

# SplitThreader: A graphical algorithm for analysis of highly rearranged and amplified cancer genomes

Maria Nattestad

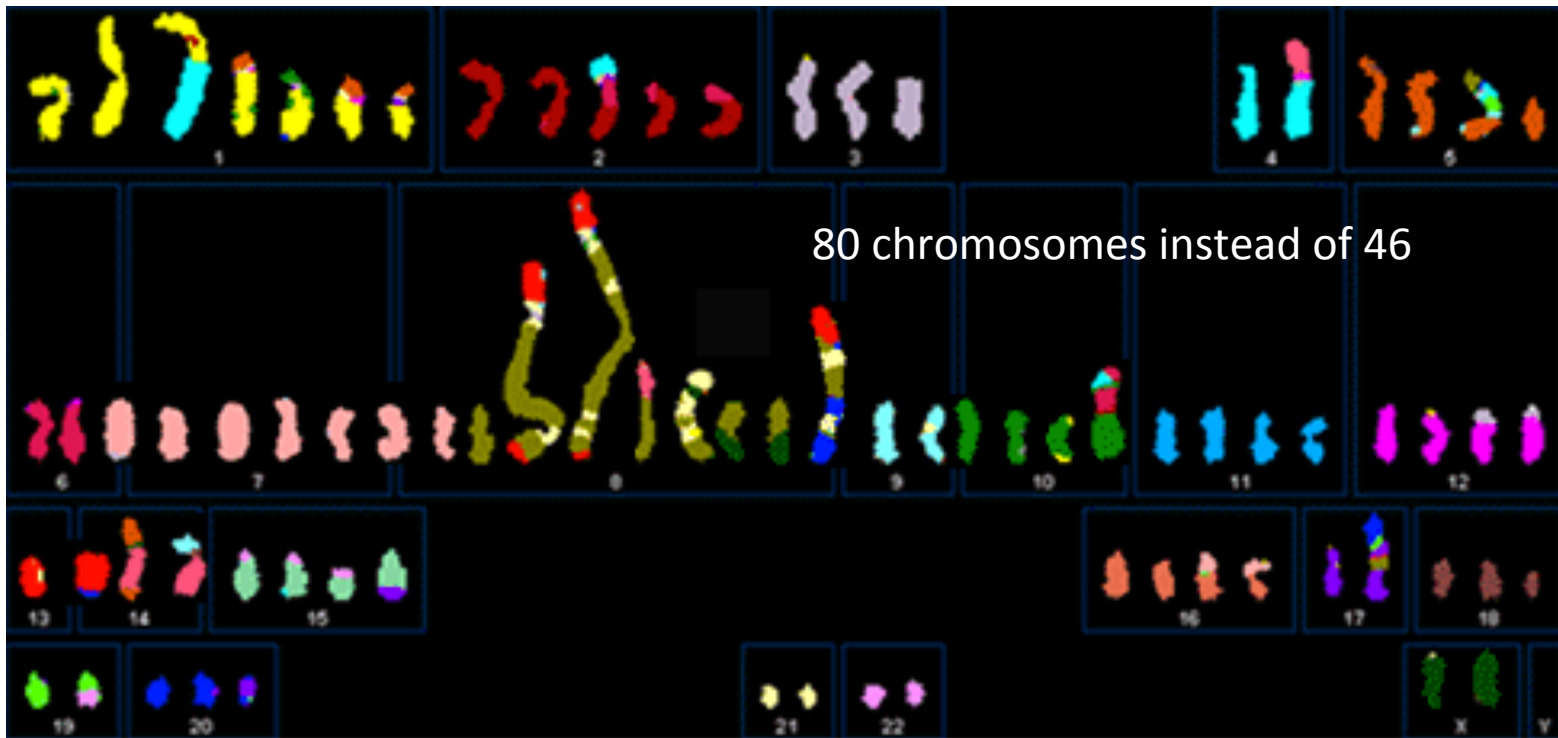
Mike Schatz lab

Cold Spring Harbor Laboratory and Johns Hopkins University



# Complex genome to study: SK-BR-3

Most commonly used Her2-amplified breast cancer cell line



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

(Davidson et al, 2000)

PacBio SMRT sequencing

Assembly

Assembly-based  
variant-calling  
using  
Assemblytics

Align to reference  
with BWA-MEM

Copy number  
analysis

Structural variant calling using  
Sniffles

SplitThreader  
graphical analysis

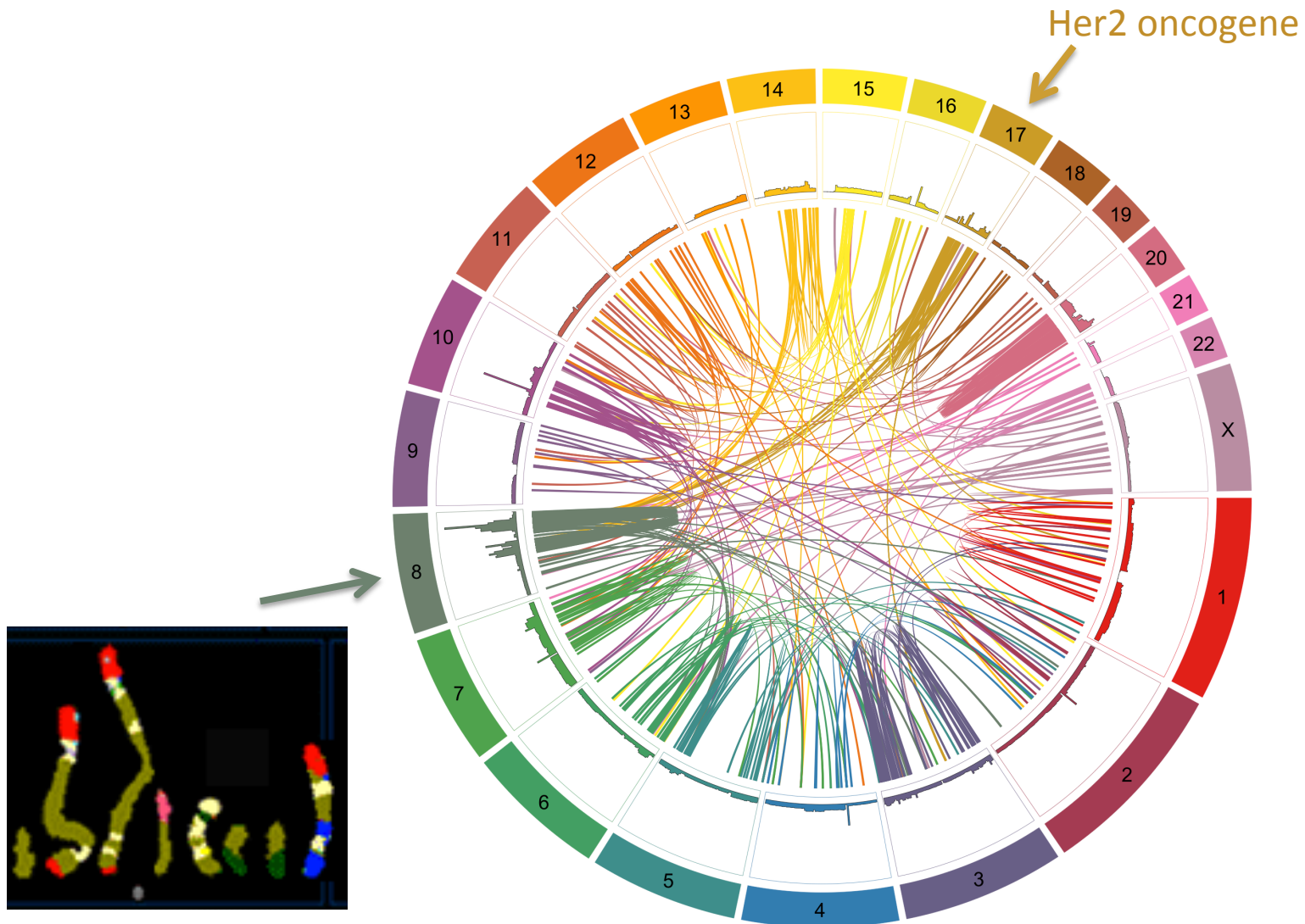
POSTER: Sniffles by Fritz  
Sedlazeck at poster  
1310

Evolution

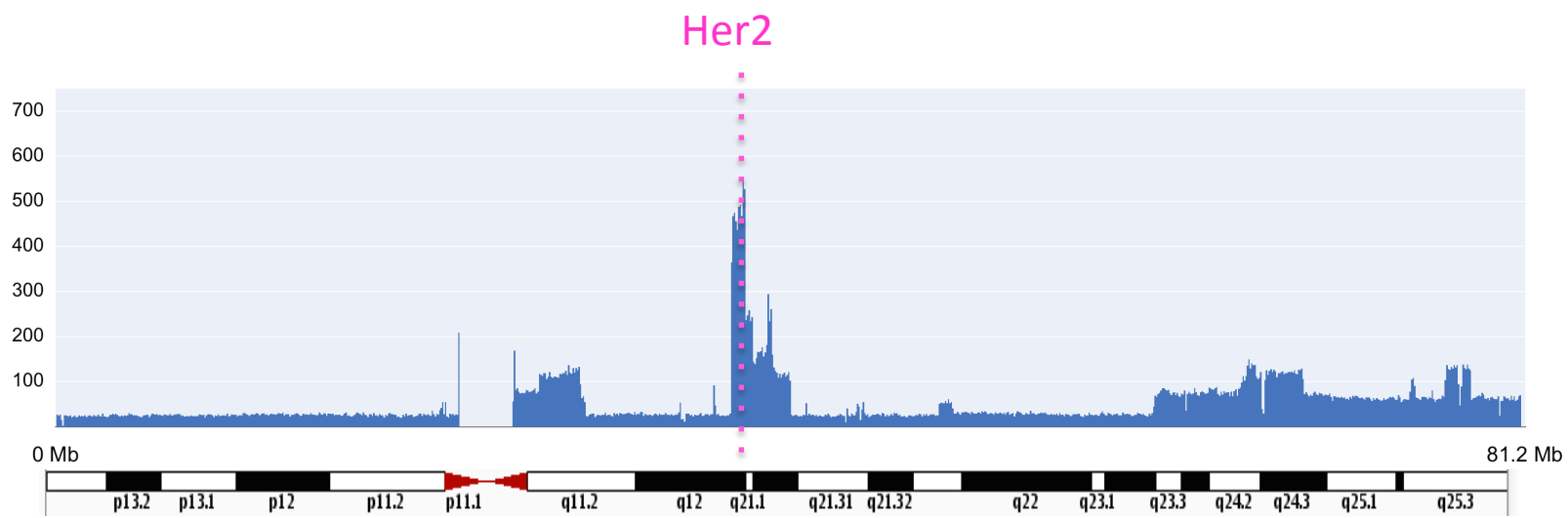
Fusion

Visualizer

# Why SplitThreader Evolution?

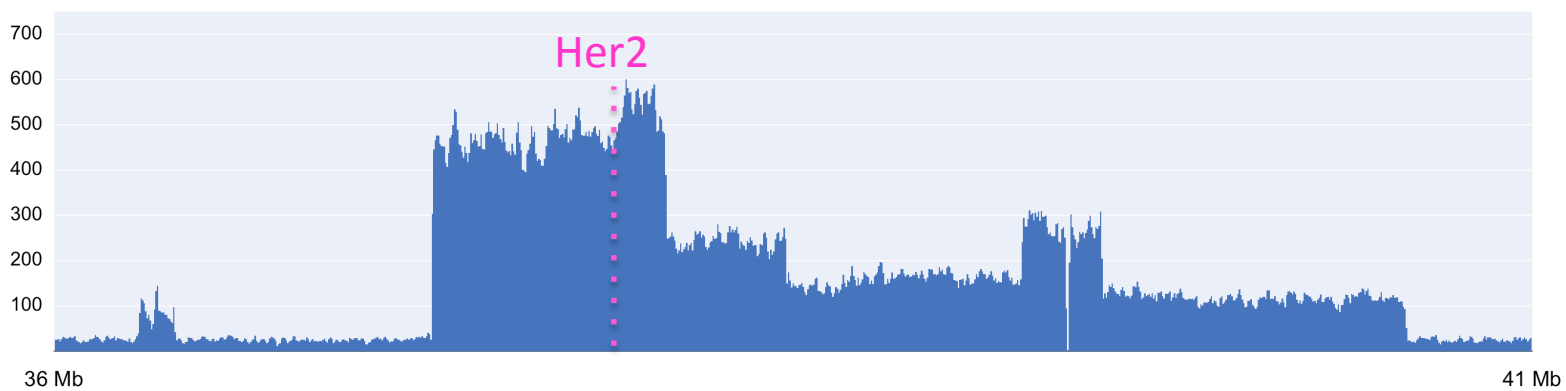
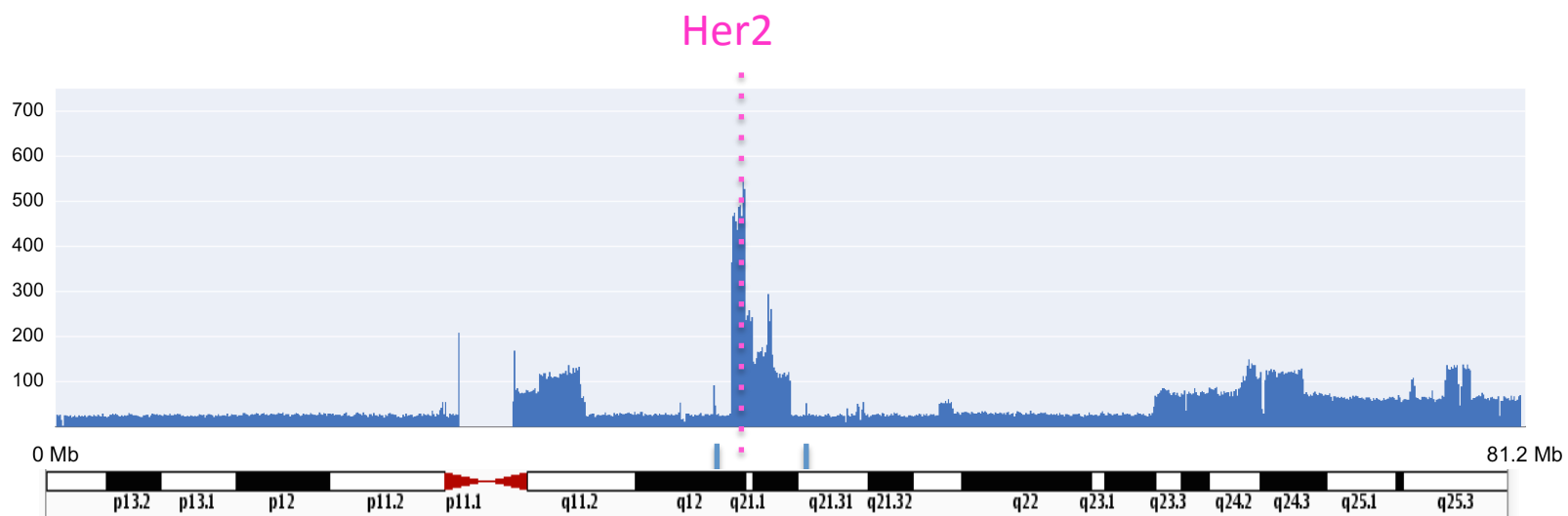


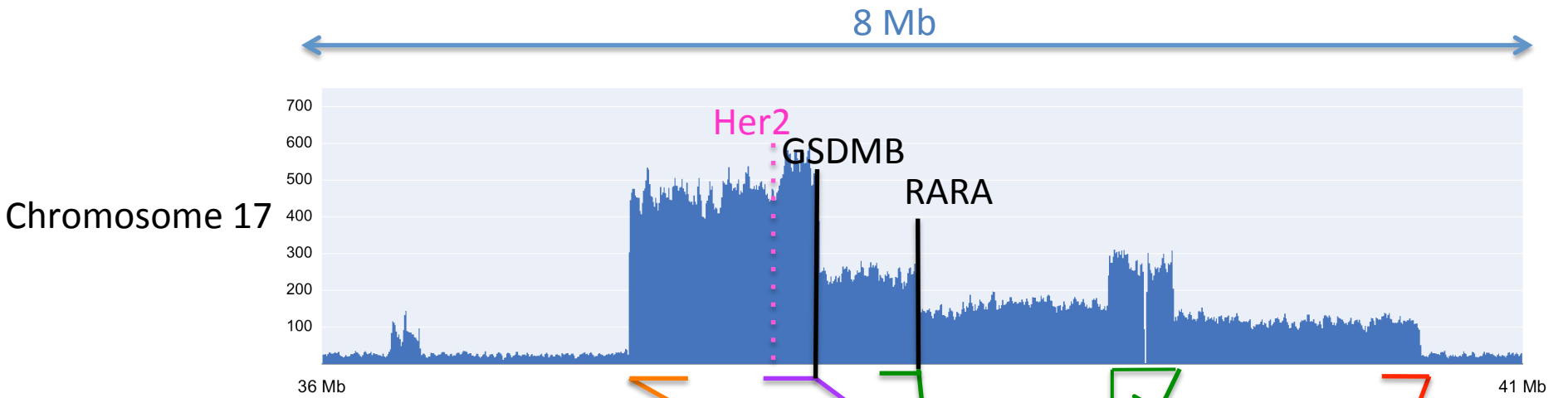
661 structural variants called by Sniffles



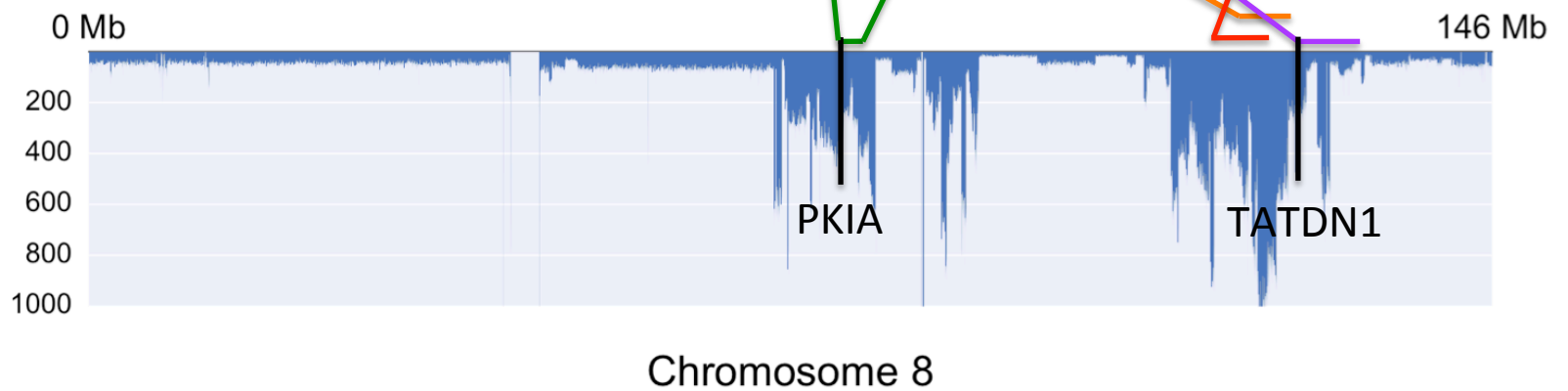
Chr 17: 83 Mb

8 Mb

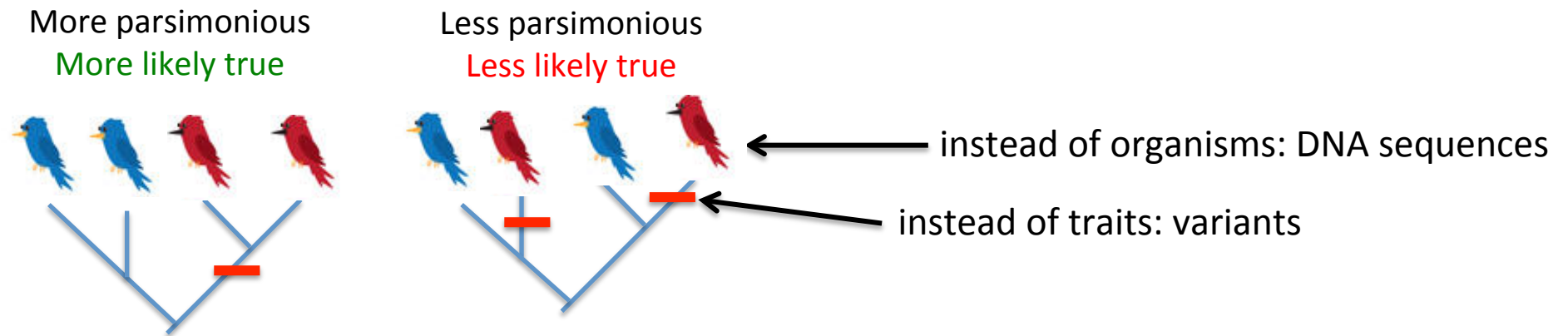




POSTER: Validation of these SV calls using BioNano – Sara Goodwin at poster 607



# Can we infer the order of events?

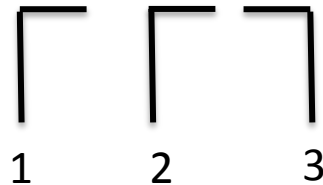


Let's apply parsimony to a toy example

Copy number:

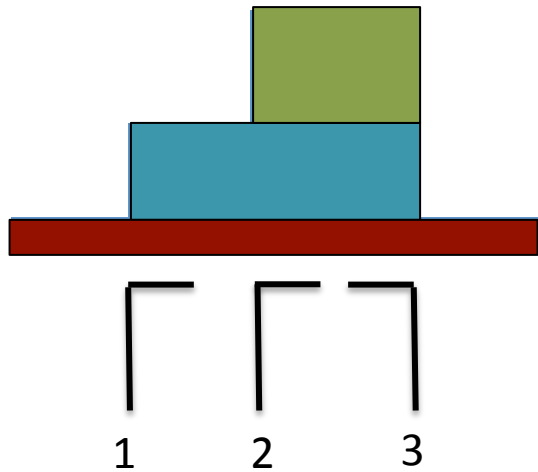


Variants:

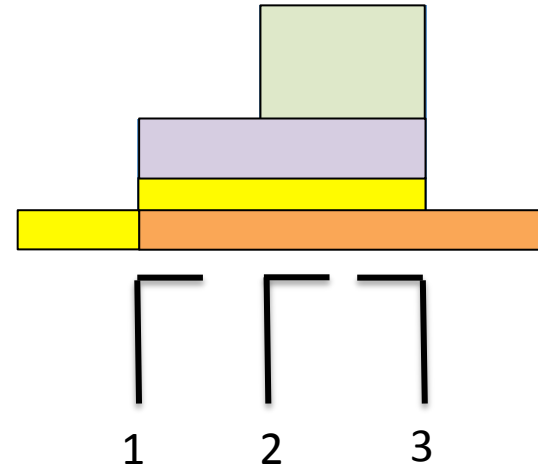




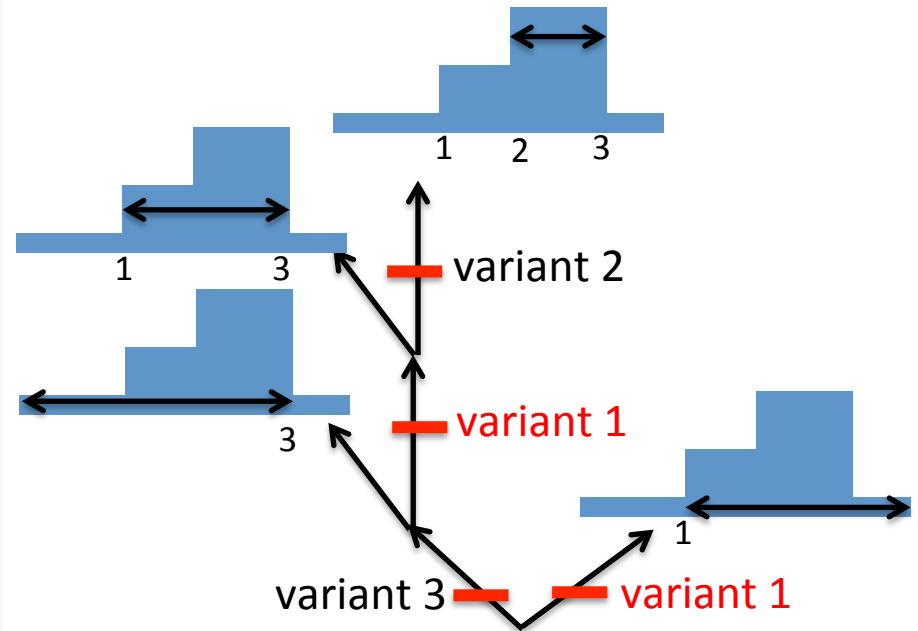
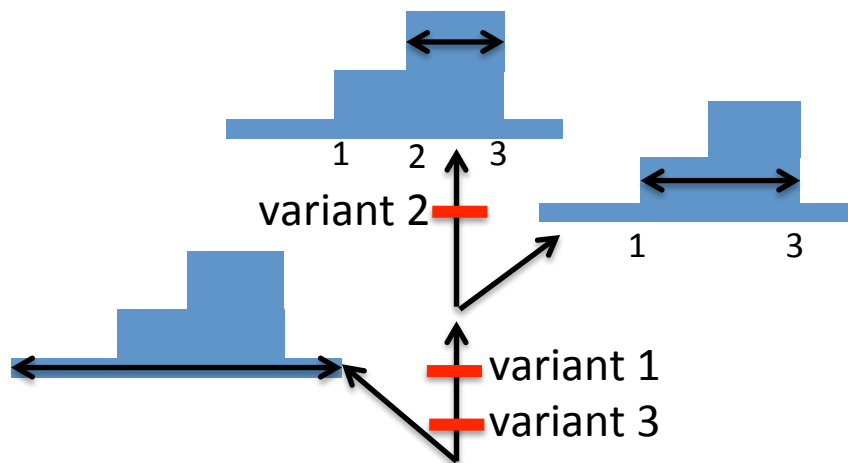
Good: Most parsimonious



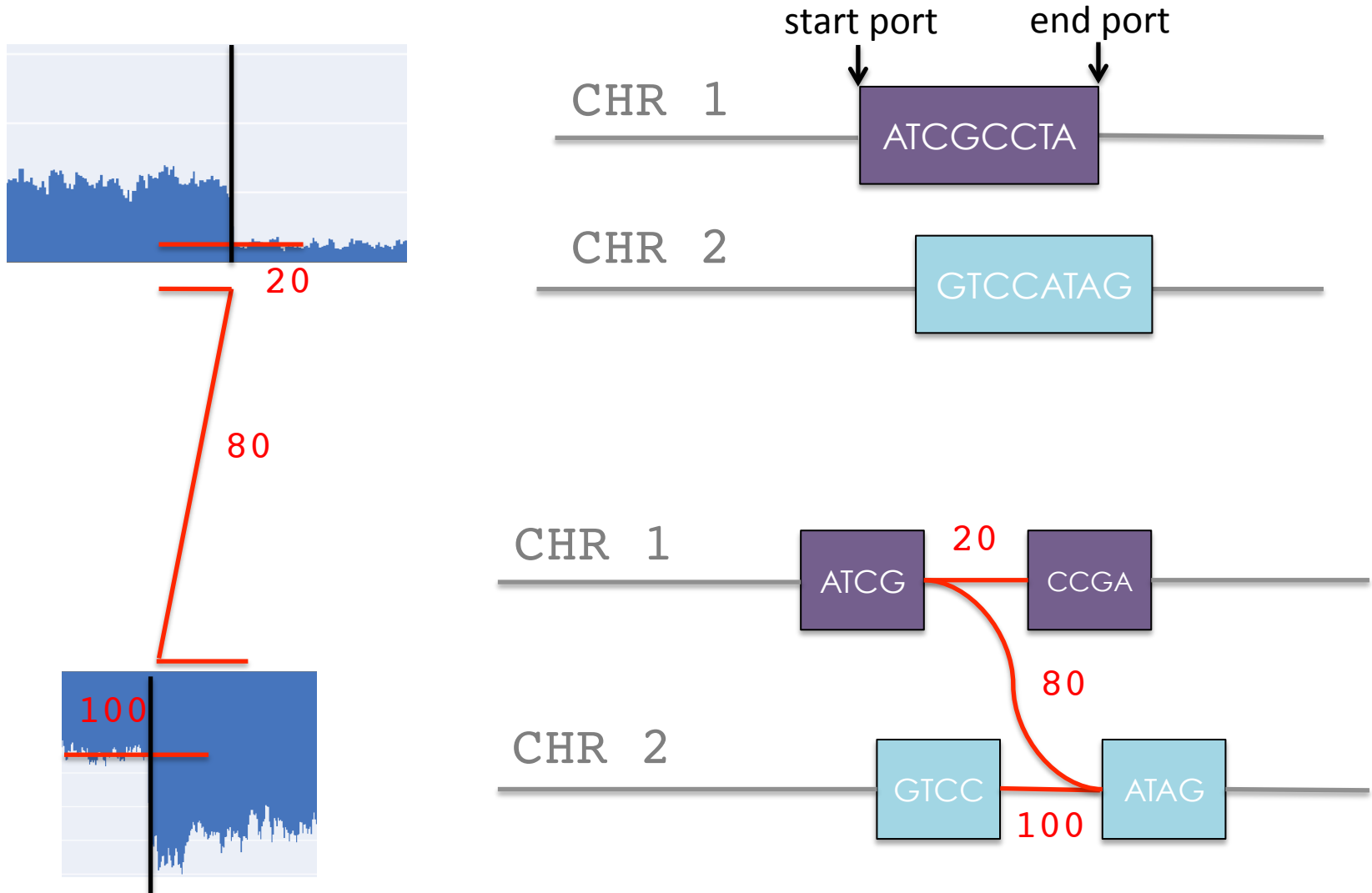
Less parsimonious



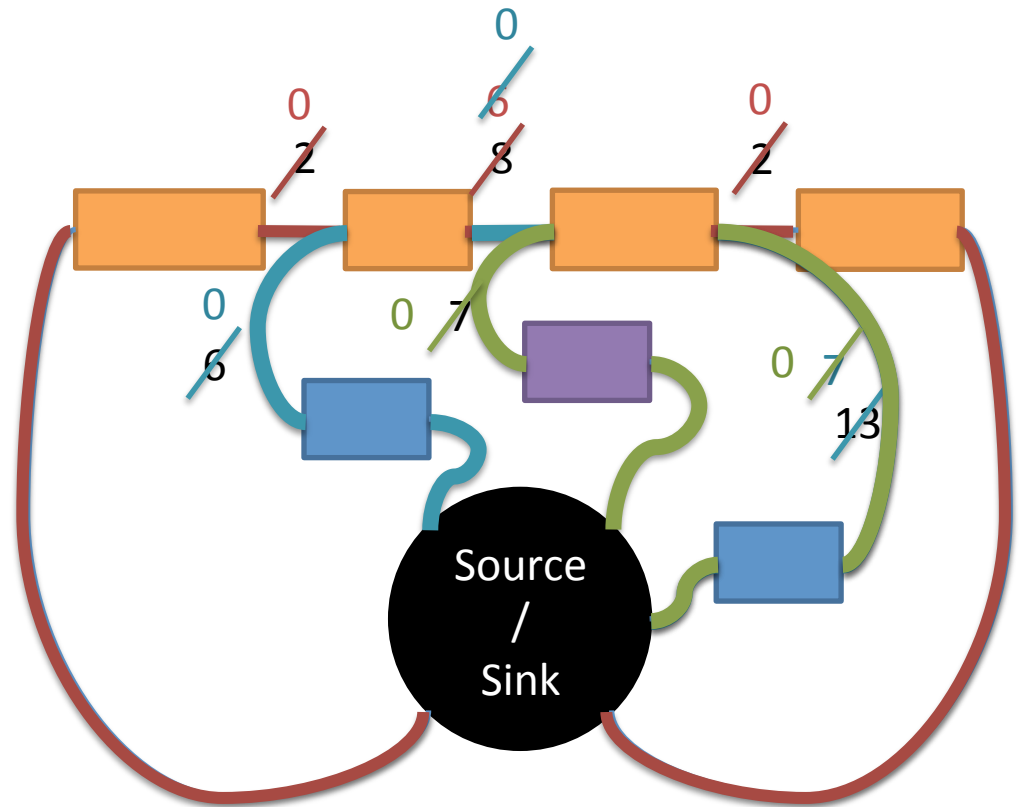
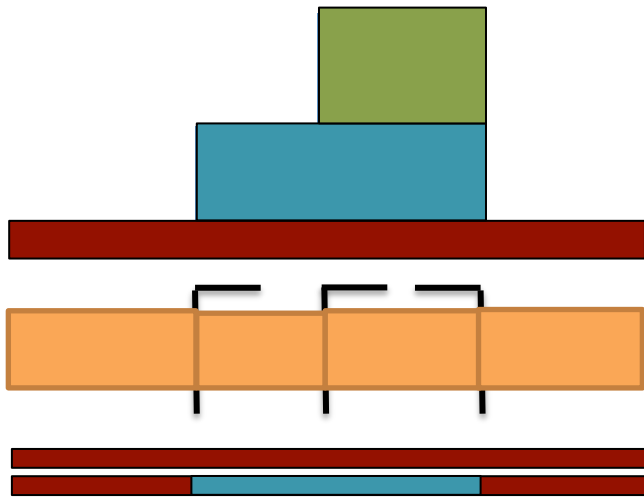
Variant 1 evolving twice is highly unlikely

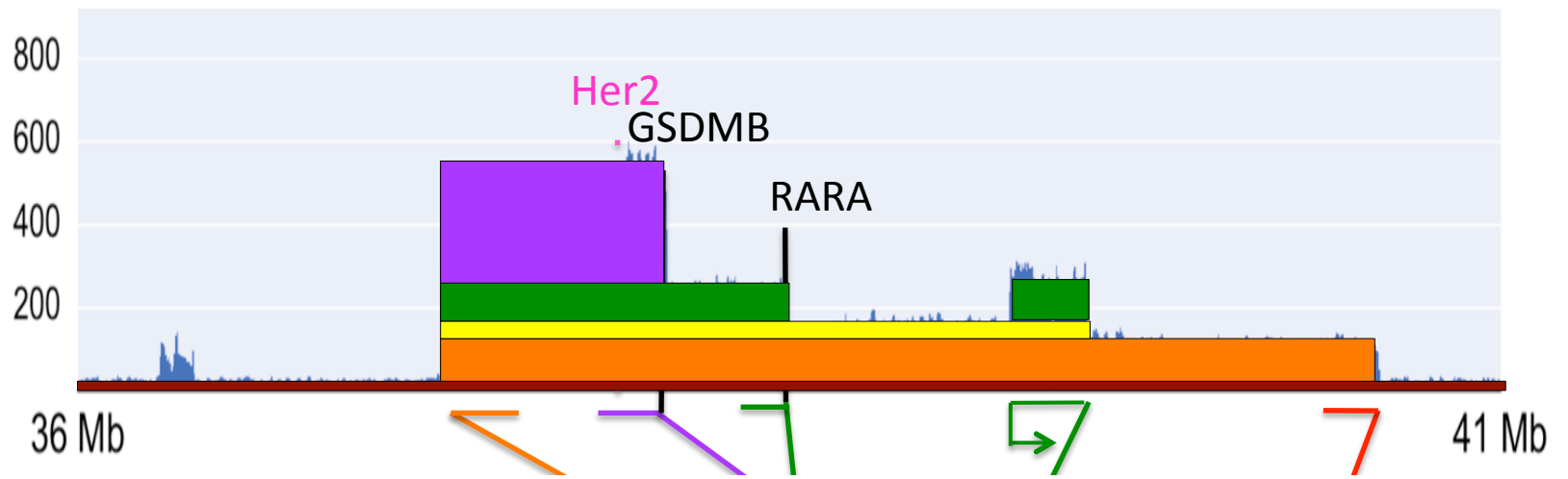


# SplitThreader: Building the graph



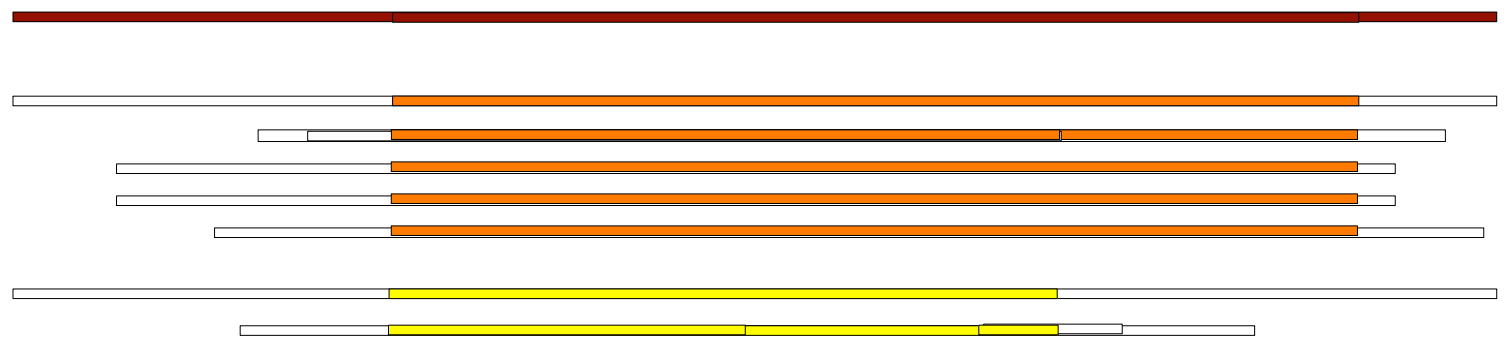
# SplitThreader Evolution



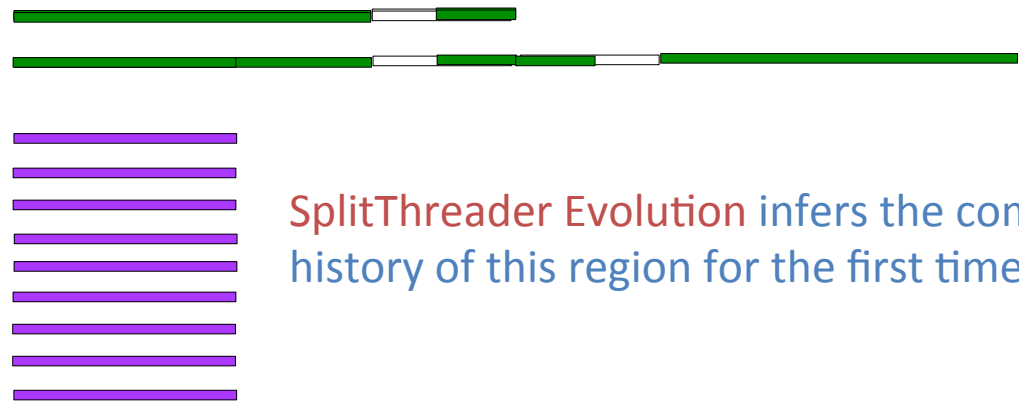


Chr 17

Chr 8



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

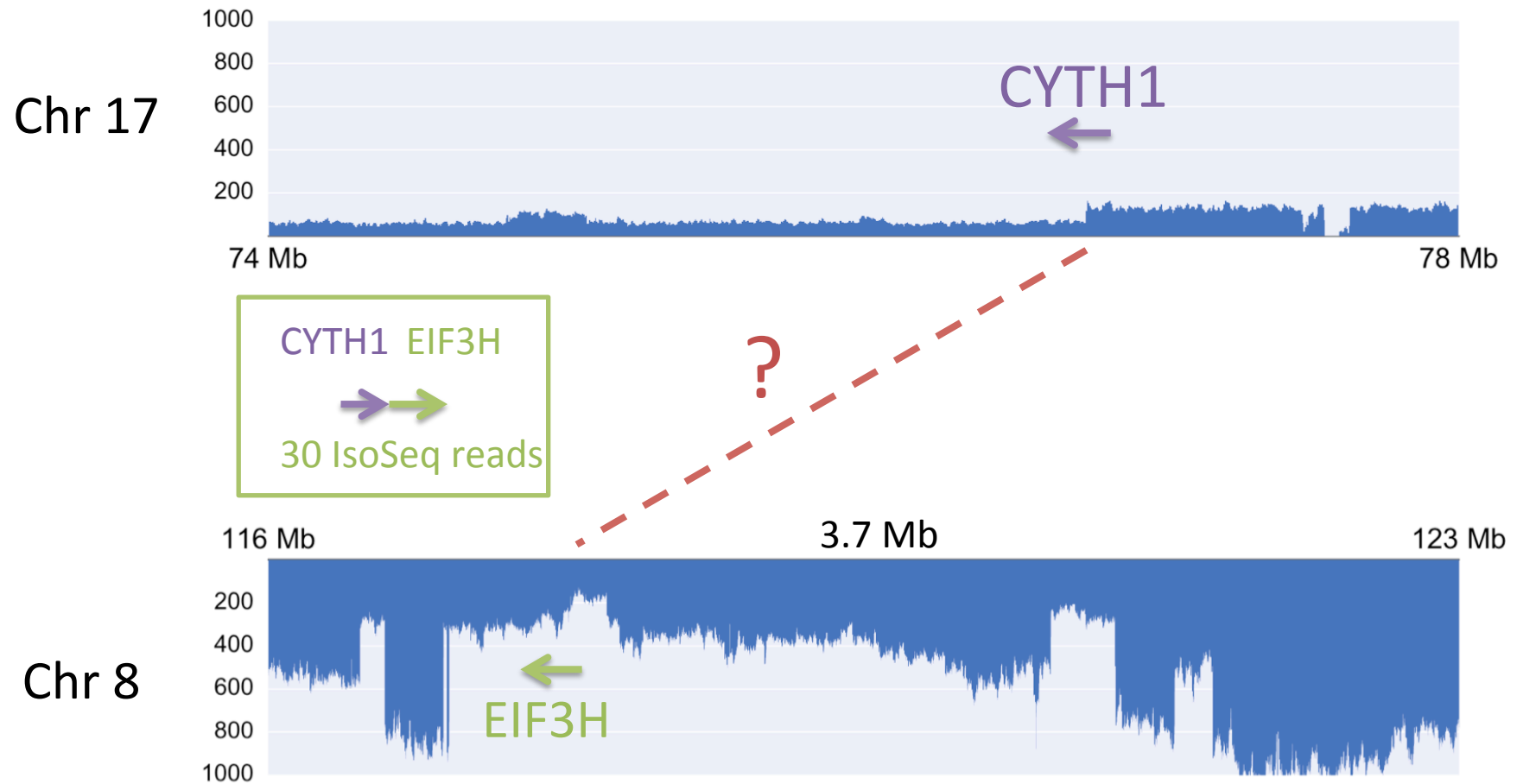


SplitThreader Evolution infers the complex history of this region for the first time

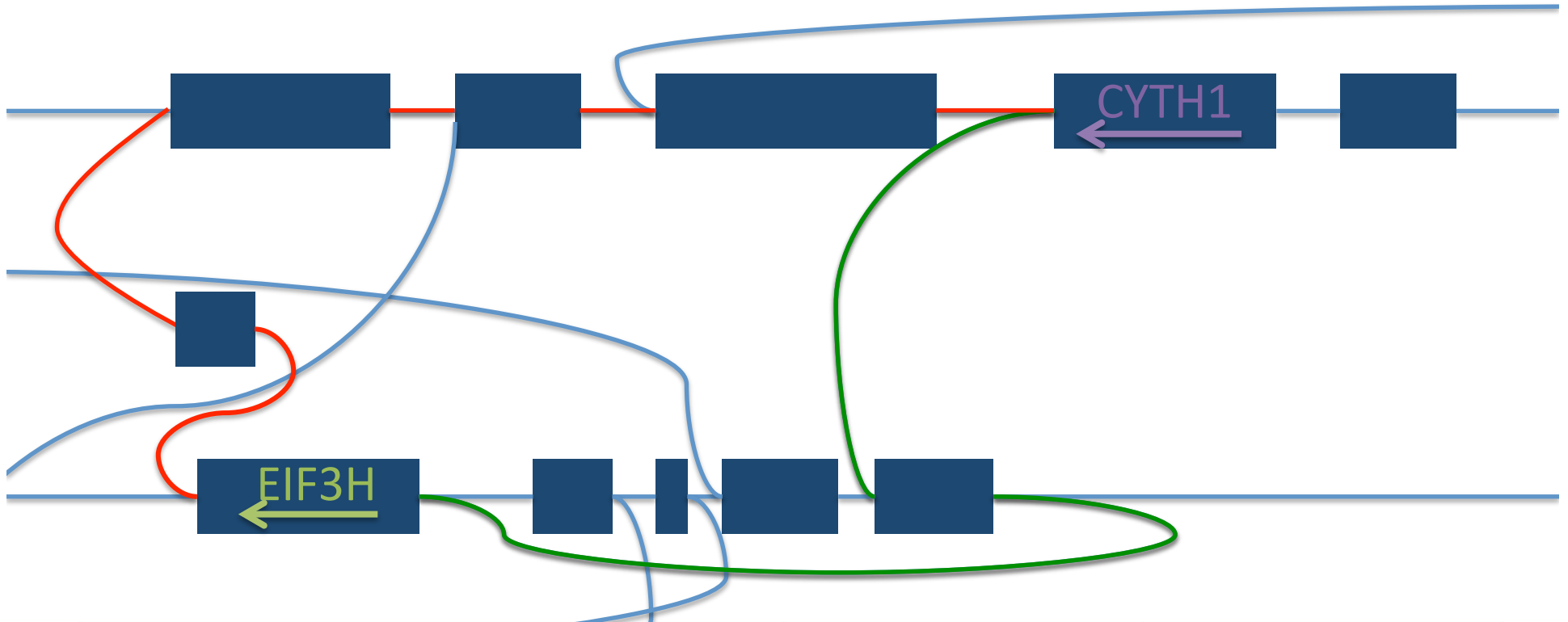
# SplitThreader Fusion

- Found candidate fusions using IsoSeq (PacBio's RNA-seq)
  - 3 million reads, mean 3.5 kb read length
- Use SplitThreader to look for DNA evidence
  - 16 gene fusions with a direct link in the DNA
  - 6 gene fusions each of which is created by threading through 2 variants

# SplitThreader Fusion: CYTH1-EIF3H example

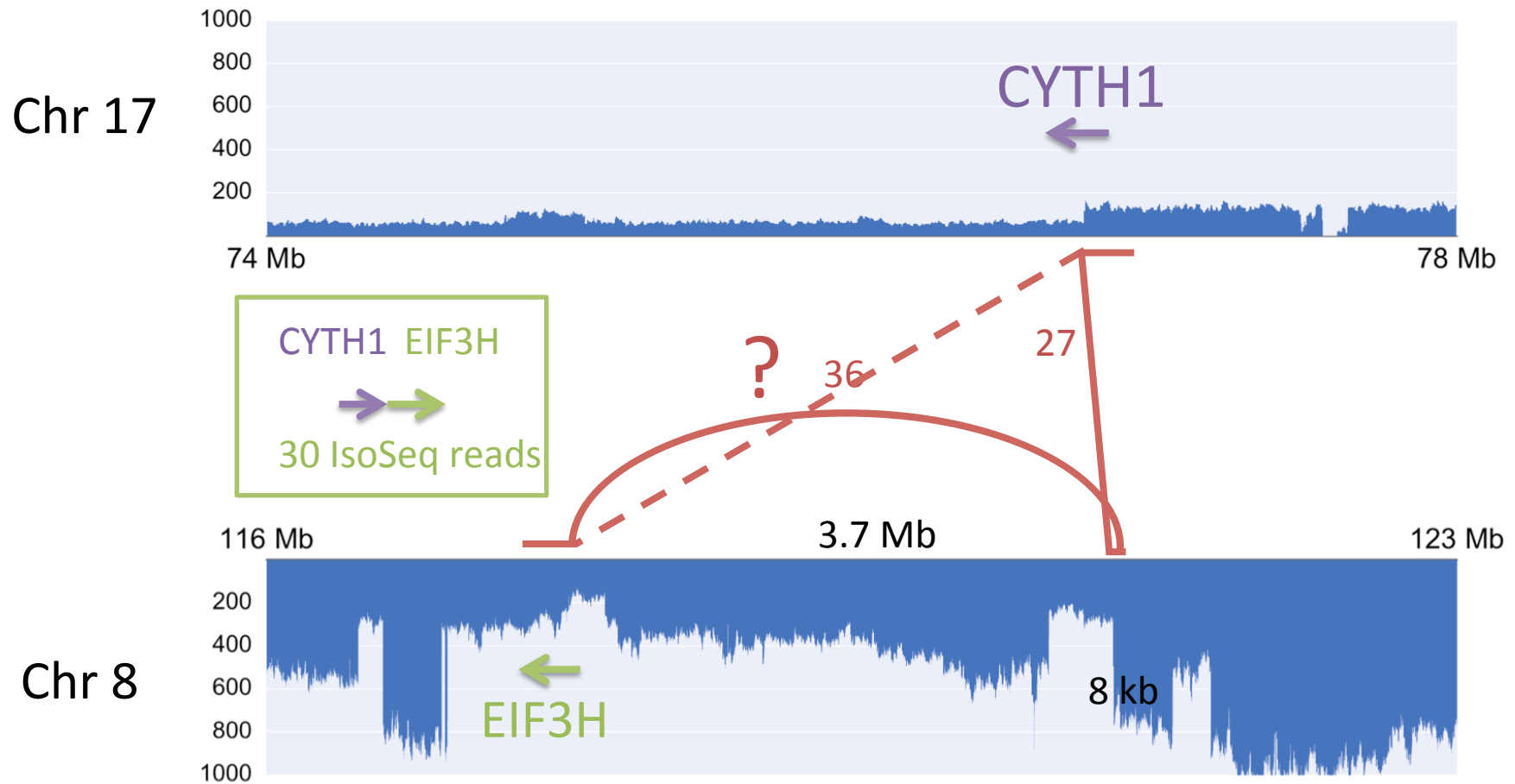


# SplitThreader Fusion: CYTH1-EIF3H



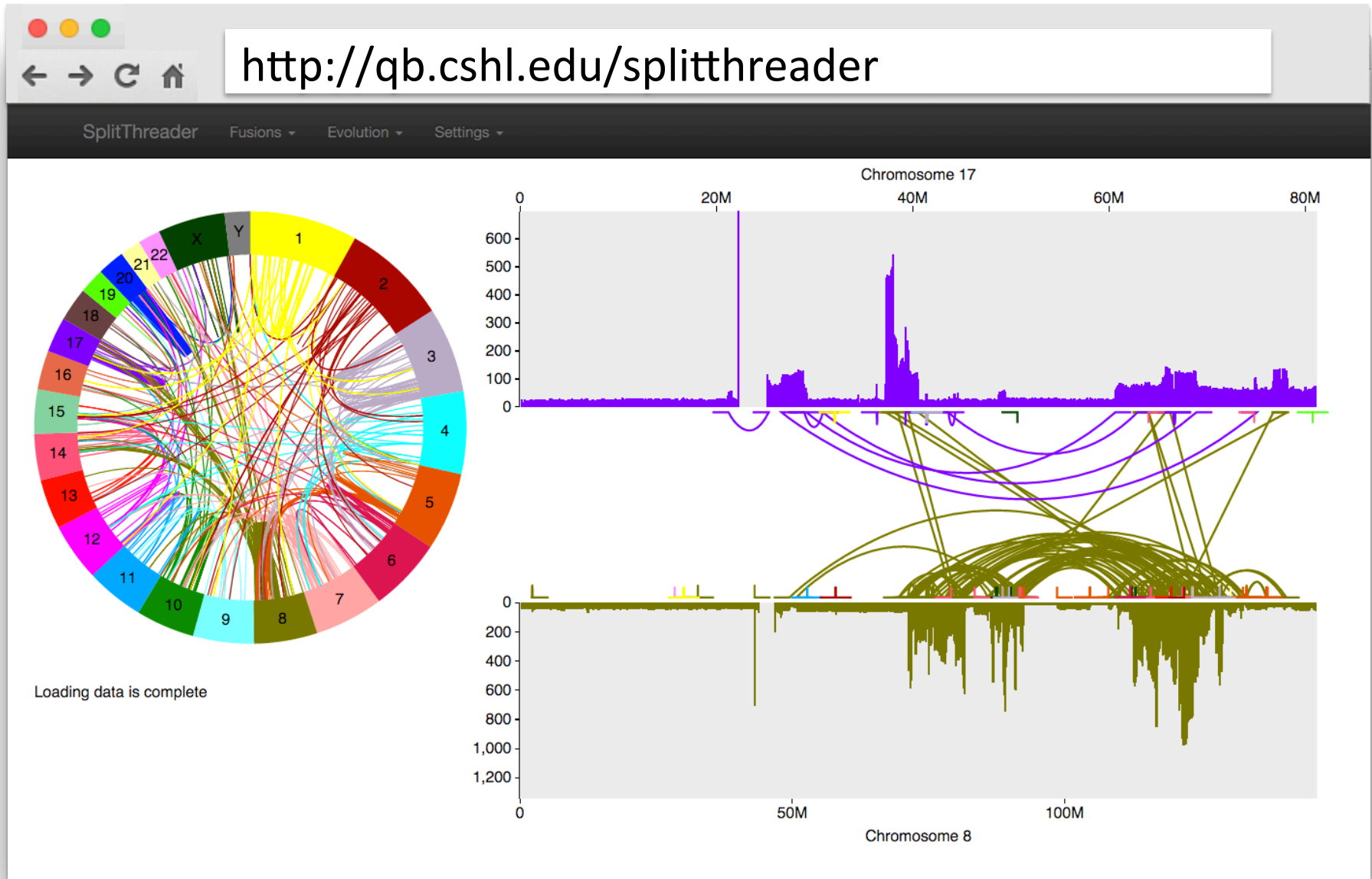
Criteria	Red path	Green path
Sense-sense stranded	No	Yes
Fewer variants	2	2
More split read DNA evidence	40, 11	27, 36
Shorter transcript (threshold < 1Mb)	1.1 Mb	164 kb

# SplitThreader Fusion: CYTH1-EIF3H results

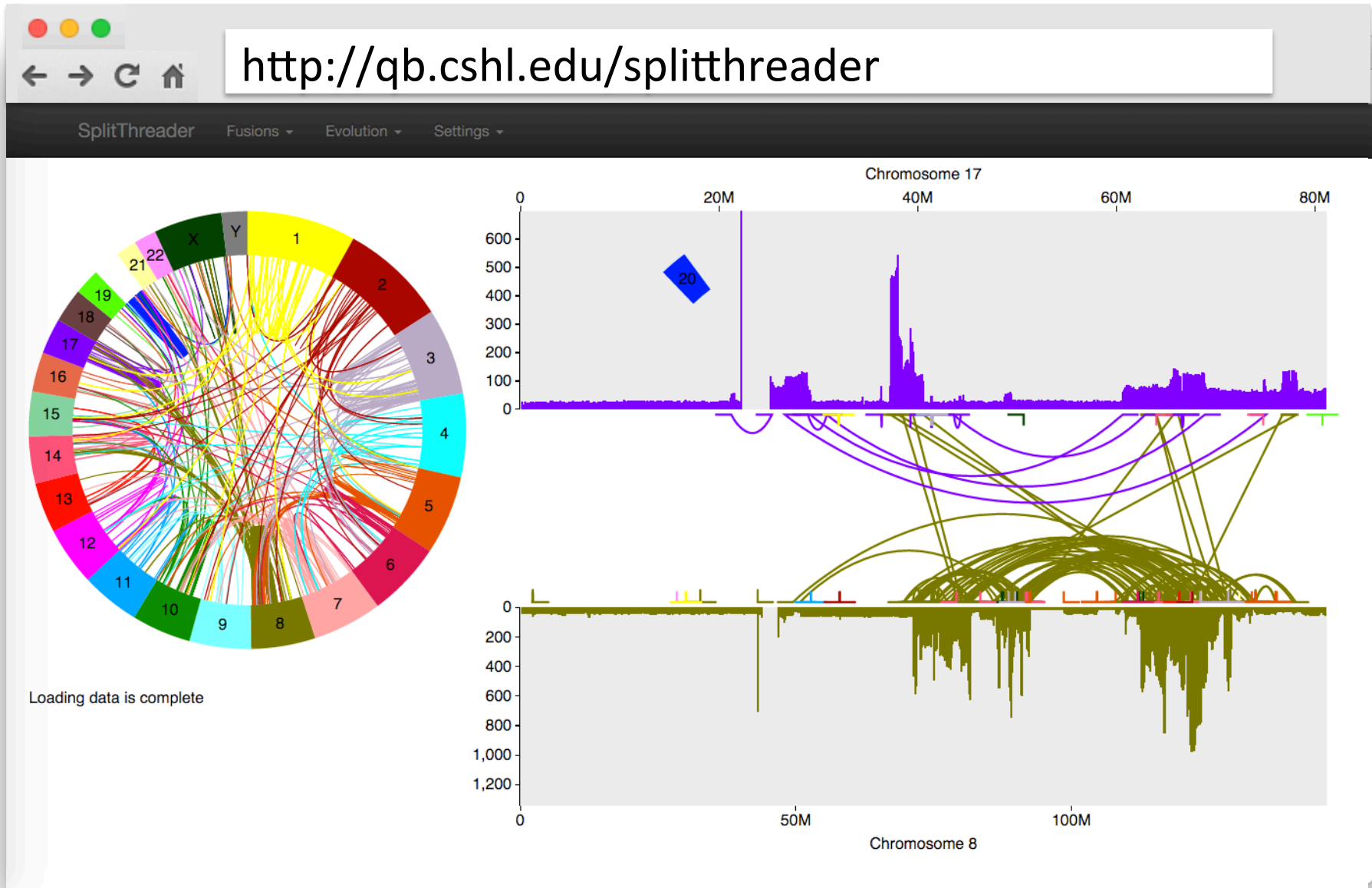




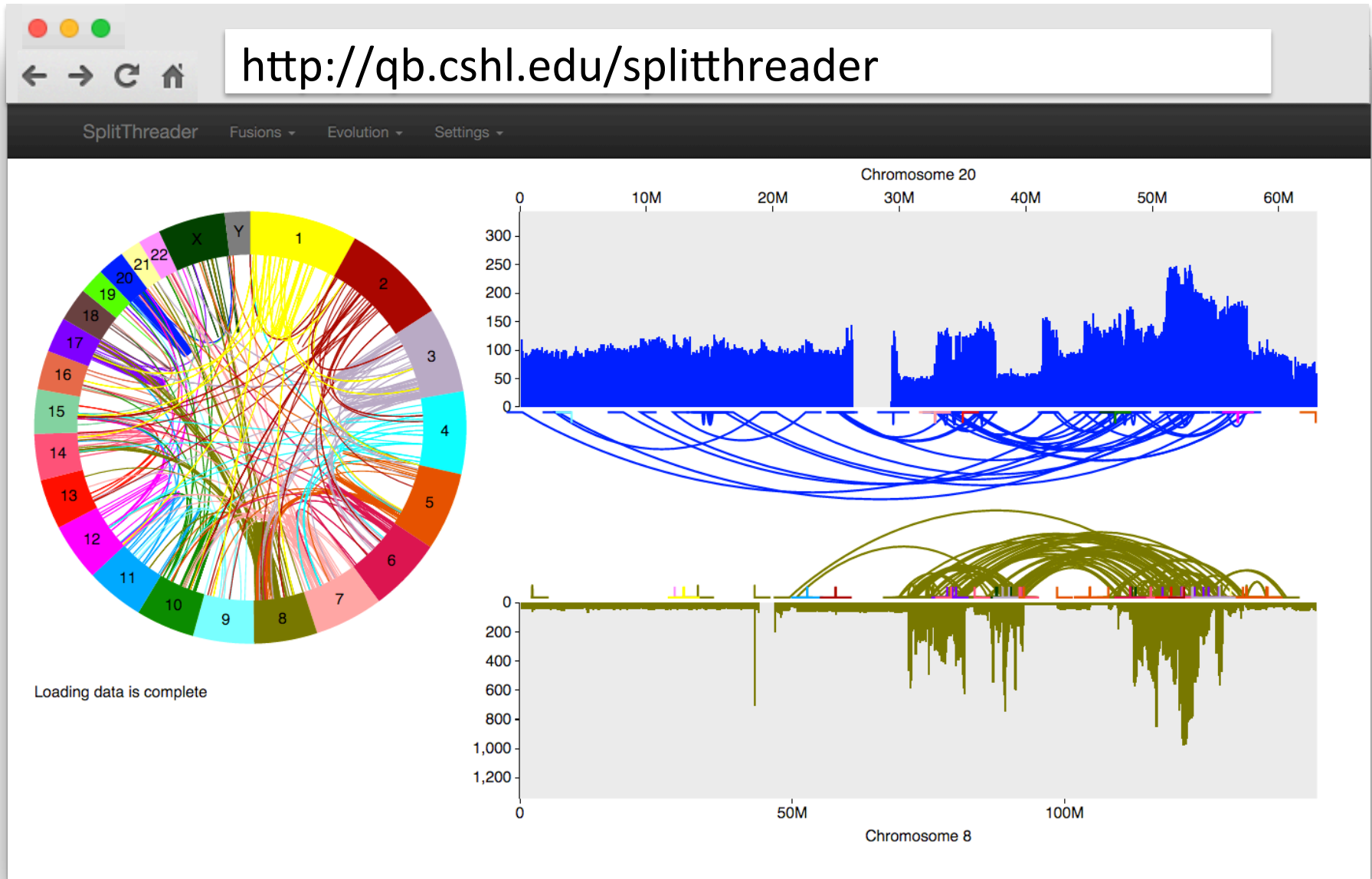
# SplitThreader Visualizer



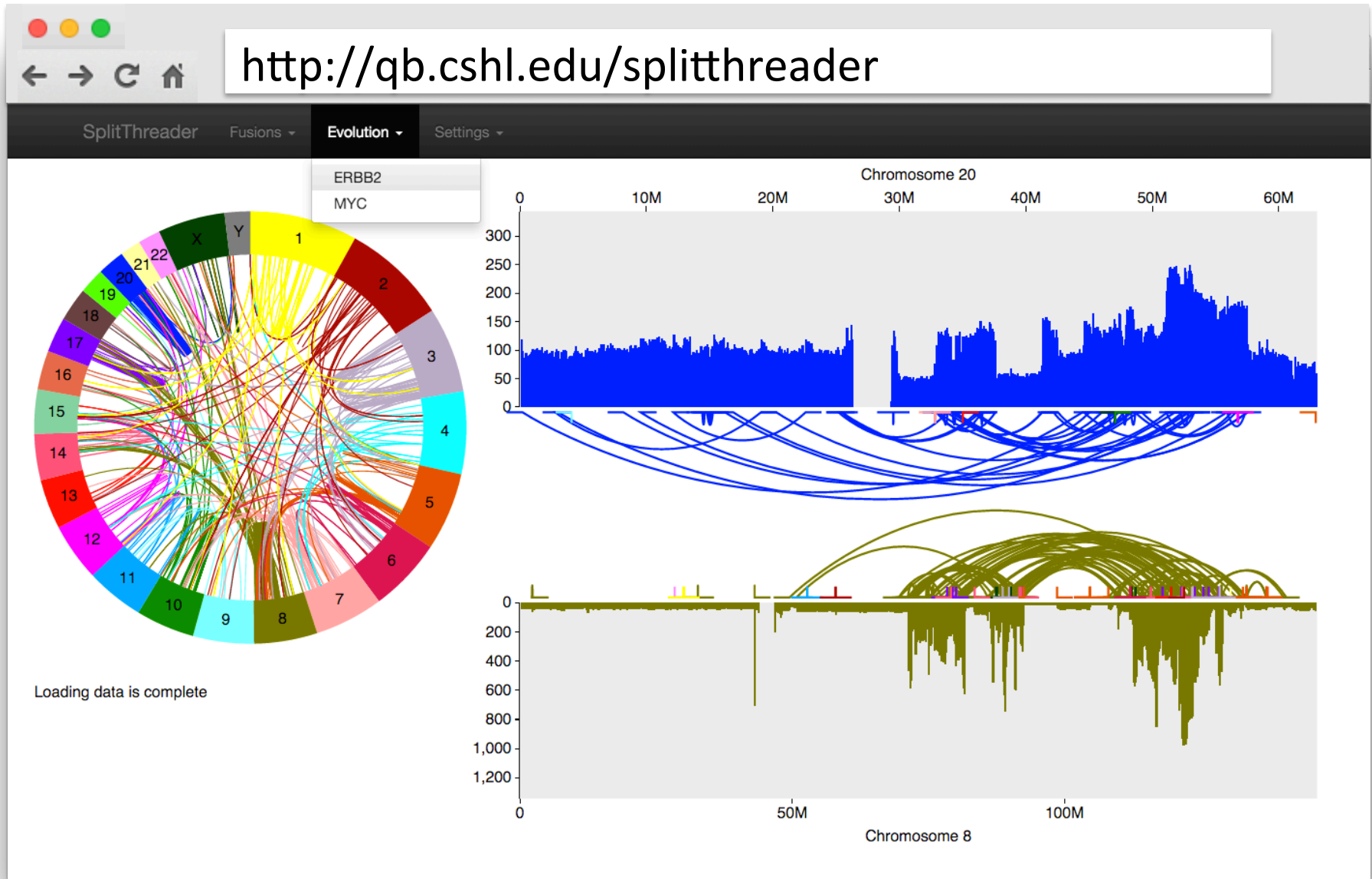
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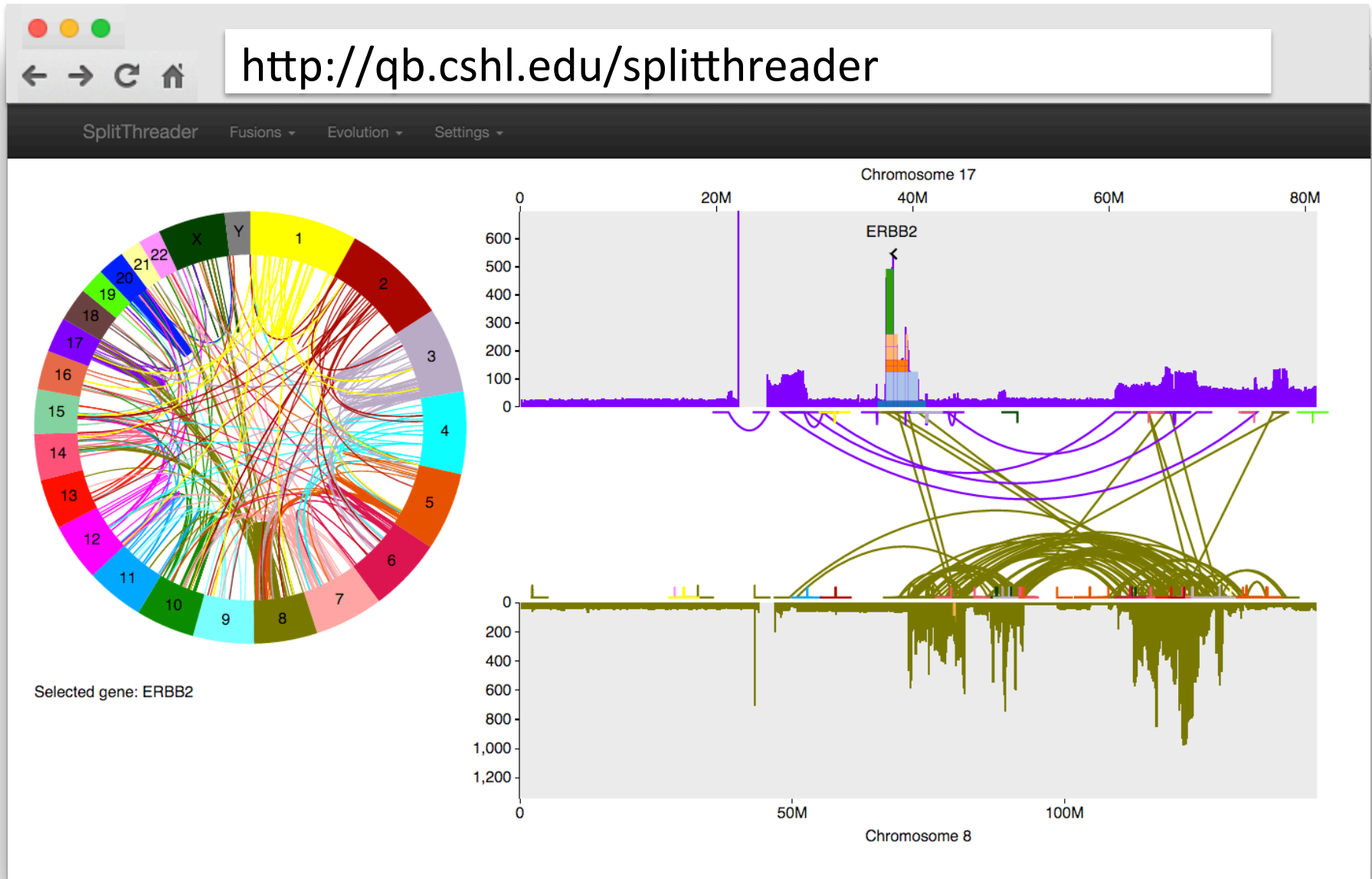
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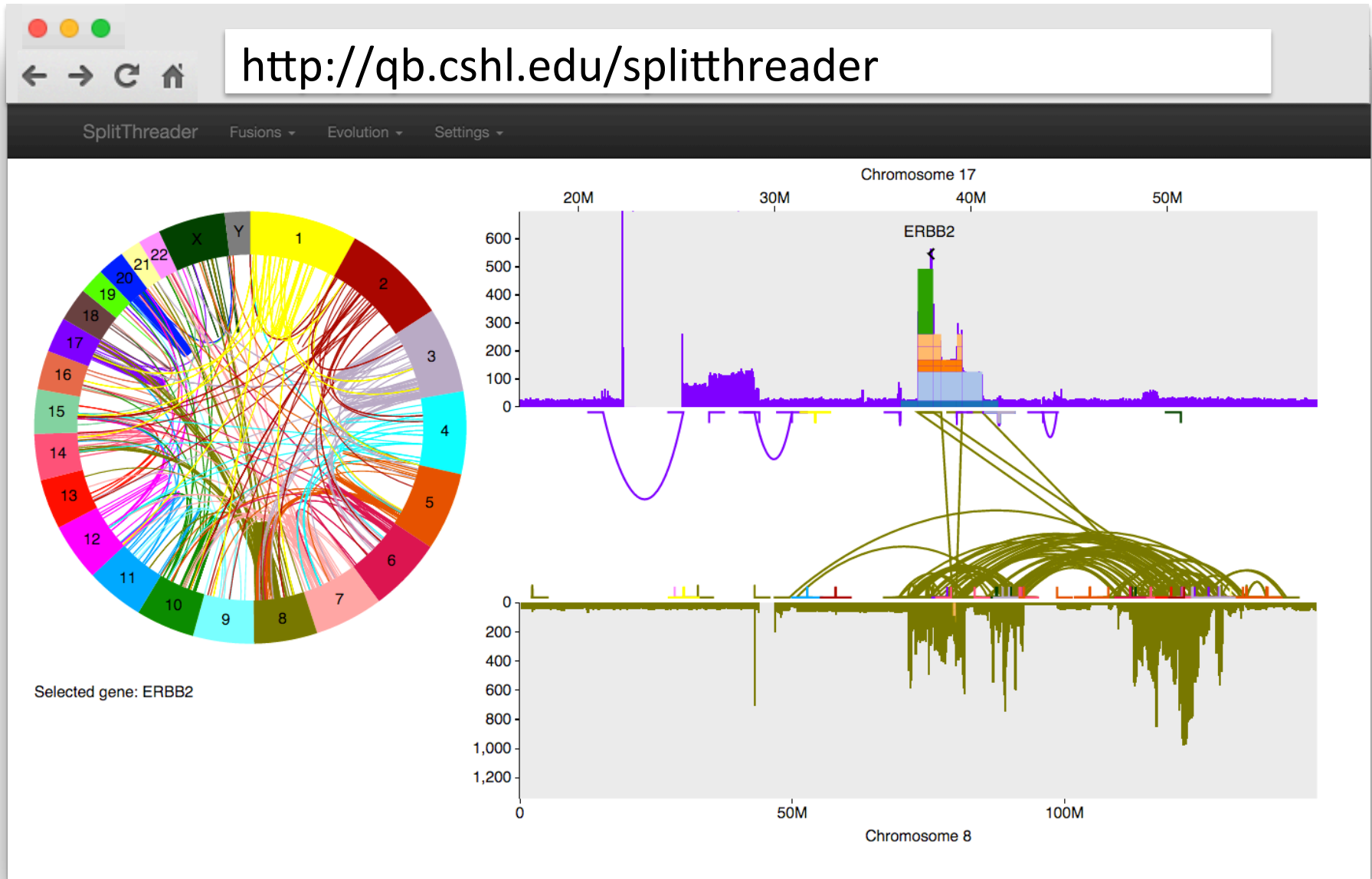
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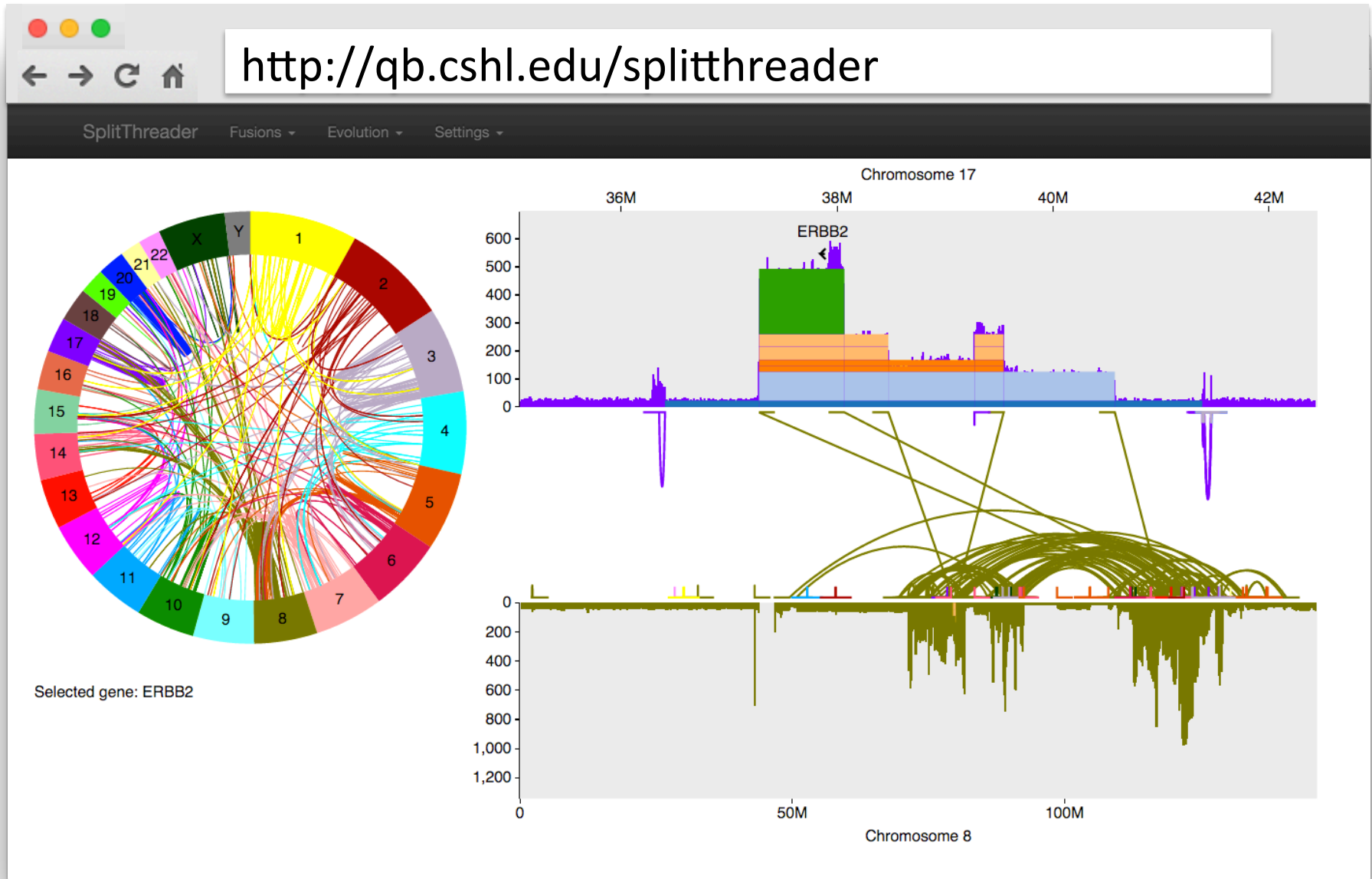
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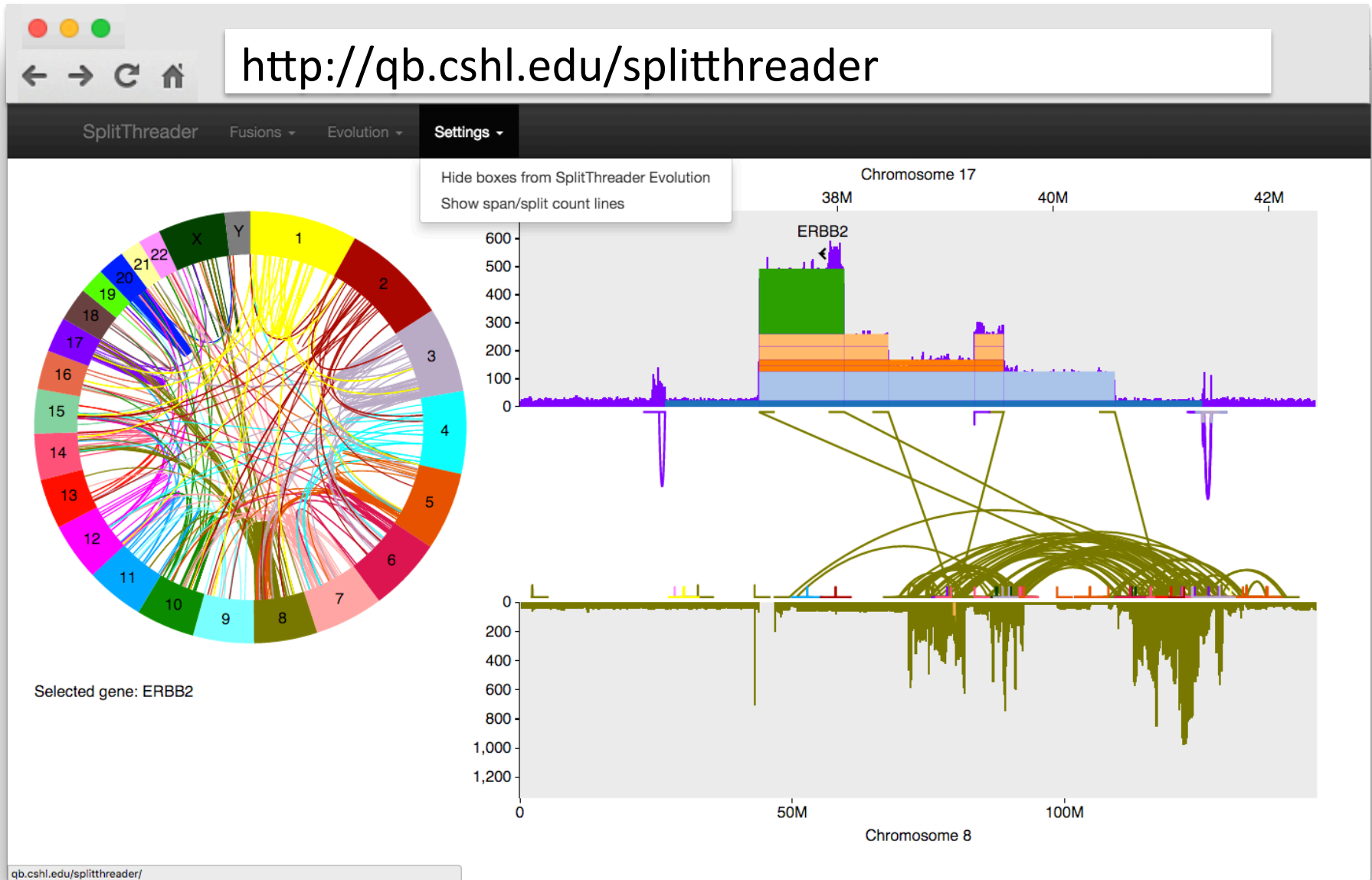
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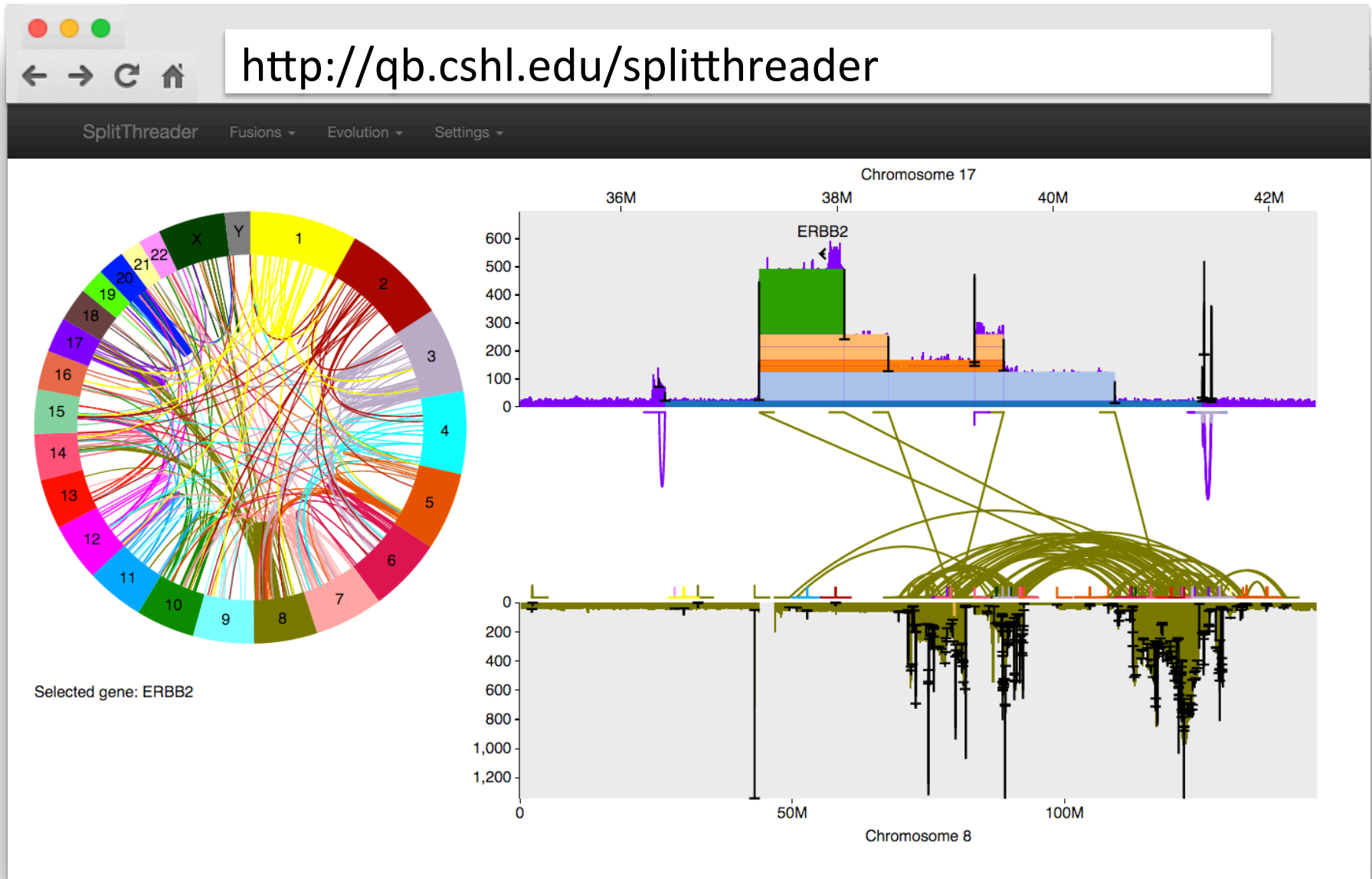


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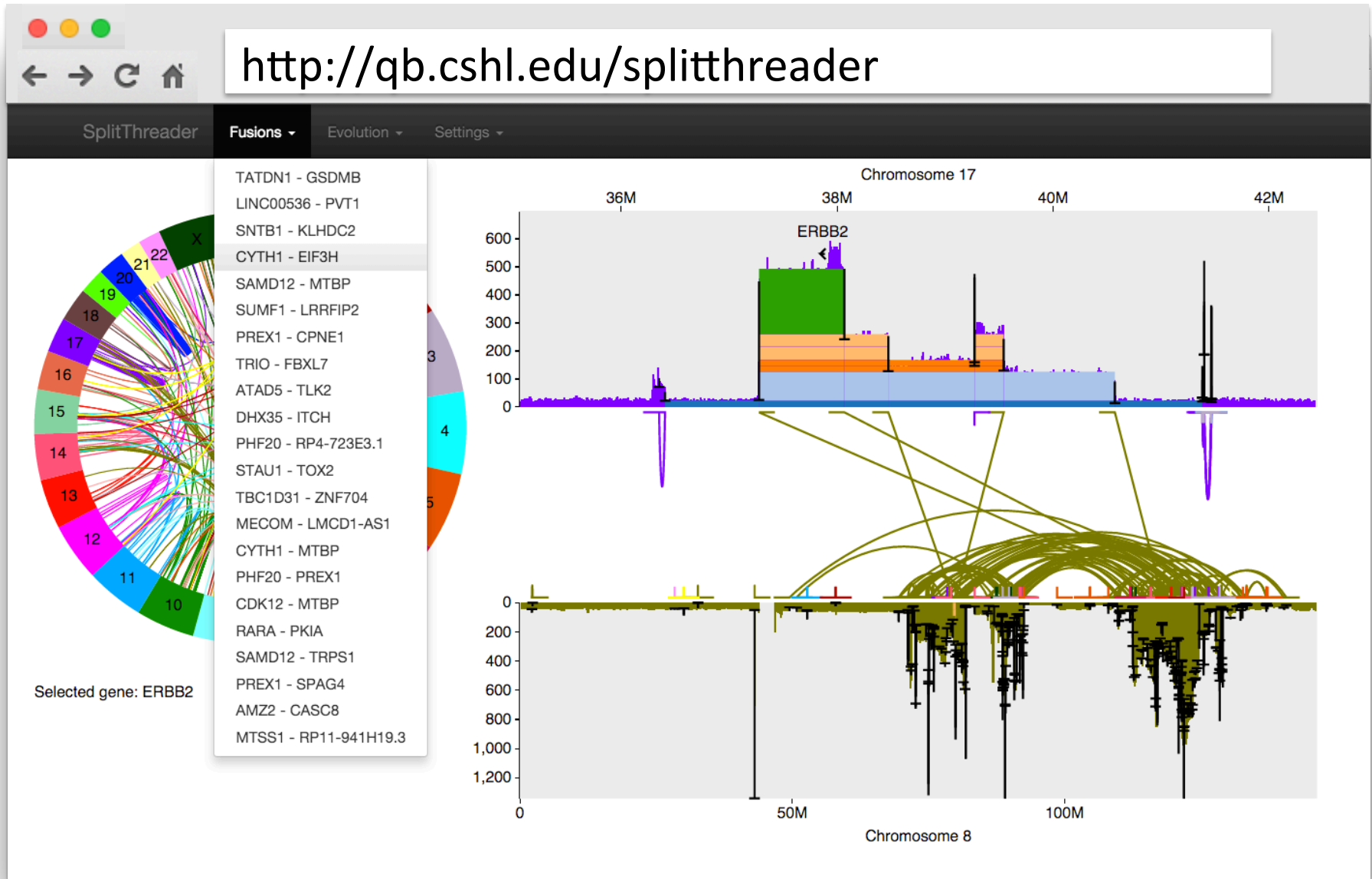




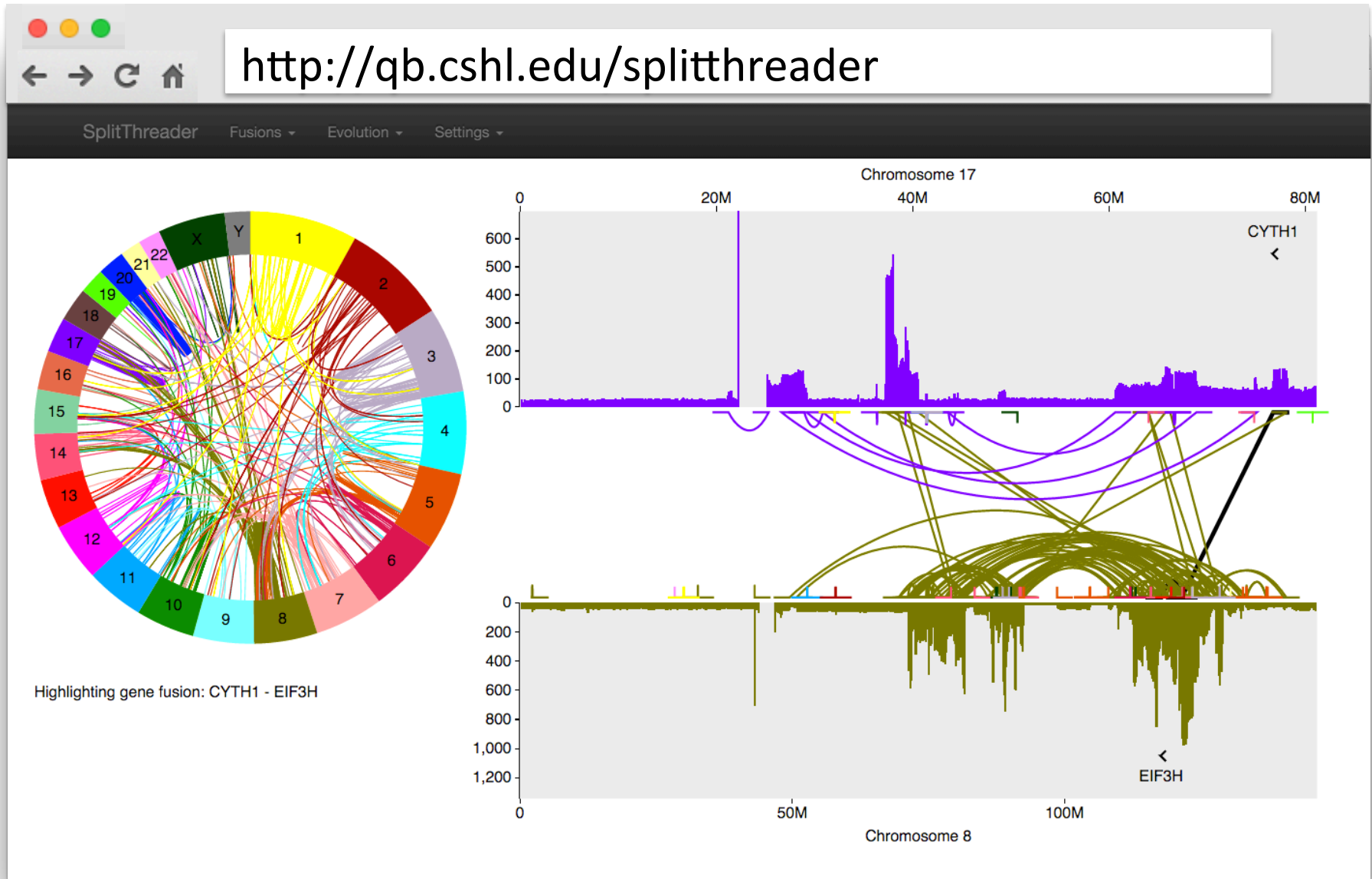
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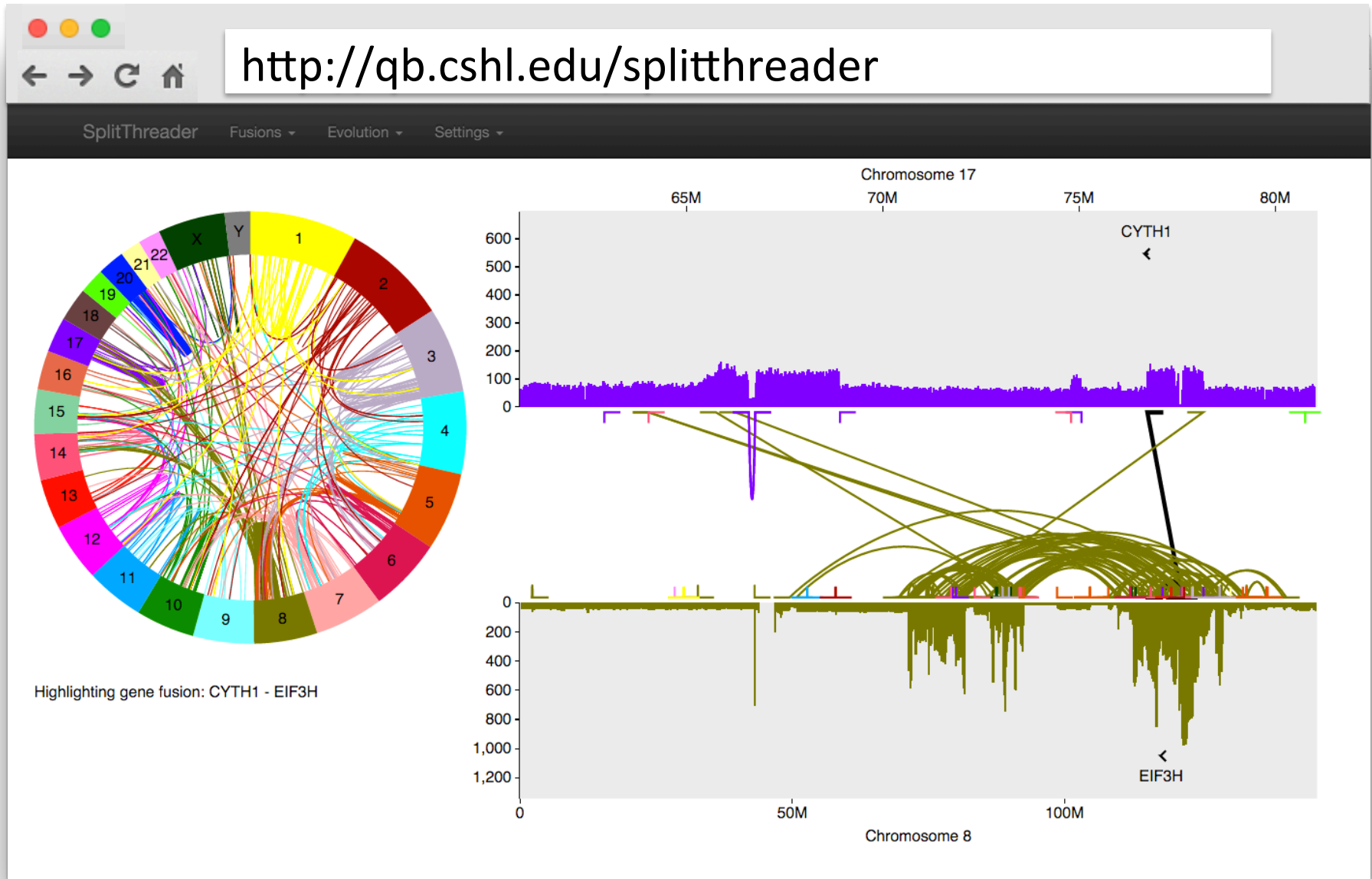
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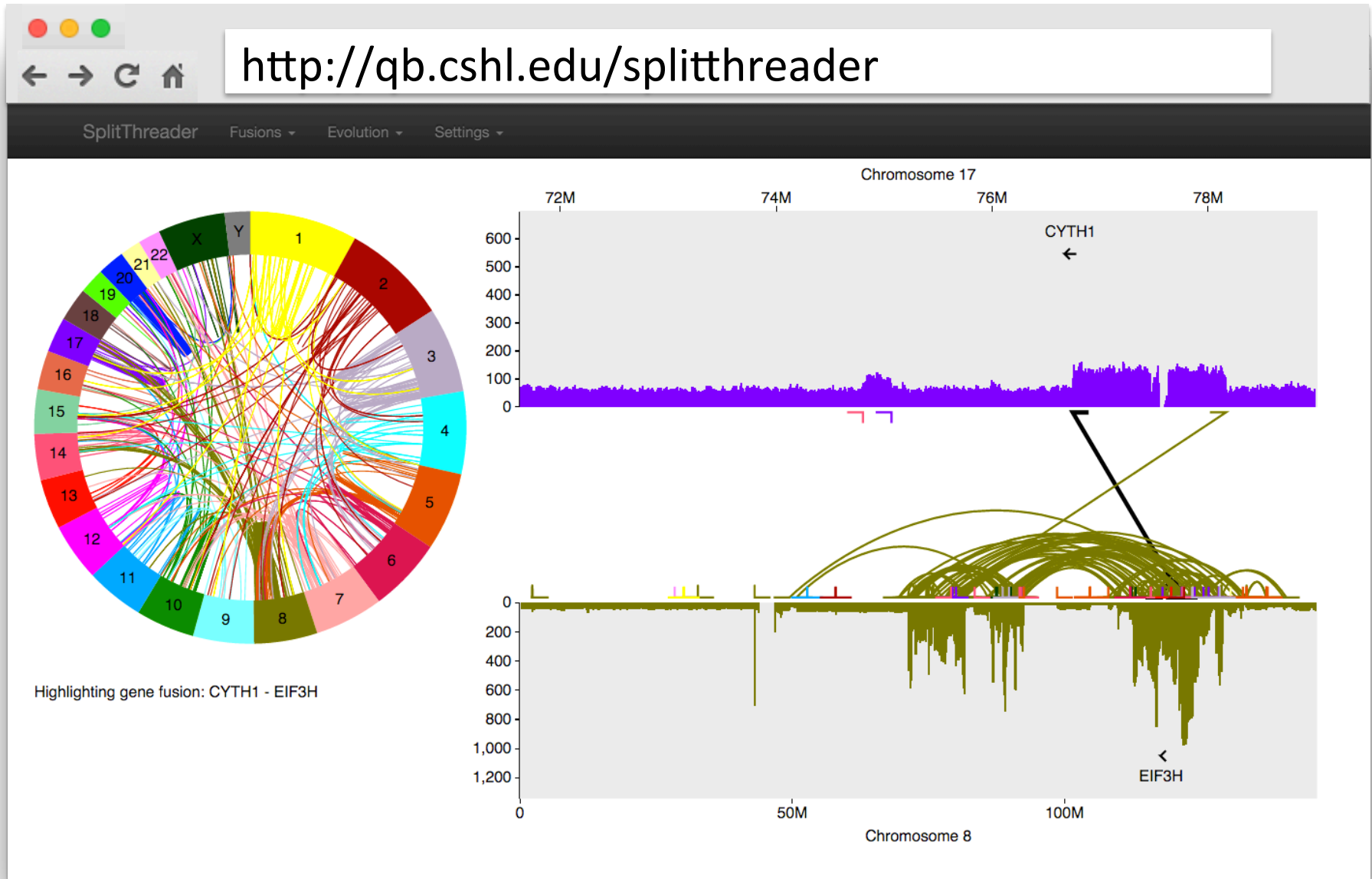
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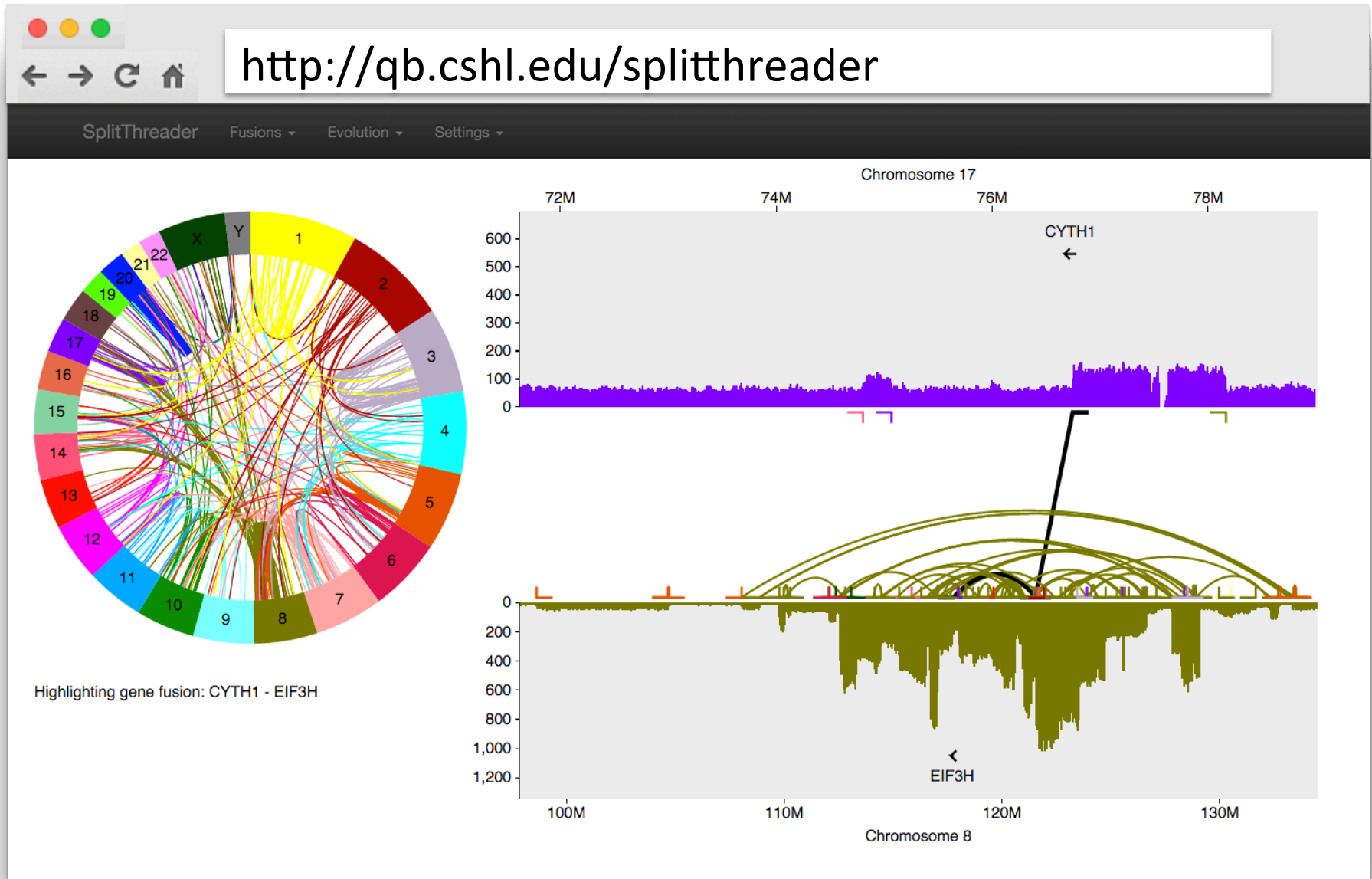
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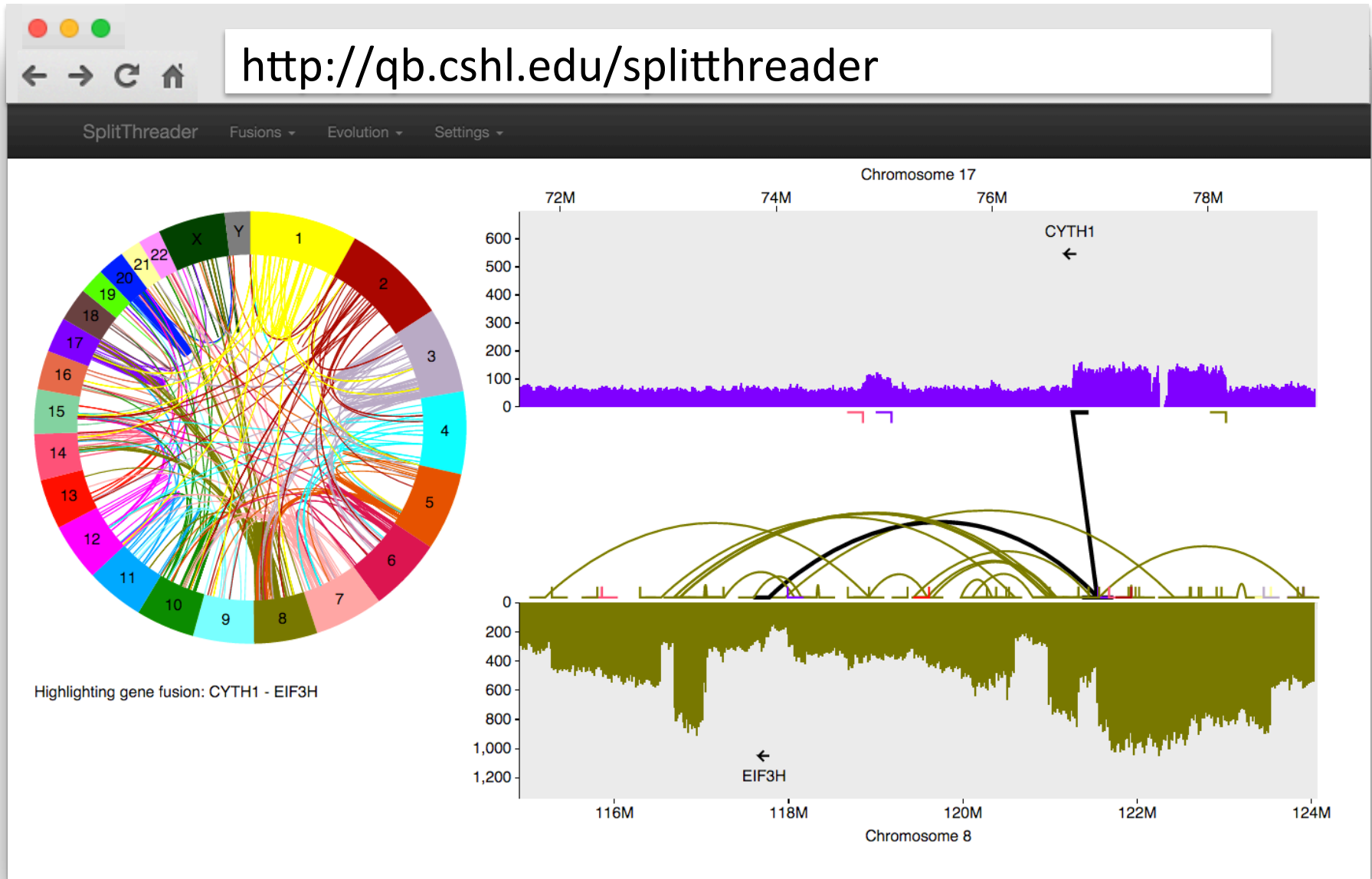
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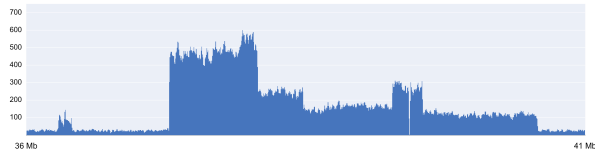
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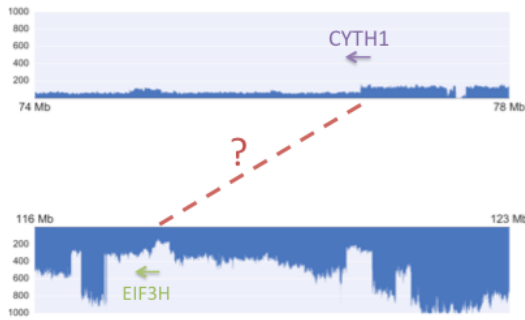
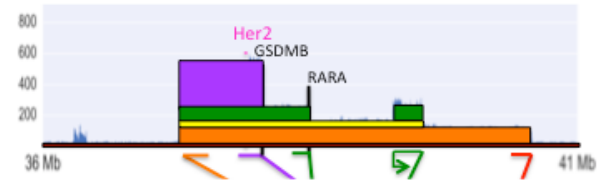


# SplitThreader



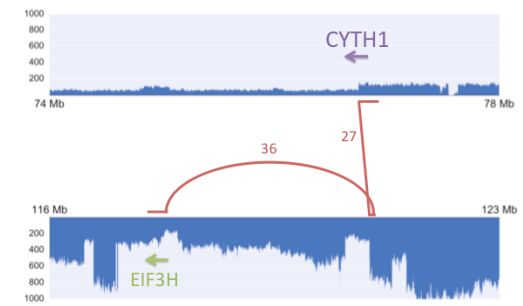
Explain oncogene amplifications

SplitThreader Evolution



Trace gene fusions

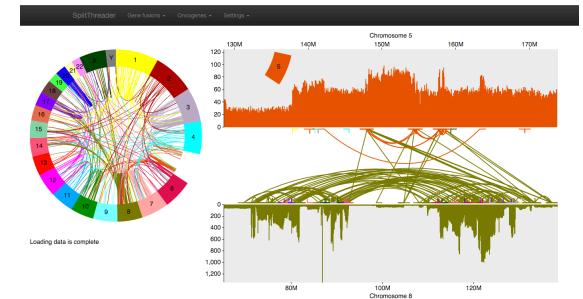
SplitThreader Fusion



[qb.cshl.edu/splitthreader](http://qb.cshl.edu/splitthreader)

Explore the genome

SplitThreader Visualizer





# Acknowledgments



Cold  
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