Variant analysis of Simplex Autism Families

Laura Jiménez
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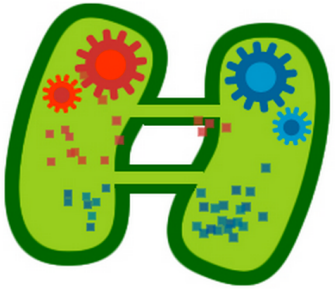
UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO



Bacillus booleanus

Acknowledgments

Gholson Lyon

Jason O'Rawe

Han Fang

Ivan Iossifov

Yiyang Wu

Autism

About 1 in 68 children has been identified with autism spectrum disorder (ASD) according to estimates from CDC's Autism and Developmental Disabilities Monitoring (ADDM) Network.

Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2010

Surveillance Summaries
March 28, 2014 / 63(SS02);1-21

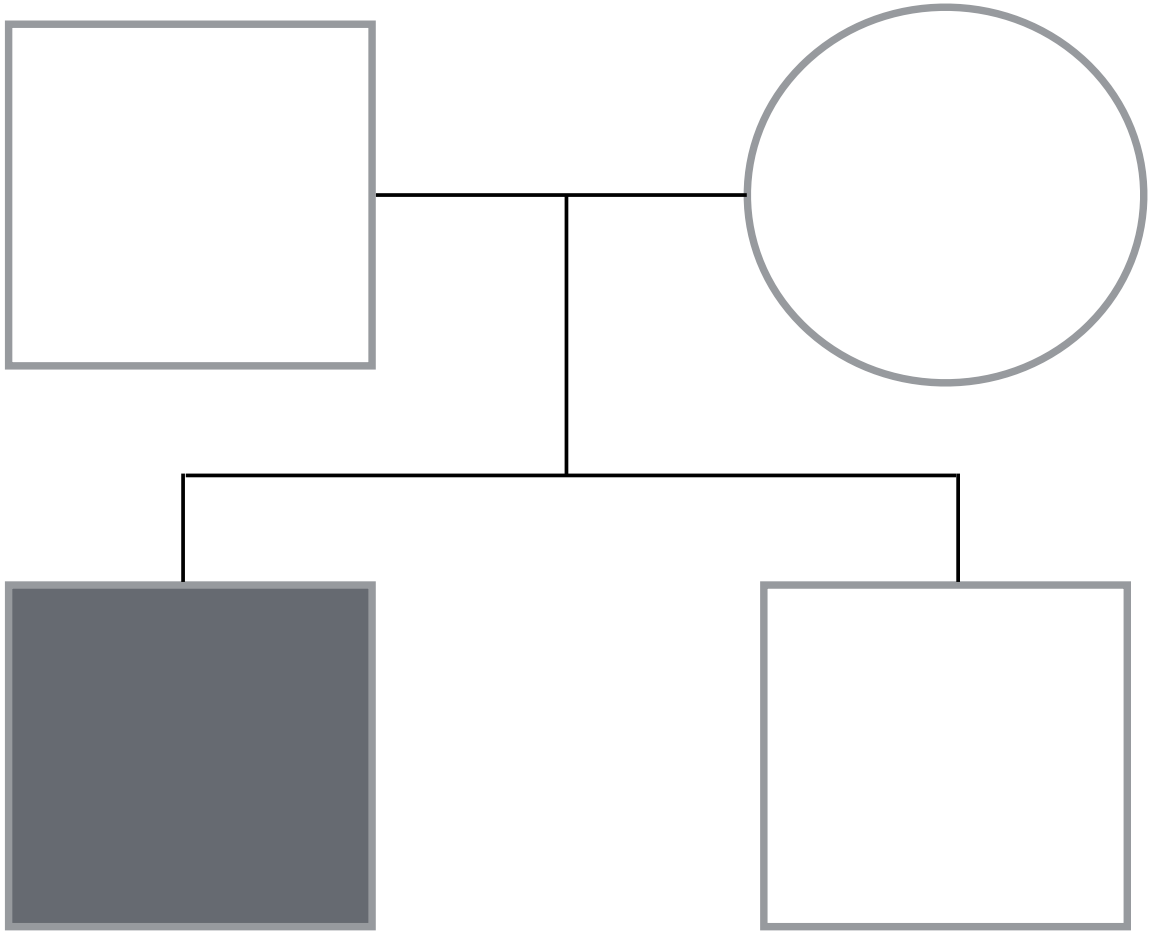
Autism and Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators

Corresponding author: Jon Baio, EdS, National Center on Birth Defects and Developmental Disabilities, CDC. Telephone: 404-498-3873; E-mail: jbaio@cdc.gov.

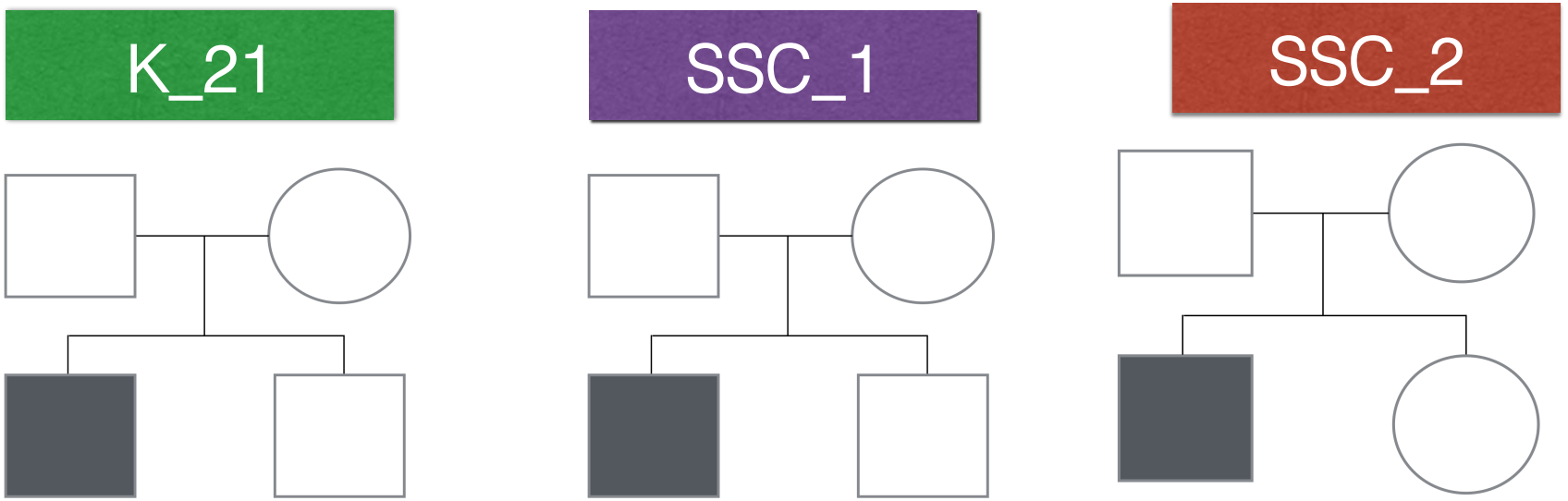
Previous Studies & Strategies

- Cytogenetic studies
- Linkage analysis, and candidate gene association analysis
- GWAS
- CNVs on Specific Regions i.e. VIP Project (16p11.2)
- WES & WGS on individuals, tríos, quads, multiplex pedigrees.

Simplex Autism Family



Analyzed Simplex Autism Families

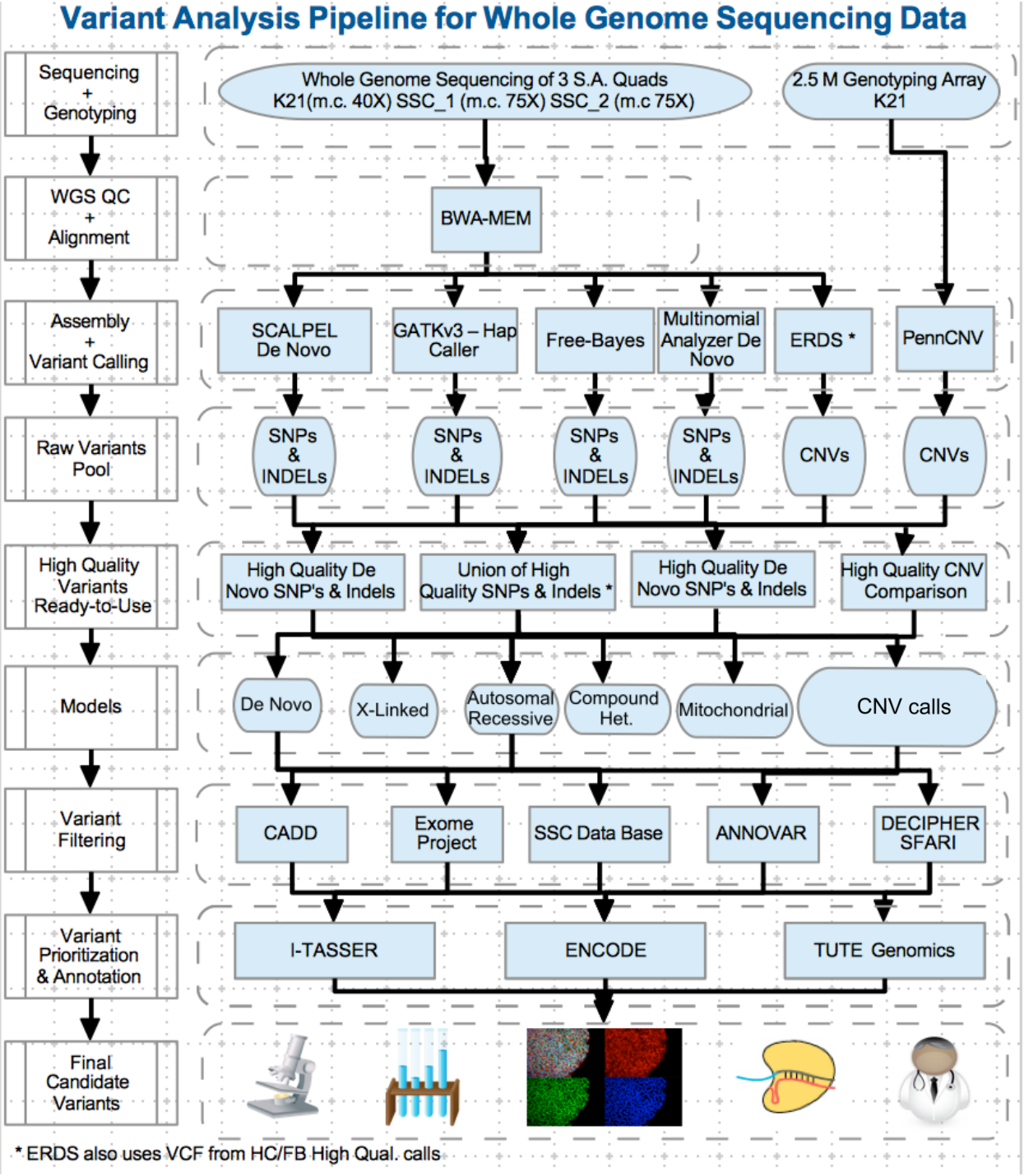


The Simons Simplex Collection

WGS ~ 40 X
Illumina HiSeq 2000
Genotyped 2.5 M
Illumina Omni2.5 array

WGS ~ 75 X
Illumina HiSeq 2000
WES ~40X
NimbleGen SeqCap EZ Exome v2.0

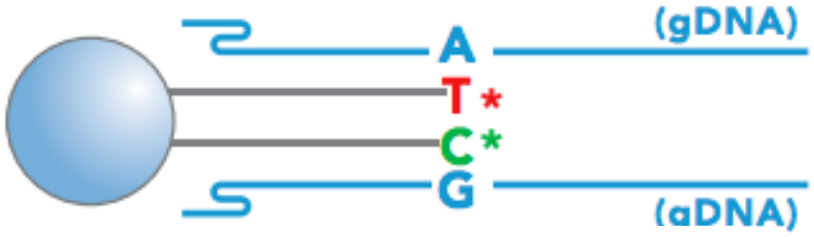
Variant Analysis Pipeline for Whole Genome Sequencing Data

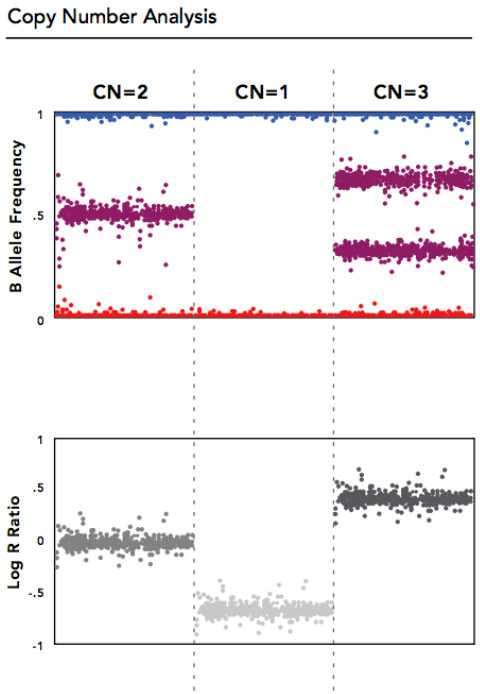


* ERDS also uses VCF from HC/FB High Qual. calls

Copy Number Variants

PennCNV calling for Microarray Data on K21





Log R Ratio = $\log_2(R_{observed}/R_{expected})$

R= (X+Y)

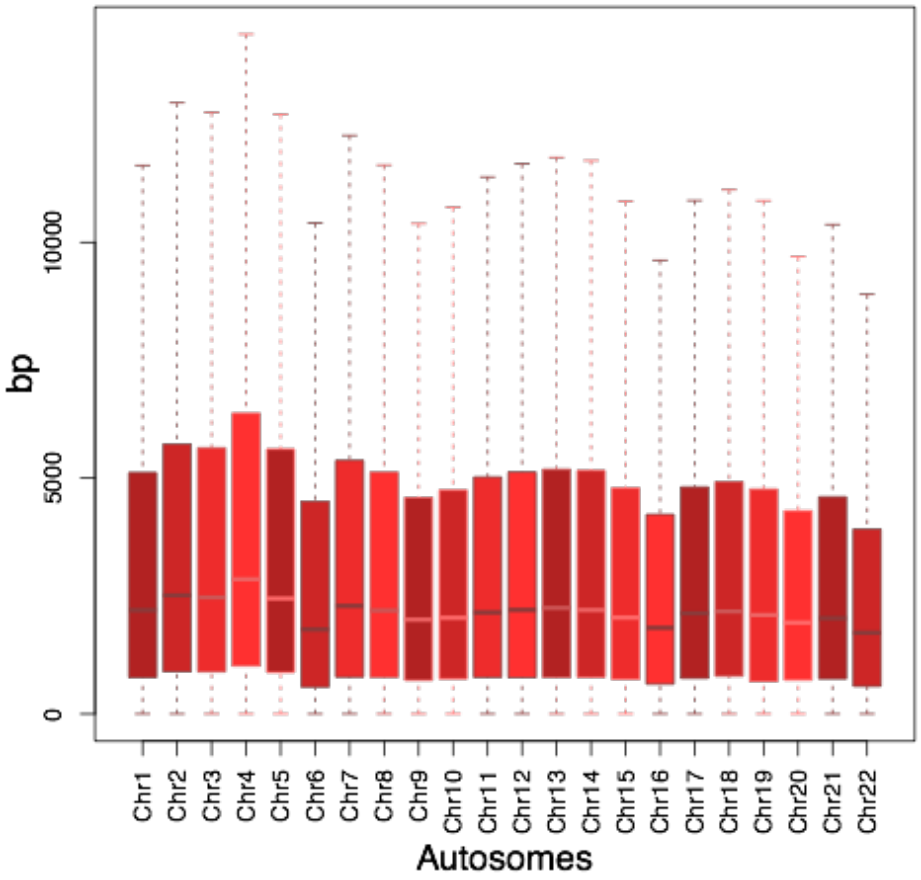
X=norm A allele Y=norm B allele

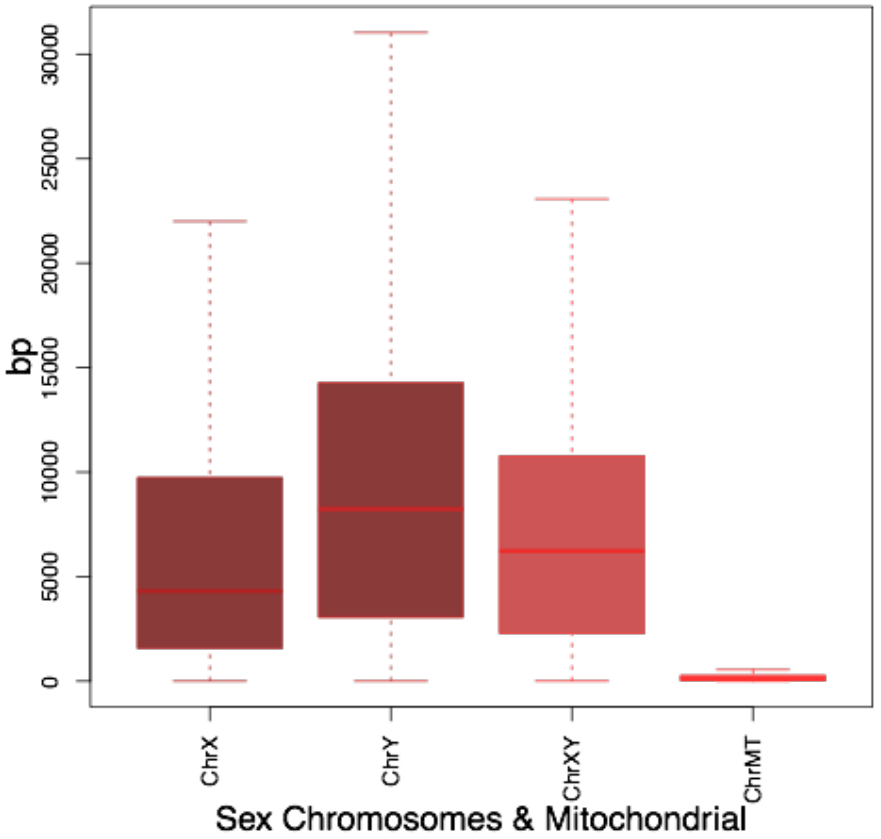
B Allele Freq. = normalized measure of relative signal intensity ratio of the B and A alleles

Population Frequency of B Allele File (generated from 600 controls)

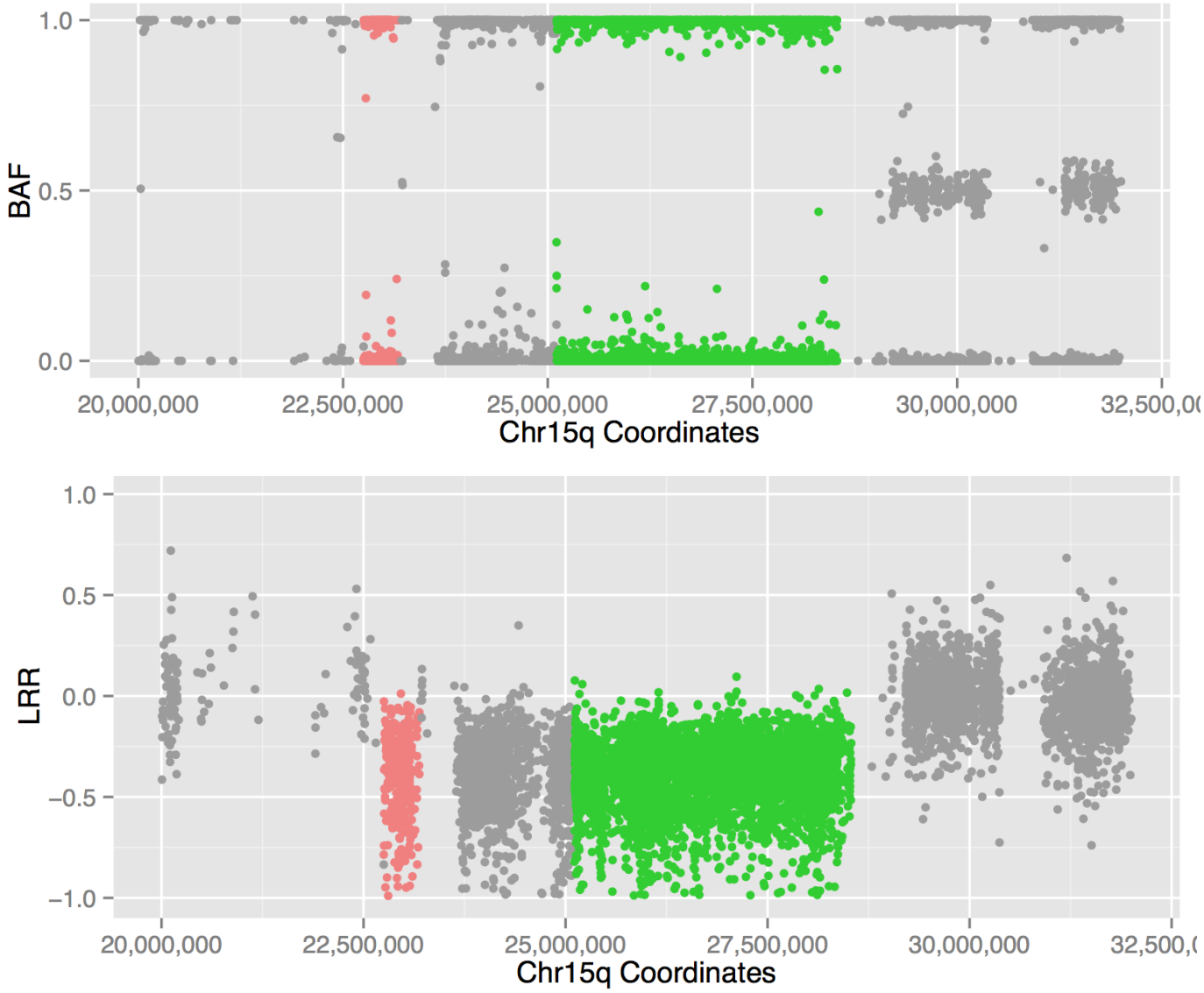
Joint Calling Algorithm

Intermarker Spacing Size by Chromosome





CNVs called by PennCNV on a Prader Willy Child



CNV calling from WGS with ERDS

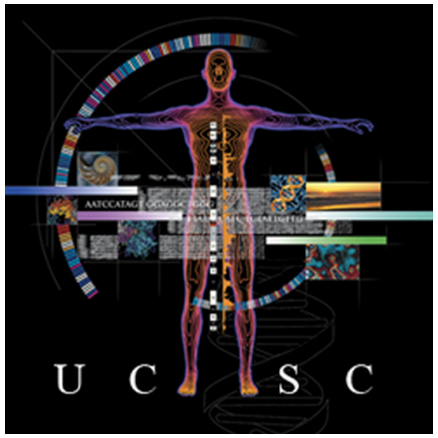
Ming Fu, et al.

Score > 300

Length > 10 Kb

Database of Genomic Variants

DECIPHER GRCh37



ANNOVAR

ClinVar

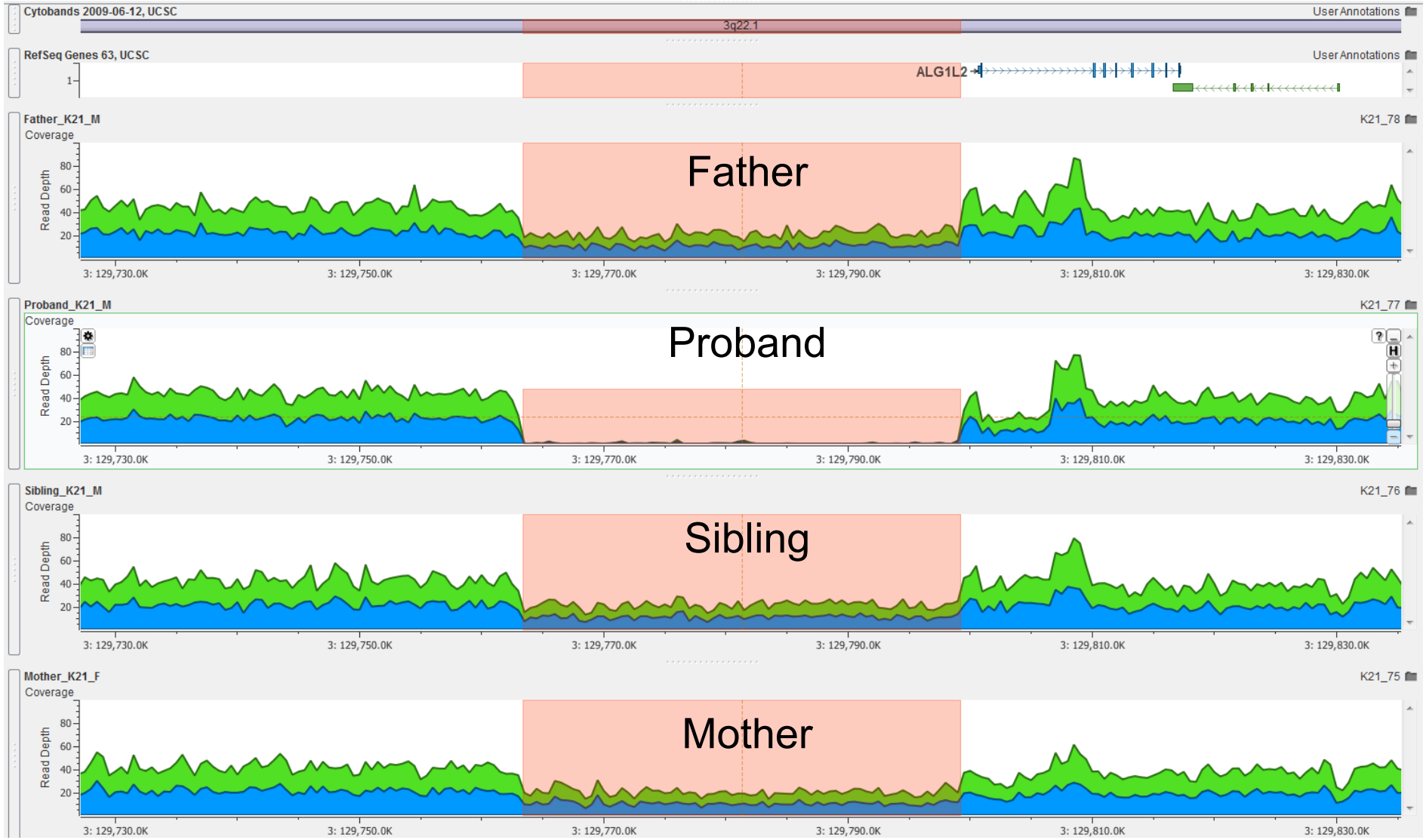
ENCODE

AutDB Autism Database

Region Alignment

K_21

Position	Size	Gene(s)	Ref/Obs. # of Copies	ERDS Score
3q22.1	~ 36 Kb.	intergenic	2/0	3026.58



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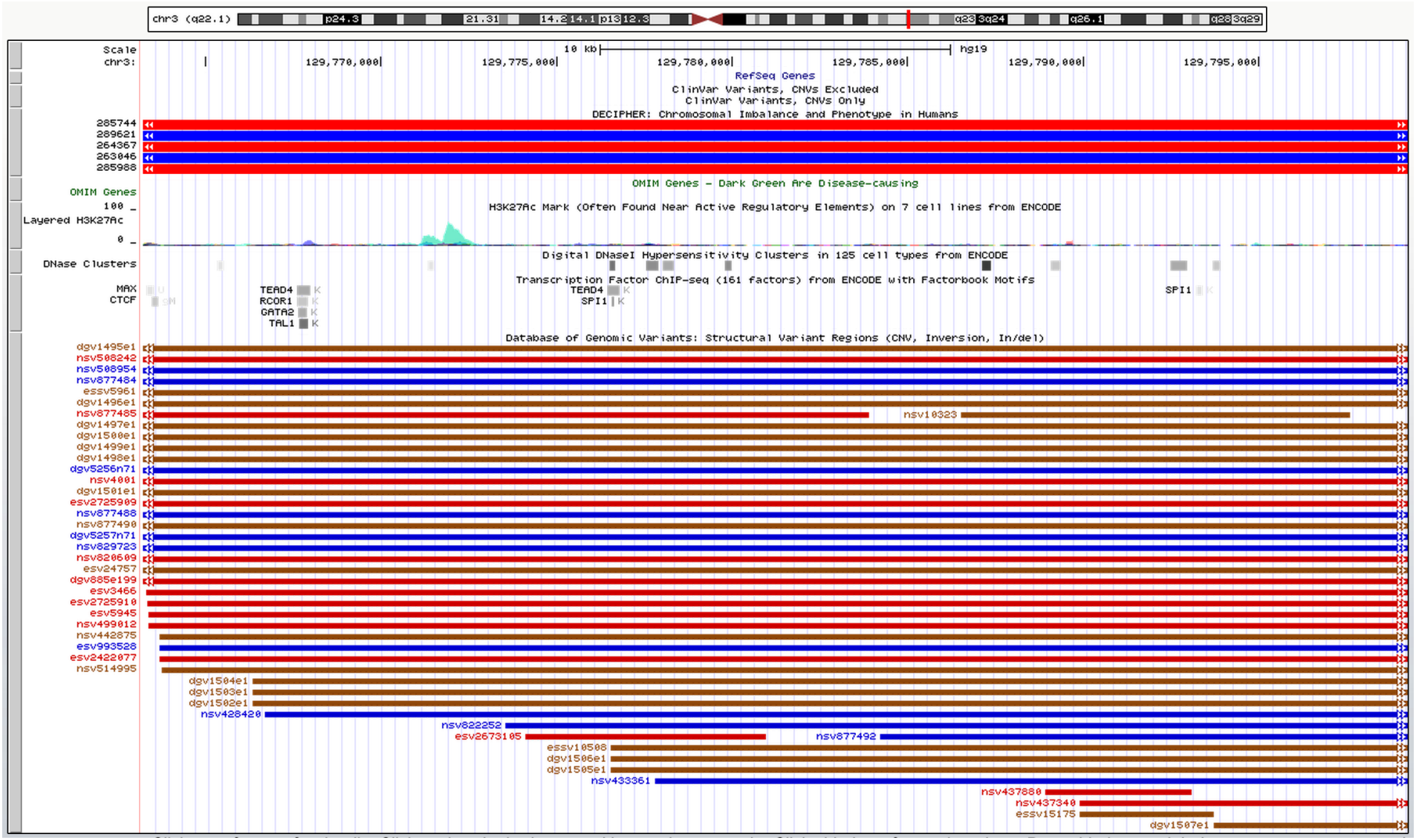

Region Annotation Zoom Out

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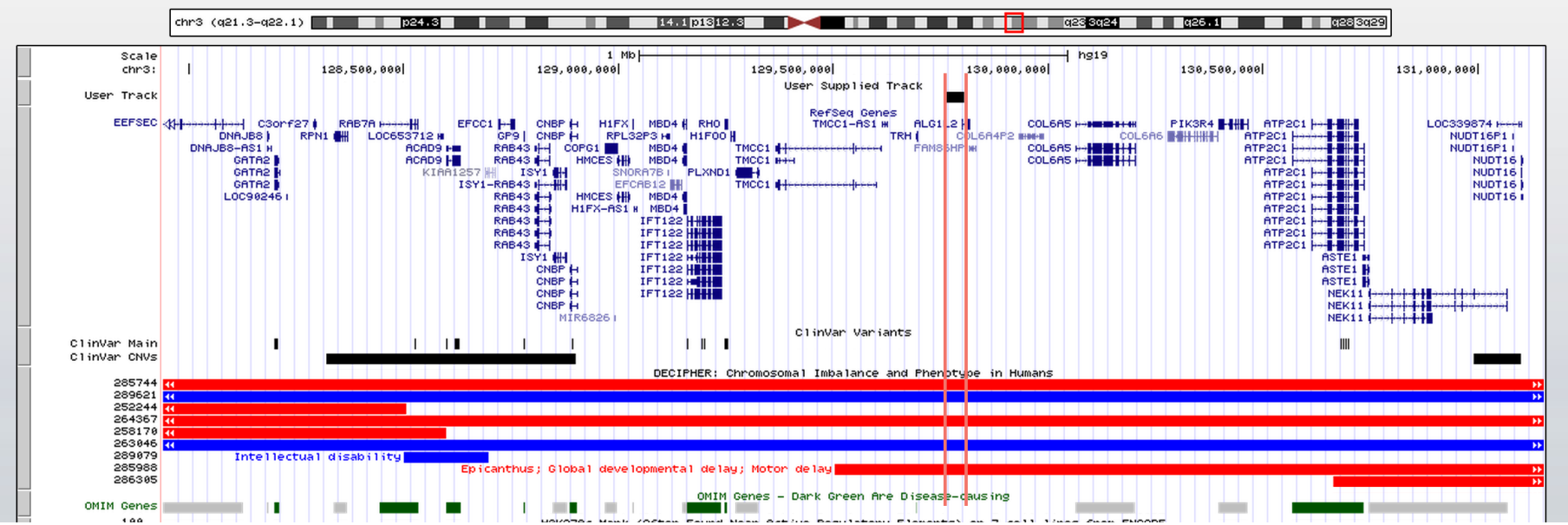

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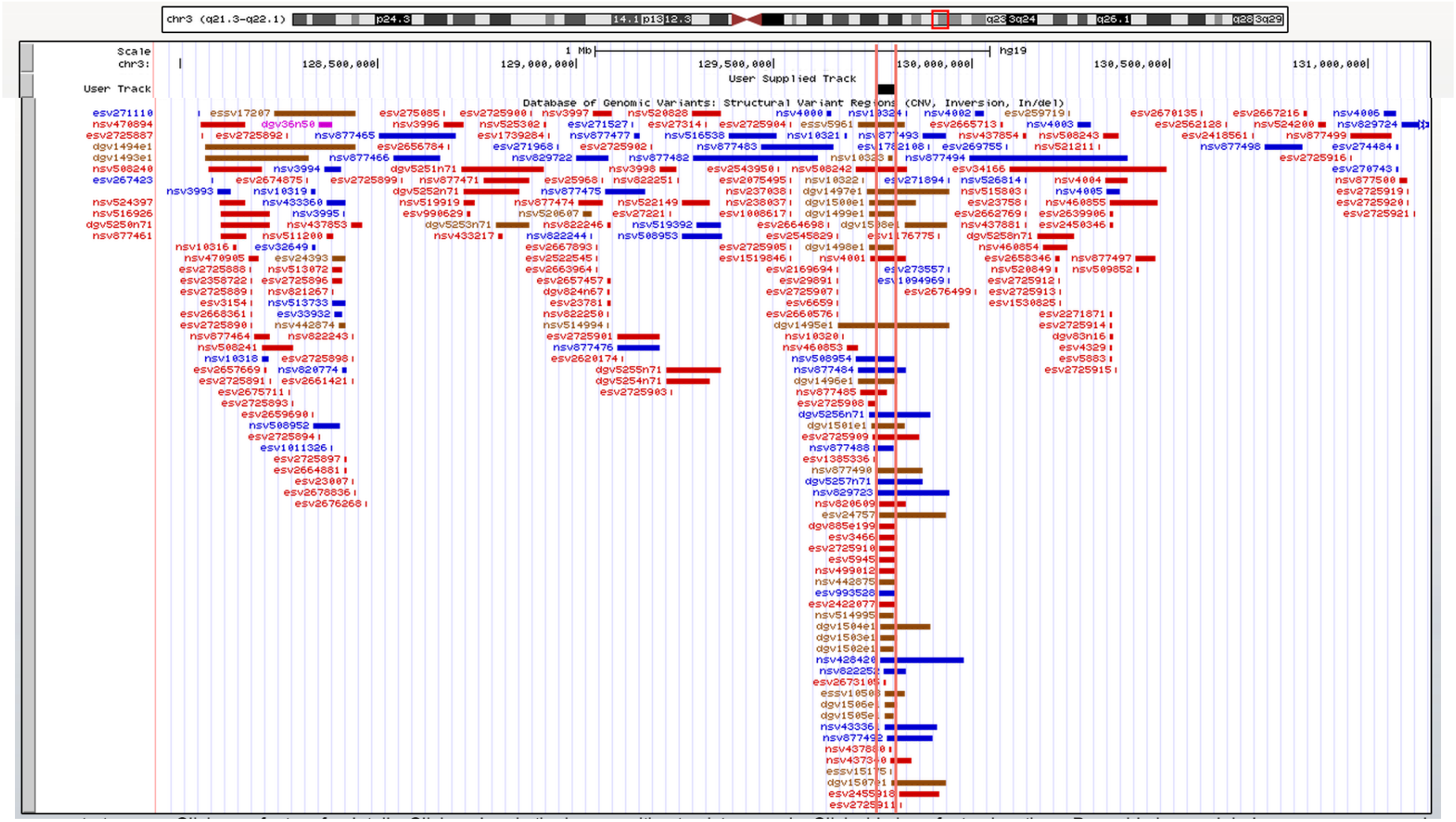

Microarray Data

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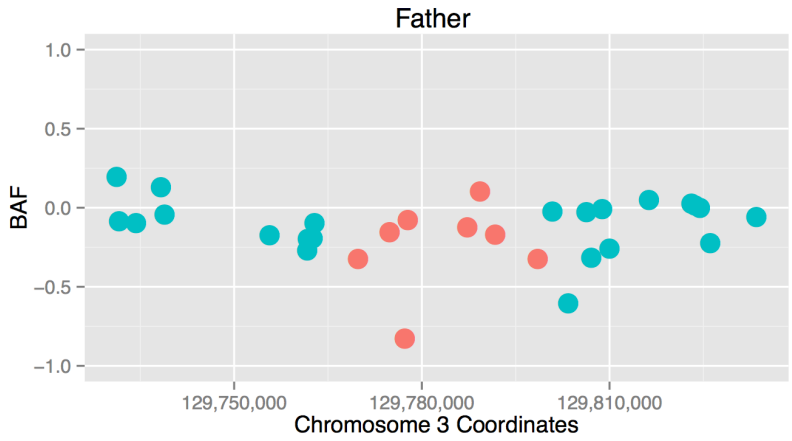

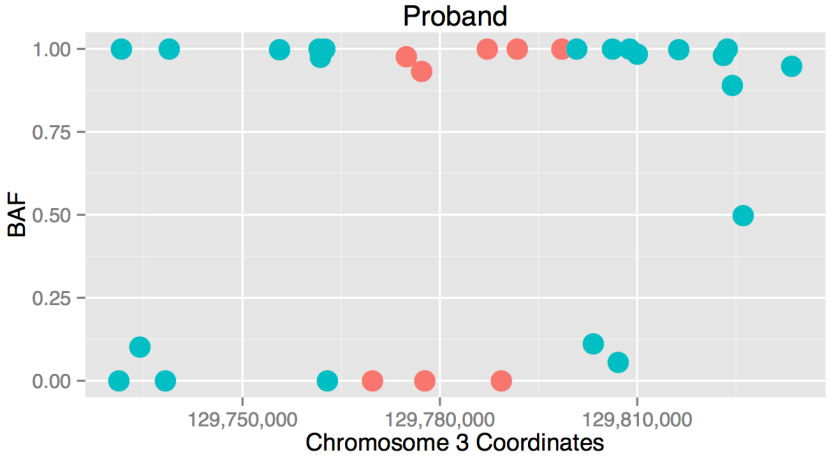

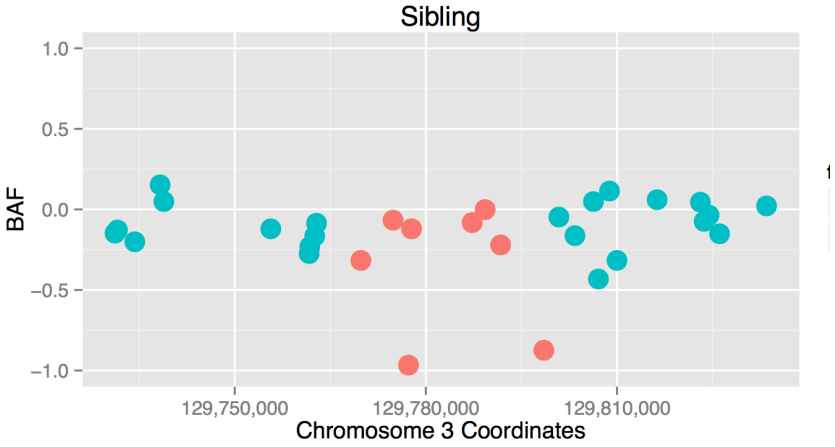

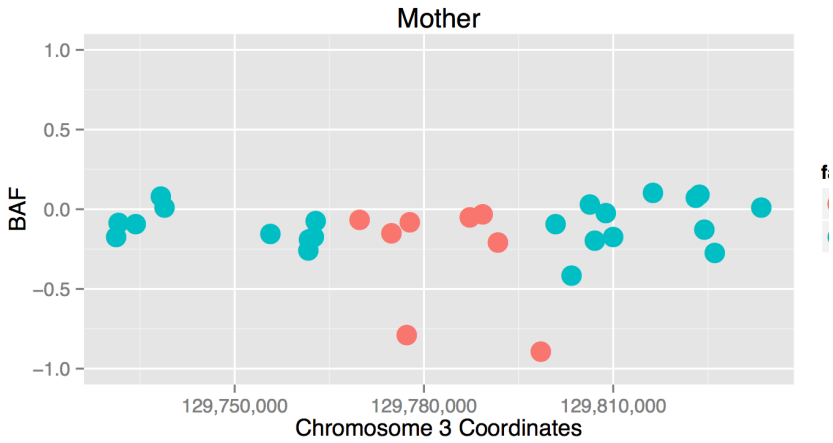

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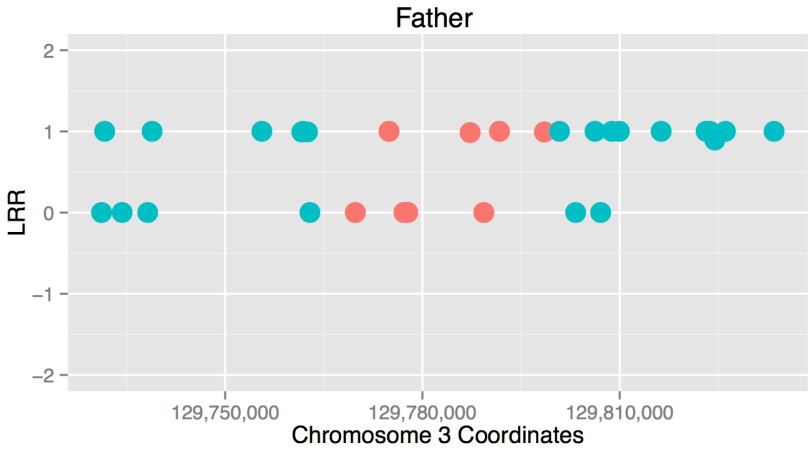
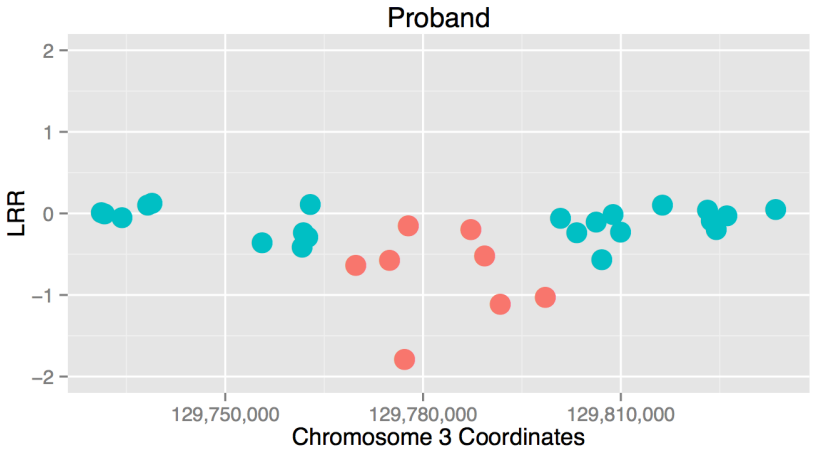
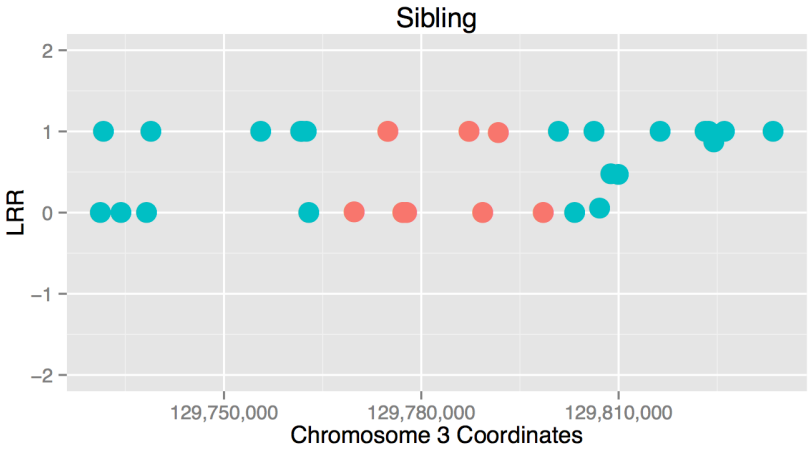
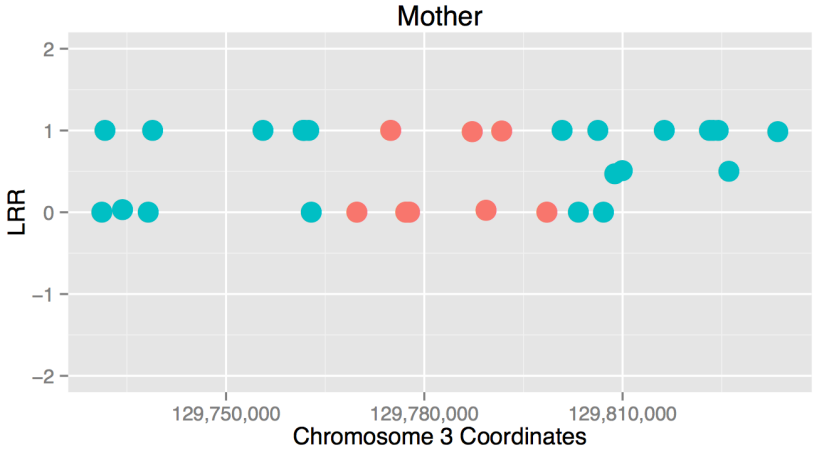




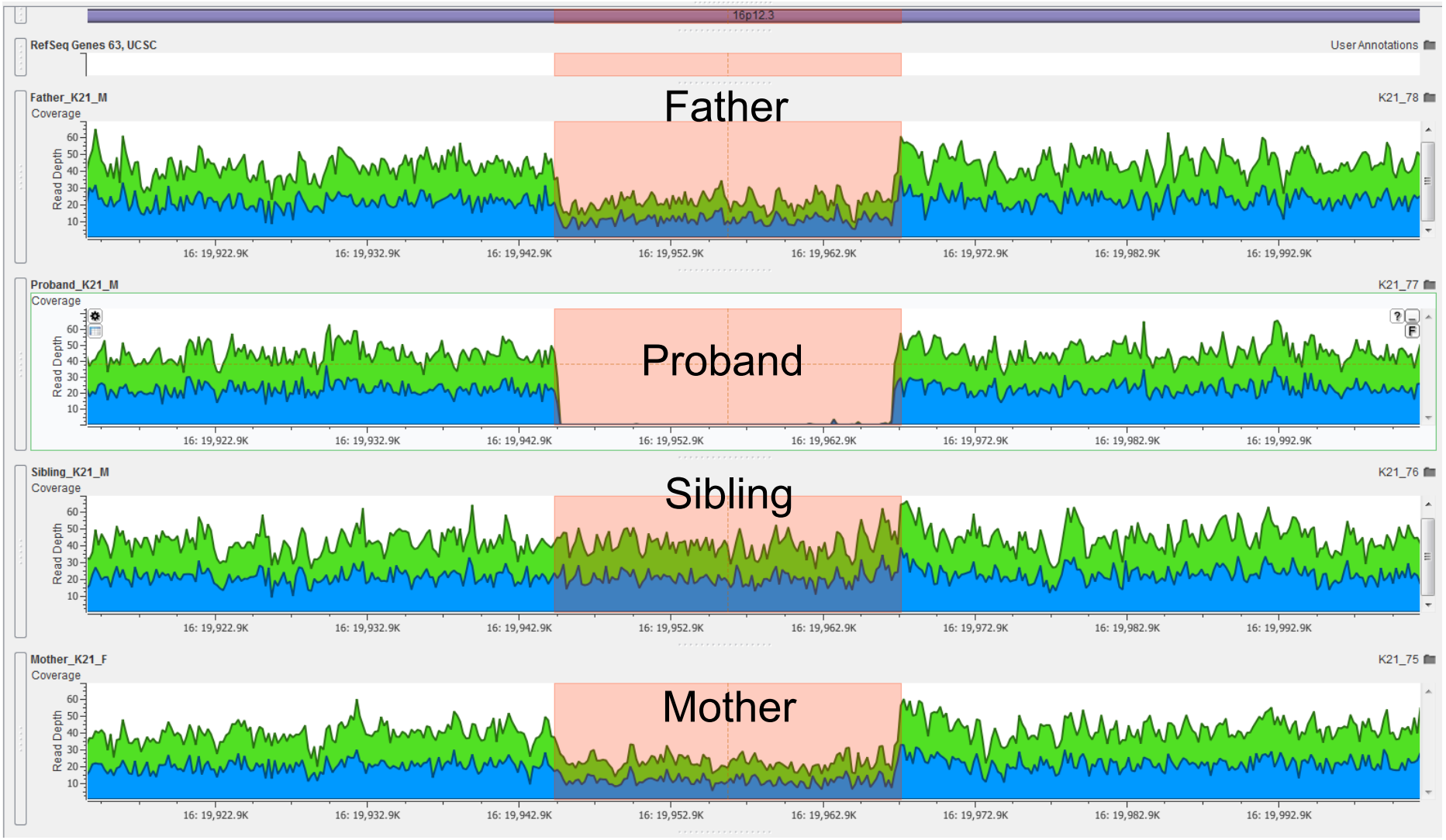




Region Alignment

K_21

Position	Size	Gene(s)	Ref/Obs. # of Copies	ERDS Score
16p12.3	~ 22 Kb.	intergenic	2/0	2475.9



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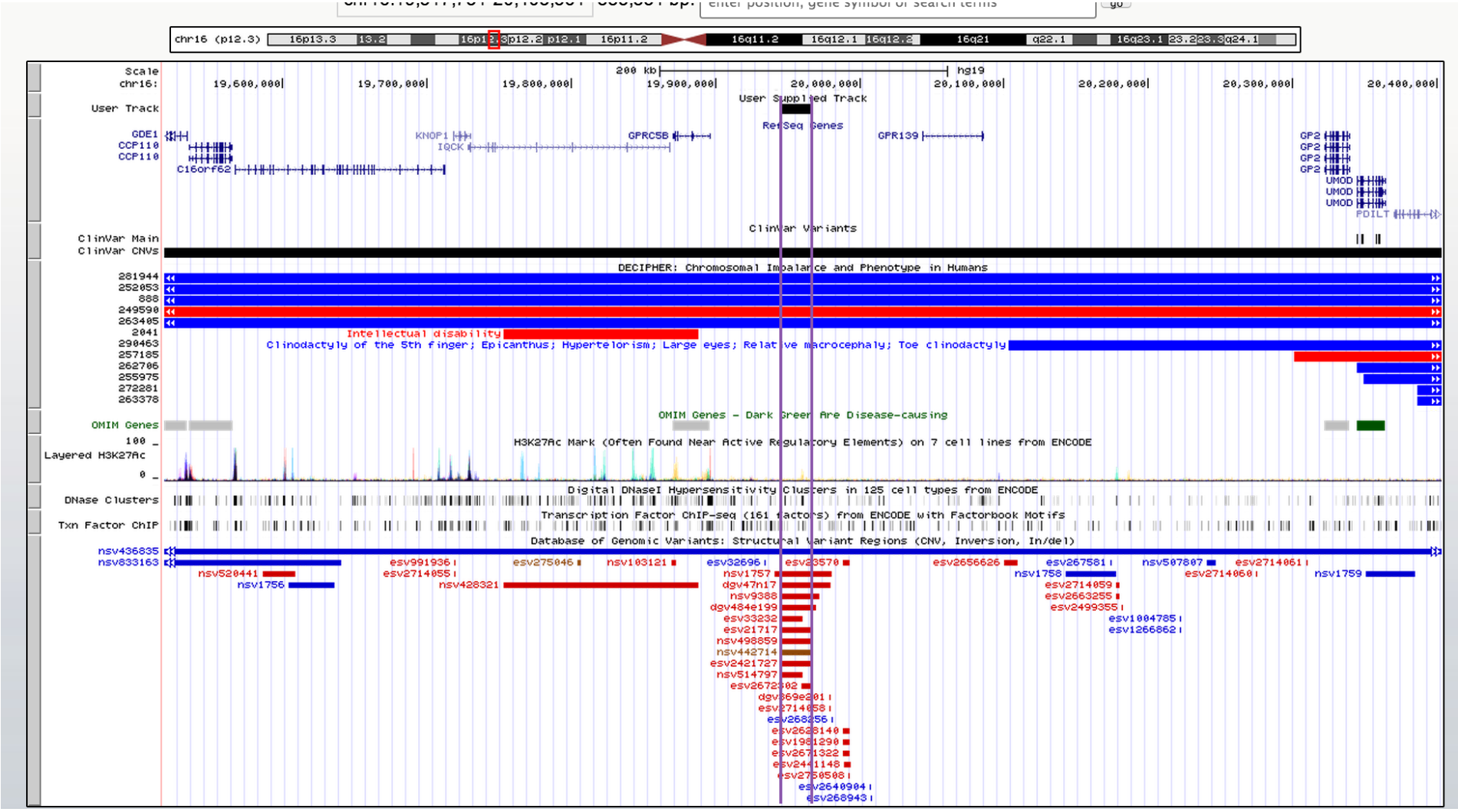

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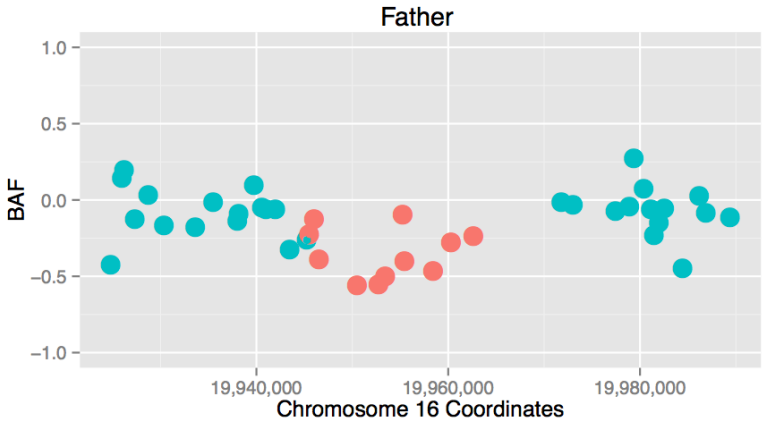

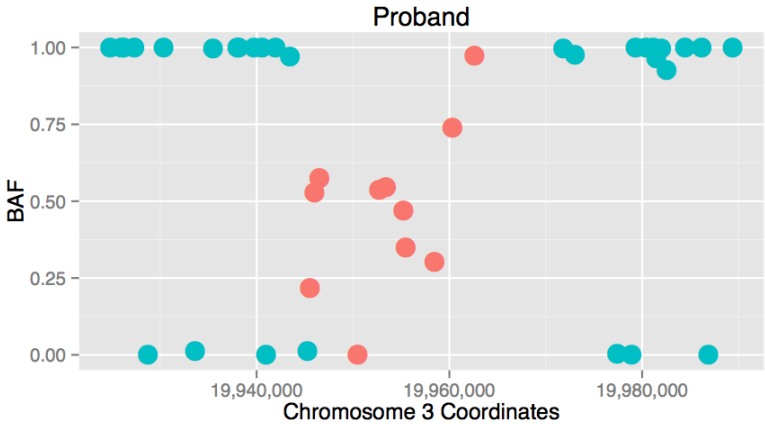

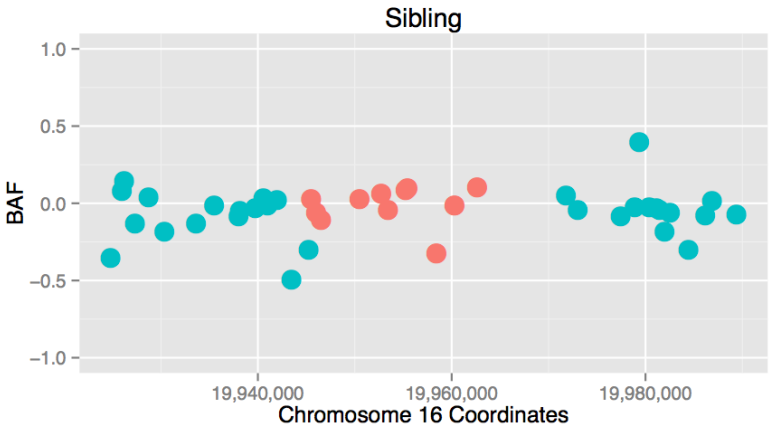

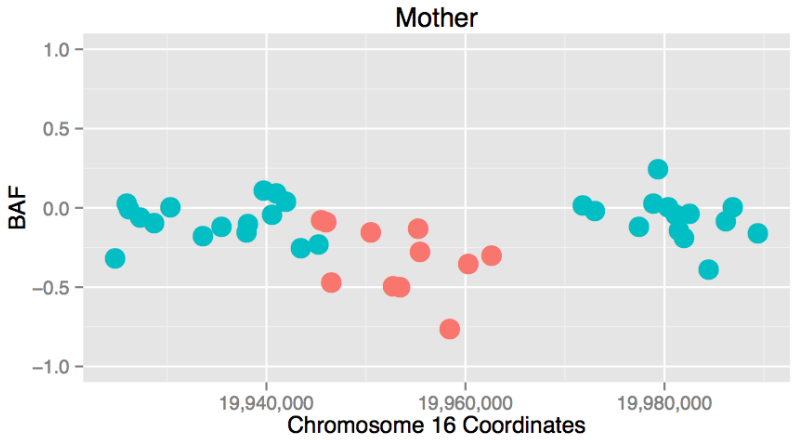

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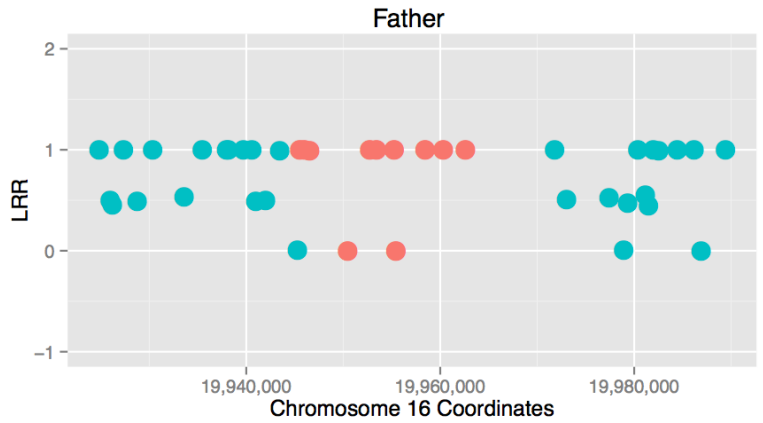
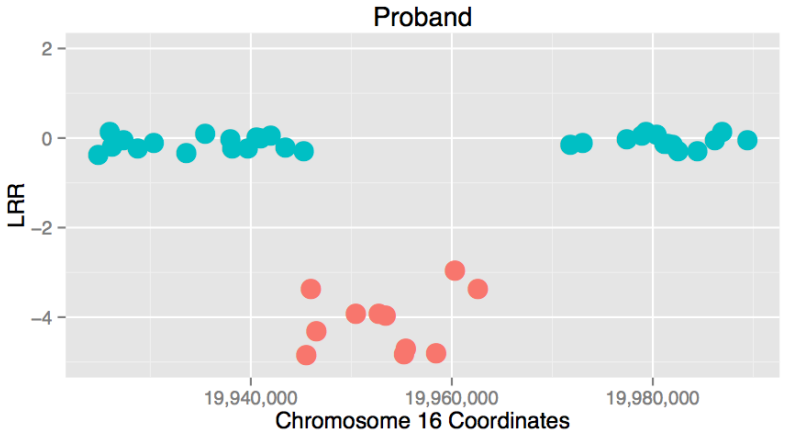
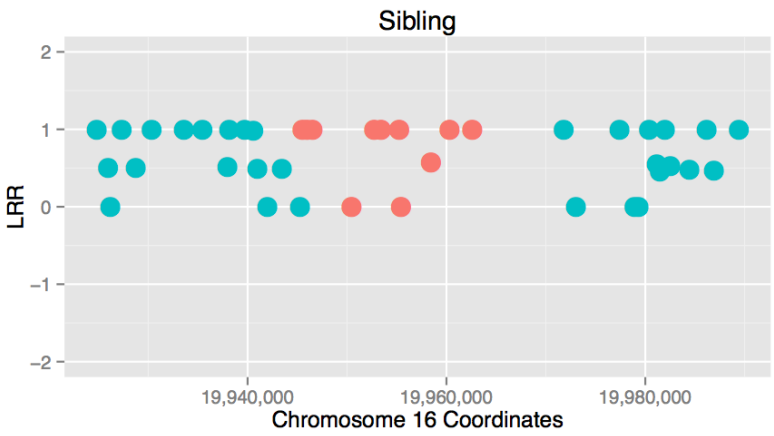
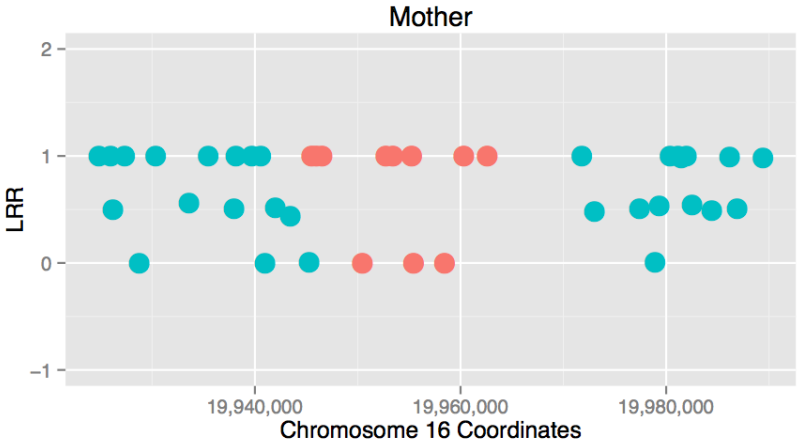




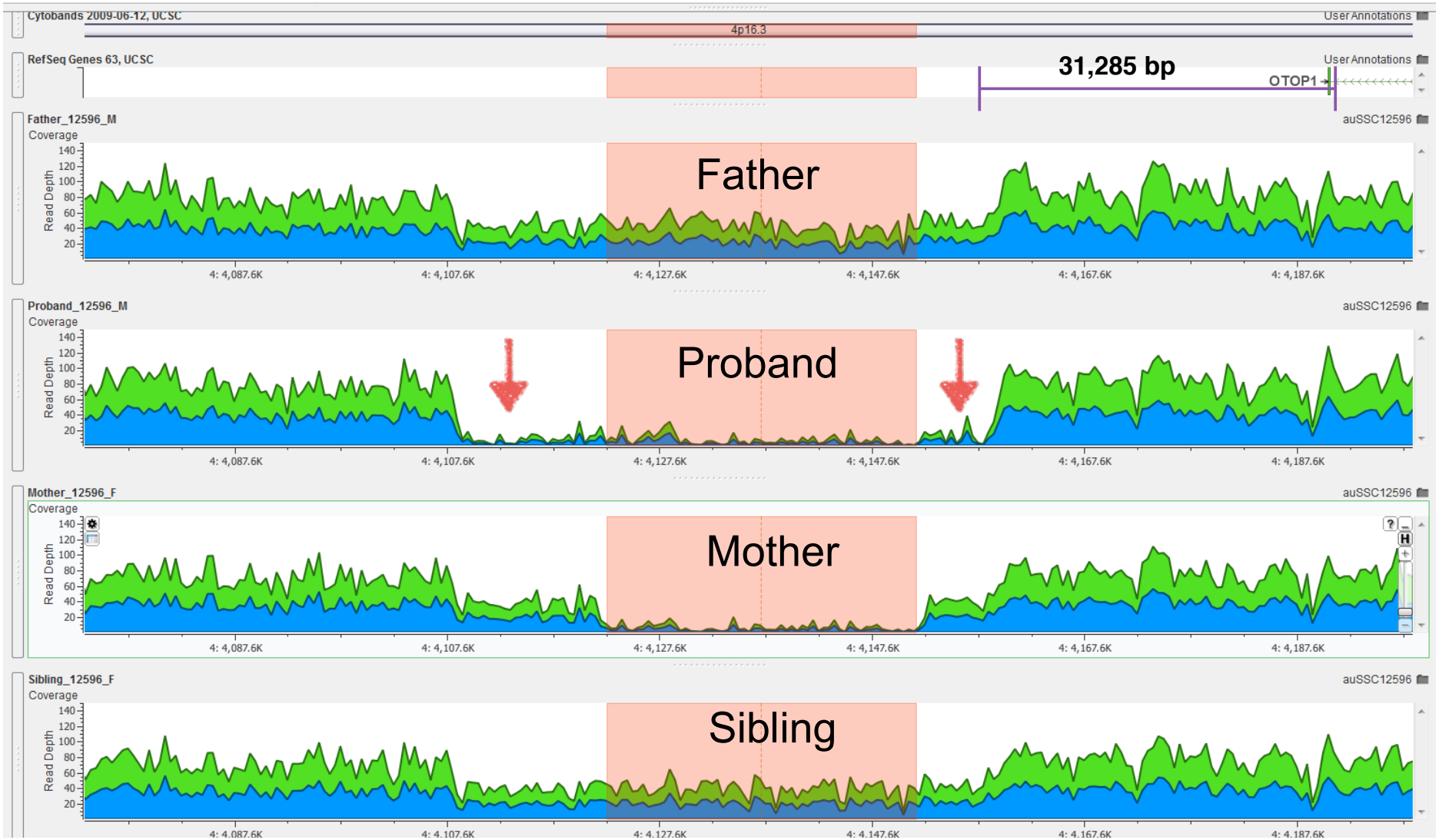




Microarray Data

SSC_2

Position	Size	Gene(s)	Ref/Obs. # of Copies	ERDS Score
4p16.3	~ 50 Kb.	intergenic	2/0	1525.08



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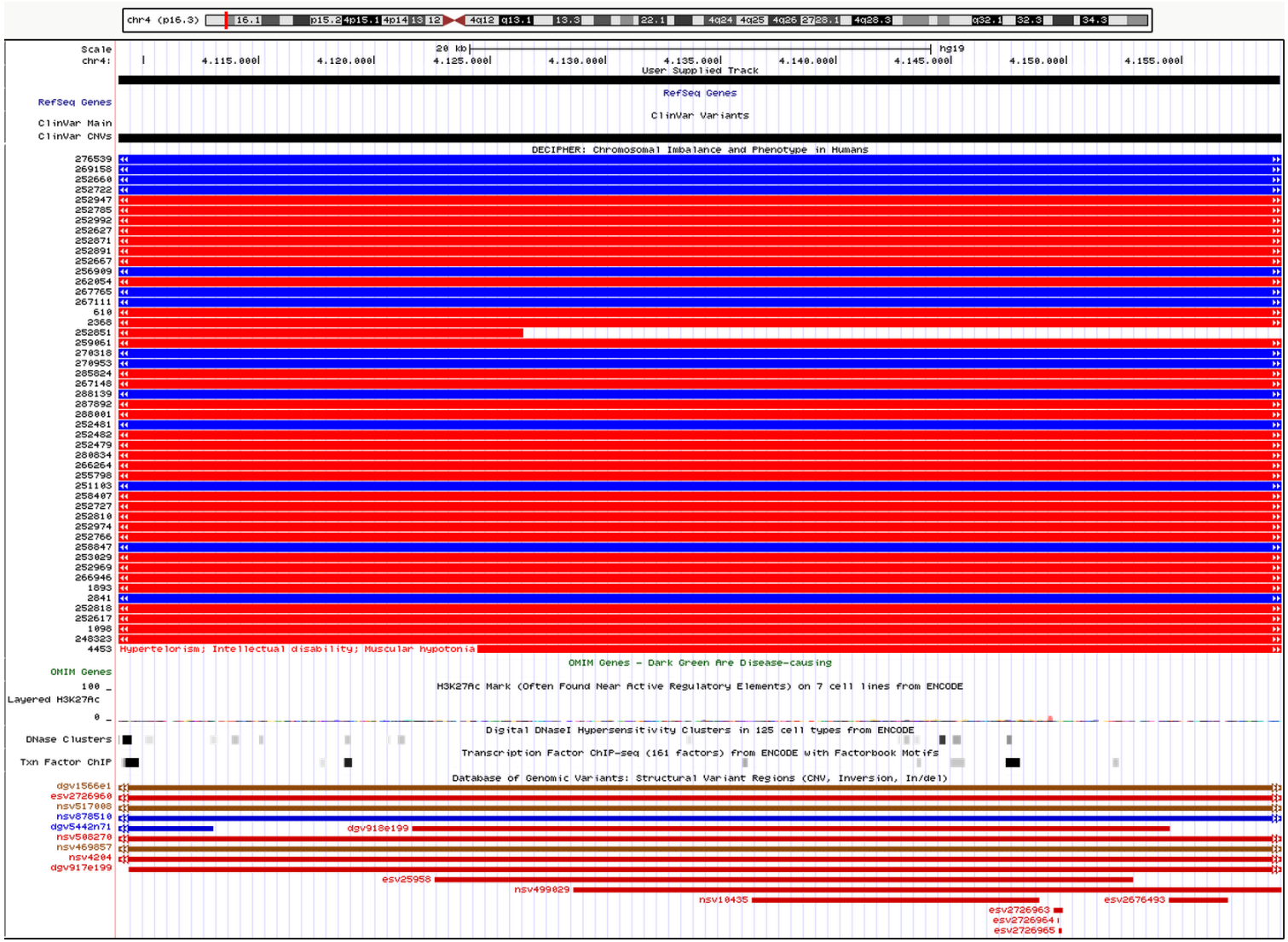

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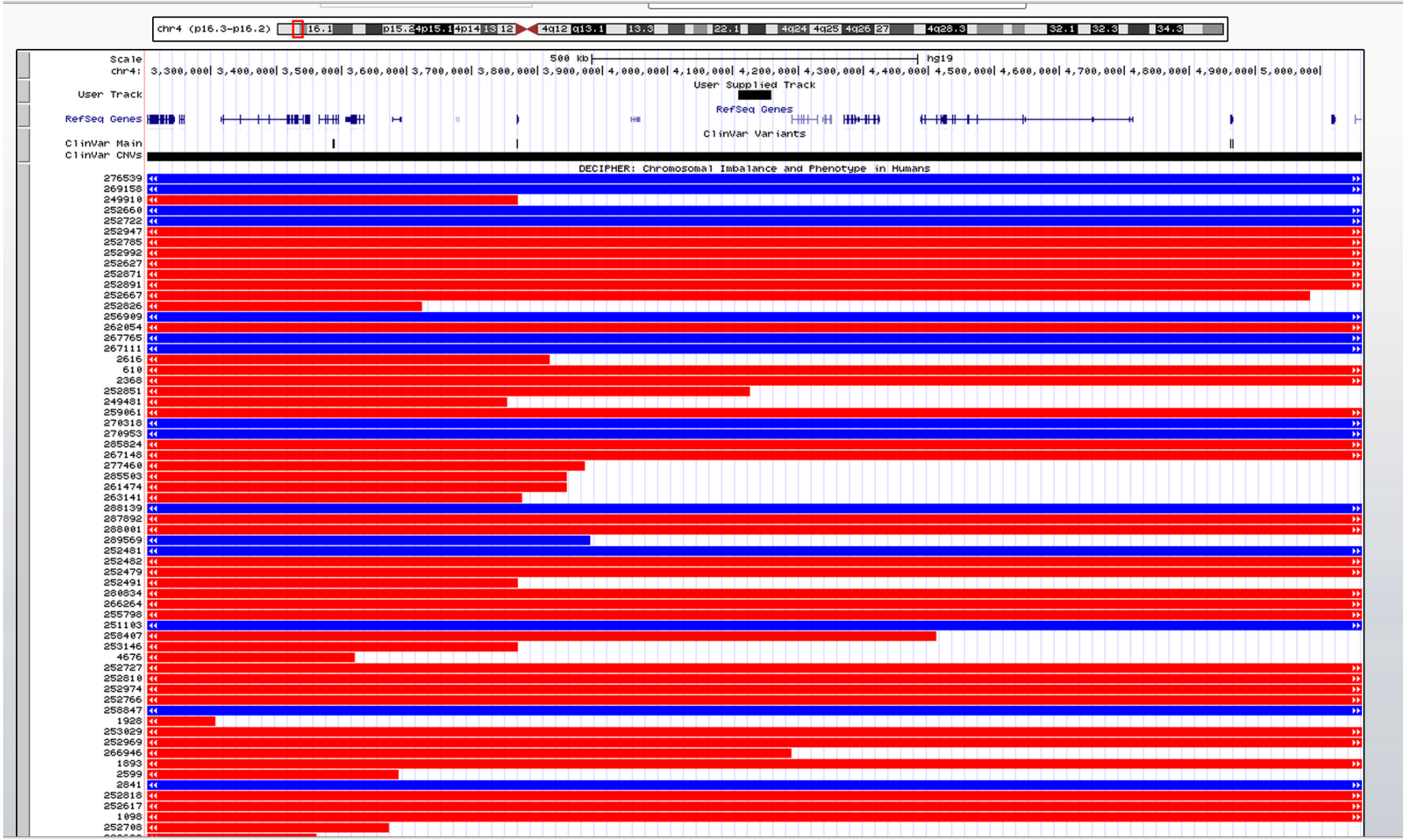

Region Annotation Zoom Out

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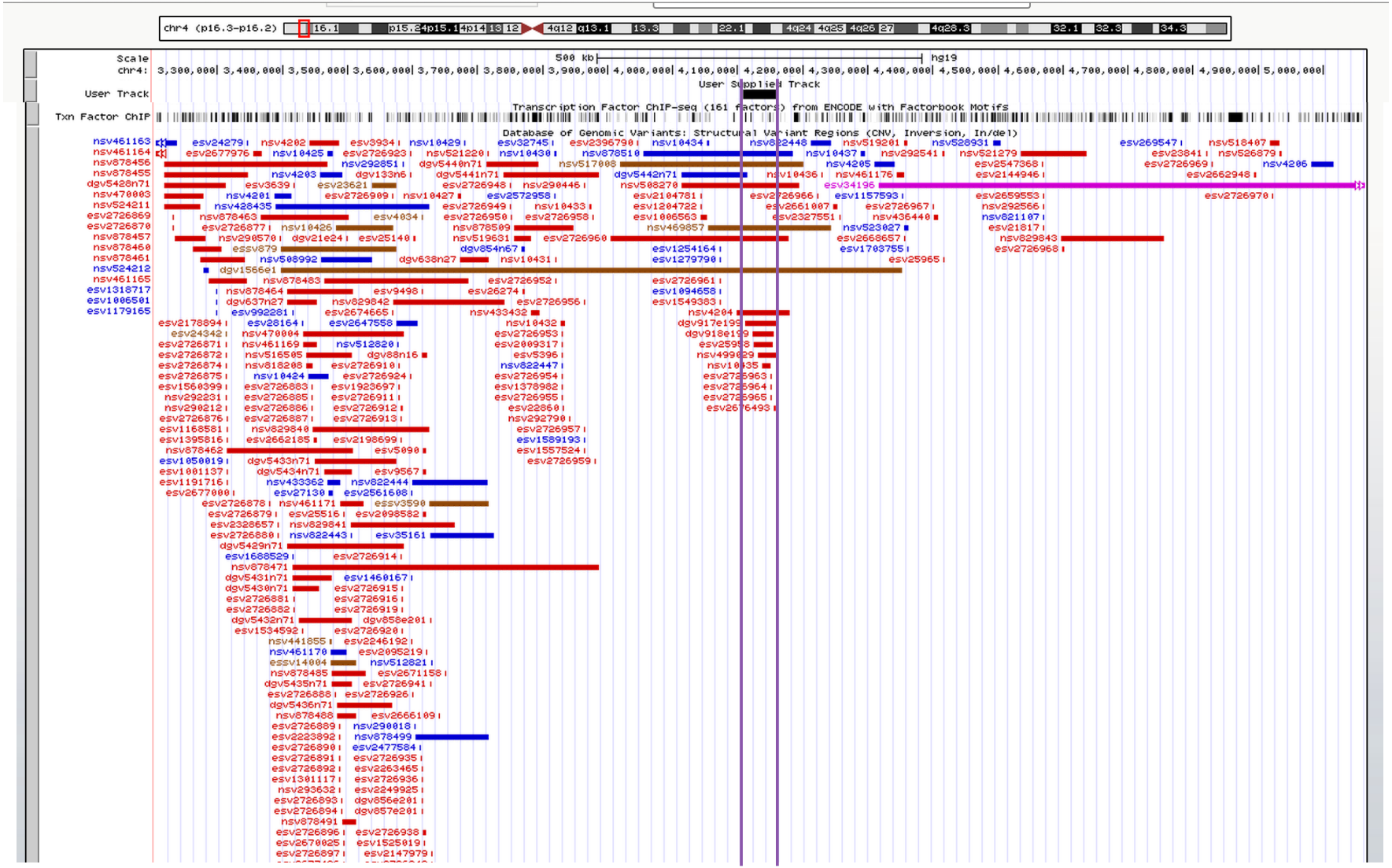
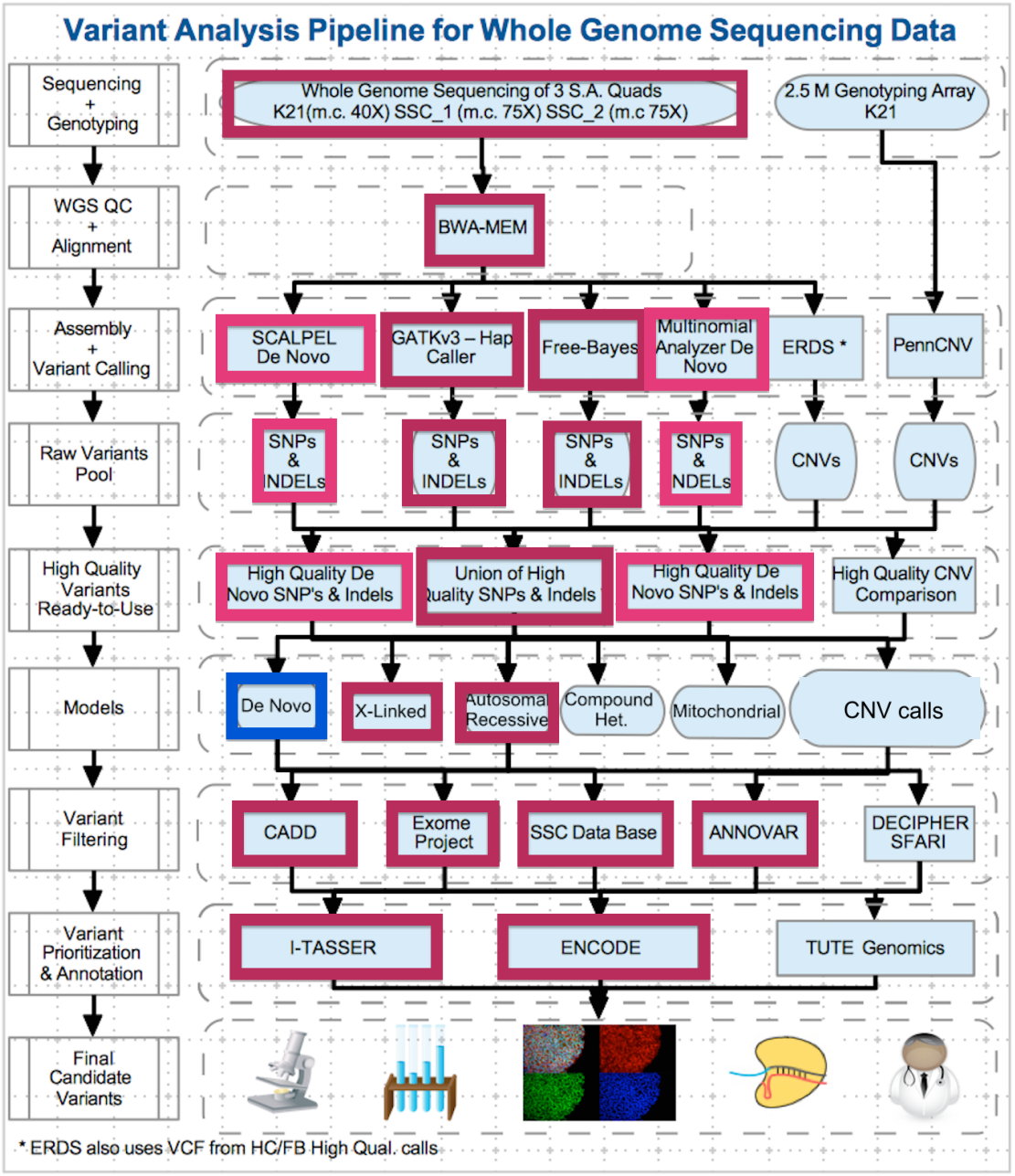


Key Points

- CNV Detection: Microarrays vs Sequencing
- Larger Amount of Samples
- Overlapping %
- Autosomal Recessive CNVs
- Regulatory Regions
- Functional Analysis
- Agregation of multiple variants

Smaller Variants



Algorithm	Parameters
BWA-MEM	Default Parameters
Picard Tools Mark Duplicates & Add Read Groups	Default Parameters
GATK Haplotype Caller 3.1-1	Default Parameters
GATK VQSR	Suggested parameters
Freebayes	Default Parameters
Filter FreeBayes	QUAL > 30
CADD	Score > 20
ANNOVAR	Ref genes / UCSC genes / DBGV / ENCODE / SSC / Exome Project

Neuron
Article

Multinomial Analyzer

Cell PRESS

De Novo Gene Disruptions in Children on the Autistic Spectrum

Ivan Iossifov,[1,6] Michael Ronemus,[1,6] Dan Levy,[1] Zihua Wang,[1] Inessa Hakker,[1] Julie Rosenbaum,[1] Boris Yamrom,[1] Yoon-ha Lee,[1] Giuseppe Narzisi,[1] Anthony Leotta,[1] Jude Kendall,[1] Ewa Grabowska,[1] Beicong Ma,[1] Steven Marks,[1] Linda Rodgers,[1] Asya Stepansky,[1] Jennifer Troge,[1] Peter Andrews,[1] Mitchell Bekritsky,[1] Kith Pradhan,[1] Elena Ghiban,[1] Melissa Kramer,[1] Jennifer Parla,[1] Ryan Demeter,[2] Lucinda L. Fulton,[2] Robert S. Fulton,[2] Vincent J. Magrini,[2] Kenny Ye,[3] Jennifer C. Darnell,[4] Robert B. Darnell,[4,5] Elaine R. Mardis,[2] Richard K. Wilson,[2] Michael C. Schatz,[1] W. Richard McCombie,[1] and Michael Wigler[1,*]
[1]Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA
[2]The Genome Institute, Washington University School of Medicine, St. Louis, MO 63108, USA
[3]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[4]Laboratory of Molecular Neuro-oncology, Rockefeller University, New York, NY 10065, USA
[5]Howard Hughes Medical Institute, Rockefeller University, New York, NY 10065, USA
[6]These authors contributed equally to this work
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DOI 10.1016/j.neuron.2012.04.009

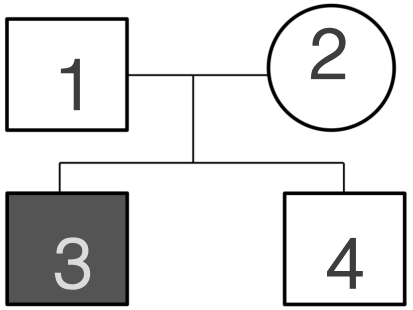
Scalpel

bioRχiv beta
THE PREPRINT SERVER FOR BIOLOGY

Accurate detection of de novo and transmitted INDELs within exome-capture data using micro-assembly

Giuseppe Narzisi, Jason A O'Rawe, Ivan Iossifov, et al.

Models



De Novo

3 – (1 ∪ (2 ∪ 4))

Family	Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
K_21	**17p13.2**	40	MYBBP1A: exon1:Stop	G/A	F44:0/ P19:22/ S28:0/ M45:0	FreeBayes/ HC/ Multinomial	SSC missense
SSC_1	**18q11.2**	36	intergenic	T/C	F8:1/ P9:11/ S9:0/ M7:0	FreeBayes/ HC/	NO

Region Alignment

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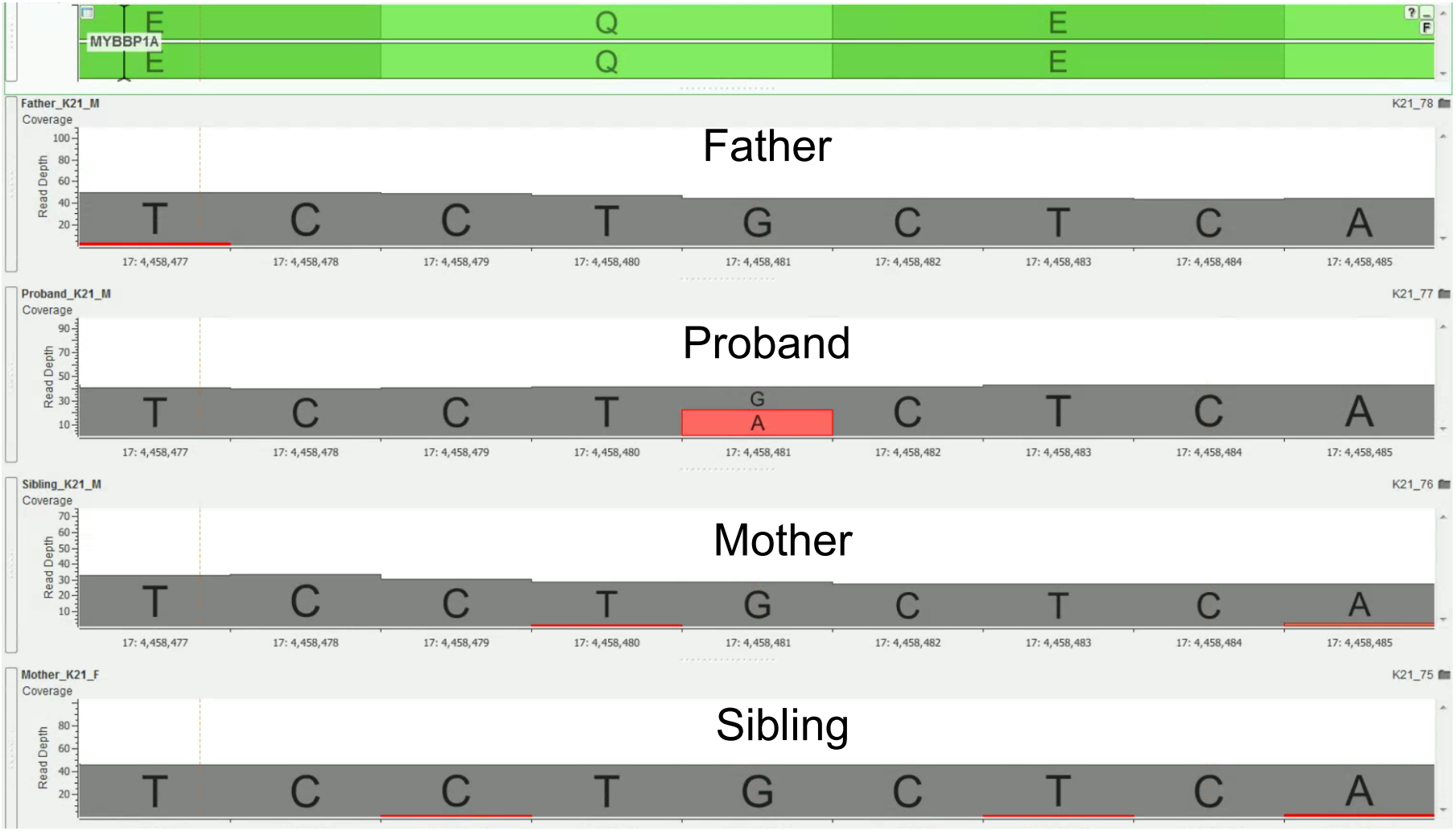
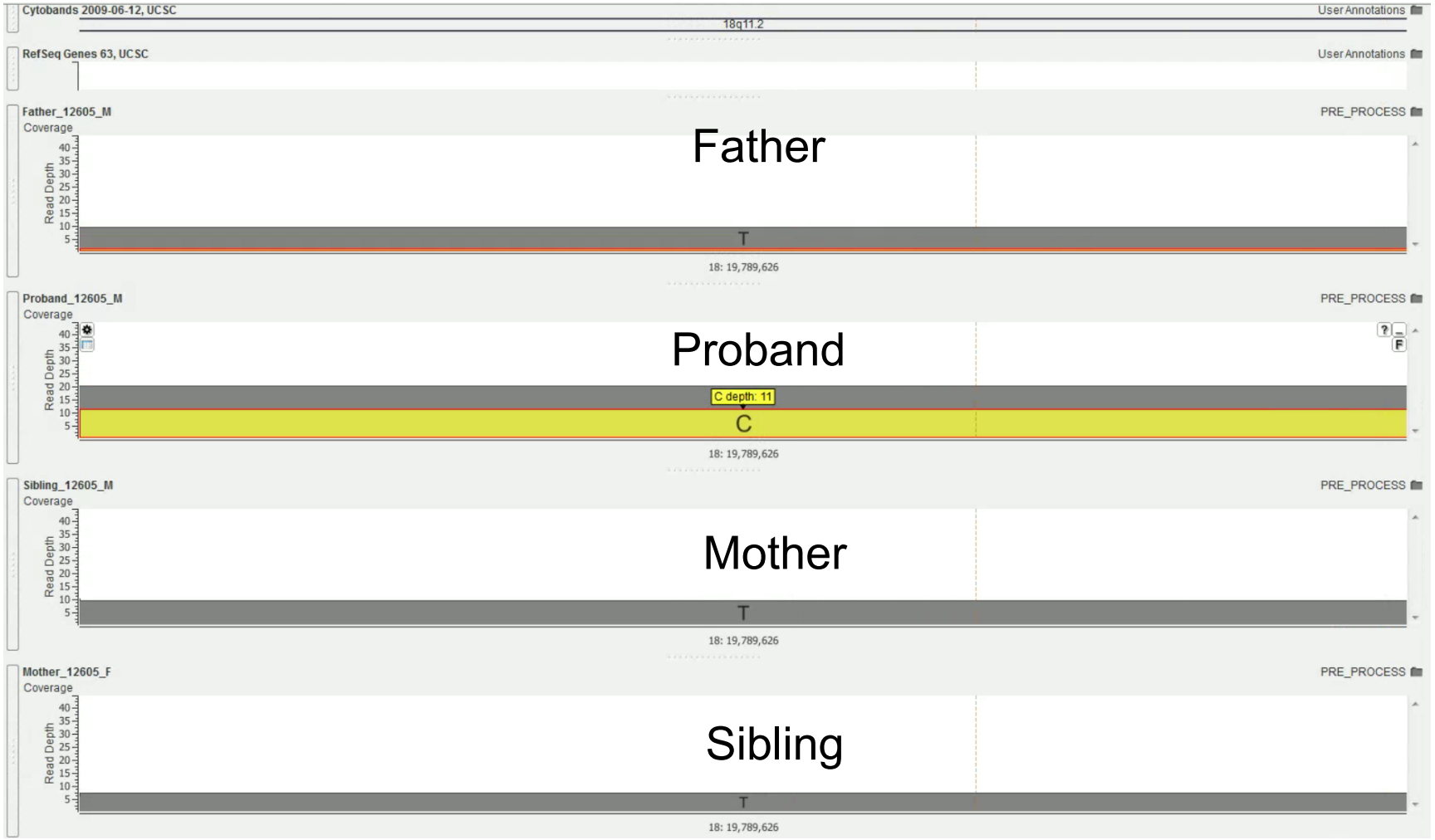
Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
17p13.2	40	MYBBP1A: exon1:Stop	G/A	F44:0/ P19:22/ S28:0/ M45:0	FreeBayes/ HC/ Multinomial	SSC missense

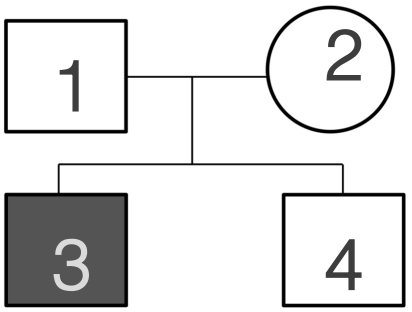


Region Alignment

SSC_1

Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
18q11.2	36	intergenic	T/C	F8:1/ P9:11/ S9:0/ M7:0	FreeBayes/ HC/ Multinomial	NO





X - Linked

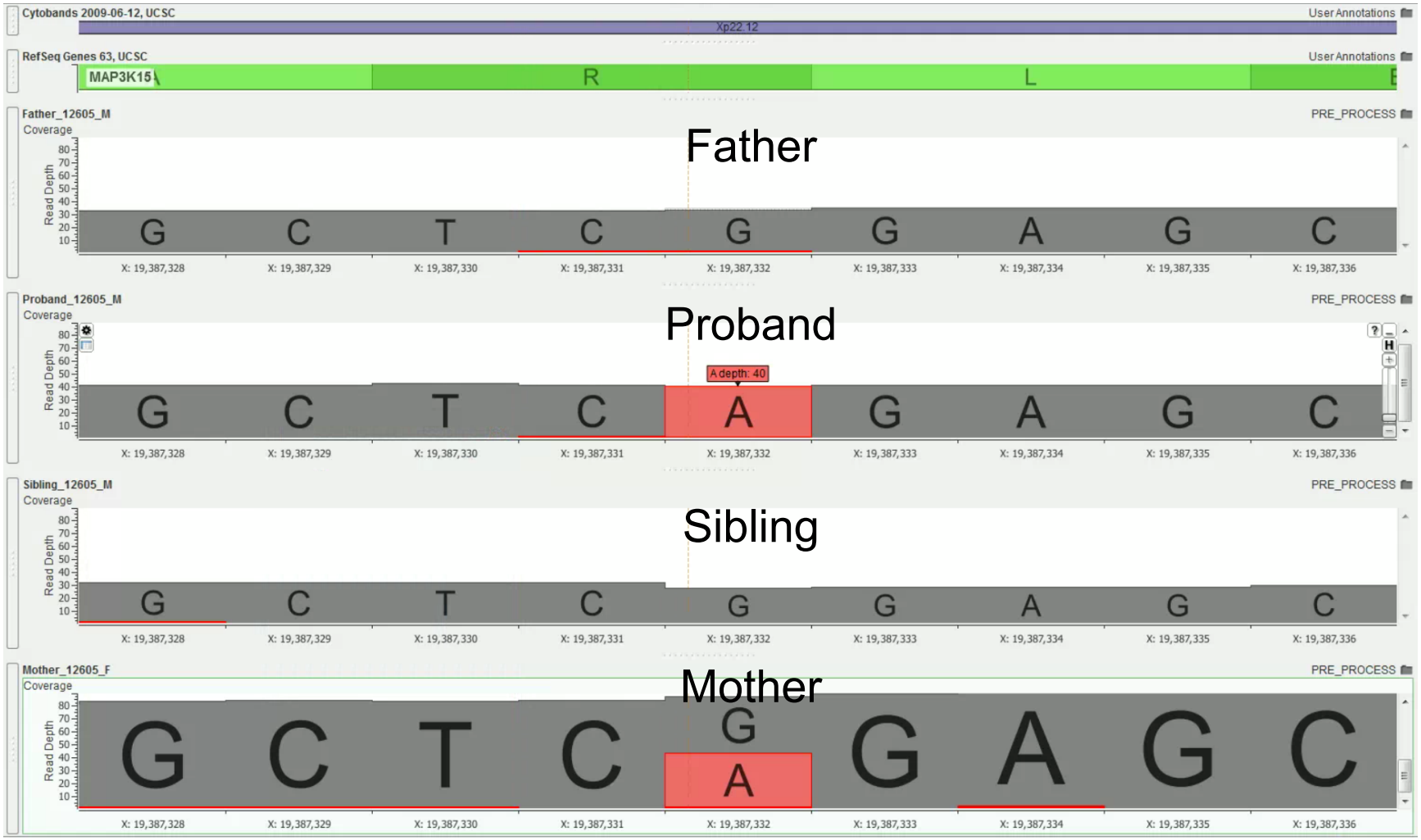
$$(1^{chrX} \cup 4^{chrX}) - (2^{chrX} \cap 3^{chrX})$$

Family	Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
SSC_1	**Xp22.12**	39	MAP3K15 exon 25 of 26 Arg-Stop	G/A	F 32:0/ P 0:40 S 31:0/ M 44:42	FreeBayes/ GATK-HC/	NO

Region Alignment

SSC_1

Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
Xp22.12	39	MAP3K15 exon 25 of 26 Arg-Stop	G/A	F 32:0/ P 0:40 S 31:0/ M 44:42	FreeBayes/ GATK-HC/	NO



Phyre²

SSC_1

3D Models

Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
Xp22.12	39	MAP3K15 exon 25 of 26 Arg-Stop	G/A	F 32:0/ P 0:40 S 31:0/ M 44:42	FreeBayes/ GATK-HC/	NO

Complete Protein

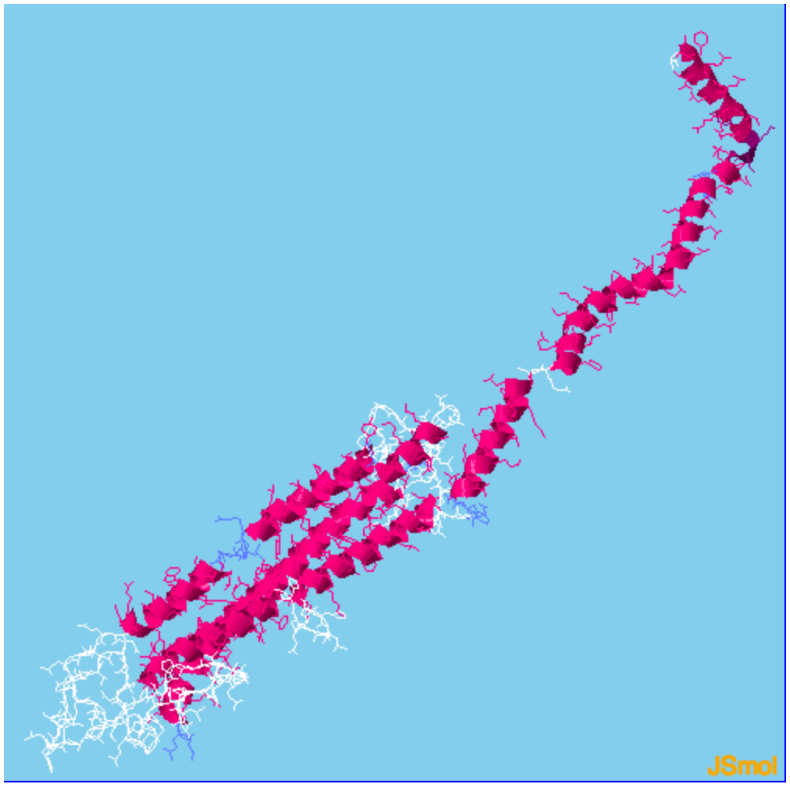
Incomplete Protein

3D Models

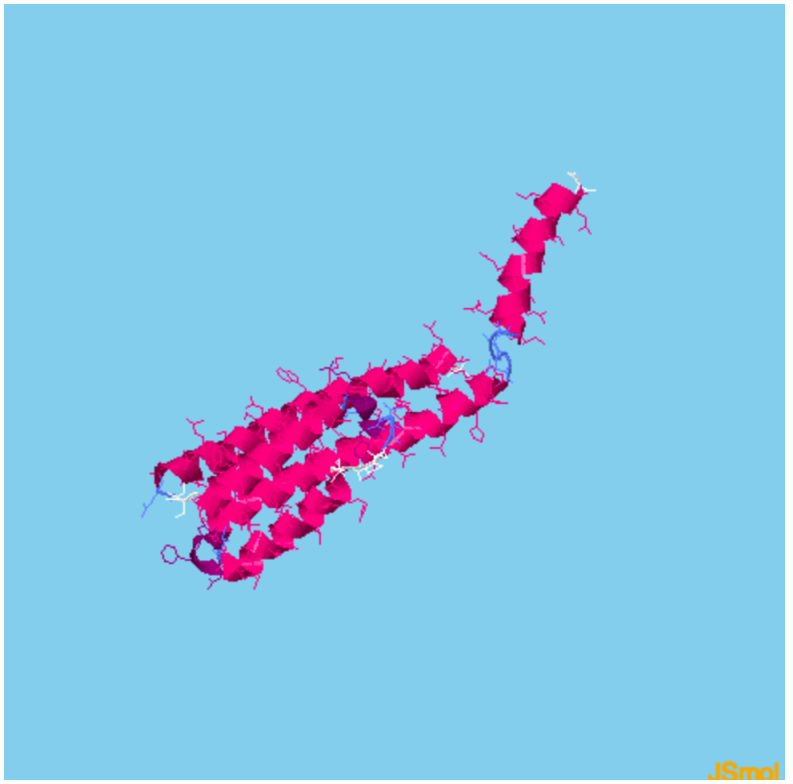
Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
Xp22.12	39	MAP3K15 exon 25 of 26 Arg-Stop	G/A	F 32:0/ P 0:40 S 31:0/ M 44:42	FreeBayes/ GATK-HC/	NO

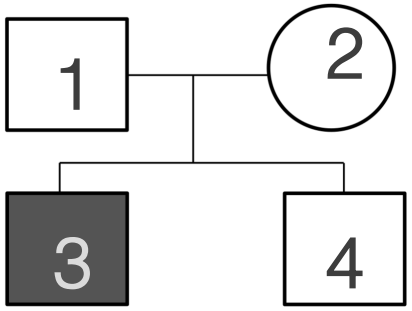
SSC_1

5th domain Complete Protein (1001-1313)



5th domain Incomplete Protein (104-1135)





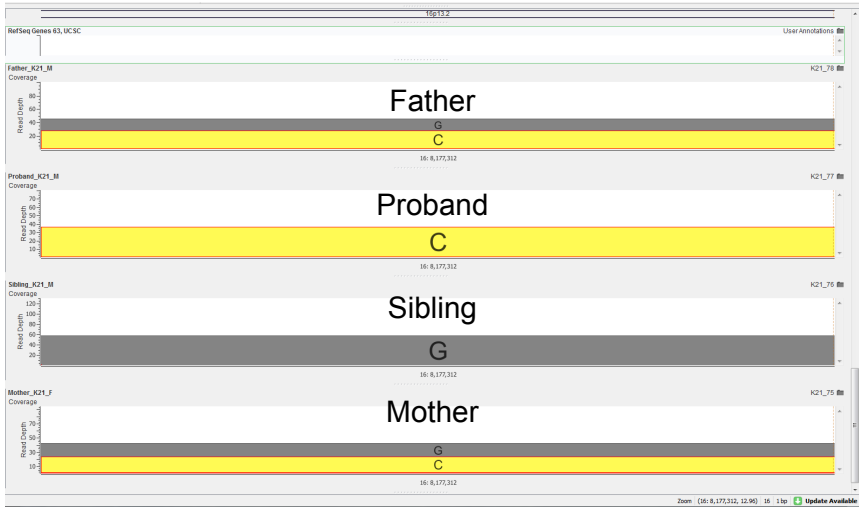
Autosomal Recessive

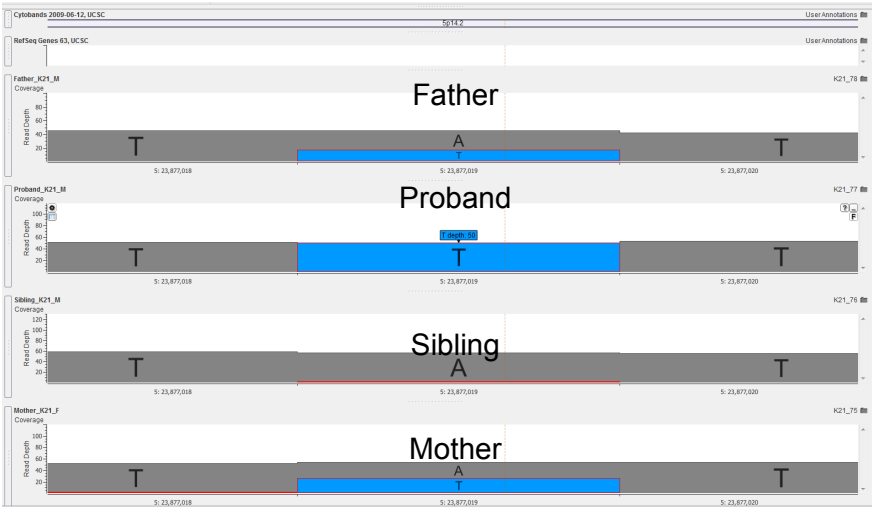
$$4 - (3_{h|hh} \cap (1_h \cap 2_h))$$

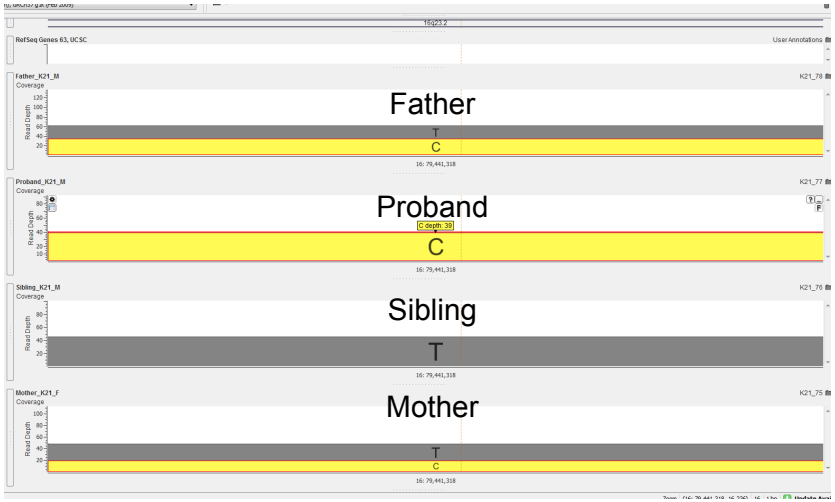
Family	Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
K_21	16p13.2	30	intergenic	G/C	F 18:27 P0:36 S57:0 M19:22	FeeeBayes/ GATK HC	NO
K_21	16q36.2	37	intergenic	T/C	F27:34 P0:39 S45:0 M29:19	FeeeBayes/ GATK HC	NO
K_21	5p14.2	34	intergenic	A/T	F28:19 P0:50 S54:0 M27:26	FeeeBayes/ GATK HC	NO
K_21	9q31.1	33	intergenic	G/A	P19:16 P0:37 S38:0 M13:24	FeeeBayes/ GATK HC	NO

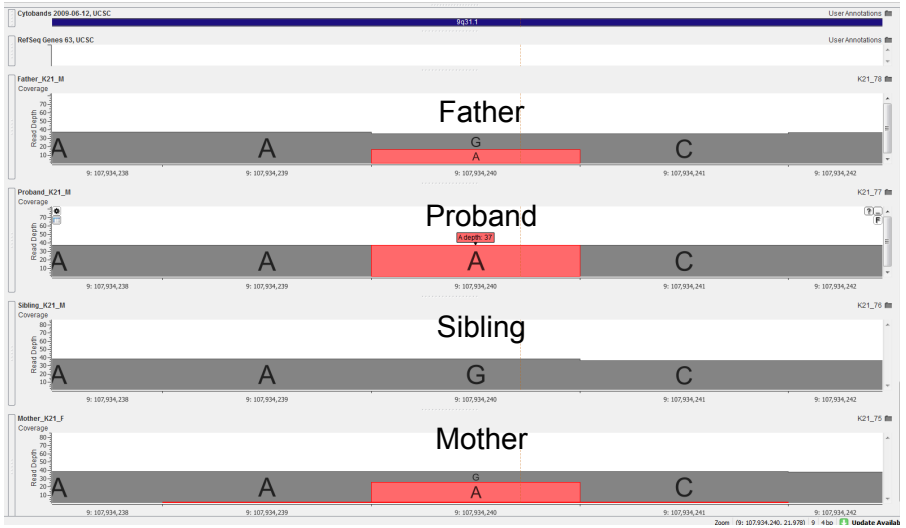
Autosomal Recessive

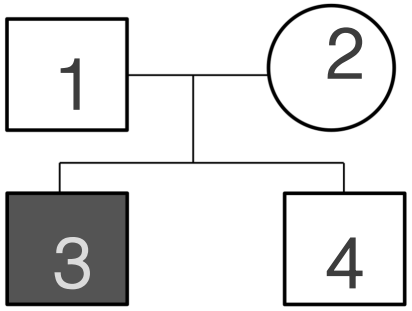
K_21











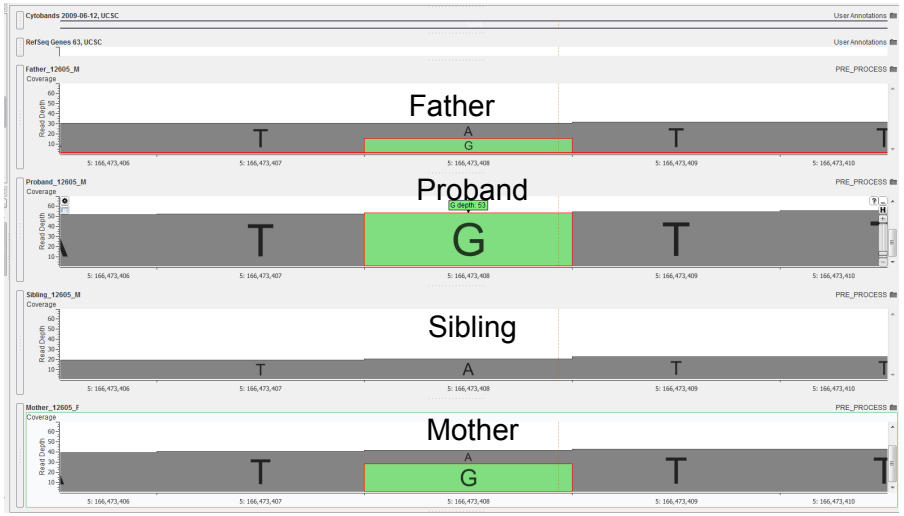
Autosomal Recessive

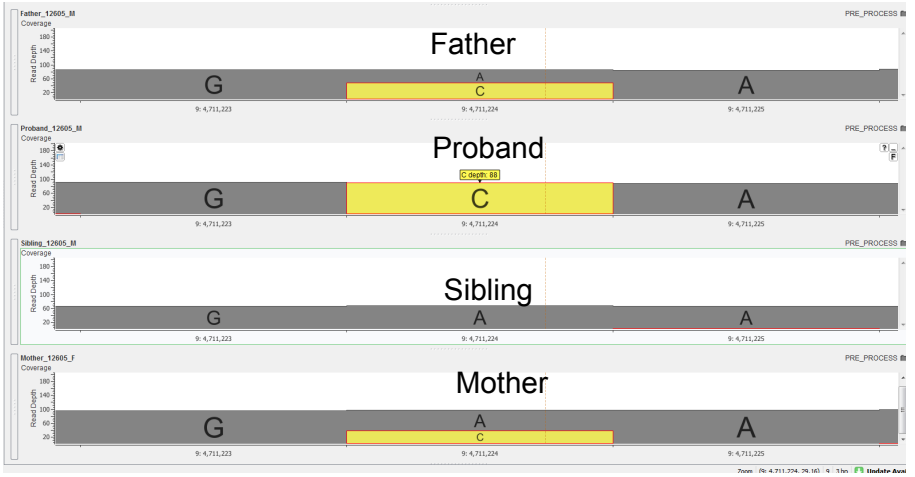
$$4 - (3_{h|hh} \cap (1_h \cap 2_h))$$

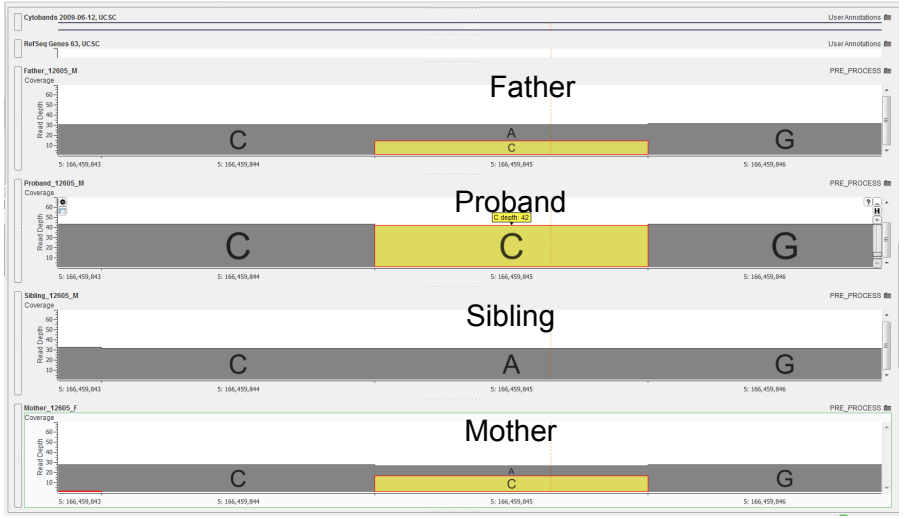
Family	Position	CAAD score	Gene Mutation	Ref/Alt	Reads	Caller	Other DB
SSC_1	5q34	32	intergenic	A/G	F15:14 P0:53 S20:0 M13:28	FeeeBayes/ GATK HC	NO
SSC_1	5q34	32	intergenic	A/C	F16:14 P0:42 S31:0 M10:16	FeeeBayes/ GATK HC	NO
SSC_1	15q26.2	32	intergenic	G/A	F26:34 P0:58 S44:0 M43:39	FeeeBayes/ GATK HC	NO
SSC_1	9p24.1	31	intergenic	A/C	F38:46 P0:88 S66:0 M59:37	FeeeBayes/ GATK HC	NO

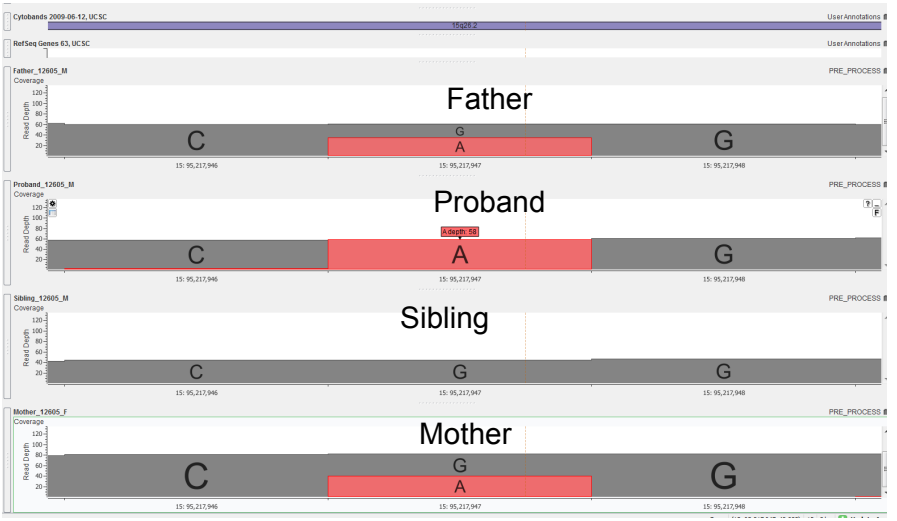
SSC_1

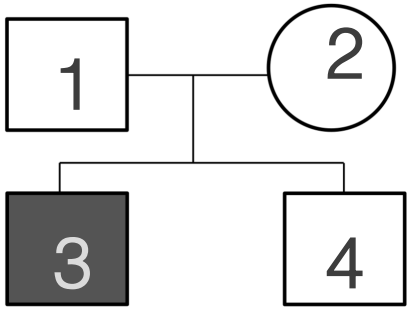
Autosomal Recessive











Compound Heterozygous

3 – (4 ∩ ((1 – 2) ∪ (2 – 1)))

Annotate all HQ variants

↓

Get variants sharing a gene

↓

Filter out exact match of combination of variants for a gene in controls

Conclussions

- Larger Amount of Samples for statistical power.
- No common pathogenic variants among different probands.
- Not only De Novo or Gene Disrupting Variants could be playing a role.
- Functional Analysis.

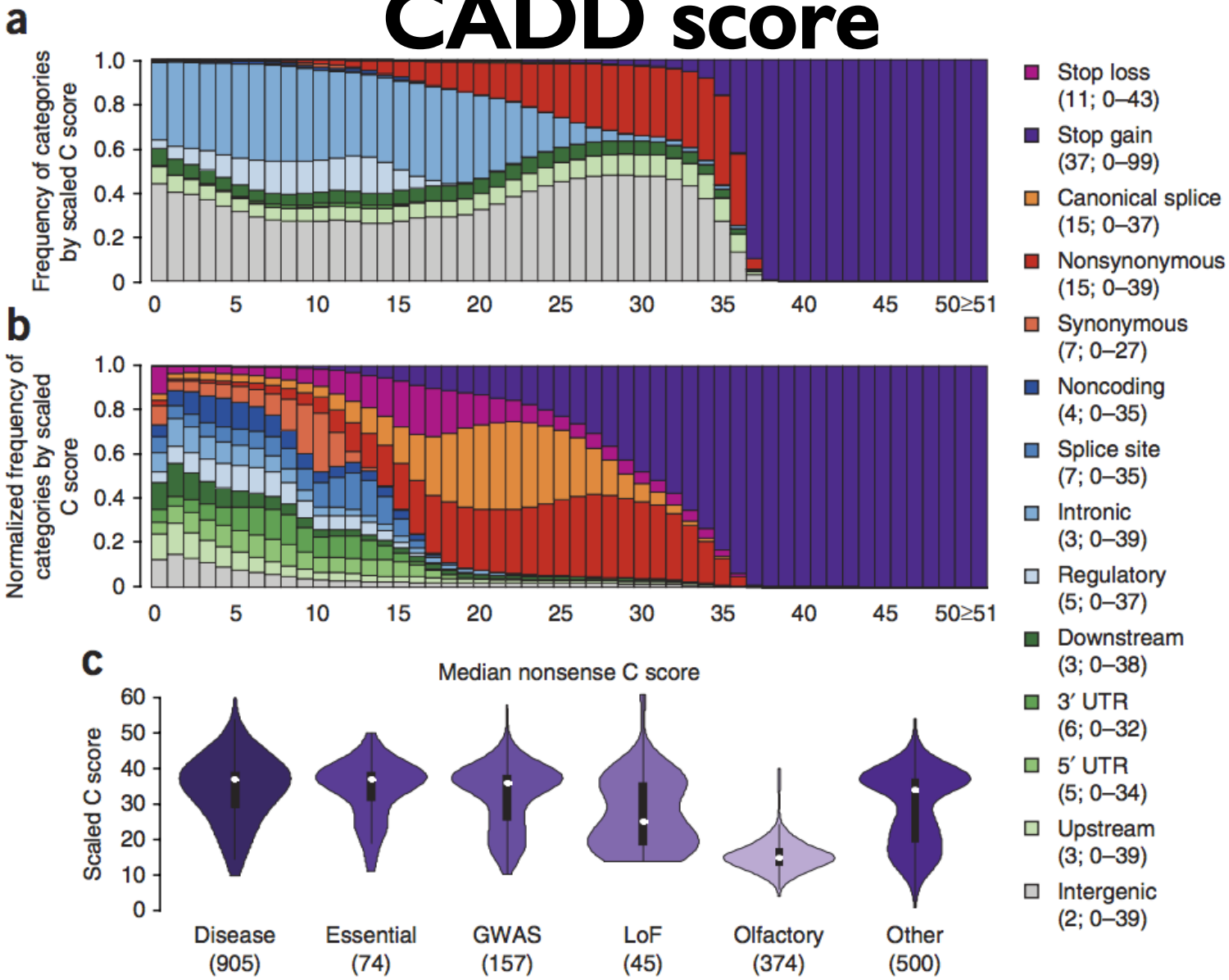
THANKS!

BAF

$$BAF = \begin{cases} 0, \text{ if } \theta < \theta_{AA} \\ 0.5(\theta - \theta_{AA})/(\theta_{AB} - \theta_{AA}), \text{ if } \theta \leq \theta < \theta_{AB} \\ 0.5 + 0.5(\theta - \theta_{AB})/(\theta_{BB} - \theta_{AB}), \text{ if } \theta_{AB} \leq \theta < \theta_{BB} \\ 1, \text{ if } \theta \geq \theta_{BB} \end{cases} \quad (1)$$

where θ_{AA}, θ_{AB}, and θ_{BB} are the θ values for three canonical genotype clusters generated from a large set of reference samples. The transformation from θ to BAF values adjusts for different chemical characteristics of each SNP so that values for different SNPs are more comparable to each other.

CADD score


a
Frequency of categories by scaled C score
1.0
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45 50≥51
b
Normalized frequency of categories by scaled C score
1.0
0.8
0.6
0.4
0.2
0
0 5 10 15 20 25 30 35 40 45 50≥51
c
Median nonsense C score
Scaled C score
60
50
40
30
20
10
0
Disease (905)
Essential (74)
GWAS (157)
LoF (45)
Olfactory (374)
Other (500)
Stop loss (11; 0–43)
Stop gain (37; 0–99)
Canonical splice (15; 0–37)
Nonsynonymous (15; 0–39)
Synonymous (7; 0–27)
Noncoding (4; 0–35)
Splice site (7; 0–35)
Intronic (3; 0–39)
Regulatory (5; 0–37)
Downstream (3; 0–38)
3′ UTR (6; 0–32)
5′ UTR (5; 0–34)
Upstream (3; 0–39)
Intergenic (2; 0–39)