Comprehensive Genome and Transcriptome Structural Analysis of a Breast Cancer Cell Line using PacBio Long Read Sequencing

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Schatz + McCombie + Hicks at Cold Spring Harbor Laboratory

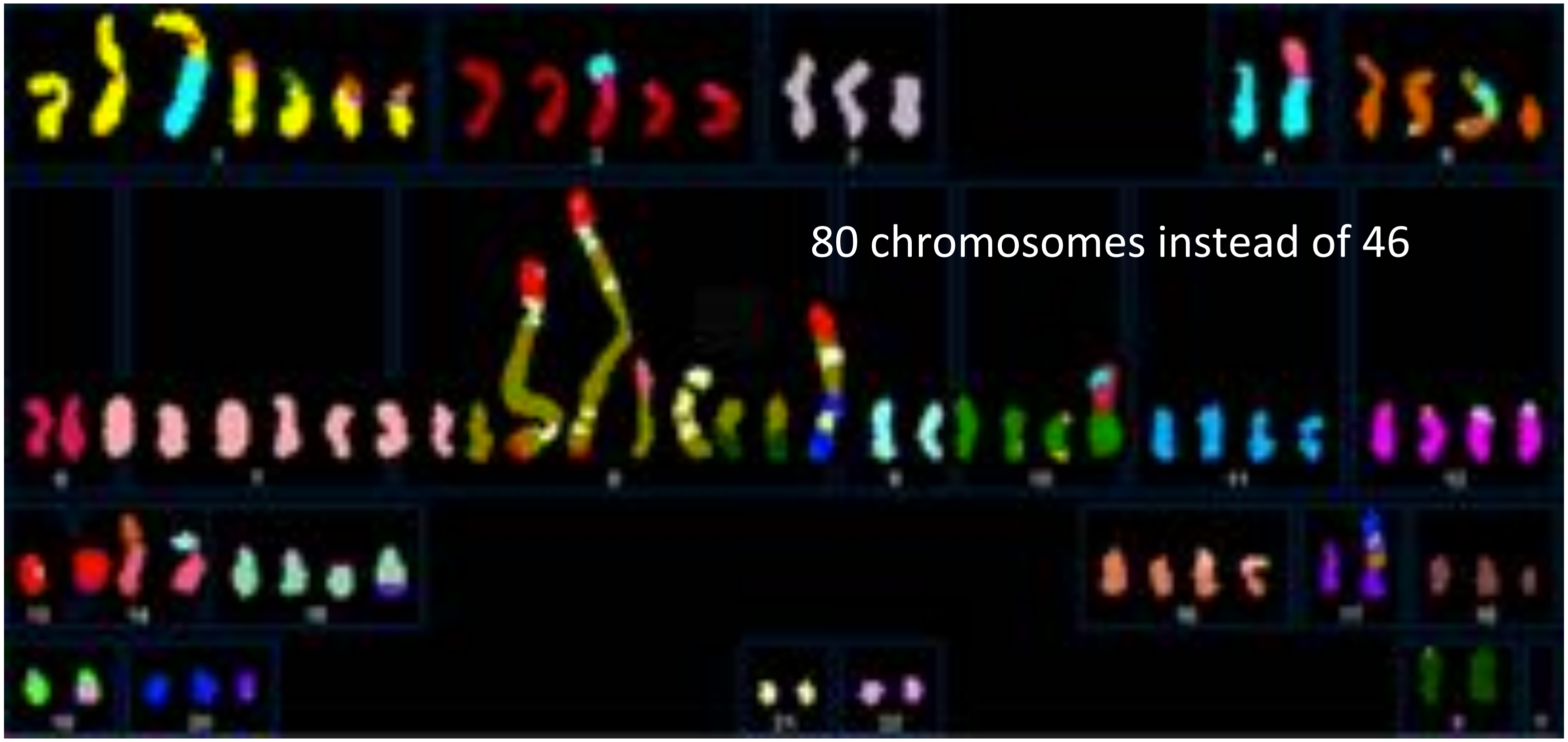
McPherson + Beck at the Ontario Institute for Cancer Research

Pacific Biosciences

DNAnexus

SK-BR-3

Most commonly used Her2-amplified breast cancer cell line



80 chromosomes instead of 46

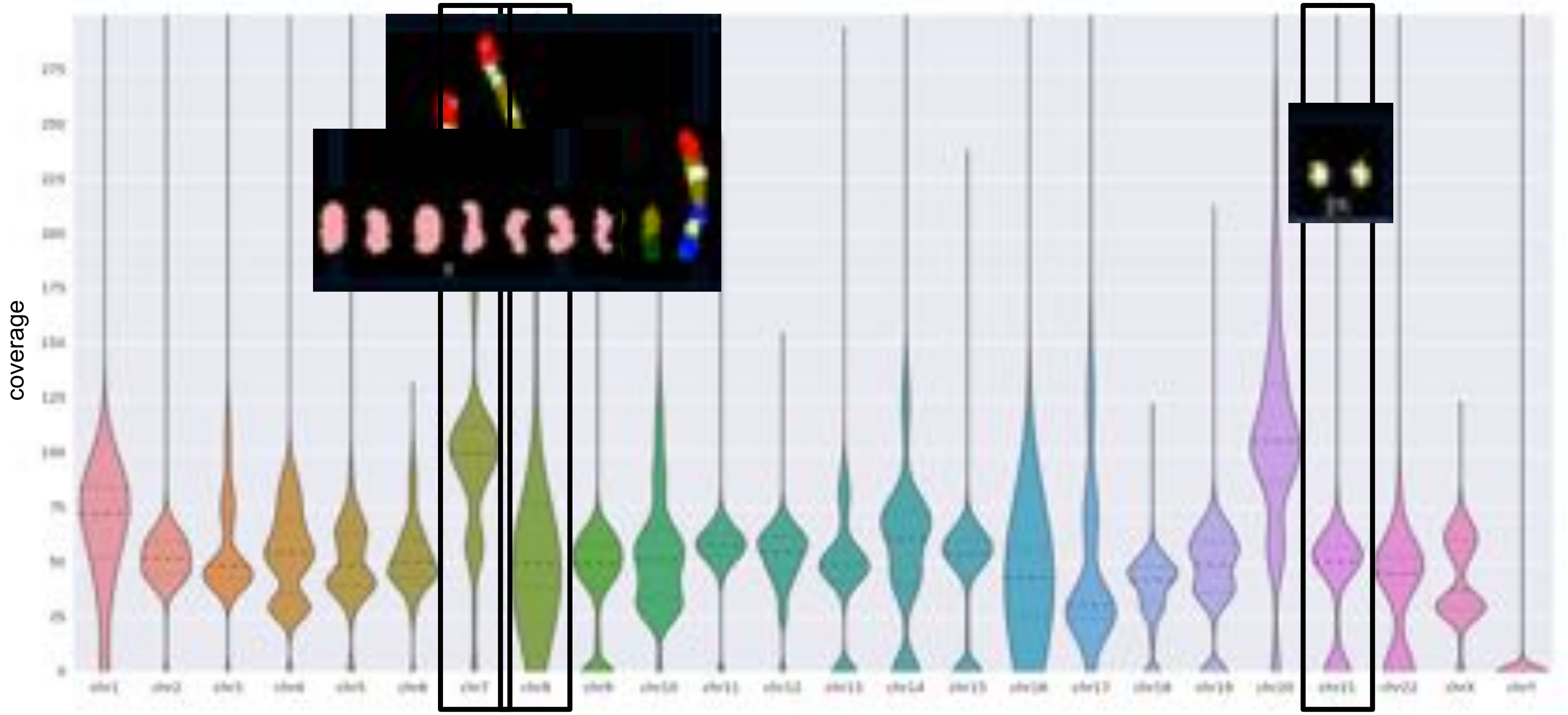
Often used for pre-clinical research on Her2-targeting therapeutics such as Herceptin (Trastuzumab) and resistance to these therapies.

(Davidson et al, 2000)

PacBio long-read DNA sequencing

mean read length: 9 kb
max read length: 71 kb

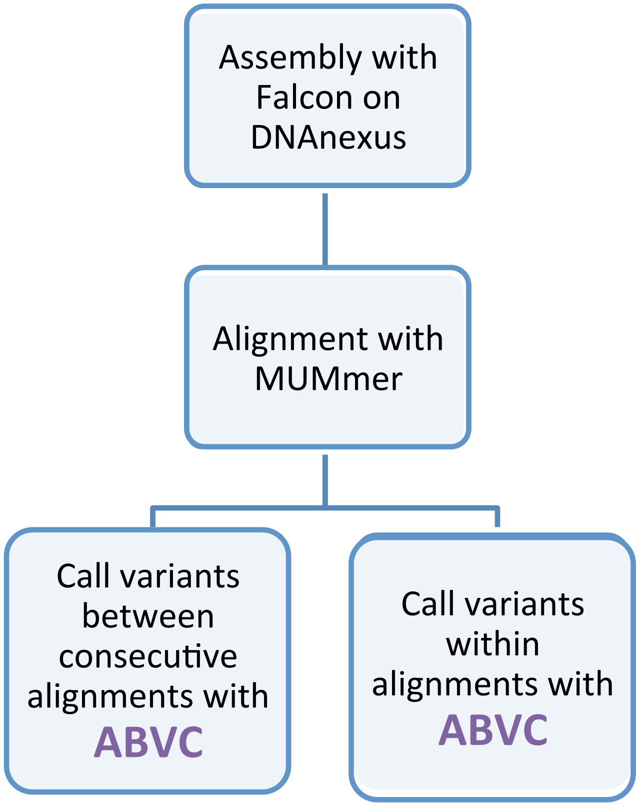
72X coverage



Genome-wide coverage averages around 54X
Coverage per chromosome varies greatly as expected from previous karyotyping results

Genome structural analysis

Assembly-based



Assembly with Falcon on DNAnexus

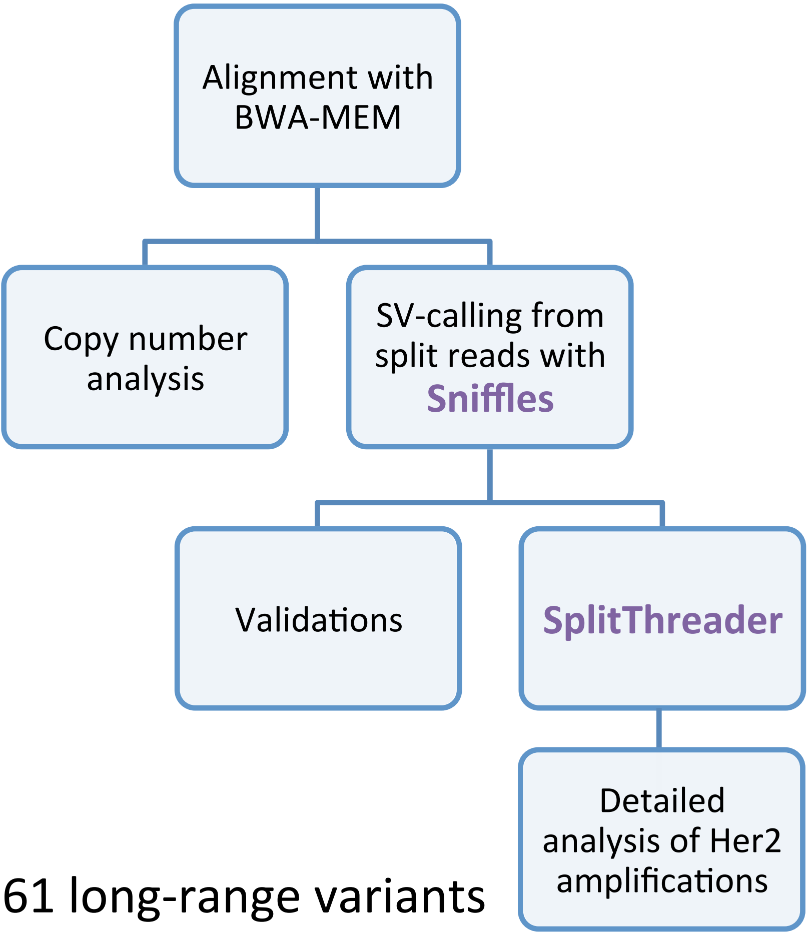
Alignment with MUMmer

Call variants between consecutive alignments with **ABVC**

Call variants within alignments with **ABVC**

~ 11,000 local variants
50 bp < size < 10 kbp

Alignment-based



Alignment with BWA-MEM

Copy number analysis

SV-calling from split reads with **Sniffles**

Validations

SplitThreader

Detailed analysis of Her2 amplifications

661 long-range variants
(>10kb distance)

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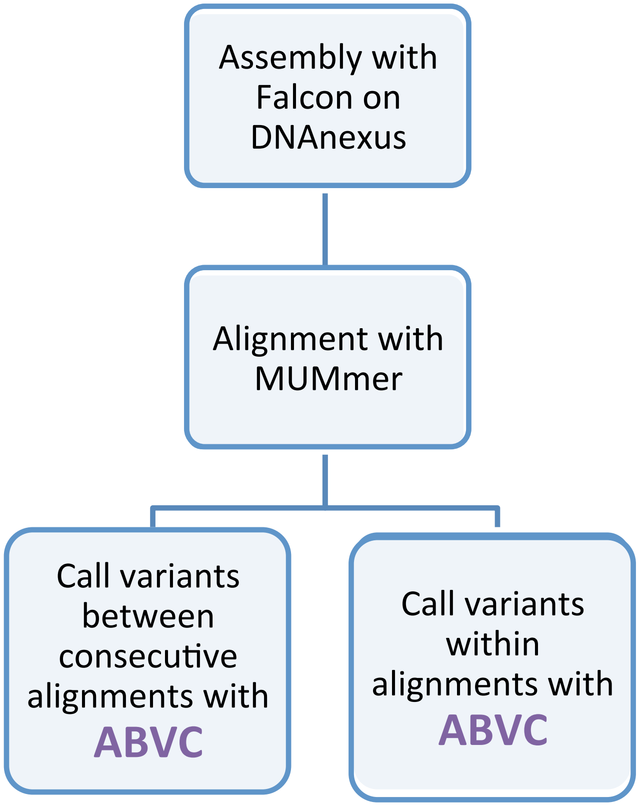

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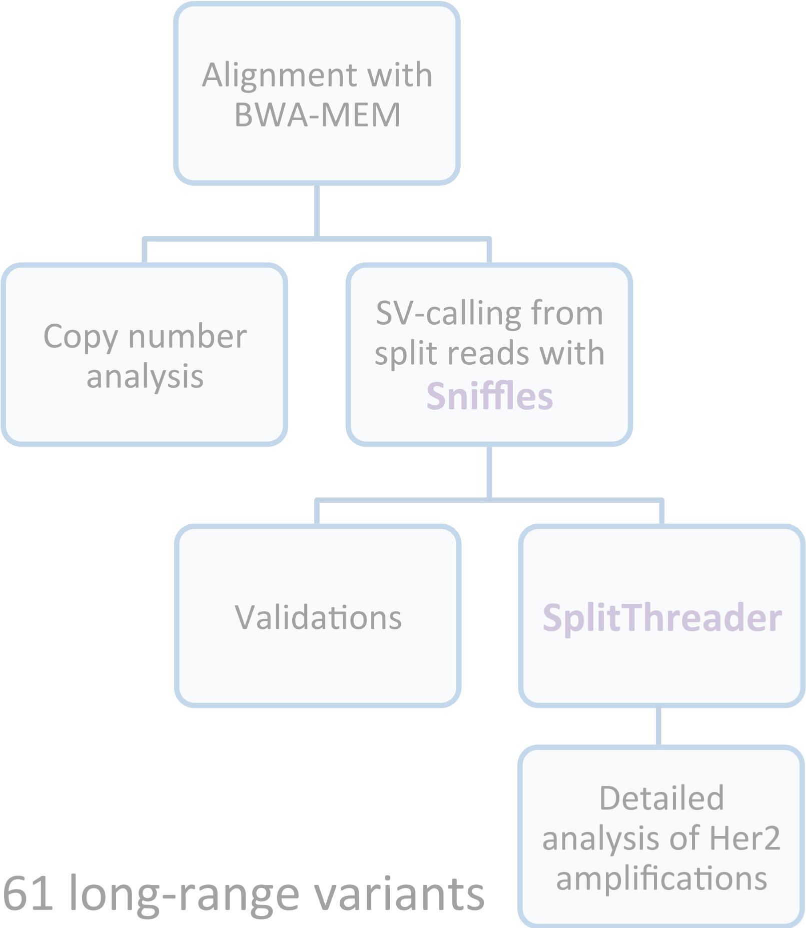

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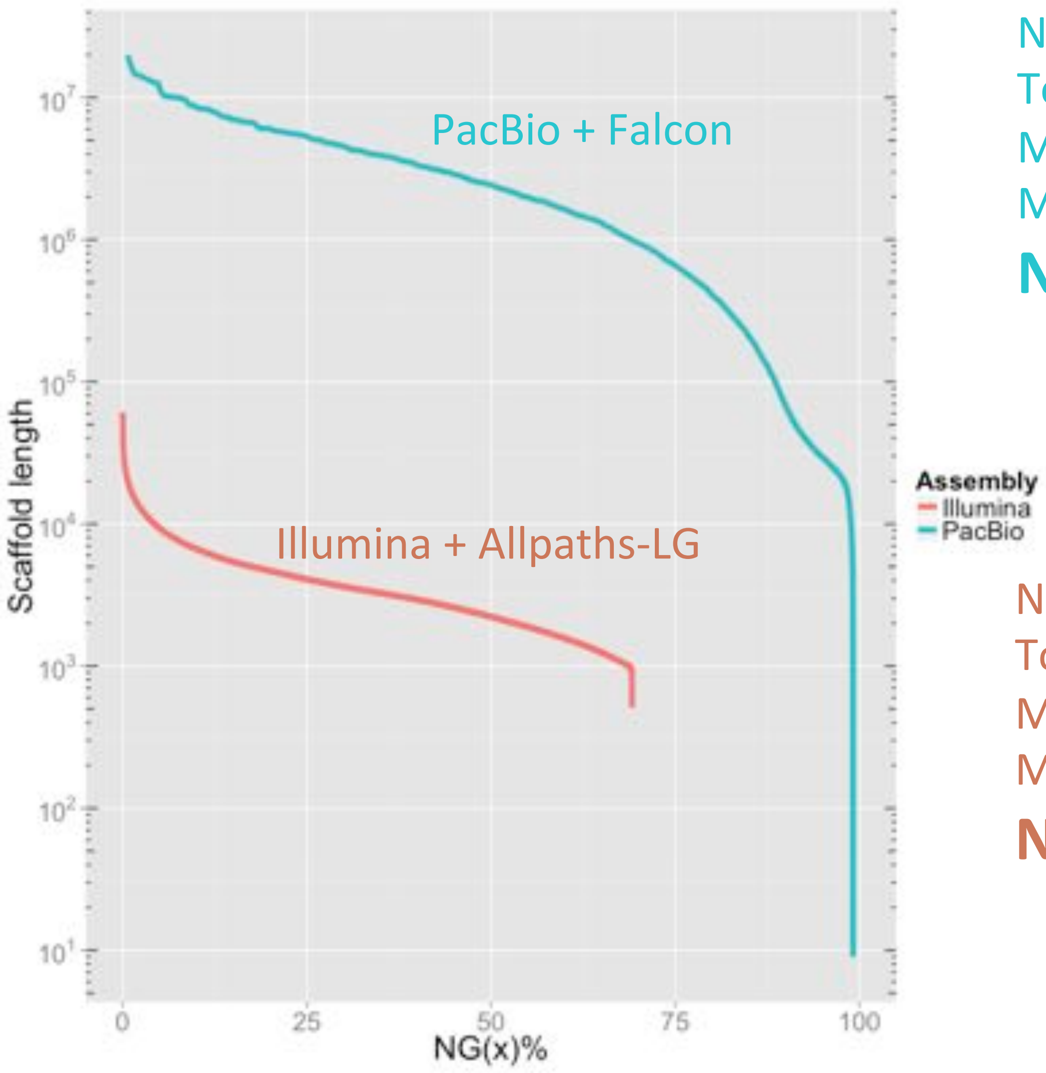
Assembly using PacBio yields far better contiguity



Number of sequences: 13,532
Total sequence length: 2.97Gb
Mean: 266 kb
Max: 19.9 Mb

N50: 2.46 Mb

Relative to a genome size of 3 Gb

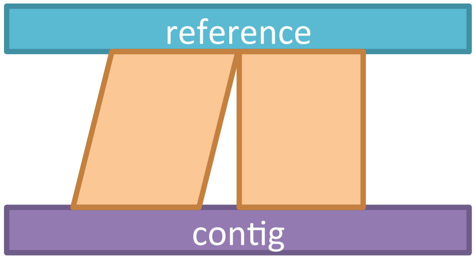
Number of sequences: 748,955
Total sequence length: 2.07 Gb
Mean: 2.8 kb
Max: 61 kb

N50: 3.3 kb

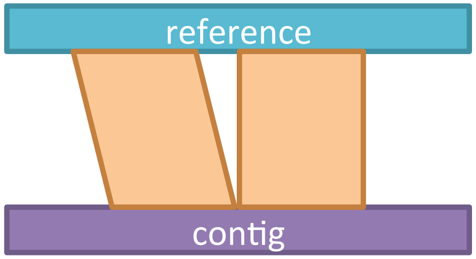
ABVC: Assembly-Based Variant-Caller

Defined point

Insertion

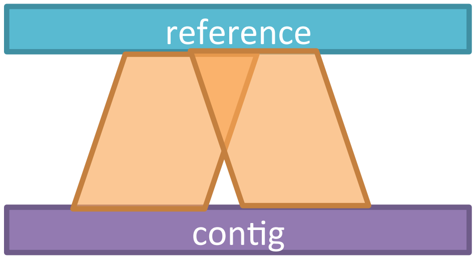


Deletion

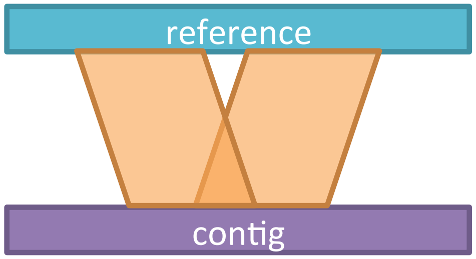


Overlapping alignments suggest tandem repeat

Tandem Expansions

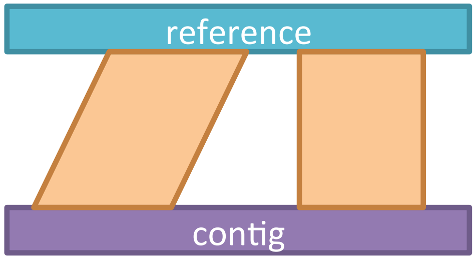


Tandem Contractions

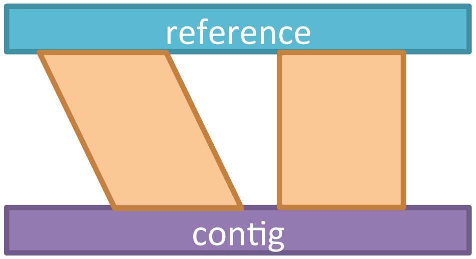


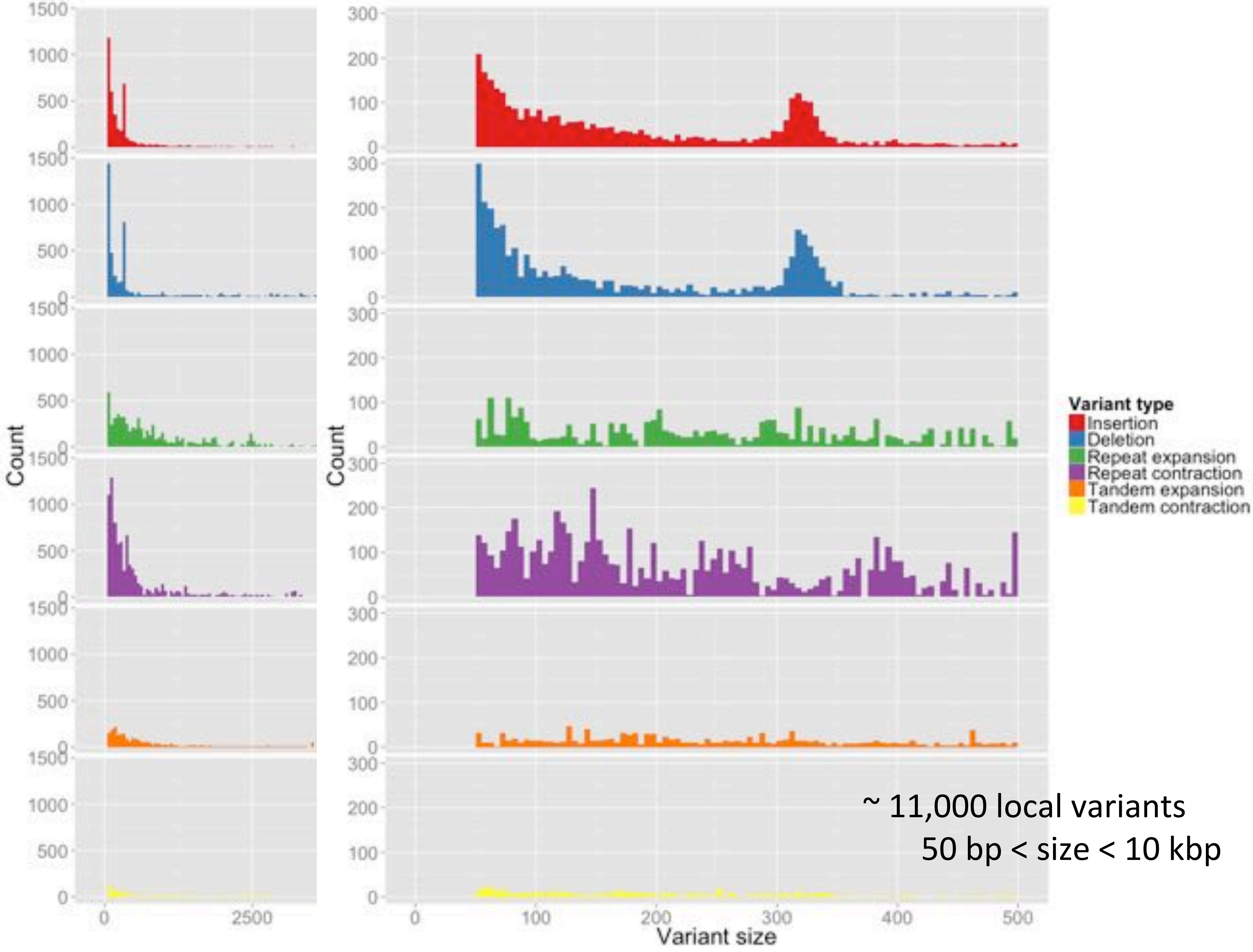
Gap where sequences do not align uniquely suggests a repeat

Repeat Expansions



Repeat Contractions





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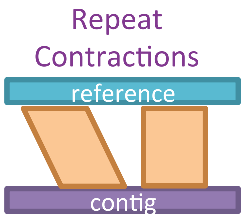
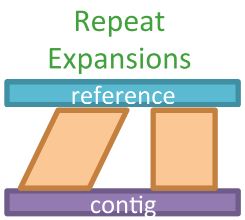
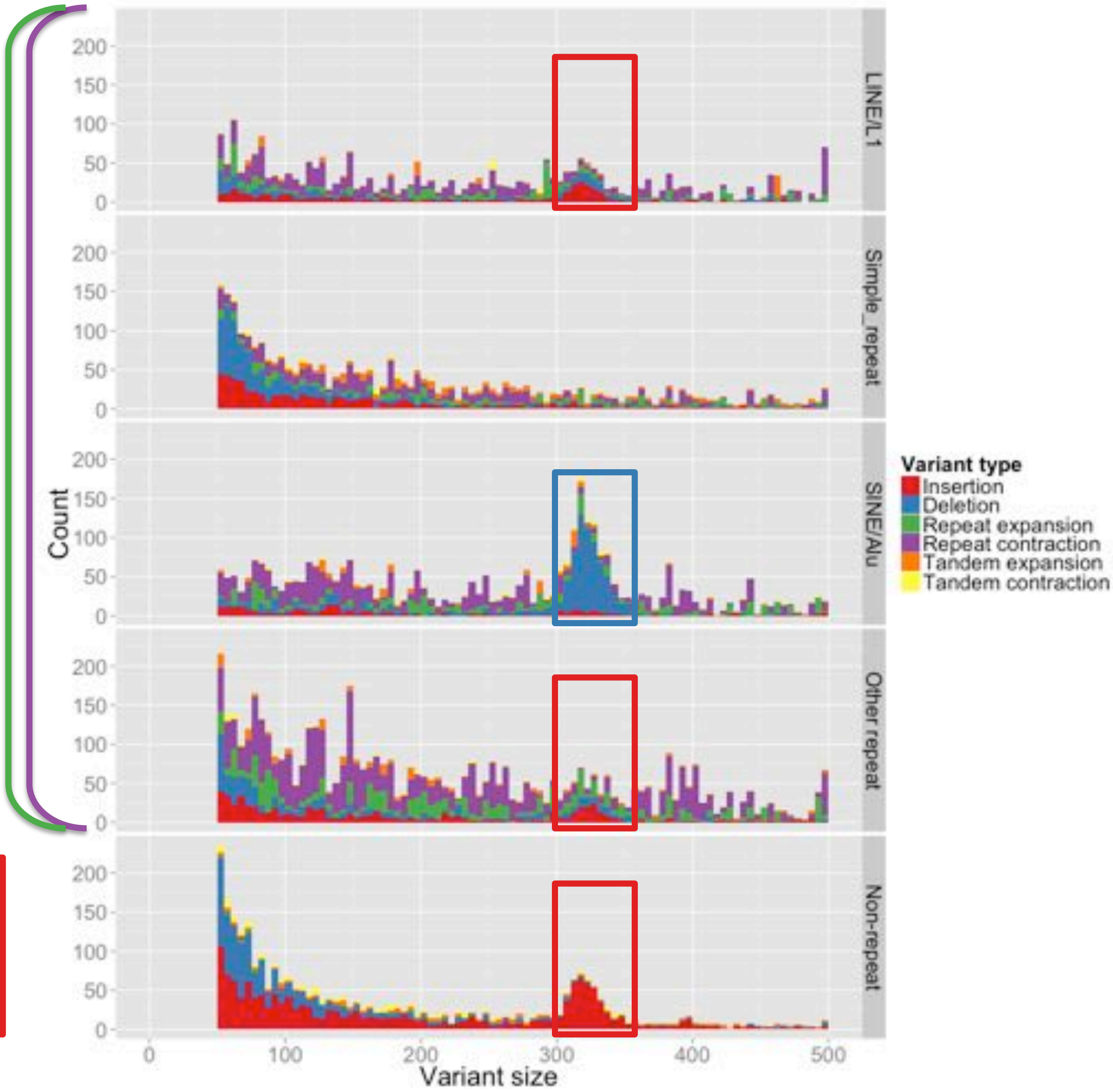
50 bp < size < 10 kbp



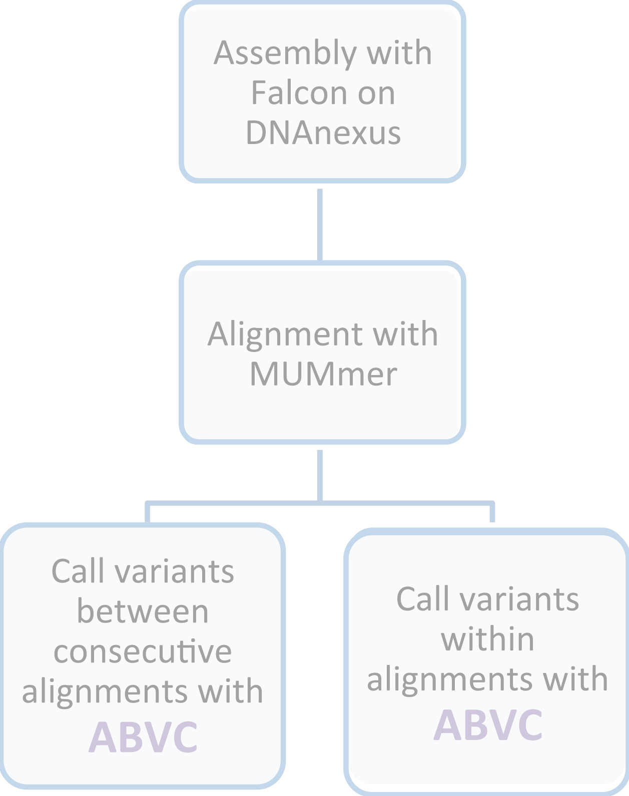




BLASTed 515 insertions:
427 (83%) of them matched Alu elements

Genome structural analysis

Assembly-based



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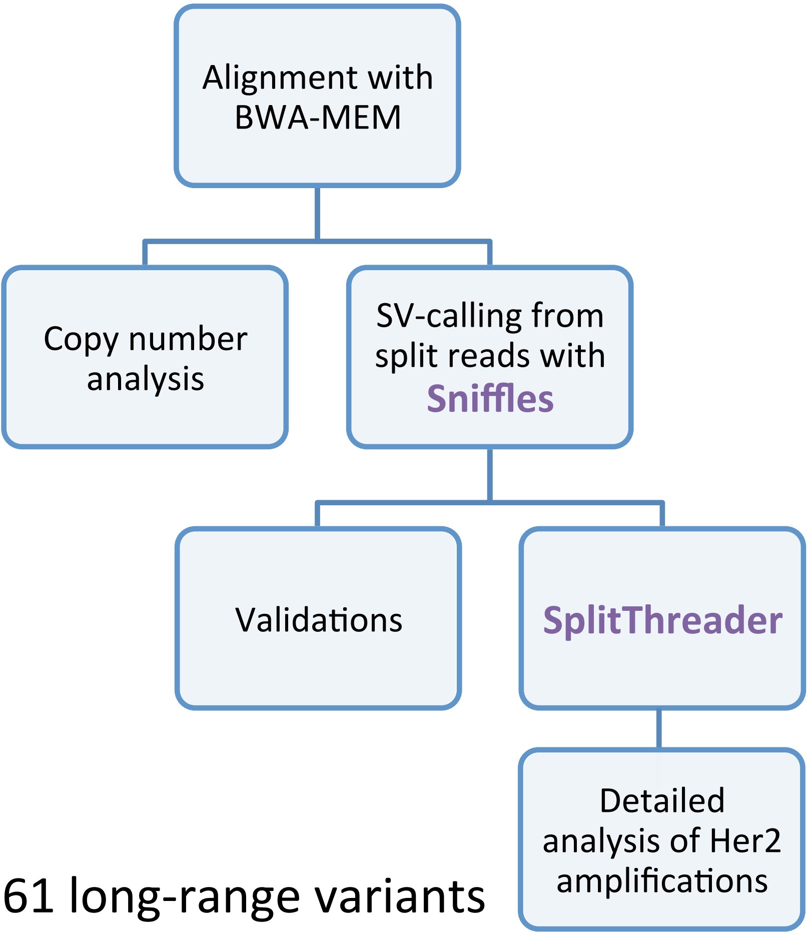

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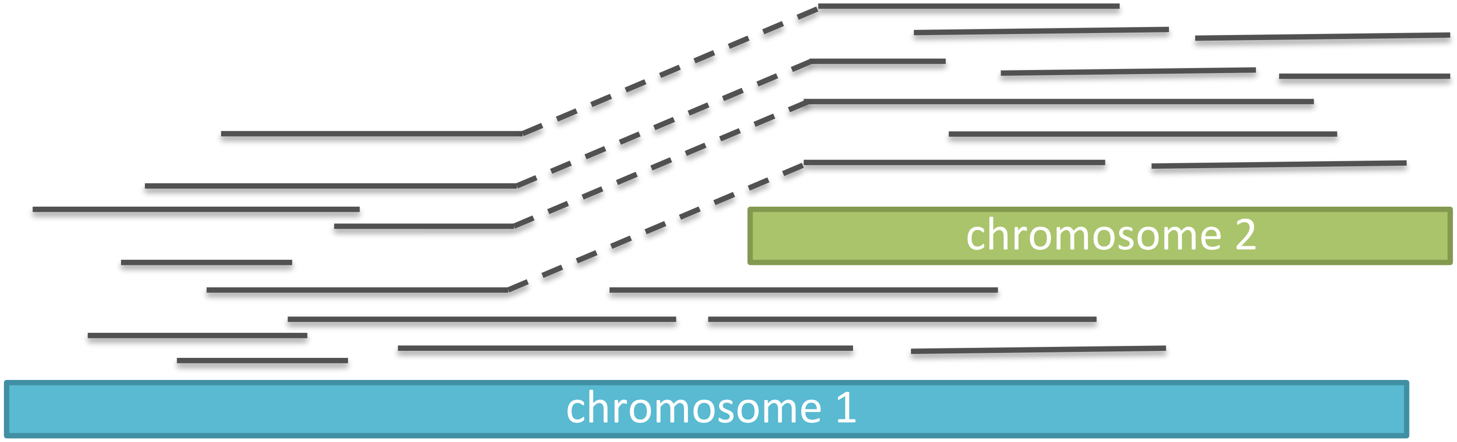
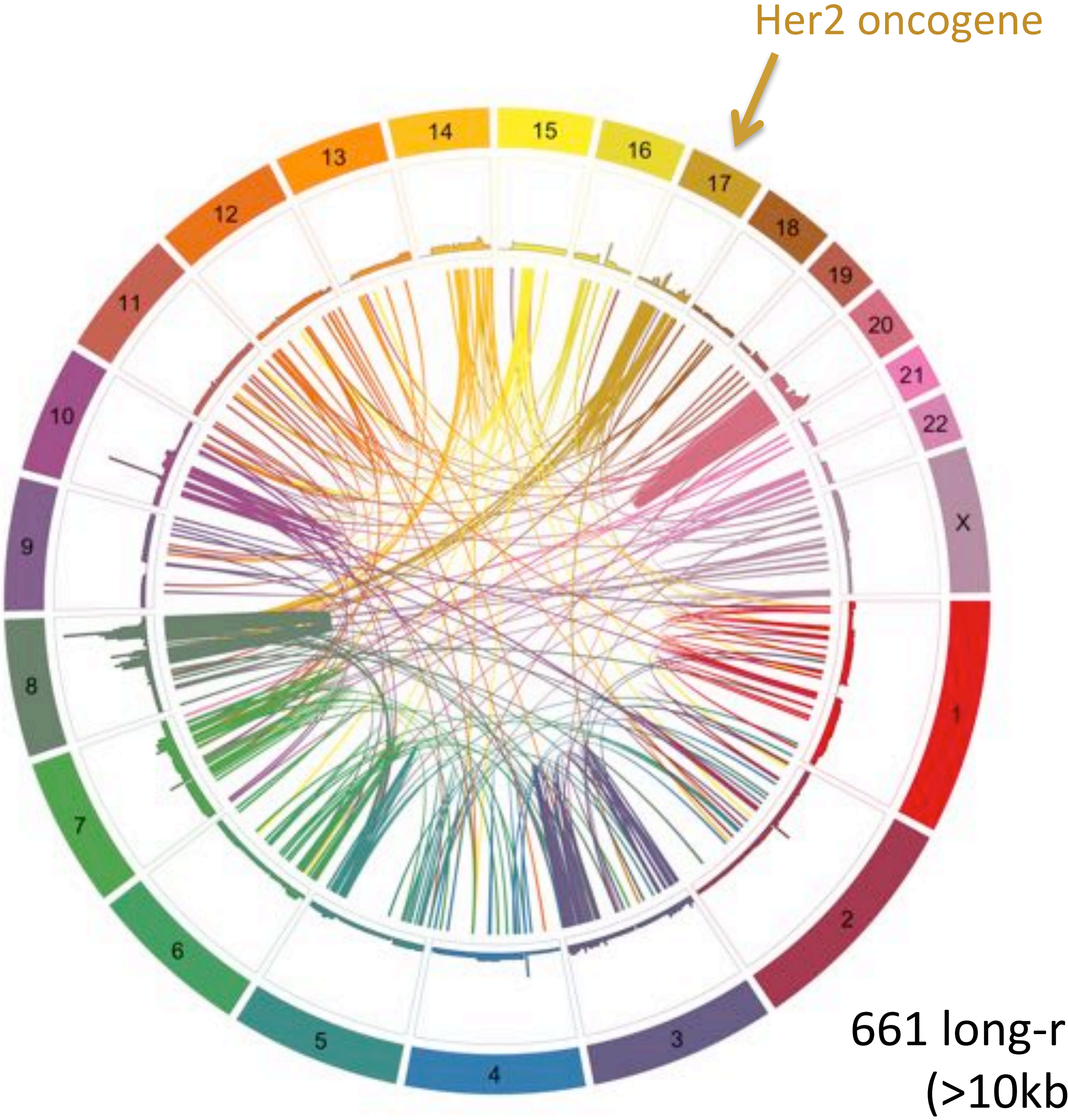
661 long-range variants
(>10kb distance)

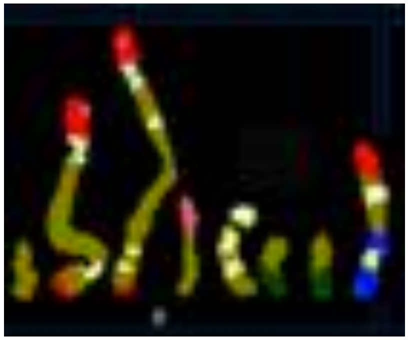
Split-read variant calling with Sniffles to capture the long-range variants



See Fritz at Poster 183

Long-range structural variants found by Sniffles





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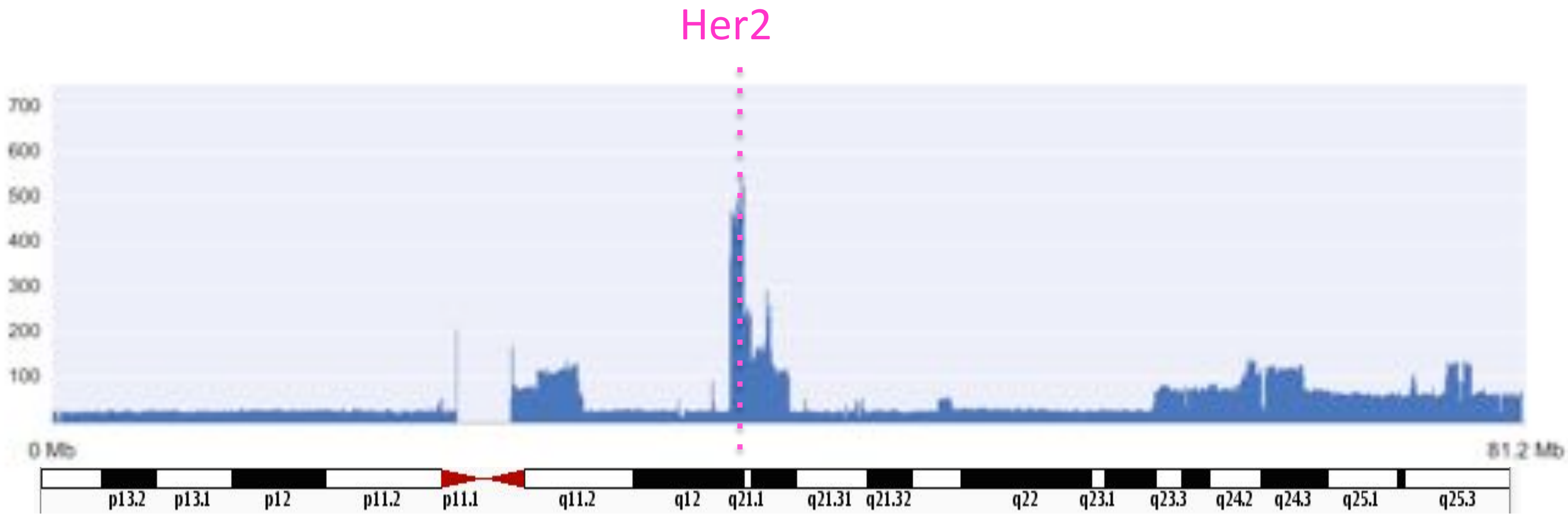
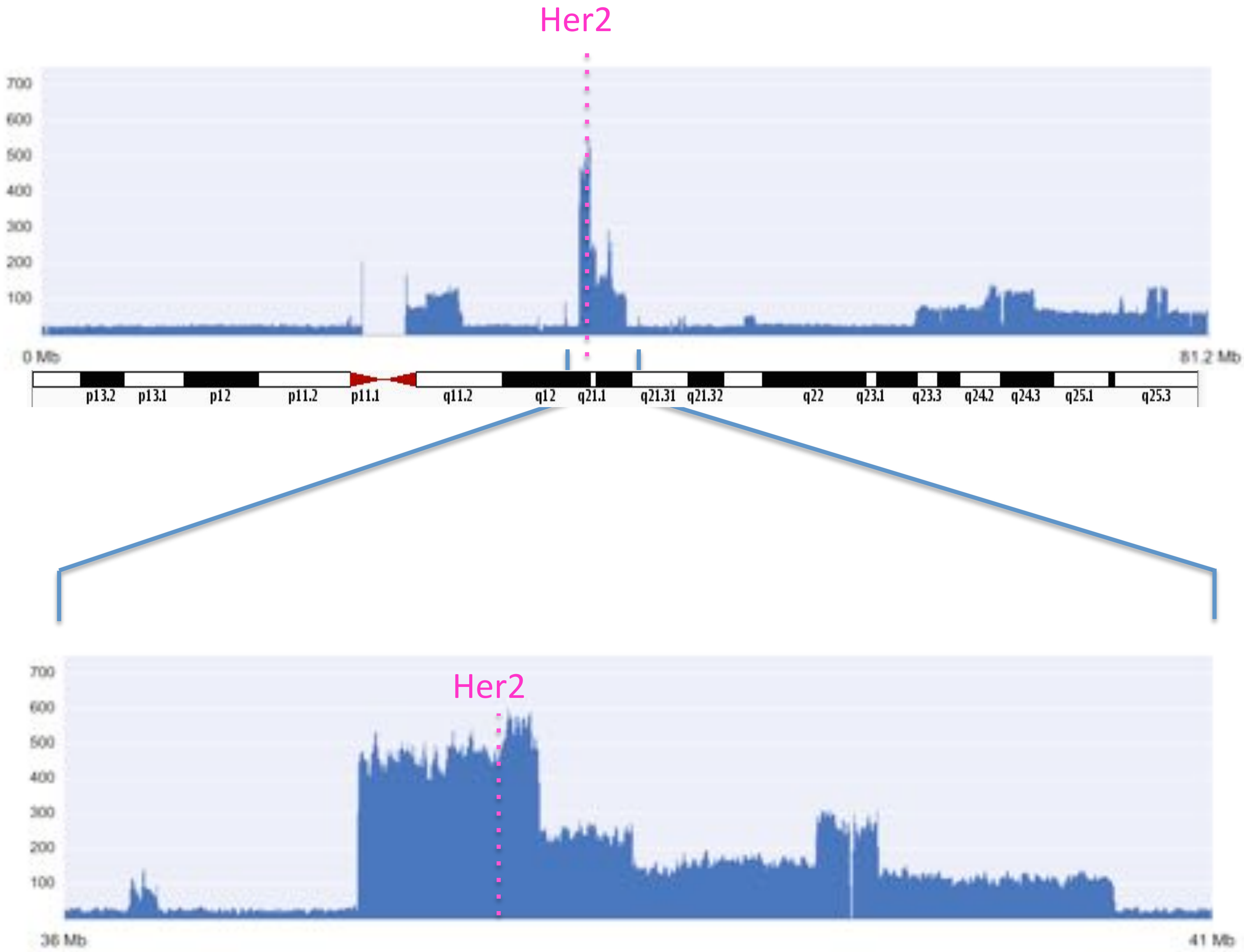
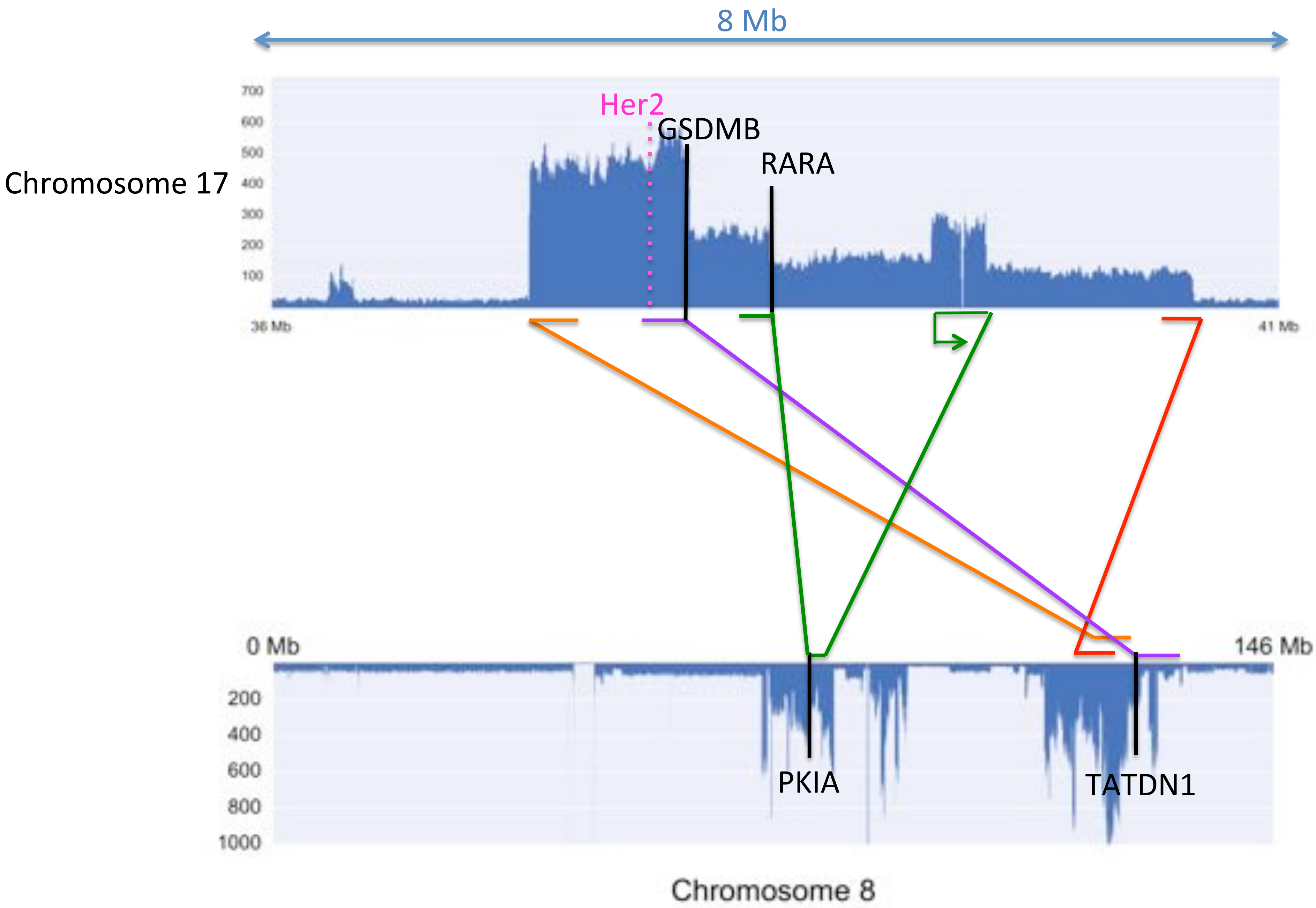


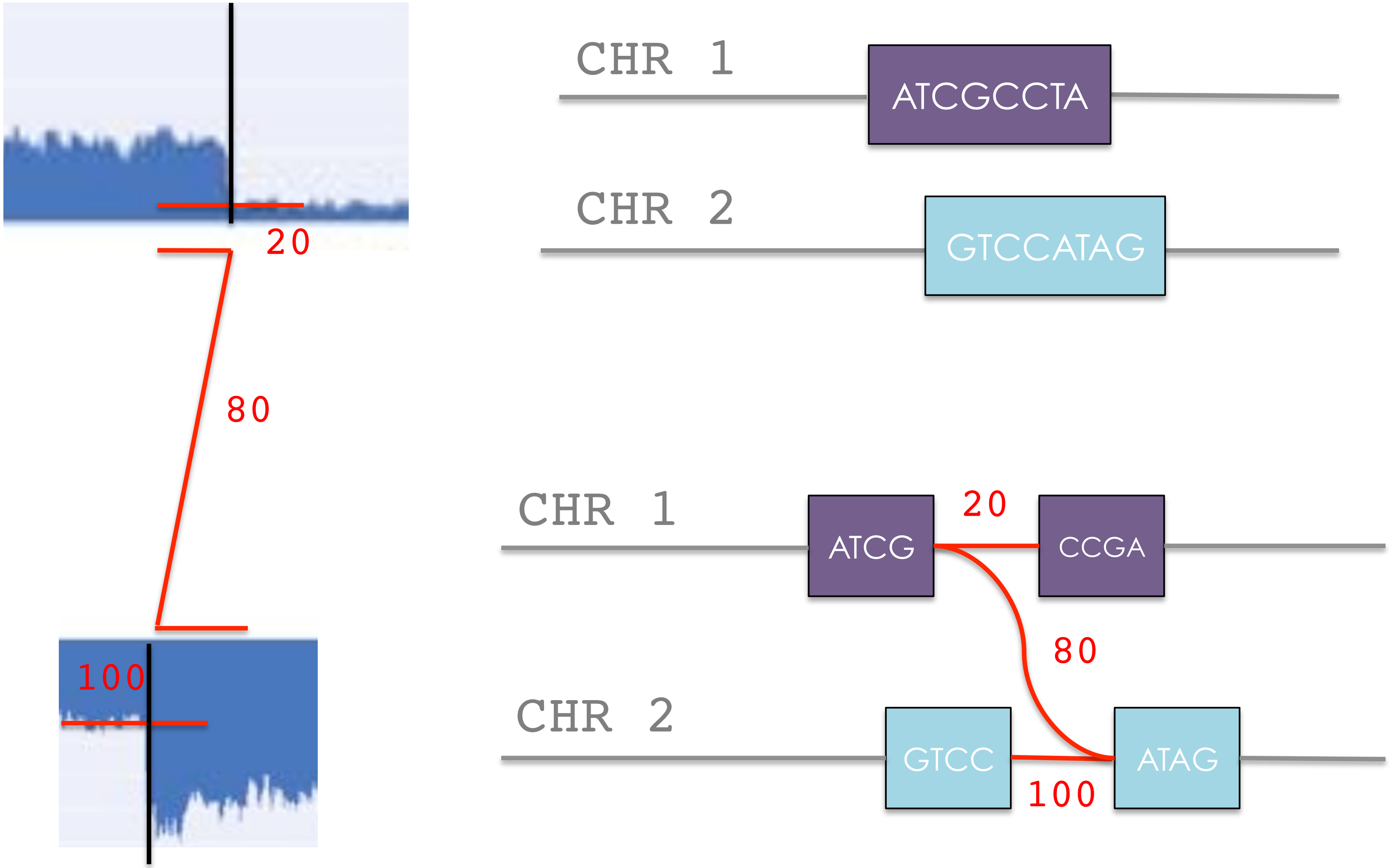
Chr 17: 83 Mb

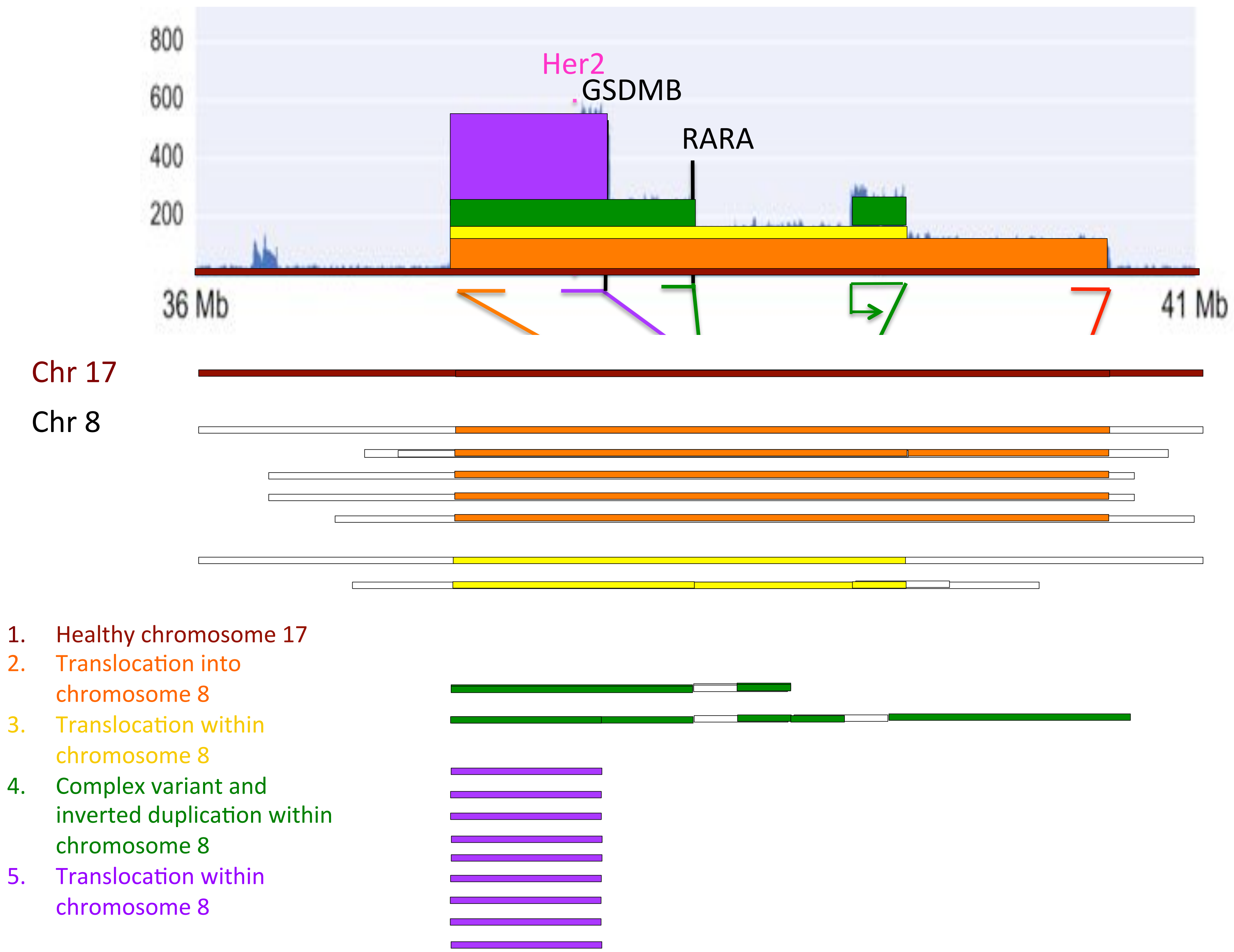
8 Mb


Her2
700
600
500
400
300
200
100
0 Mb
81.2 Mb
p13.2
p13.1
p12
p11.2
p11.1
q11.2
q12
q21.1
q21.31
q21.32
q22
q23.1
q23.3
q24.2
q24.3
q25.1
q25.3
Her2
700
600
500
400
300
200
100
36 Mb
41 Mb


8 Mb
Chromosome 17
700
600
500
400
300
200
100
Her2
GSDMB
RARA
36 Mb
41 Mb
0 Mb
146 Mb
200
400
600
800
1000
PKIA
TATDN1
Chromosome 8

SplitThreader: Graphical threading to retrace complex history of rearrangements in cancer genomes



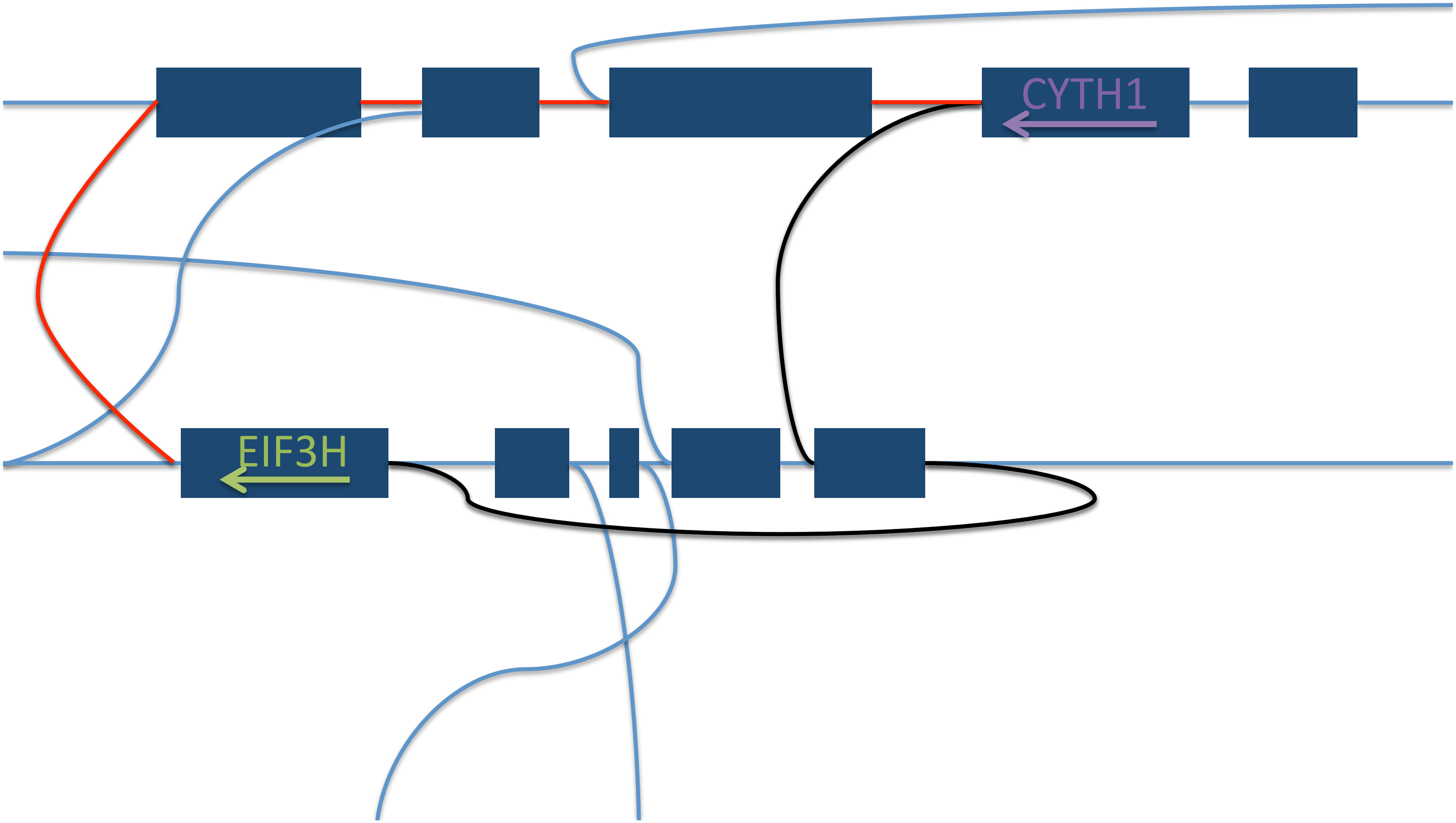


1. Healthy chromosome 17
2. Translocation into chromosome 8
3. Translocation within chromosome 8
4. Complex variant and inverted duplication within chromosome 8
5. Translocation within chromosome 8

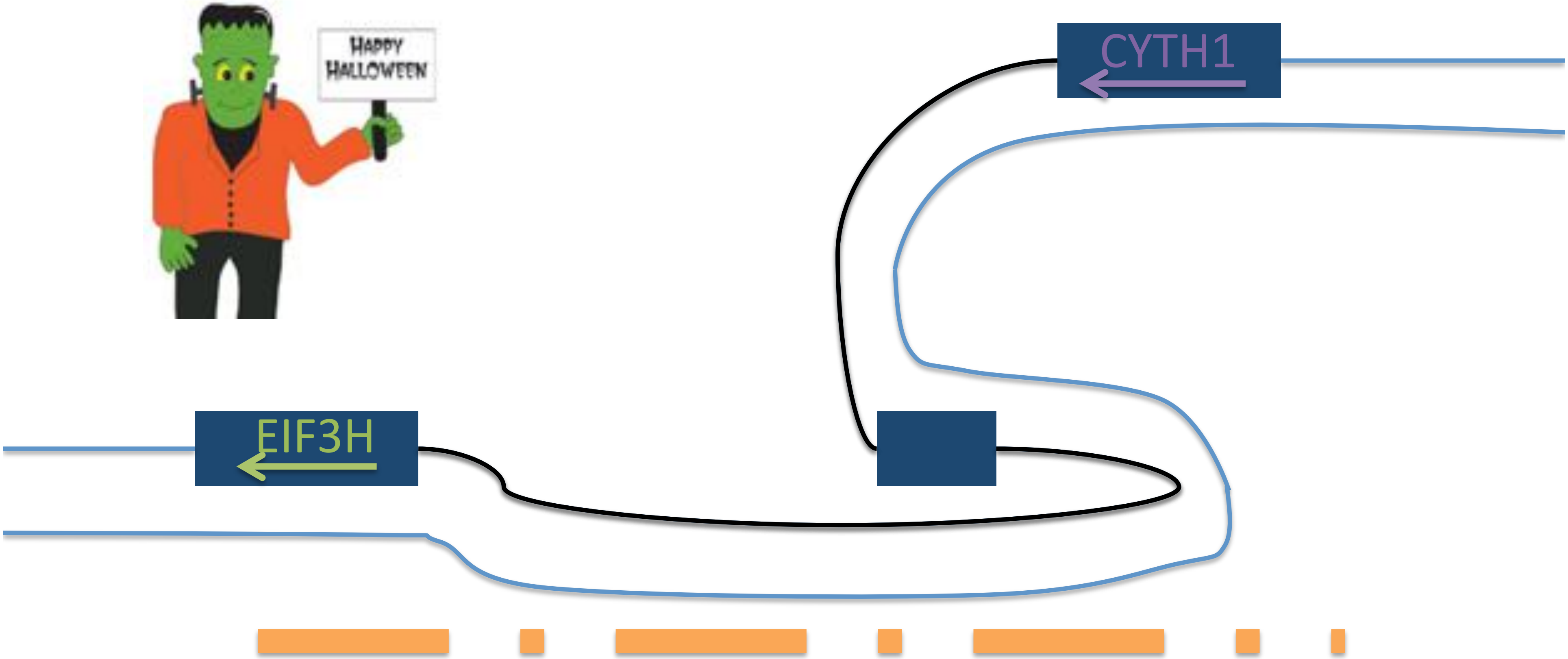
Transcriptome analysis with IsoSeq: Long-read RNA sequencing

- Full-length transcripts
- Found 17 gene fusions with both DNA and RNA evidence
  - 13 seen in previous RNA-seq literature
  - 4 novel fusions
- 2 previously observed fusions had RNA evidence but no direct link in the DNA
  - Confirmed using SplitThreader

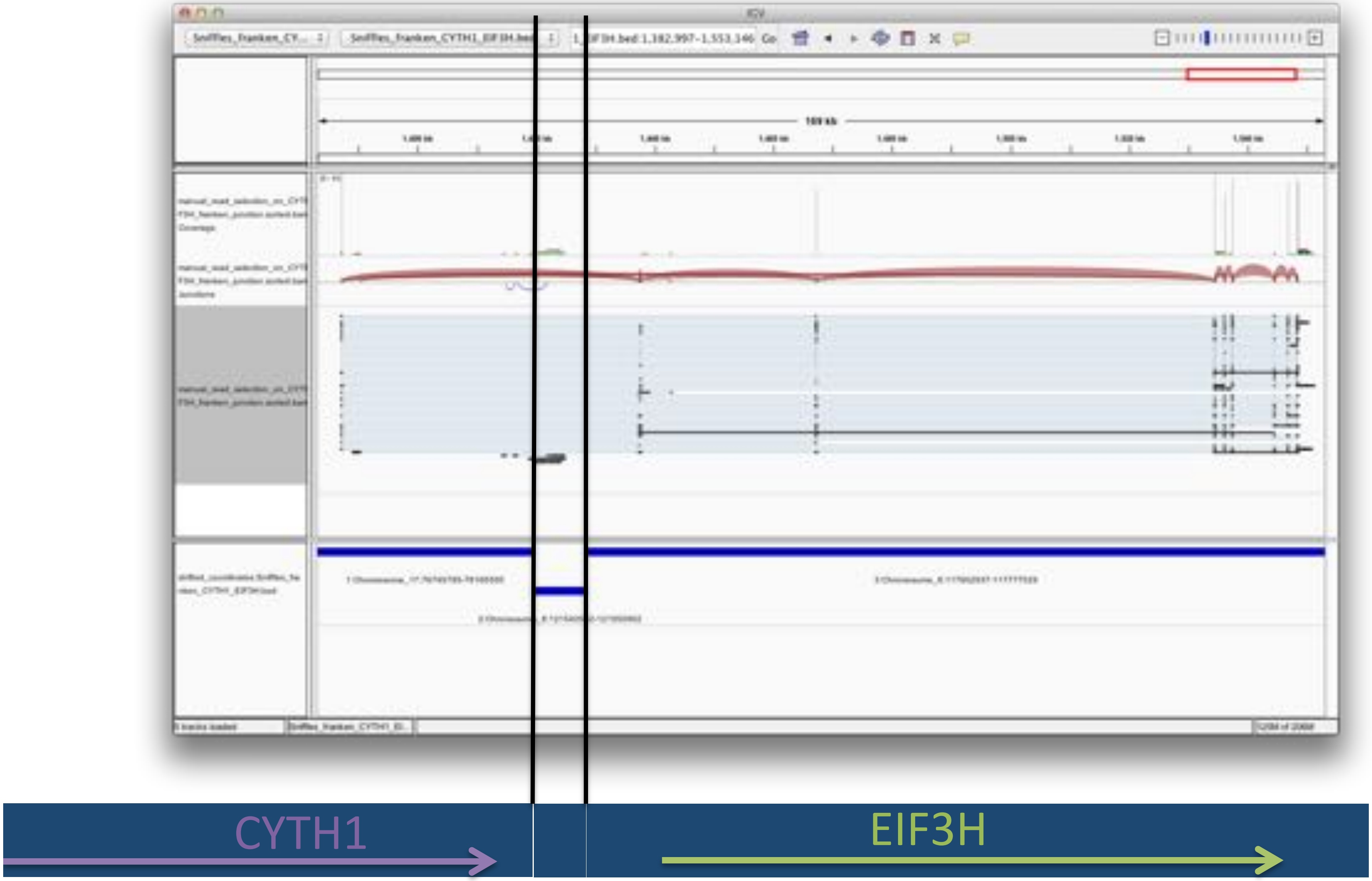
CYTH1-EIF3H gene fusion in the SplitThreader graph



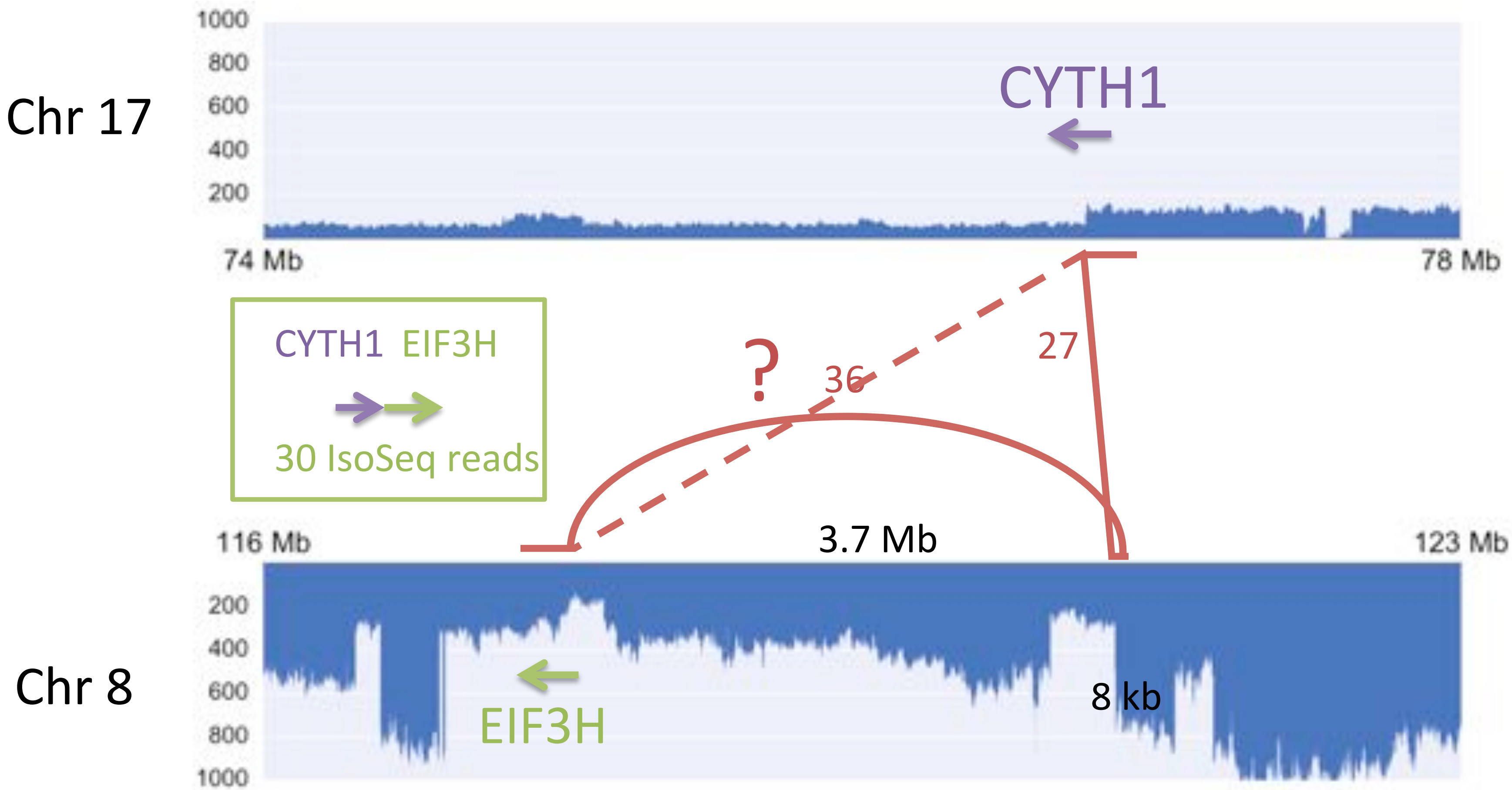
CYTH1-EIF3H gene fusion in the SplitThreader graph



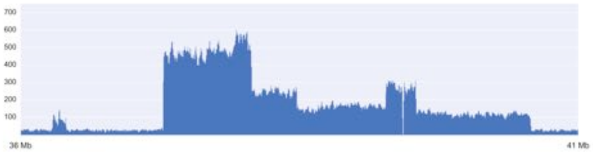
Frankensteining the CYTH1-EIF3H gene fusion


IGV
Sniffles_Franken_CY...
Sniffles_Franken_CYTH1_EIF3H.bed
1_EIF3H.bed:1,382,997-1,553,146
Go
169 kb
CYTH1
EIF3H

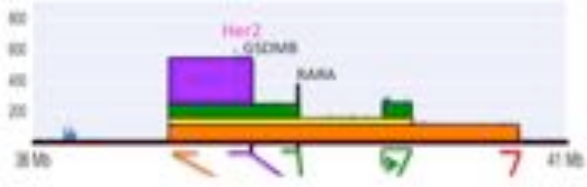
CYTH1-EIF3H gene fusion

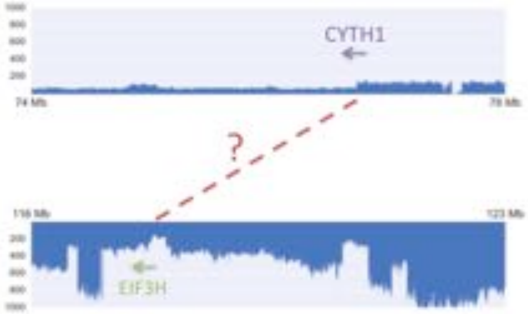


The genome informs the transcriptome

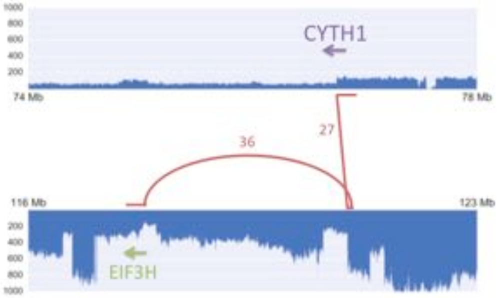


Explain amplifications





Trace gene fusions



More genomes coming soon!

Data and additional results: http://schatzlab.cshl.edu/data/skbr3/

Acknowledgments

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Fritz Sedlazeck
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