GLOBAL HAPLOTYPE RECONSTRUCTION OF HIV-1

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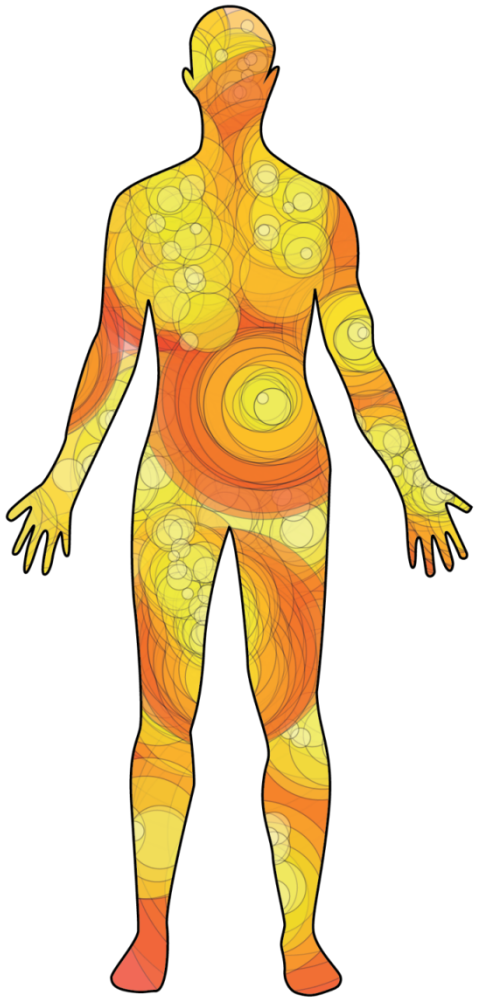
SANGER-SEQUENCING VS. NEXT-GENERATION SEQUENCING (NGS)

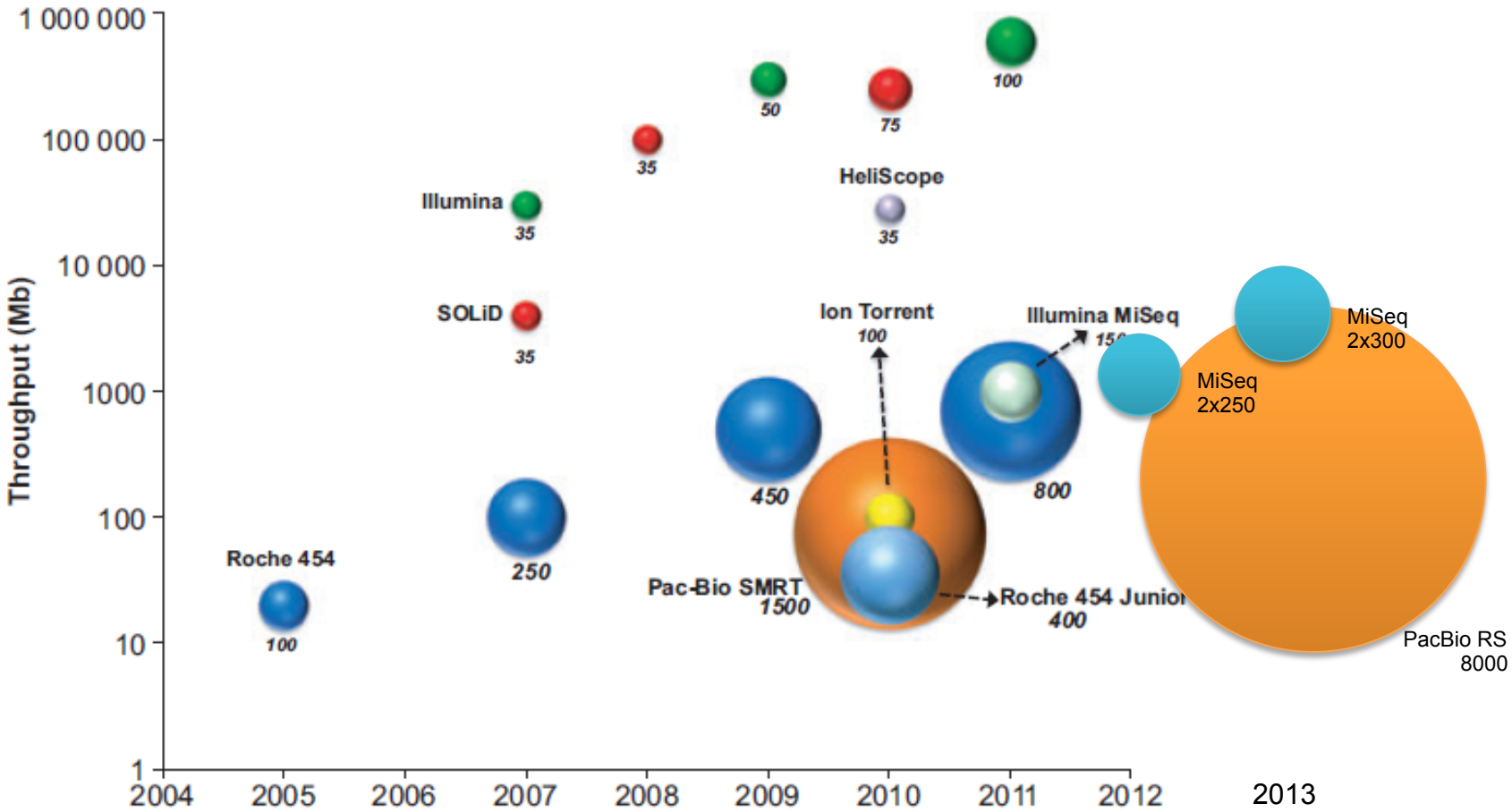


INFECTED WITH A POPULATION OF STRAINS

SANGER - CONSENSUS

NGS – INDIVIDUAL SEQUENCES

OVERVIEW OF NGS PLATFORMS



Shokralla S *et al*, Mol Ecol. 2012 Apr;21(8):1794-805

LOCAL AND GLOBAL HAPLOTYPE RECONSTRUCTION

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      ↓     ↓     ↓     ↓     ↓       ↓     ↓       ↓
A:  CCTGAAATCACTCTATGGCAACGACCCATCGTCACAATAAAGATAGGG  60%
B:  CCTCAAATCACTCTTTGGCAACGACGCATCGTCACAATATAGATAGGA  30%
C:  CCTCAAATCTCTCTTTGGCACCGACCCATCGTCCCAATAAAGATAGGG  10%
```

NGS

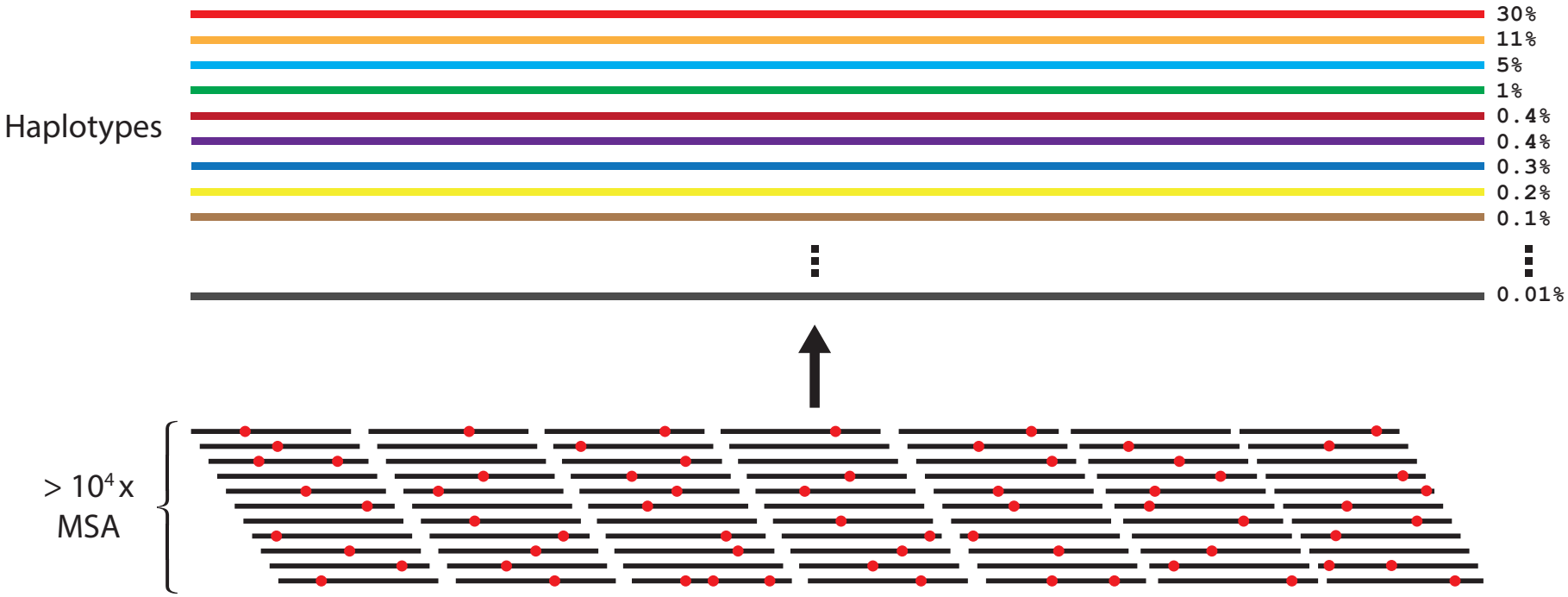
```
 1  CCTGAAATCACTCTATGGCA
 2     GAAAACACTCTATGGCAACG
 3        ATCACTCTTTGGCAAGGCCG
 4         TCACTCTATGGCAACGACCC
 5          CTCTTTTGGGCACCGACCCA
 6              CTATGGTAACGACCCATCGT
 7               TATGGCAACGAGCCATCGTC
 8                ATGGCACGGACCCATCCCCC
 9                 TGGCAACGACGCATCGTCAC
10                    CAACGACGCATCGTCACAAT
11                    CAACGACGCATCGTCACGAT
12                      AACGACCCTTCGTCACAATA
13                        CGACCCATCGTCTCAATAAA
14                            GCATCGTCACAATATAGAGA
15                             CATCGTCACAAAATAGATAG
16                               TCGTCACAATAAAGATAGGG
17                                  TCACAATAAAGATGGGG
18                                    CCAATAAAGATAGGG
19                                       AATAAGGATGGGG
20                                          ATAGATAGGA
```

errors

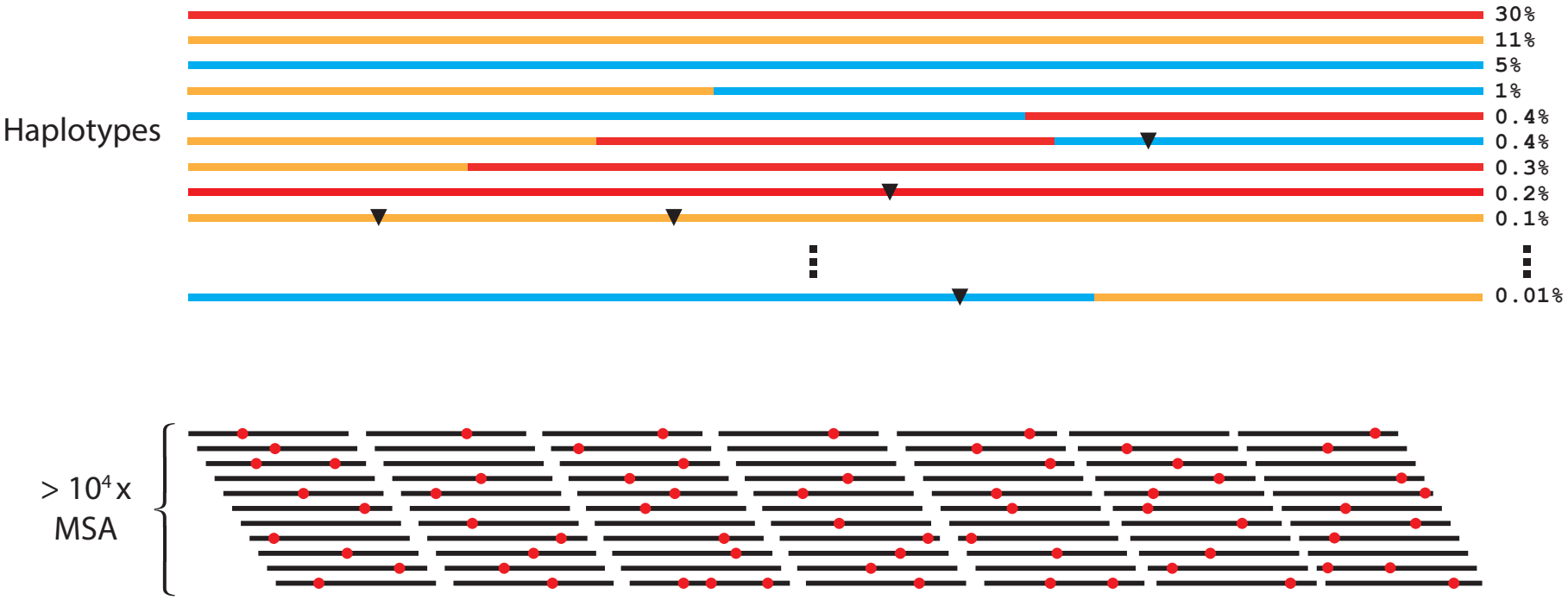
···· global
--- local
— SNV

Beerenwinkel et al. Front Microbiol. 2012;3:329

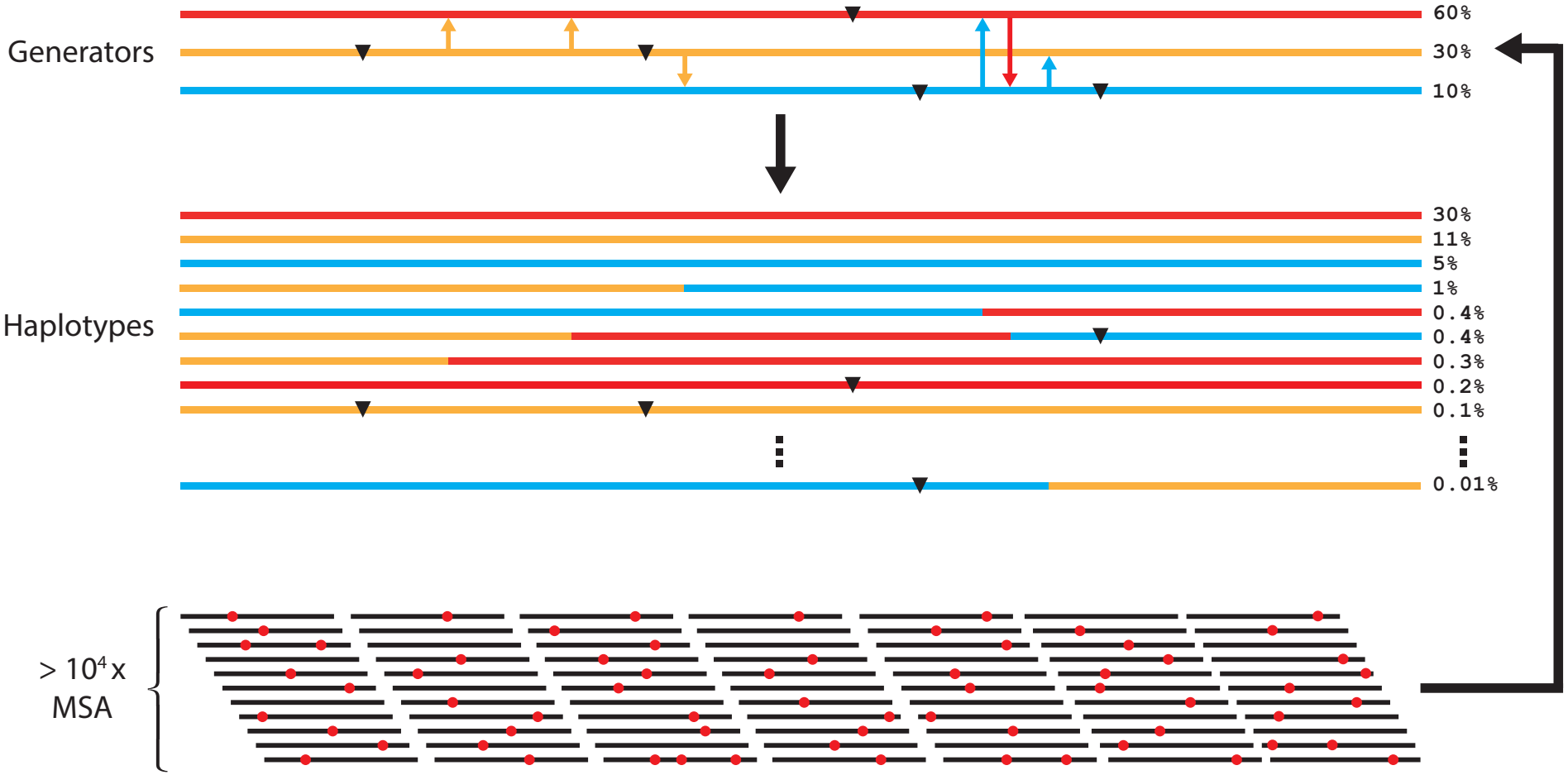
GLOBAL PHASING STRATEGY, OTHER APPROACHES



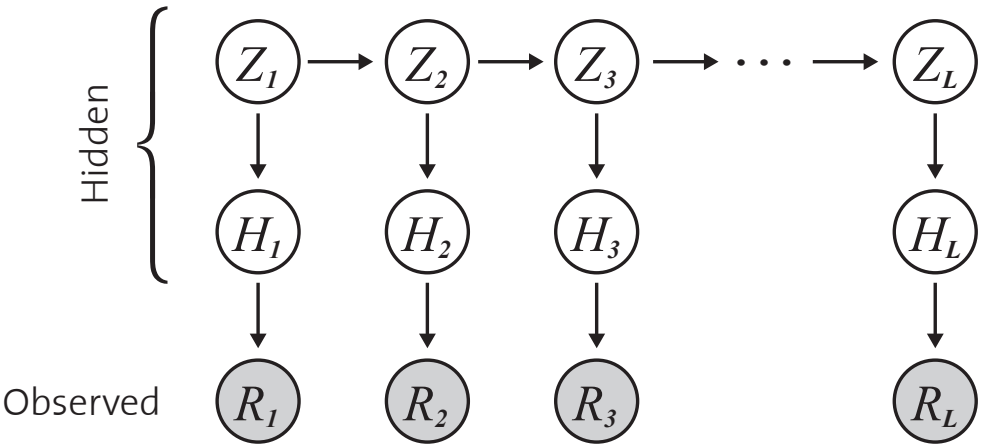
GLOBAL PHASING STRATEGY, NEW

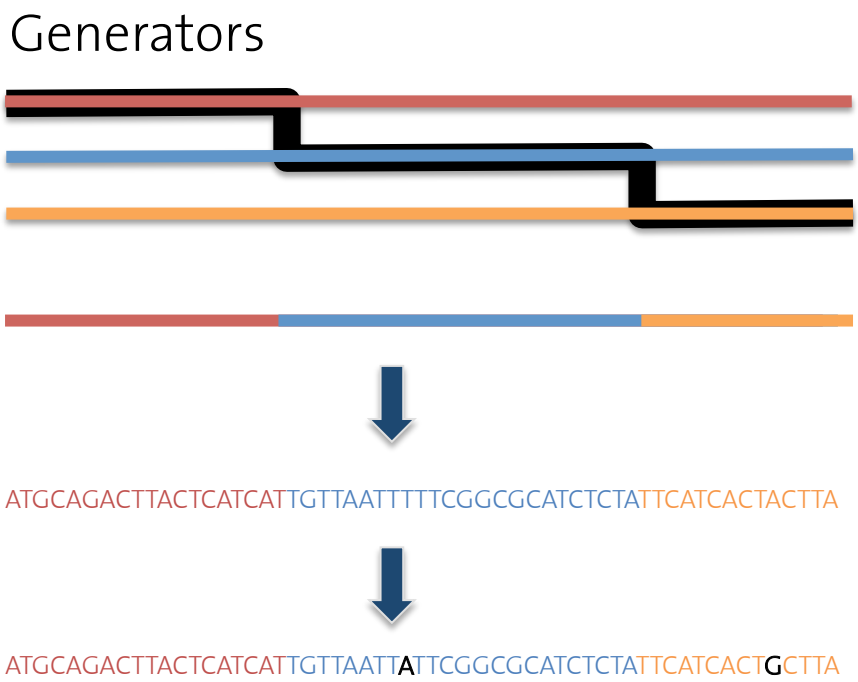


GLOBAL PHASING STRATEGY, NEW



MODEL

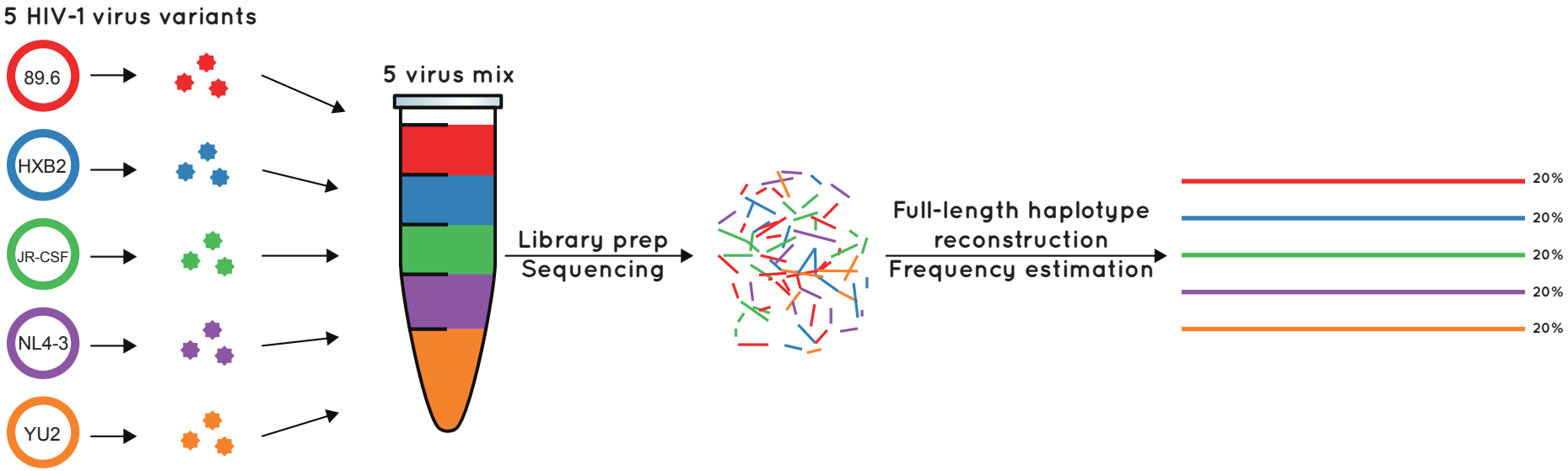




- Maximum a posteriori estimation:

$$P(\theta \mid R) \propto P(R \mid \theta) P(\theta)$$

5-VIRUS-MIX


5 HIV-1 virus variants
89.6
HXB2
JR-CSF
NL4-3
YU2
5 virus mix
Library prep
Sequencing
Full-length haplotype
reconstruction
Frequency estimation
20%
20%
20%
20%
20%

METHOD: SAMPLE PREPARATION FOR FULL-LENGTH SEQUENCING

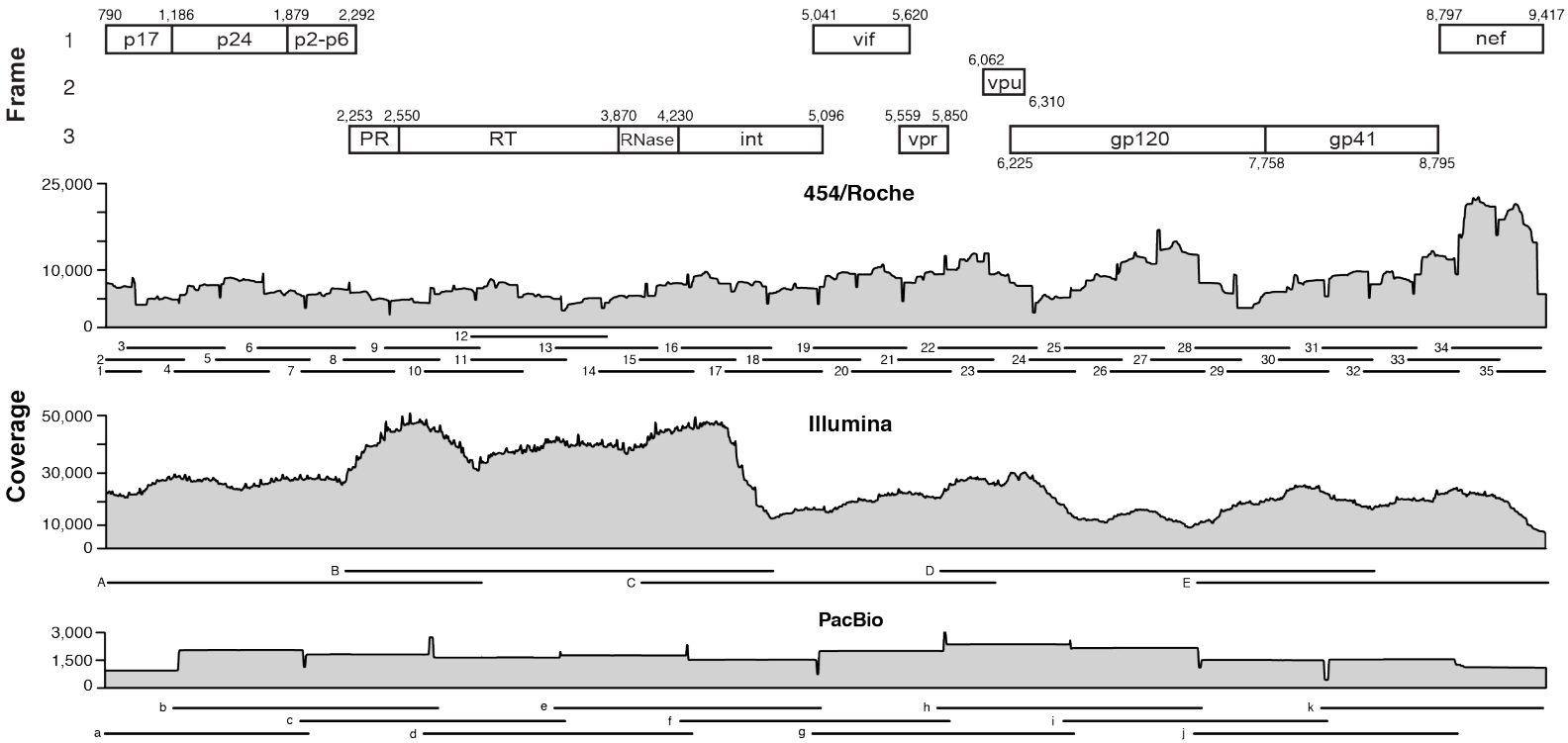
RNA isolation

cDNA synthesis of protease only	cDNA synthesis with 35 RT primers	cDNA synthesis with 5 RT primers	cDNA synthesis with 6 RT primers
End-point dilution	35 separate PCRs	5 separate PCRs	11 separate PCRs
qPCRs (one of five positive)		random fragmentation and adaptor ligation	
Sanger sequencing	454/Roche pyrosequencing	Illumina paired-end sequencing	Pacific Biosciences Single-molecule sequencing

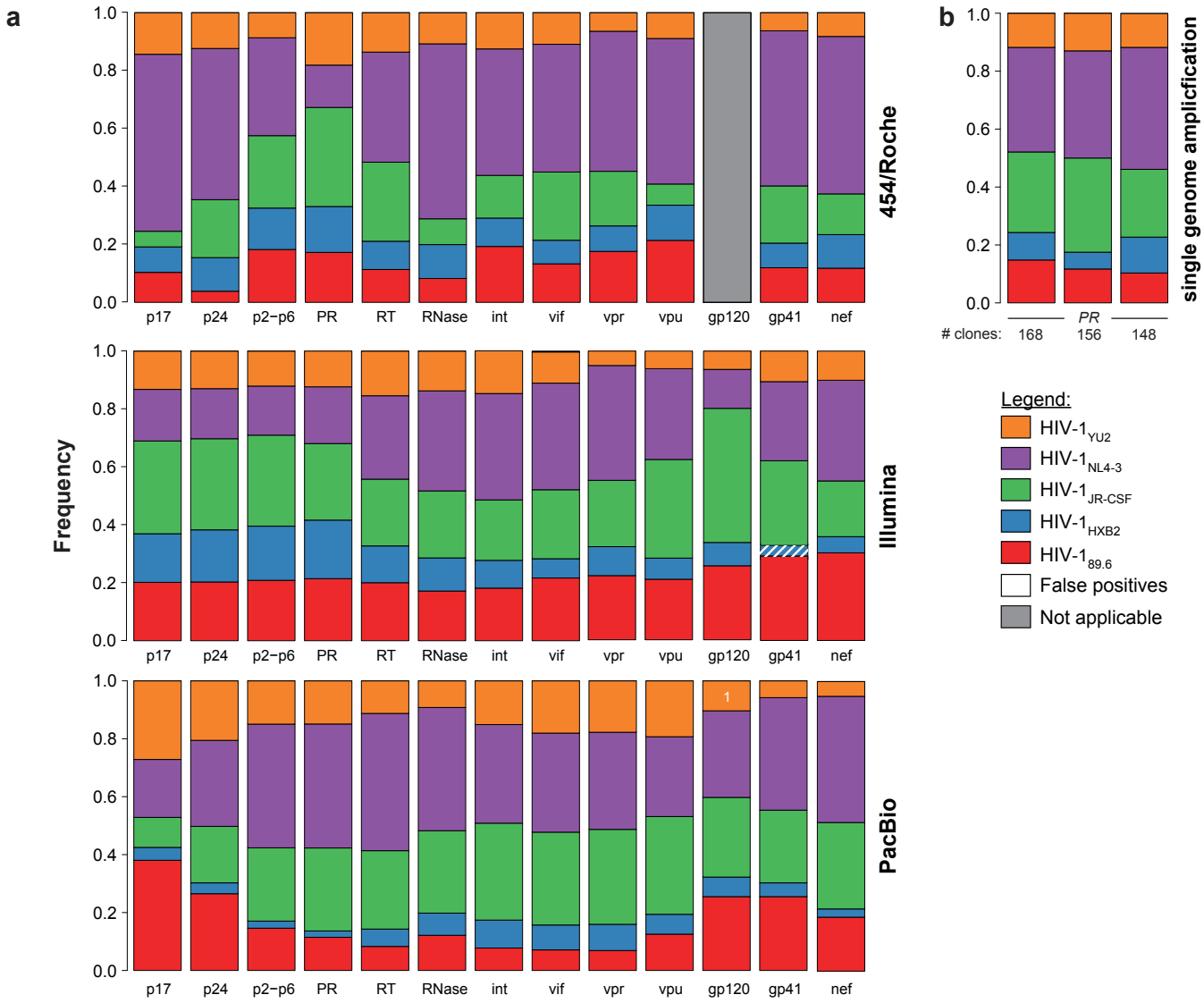
GOLD STANDARD

AMPLICON LAYOUT AND READ COVERAGE

Technology	Amplicons	~ Length (bp)	~ Overlap (bp)
454/Roche	35	510	250
Illumina MiSeq	5	2,500	750
PacBio RS	11	1,500	750

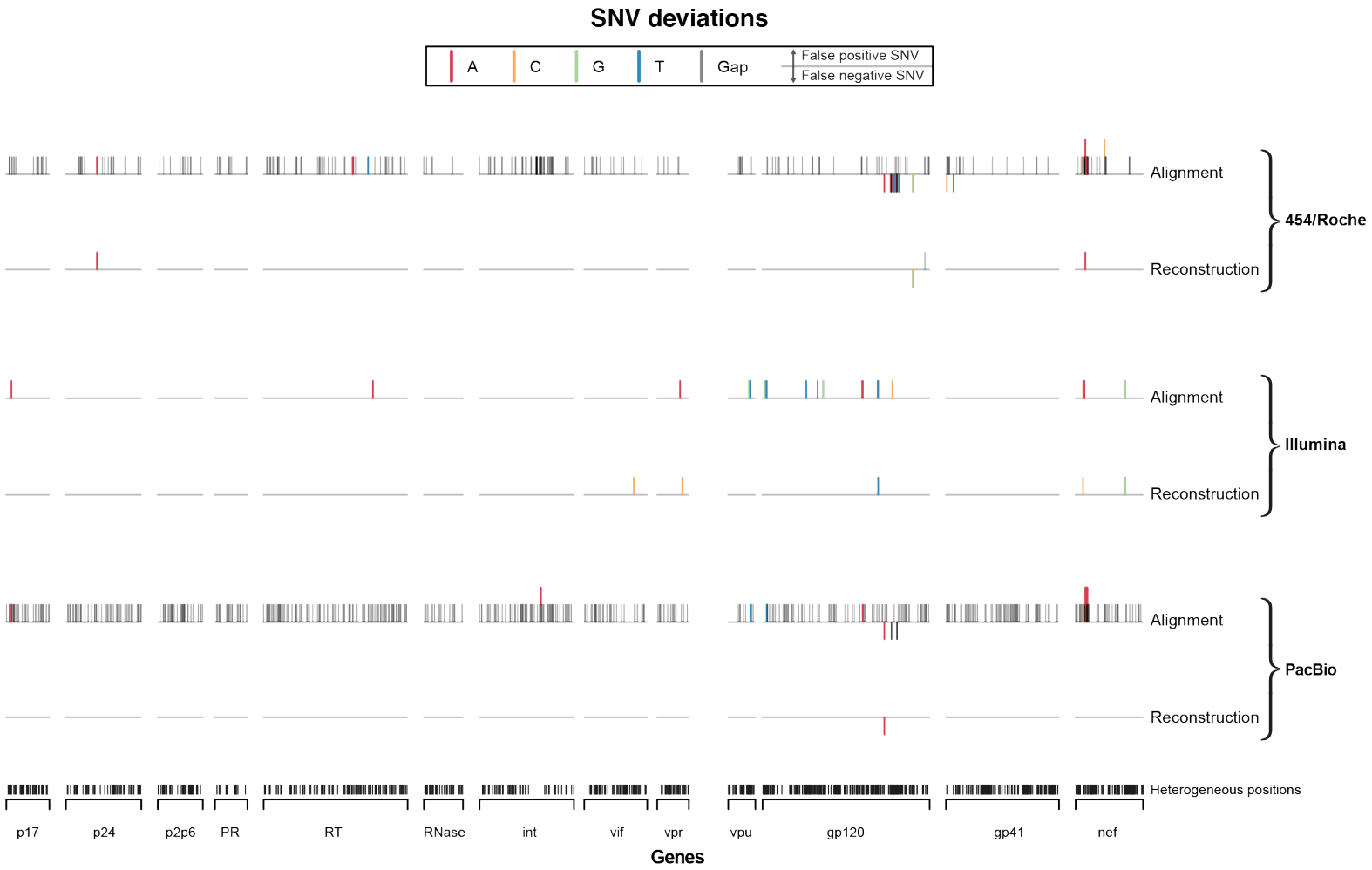


GENE-WISE RECONSTRUCTION



	p17	p24	p2−p6	PR	RT	RNase	int	vif	vpr	vpu	gp120	gp41	nef
Gene length (bp):	396	693	413	297	1,320	350	866	578	291	248	1,533	1,037	620
Min distance (%):	3.77	1.72	2.17	1.34	2.49	2.49	1.73	3.45	3.08	2.82	1.89	0.96	1.44
Max distance (%):	6.80	3.60	4.34	4.69	3.33	5.54	2.88	5.52	6.50	16.93	11.60	8.09	13.36
Mean distance (%):	5.23	2.82	3.30	2.75	2.88	4.62	2.29	4.81	4.96	12.82	9.50	6.29	8.06

NAÏVE SNV CALLING



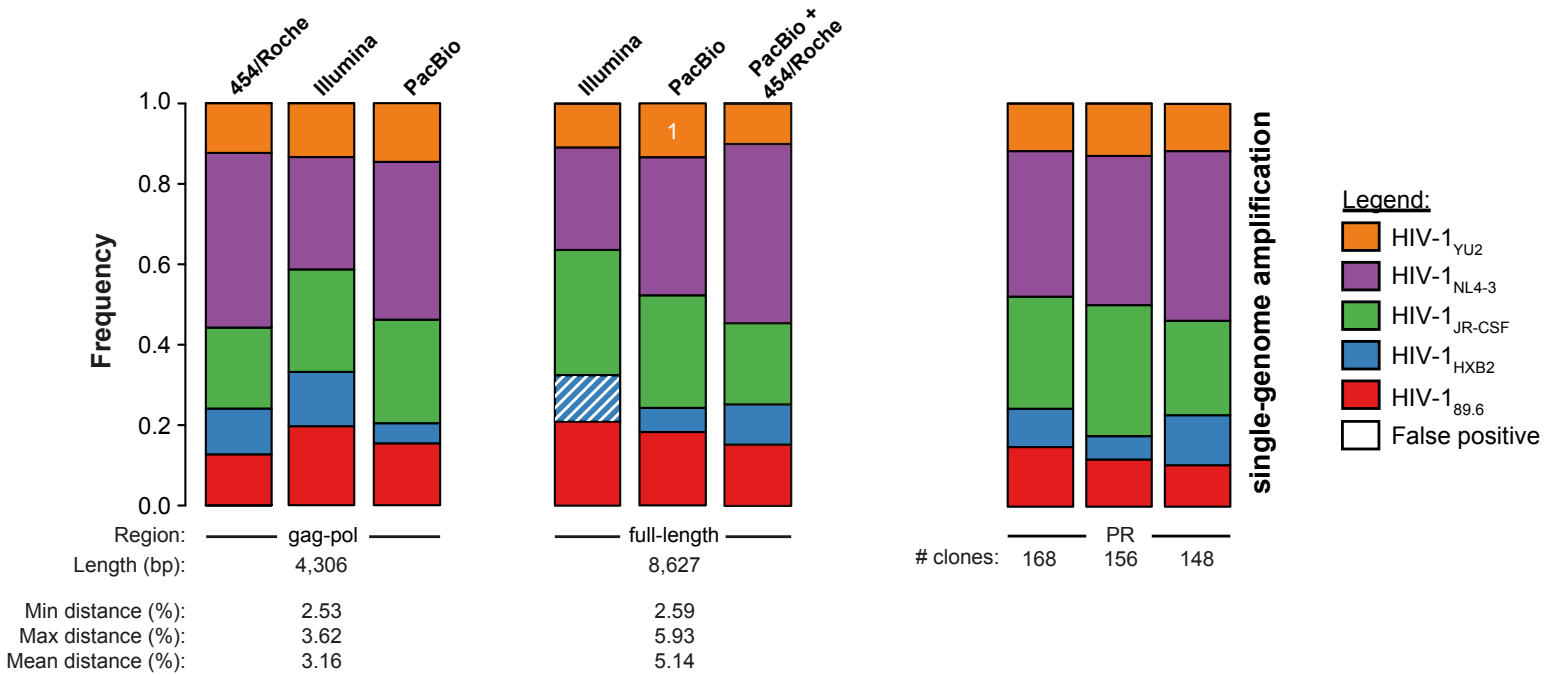
GENE-WISE RECONSTRUCTION

Estimated frequencies of reconstructed haplotypes across genes

	estimated frequencies % (mean±sd)				
Sequencing technique	HIV-$1_{89.6}$	HIV-1_{HXB2}	HIV-1_{JR-CSF}	HIV-1_{NL4-3}	HIV-1_{YU2}
454/Roche	13.7±5.1	11.0±2.4	18.2±8.6	46.2±12.9	10.9±3.5
Illumina	22.1±3.9	11.2±5.9	28.0±7.3	27.3±9.2	11.1±3.2
PacBio	16.5±9.7	5.8±2.6	27.2±6.4	35.9±7.9	14.6±6.2
SGA	12.3±2.3	9.2±3.3	27.9±4.6	38.4±3.2	12.2±0.7

NGS estimates **do not differ significantly** from SGA estimates

GLOBAL RECONSTRUCTION OF FUNCTIONAL REGIONS



Full-length variance of **0.4%** for 100 bootstrap samples

LESSONS LEARNED

- Naïve SNV calling is insufficient
- Experimental design:
  - Amplicon layout & overlap
  - Read length
  - Coverage
- Full-length reconstruction **is possible** with 454/Roche, Illumina, and PacBio
- Global frequency **estimates are reliable**

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