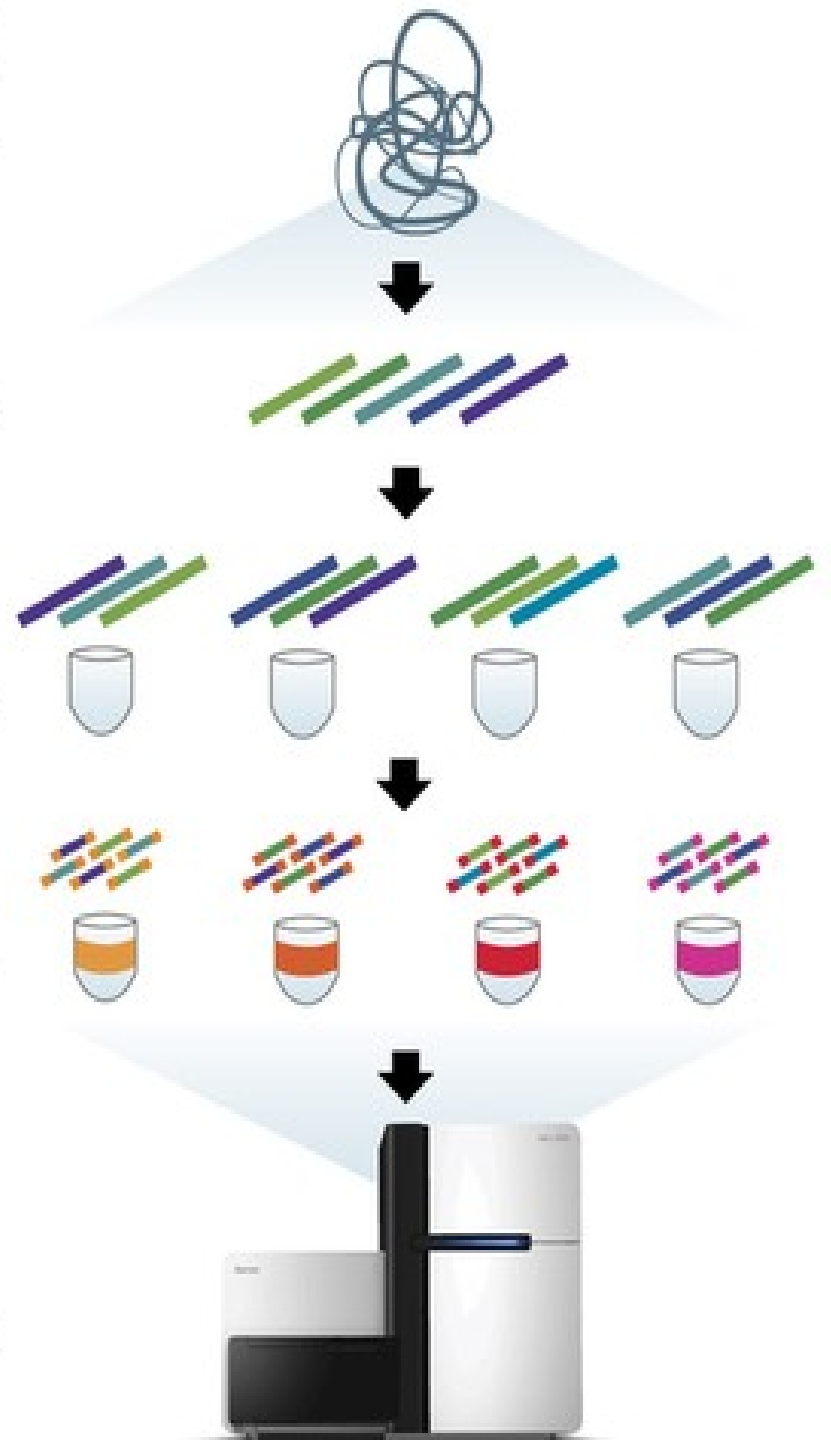


Moleculo Overview

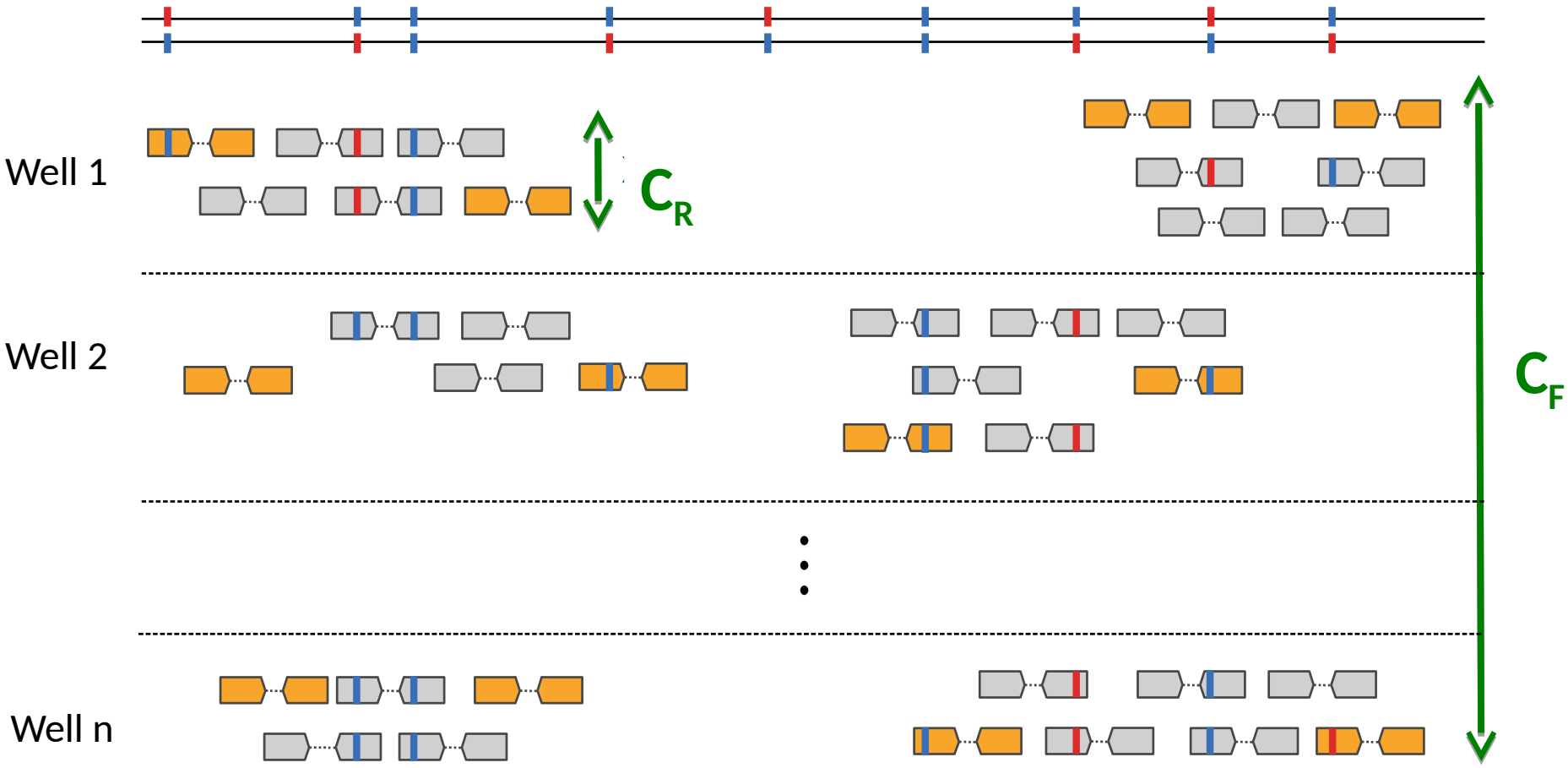
1. Sample DNA is sheared into fragments of about 10 kbp

2. Fragments are diluted and placed into 384 wells

3. Fragments are amplified through long-range PCR, cut into short fragments and barcoded

4. Short fragments are pooled together and sequenced

Read Clouds



Coverage = $C_F C_R$

- Normal (FFPE)
- Sequenced by:
  - Shotgun (40X)

- IDC (Fresh Frozen)
- Sequenced by:
  - Shotgun (40X)
  - Moleculo (78X)

C_F=43, C_R=1.8

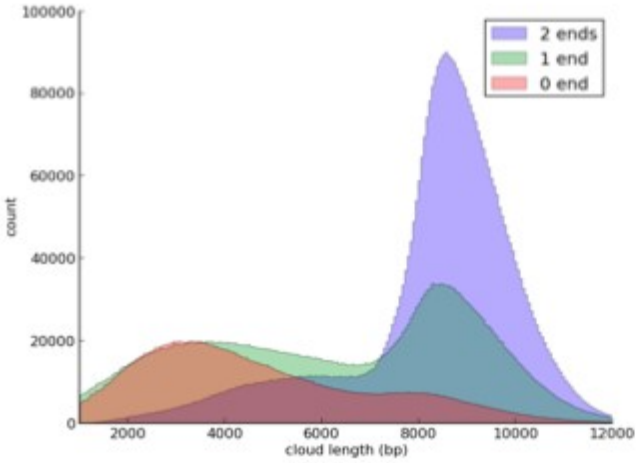
Align reads

Create clouds

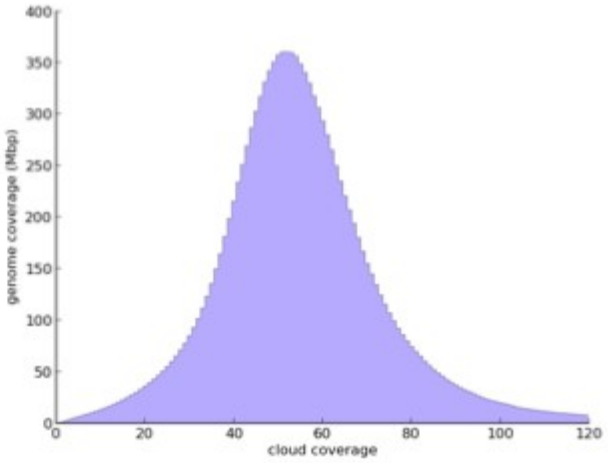
Realign reads

Call Variants (GATK)

Phase



(a) Read cloud sizes



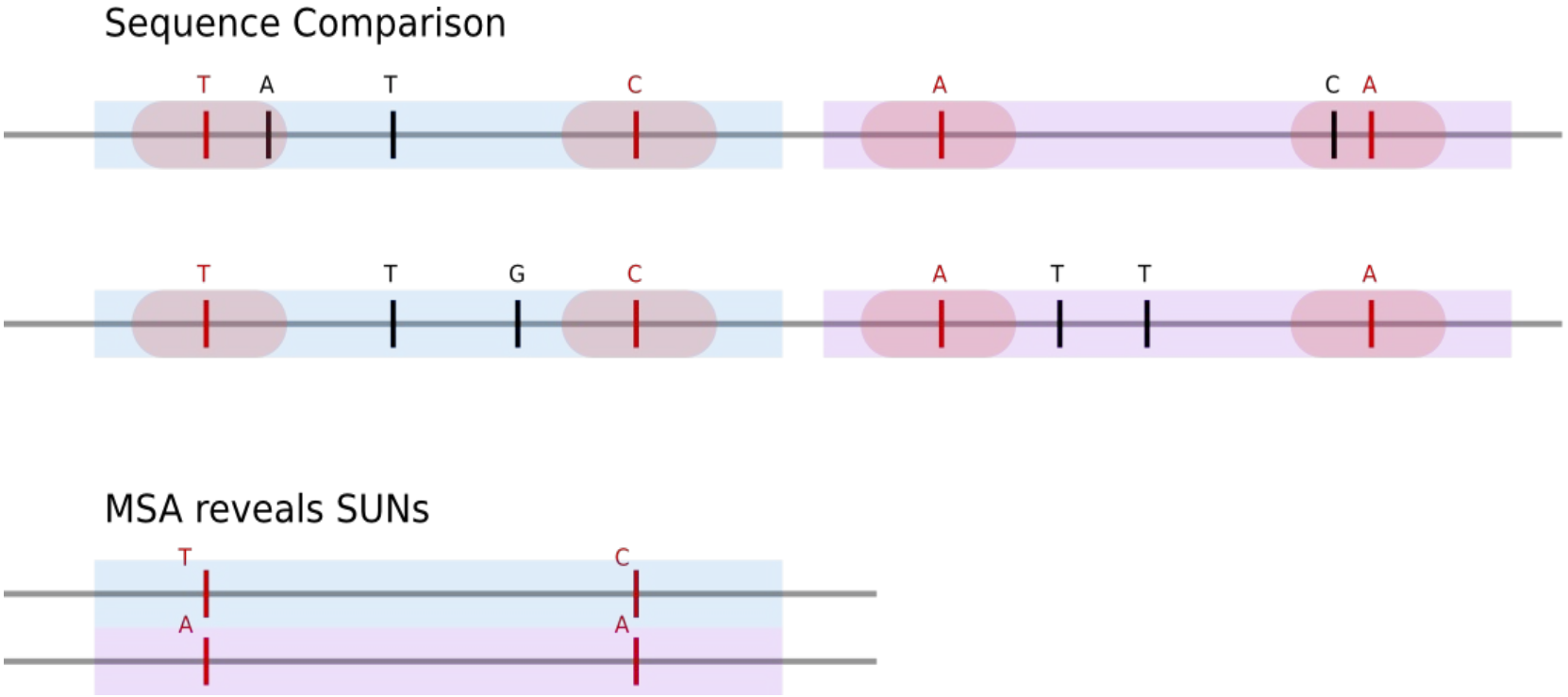
(b) Read Cloud Genome Coverage

Identifying Variation in Segmental Duplications

~180 Mbp of human in almost exact repeats

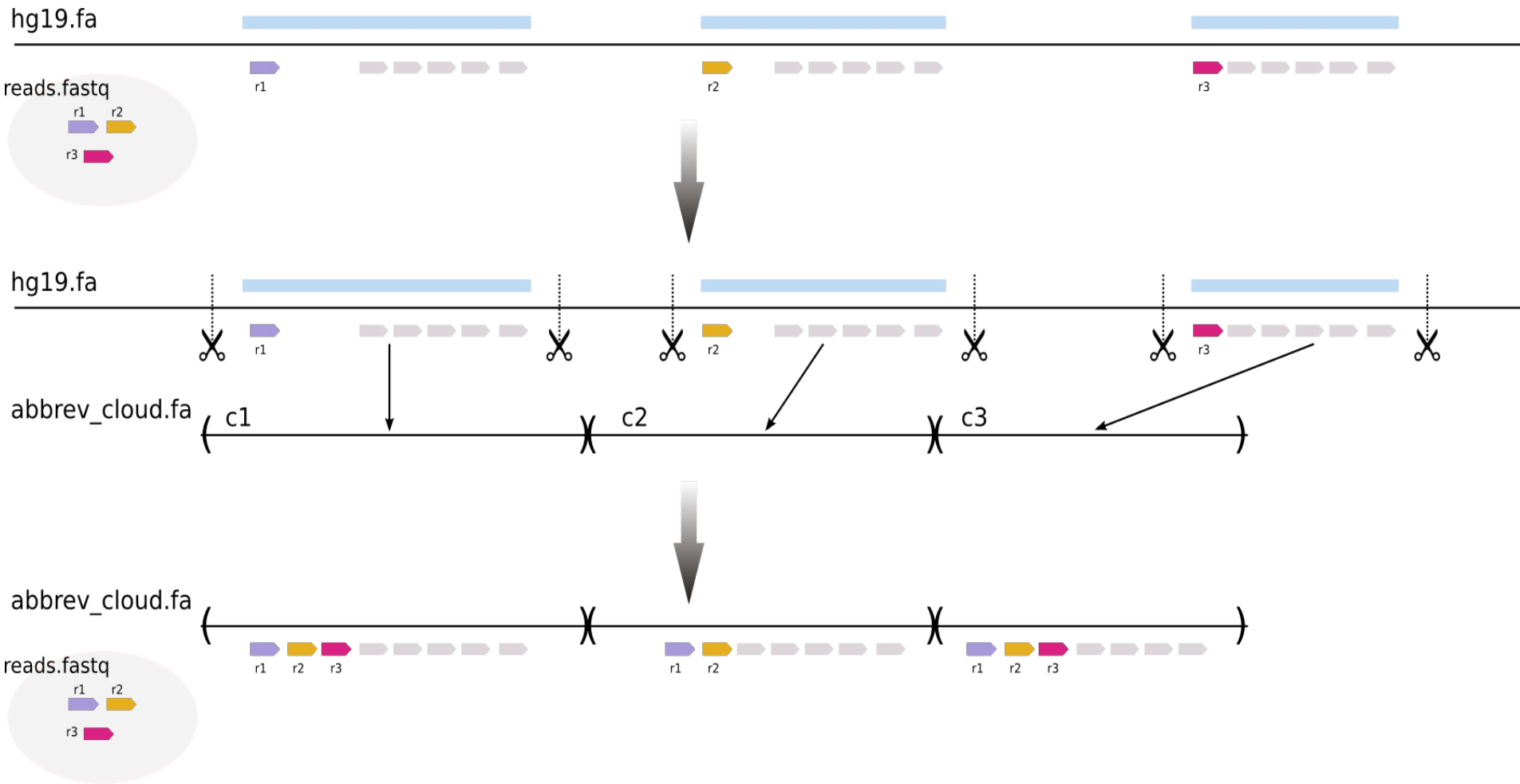
Novel variation impossible to detect with short reads alone

Single unique nucleotides (SUNs) in segdups can be leveraged by read clouds



Candidate Cloud and Alignment Generation

- Use Bowtie2 to align each well separately to the reference



MRF-based Realignment

Generative model of read cloud generation of set of reads R

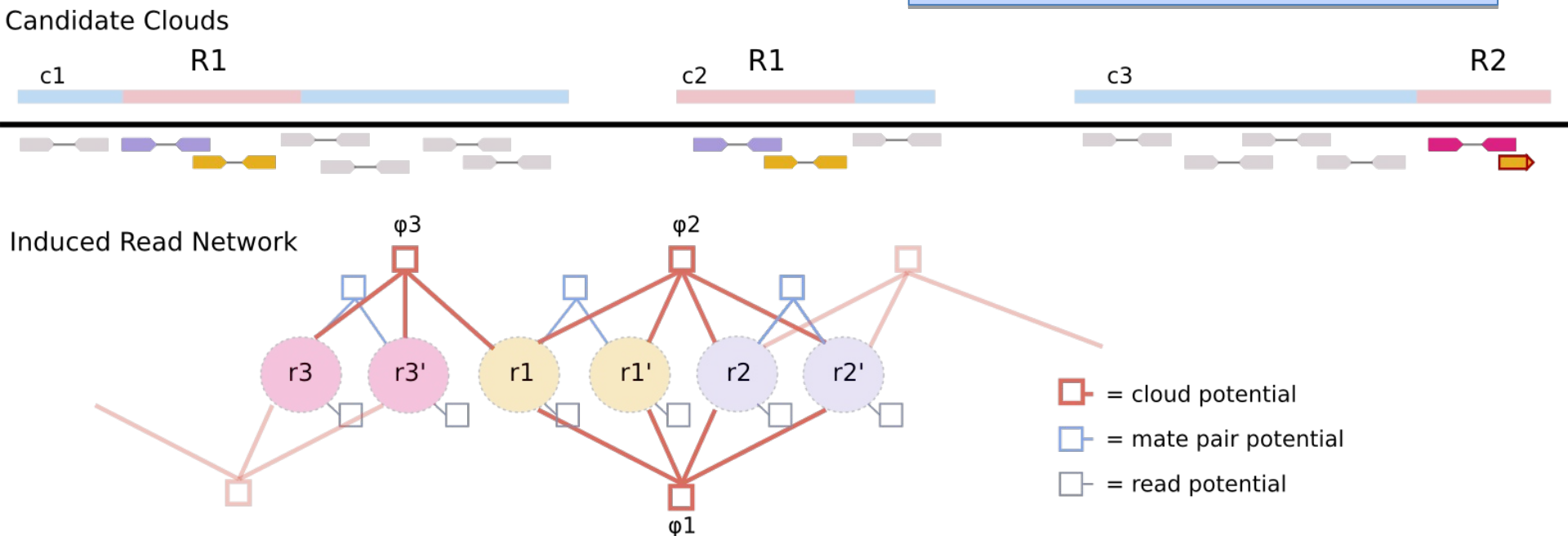
$$P(R) = \sum_{\{\text{Underlying molecule set M}\}} P(M)\, P(R \mid M)$$

Markov Random Field (MRF)

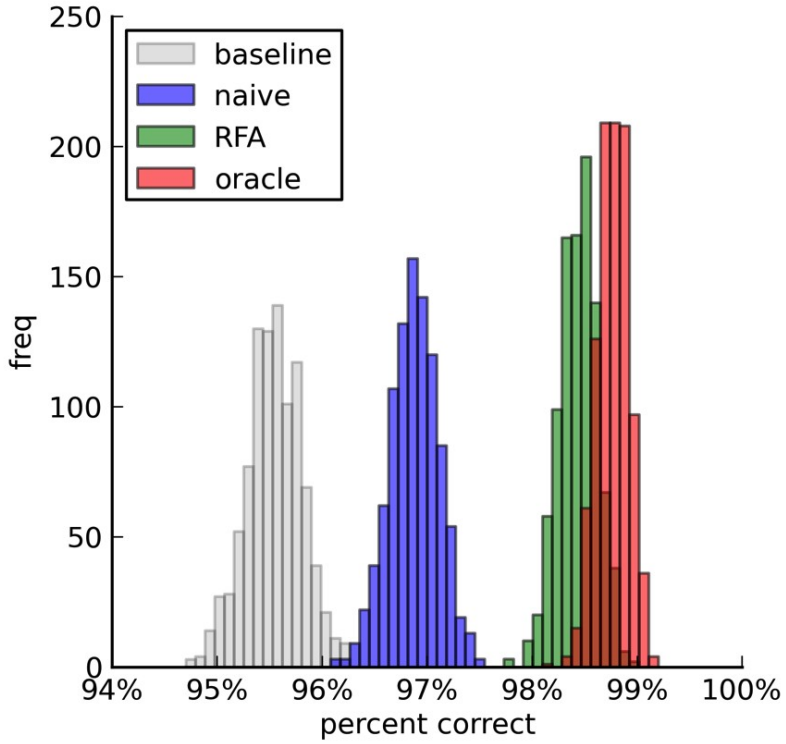
- Read alignment quality
- Mate pairs biased to map together
- Reads biased to form clouds

Optimize with Simulated Annealing

- Move a read
- Move a pair of reads
- Move a cloud of reads



Results of 1000 simulated read cloud wells



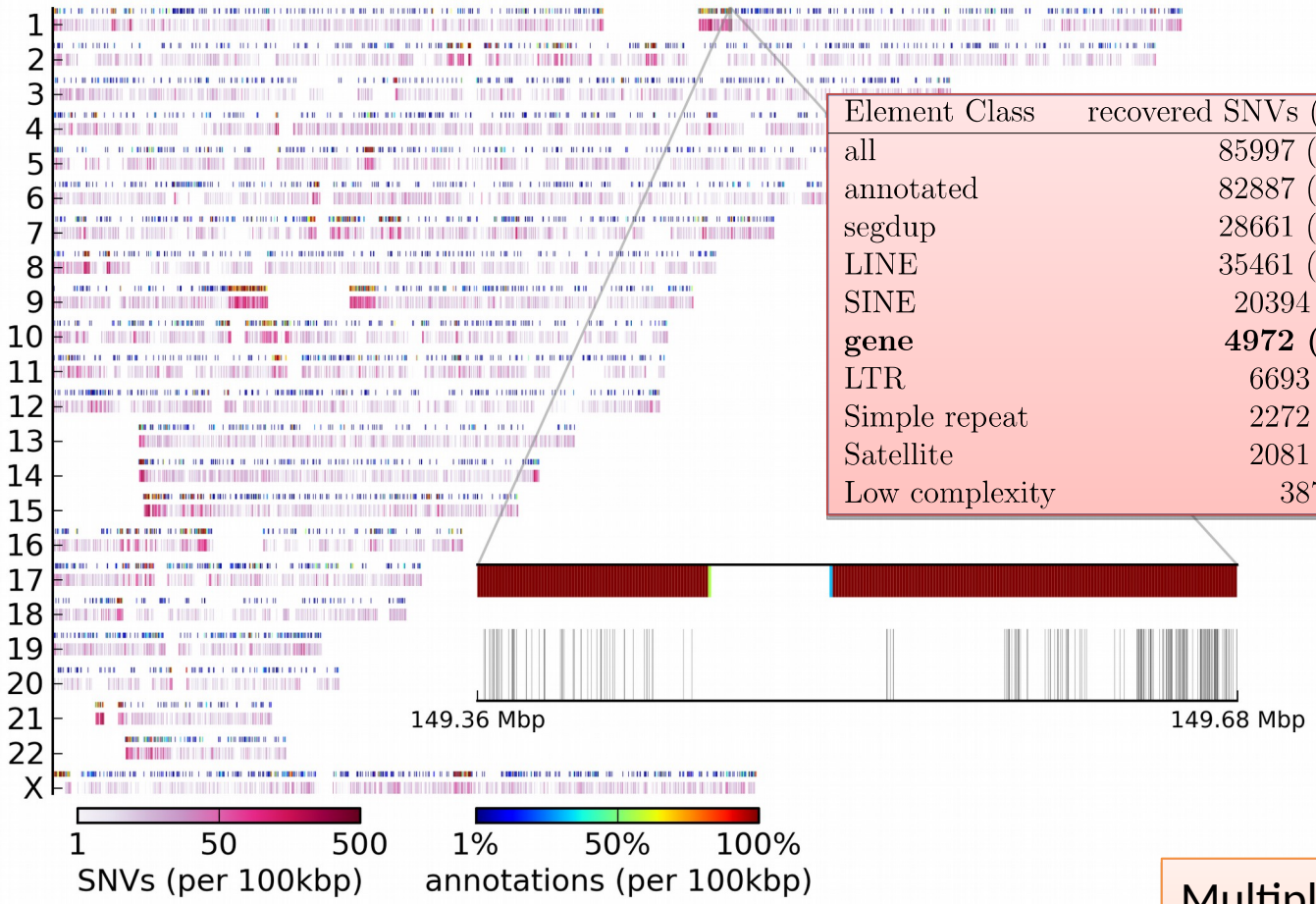
Element Class	frequency (%)	illuminated (%)
all	100.0	90.6
annotated	88.4	90.5
segdup	43.4	93.8
LINE	35.2	88.3
SINE	14.2	92.7
gene	**7.0**	**95.5**
LTR	6.3	92.3
Simple repeat	4.6	85.9
Satellite	2.3	88.1
Low complexity	1.6	89.0

Baseline: Bowtie2 baseline

Naive: Naive policy of using alignments to abbreviated reference

Oracle: Pick the true alignment among Bowtie2 alignments

Results on IDC Sample

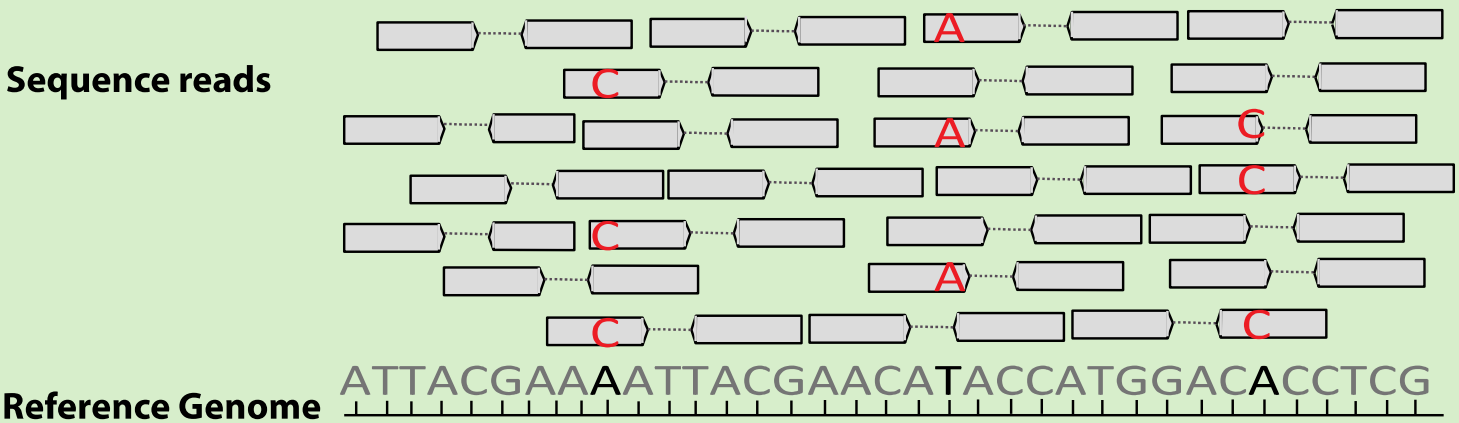


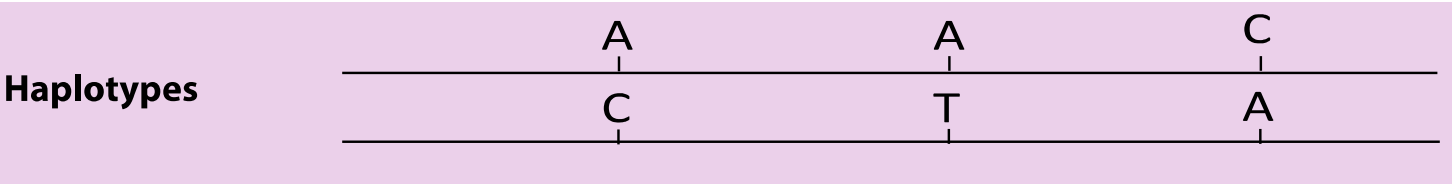
Element Class	recovered SNVs (hetero)	unique SNVs (hetero)
all	85997 (44503)	19408 (12747)
annotated	82887 (42625)	19033 (12552)
segdup	28661 (16970)	16421 (11168)
LINE	35461 (17054)	2961 (1983)
SINE	20394 (9945)	3351 (2075)
gene	**4972 (2915)**	**2592 (1869)**
LTR	6693 (3582)	2018 (1289)
Simple repeat	2272 (1271)	1376 (755)
Satellite	2081 (1270)	388 (238)
Low complexity	387 (231)	187 (123)

Multiplex PCR
346 SNVs, 94% validated

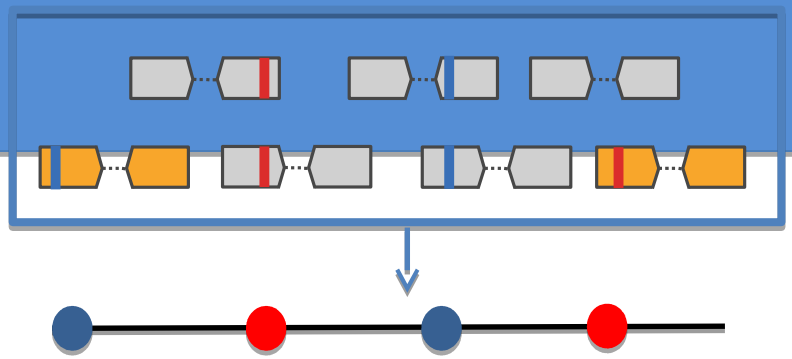
Phasing

Dorna Kashef

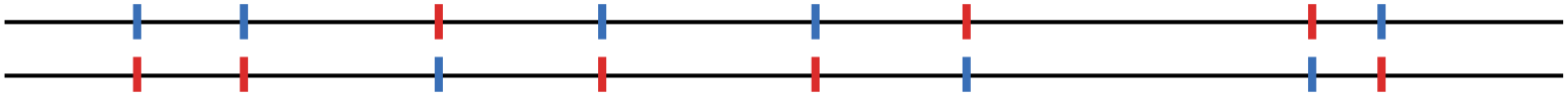




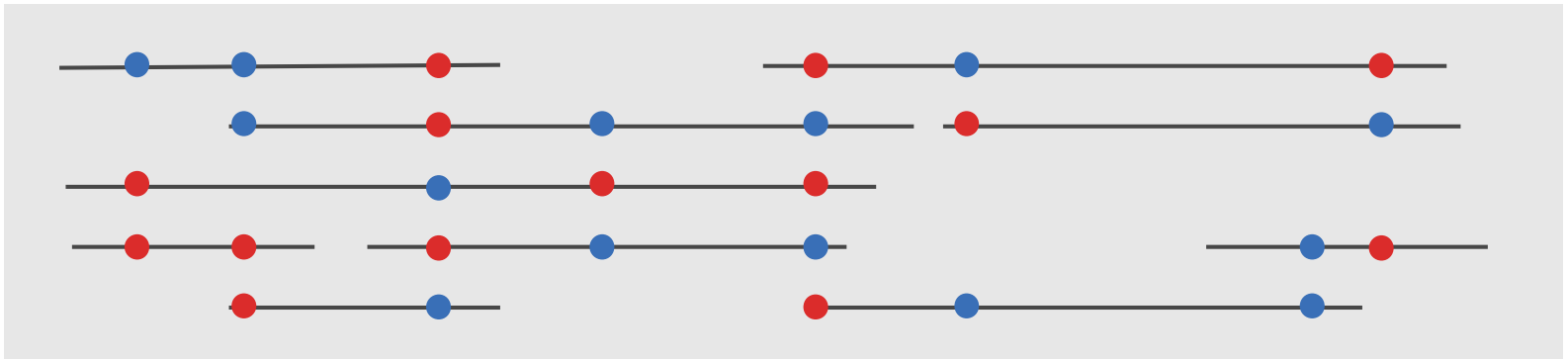
Germline		Somatic
Het	Hom	
1,948,144	1,266,460	4,689



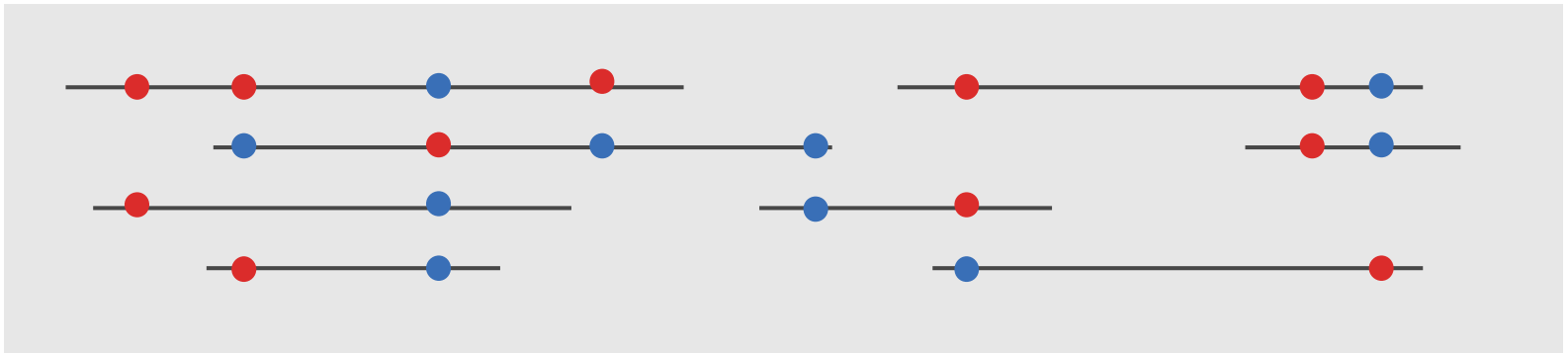
Diploid Genome



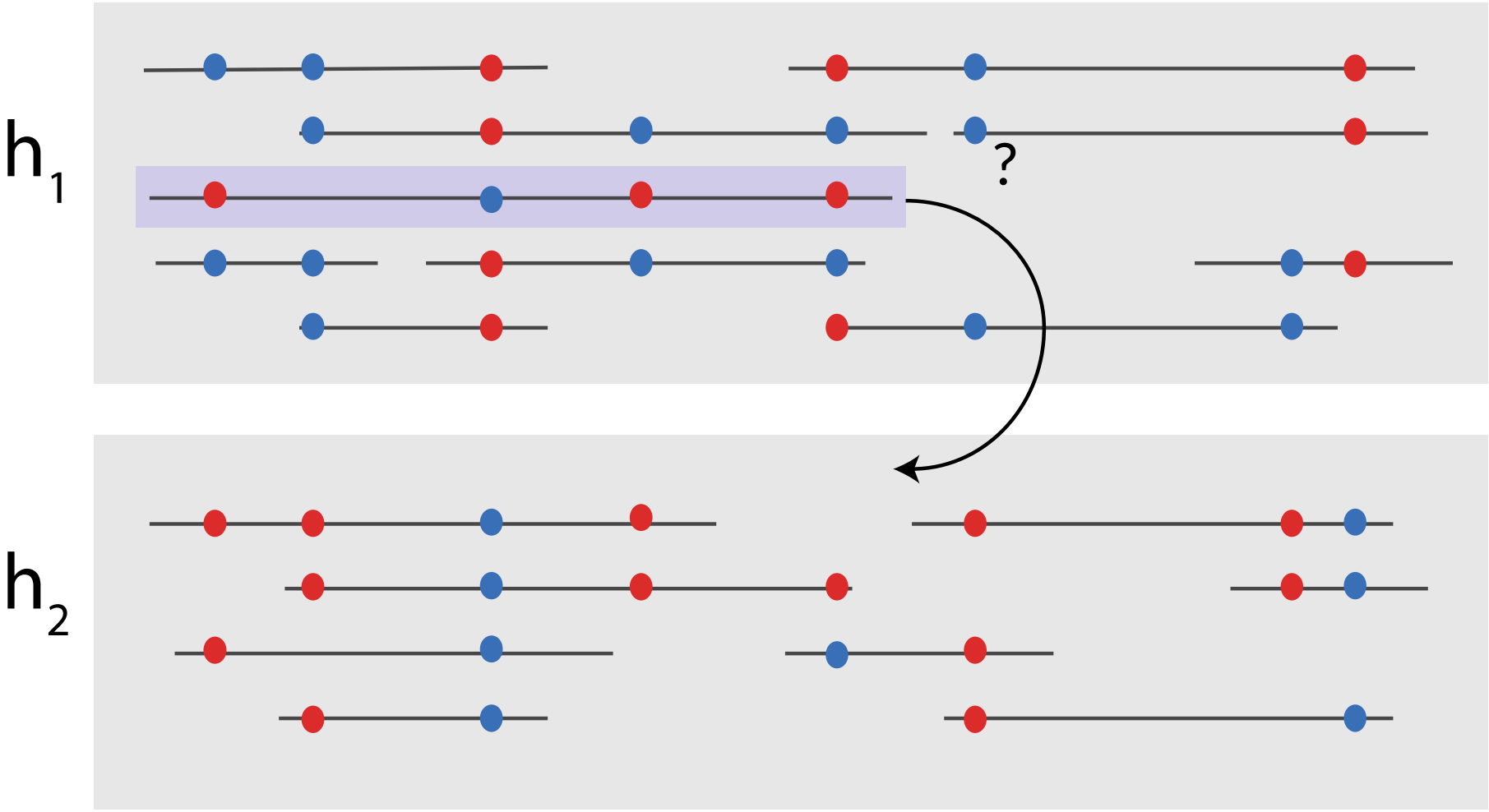
h_1



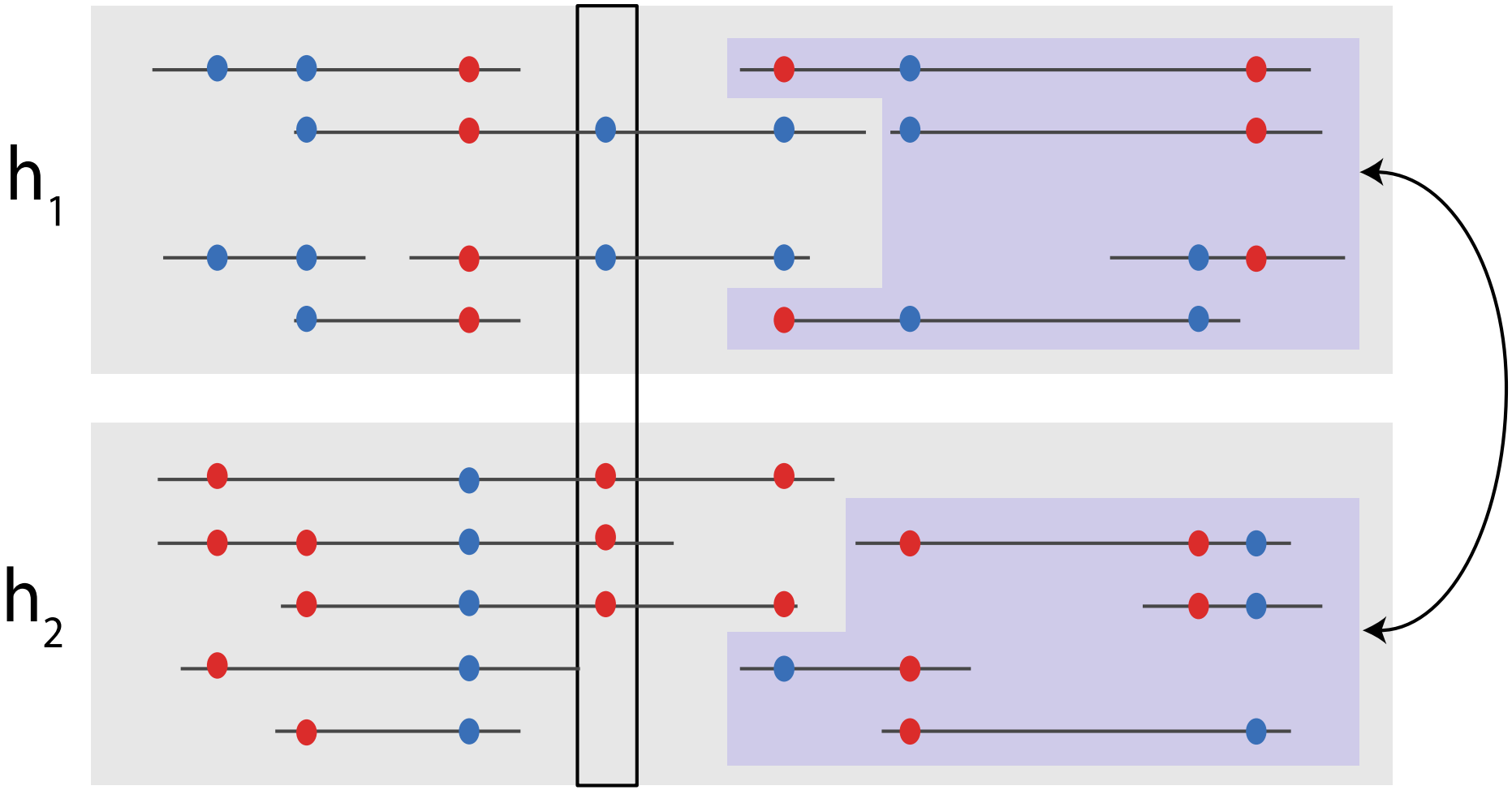
h_2



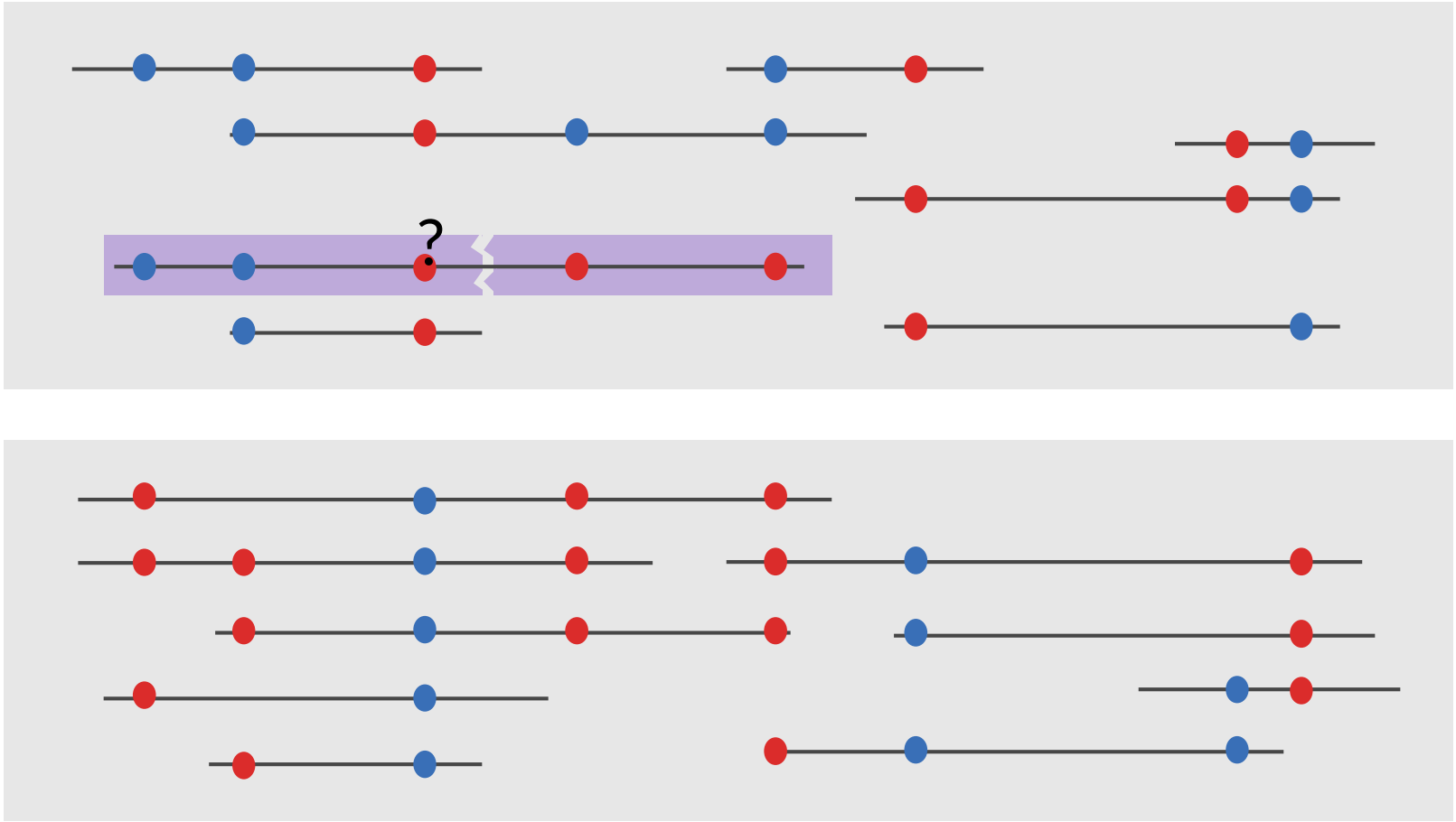
Move 1: assign a cloud to a haplotype



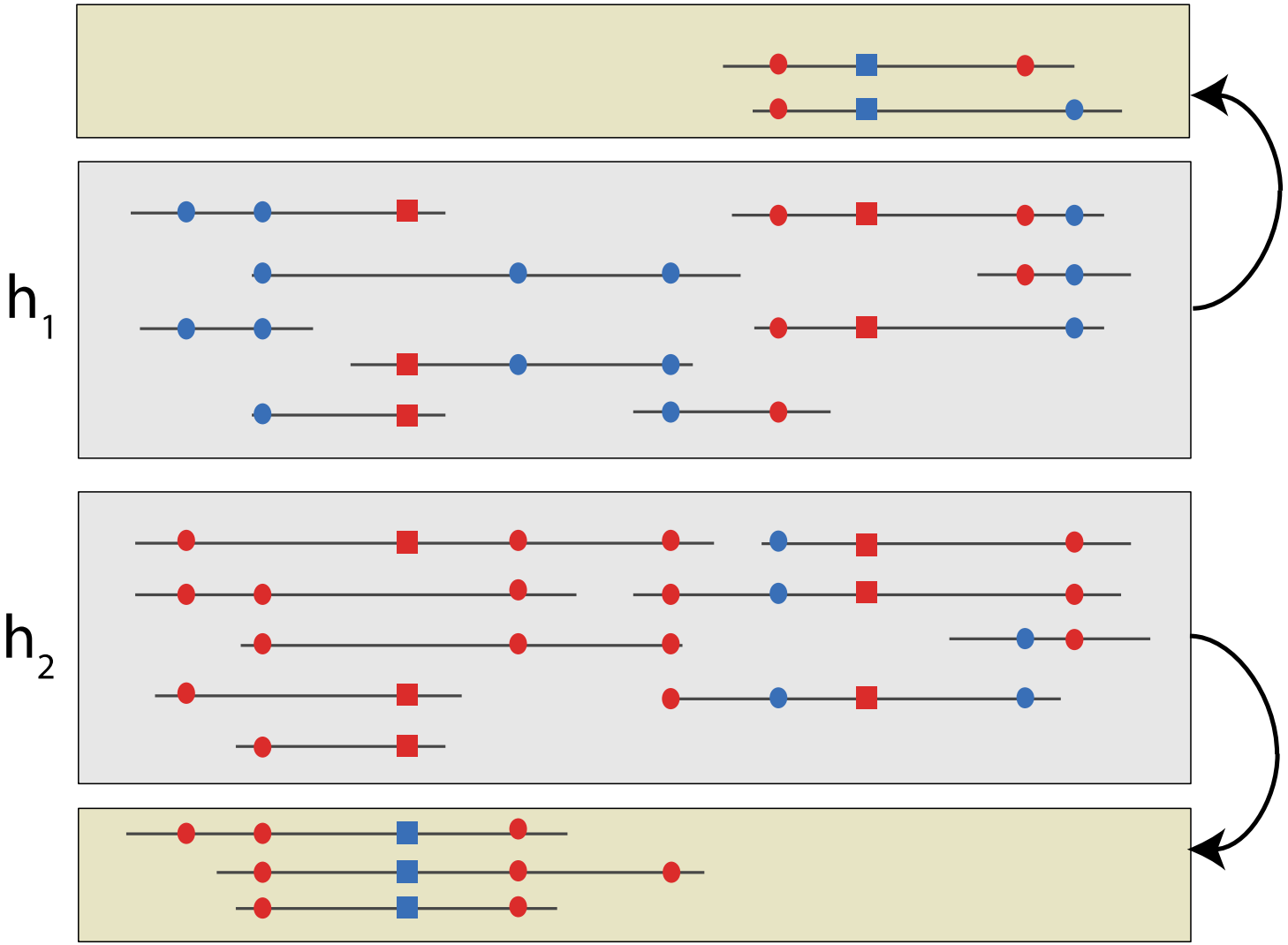
Move 2: unwind a switch error



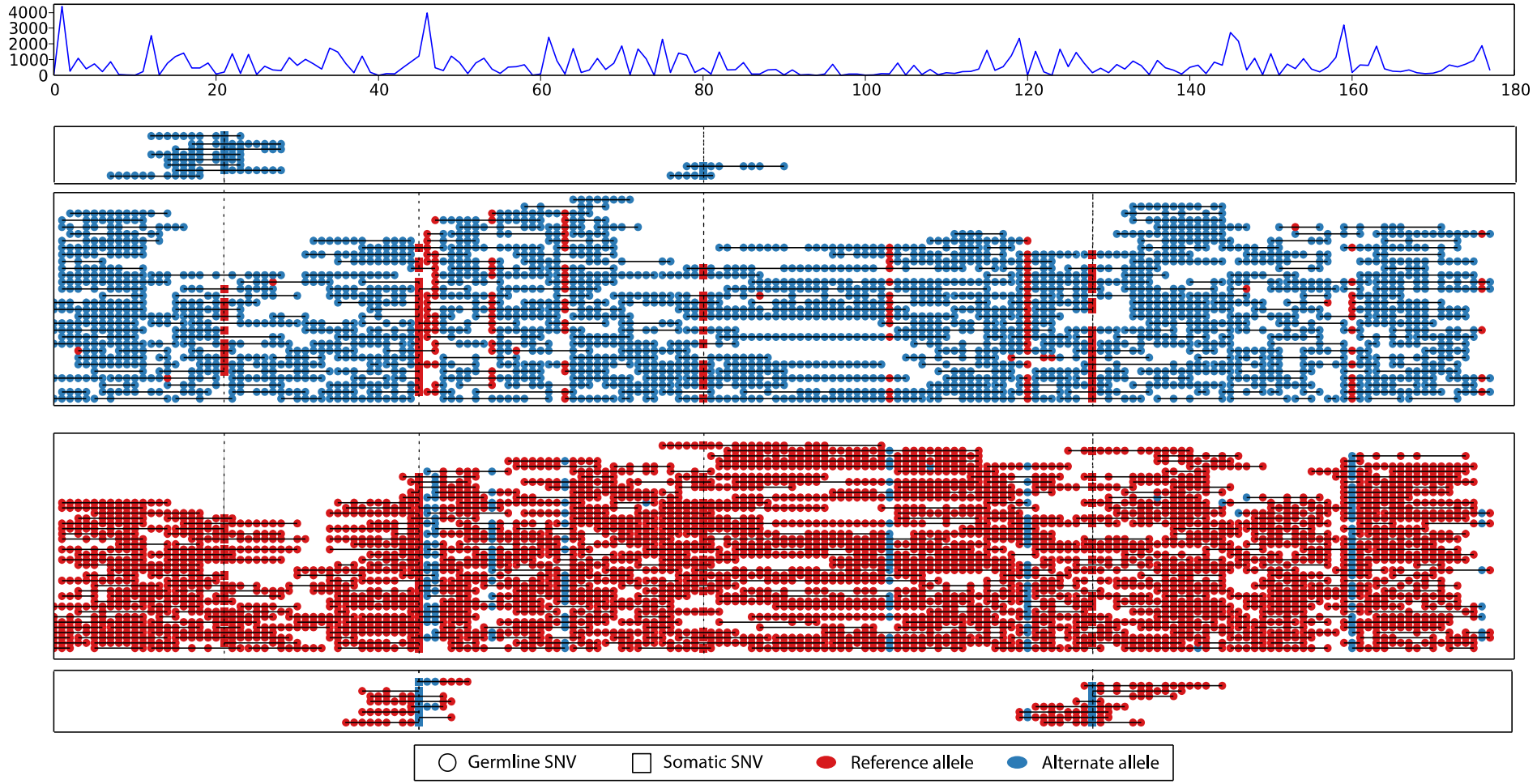
Move 3: flag/unflag clouds as mixed



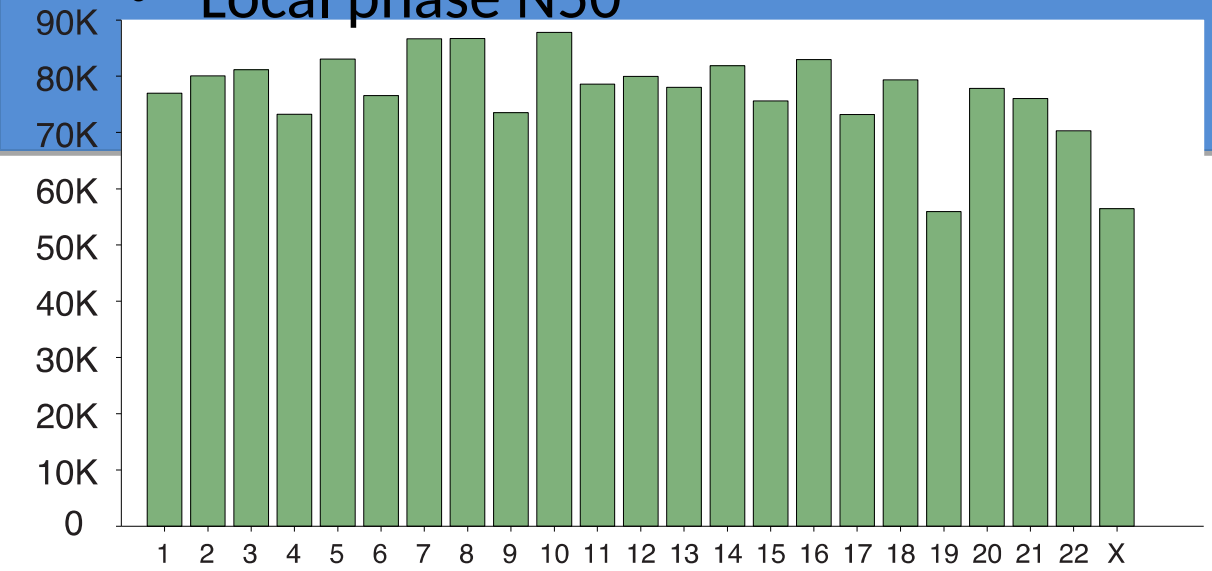
Separate somatic haplotypes



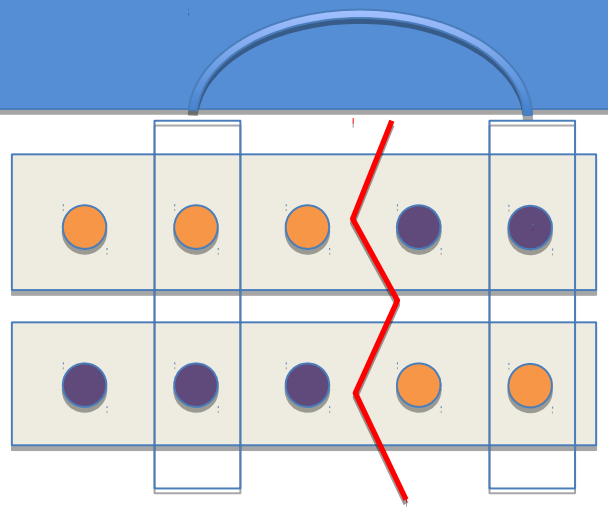
Sample Output



- Local phase N50



LD=1

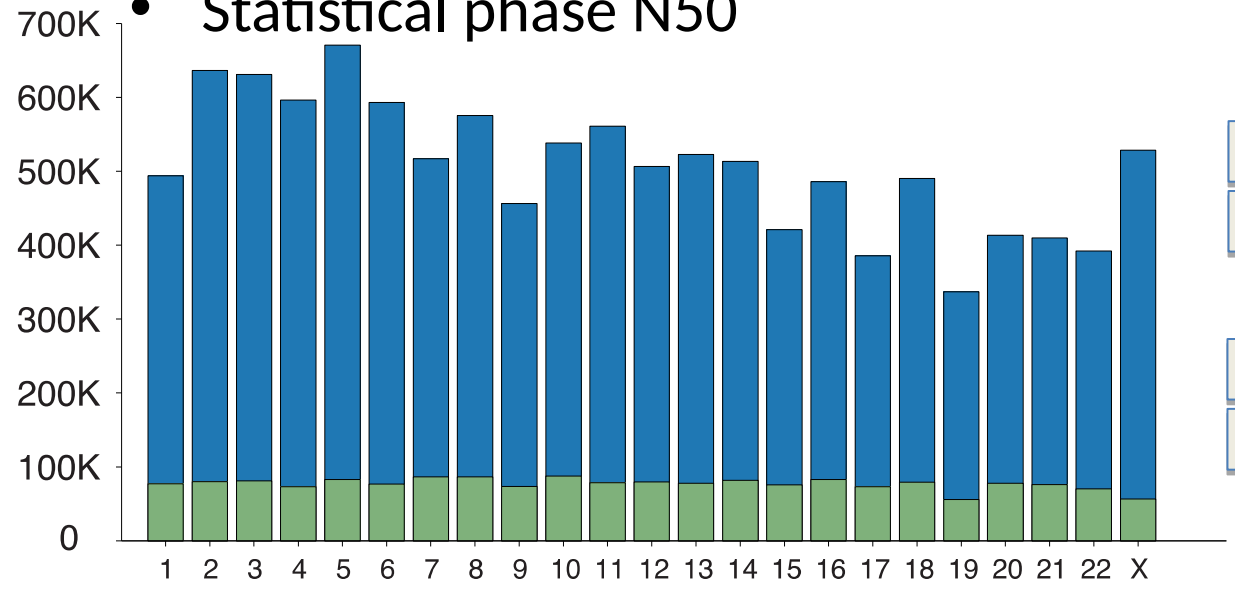


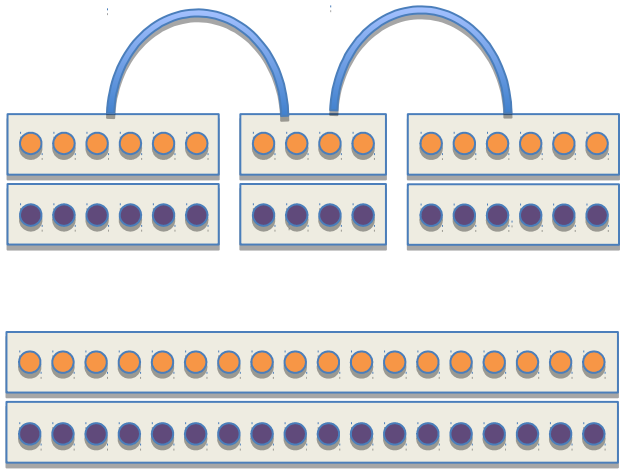
Switch error

- Switch Errors

2	-	-	-	-	1	1	-	-	-	1	2	1	-	-	-	2	1	-	-	-	-	-

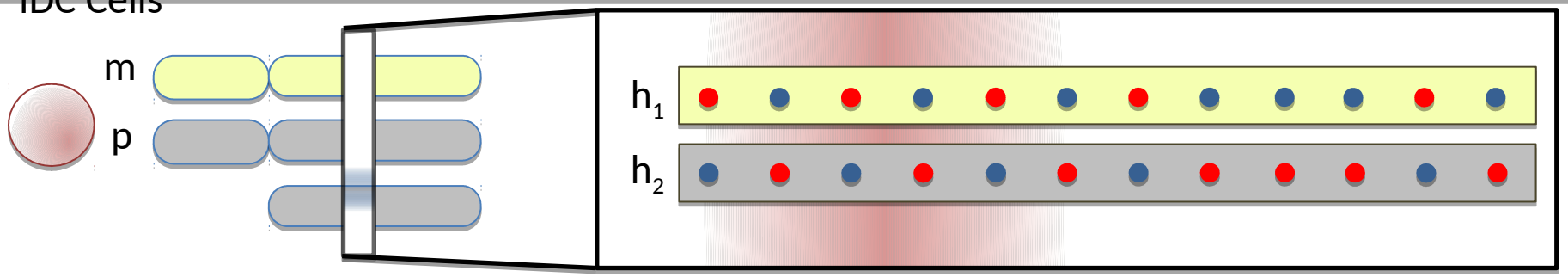
- Statistical phase N50



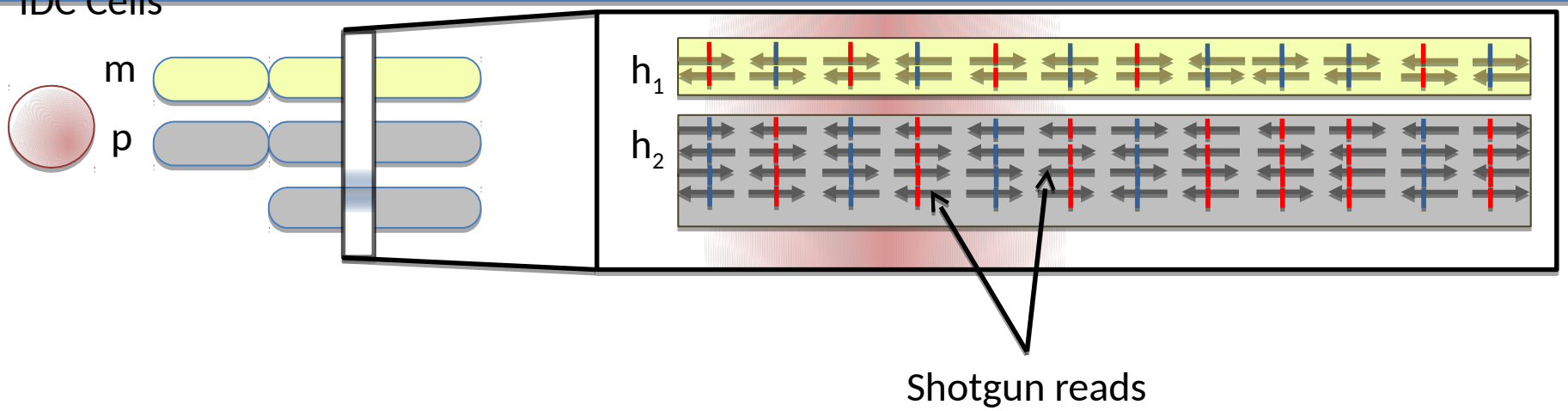


- We can use aneuploidy information to detect switch errors

IDC Cells

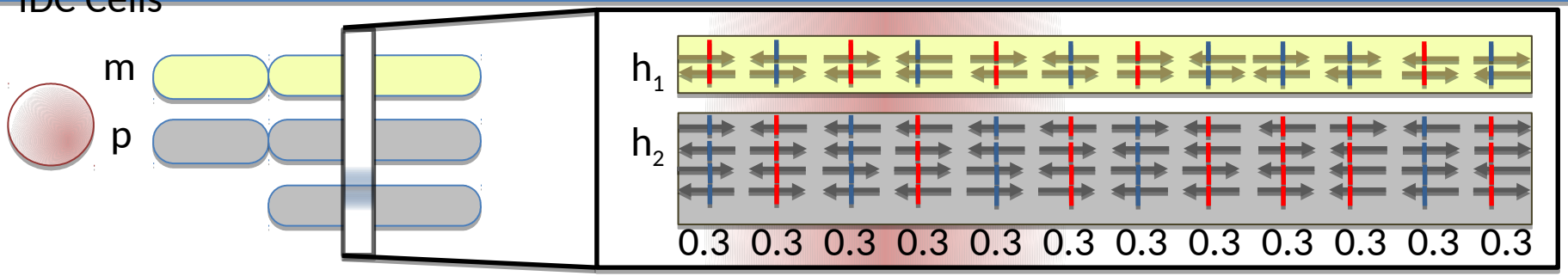


- We can use aneuploidy information to detect switch errors

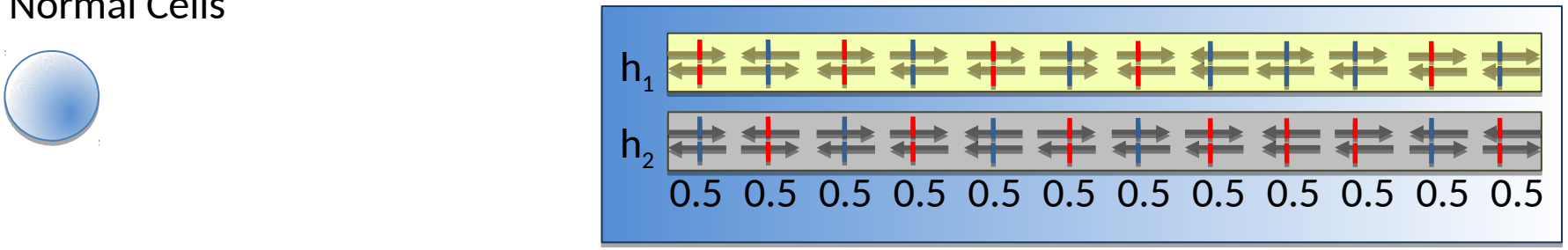


- We can use aneuploidy information to detect switch errors

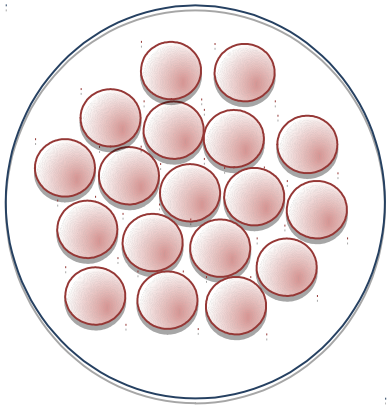
IDC Cells

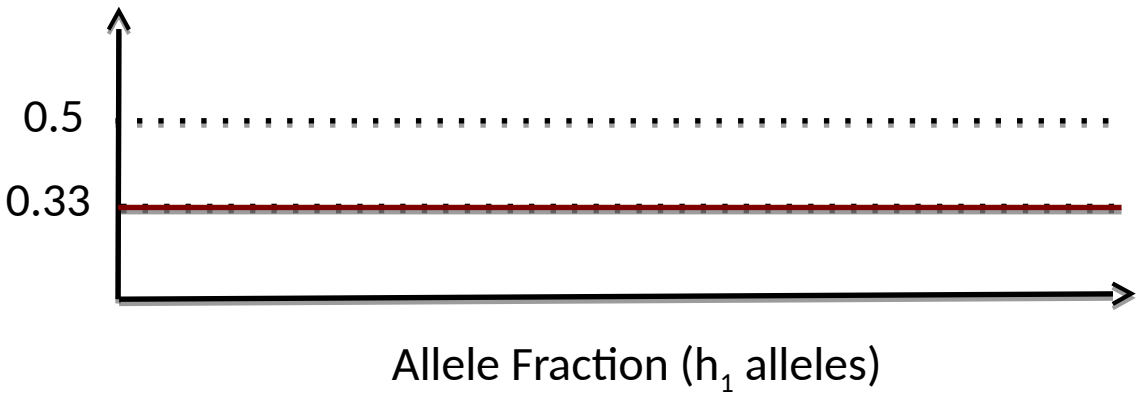


Normal Cells



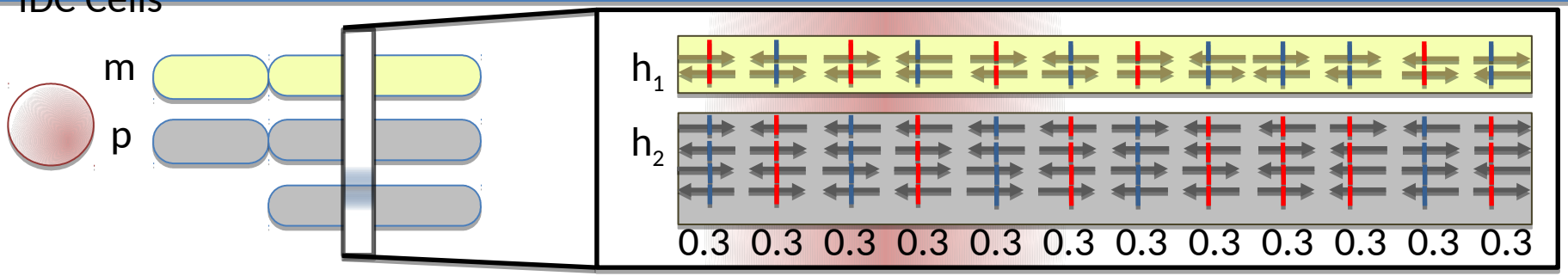
IDC Sample



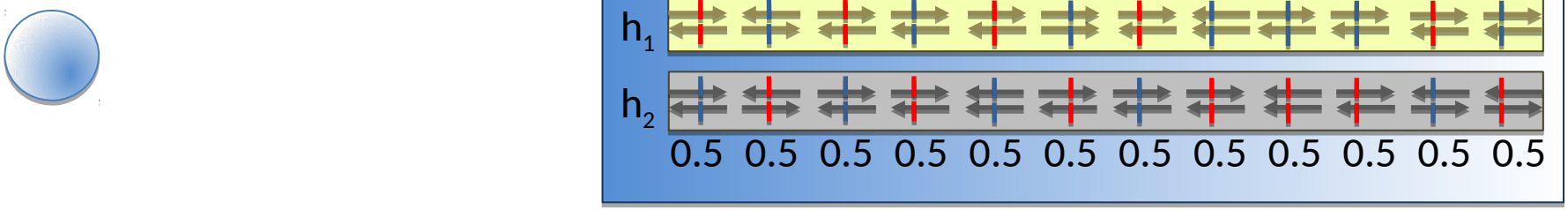


- We can use aneuploidy information to detect switch errors

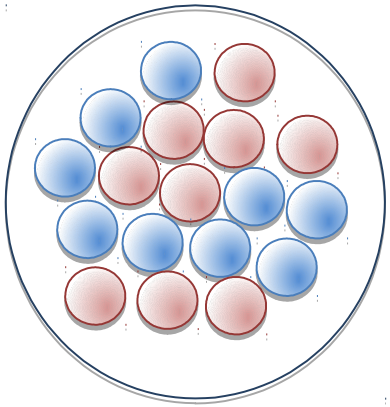
IDC Cells

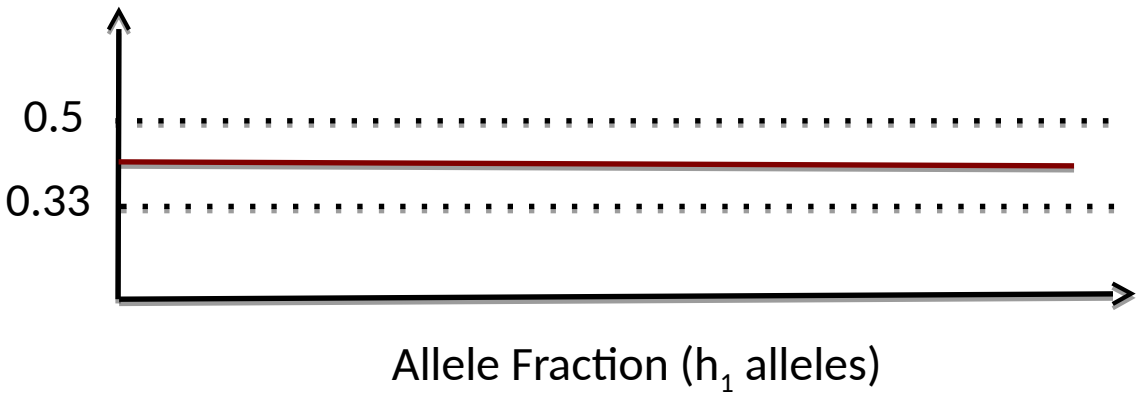


Normal Cells

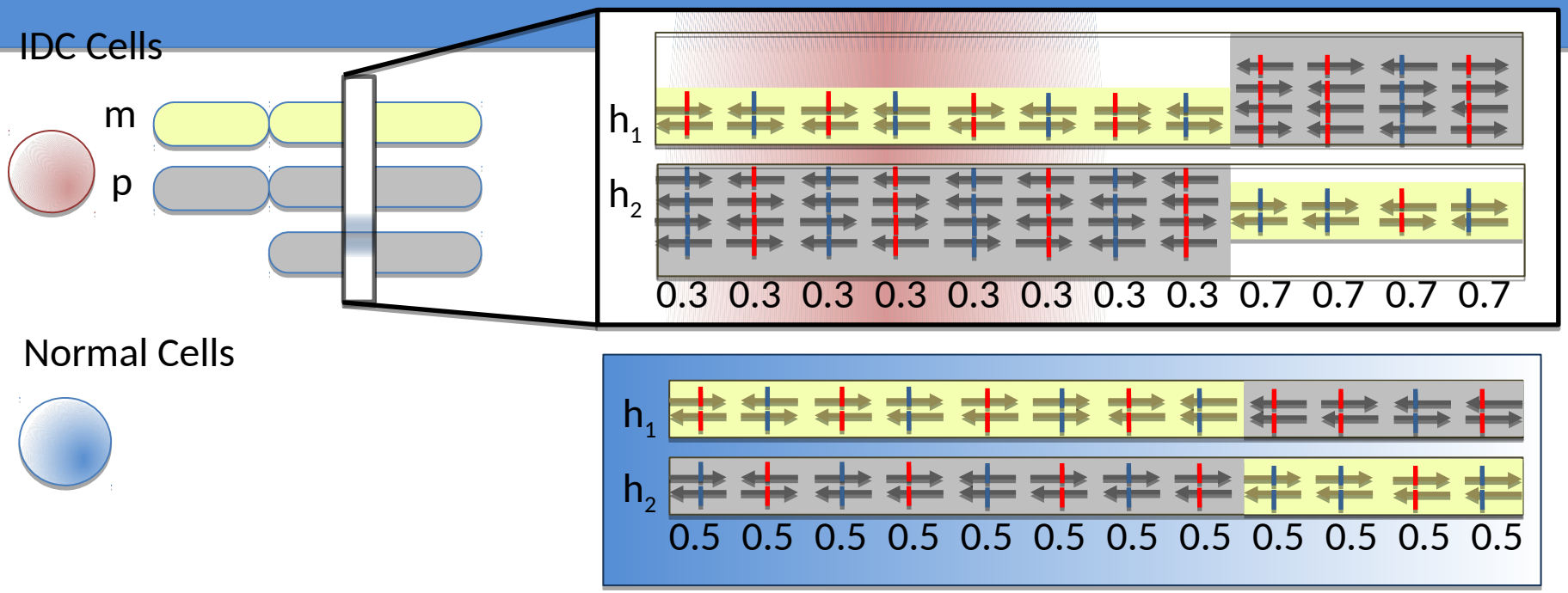


IDC Sample

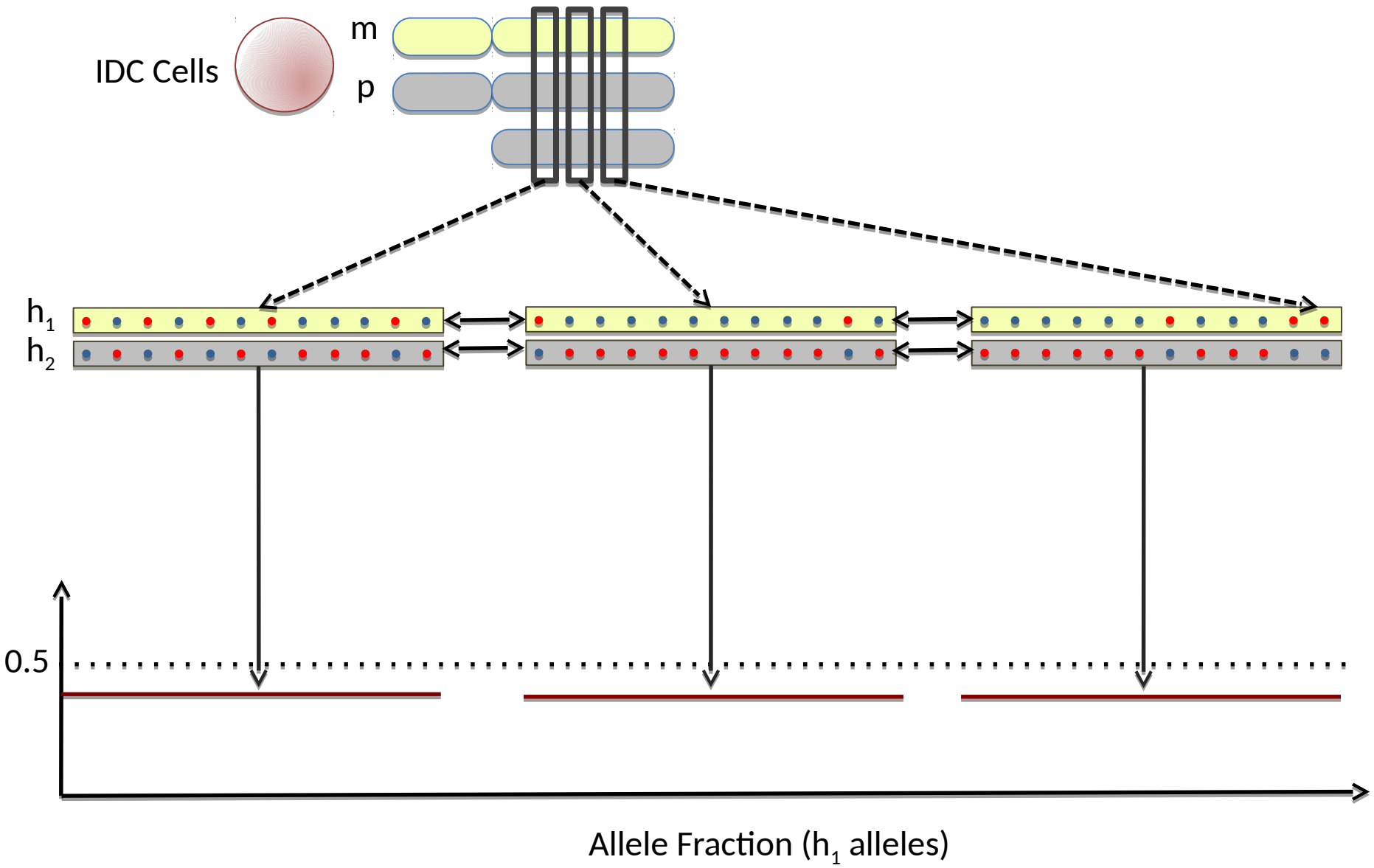


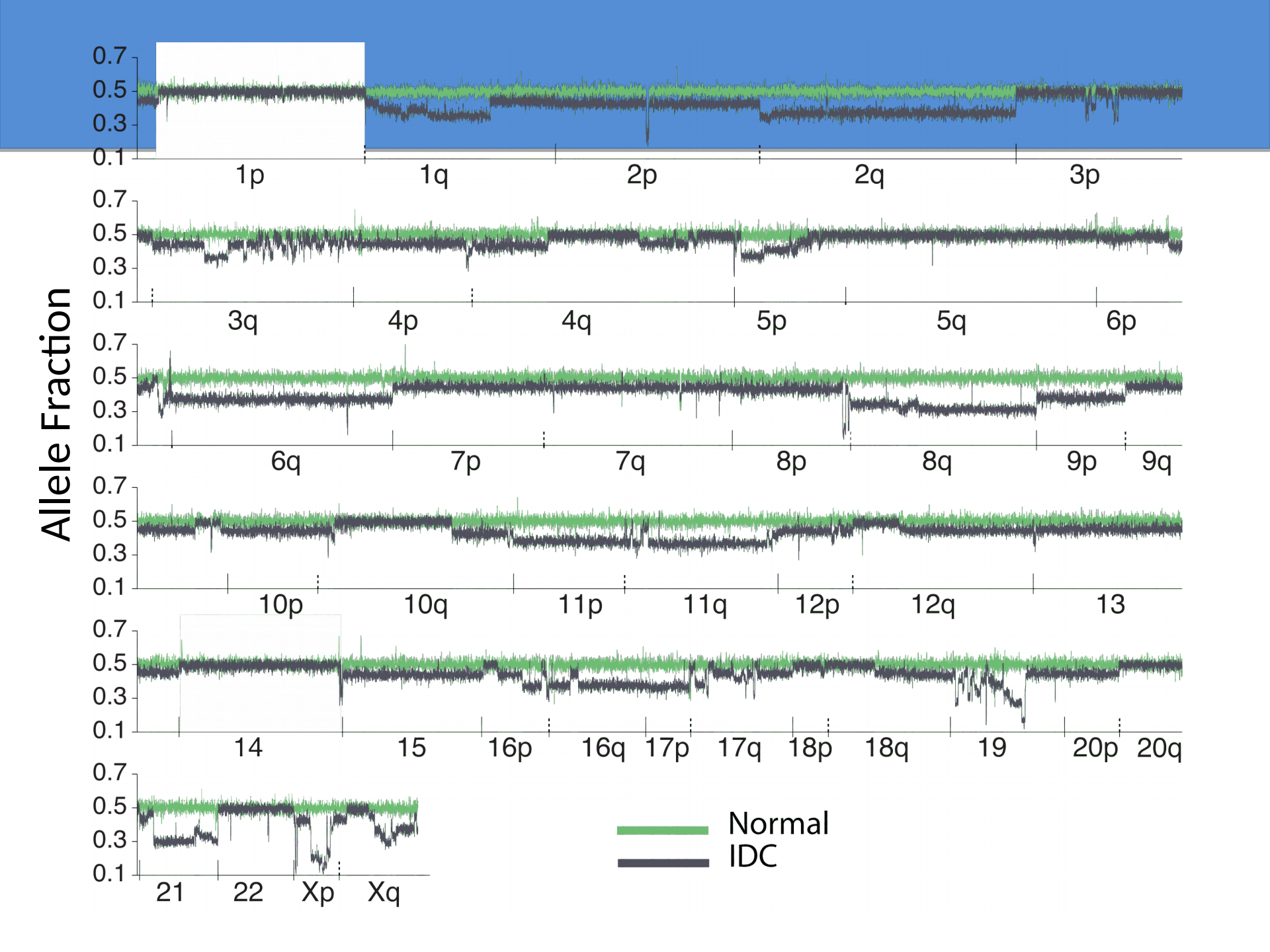


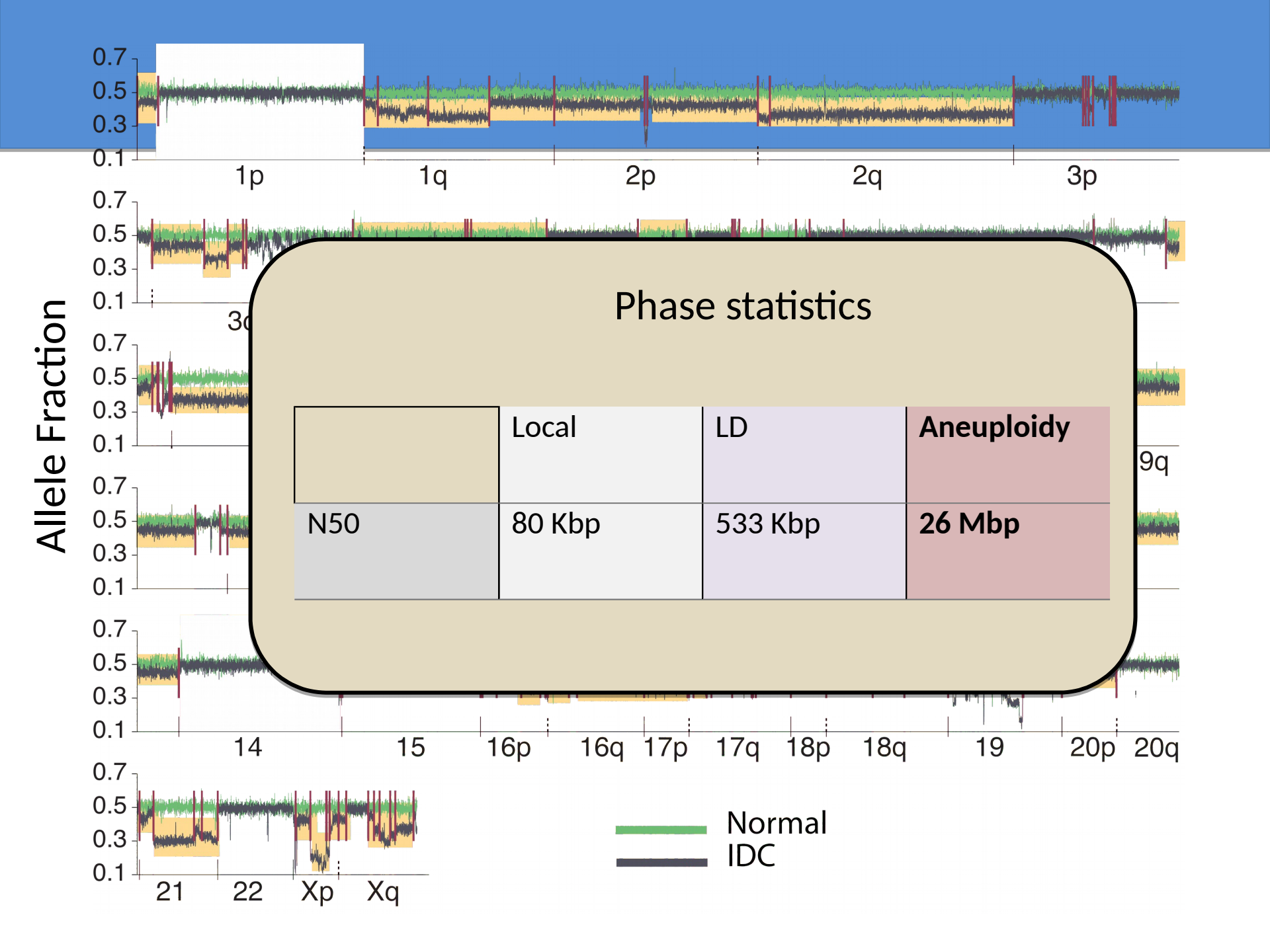
- We can use aneuploidy information to detect switch errors



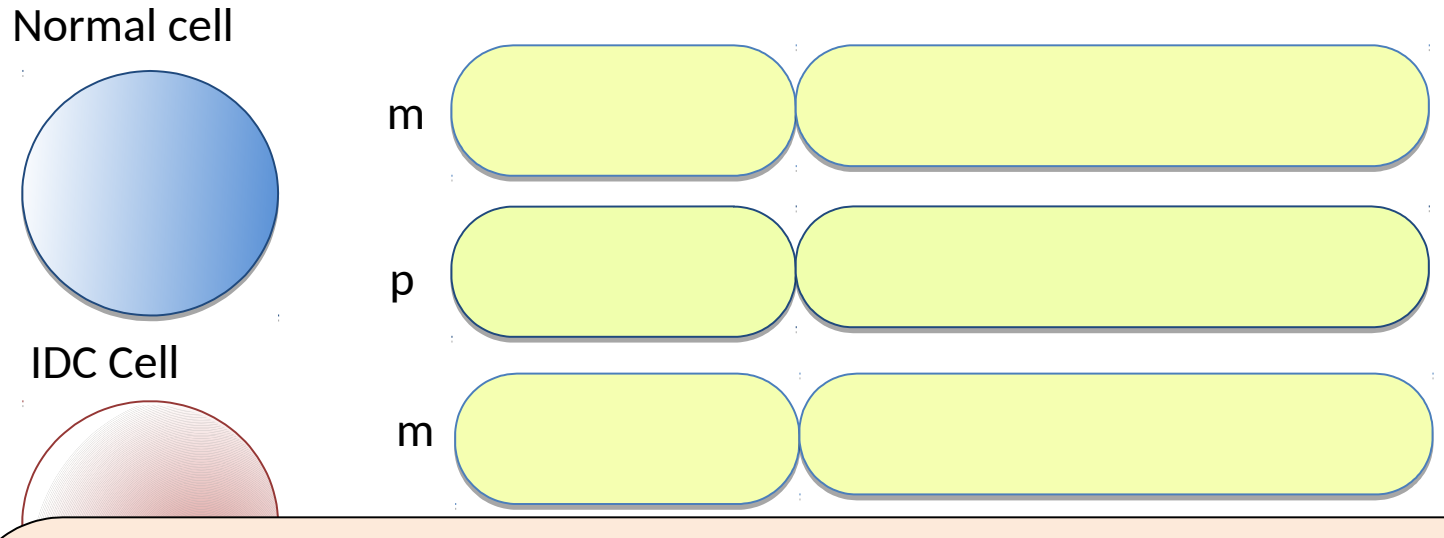
- We can connect haplotype blocks in regions of aneuploidy.



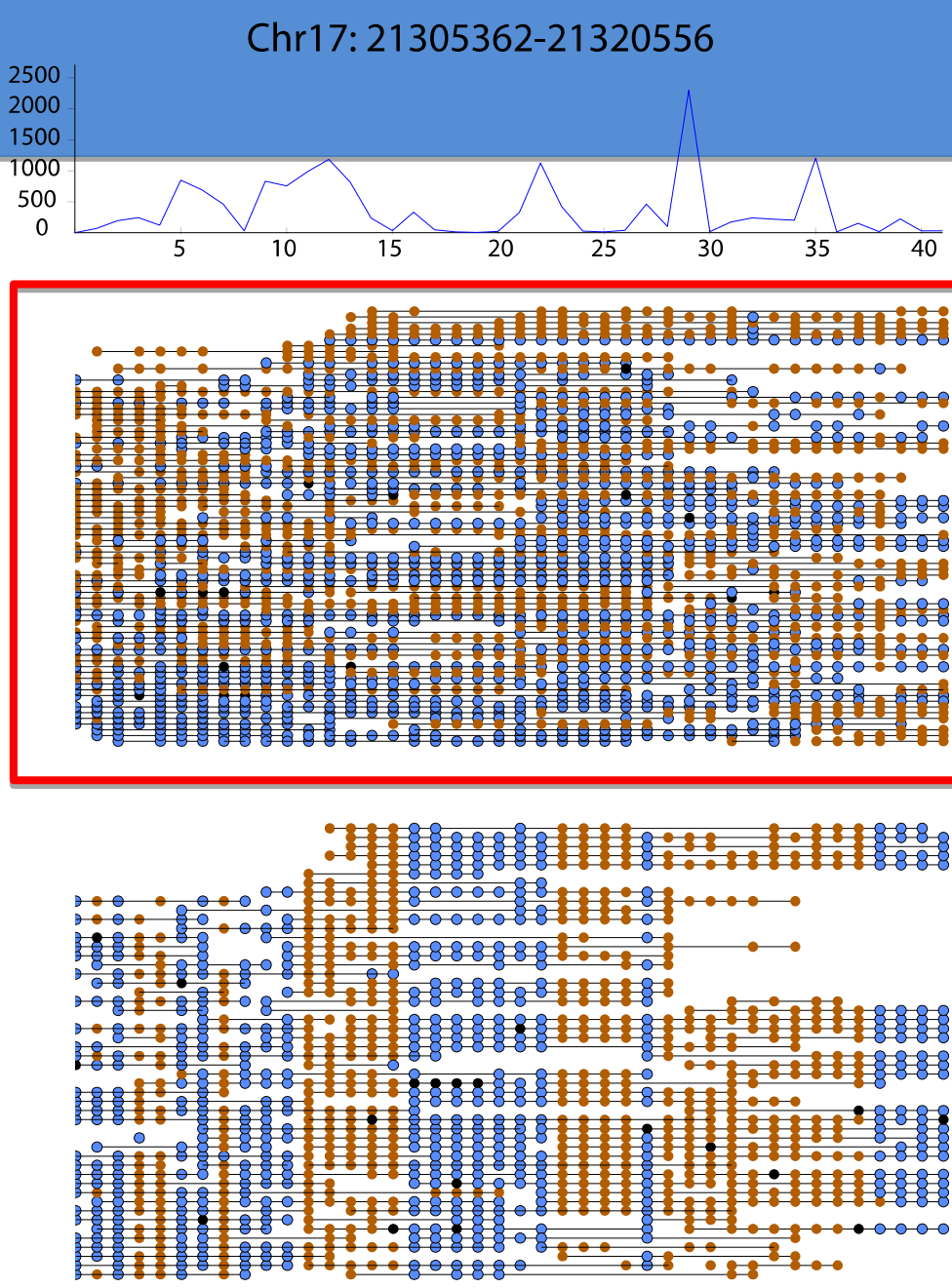

Allele Fraction
0.7
0.5
0.3
0.1
1p
1q
2p
2q
3p
3q
4p
4q
5p
5q
6p
6q
7p
7q
8p
8q
9p
9q
10p
10q
11p
11q
12p
12q
13
14
15
16p
16q
17p
17q
18p
18q
19
20p
20q
21
22
Xp
Xq
Normal
IDC

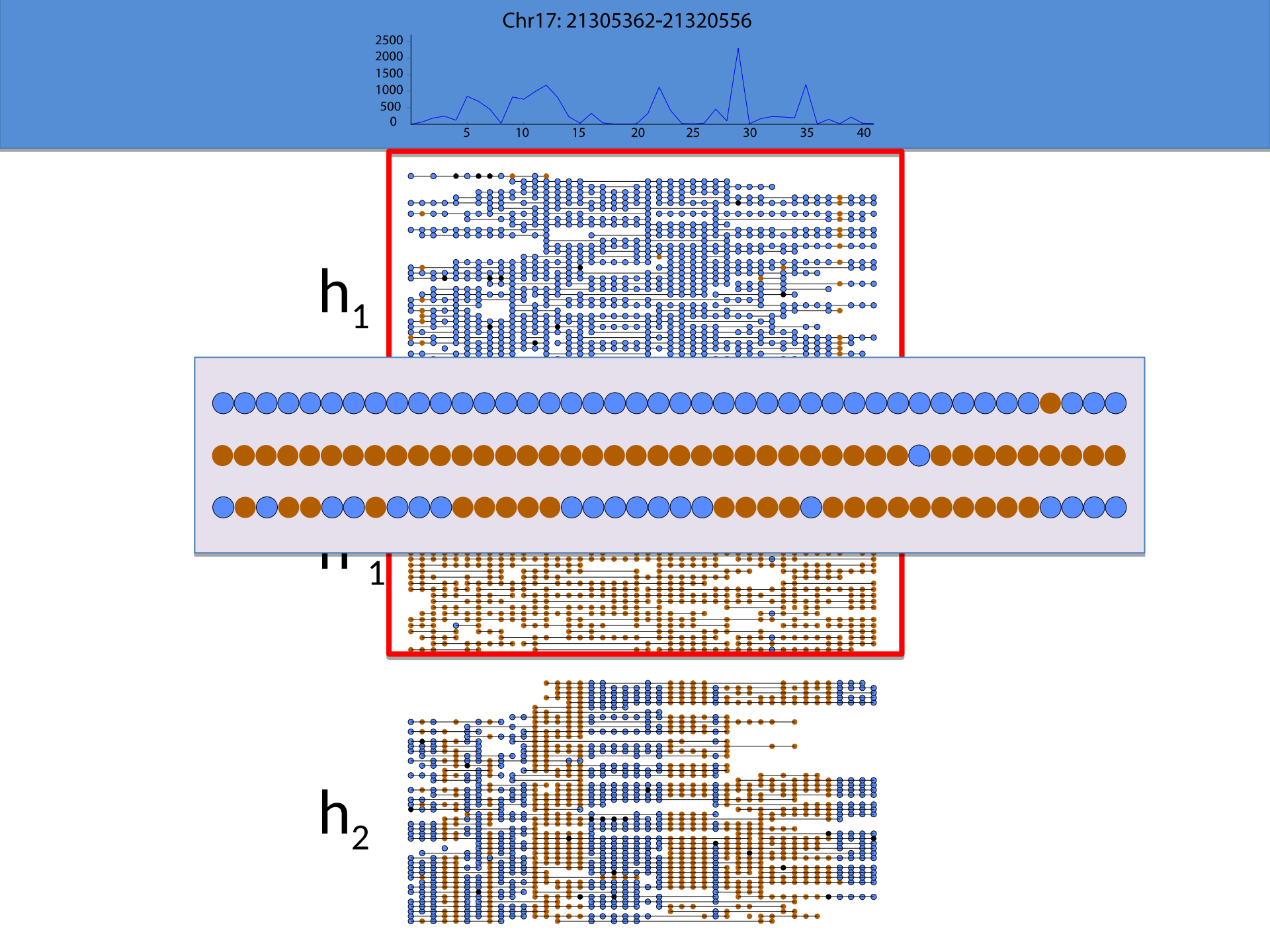

Allele Fraction
0.7
0.5
0.3
0.1
1p
1q
2p
2q
3p
9q
14
15
16p
16q
17p
17q
18p
18q
19
20p
20q
21
22
Xp
Xq
Phase statistics
Local
LD
Aneuploidy
N50
80 Kbp
533 Kbp
26 Mbp
Normal
IDC

Validation: Somatic SNVs in LOH



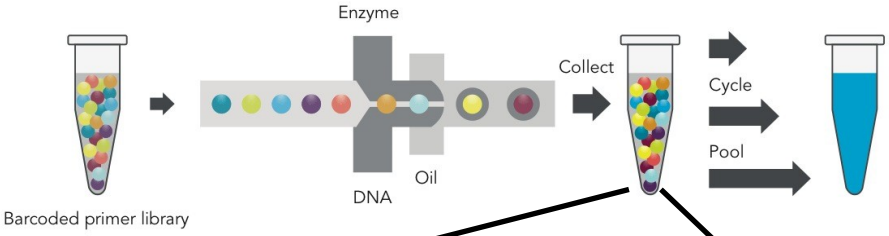
- 628 Mbp (20% of genome) exhibit LOH
- 308 somatic SNVs
  - 304 are assigned correctly

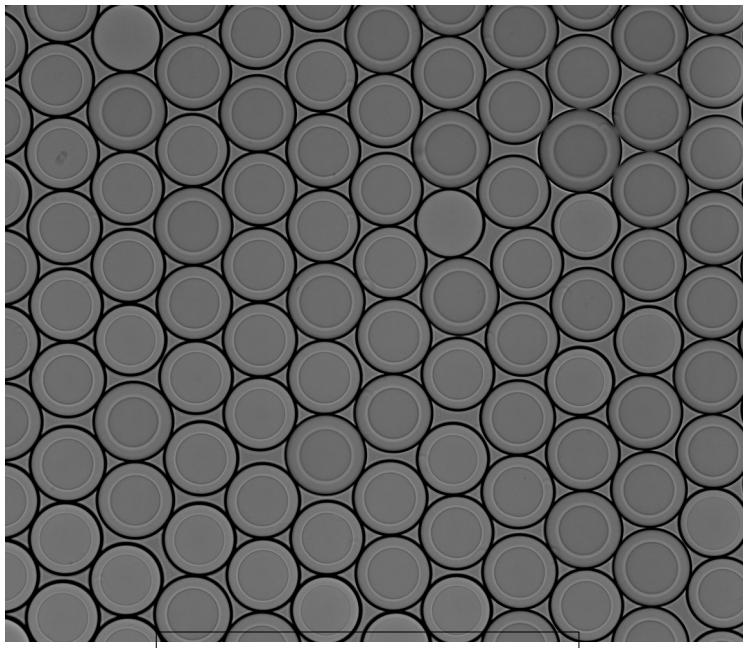

Chr17: 21305362-21320556
2500
2000
1500
1000
500
0
5
10
15
20
25
30
35
40
h1
h2


Chr17: 21305362-21320556
2500
2000
1500
1000
500
0
5
10
15
20
25
30
35
40
h1
h1
h2

10x System

Massively Parallel Partitioning

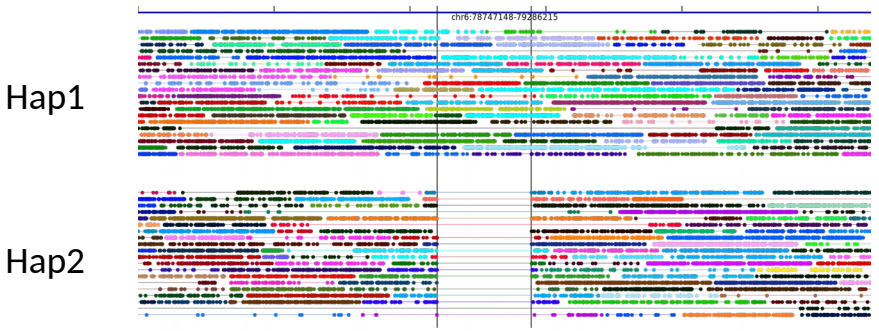




X 100,000+

10X Instrument & Reagents

Read Clouds ("linked reads")



Phased 60Kb deletion

Further Readings for the Curious

- **Fantastic Cancer Reviews**
  - Hanahan and Weinberg. 2000. The hallmarks of cancer. Cell 100: 57-70.
  - Hanahan and Weinberg. 2011. Hallmarks of cancer: the next generation. Cell 144, 646–74.
- **Reviews of Cancer Genomics**
  - Meyerson, Matthew, Stacey Gabriel, and Gad Getz. 2010. Advances in understanding cancer genomes through second-generation sequencing. Nature Reviews Genetics 11, no. 10 (October): 685-696. doi:10.1038/nrg2841. http://www.nature.com/doifinder/10.1038/nrg2841.
  - Yates, L. R. & Campbell, P. J. Evolution of the cancer genome. Nat. Rev. Genet. 13, 795–806 (2012).
- **Variant Calling**
  - Dalca, Adrian V, and Michael Brudno. 2010. Genome variation discovery with high-throughput sequencing data. Briefings in bioinformatics 11, no. 1 (January): http://www.ncbi.nlm.nih.gov/pubmed/20053733.
  - Medvedev, Paul, Monica Stanciu, and Michael Brudno. 2009. Computational methods for discovering structural variation with next-generation sequencing. nature methods 6, no. 11 http://www.nature.com/nmeth/journal/v6/n11s/full/nmeth.1374.html.