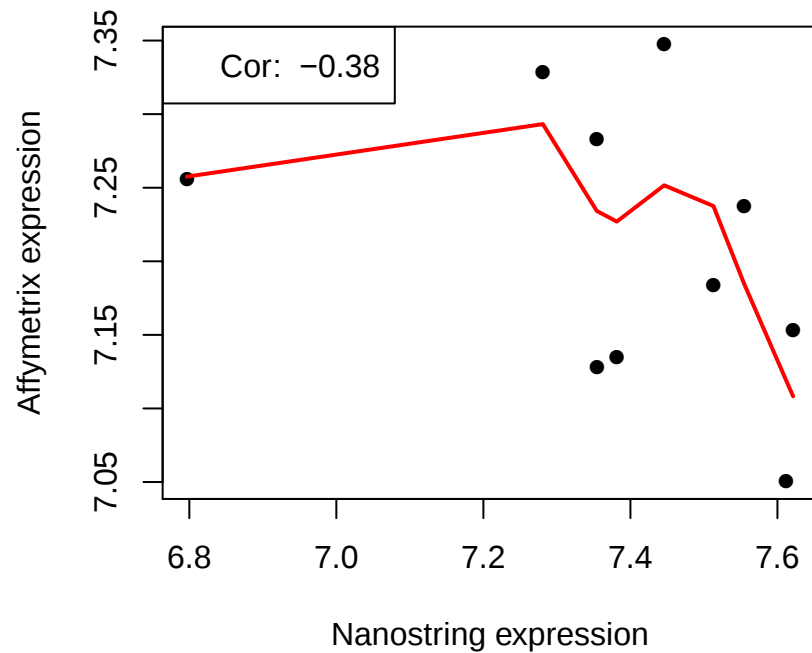
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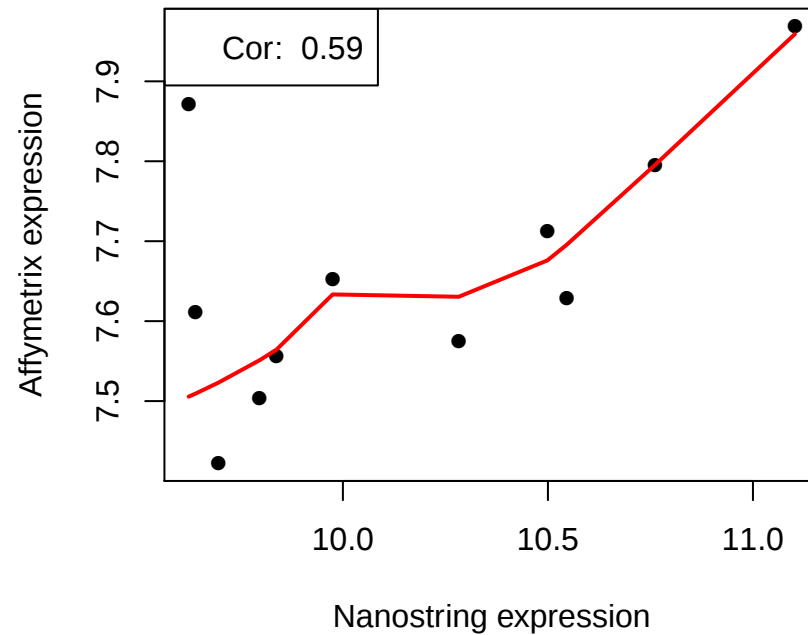
Ovarian data



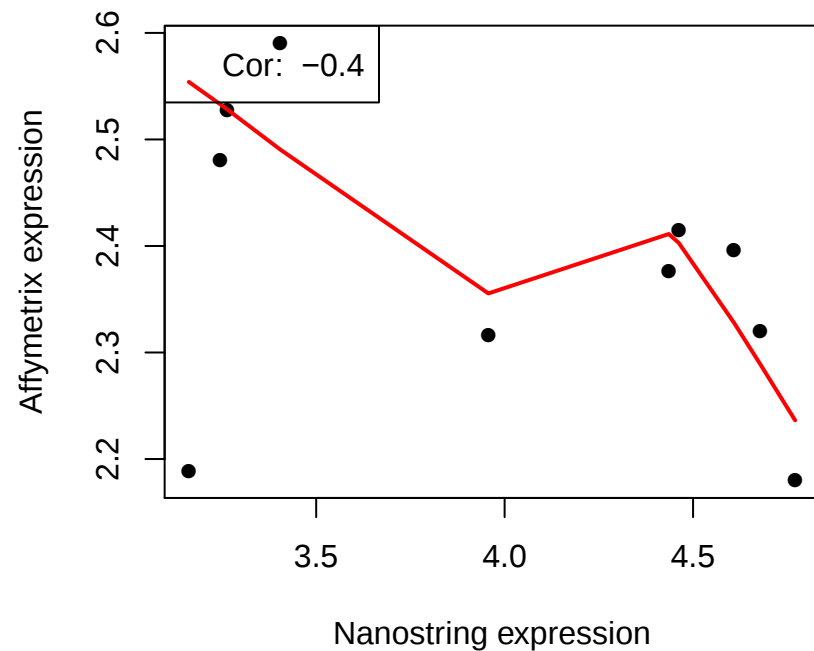
Prostate data



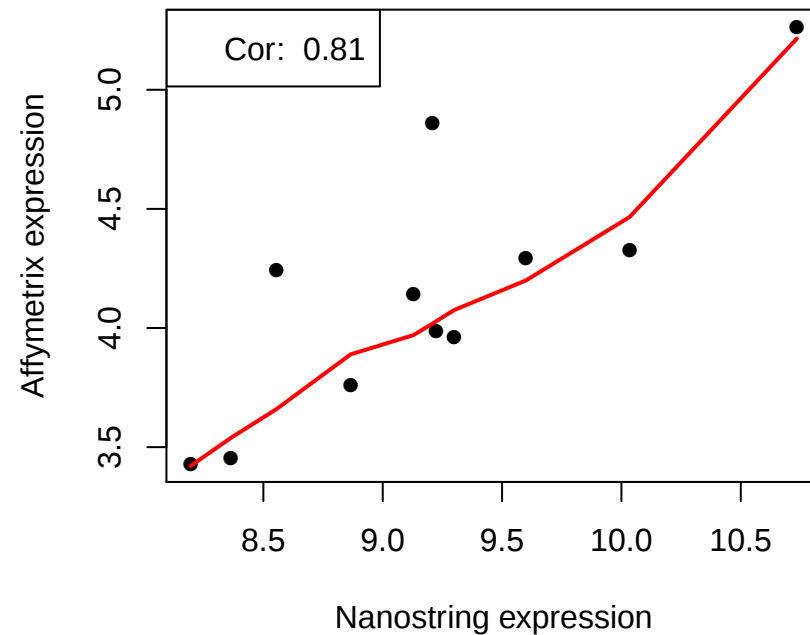
Ovarian data



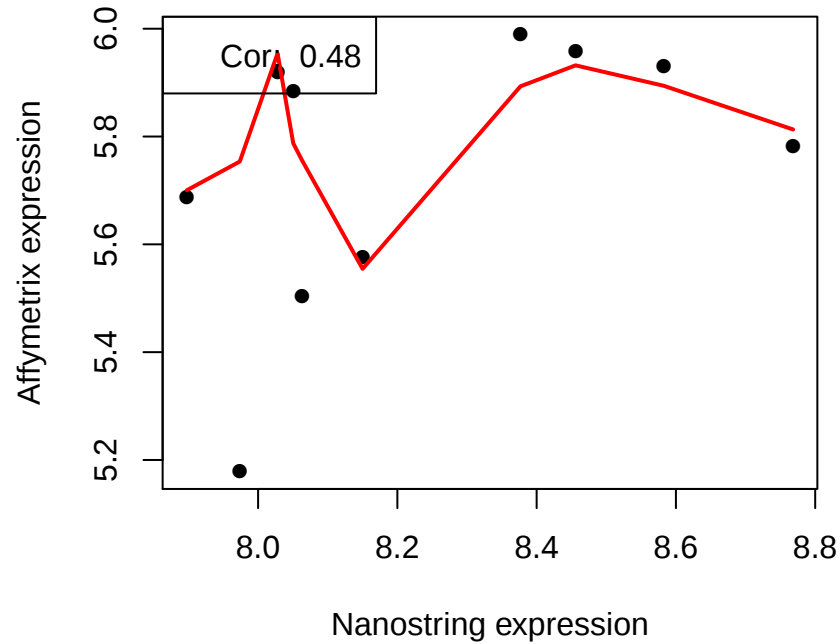
Prostate data



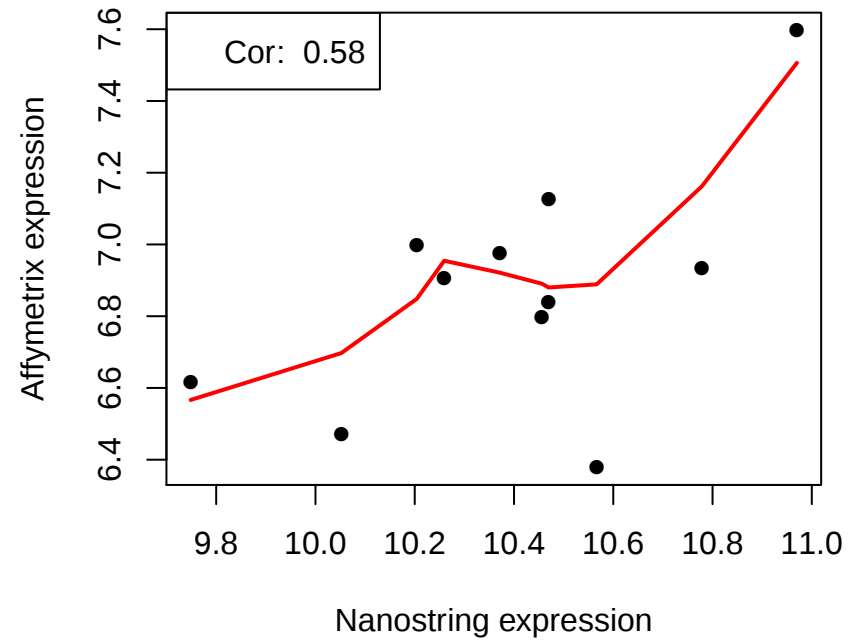
Ovarian data



Prostate data



Ovarian data



	case	Block.age	Tissue	Gleason	Amount	
ES2012020201.CEL	p8	18	Tumor		8	50
ES2012020202.CEL	p31	19	Normal	na		50
ES2012020203.CEL	p16	11	Tumor		8	50
ES2012020204.CEL	p8	18	Tumor		8	100
ES2012020205.CEL	p16	11	Tumor		8	50
ES2012020206.CEL	p7	16	Tumor		6	100
ES2012020207.CEL	p8	18	Normal	na		100
ES2012020208.CEL	p31	19	Tumor		6	50
ES2012020209.CEL	p19	12	Normal	na		100
ES2012020210.CEL	p31	19	Tumor		6	100
ES2012020211.CEL	p1	14	Tumor		7	100
ES2012020212.CEL	p19	12	Tumor		6	100
ES2012020213.CEL	p14	21	Normal	na		50
ES2012020214.CEL	p7	16	Tumor		6	100
ES2012020215.CEL	p7	16	Normal	na		100
ES2012020216.CEL	p14	21	Tumor		8	50
ES2012020217.CEL	p31	19	Normal	na		100
ES2012020218.CEL	p1	14	Normal	na		100
ES2012020219.CEL	p1	14	Tumor		7	100
ES2012020220.CEL	p19	12	Normal	na		50
ES2012020221.CEL	p14	21	Normal	na		100
ES2012020222.CEL	p14	21	Tumor		8	100
ES2012020223.CEL	p19	12	Tumor		6	100
ES2012020224.CEL	p16	11	Tumor		8	100
ES2012020225.CEL	p31	19	Normal	na		50
ES2012020226.CEL	p16	11	Normal	na		50
ES2012020227.CEL	p19	12	Tumor		6	50
ES2012020228.CEL	p14	21	Tumor		8	100
ES2012020229.CEL	p31	19	Tumor		6	50
ES2012020230.CEL	p16	11	Normal	na		100

Prostate tumor vs normal	High vs low Gleason	Clear cell vs serous ovarian adenocarcinoma
AMACR	BCAS1	ADM
SMPDL3B	ALOX15B	CD59
MS4A8B	EYA1	CP
MTHFD2	PAGE4	CRK
SDK1	BIRC5	CXCR4
TMTC4	MYBPC1	EN01
RPL22L1	MT1G	EN02
SLC43A1	EHHADH	F3
GCNT1	MPPED2	FLT1
UAP1	PTTG1	HIF1A
C4orf14	CRIP2	HK1
PPAT	CXCL13	HK2
VSTM2L	UBE2C	HSPCA
PPP1R14B	SERPINA3	INHBA
FNIP2	ATP8A2	JAG1
DCID1	INHBA	MDM2
GLYATL1	LMNB1	NOTCH1
DNAH5	CDKN3	PDHA1
IMPDH2	MCM4	PFKP
SOX4	FMO5	PGF
DLX1	NOTCH3	PLCG1
RGS10	NEK2	PROCR
C14orf104	PRKCB1	PROS1
MARCKSL1	MT1F	PTEN
MYO6	CHRNA2	SDHD
TOX3	SCUBE2	SLC2A1
POLR2H	TOP2A	TFP12
GMDS	RRM2	THBD
C7orf46	JAG1	TIMP3
FRMPD3	CD38	VEGF
CAMKK2	MRPS12	GPX3
PTPRT	BUB1B	GLRX
CBX3	RGS4	LBP
TSPAN13	BGN	CRYAB
EPCAM	ANPEP	DEFB1
SLIT1	GNG4	HCLS1
CLDN8	ASPN	SOD2
PAICS	CYP27A1	HSPA2
ZNF664	FKBP1B	ORM1
UCK2	KCNN2	CSPG2

DAPK1	SPAG5	IGFBP1
SH3RF1	GPR116	PTHLH
SIM2	MT1A	TCF2
CACNA1D	VEGF	DRIL1
E2F5	ARG2	GPC3
MMP26	DPP4	IGFBP3
MICALCL	SFTPA2	TOB1
NLRP12	CENPF	MITF
CNHG3-RCC1	DLG7	NDRG1
ABCC4	SMPDL3A	NR1H4
LOC440335	SERPINE1	FGFR3
HLA-DMB	PGM5	PVR
GNL3	SC65	PIG7
MAP2K6	NTRK3	IL6
DKC1	CYP4F12	PTPRM
ROR2	SATB1	FOXO1A
TP63	ERG	ERBB2
C5orf4	NELL2	C5R1
COL4A6	HSD17B6	MIG2
EFS	KHDRBS3	PRX2
MYOF	TFF3	NID2
MEIS2	PENK	LAMB1
NAV2	F2R	COMP
GSTP1	ABAT	MAGP2
DUOX1	SLC15A2	PLS3
ALDH2	PROK1	MCAM
FGFR2	COL4A1	SPP1
ACSF2	TYMS	LAMC1
GPRC5B	ITPR2	COL4A2
KRT5	CDC42BPA	E48
SMAD3	FXD1	PSCDBP
STOX2	NUSAP1	COL4A1
DOK4	CACNA1D	MYOC
KLHL29	BMP6	ANXA4
C9orf125	DHFR	TFPI2
PCDH7	XRCC2	CST6
SLC16A2	PTN	SLPI
TLE2	TK1	TIMP2
WFDC2	KLF5	CPM
HOXD11	RARRES1	GGT1
S100A16	ESM1	NNMT

ETNK2	TRIP13	MAL
PPARGC1A	PAH	EEF1A2
AMT	PTK7	HGD
NRG1	CYB5A	TCN2
FBXO17	PTPRN2	CDA
COL17A1	MGST1	PCCA
KLF8	IGFBP6	CRYM
ATP2B4	MYLK	PDXK
HSPB1	PLA2G7	CYP1B1
CPA6	GRIA3	STC1
ARSJ	MT1X	NP
SH3PXD2B	SLC4A4	WARS
AOX1	DLGAP1	HMOX1
ITGB4	C2	FXD2
SLC16A5	FOXO1	RBP4
BCL11A	DDEF2	SLC6A12
GSTM2	CSPG2	VATI
LDHB	F5	GP36B
ARMCX1	CUL7	RAB9
MYLK	SEMA3F	KDEL3
CAV1	COPS5	C3F
SNAI2	SCGB1D2	ITM2B
IQCA1	CCNB1	TFPI2
TCEAL2	GREB1	ANXA4
LOC399783	PHC2	UGT1A1
ASXL3	GMNN	FXD2
TMEM35	CES1	GLRX
RAB34	TBXAS1	KIAA1922
GABRE	HERC3	MAP3K5
C1orf175	RGS16	CXADR
ZNF516	FLRT2	RFX5
STXBP5L	E2F3	MITF
JUB	KIAA0040	ANXA4
APOBEC3C	TGFB2	UGT1A1
CSRP1	ESRRG	FXD2
	ARMCX2	GLRX
	PLA2G10	DEGS
	RNASE4	MAP3K5
	HIST1H1T	NAB1
	ENTPD1	BHLHB3
	ELK4	

SHMT2
NAT1
EVPL
LIPH
MAP3K5
LCN2
RPE65
TPST1
CRISP3
LAMC1
ASNS
MEOX2
SEMA3C
FST
FGF7
HRAS
PLCG1
AOX1
ANG
UPP1
MYBL2
TLE1
IL1R1
OCLN
MMP11
SPARCL1
AZGP1
GSTA4
RAB27A
GMDS
EPHB6
NME3
KIAA0907
CPT1A
HOXB7