Quality Control in Sequence Data

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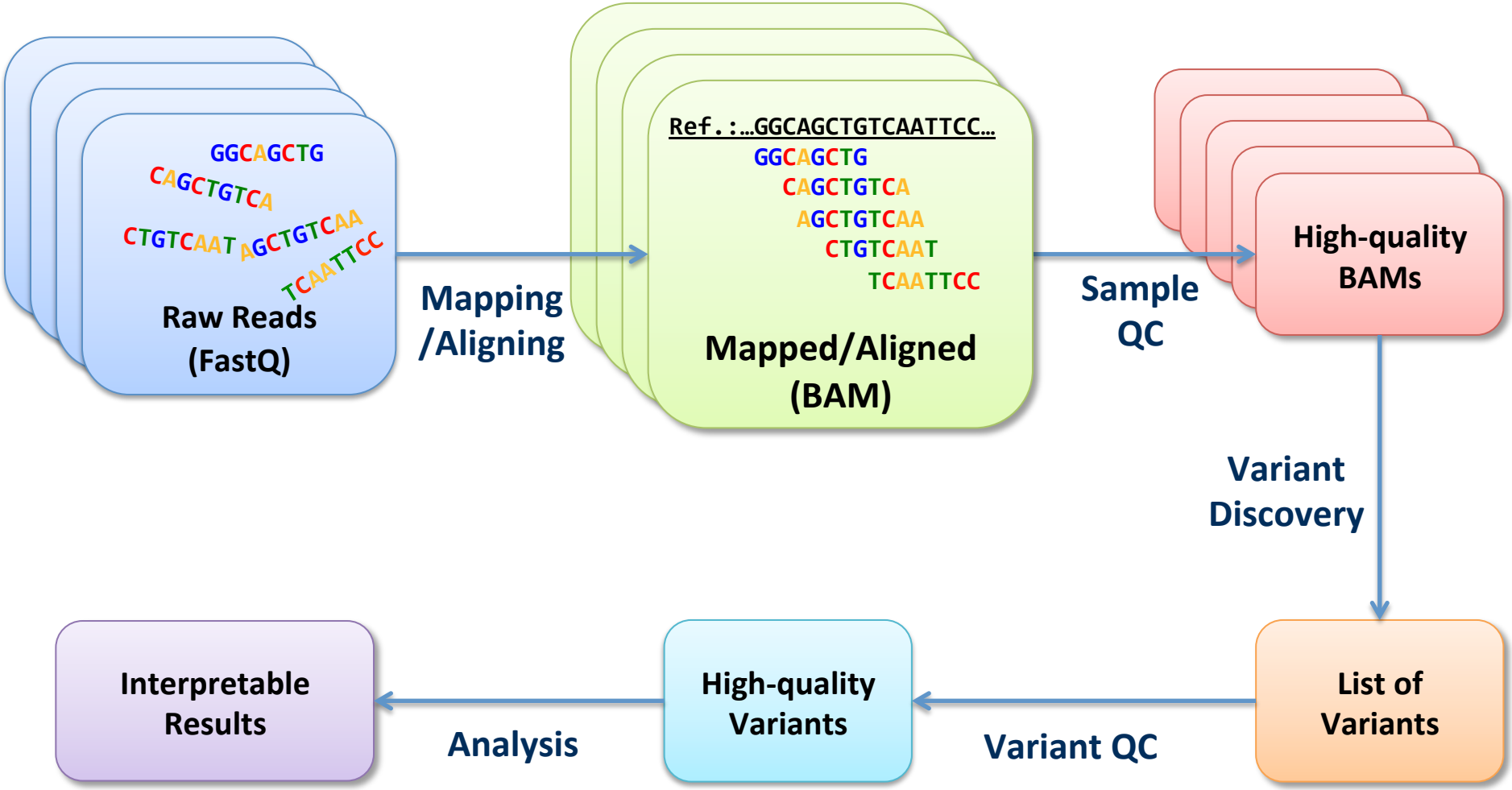
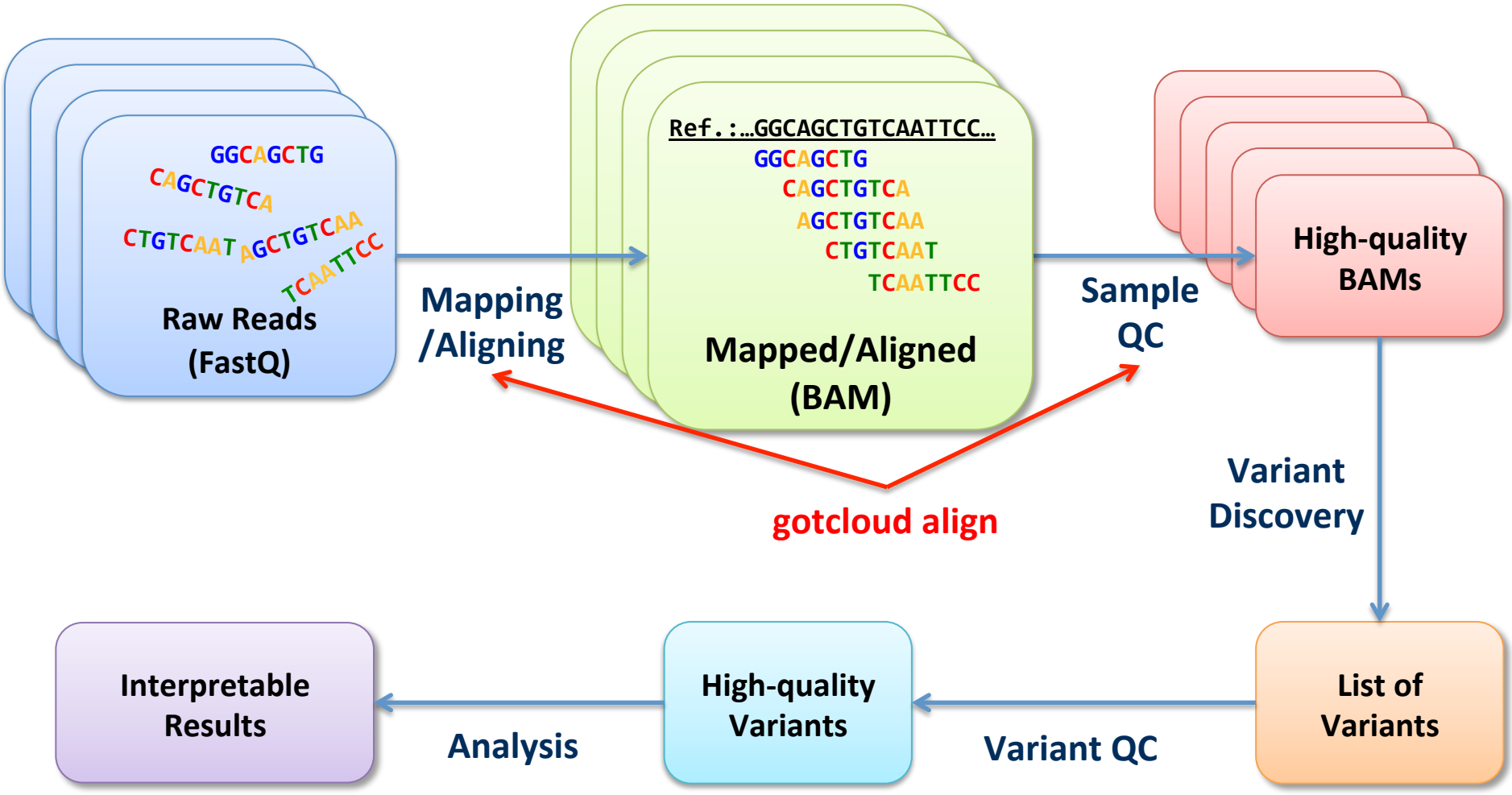
MICHIGAN

CENTER FOR STATISTICAL GENETICS

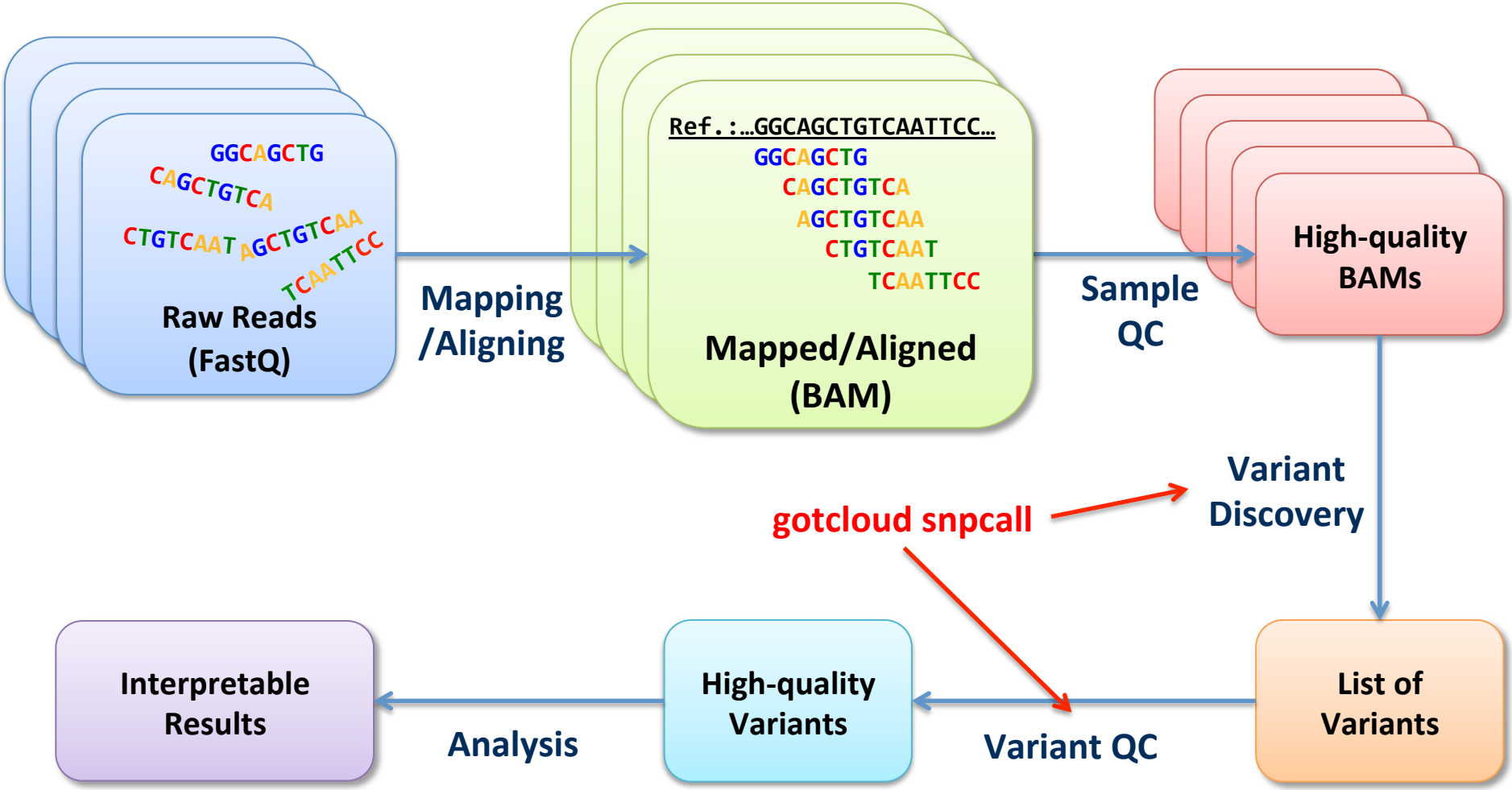
(Re)sequencing Data Analysis Flow



(Re)sequencing Data Analysis Flow



(Re)sequencing Data Analysis Flow



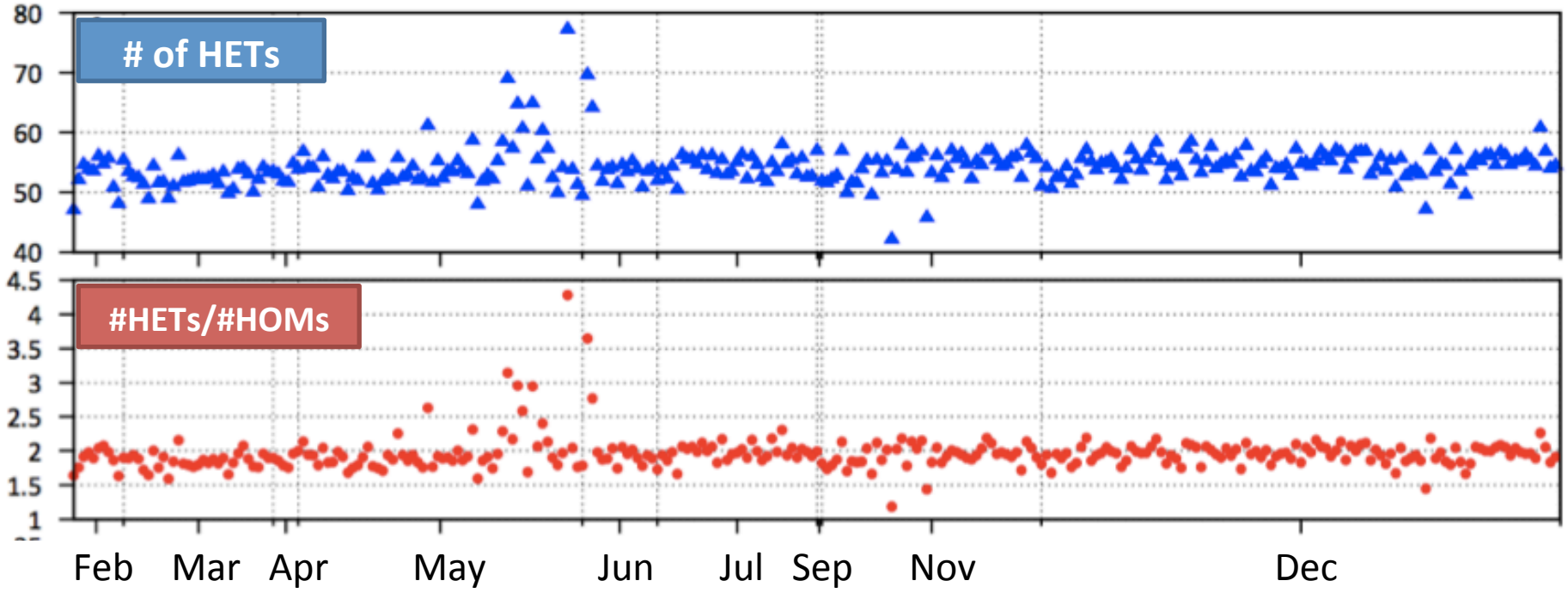
Part 1.

Detecting and estimating sample contamination

DNA Sample Contamination

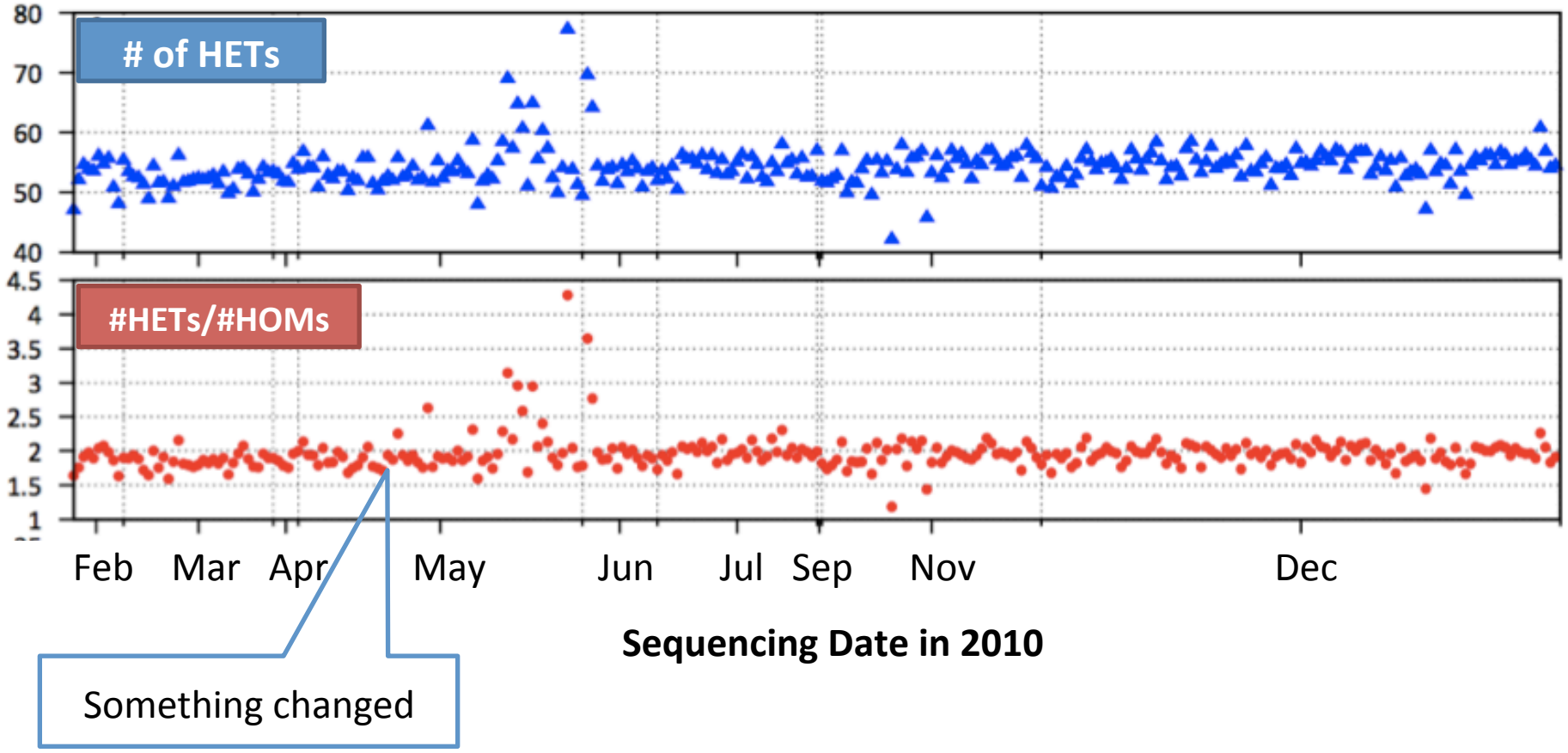
- Sample contamination is a *common* problem.
- Sample contamination degrades genotype accuracy and power of analysis.
- Timely feedback about contamination is important.

Contamination in Sequence Data



Sequencing Date in 2010

Contamination in Sequence Data



Reference-Aligned Sequence Reads

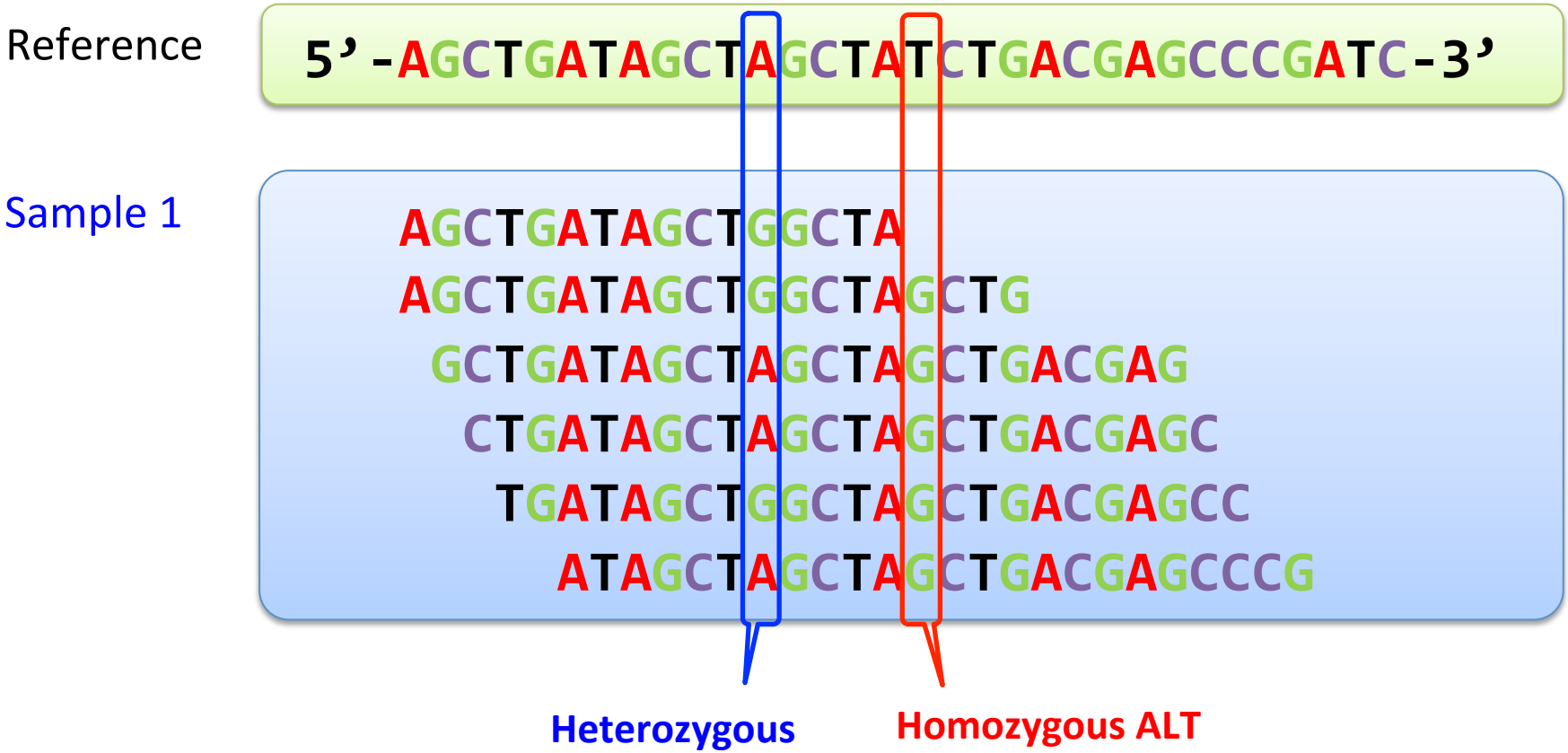
Reference

5’-AGCTGATAGCTAGCTATCTGACGAGCCCGATC-3’

Sample 1

AGCTGATAGCTGGCTA
AGCTGATAGCTGGCTAGCTG
GCTGATAGCTAGCTAGCTGACGAG
CTGATAGCTAGCTAGCTGACGAGC
TGATAGCTGGCTAGCTGACGAGCC
ATAGCTAGCTAGCTGACGAGCCCG

Identifying SNPs



Base Distribution in Two Samples

Reference

5’-AGCTGATAGCTAGCTATCTGACGAGCCCGATC-3’

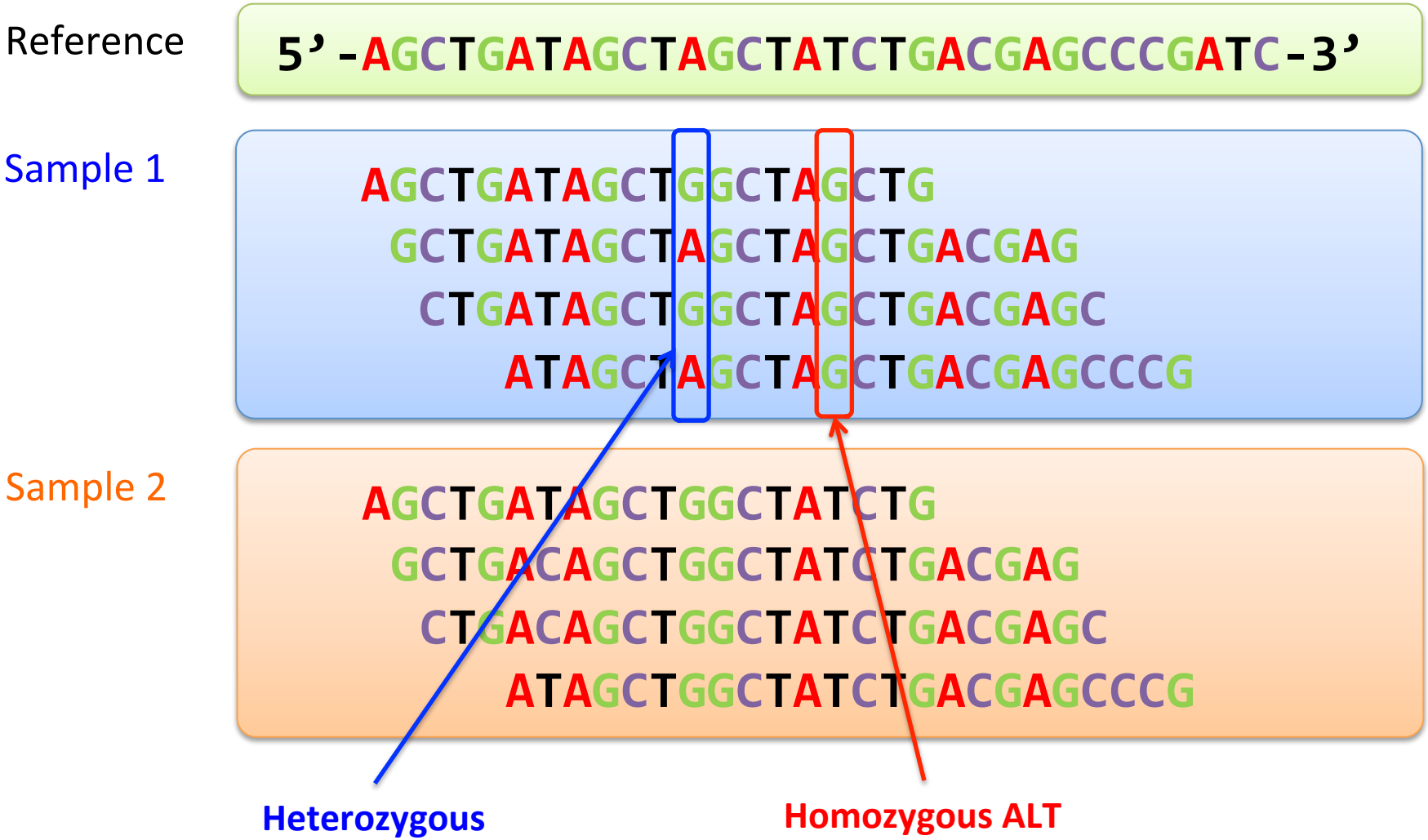
Sample 1

AGCTGATAGCTGGCTAGCTG
GCTGATAGCTAGCTAGCTGACGAG
CTGATAGCTGGCTAGCTGACGAGC
ATAGCTAGCTAGCTGACGAGCCCG

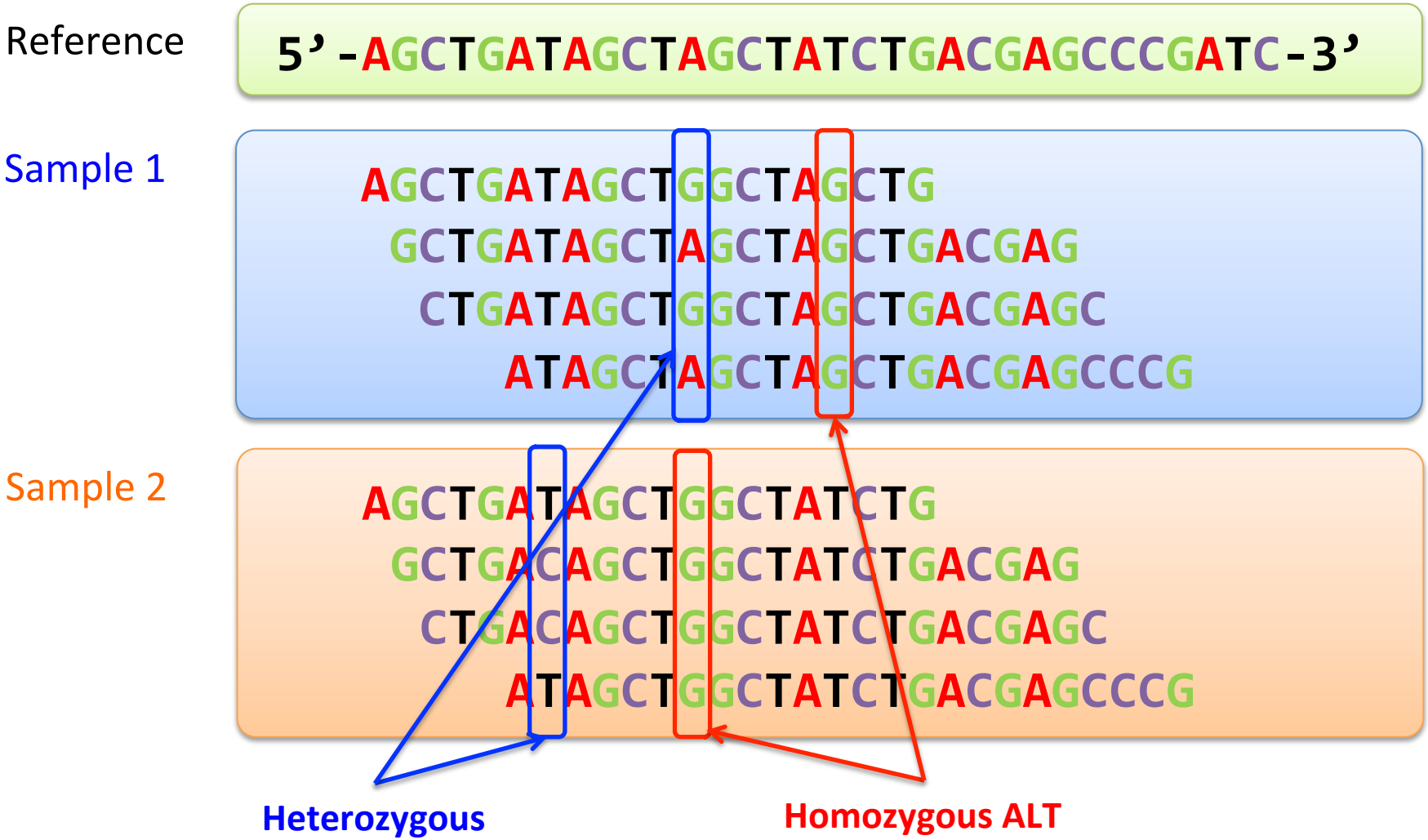
Sample 2

AGCTGATAGCTGGCTATCTG
GCTGACAGCTGGCTATCTGACGAG
CTGACAGCTGGCTATCTGACGAGC
ATAGCTGGCTATCTGACGAGCCCG

Base Distribution in Two Samples



Base Distribution in Two Samples



Contamination: Mixture of Samples

Reference

5’-AGCTGATAGCTAGCTATCTGACGAGCCCGATC-3’

Sample 1+2

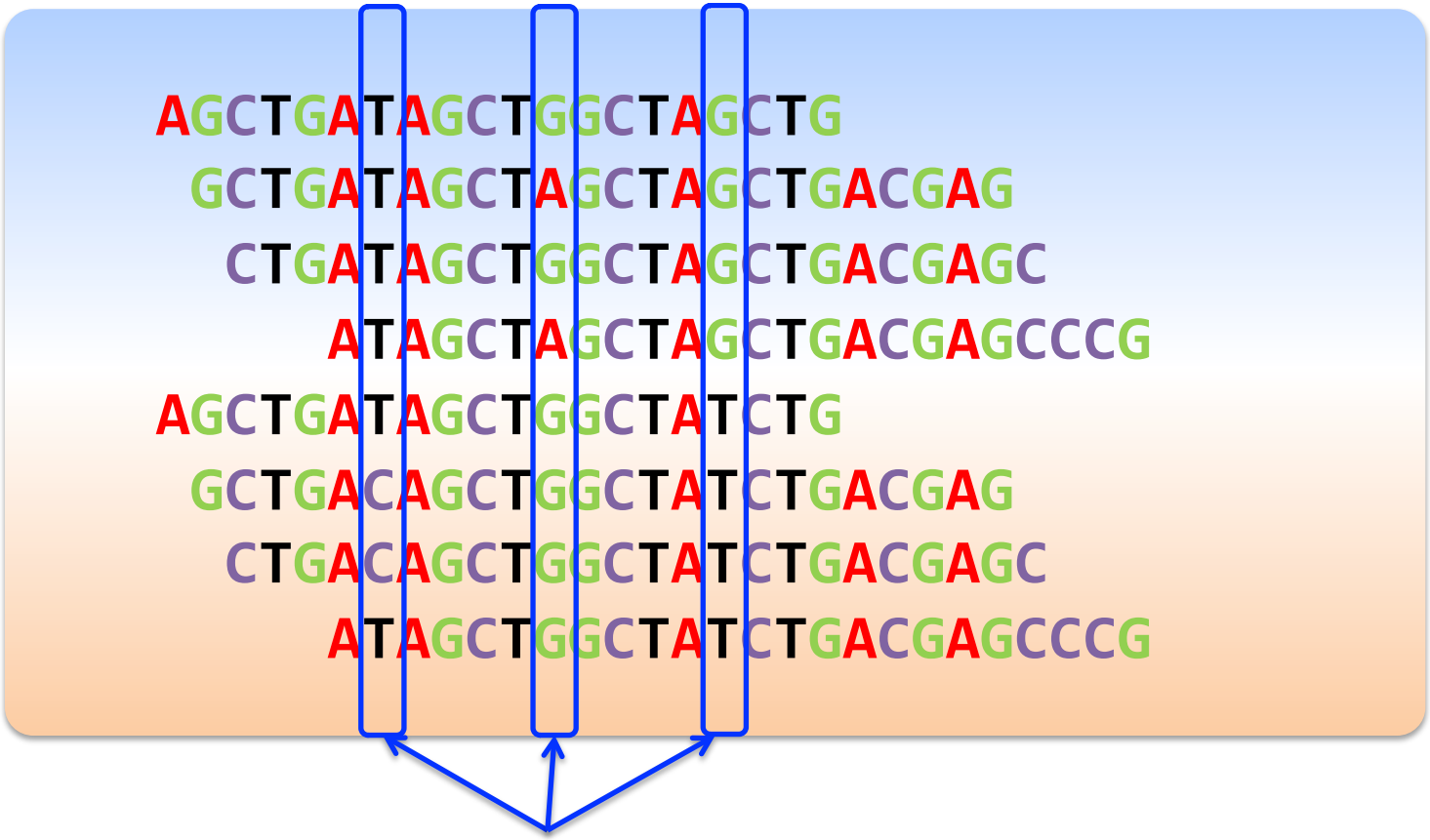
AGCTGATAGCTGGCTAGCTG
GCTGATAGCTAGCTAGCTGACGAG
CTGATAGCTGGCTAGCTGACGAGC
ATAGCTAGCTAGCTGACGAGCCCG
AGCTGATAGCTGGCTATCTG
GCTGACAGCTGGCTATCTGACGAG
CTGACAGCTGGCTATCTGACGAGC
ATAGCTGGCTATCTGACGAGCCCG

Contamination: Excessive Heterozygous SNPs

Reference

5’-AGCTGATAGCTAGCTATCTGACGAGCCCGATC-3’

Sample 1+2



Sample mixture leads to more heterozygosity

Quantifying Sample Contamination

- (# HETs) / (# HOMs)
  - No clear way to quantify the amount of contamination
  - Varies by population (admixed samples)
  - Requires variant calling
- Better solution
  - Estimate amount of contamination directly from base reads.

Likelihood of Sequence Data

- For M SNPs with known genotypes

$$L = \prod_{i}^{M} P(\mathbf{b}_i | G_i)$$

$$= \prod_{i}^{M} \prod_{j=1}^{N_i} P(b_{ij} | G_i)$$

  - Bases at i-th SNP site with N_i reads: $\mathbf{b}_i = (b_{i1}, b_{i2}, ..., b_{iN_i})$
  - Genotype of i-th marker: $G_i \in \{\mathrm{AA}, \mathrm{AB}, \mathrm{BB}\}$

Two-sample Mixture Model

- Likelihood with mixing proportion α

$$L(\alpha) = \prod_{i}^{M} \prod_{j=1}^{N_i} P(b_{ij}|G_i;\alpha)$$

$$= \prod_{i}^{M} \sum_{g_i \in \{AA,AB,BB\}} \prod_{j=1}^{N_i} P(b_{ij}|G_i,g_i;\alpha)P(g_i)$$

$$= \prod_{i}^{M} \sum_{g_i} \prod_{j=1}^{N_i} \{(1-\alpha)P(b_{ij}|G_i) + \alpha P(b_{ij}|g_i)\} P(g_i)$$

  - Estimated contamination: MLE of α

Two-sample Mixture Model

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From population allele frequency under HWE

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Probability of each base read given genotype

Probability of Each Base Read

- Base read observation
  - Reference (A), Alternative (B), or Other (O)
- Probability of observing a base depends on
  - Underlying (true) genotype
  - Occurrence of base read error
- Example
  - P(read = A | genotype = AA, **no error**) = 1
  - P(read = O | genotype = BB, ***error***) = 2/3

(In case of base read error, assume all possibilities are equally likely)

Likelihood Table

- b_{ij} : j-th read at i-th marker ($j = 1 \,..\, N_i$)
- G_i : true genotype of i-th marker
- e_{ij} : occurrence of error (1: error, 0: no error)

G_i	e_{ij}	$P(b_{ij} \mid G_i, e_{ij})$		
		b_{ij} =A	b_{ij} =B	b_{ij} =O
AA	0	1	0	0
	1	0	1/3	2/3
AB	0	1/2	1/2	0
	1	1/6	1/6	2/3
BB	0	0	1	0
	1	1/3	0	2/3

Computing Full Likelihood

- Likelihood over M markers :

$$L(\alpha) = \prod_{i}^{M} \sum_{g_i} \prod_{j=1}^{N_i} P(b_{ij}|G_i, g_i; \alpha) P(g_i)$$

$$= \prod_{i}^{M} \sum_{g_i} \prod_{j=1}^{N_i} \sum_{e_{ij} \in \{0,1\}} P(b_{ij}|G_i, g_i, e_{ij}; \alpha) P(e_{ij}) P(g_i)$$

- $P(e_{ij})$: probability of base read error, from Phred score Q_{ij}

$$P(e_{ij} = 1) = 10^{-\frac{Q_{ij}}{10}}$$

Estimation with Sequence Data Only

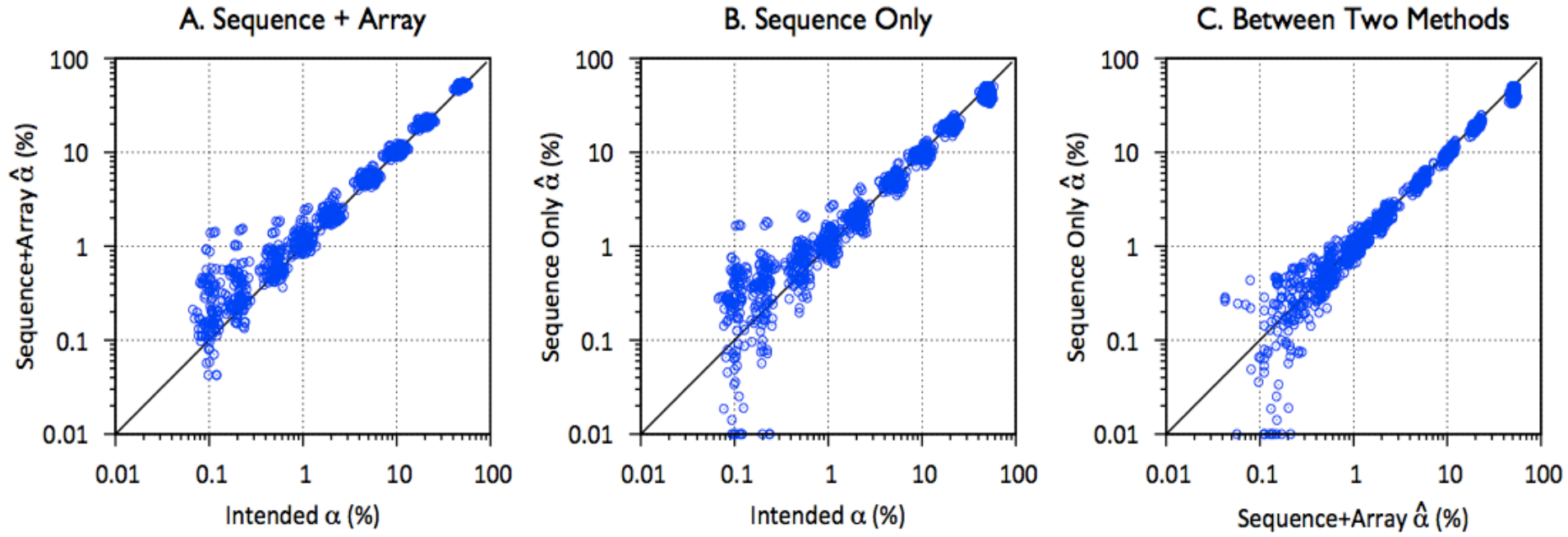
- If sequenced sample does not have external genotypes
  - Model both genotypes from population allele frequency

- Latent variables
  - G_i : true genotype of the contaminated sample
  - g_i : true genotype of the contaminating sample

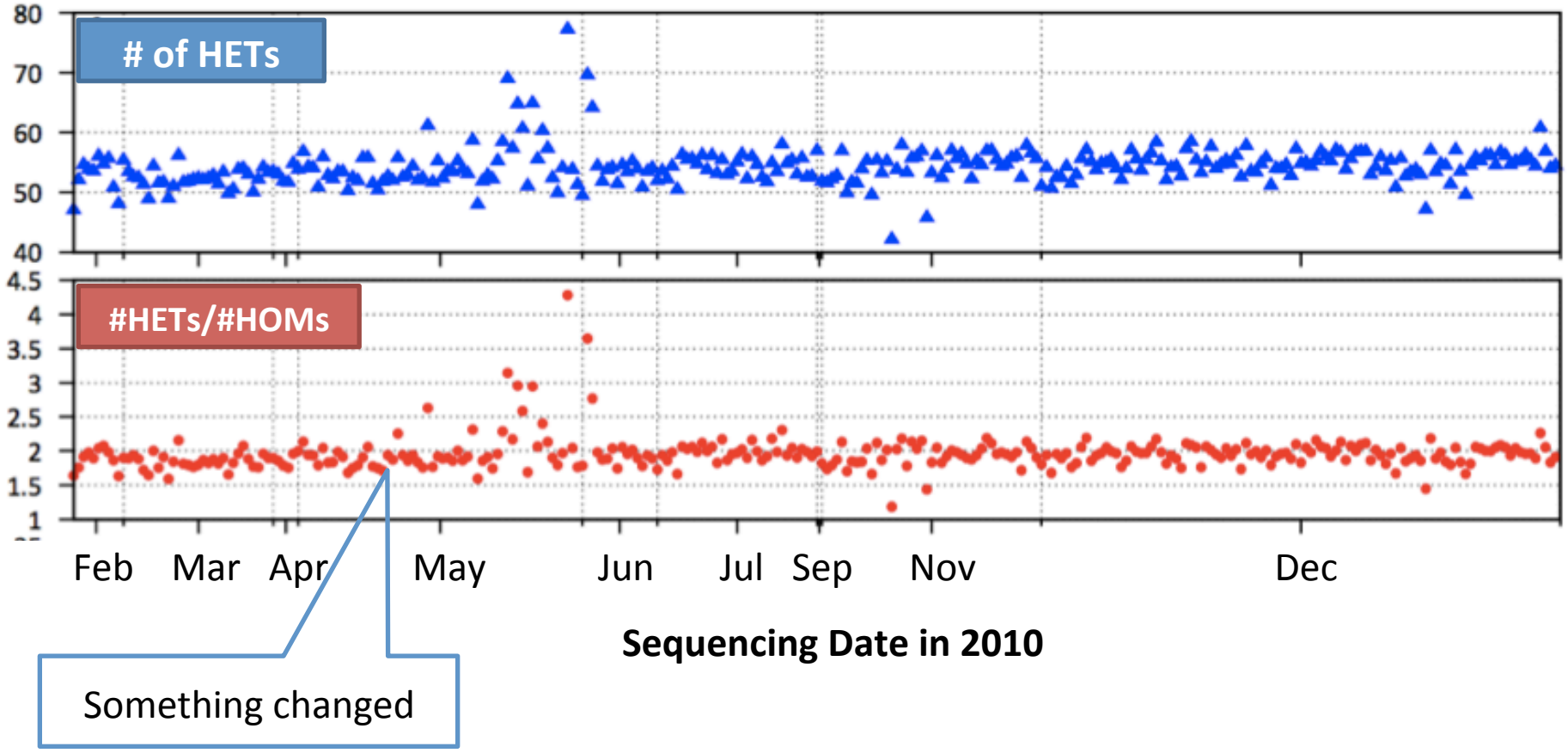
$$L(\alpha) = \prod_{i}^{M} \sum_{G_i} \sum_{g_i} \prod_{j}^{N_i} P(b_{ij}|G_i, g_i; \alpha) P(G_i) P(g_i)$$

Results: Simulation

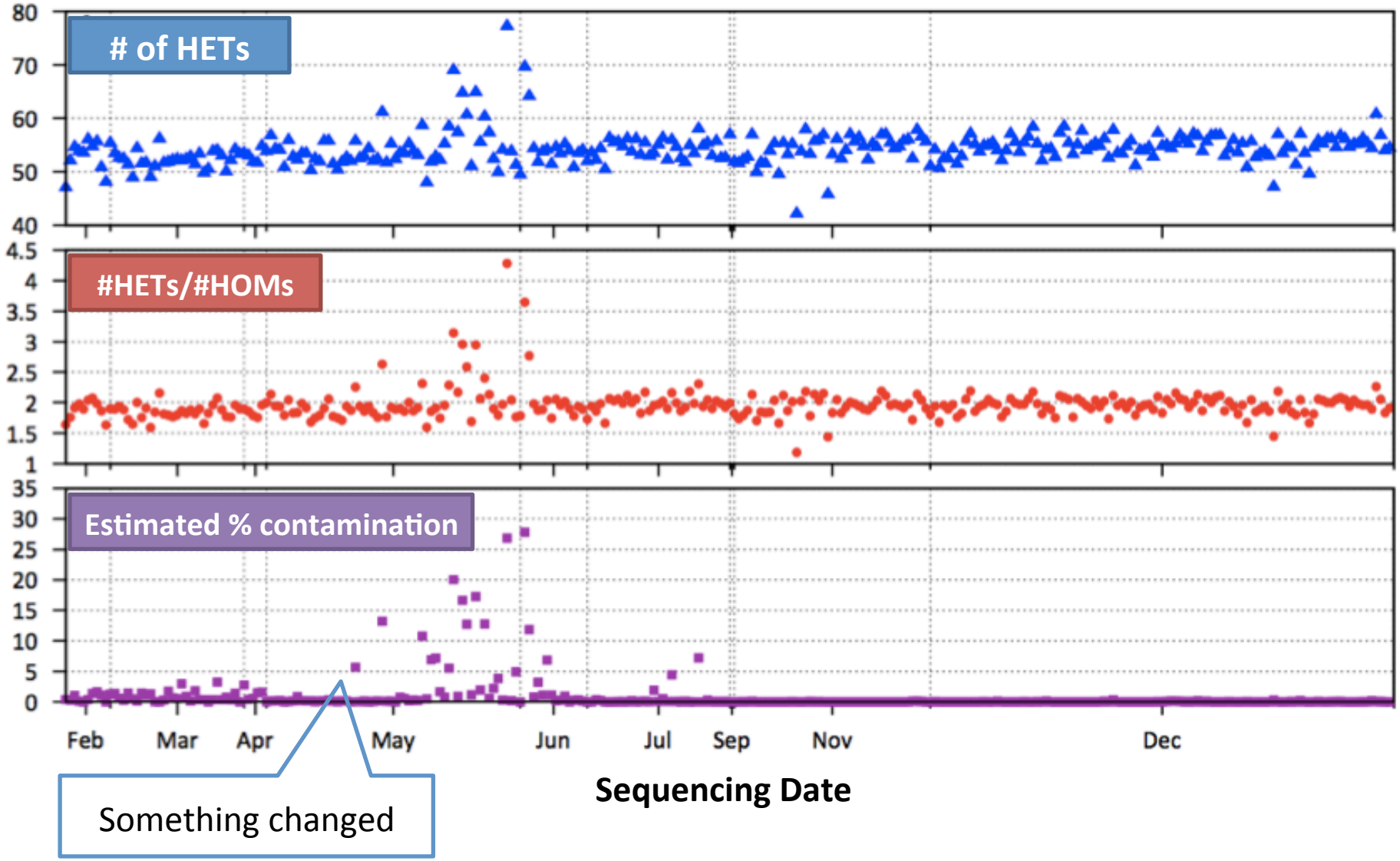
- Simulated contamination
  - Randomly mixed reads from two samples in 1000 Genomes
  - Simulated from 0.1% to 50% contamination



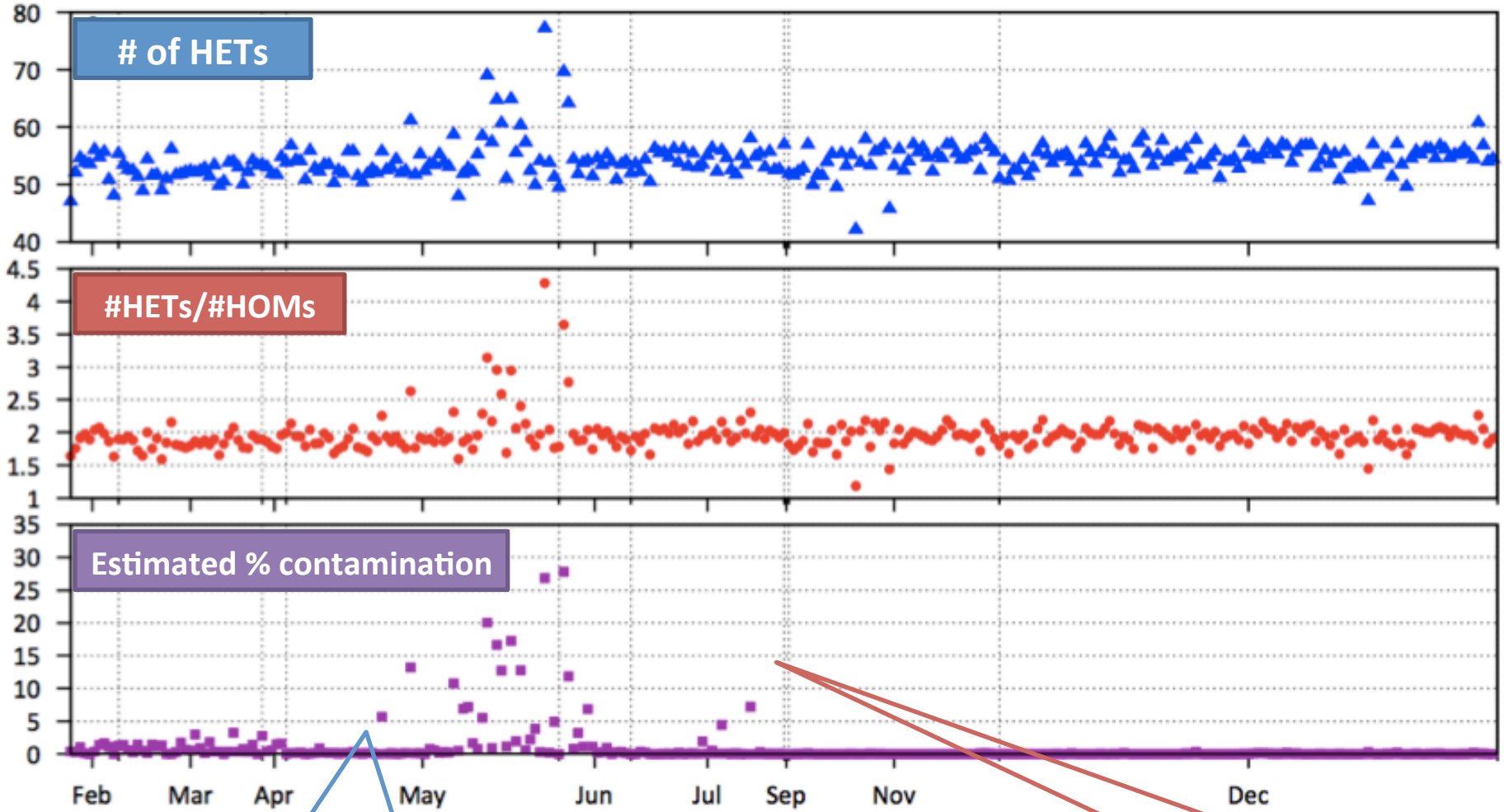
Contamination in Sequence Data



Results: Real Data



Results: Real Data



gotcloud Sample QC Steps after Alignment

- Remove duplicate reads
- Base quality recalibration
  - Adjust reported base quality with empirically measured quality
- BAM quality report (QPLOT)
- Check sample contamination (verifyBamID)

```
cd ~goo/gotcloudTutorial/QCFiles
ls *.selfSM
tail *.selfSM
```

gotcloud Example

```
> tail ~goo/gotcloudTutorial/QCFiles/*.selfSM
#SEQ_ID         RG      CHIP_ID         #SNPS   #READS  AVG_DP  FREEMIX
HG00096         ALL     NA              36298   392     0.01    0.00000

FREELK1         FREELK0         FREE_RH         FREE_RA
86.22           86.22           NA              NA

CHIPMIX         CHIPLK1         CHIPLK0         CHIP_RH         CHIP_RA
NA              NA              NA              NA              NA

DPREF   RDPHET  RDPALT
NA      NA      NA
```

http://genome.sph.umich.edu/wiki/VerifyBamID

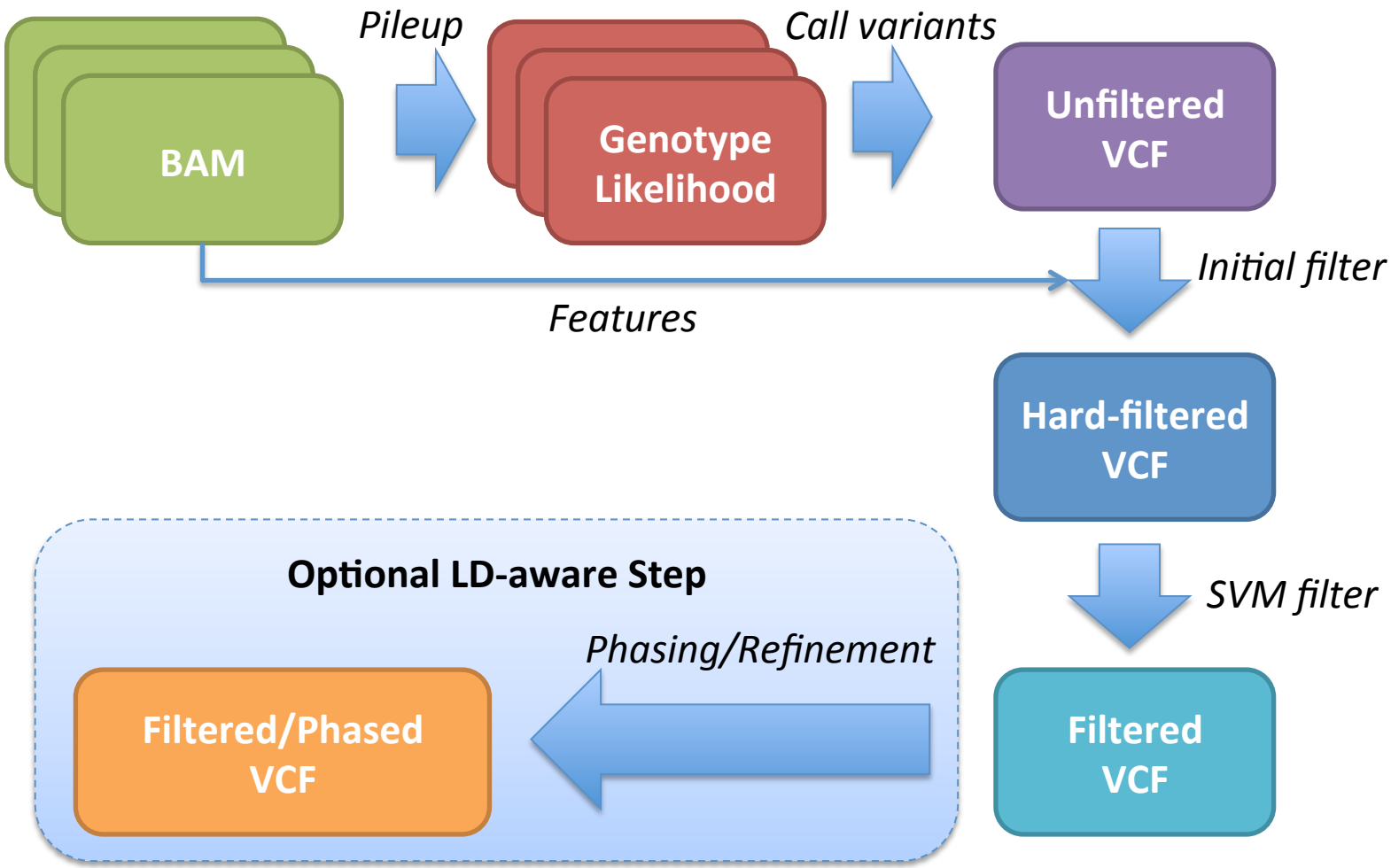
Summary: Part 1

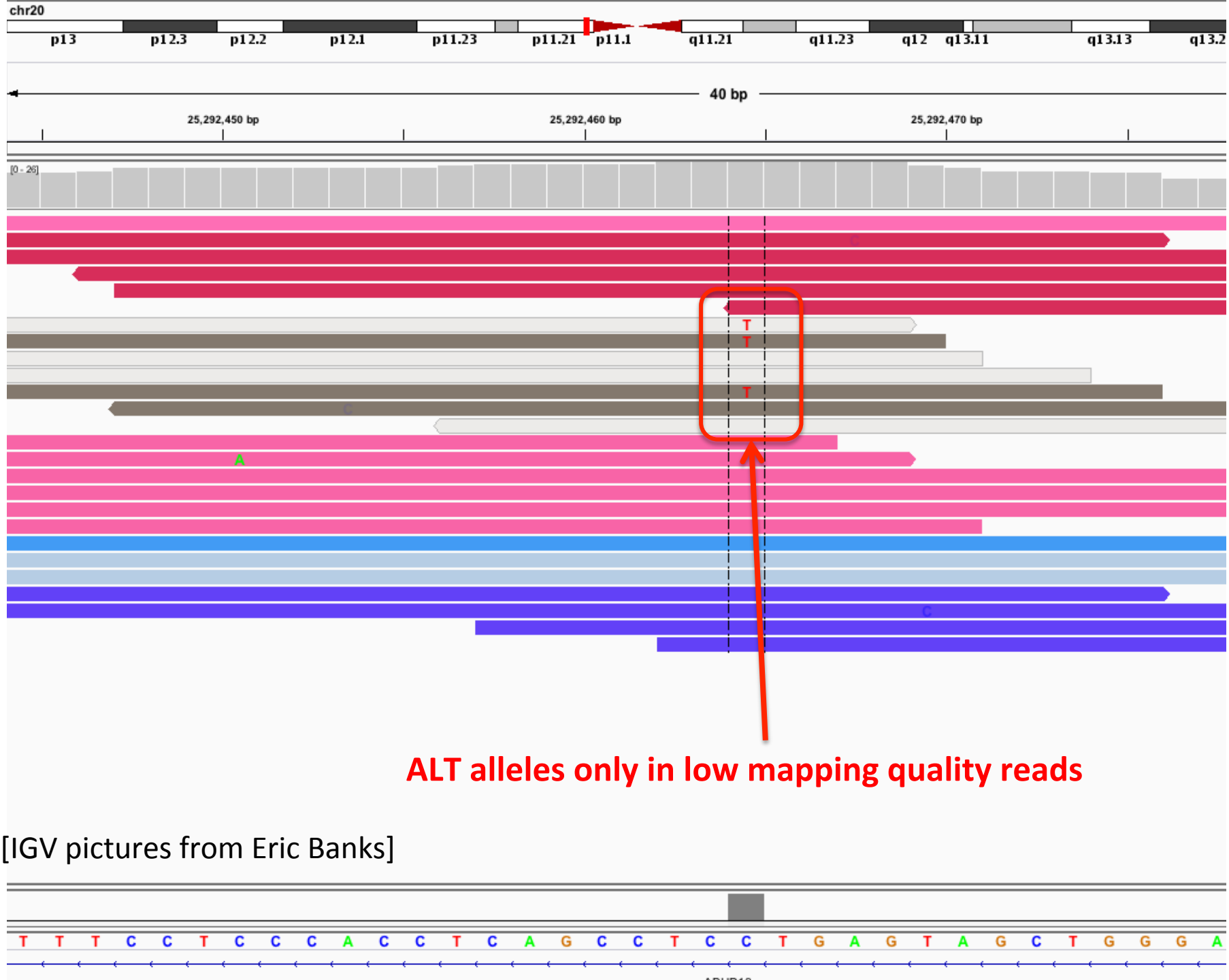
- Likelihood-based model accurately estimates of % of potential sample contamination.
- The sample likelihood model can be used to correct genotype likelihoods, which greatly improves genotype accuracies. (skipped)
- Stand-alone tools to check contamination:
  - http://genome.sph.umich.edu/wiki/VerifyBamID
  - http://genome.sph.umich.edu/wiki/VerifyIDintensity

Part 2.

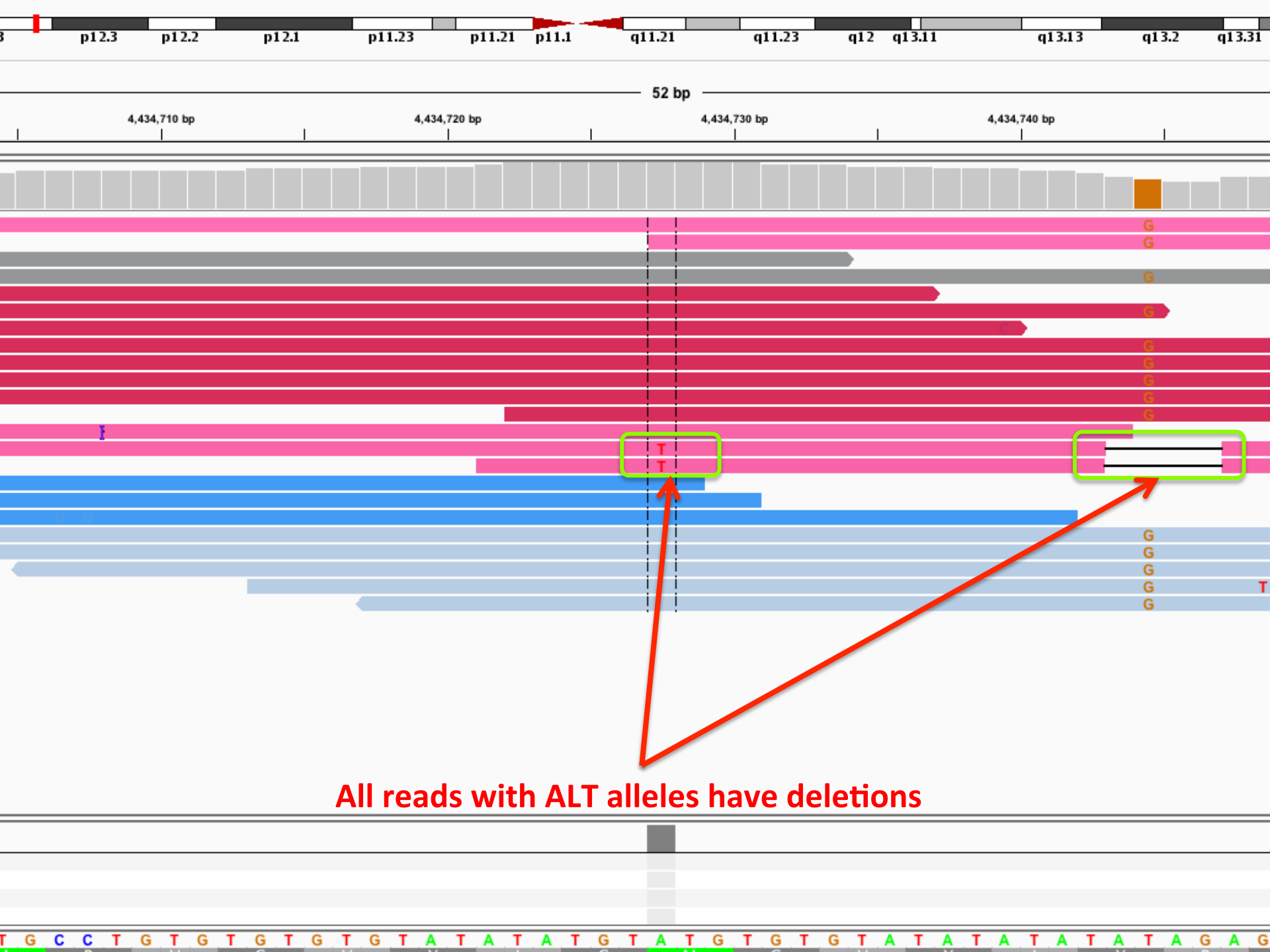
Variant Calling and Filtering

Michigan SNP Calling Pipeline

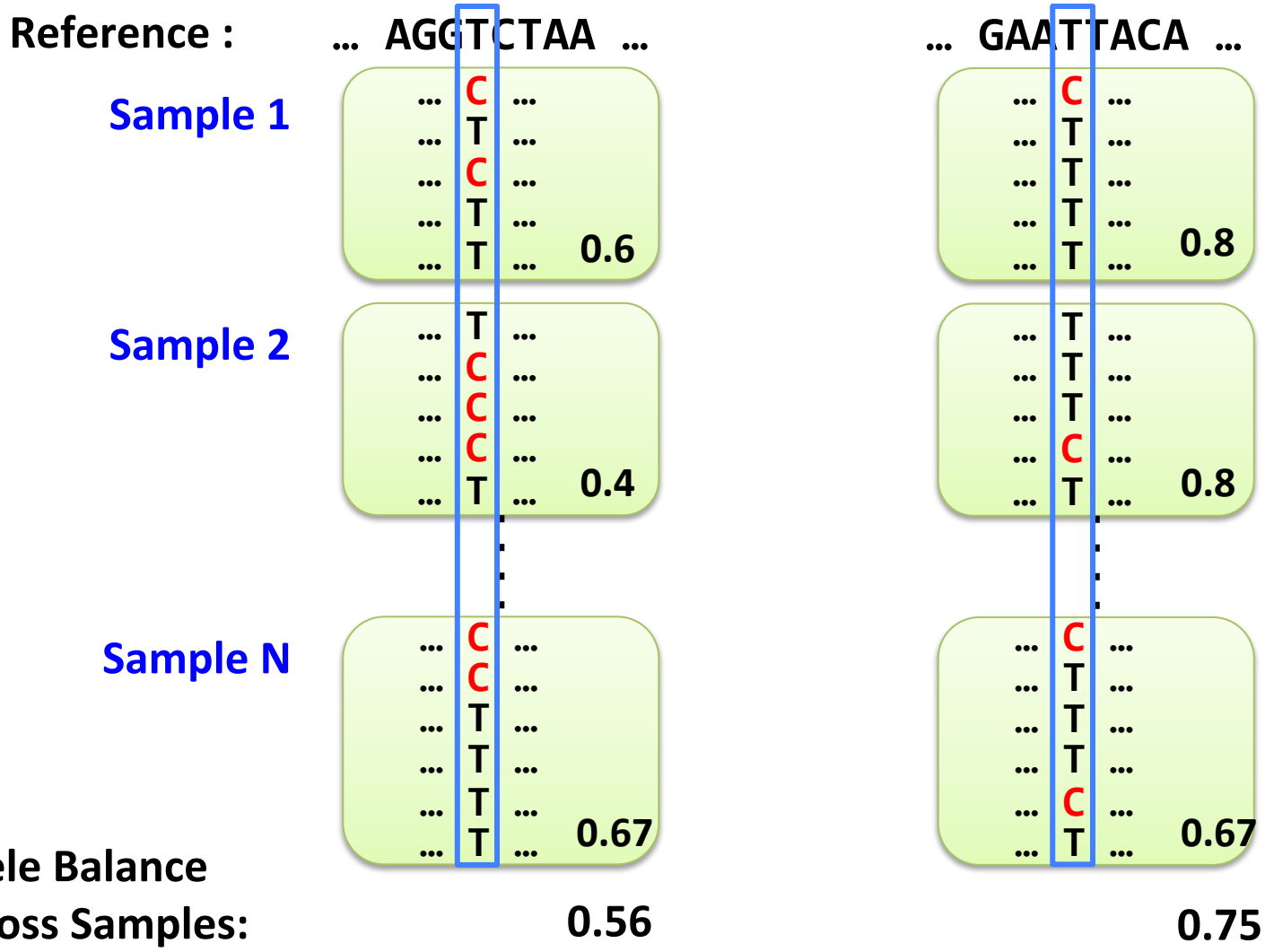




[IGV pictures from Eric Banks]


p12.3
p12.2
p12.1
p11.23
p11.21
p11.1
q11.21
q11.23
q12
q13.11
q13.13
q13.2
q13.31
52 bp
4,434,710 bp
4,434,720 bp
4,434,730 bp
4,434,740 bp
All reads with ALT alleles have deletions

Multi-sample Filtering is Effective



Filtering Criteria Examples

Feature	Description
Depth	Overall depth across samples
QUAL	Overall genotype confidence
Call Rate	Proportion of genotyped samples
Allele Balance	(# REF)/(# ALT) in HET sites
Strand Bias	Correlation of ALT allele with +/- strand
Cycle Bias	Correlation of ALT allele with read cycle
Etc.	And many more…

gotcloud Example: SNP Filtering Results

- Let's check out SNP calling results from gotcloud

```
cd ~goo/gotcloudTutorial
cd vcfs/chr20
```

- Filtering summary file

```
less chr20.filtered.sites.vcf.summary
```

- Let's see what sites are filtered out

```
grep AB70 chr20.filtered.sites.vcf|less -S
```

Dig into VCF

- Check which sample has ALT alleles in those SNPs

```
tabix -h chr20.filtered.vcf.gz 20:42925764-42925764
tabix -h chr20.filtered.vcf.gz 20:42925764-42925764|tail -2
tabix -h chr20.filtered.vcf.gz 20:42925764-42925764|tail -2|cut –f10
```

- (Don’t need to do this yourself)

```
for i in {10..69};do tabix -h chr20.filtered.vcf.gz 20:42925764-42925764 |tail -2|cut -f$i;done
```

```
HG00103 HG00111 HG00112 HG00114 HG00125 HG00127 HG00131 HG00137
HG00142 HG00239 HG00246
```

Check BAM Files at Problematic Sites

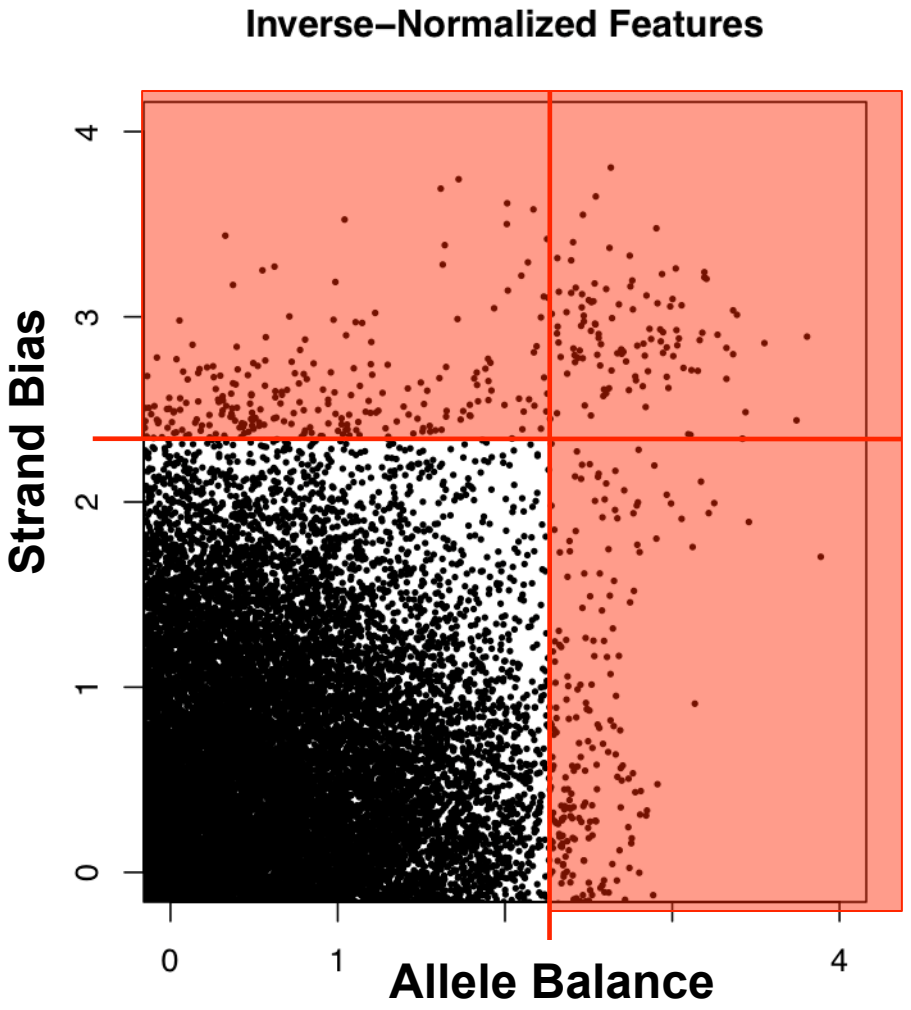
- Use samtools to see original BAM files

```
cd /opt/gotcloudExample/
cd bams

samtools tview HG00103.bam ../chr20Ref/humak_g1k_v37_chr20.ref
(type g) 20:42925764
samtools tview HG00111.bam ../chr20Ref/humak_g1k_v37_chr20.ref
(type g) 20:42925764
```

Hard Filtering by Individual Thresholds

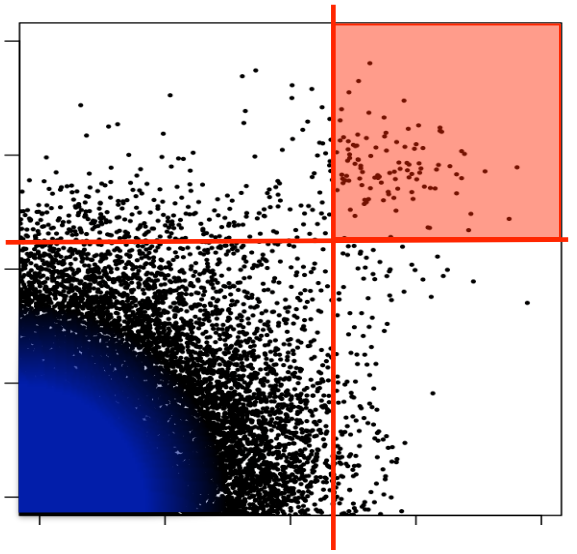
- Problems
  - False negative increases with number of filters
  - Too many knobs to turn (thresholds)



Filtering by Supervised Learning

- Use features to train a support vector machine (SVM)
  - Can be trained using suspected positive/negative examples
  - Provides single score from all features combined

- Training
  - Positive examples
    - Known polymorphic sites
  - Negative examples
    - Filtered out by multiple hard filters
  - Input
    - All individual features collected for each site



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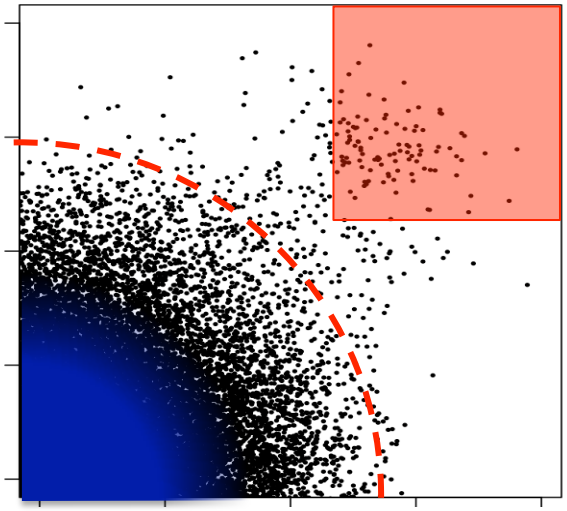
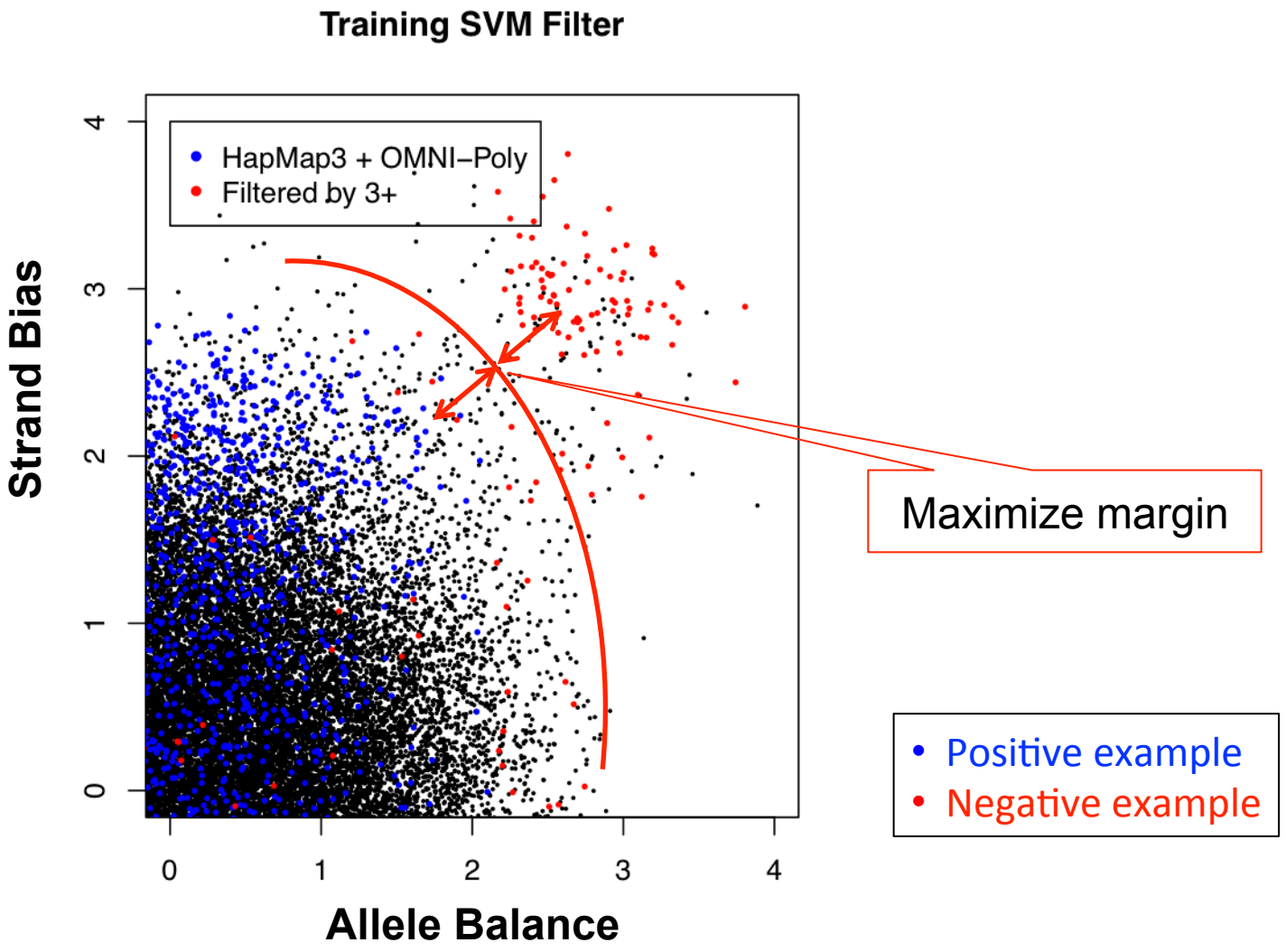
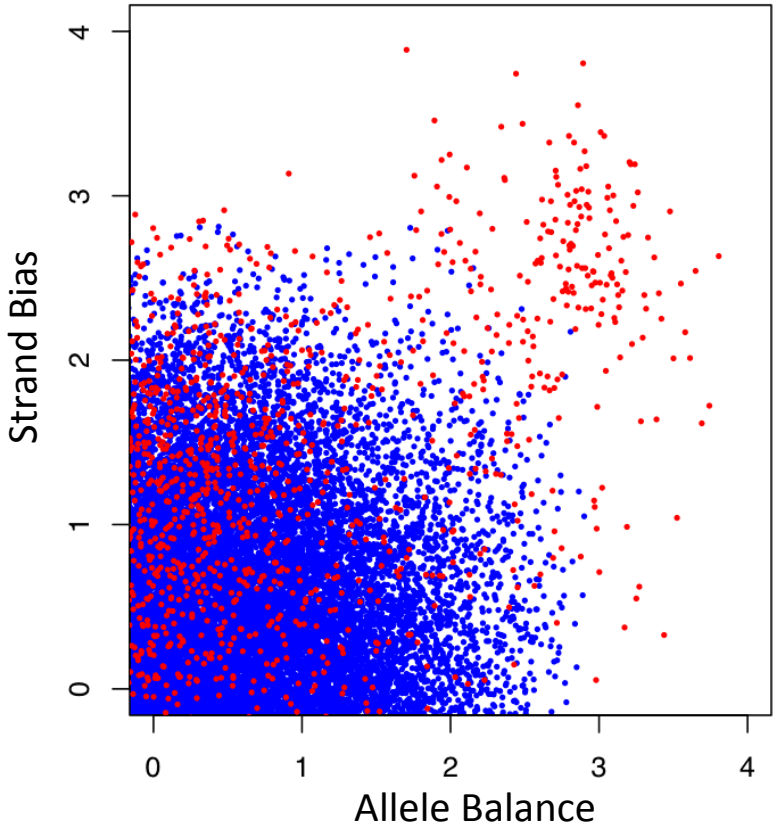


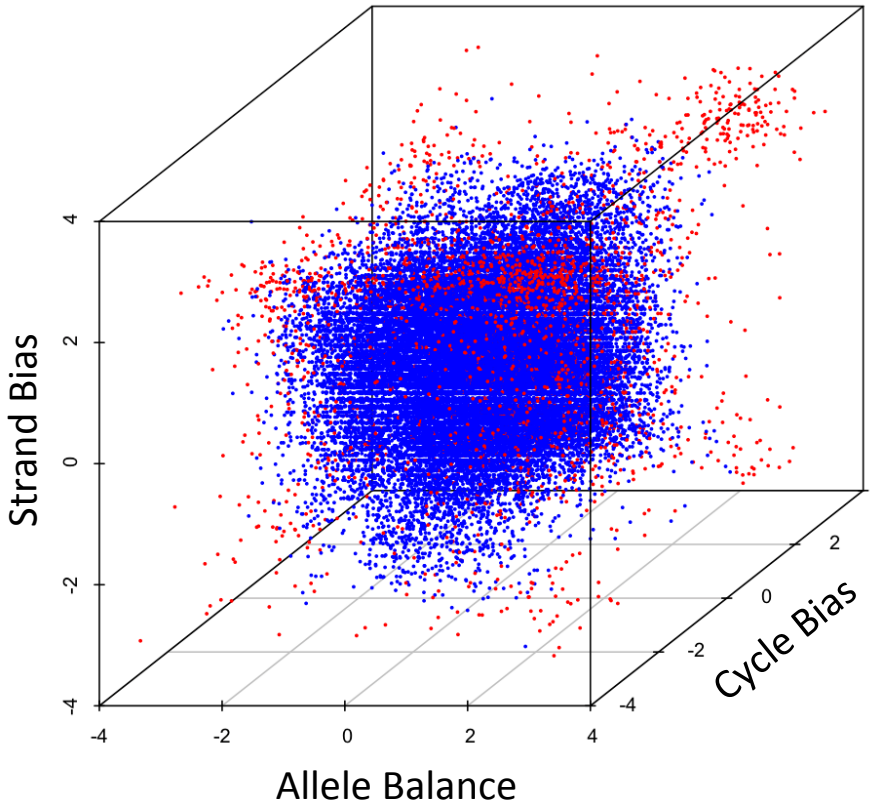
Training SVM with Examples



>20 dimensional feature set was used for final filtering under nonlinear kernel space

SVM Output in Multi-dimensional Space



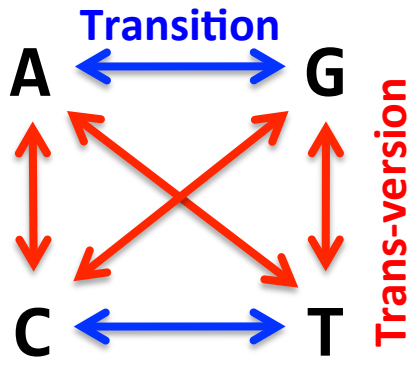


- Filter PASS
- Filter FAIL

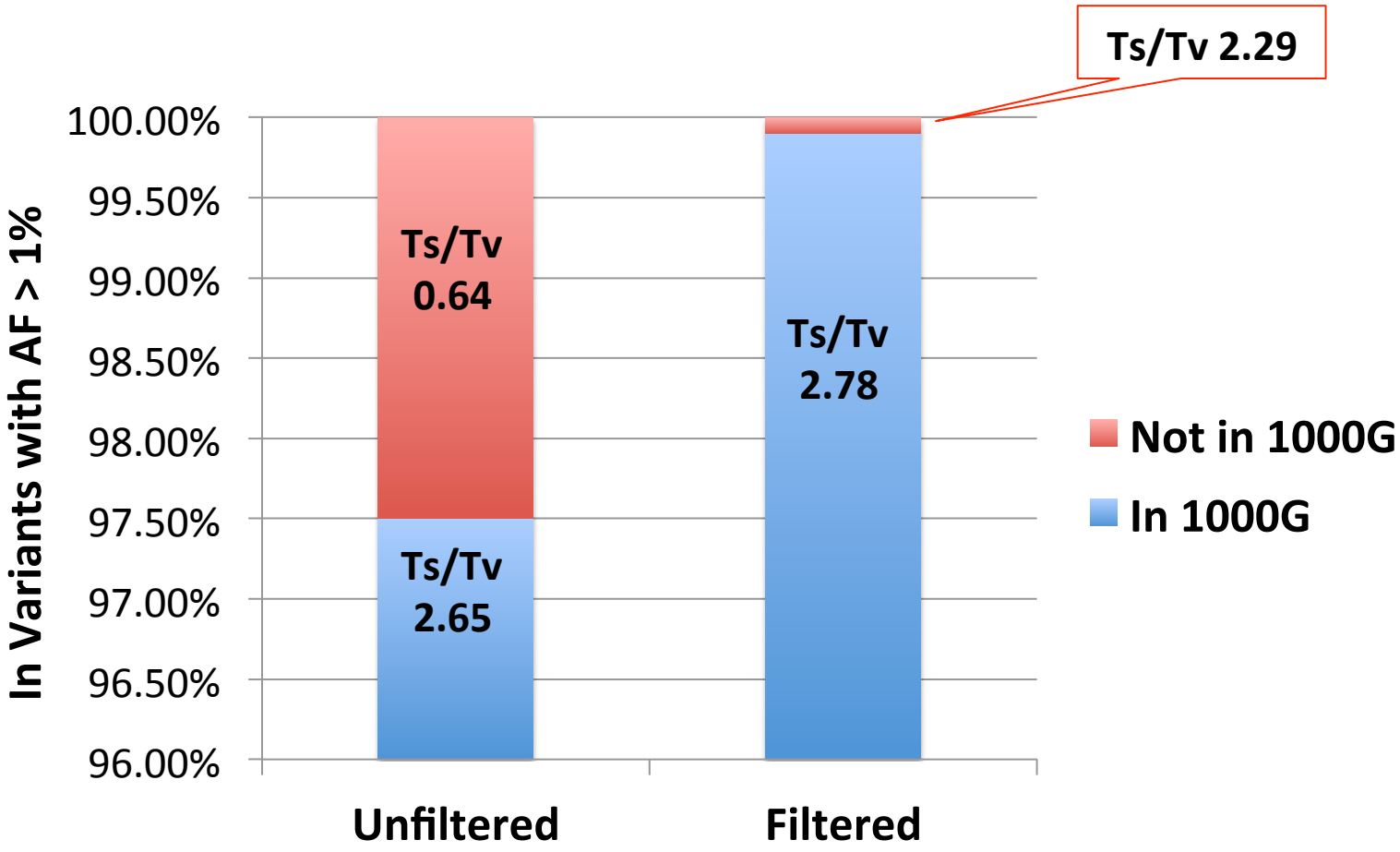
Most of FAIL SNPs are outliers in higher-dimensional view

Evaluation of SNP Callsets

- Sensitivity on known SNP data
  - dbSNP, HapMap, 1000G, etc.
- Transition to trans-version ratio
  - Transition is easier to occur.
  - Typical Ts/Tv values
    - Whole genome: 2.2~2.4
    - Whole exome: 2.7~3.0



Results: Exome Sequencing Project (GO-ESP)



Summary: Part 2

- Multi-sample strategy delivers high-quality variants
- SVM-based filtering provides effective and flexible methods for variant filtering
  - Applicable to GATK-generated variants and features
  - Trained SVM models are re-usable
  - Applicable to sequencing data from multiple platforms
- Michigan Mapping/Variant calling pipeline on the cloud
  - http://genome.sph.umich.edu/wiki/GotCloud

Backup

Genotype Calling with Contamination Parameter

- SNP calling with genotype likelihood model

$$\arg\max_{G_i} L = \arg\max_{G_i} \prod_{j=1}^{N_i} P(b_{ij}|G_i)$$

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- Genotype likelihood model with contamination

$$\arg\max_{G_i} L(\alpha) = \arg\max_{G_i} \sum_{g_i} \prod_{j=1}^{N_i} \{(1-\alpha)P(b_{ij}|G_i) + \alpha P(b_{ij}|g_i)\} P(g_i)$$

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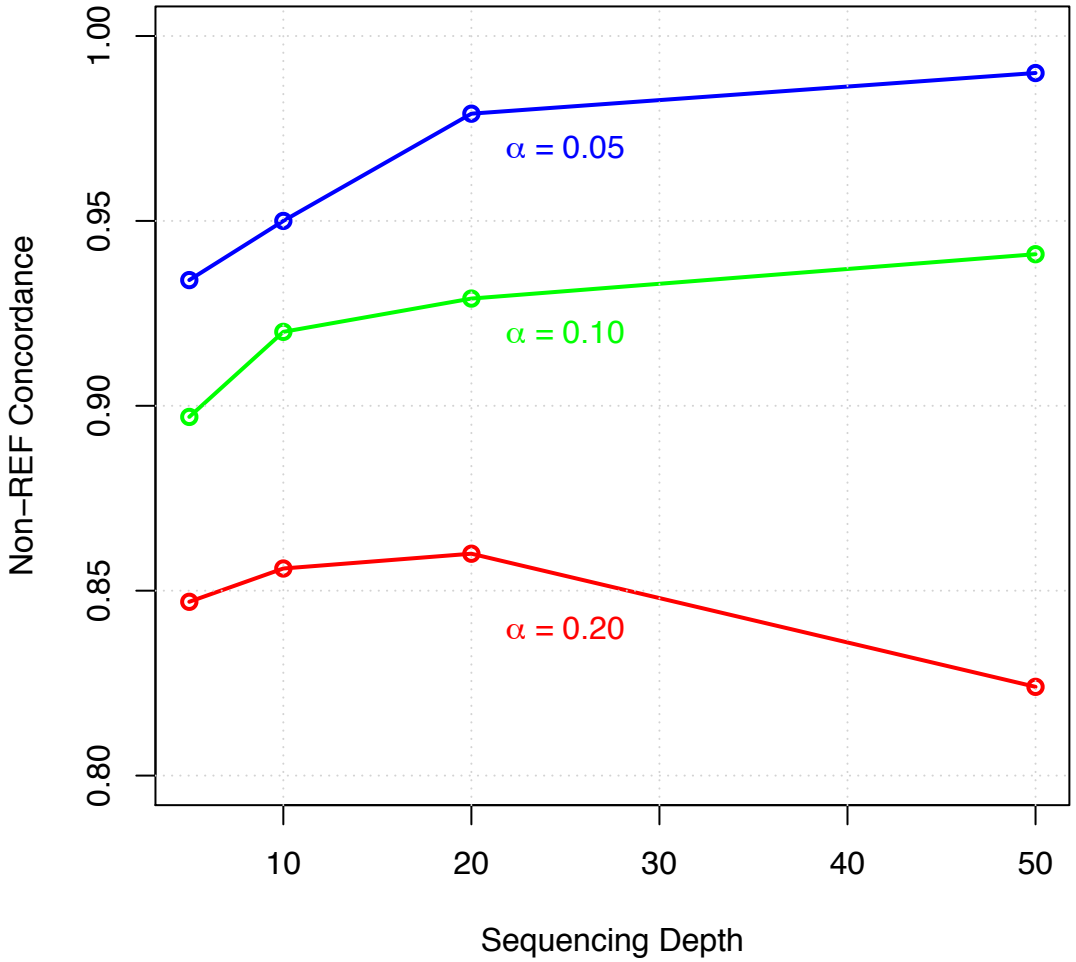
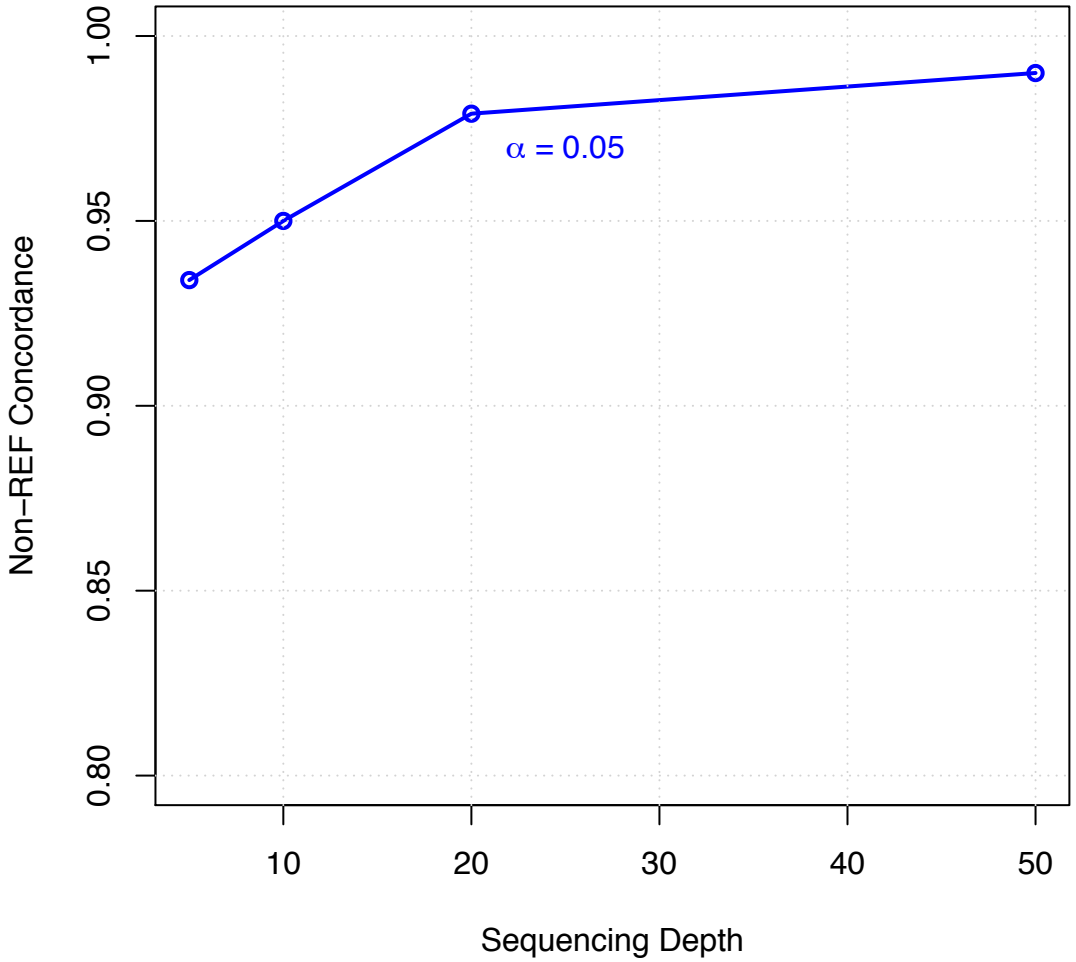
Estimated Contamination

Simulation: Correcting Contamination



Simulation: Correcting Contamination



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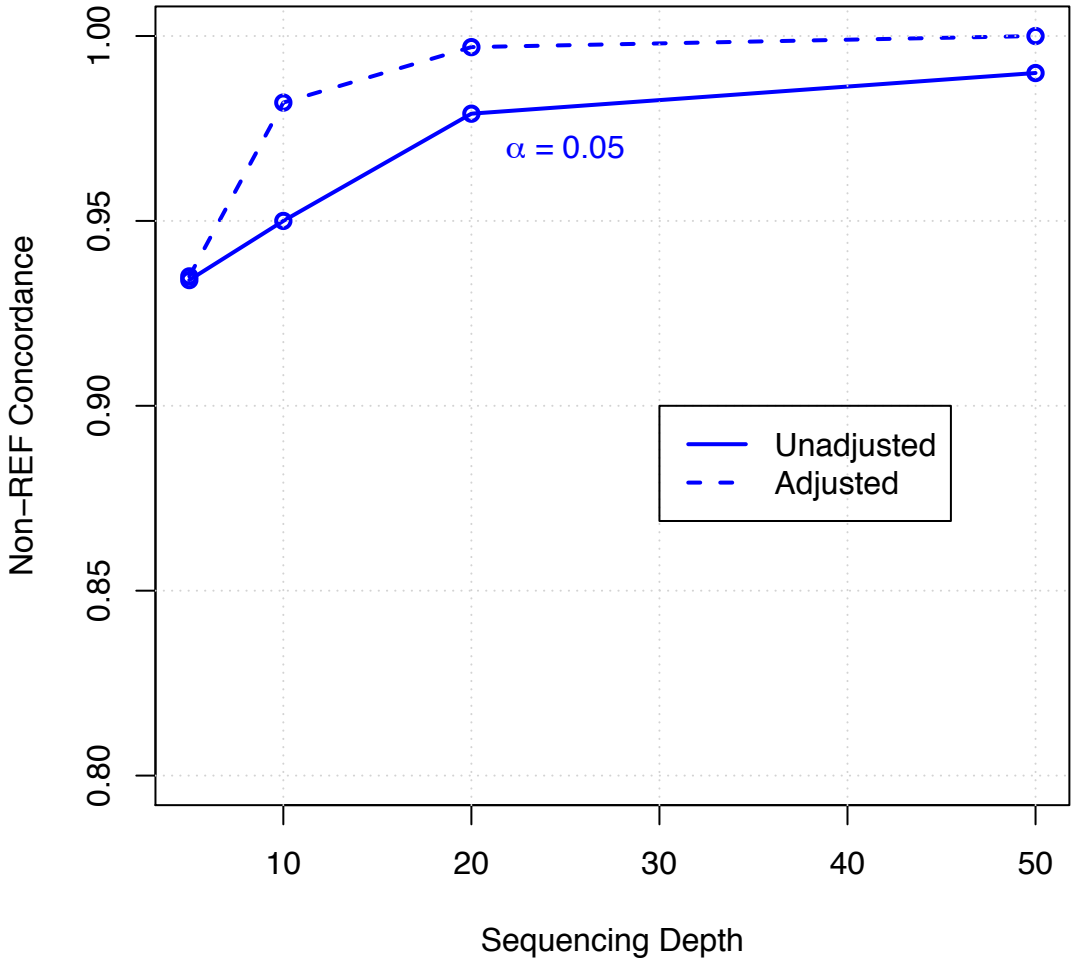

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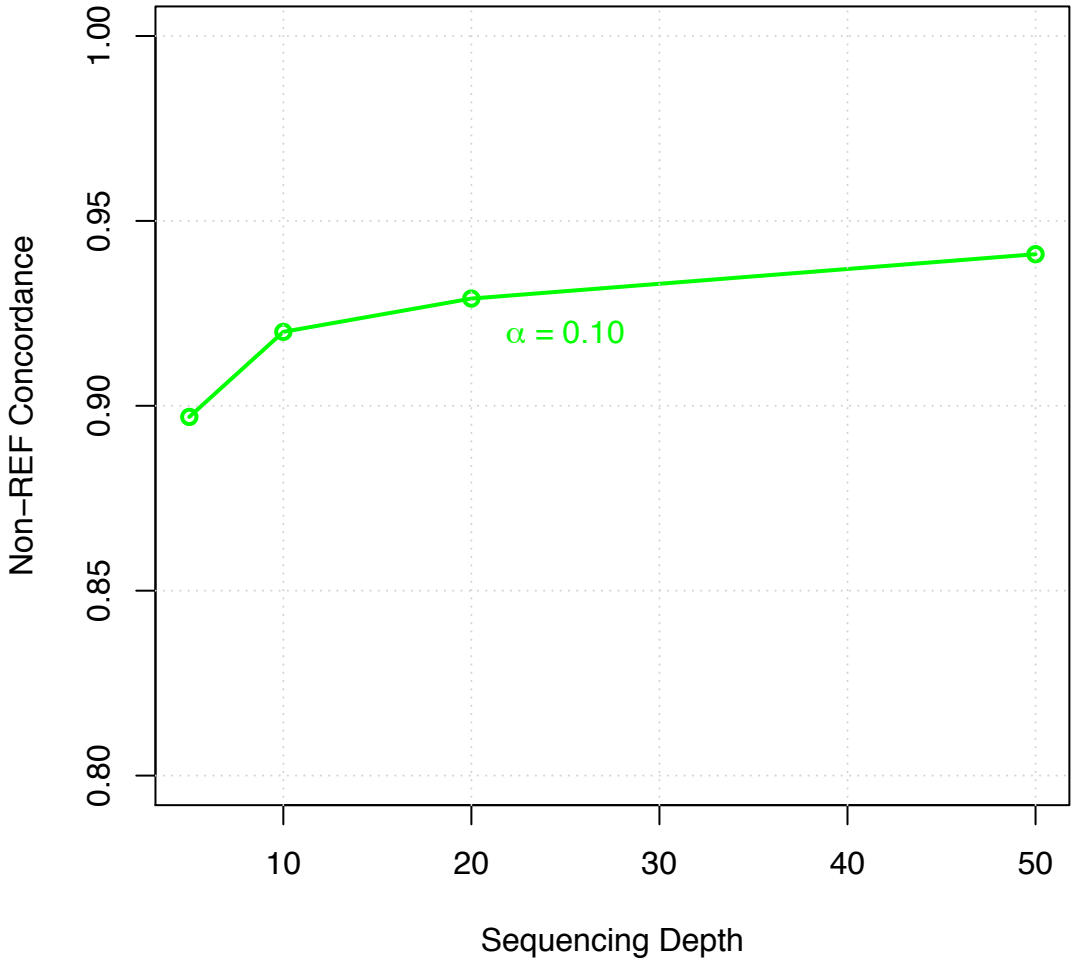

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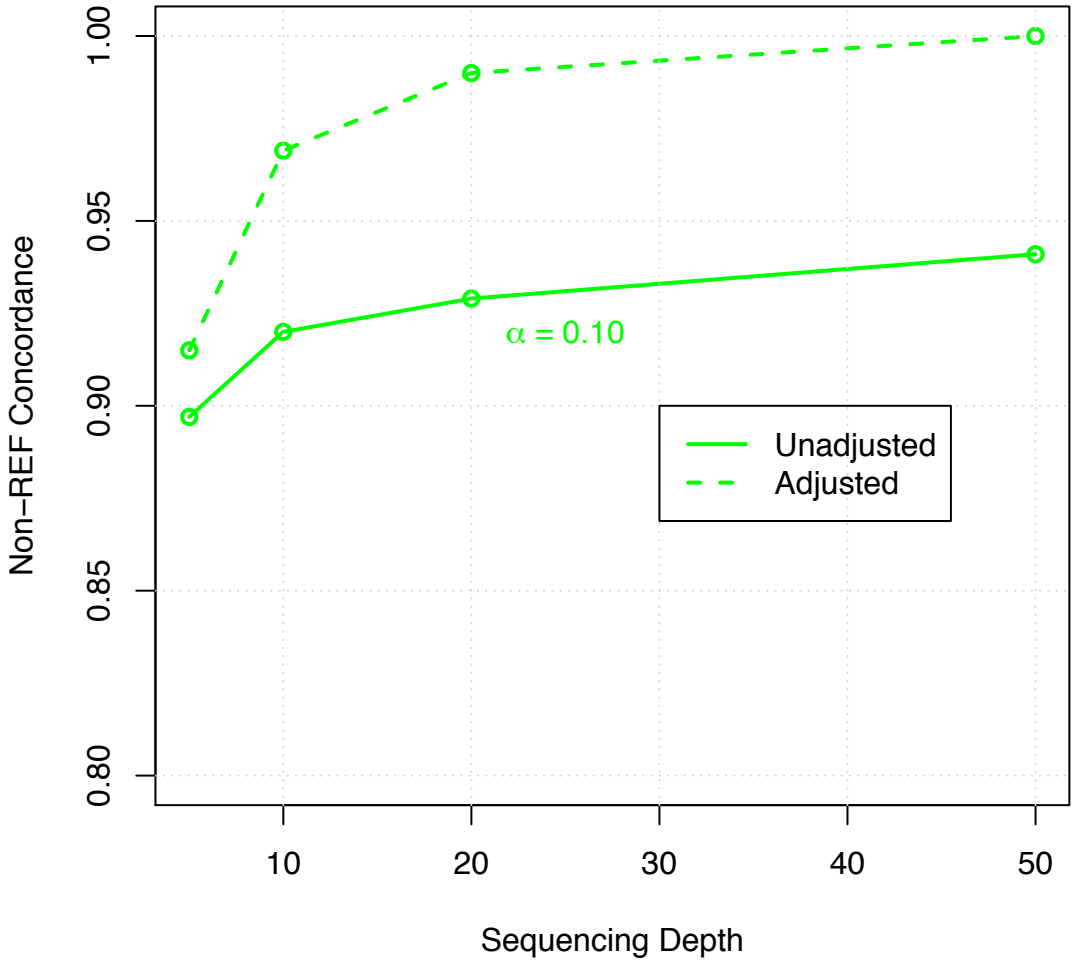
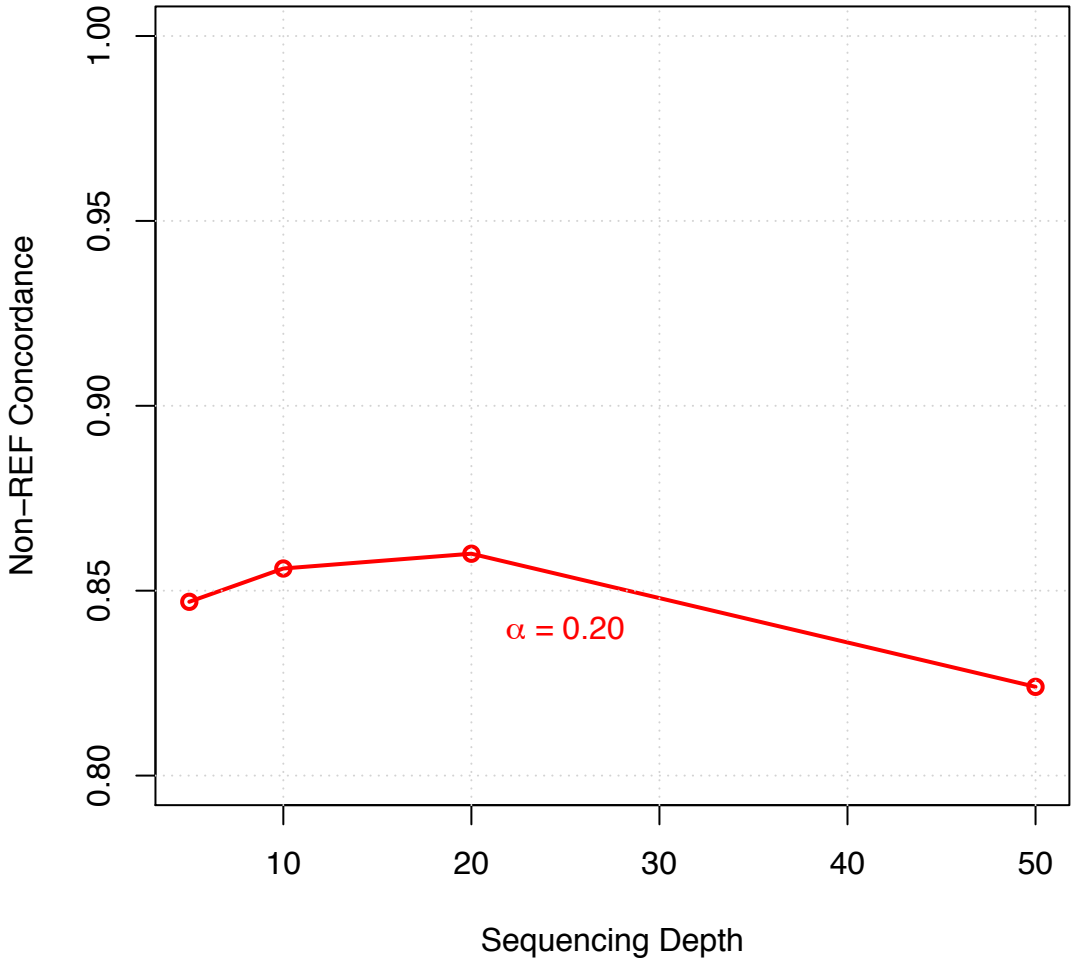
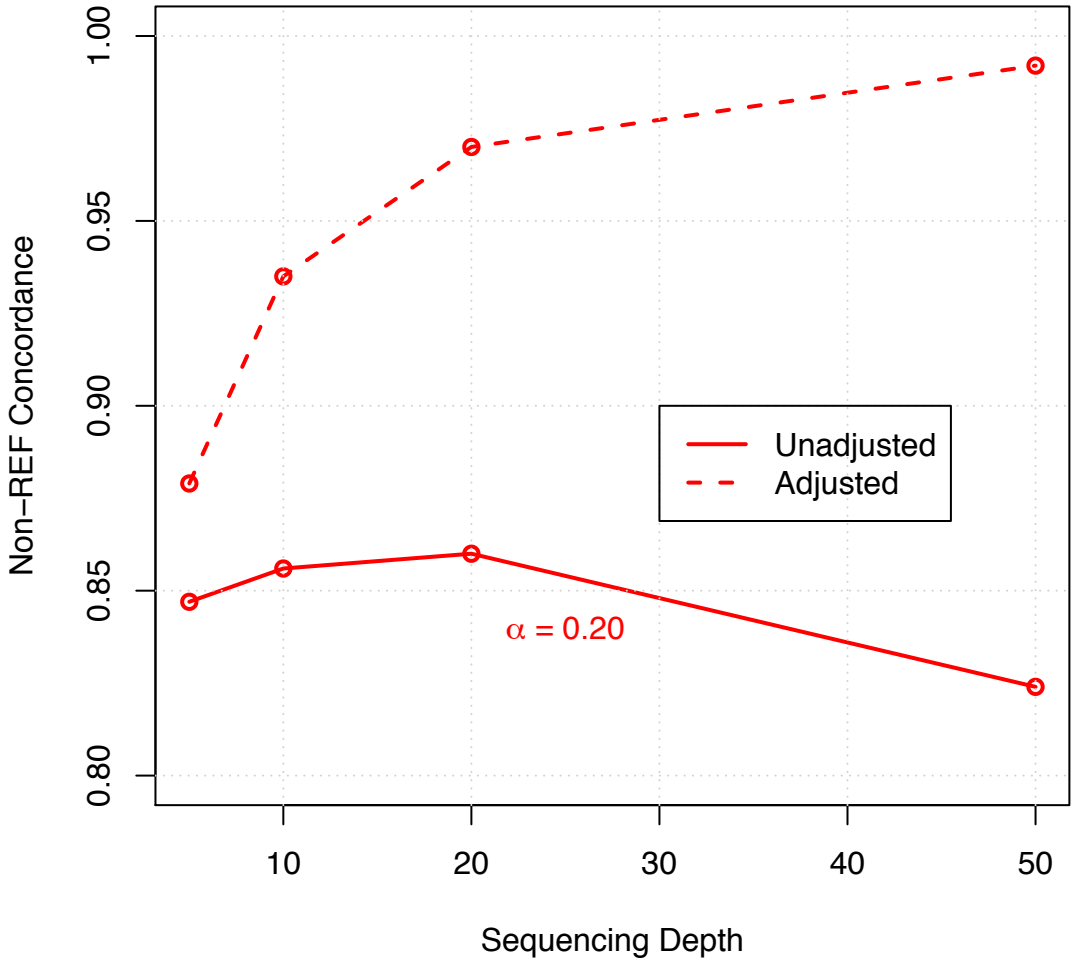


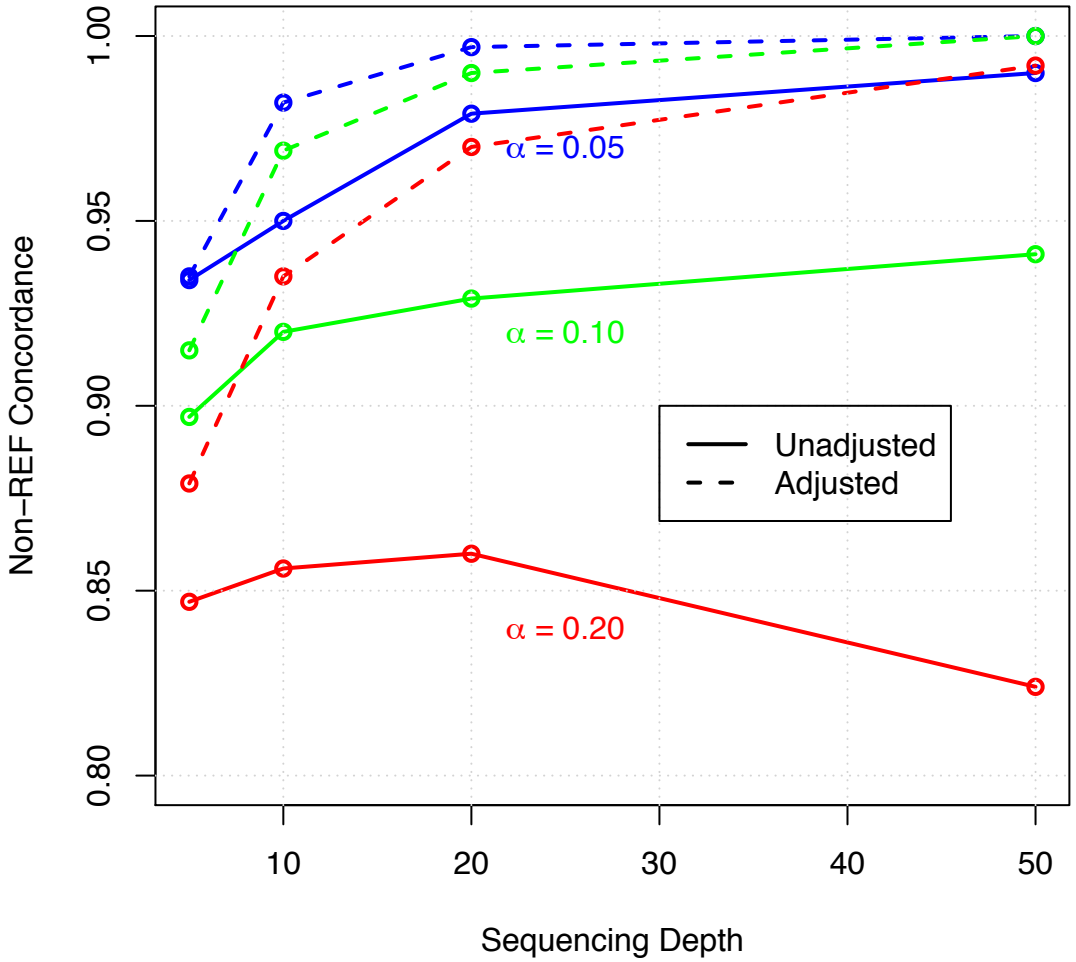
Simulation: Correcting Contamination



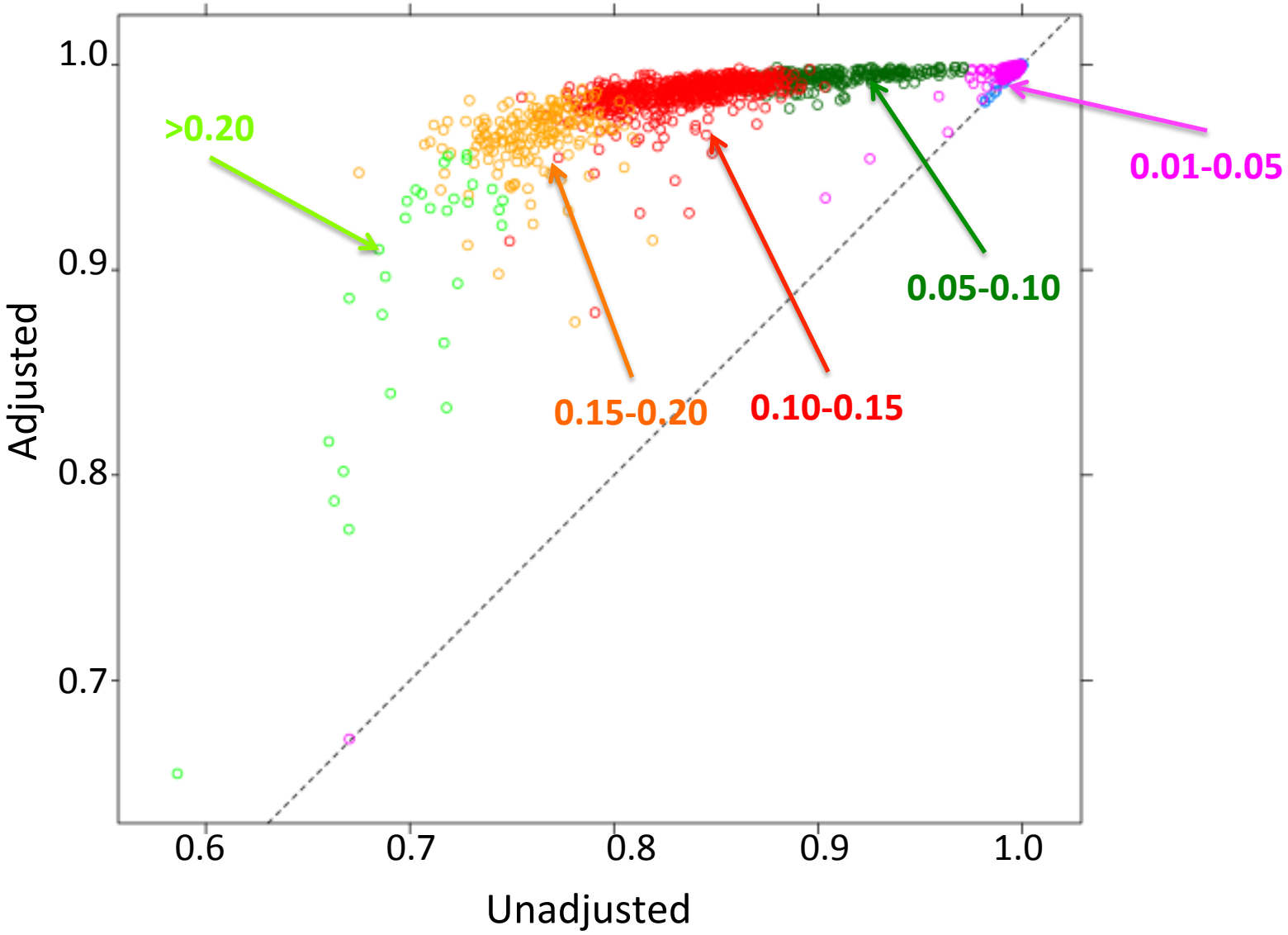
Simulation: Correcting Contamination



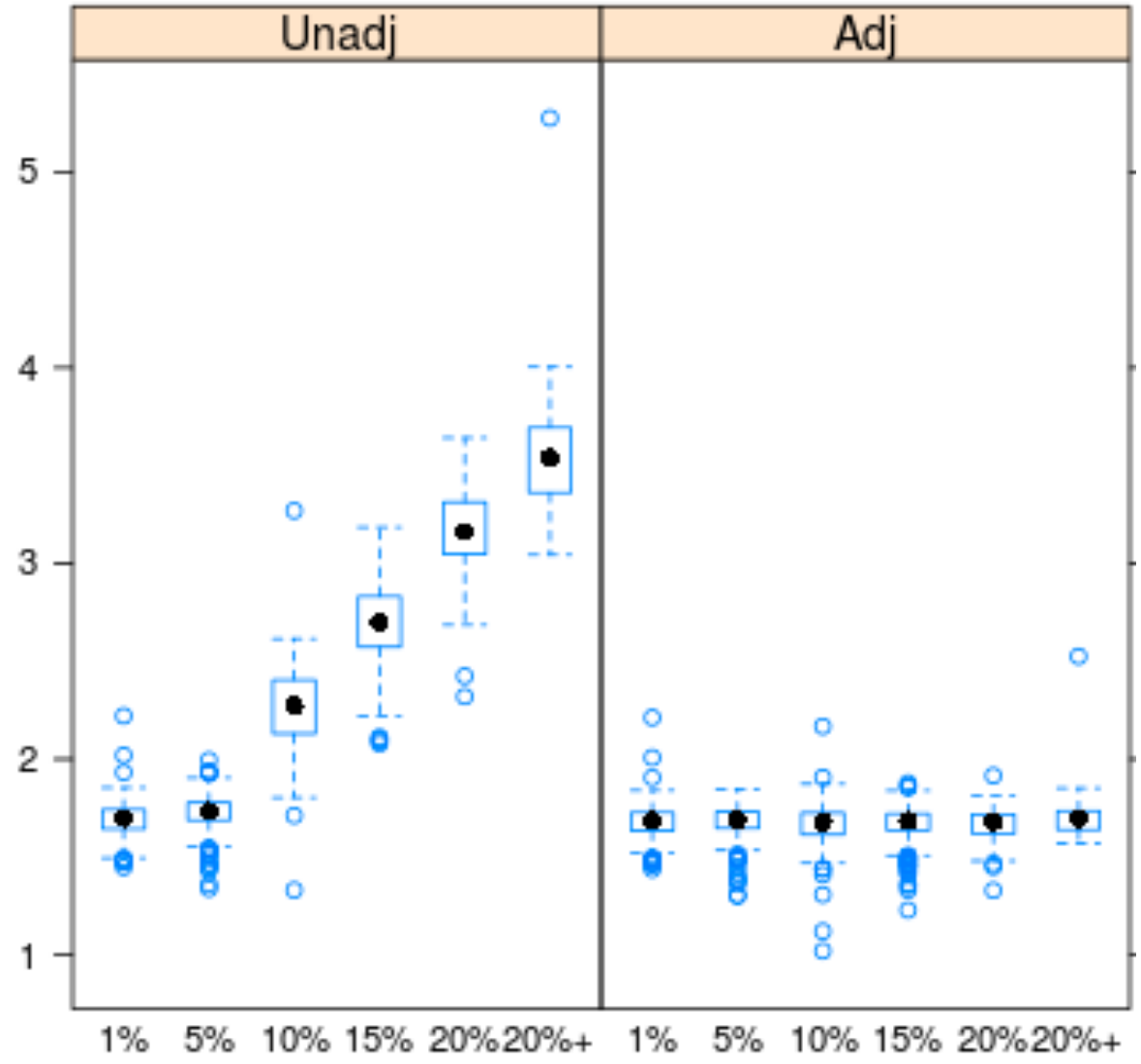
Simulation: Correcting Contamination



Results: Non-REF Genotype Concordance

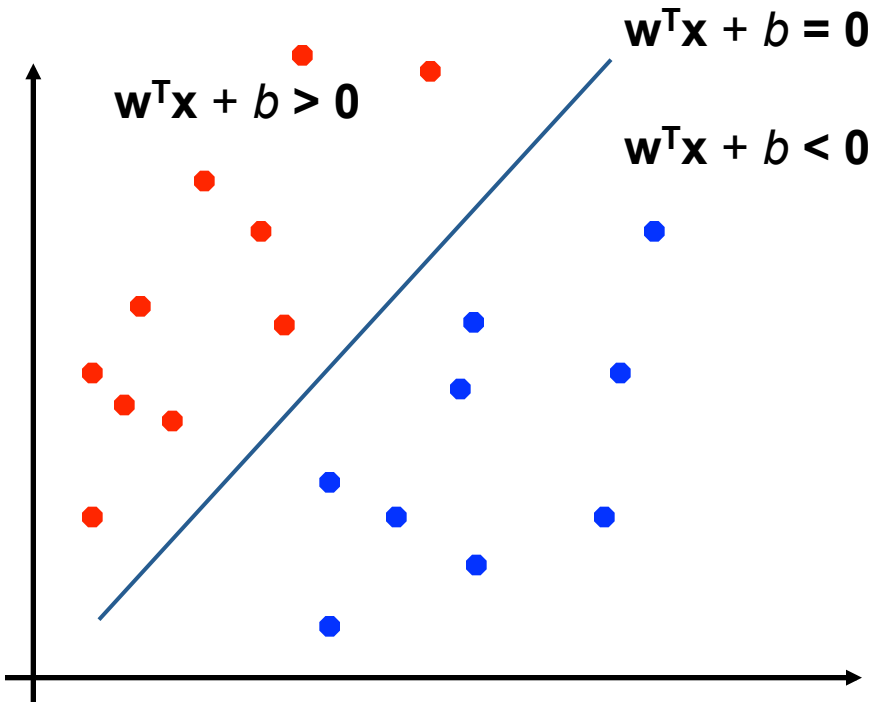


Results: HET/HOM Change



SVM Basics: Linear Separators

- Binary classification can be viewed as the task of separating classes in feature space:

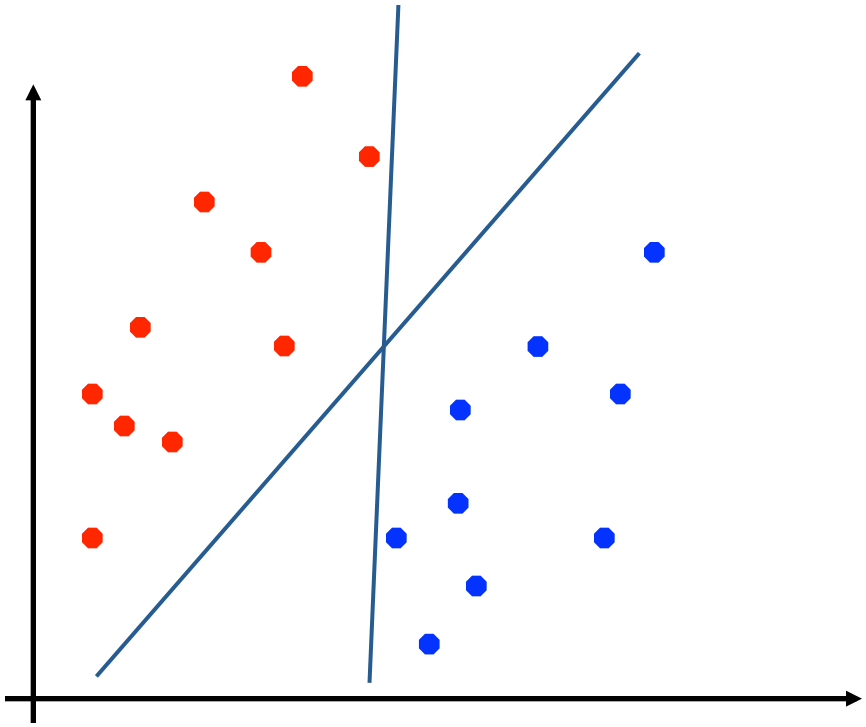


$$f(\mathbf{x}) = \text{sign}(\mathbf{w}^T\mathbf{x} + b)$$

[Figures: R. Mooney]

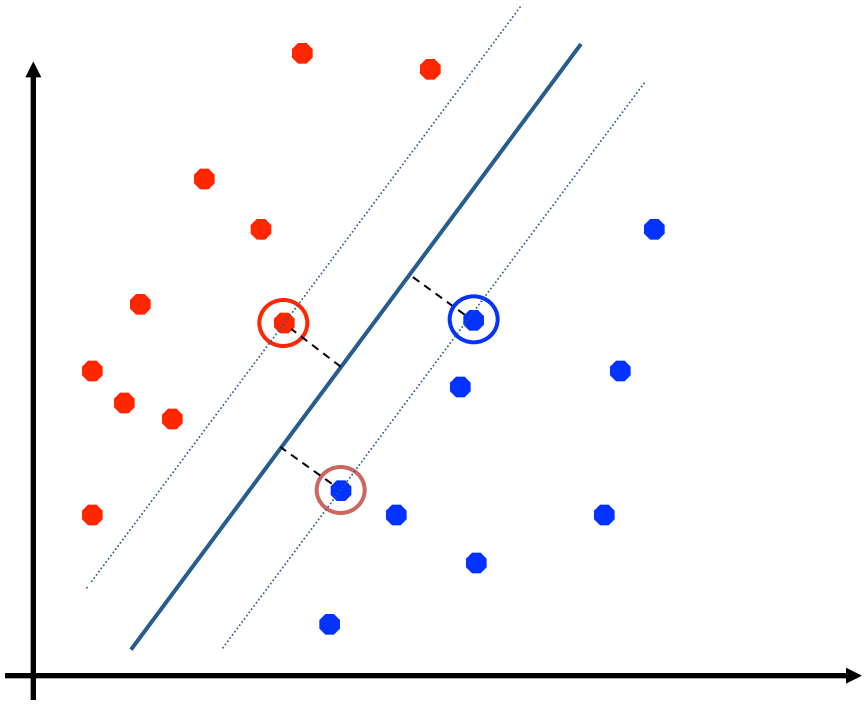
SVM Basics: Optimal Linear Separator

- Which of the linear separators is optimal?



SVM Basics: Maximum Margin

- Maximizing the margin is good according to intuition and PAC theory.
- Implies that only support vectors matter; other training examples are ignorable.



Non-linear SVMs: Kernel Trick

- General idea: the original feature space can always be mapped to some higher-dimensional feature space where the training set is separable: