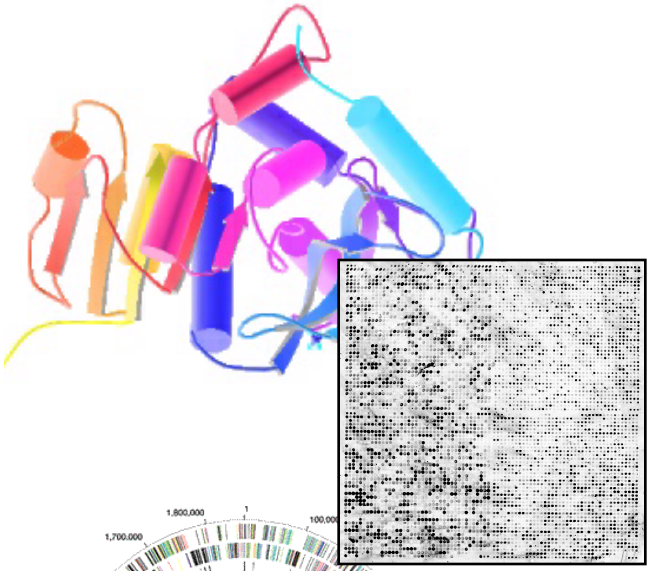
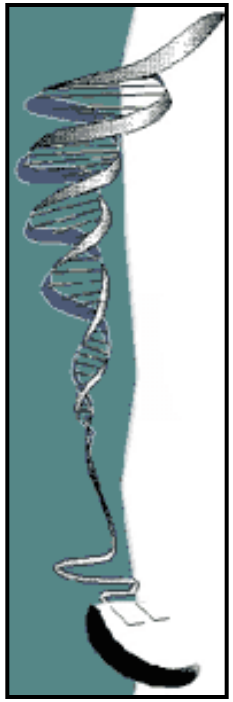
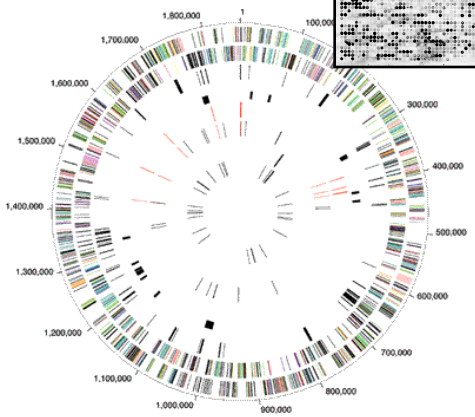
Biomed. Data Sci:

Variant Identification, Focusing on SVs







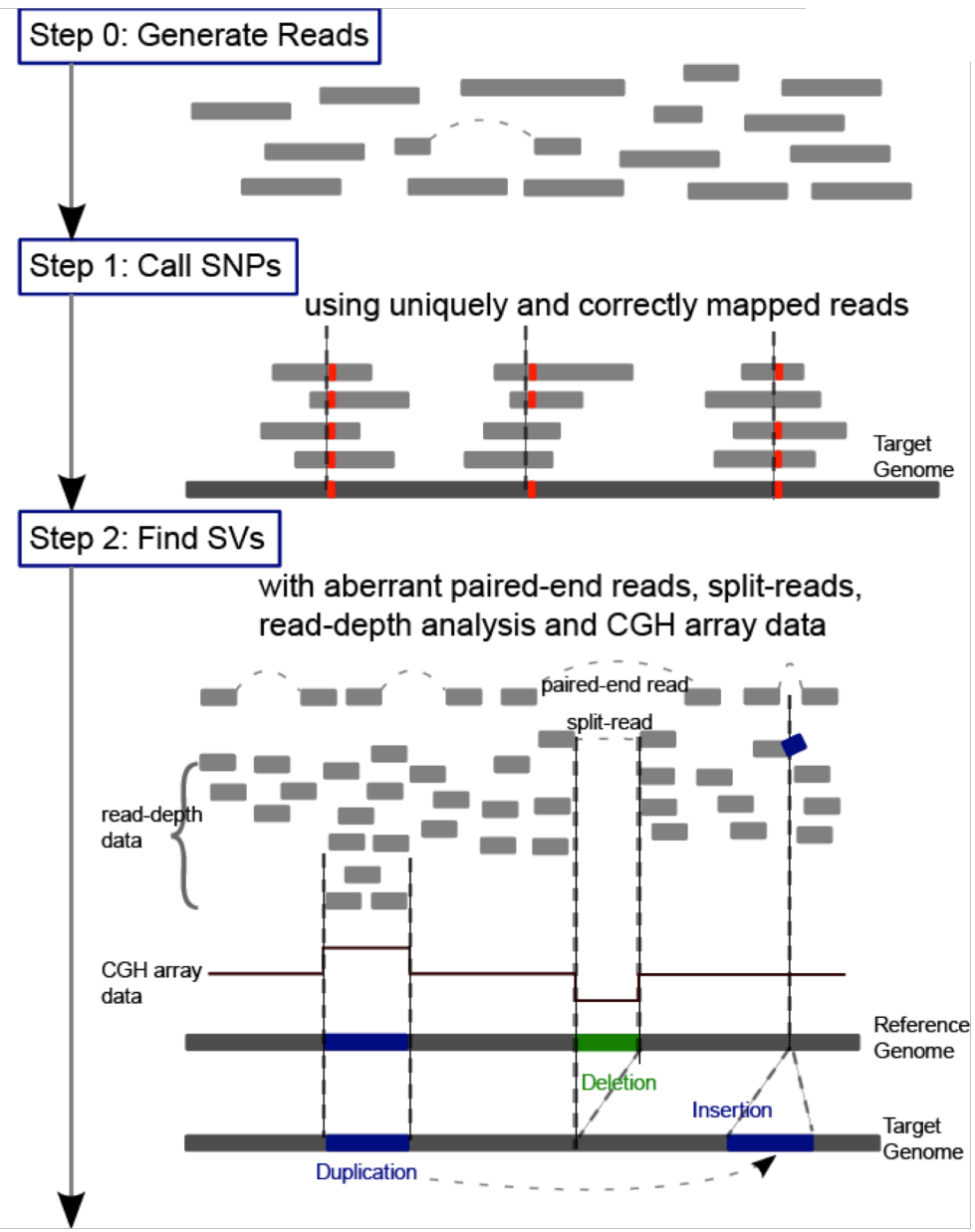
Mark Gerstein, Yale University

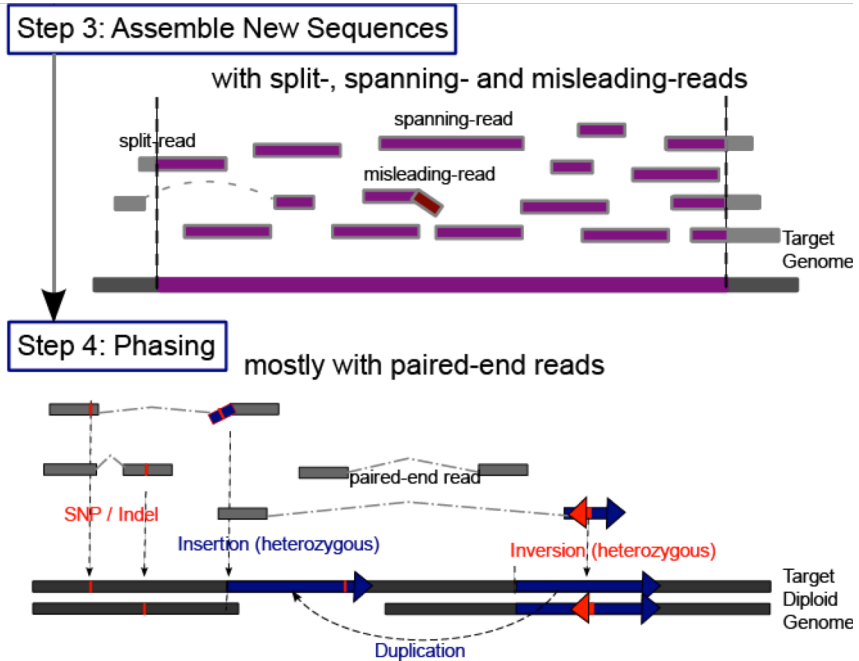
gersteinlab.org/courses/452

(last edit in spring ’19, pack #6)

Main Steps in Genome Resequencing

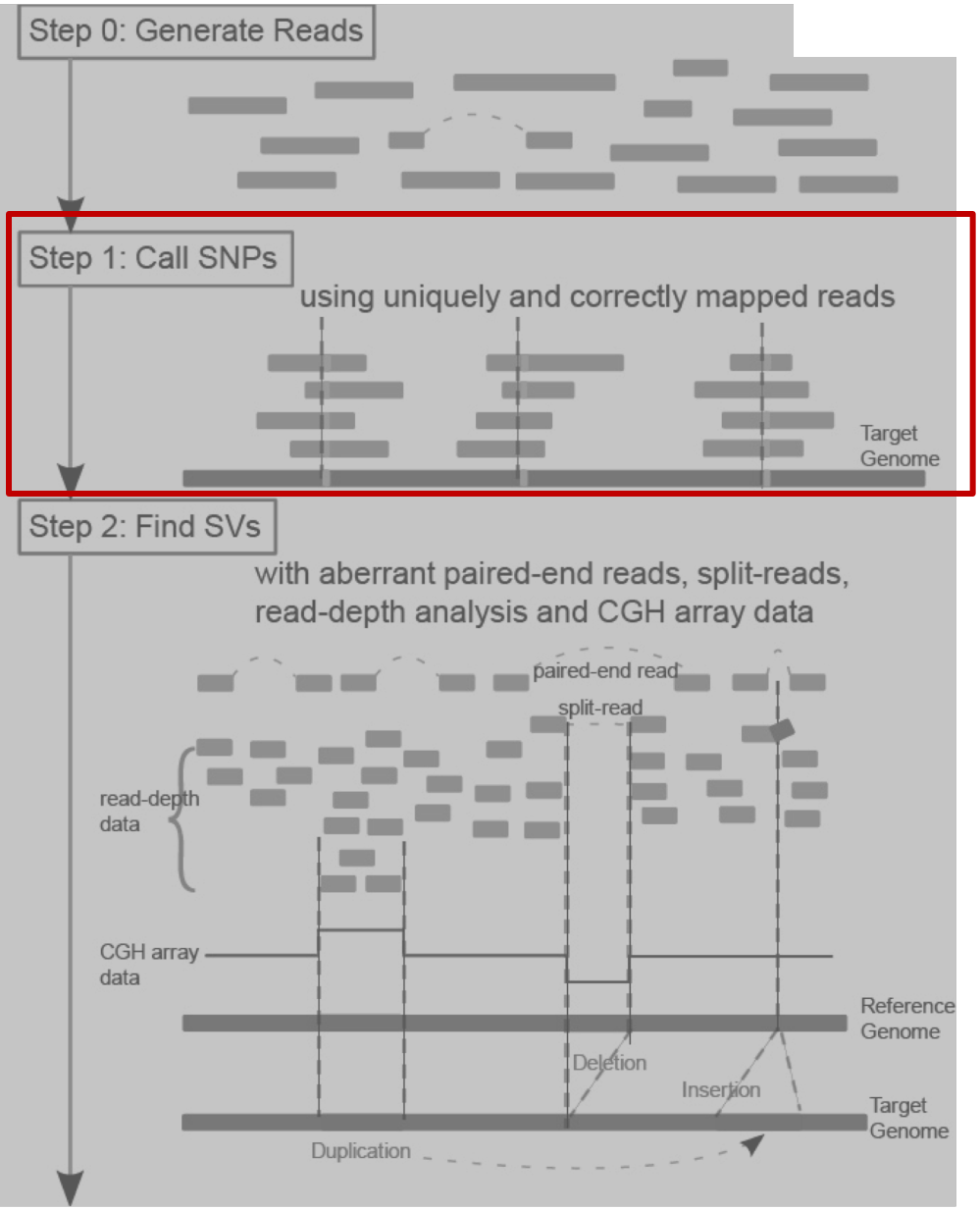
[Snyder et al. Genes & Dev. ('10)]

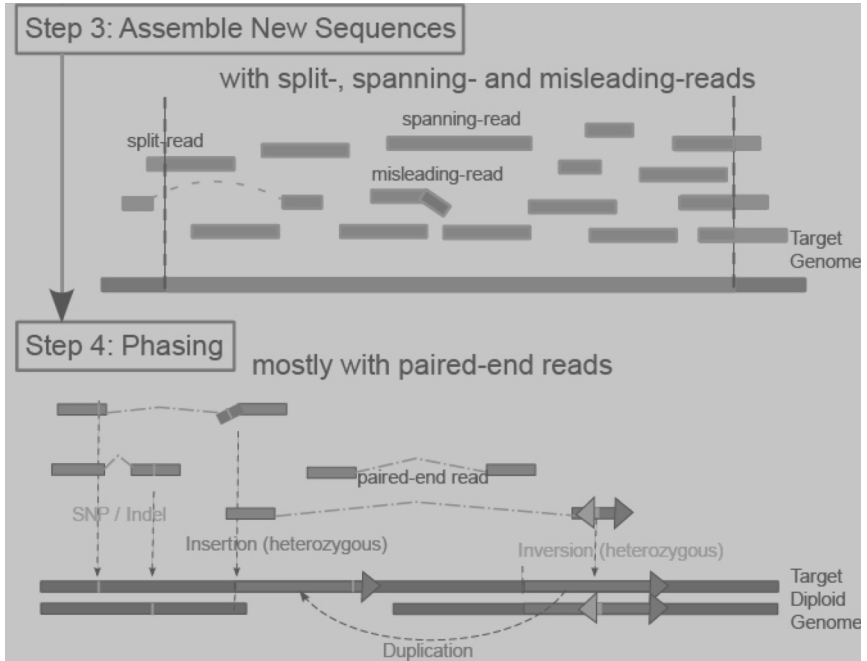




Main Steps in Genome Resequencing

[Snyder et al. Genes & Dev. ('10)]





Bayes' Theorem to detect genomic variant

$$P(G|D) = \frac{P(D|G)P(G)}{P(D)}$$

$$= \frac{P(D|G)\,P(G)}{\sum_{i=1}^{n} P(D|G_i)\,P(G_i)}$$

In the above equation:

- D refers to the observed data
- G is the genotype whose probability is being calculated
- G_i refers to the ith possible genotype, out of n possibilities

[You et al. ('12) Bioinformatics 28: 643 doi: 10.1093/bioinformatics/bts001]

Calculating the conditional distribution $P(D|G)$:

Assuming an error free model, for each heterozygous SNP site of the diploid genome, covered by K reads, the number of reads i representing one of the two alleles follows binomial distribution.

$$\boldsymbol{P_{err\_free}(D|G) = f(i|k, 0.5) = \binom{k}{i} 0.5^k}$$

With errors, the calculation is more complicated.

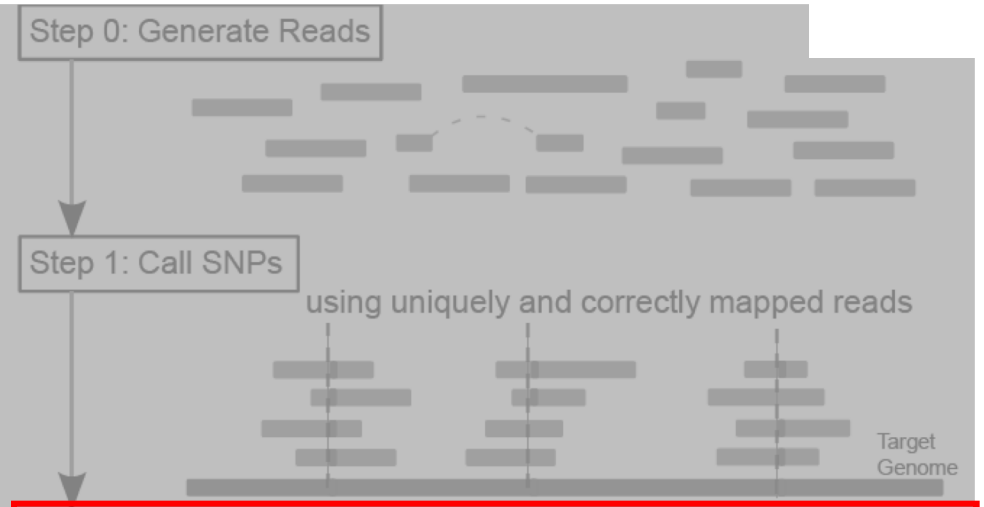
In general:

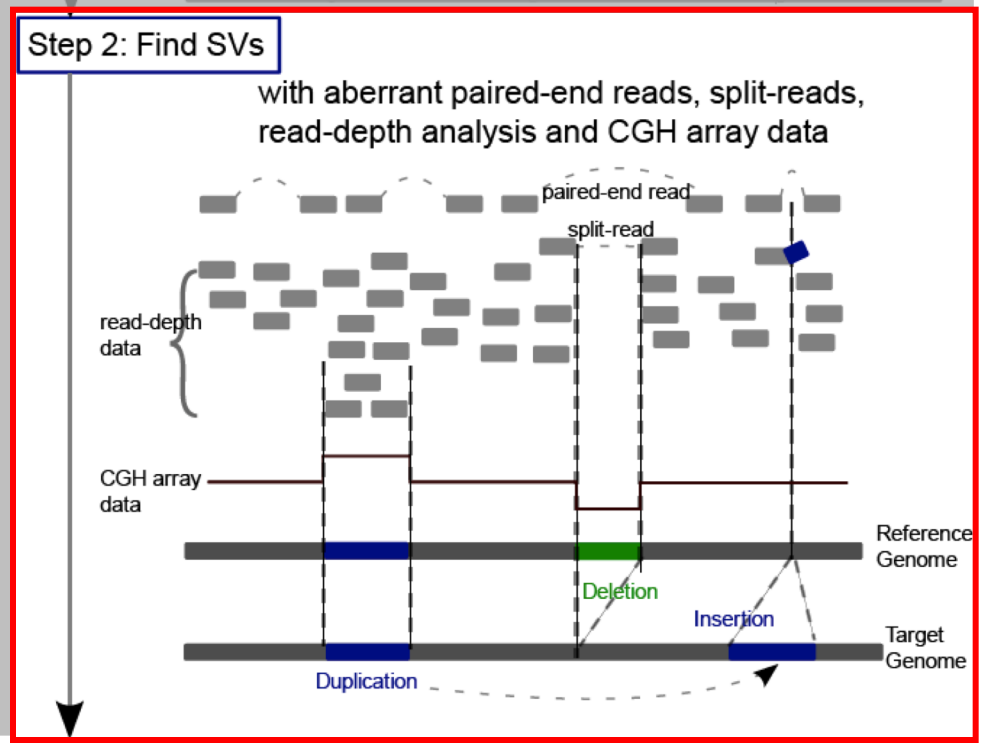
$$\boldsymbol{P(D|G) = P_{err\_free}(D|G) + P_{err}(D|G)}$$

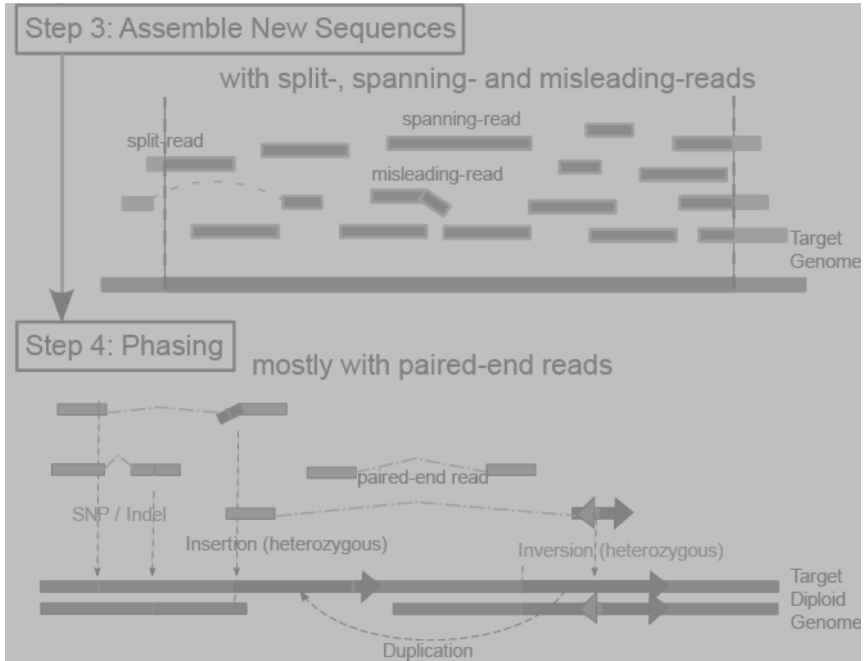
[You et al. ('12) Bioinformatics 28: 643 doi: 10.1093/bioinformatics/bts001]

Main Steps in Genome Resequencing

[Snyder et al. Genes & Dev. ('10)]



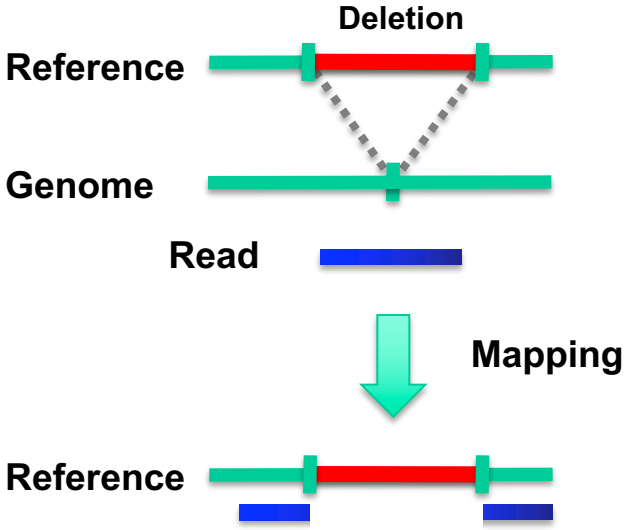




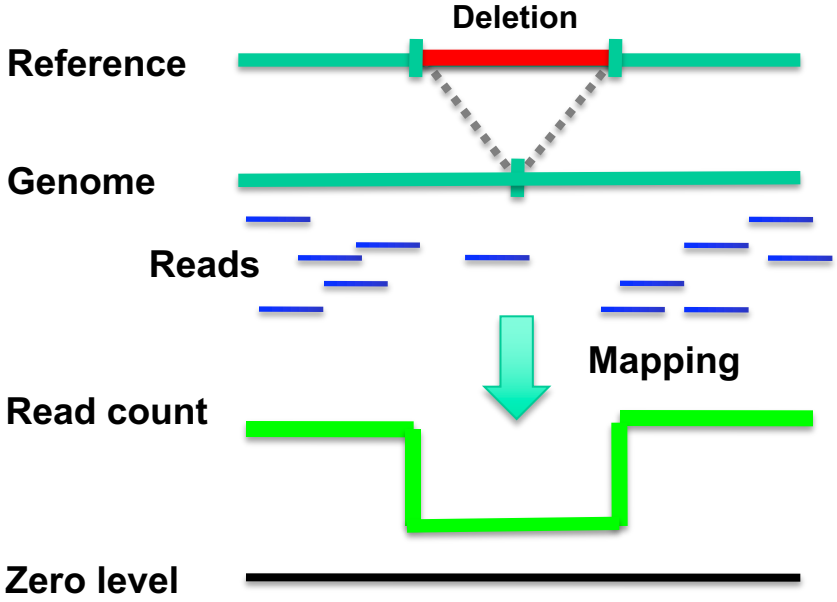
Methods to Find SVs

1. Paired ends

2. Split read



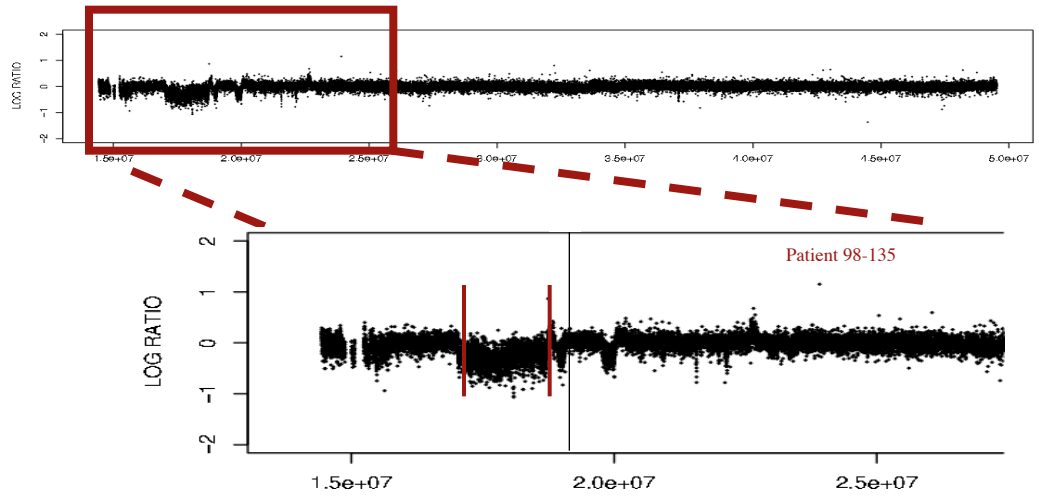
3. Read depth (or aCGH)



4. Local Reassembly

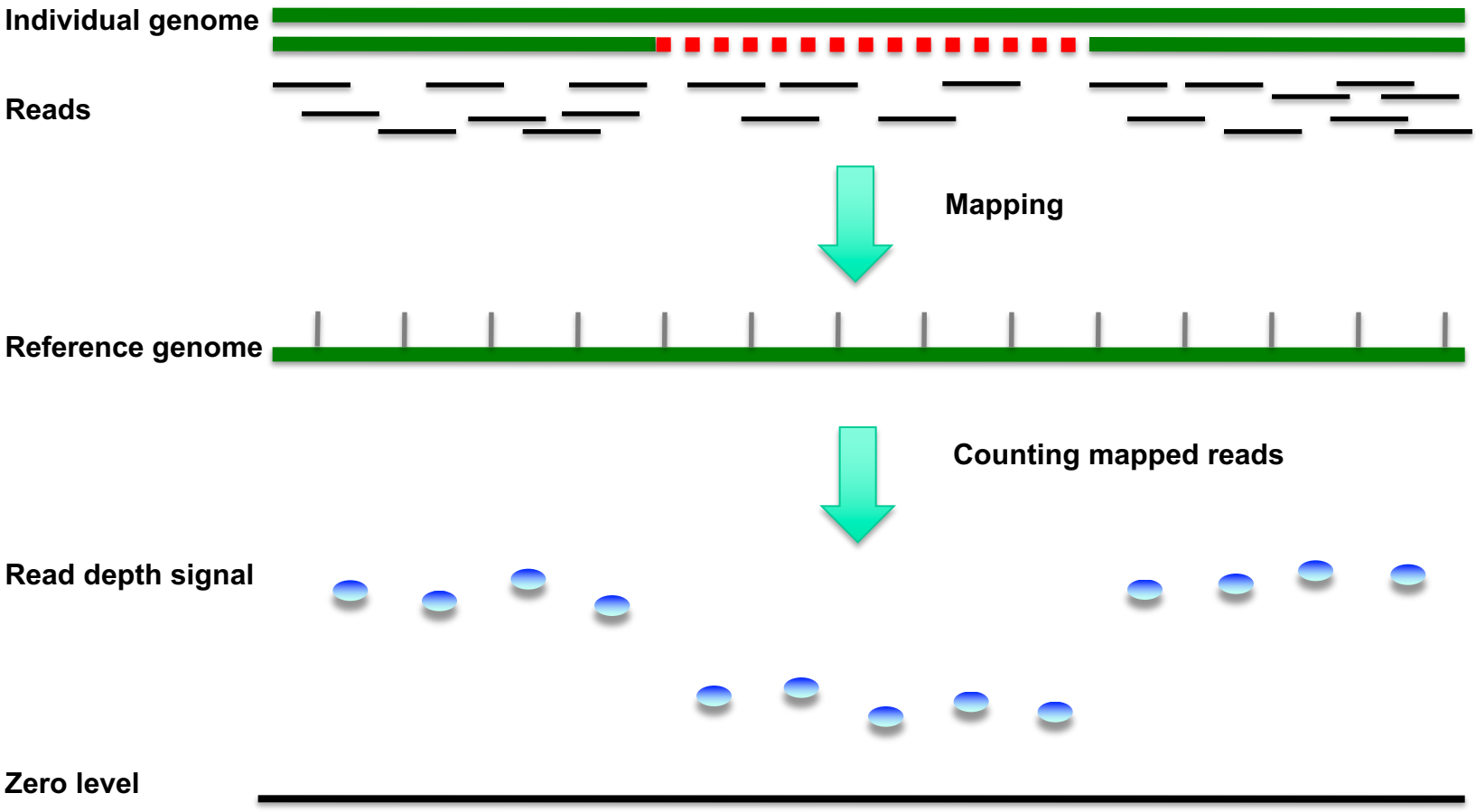
[Snyder et al. Genes & Dev. ('10)]

Read Depth



Array Signal

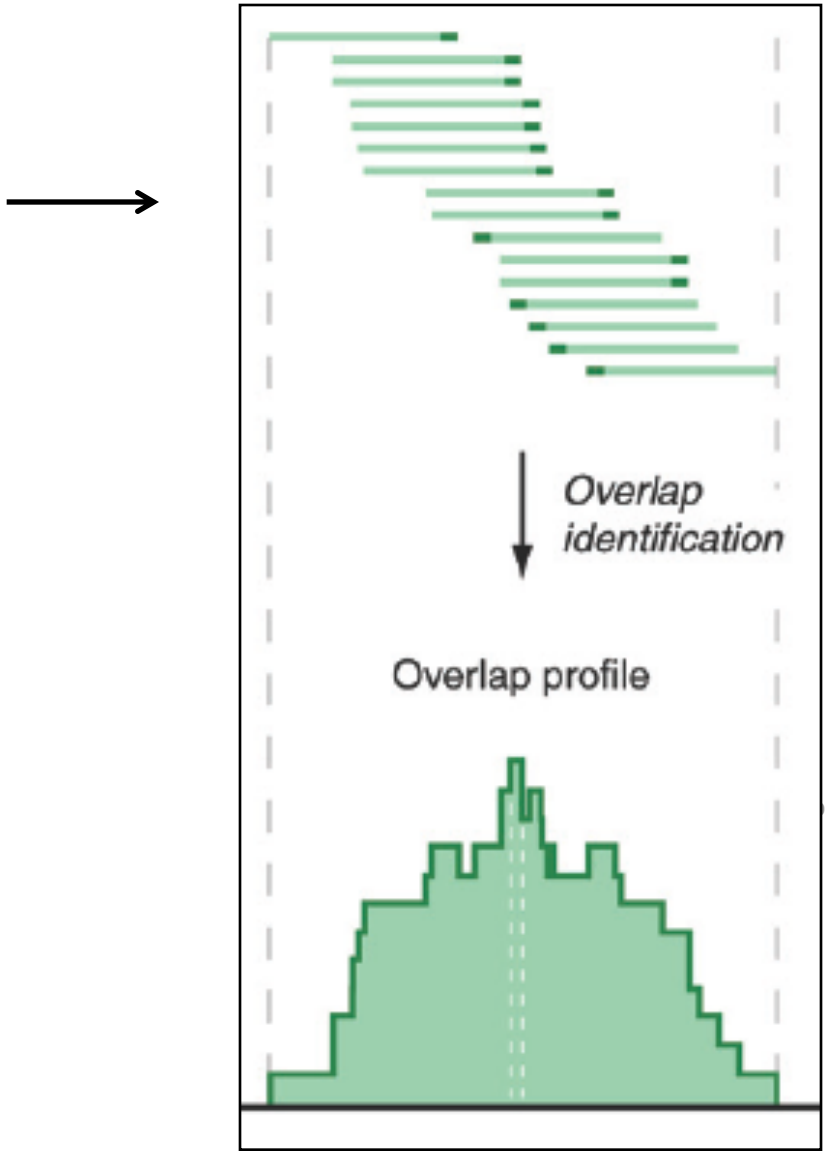
Read depth



[Urban et al. ('06) PNAS; Wang et al. Gen. Res. ('09); Abyzov et al. Gen. Res. ('11)]

Reads to Signal Track

```
@ILMN-GA001_3_208HWAAXX_1_1_110_812
ATACAAGCAAGTATAAGTTCGTATGCCGTCTT
+ILMN-GA001_3_208HWAAXX_1_1_110_812
hhhYhh]NYhhhhhhYIhhaZT[hYHNSPKXR
@ILMN-GA001_3_208HWAAXX_1_1_111_879
GGAGGCTGGAGTTGGGGACGTATGCGGCATAG
+ILMN-GA001_3_208HWAAXX_1_1_111_879
hSWhRNJ\hFhLdhVOhAIB@NFKD@PAB?N?
```



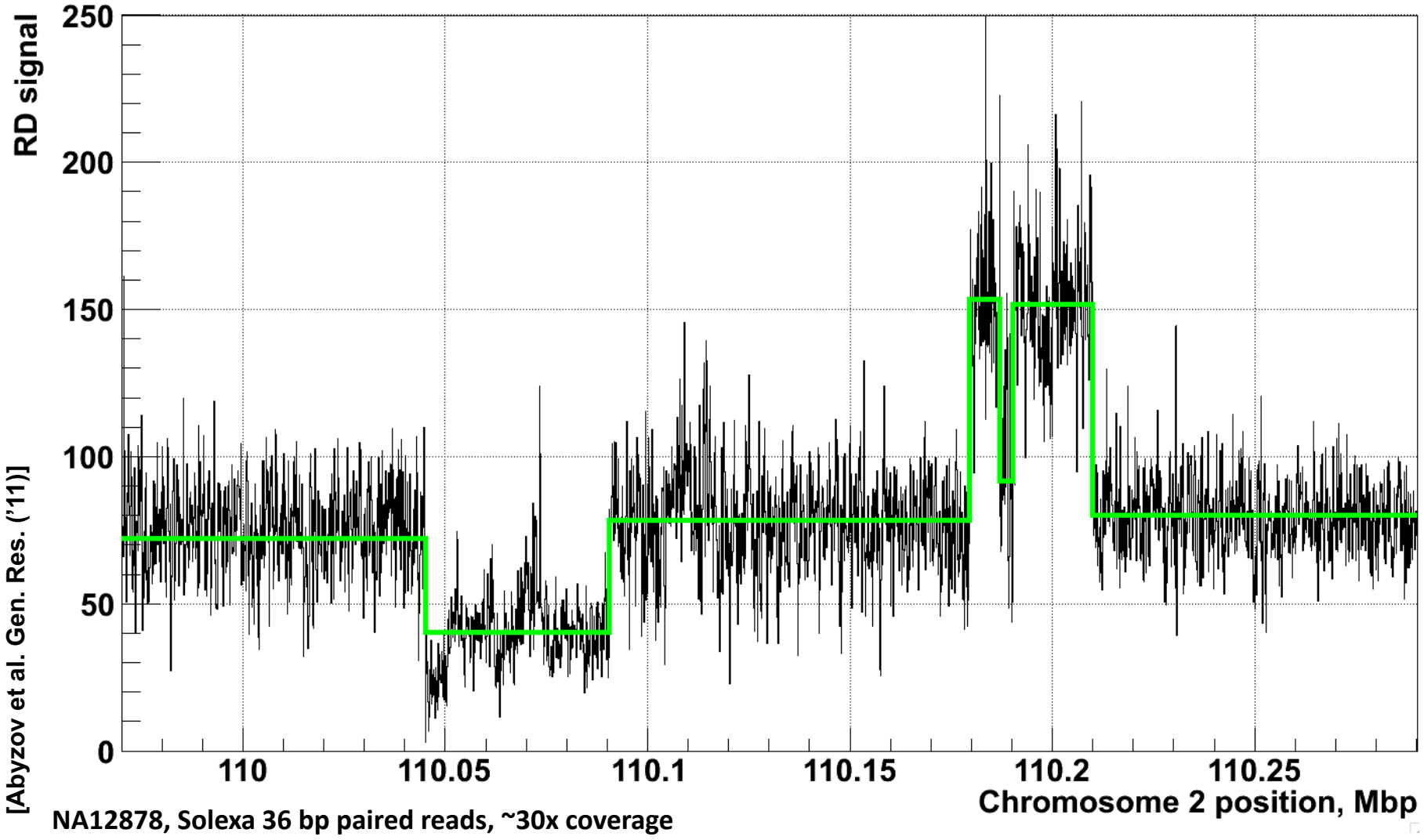
Reads (fasta)
+ quality scores (fastq)
+ mapping (BAM)

Reads => Signal (Intermediate file)

Accumulating @ >1 Pbp/yr (currently),
~20% of tot. HiSeq output

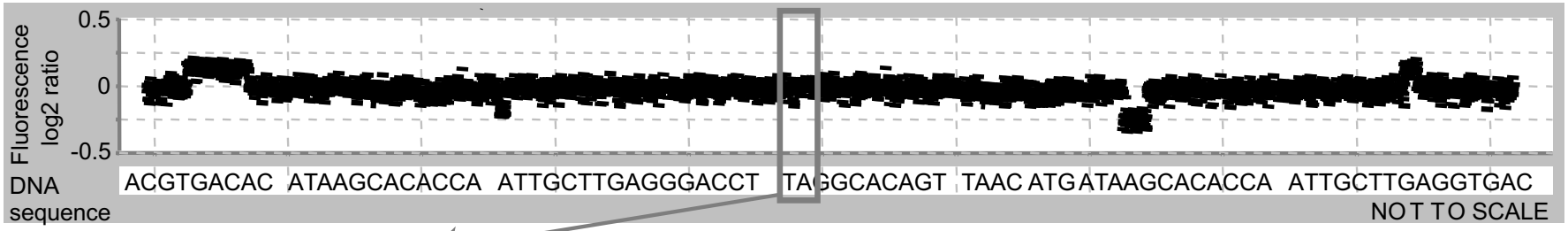
[*PLOS CB* 4:e1000158]

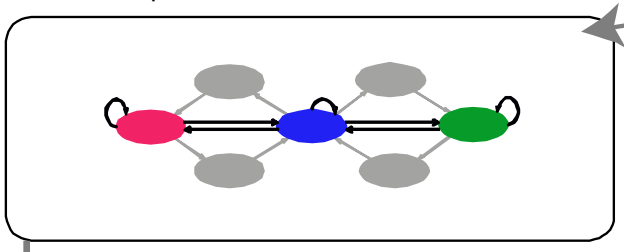
Example of Application to RD data

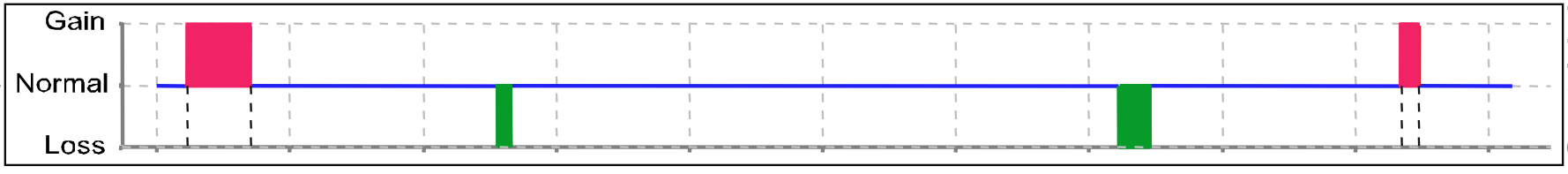


HMM

- To get highest resolution on breakpoints need to smooth & segment the signal
- BreakPtr: prediction of breakpoints, dosage and cross-hybridization using a system based on Hidden Markov Models

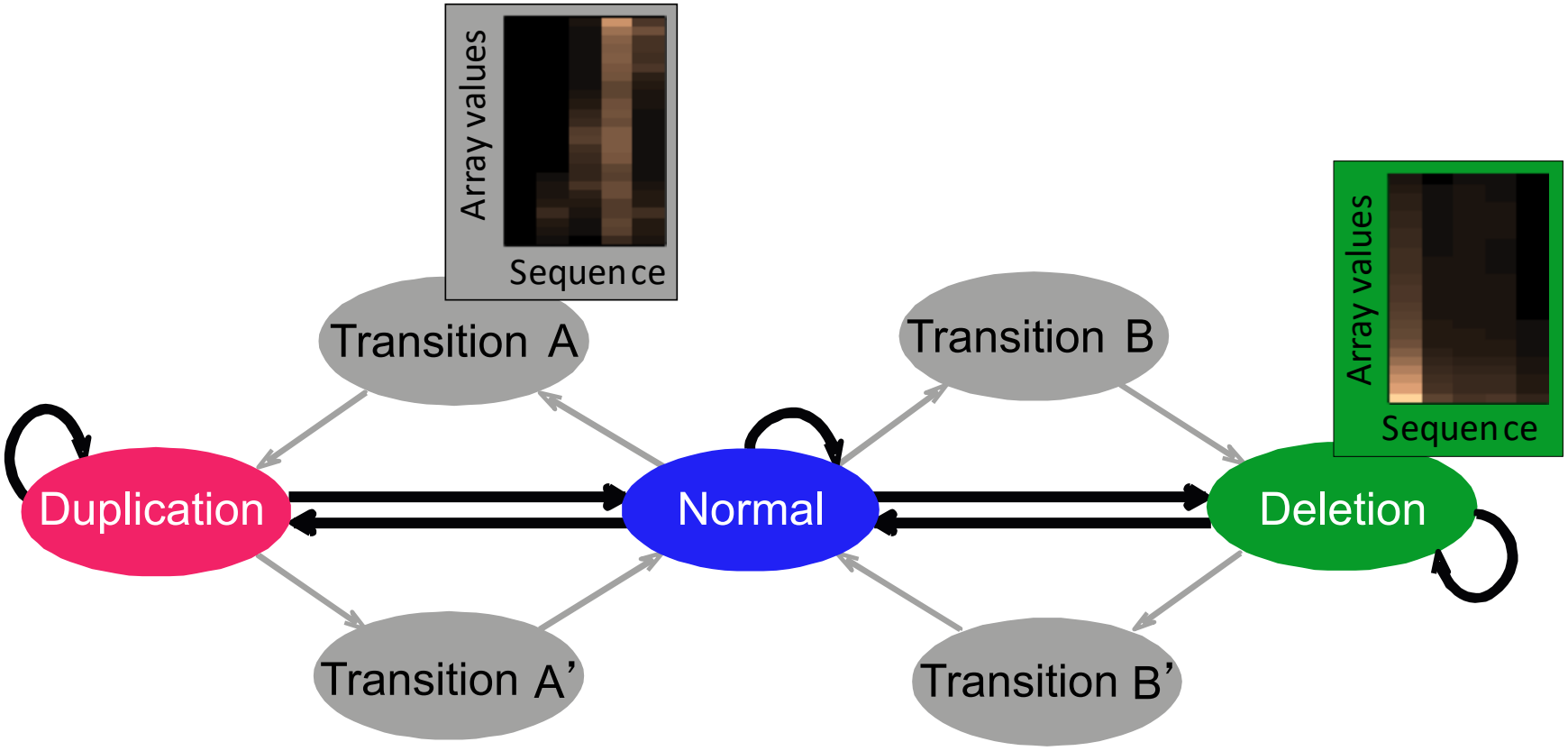






Korbel*, Urban* *et al.*, PNAS (2007)

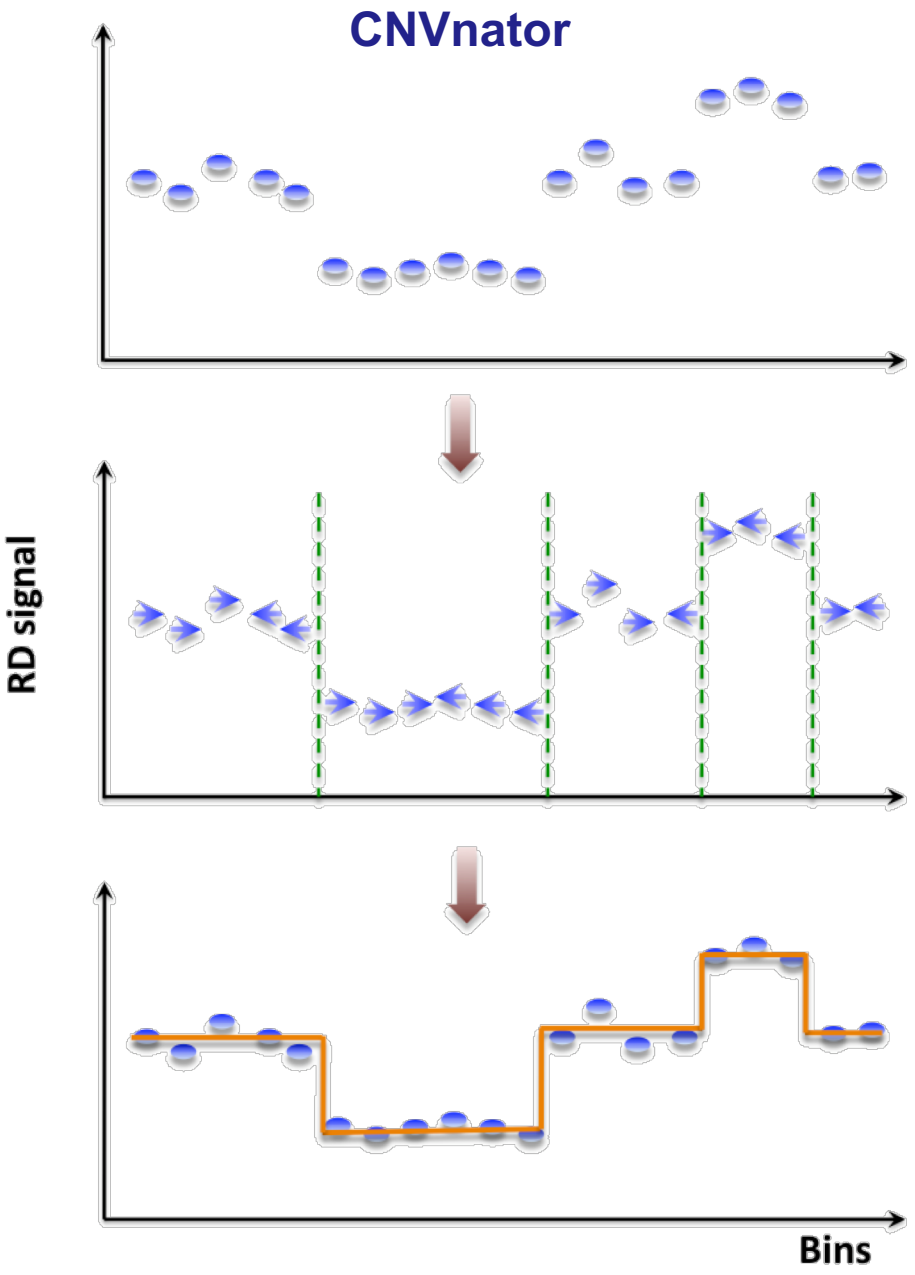
Statistically integrates array signal and DNA sequence signatures (using a discrete-valued bivariate HMM)



Korbel*, Urban* *et al.*, PNAS (2007)

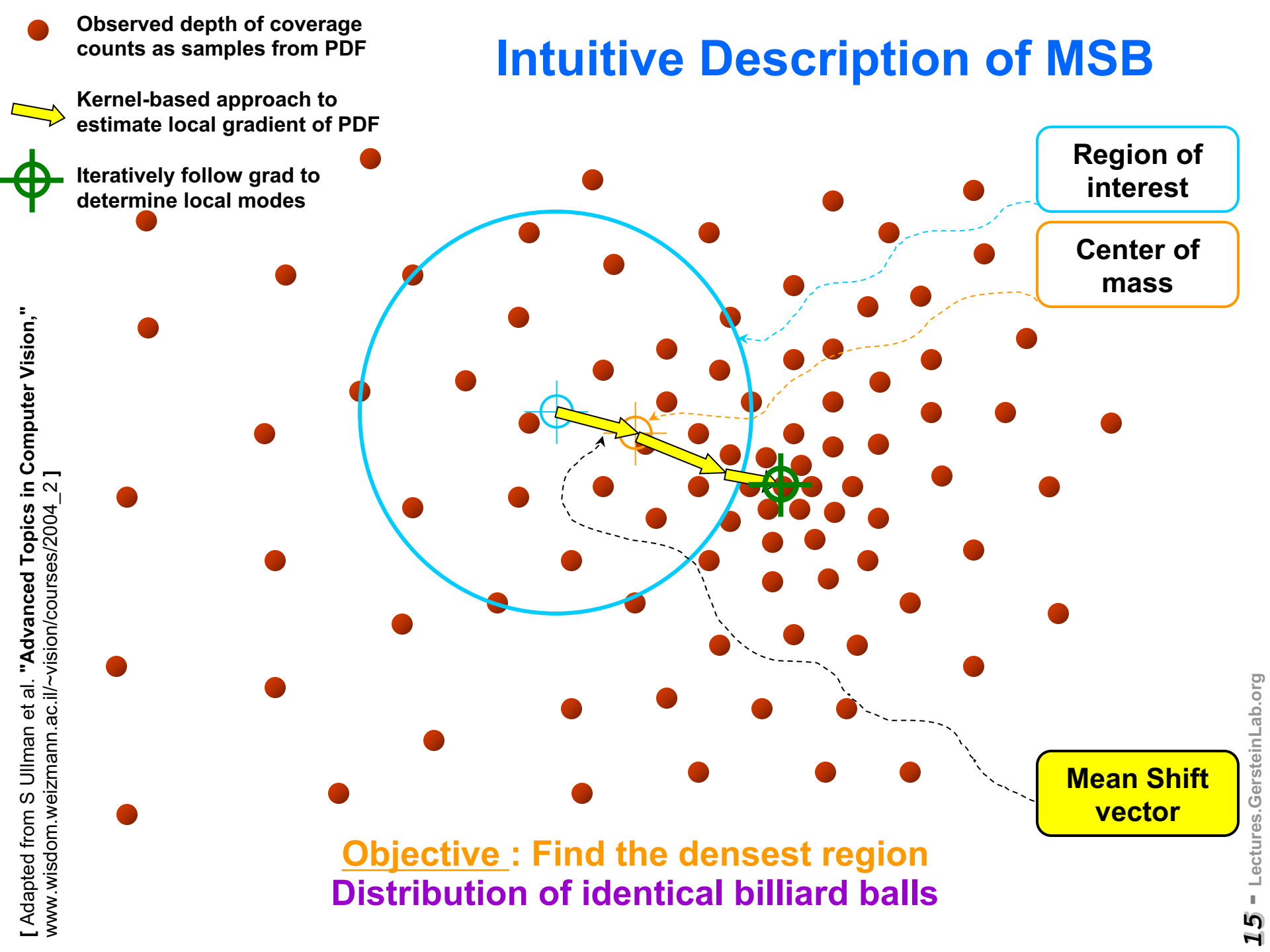
Mean-shift-based (MSB) segmentation: no explicit model

- For each bin attraction (mean-shift) vector points in the direction of bins with most similar RD signal
- No prior assumptions about number, sizes, haplotype, frequency and density of CNV regions
- Not Model-based (e.g. like HMM) with global optimization, distr. assumption & parms. (e.g. num. of segments).
- Achieves discontinuity-preserving smoothing
- Derived from image-processing applications



[Abyzov et al. Gen. Res. ('11)]

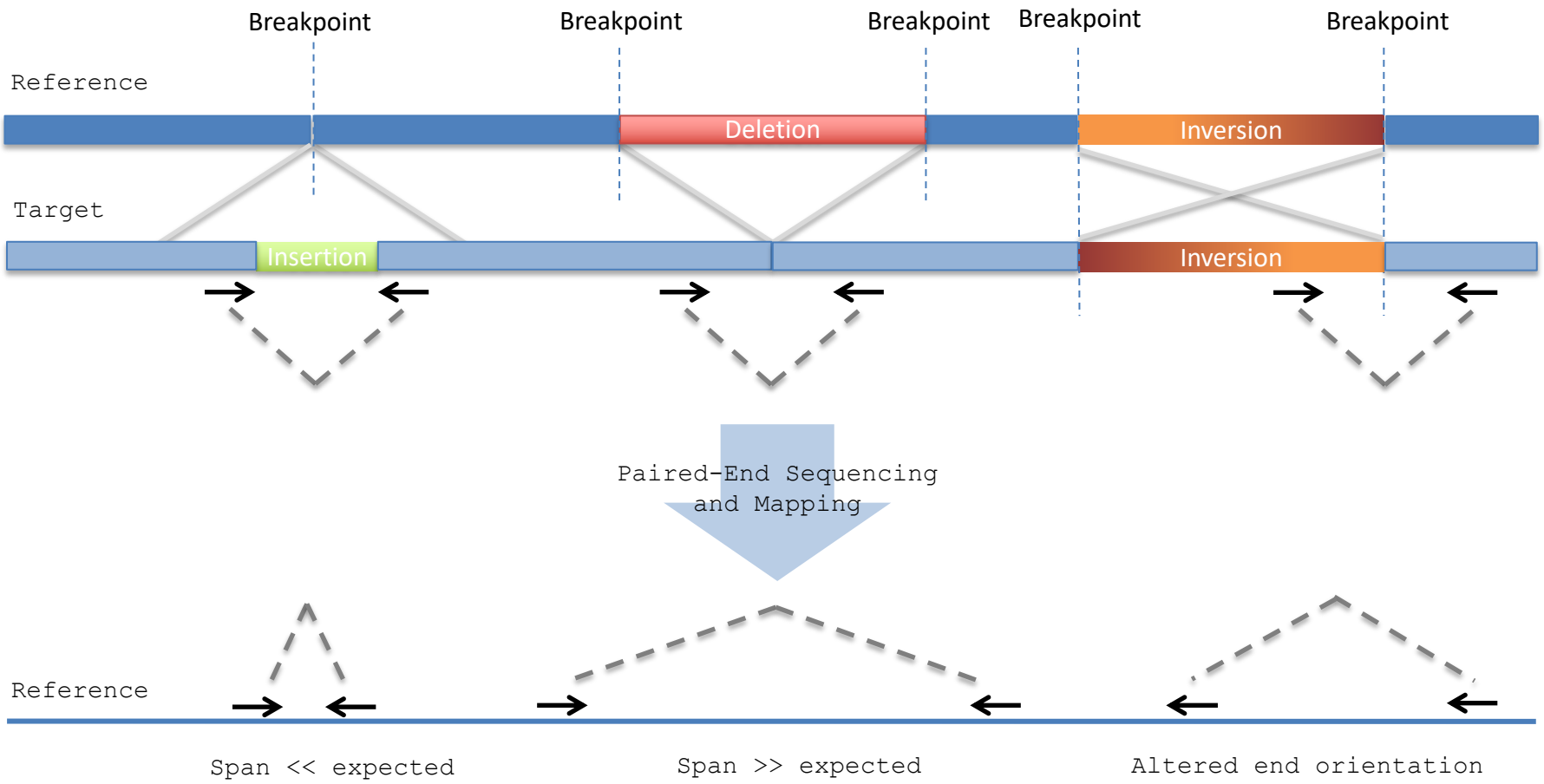
Intuitive Description of MSB



[Adapted from S Ullman et al. **"Advanced Topics in Computer Vision,"** www.wisdom.weizmann.ac.il/~vision/courses/2004_2]

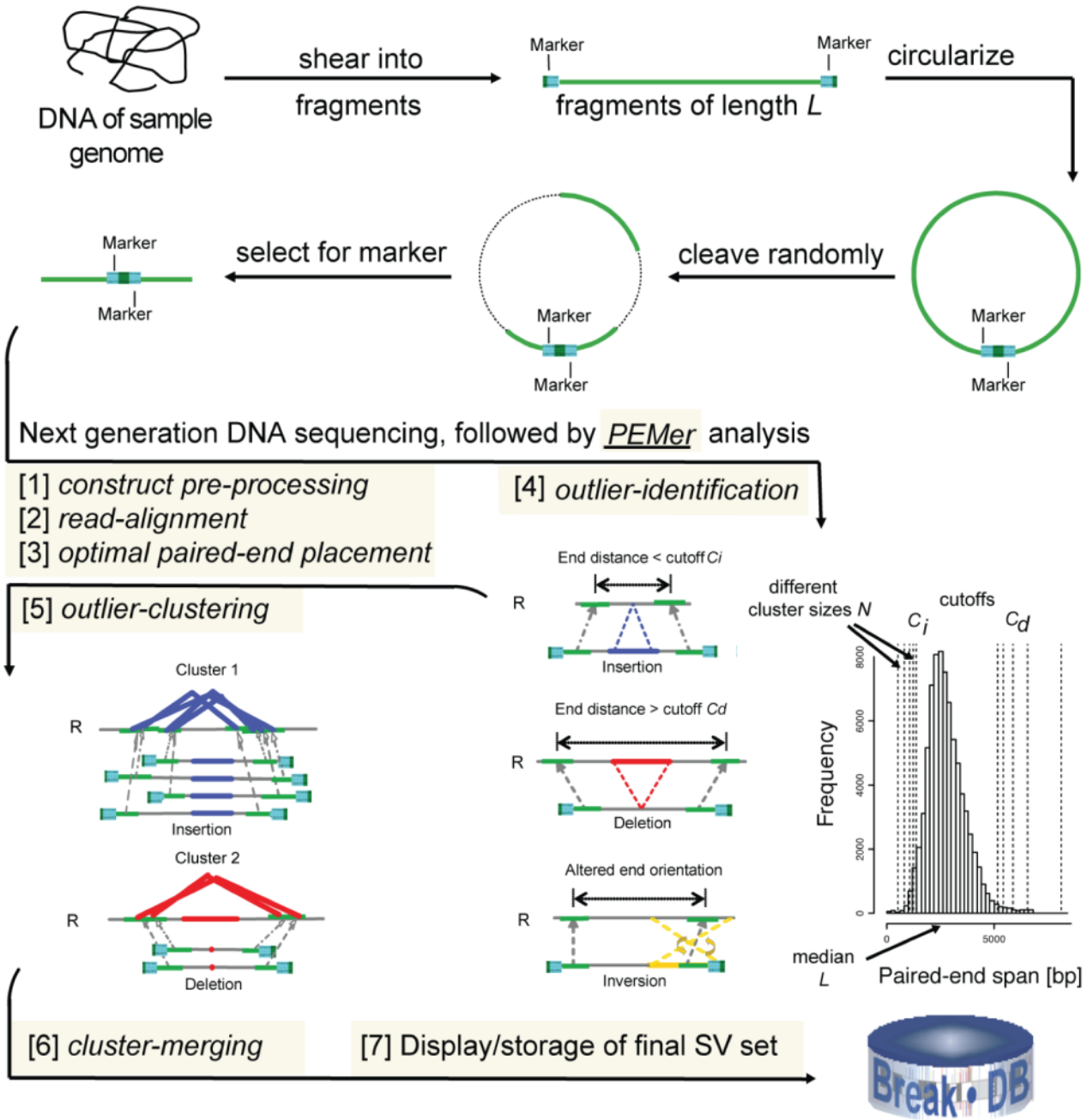
Paired-End

Paired-End Mapping



- Both paired-ends map within repeats.
- Limited the distance between pairs; therefore, neither large nor very small rearrangements can be detected

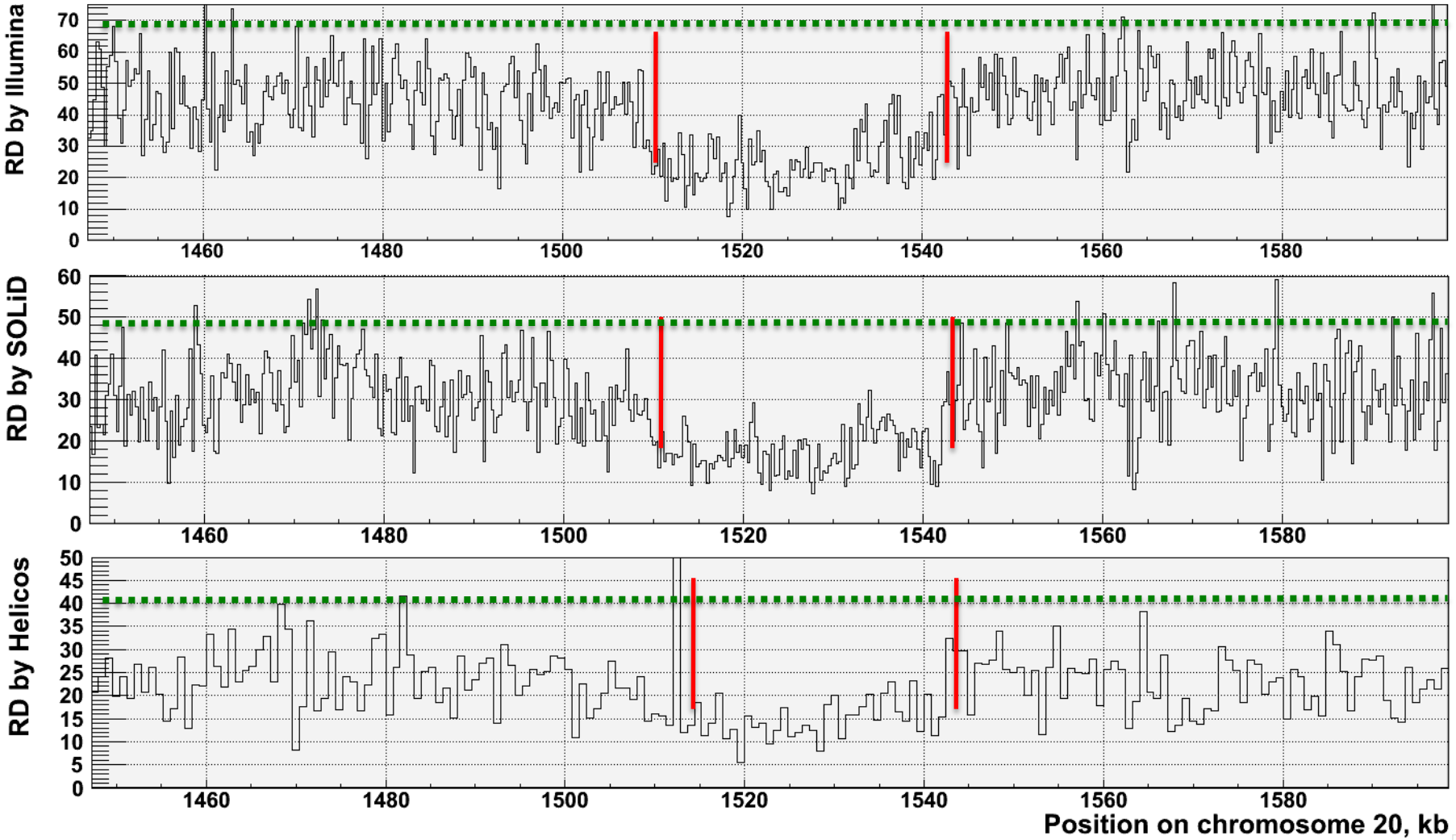
Overall Strategy for Analysis of NextGen Seq. Data to Detect Structural Variants



[Korbel et al., Science ('07); Korbel et al., GenomeBiol. ('09)]

Split Read

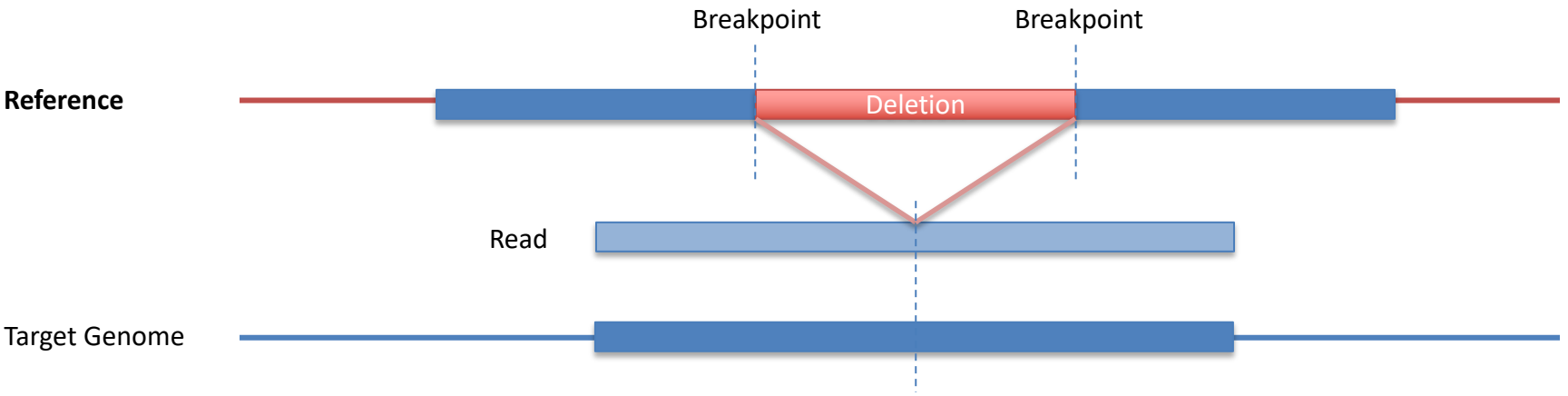
Read-depth works well on a variety of sequencing platforms but provides imprecise breakpoints

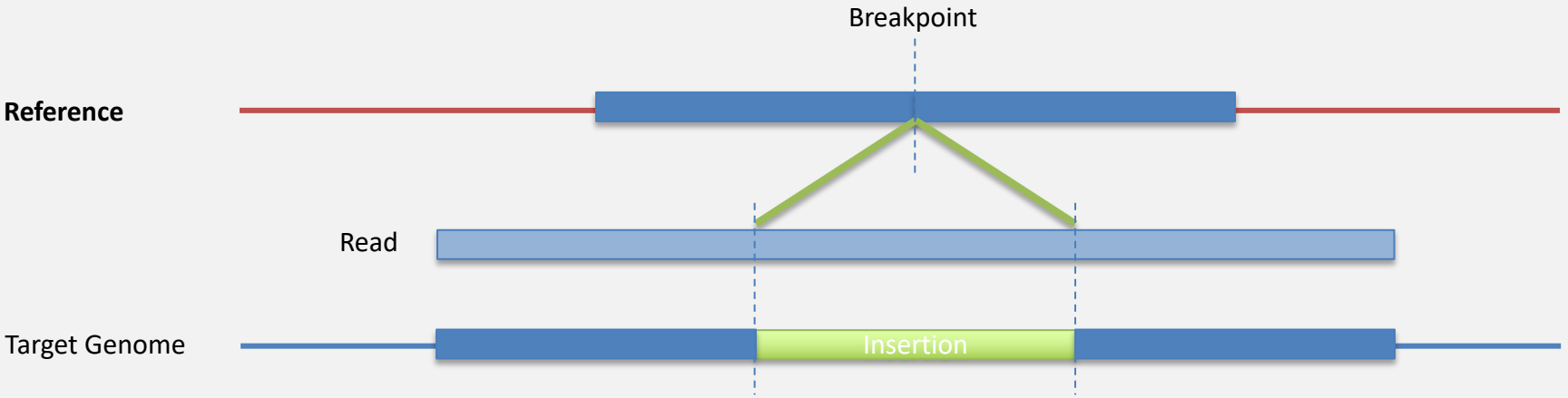


[Abyzov et al. Gen. Res. ('11)]

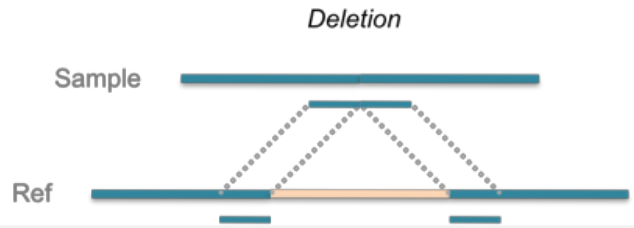
[NA18505]

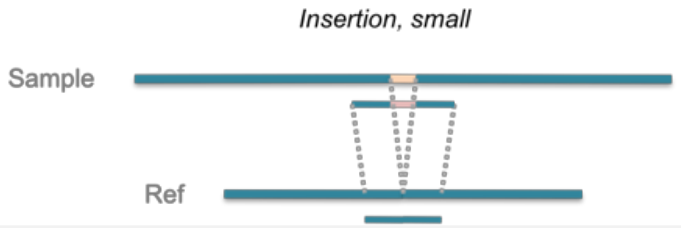
Split-read Analysis

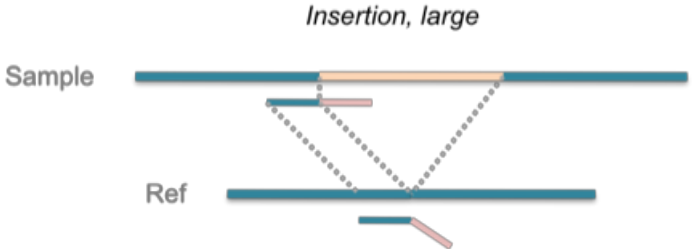




Simple SVs

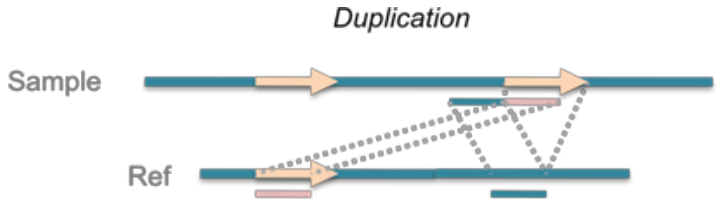


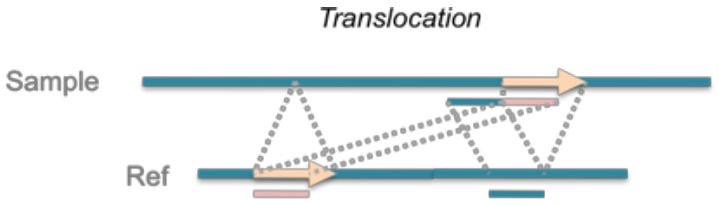


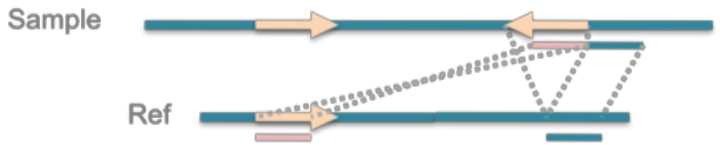


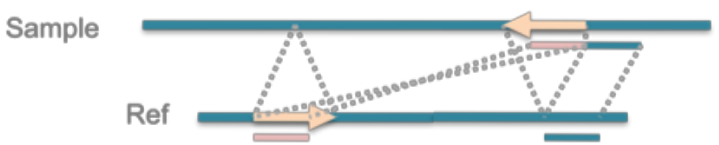
Deletions are the Easiest to Identify

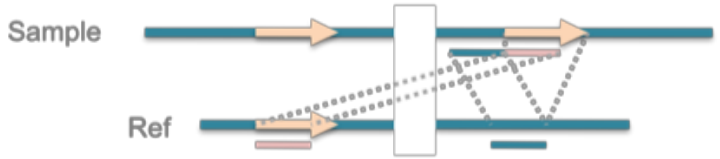
Complex SVs

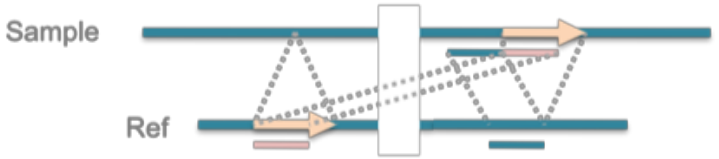








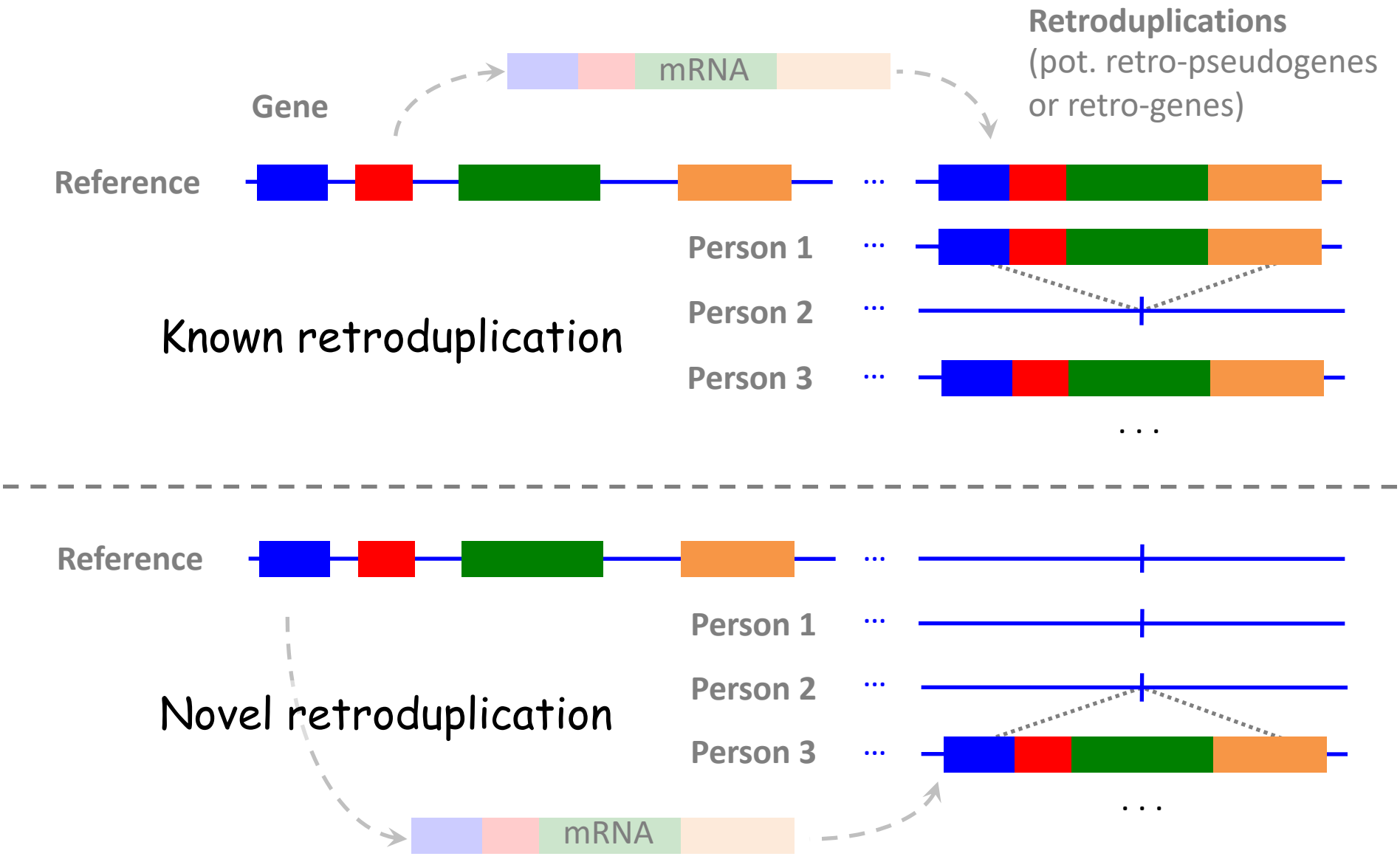




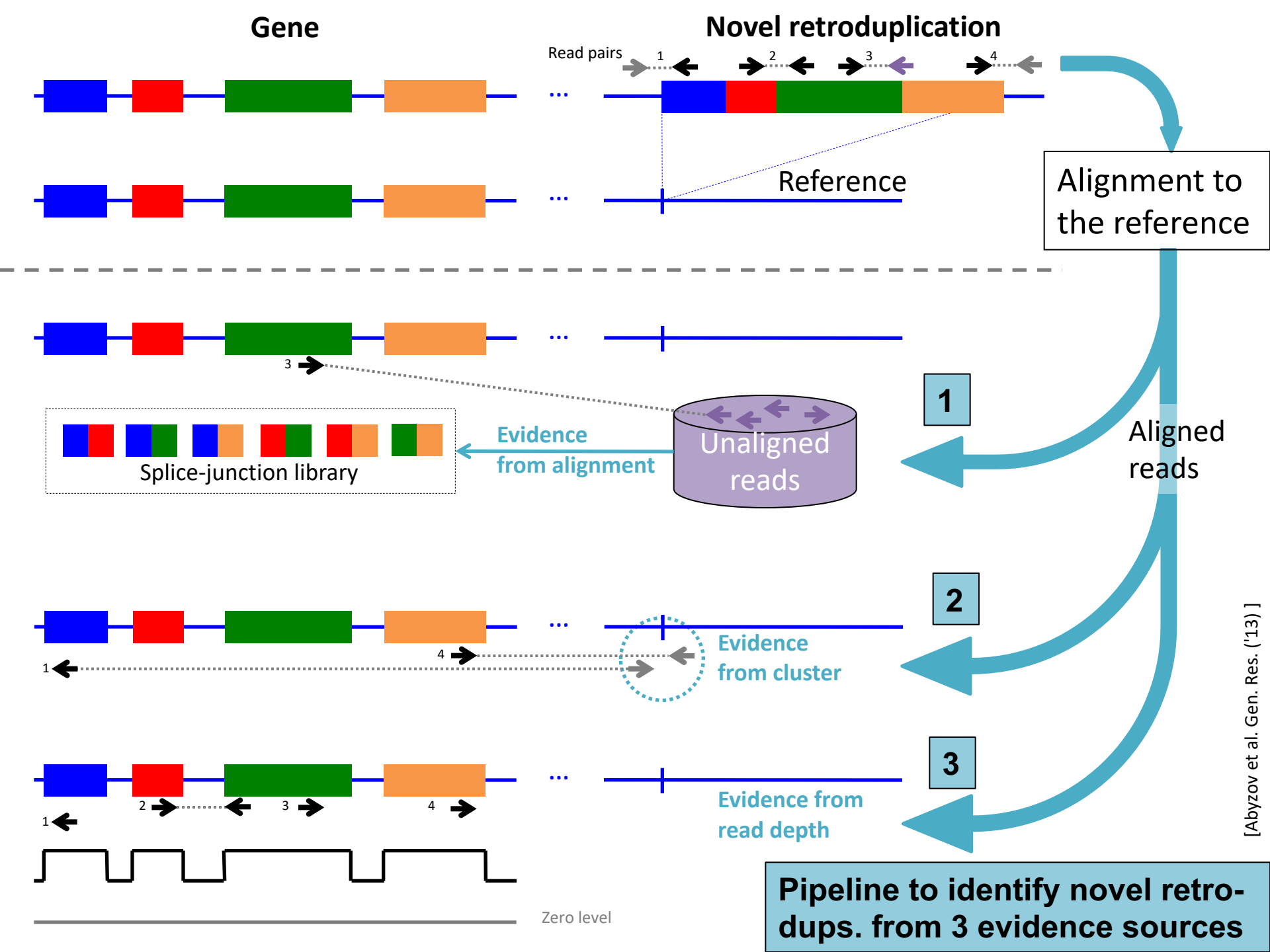
[Zhang et al. ('11) *BMC Genomics*]

RDV & Mobile Elements

Retroduplication variation (RDV)



[Abyzov et al. Gen. Res. ('13)]


Gene
Novel retroduplication
Read pairs
1
2
3
4
Reference
Alignment to the reference
Aligned reads
Splice-junction library
Evidence from alignment
Unaligned reads
1
2
Evidence from cluster
3
Evidence from read depth
Zero level
[Abyzov et al. Gen. Res. ('13)]
Pipeline to identify novel retro-dups. from 3 evidence sources

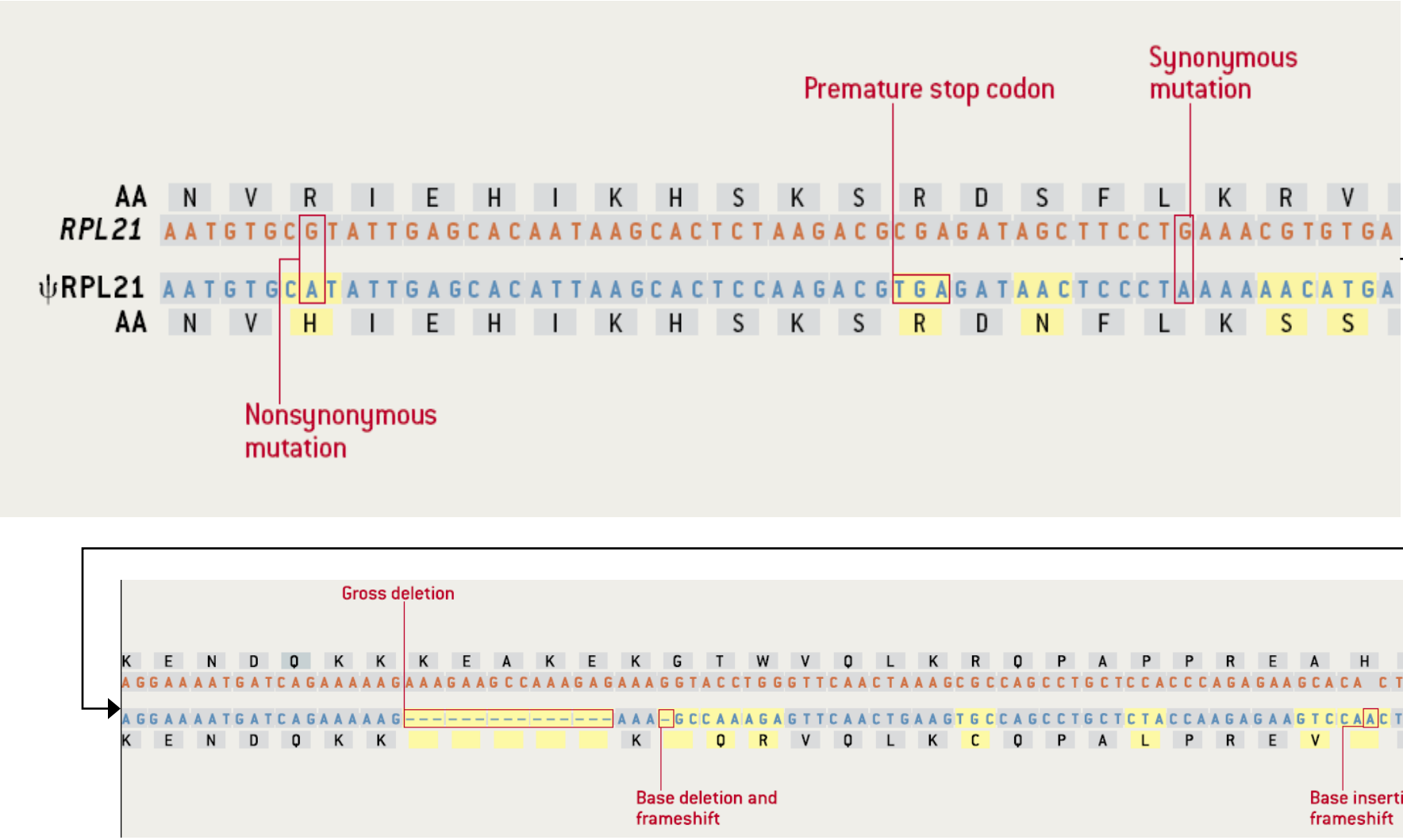
Pseudogenes & Genomic Duplications

Pseudogenes are among the most interesting intergenic elements

- Formal Properties of Pseudogenes (ΨG)
  - Inheritable
  - Homologous to a functioning element – ergo a repeat!
  - Non-functional
    - No selection pressure so free to accumulate mutations
      - Frameshifts & stops
      - Small Indels
      - Inserted repeats (LINE/Alu)
    - **What does this mean?** no transcription, no translation?…

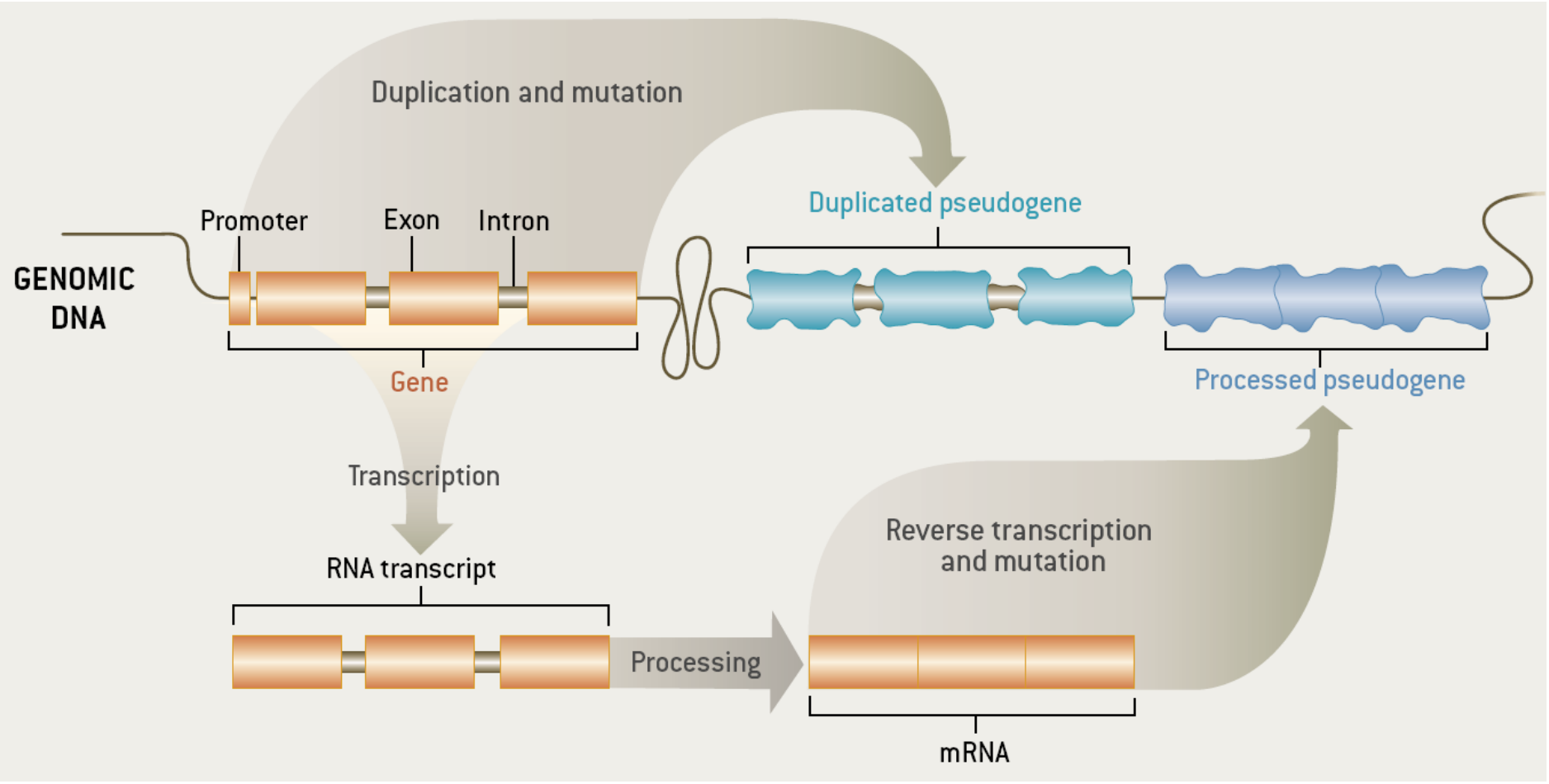
[Mighell et al. *FEBS Letts*, 2000]

Identifiable Features of a Pseudogene (ψRPL21)



[Gerstein & Zheng. Sci Am 295: 48 (2006).]

Two Major Genomic Remodeling Processes Give Rise to Distinct Types of Pseudogenes



[Gerstein & Zheng. Sci Am 295: 48 (2006).]

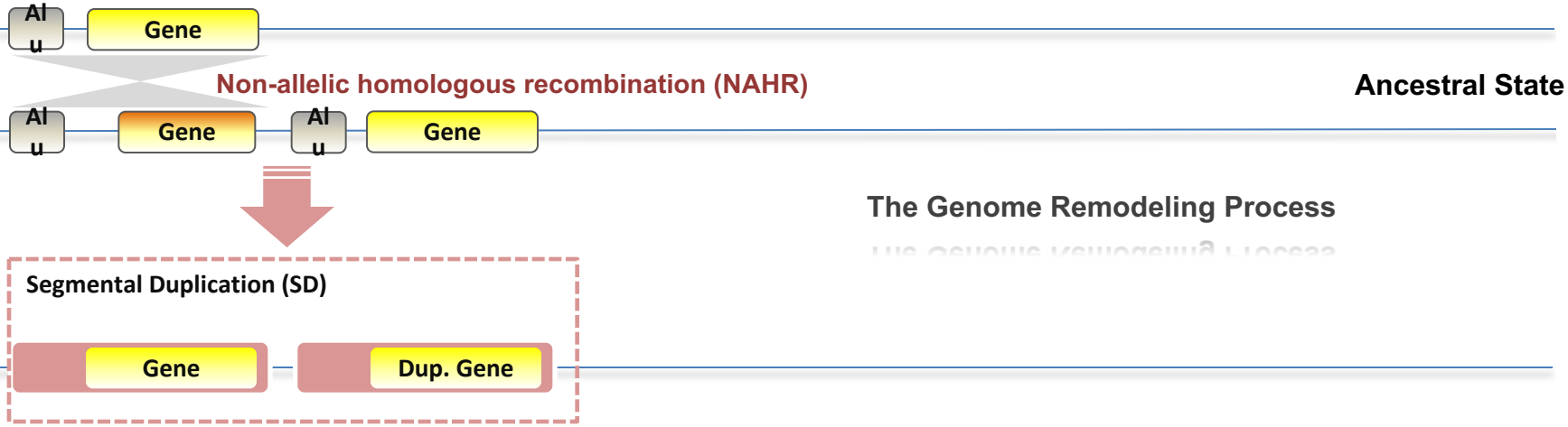
Impact of Genetic Variability: Loss-of-function

- - Truncating nonsense SNPs
- - Splice-disrupting SNPs
- - Frameshift-causing indels
- - Disrupting structural variants

- Previous LoFs are considered as having high probability of being deleterious
- Surprisingly, ~ 100 LoF variants per genome, 20 genes are completely inactivated
- Among ~100 LoFs, we estimate 2 recessive, close to 0 dominant disease nonsense variants per healthy genome.

Genomic Variation

Genomic Variation



Genomic Variation

Alu Gene

Non-allelic homologous recombination (NAHR)

Ancestral State

Alu Gene Alu Gene

The Genome Remodeling Process

Segmental Duplication (SD)

Gene Dup. Gene

Syntenic Ortholog

SD

Gene Dup. Gene

Paralog

duplicate

Gene Dup. Gene Dup. Gene

family

Gene Dup. ψgene

Genomic Variation

Alu Gene

Non-allelic homologous recombination (NAHR)

Ancestral State

Alu Gene Alu Gene

The Genome Remodeling Process

Segmental Duplication (SD)

Gene Dup. Gene

Syntenic Ortholog

SD

Gene Dup. Gene

Paralog

duplicate

Gene Dup. Gene Dup. Gene

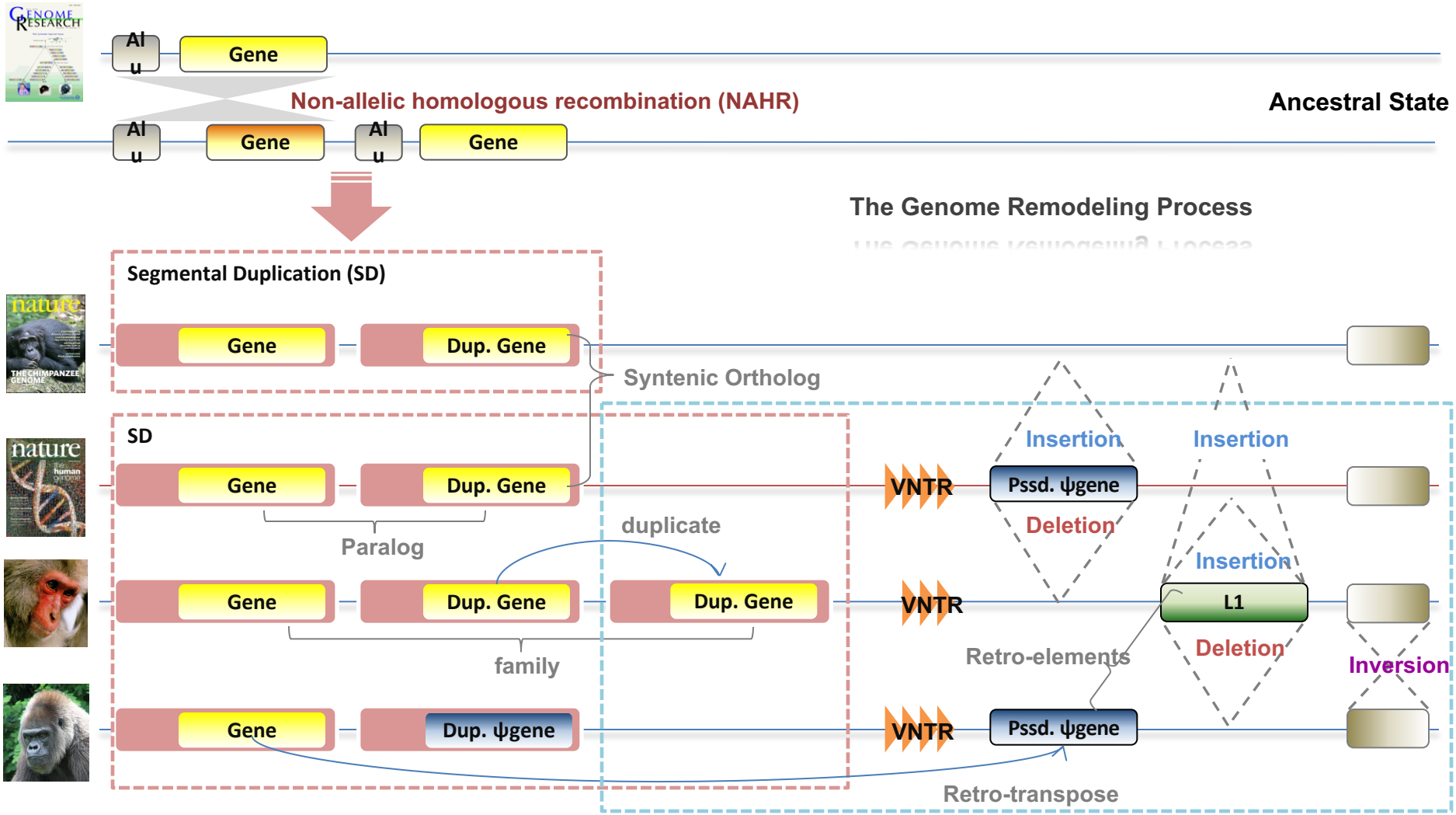
family

Gene Dup. ψgene

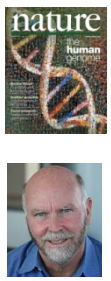
Pssd. ψgene

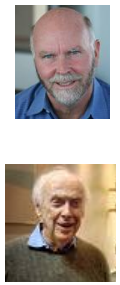
Retro-transpose

Genomic Variation



Genomic Variation





Alu Gene

Non-allelic homologous recombination (NAHR)

Ancestral State

Alu Gene Alu Gene

The Genome Remodeling Process

Segmental Duplication (SD)

Gene Dup. Gene

Syntenic Ortholog

CNV (type of SV)

Gene Dup. Gene

duplicate

Gene Dup. Gene Dup. Gene

Gene Dup. ψgene

VNTR

Pssd. ψgene

Insertion

Deletion

Insertion

VNTR

L1

Insertion

Deletion

Retro-elements

Inversion

VNTR

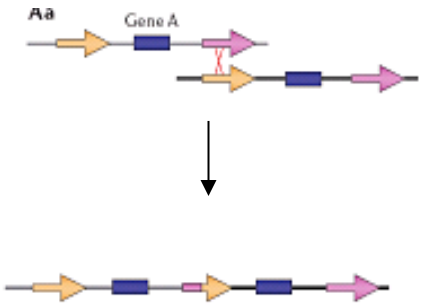
Pssd. ψgene

Retro-transpose

"Polymorphic" Genes & Pseudogenes

Exact Breakpoints & Mechanism Classification

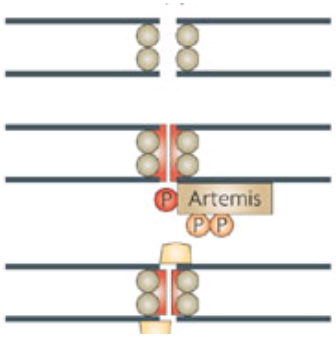
4 mechanisms for SV formation



NAHR

(Non-allelic homologous recombination)

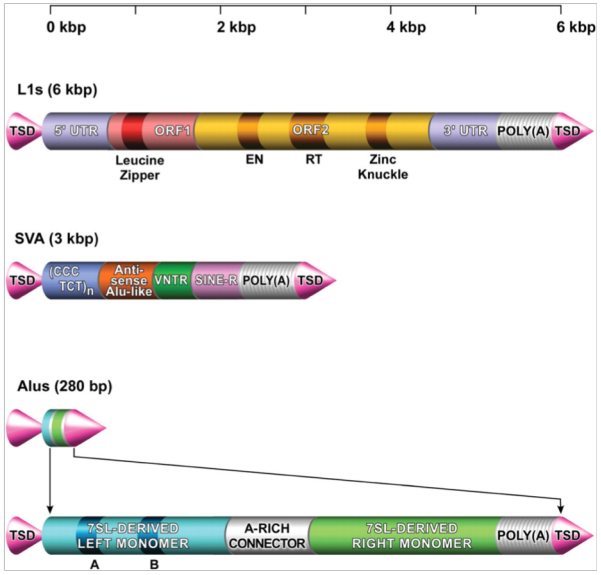
Flanking repeat
(e.g. Alu, LINE…)



NHEJ (NHR)

(Non-homologous-end-joining)

No (flanking) repeats.
In some cases <4bp microhomologies



TEI

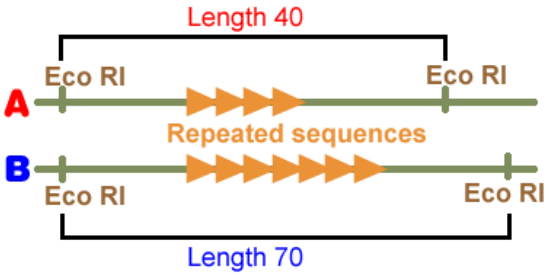
(Transposable element insertion)

L1, SVA, Alus

VNTR

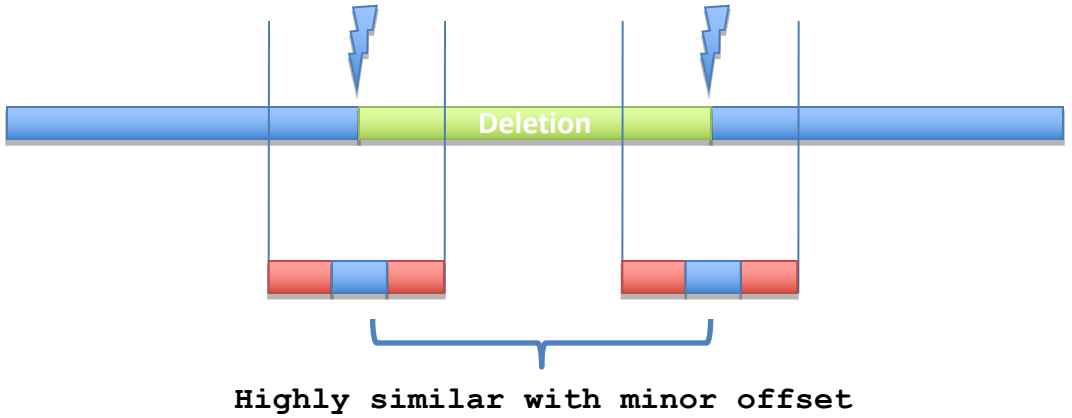
(Variable Number Tandem Repeats)

Number of repeats varies between different people



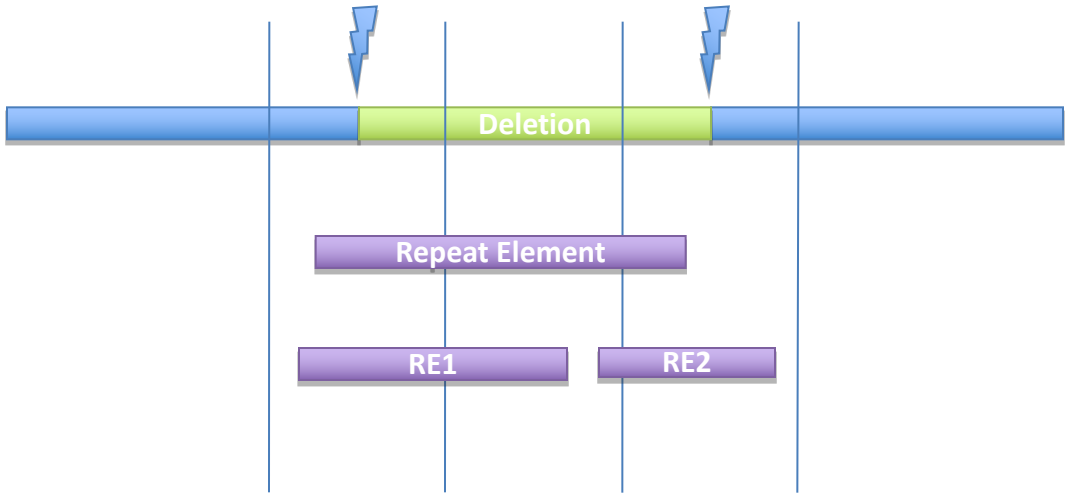
SV Mechanism Classification

NAHR



Single RETRO

Multiple RETRO

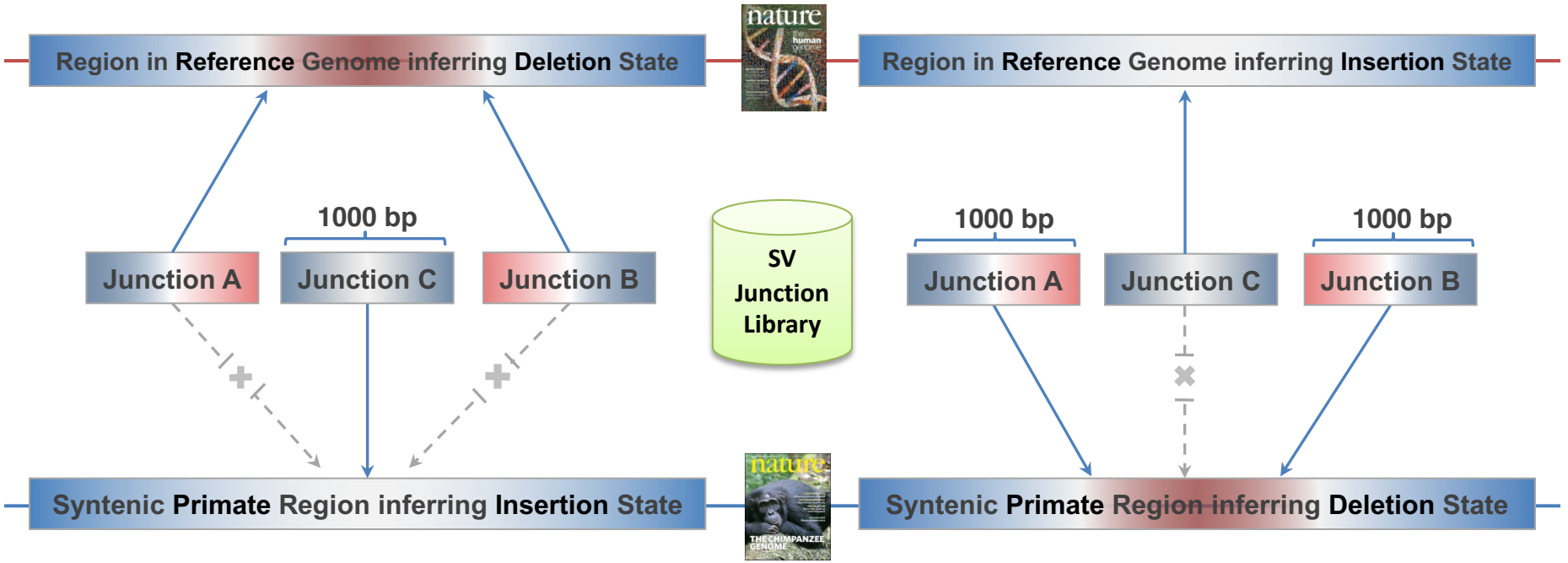


[Lam et al., ('10) *Nat. Biotech.*]

SV Ancestral State Analysis

Inferring Insertion according to Ancestral State

Inferring Deletion according to Ancestral State



[Lam et al., ('10) *Nat. Biotech.*]

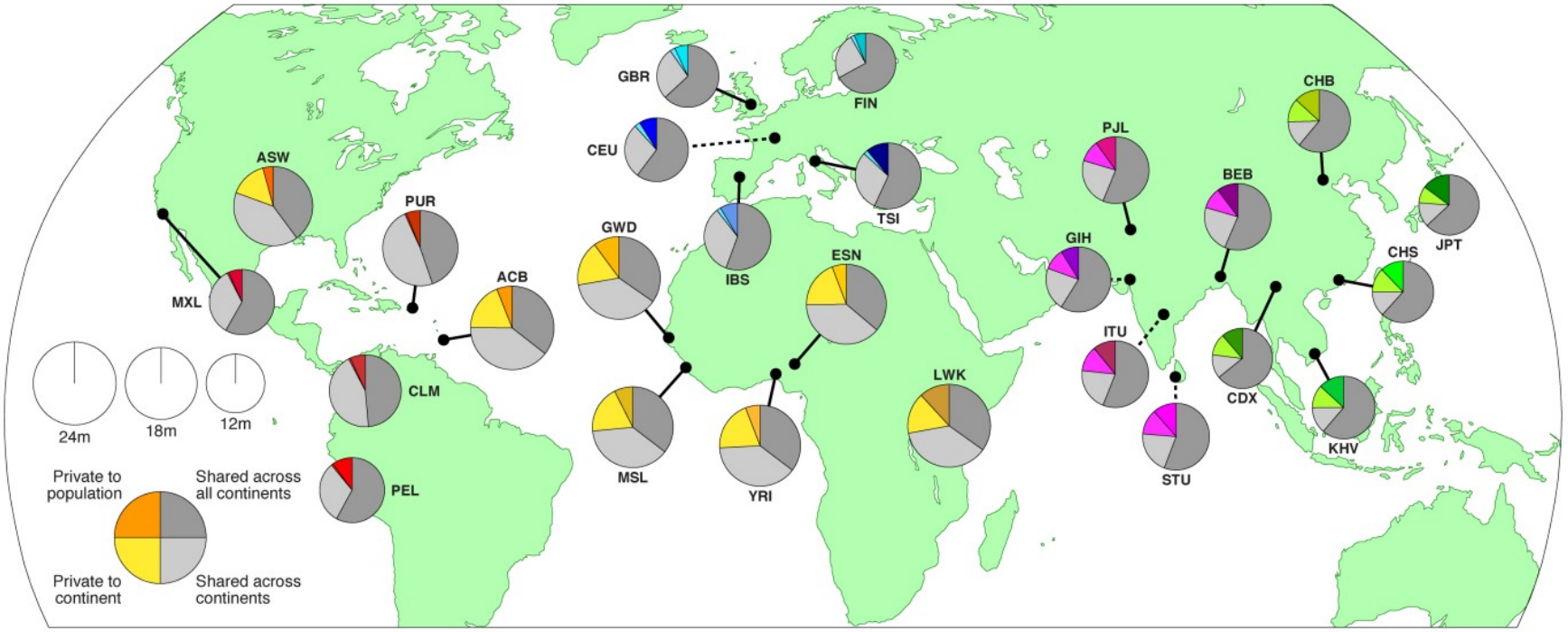
1000G summary

1000G SV (Pilot, **Phase I** & III)

- **Many** different callers compared & used
  - including SRiC & CNVnator but also VariationHunter, Cortex, NovelSeq, PEMer, BreakDancer, Mosaik, Pindel, GenomeSTRiP, mrFast….
- Merging
- Genotyping (GenomeSTRiP)
- Breakpoint assembly (AGE & Tigra_SV)
- Mechanism Classification

[1000 Genomes Consortium, Nature (2010, 2012); Mills et al., Nature (2011)]

Summary Stats of 1000GP SV Phase3



- 68,818 SVs
- 2,504 unrelated individuals
- 26 populaSons
- 37,250 SVs with resolved breakpoints

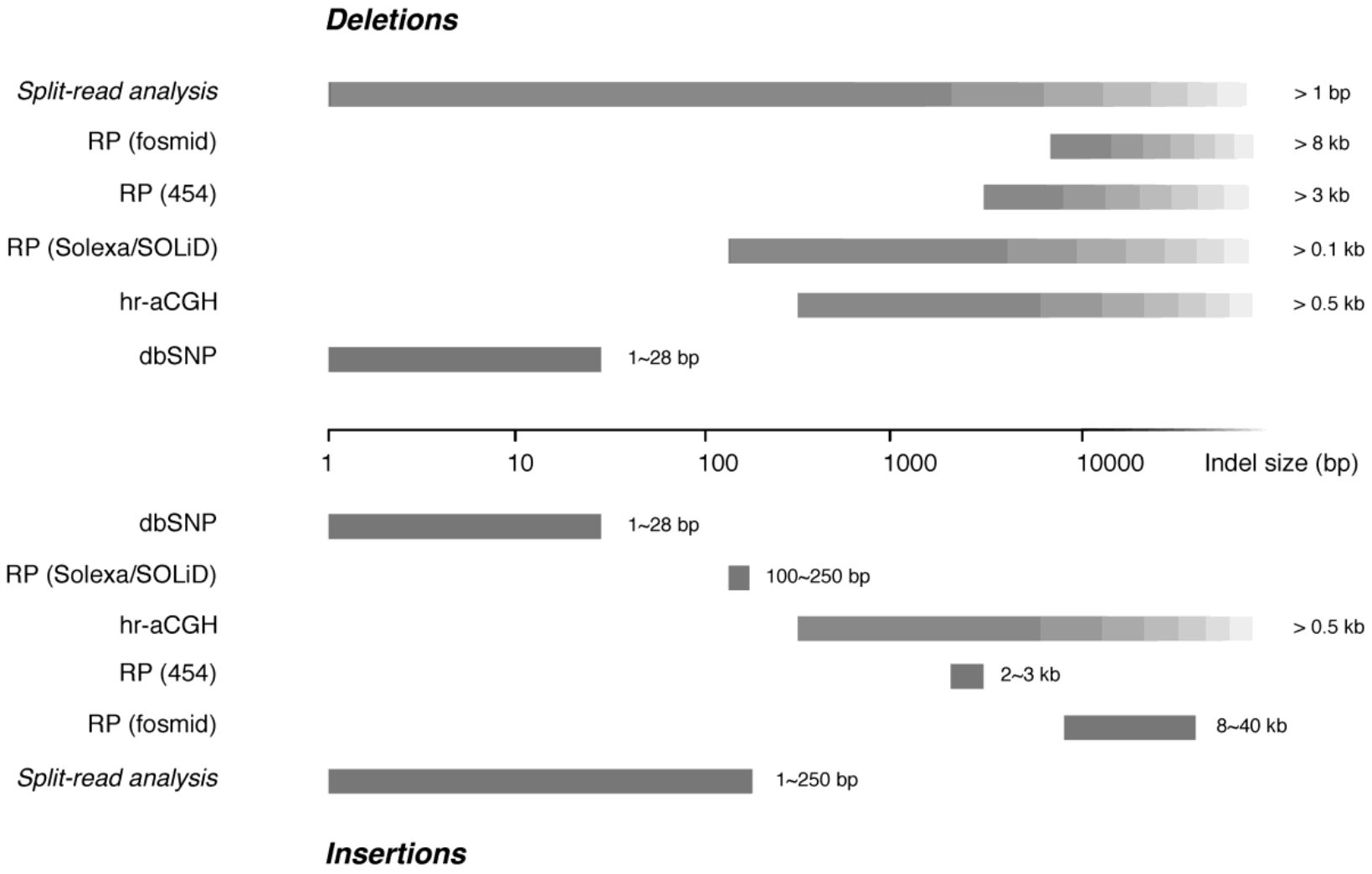
[2] 1000GP Phase3 SV paper. Submided to Nature, 2015.
[3] 1000GP ConsorSum. Submided to Nature, 2015.

Phase 3: Median Autosomal Variant Sites Per Genome

	AFR		AMR		EAS		EUR		SAS	
Samples	661		347		504		503		489	
Mean Coverage	8.2		7.6		7.7		7.4		8.0	
	Var. Sites	**Singletons**	**Var. Sites**	**Singletons**	**Var. Sites**	**Singletons**	**Var. Sites**	**Singletons**	**Var. Sites**	**Singletons**
SNPs	4.31M	14.5k	3.64M	12.0k	3.55M	14.8k	3.53M	11.4k	3.60M	14.4k
Indels	625k	-	557k	-	546k	-	546k	-	556k	-
Large Deletions	1.1k	5	949	5	940	7	939	5	947	5
CNVs	170	1	153	1	158	1	157	1	165	1
MEI (Alu)	1.03k	0	845	0	899	1	919	0	889	0
MEI (LINE1)	138	0	118	0	130	0	123	0	123	0
MEI (SVA)	52	0	44	0	56	0	53	0	44	0
MEI (MT)	5	0	5	0	4	0	4	0	4	0
Inversions	12	0	9	0	10	0	9	0	11	0
NonSynon	12.2k	139	10.4k	121	10.2k	144	10.2k	116	10.3k	144
Synon	13.8k	78	11.4k	67	11.2k	79	11.2k	59	11.4k	78
Intron	2.06M	7.33k	1.72M	6.12k	1.68M	7.39k	1.68M	5.68k	1.72M	7.20k
UTR	37.2k	168	30.8k	136	30.0k	169	30.0k	129	30.7k	168
Promoter	102k	430	84.3k	332	81.6k	425	82.2k	336	84.0k	430
Insulator	70.9k	248	59.0k	199	57.7k	252	57.7k	189	59.1k	243
Enhancer	354k	1.32k	295k	1.05k	289k	1.34k	288k	1.02k	295k	1.31k
TFBS	927	4	759	3	748	4	749	3	765	3
Filtered LoF	182	4	152	3	153	4	149	3	151	3
HGMD-DM	20	0	18	0	16	1	18	2	16	0
GWAS	2.00k	0	2.07k	0	1.99k	0	2.08k	0	2.06k	0
ClinVar	28	0	30	1	24	0	29	1	27	1

[3] 1000GP Consortium. Submitted to Nature,

Different Approaches Work Differently on Different Events



[Zhang et al. ('11) *BMC Genomics*]