Genome resolved metagenomics II: De novo Extraction of Strains from MetAgeNomes

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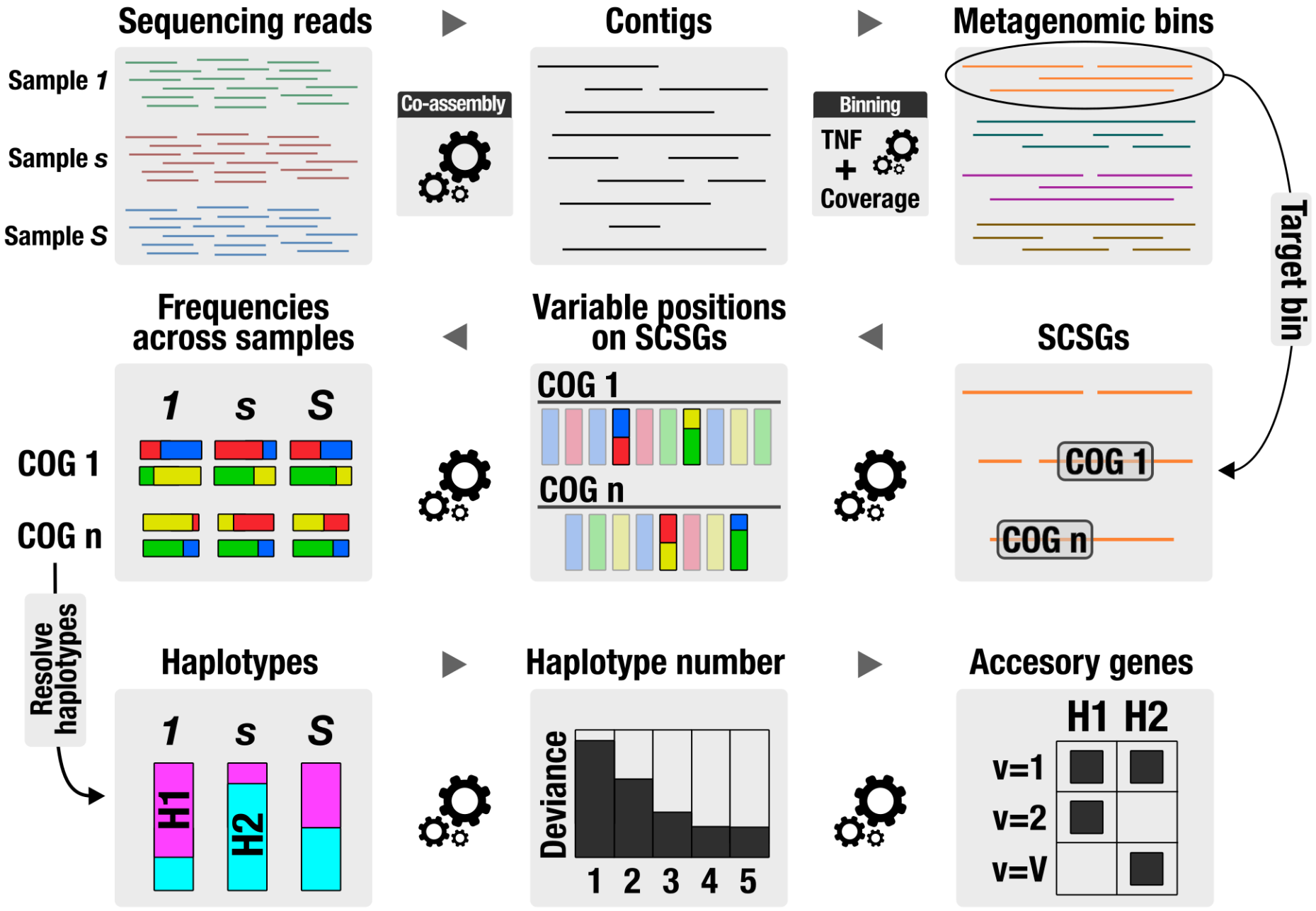
Strain resolution from short read metagenome data

- Co-occurrence binners can cluster contigs into species genomes
- Additional variation exists within species both nucleotide variants on shared genes and variation in the accessory genome
- Can call per sample consensus e.g. MIDAS and StrainPhlAn (Nayfach et al. Genome Res 2016, Truong et al. Genome Biology 2017)
- Can use co-occurrence across multiple samples to resolve strain mixtures *de novo* after mapping to references:
  - Constrains identifies microbial strains in metagenomic datasets (Luo et al. Nature Biotech. 2015)
  - A Bayesian Approach to Inferring the Phylogenetic Structure of Communities from Metagenomic Data (O'Brien et al. Genetics 2014)
- Can we do this entirely *de novo* using contigs and MAGs?

DESMAN: De novo Extraction of Strains from MetAgeNomes

- **DESMAN** https://github.com/chrisquince/DESMAN:
  1) Map reads onto core genes on contigs
  2) Gibbs sampler to infer strain haplotypes and abundances
  3) Determine copy count of accessory genome

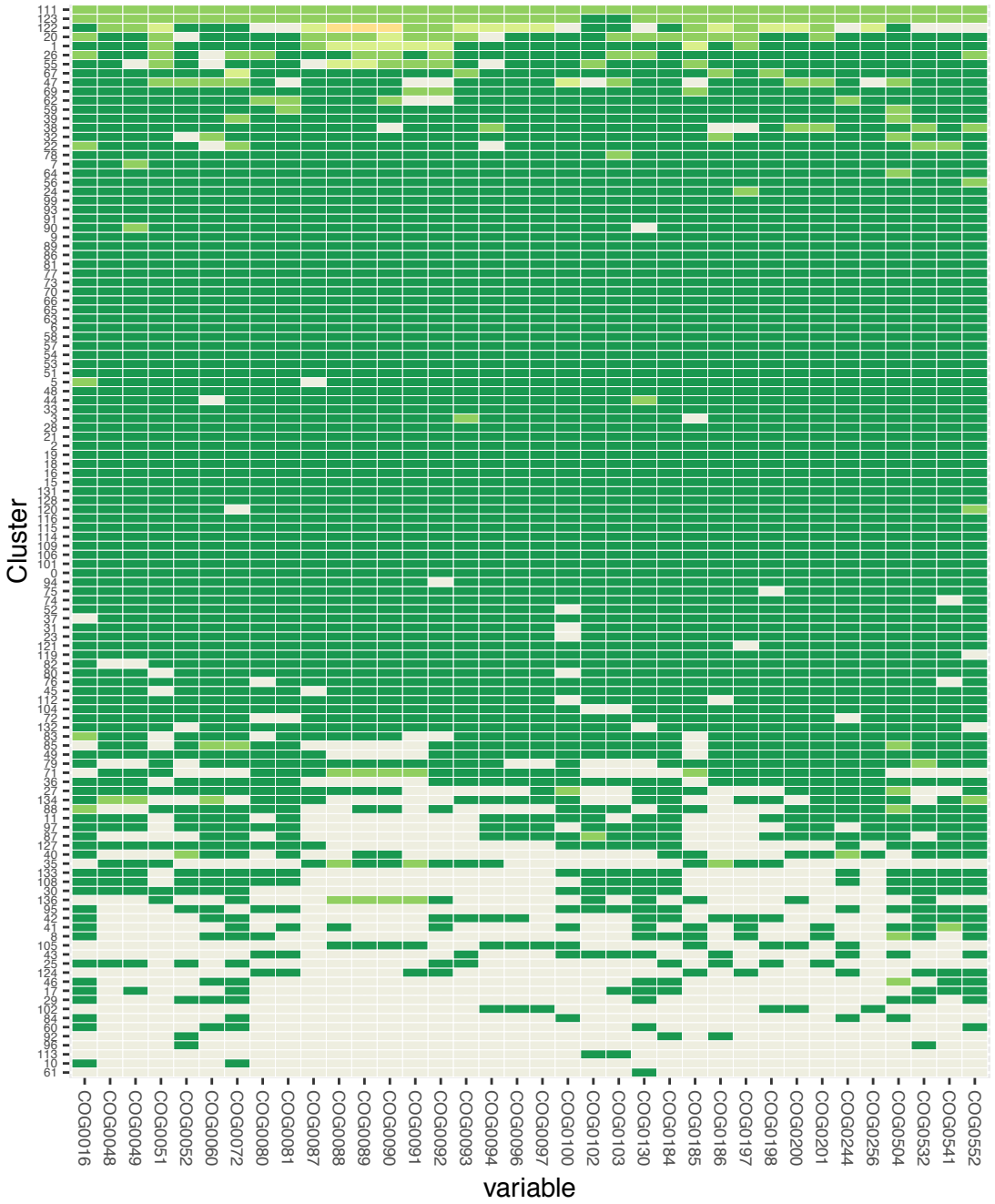
bioRxiv: http://biorxiv.org/content/early/2016/09/06/073825


Sequencing reads
Sample 1
Sample s
Sample S
Co-assembly
Contigs
Binning
TNF
+
Coverage
Metagenomic bins
Target bin
SCSGs
COG 1
COG n
Variable positions on SCSGs
COG 1
COG n
Frequencies across samples
1 s S
COG 1
COG n
Resolve haplotypes
Haplotypes
1 s S
H1
H2
Haplotype number
Deviance
1 2 3 4 5
Accesory genes
H1 H2
v=1
v=2
v=V

Complex synthetic community

- 100 different species and 210 NCBI genomes
- 10 ten separate phyla, 49 families, and 74 genera
- Species strain frequency distribution of (1:50, 2:20, 3:10, 4:10, 5:10)
- Simulated 96 samples of 6.25 million 2X150 bp paired end reads using ART: 1 HiSeq 2500 high output: https://github.com/chrisquince/StrainMetaSim

Concoct binning

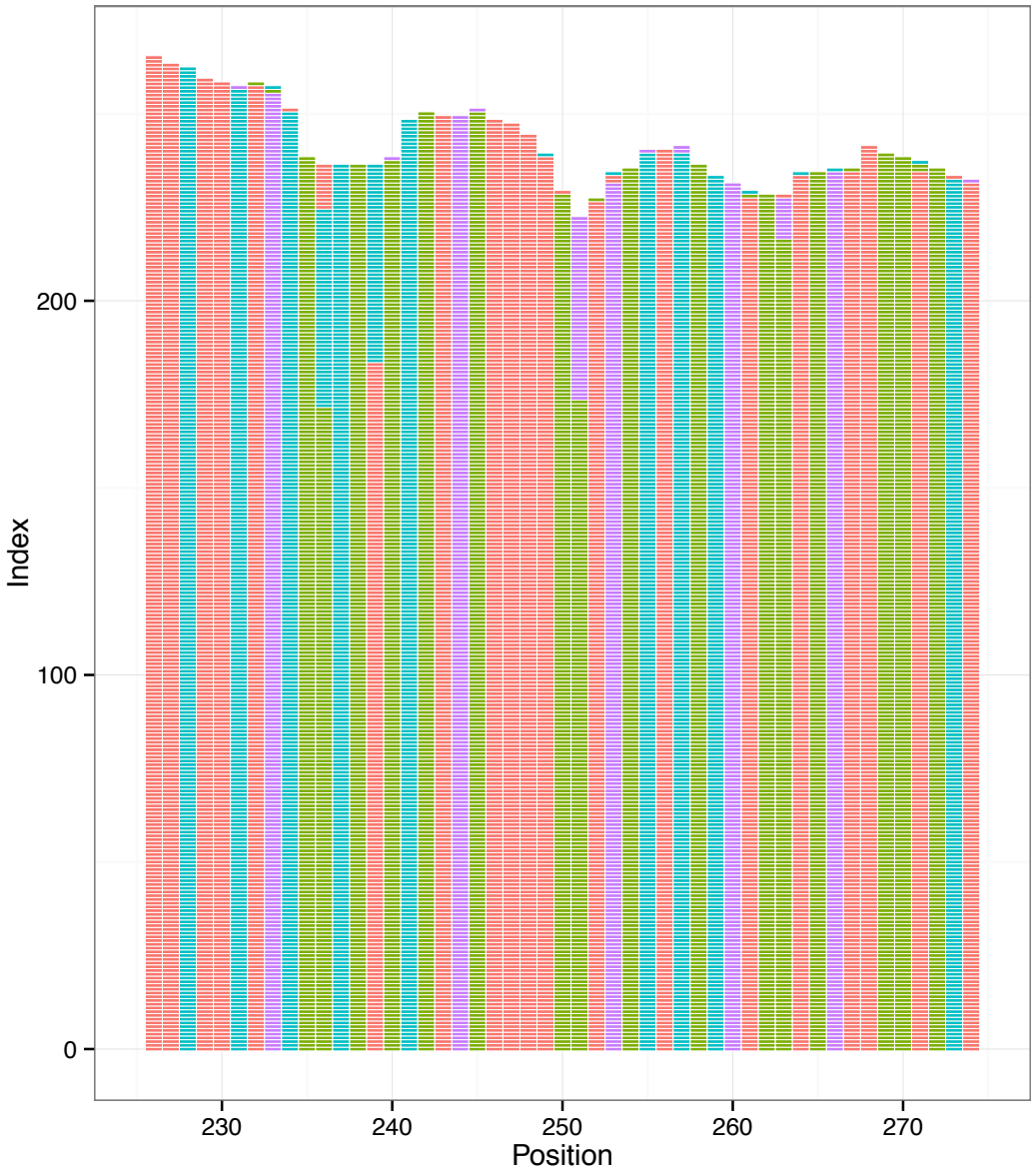


- Coassembly gave 74,580 contig fragments with a total length of 409 Mbp as compared to 687 Mbp for all 210 genomes
- CONCOCT2 generated 137 clusters (species recall of 86.1% and a precision of 98.2%)
- 75 clusters with 75% of SCGs in single copy
- Map clusters onto reference species

DESMAN analysis using single-copy core genes

- Process each of the 75 – 75% complete clusters separately
- Map reads onto 36 SCGs
- If target taxa is known and cultured custom set of species specific single-copy core genes (SSCGs) could be used instead

Determining variant positions on SCGs

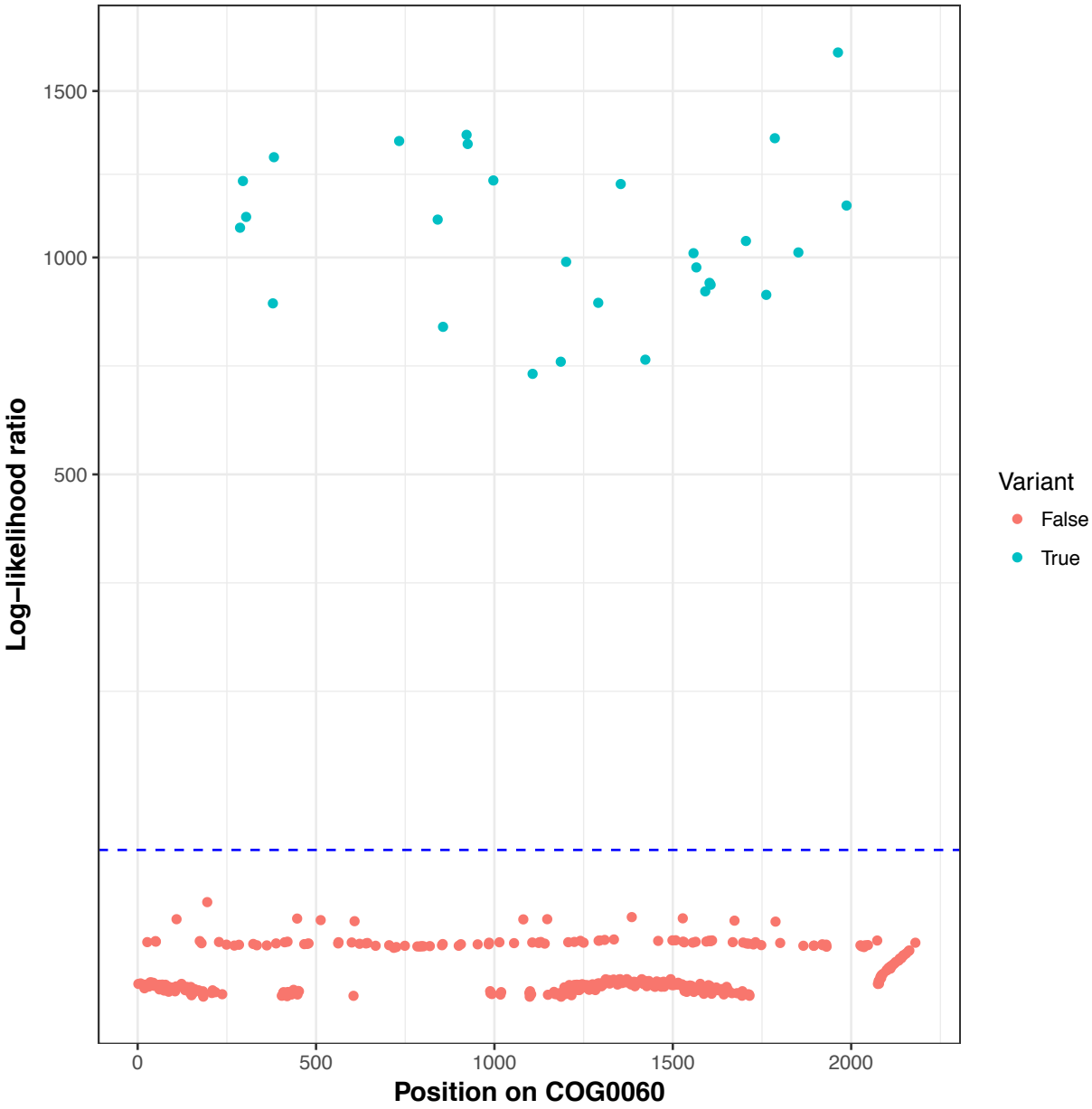


- Ignore distribution across samples just ask if minority bases could be created by errors alone
- Assume errors are position independent with true base *a* generating observed base *b* with probability:

$$\varepsilon_{a,b}$$

- Likelihood ratio test comparing null hypothesis that there is one variant present against two variants

Variant prediction for Cluster 37 -> Rhodococcus erythropolis



COG0060 predict 28 variant positions all correctly

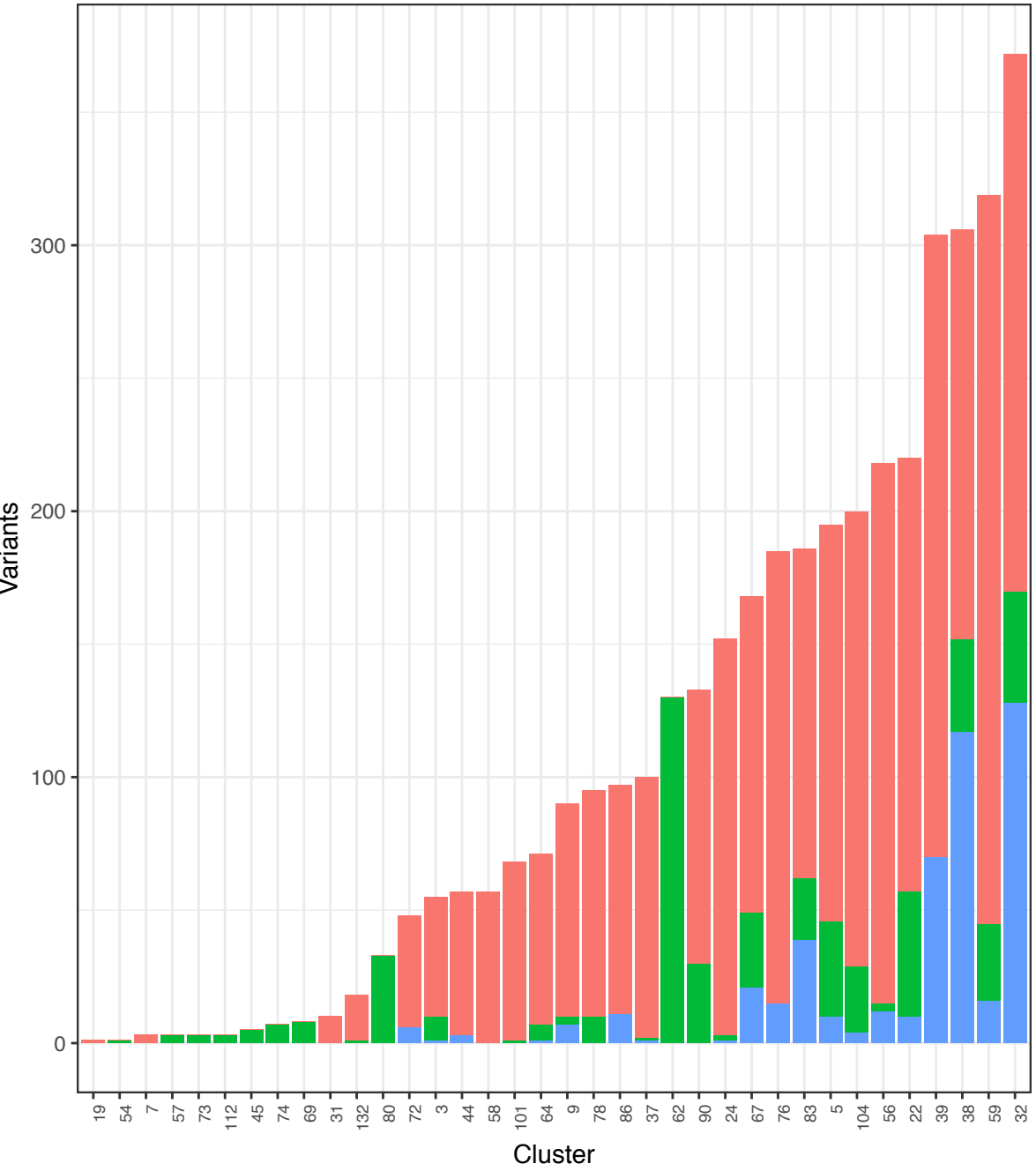
Over all 27 SCGs and 26015 positions with q < 0.001

Predicted

	False	True
False	25015	1
True	1	98

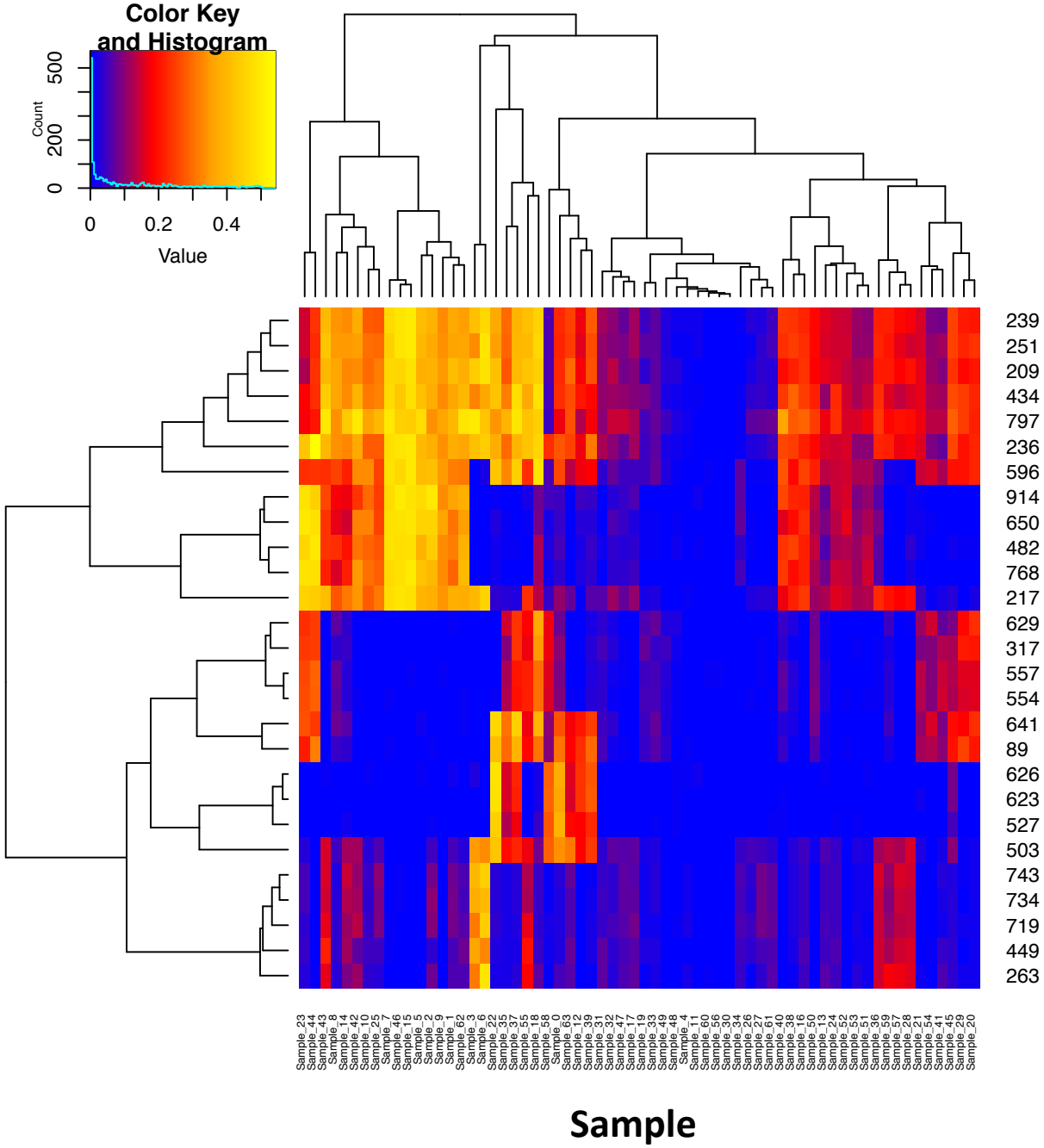
Recall = 98.9%, Precision = 98.9%

Complex mock variant results



- Filtered SCGs for coverage outliers (medians SCGs 35 to 30)
- Of the 75 clusters we predicted variants in 36, including 27 of the 29 that should have had SNPs
- Over those 27 clusters we predicted a median of 99 variants per cluster, with a mean precision of 92.32% and a mean recall of 91.85%
- 25 of the 27 clusters had at least five variants (DESMAN)

Linking variants by co-occurrence



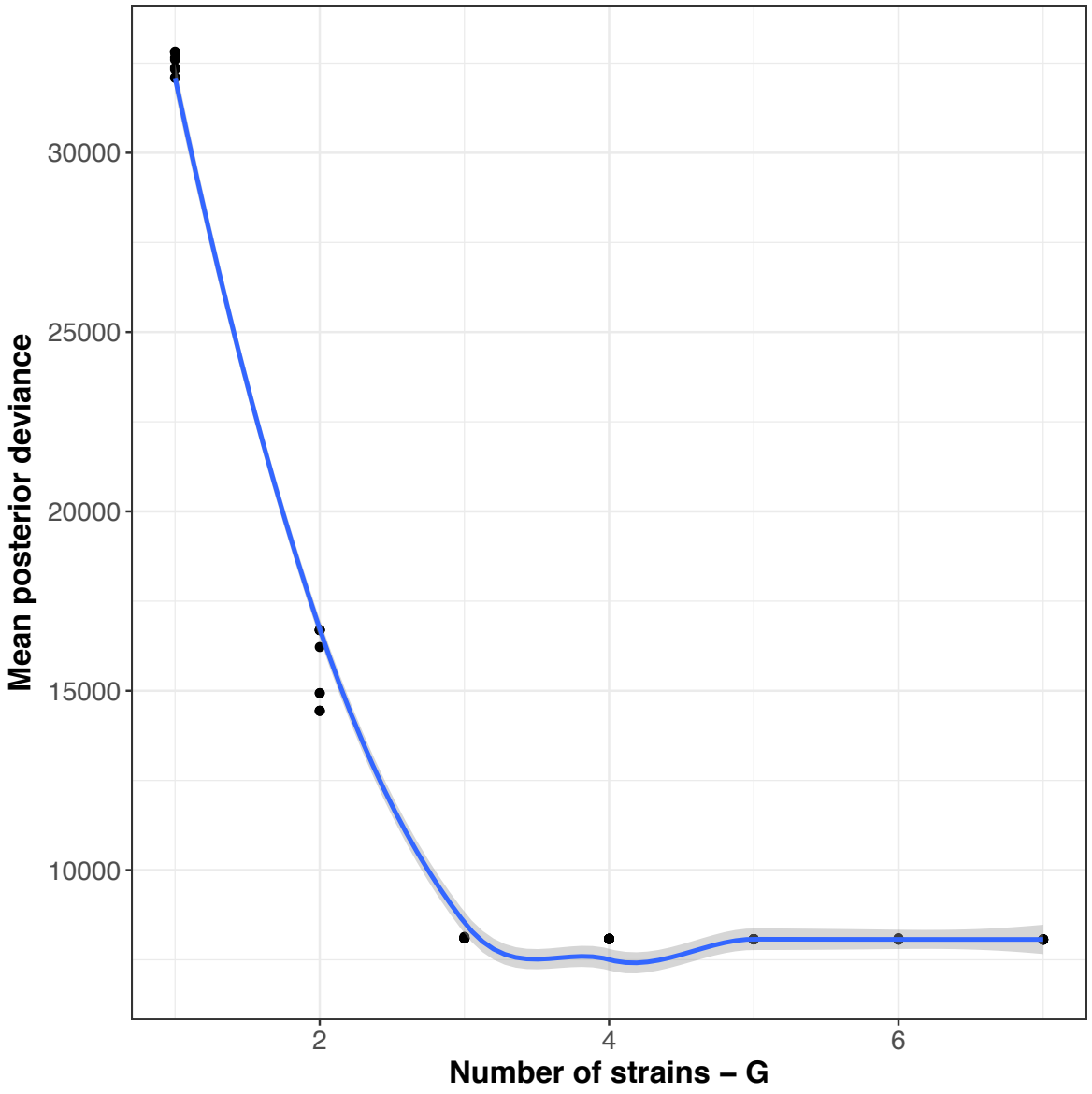
Resolving haplotypes and their abundance (intuitive)

- Assume that each haplotype has a characteristic frequency profile across samples
- Cannot cluster variants into haplotypes since clustering assumes that each sample derives from a single cluster
- Instead the variant frequencies at a given position is a summation of underlying haplotype profiles

Gibbs sampling algorithm

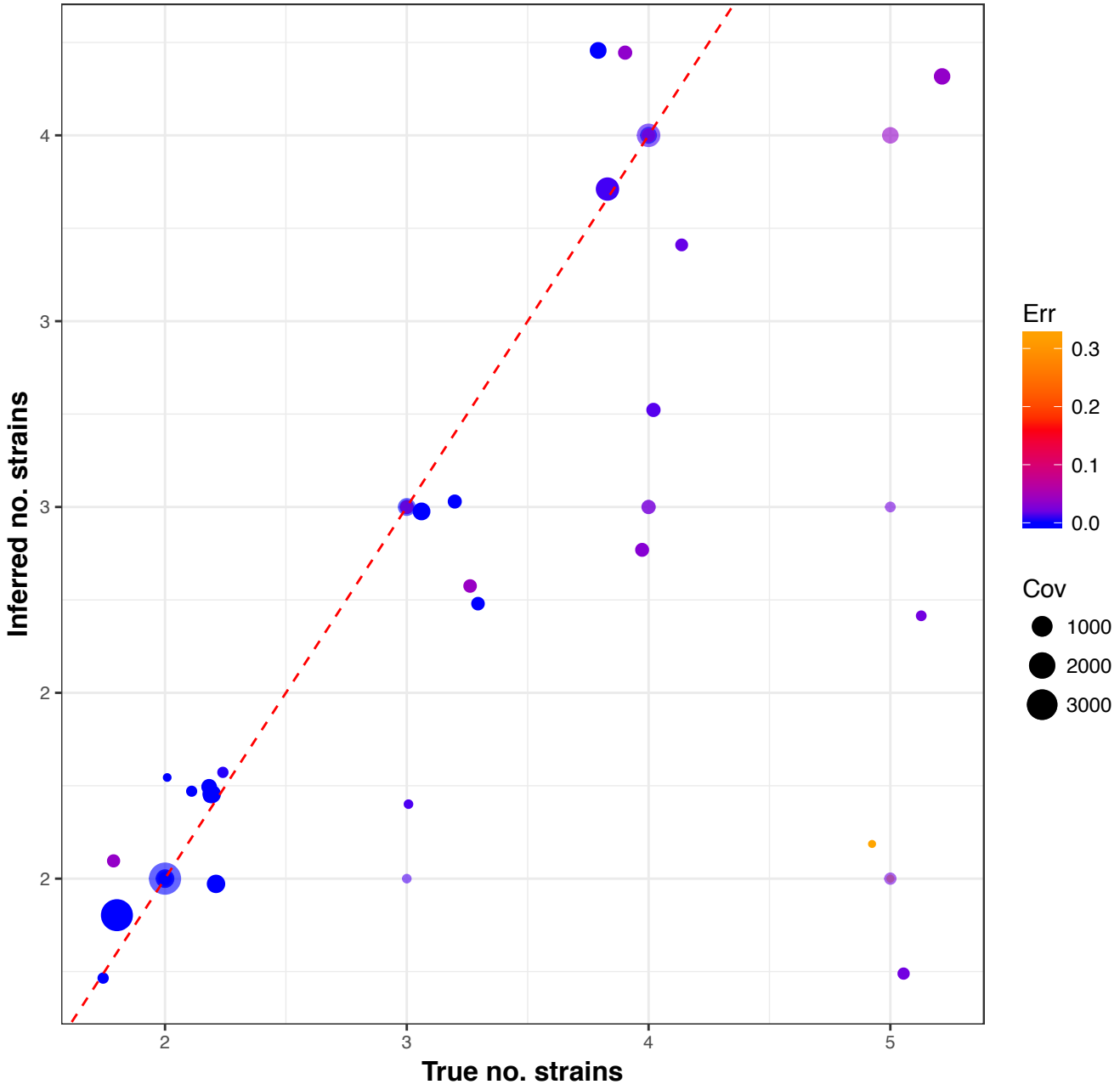
- Assume bases are independent leads to binomial probability at each position
- Devise an iterative algorithm that successively samples:
  - Haplotypes
  - Relative frequencies
  - Error rates
- Bayesian algorithm generates distribution of fitted values
- Negative log-likelihood describes overall fit
- Heuristic for determining optimum haplotype number:
  - Determine haplotype number when fractional reduction in deviance below some value
  - Find value below or equal to this which gives the most strains with relative abundance > 5% and mean SNP uncertainty < 10%

Results for Cluster 37

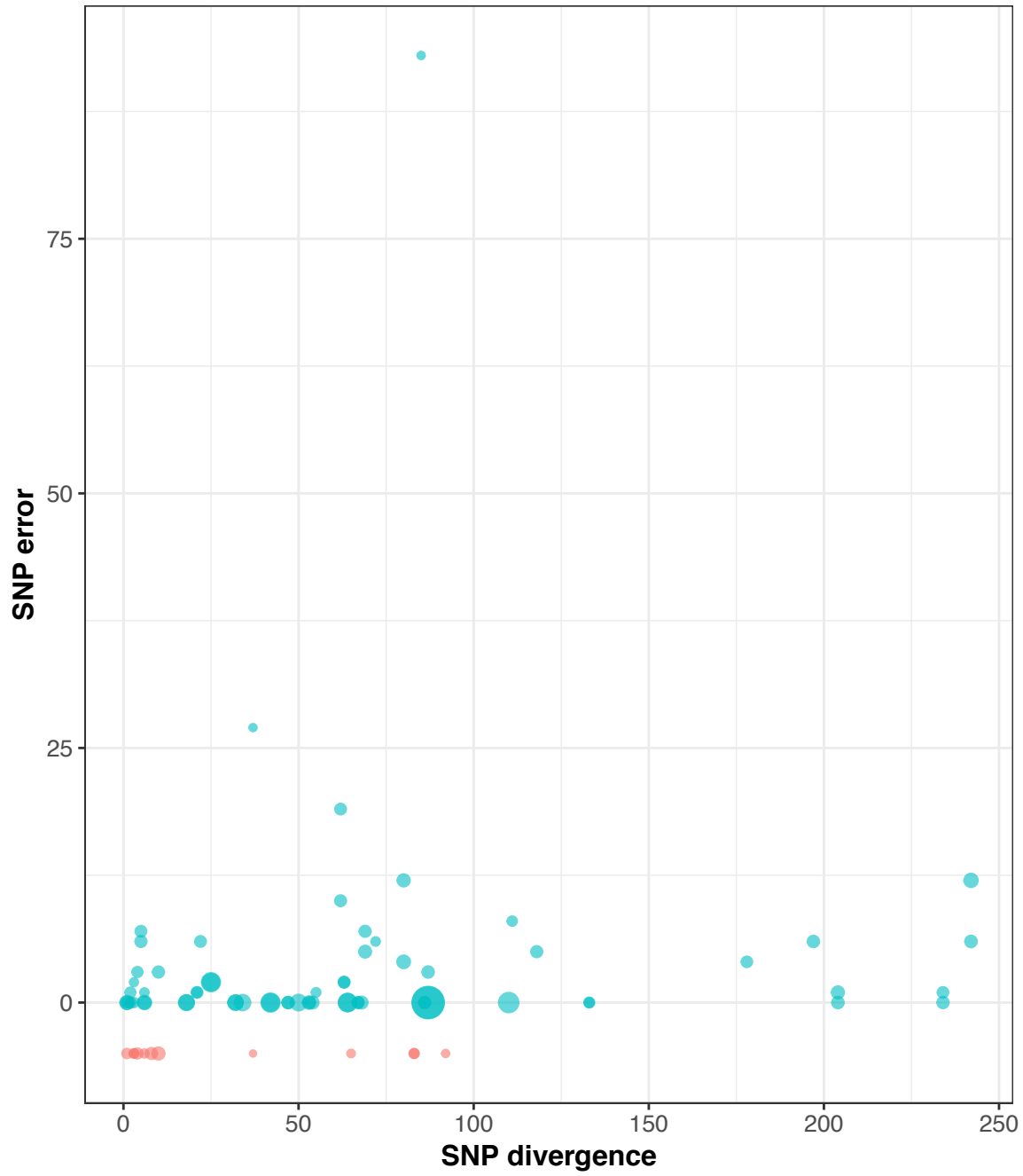


Model fit fails to improve after 3 haplotypes

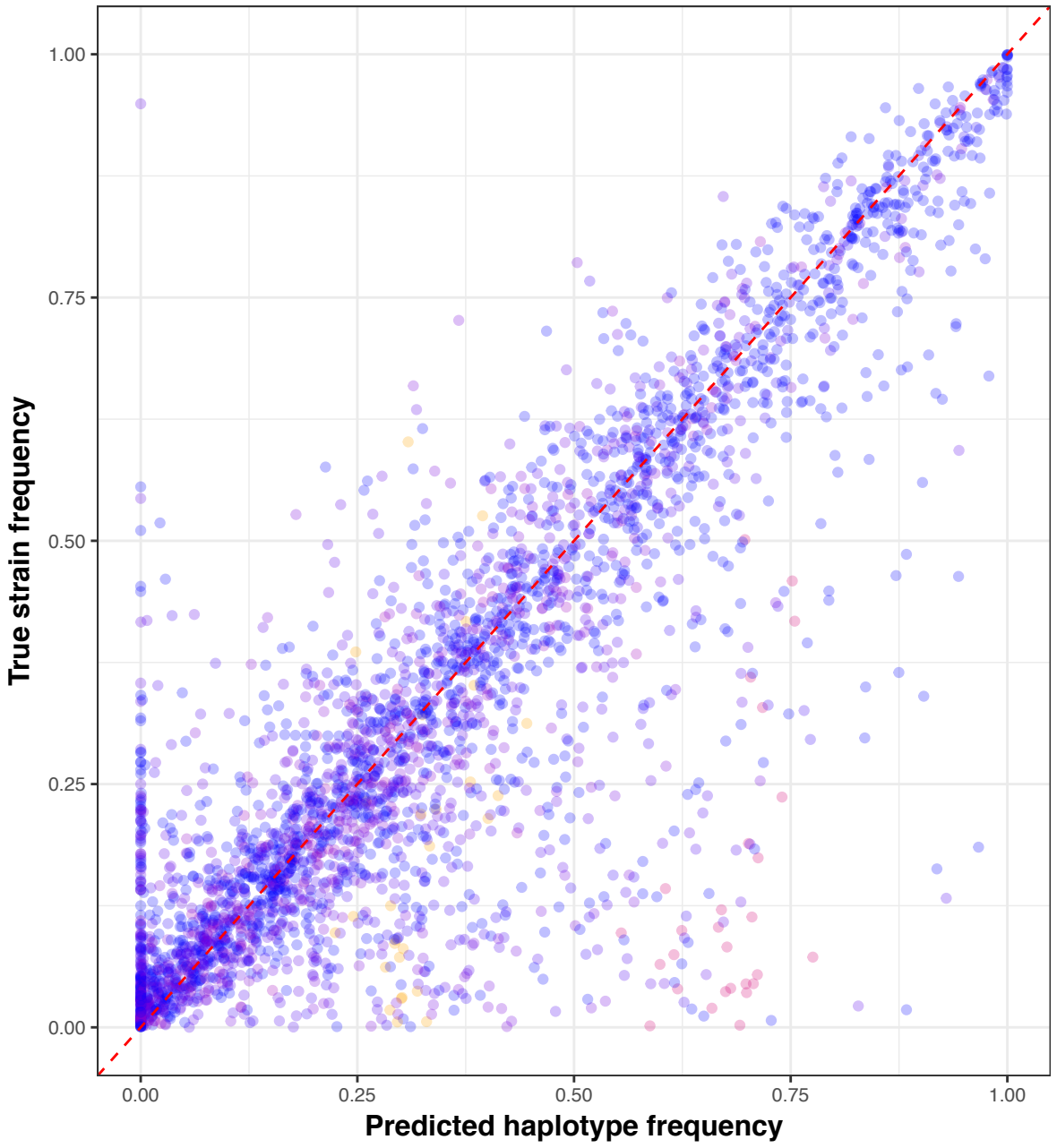
The best fit 3 haplotype run had each haplotype map onto a different reference strain with no errors



- Predicted the correct haplotype number for 18/25 (72%) of the clusters
- For 22/25 (88%) prediction was within one of true value



- Mean SNV error rate below 1% for 15/25 (60%) of clusters
- Median of 0.25% and and a mean of 2.38%
- No correlation between error rate and either the number of variants in the cluster or coverage
- We find a positive relationship between detection (67 out of 79 detected) and individual strain coverage (p-value = 0.0035)



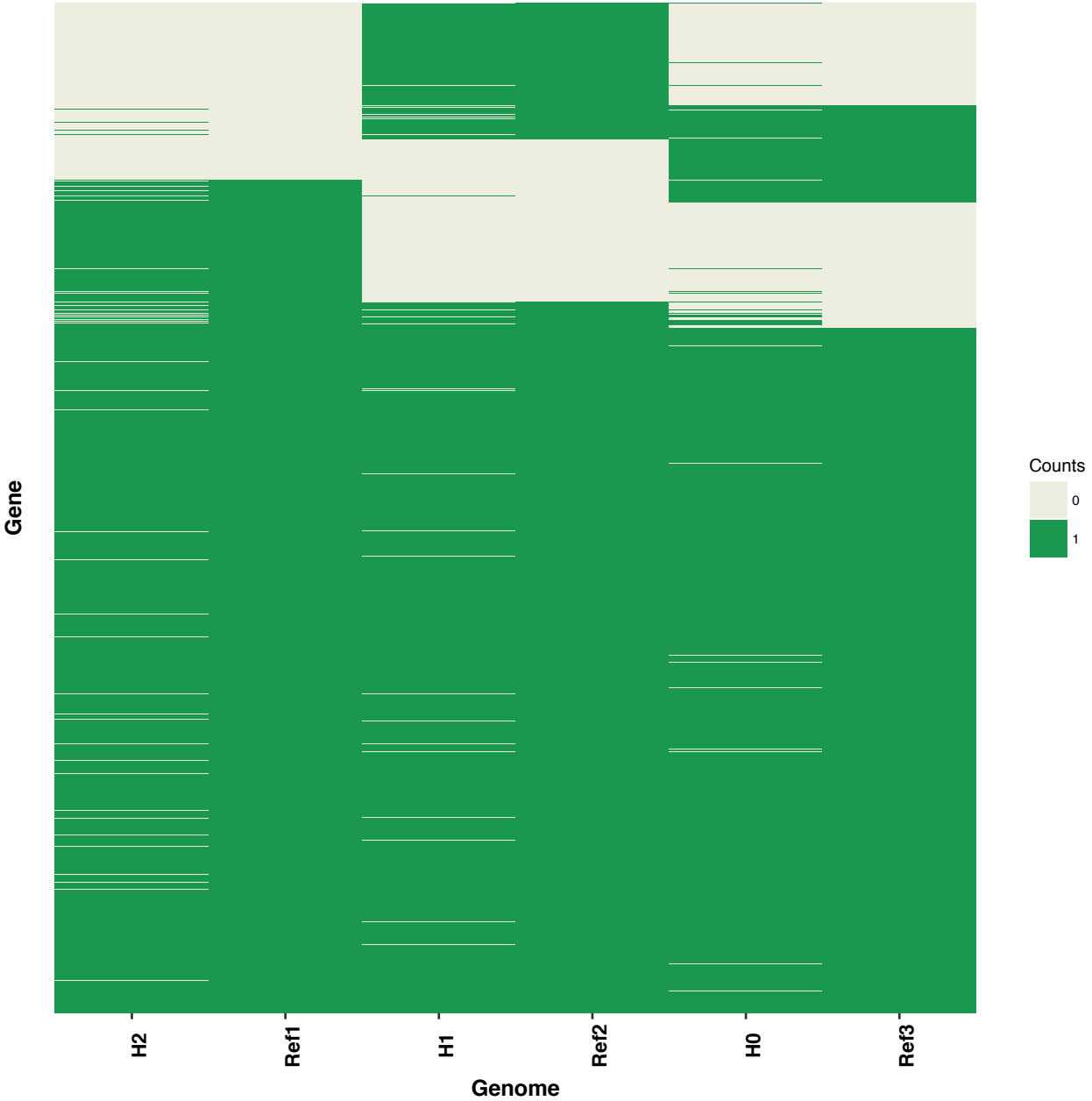
Linear regression: slope 0.820, adjusted R-squared 0.741, p-value: < 2.2e-16

Haplotypes with E < 0.01, slope 0.853, adjusted R-squared 0.810, p-value: < 2.2e-16

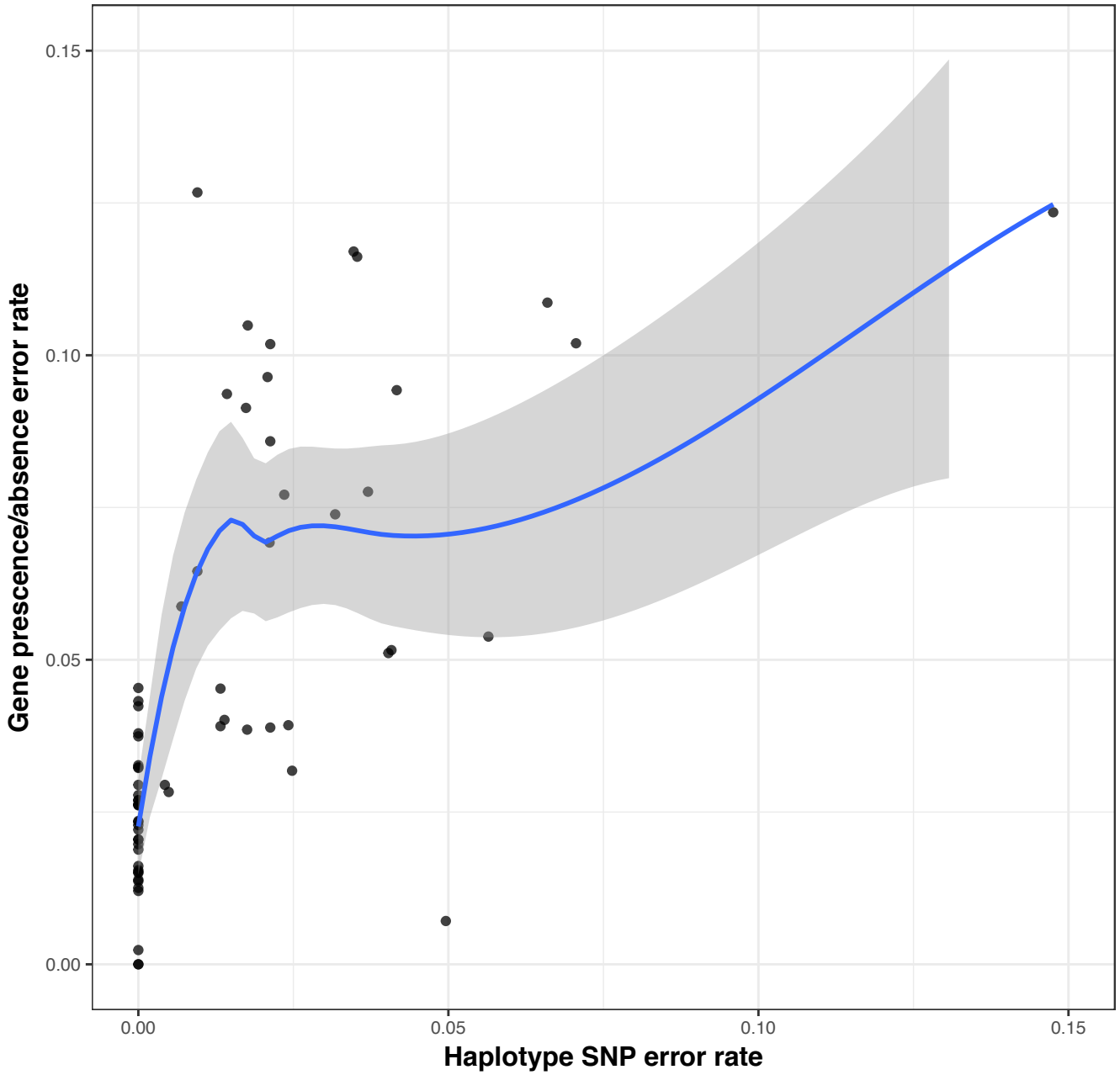
Performance of Lineage (O'Brien et al. Genetics 2014)

- On the complex mock community applied to SCGs DESMAN consistently outperformed Lineage:
  - Haplotype number correct in 18 rather than 15 MAGs
  - SCG SNV median and mean error rates were also lower at 0.25% and 2.38% for DESMAN vs. 0.641% and 3.583% respectively for Lineage (p-value = 0.06)
- On a simpler 5 strain E. coli 20 genome mock DESMAN did dramatically better 99.58% vs 76.32% accuracy
- Difference more complex problem 6,444 variants on 372 single-copy species genes
- Constrains would not run with more than ~30 samples

Cluster37: Resolving the accessory genome

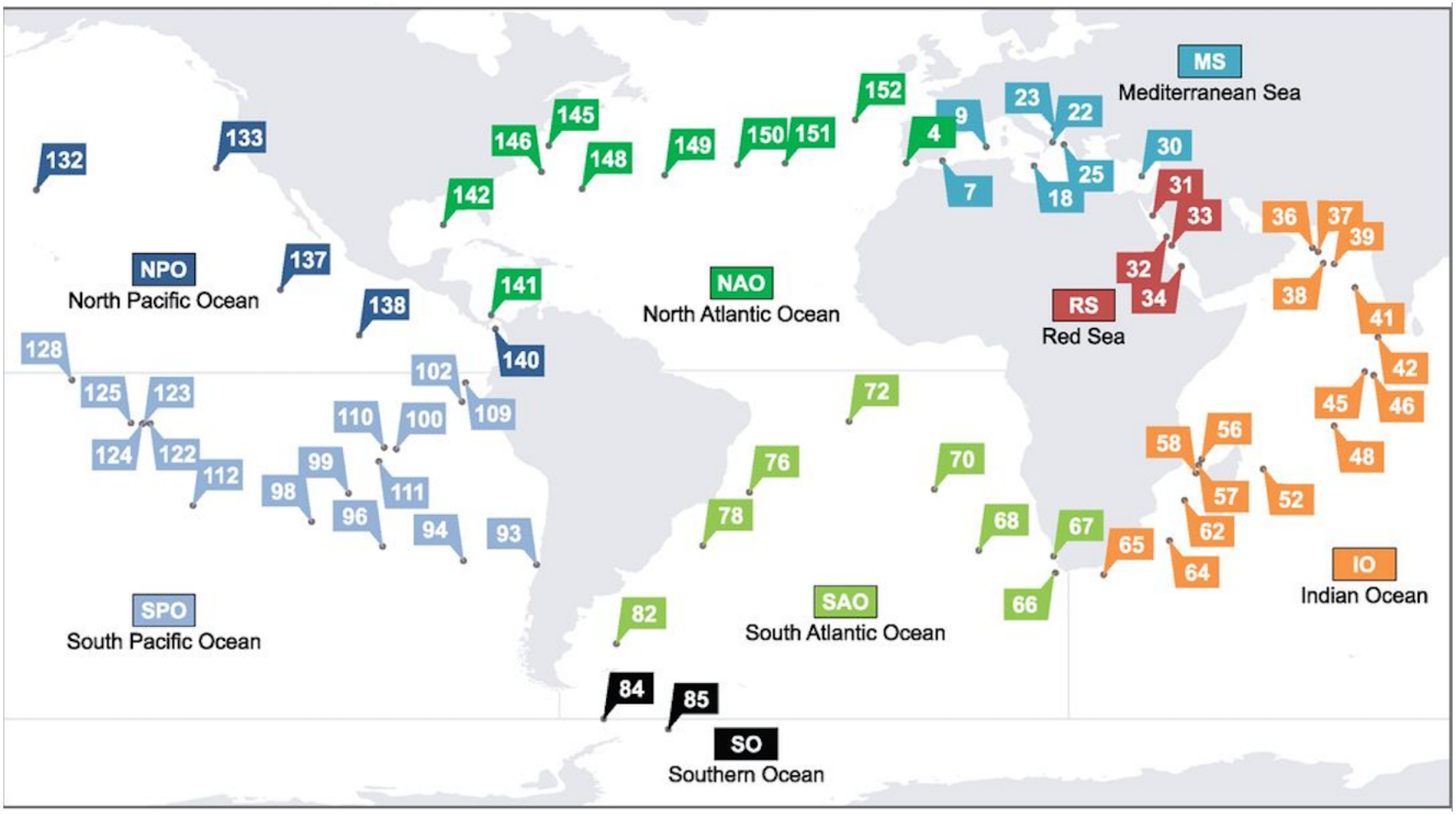


- Don’t know whether an accessory gene is present or absent in a strain
- This changes the effective strain proportions
- We infer the gene presence/absences assuming the strain proportions and error matrix are fixed at their posterior mean values from core genes
- Overall accuracy: 96-97% of gene presence/absence correct for three haplotypes

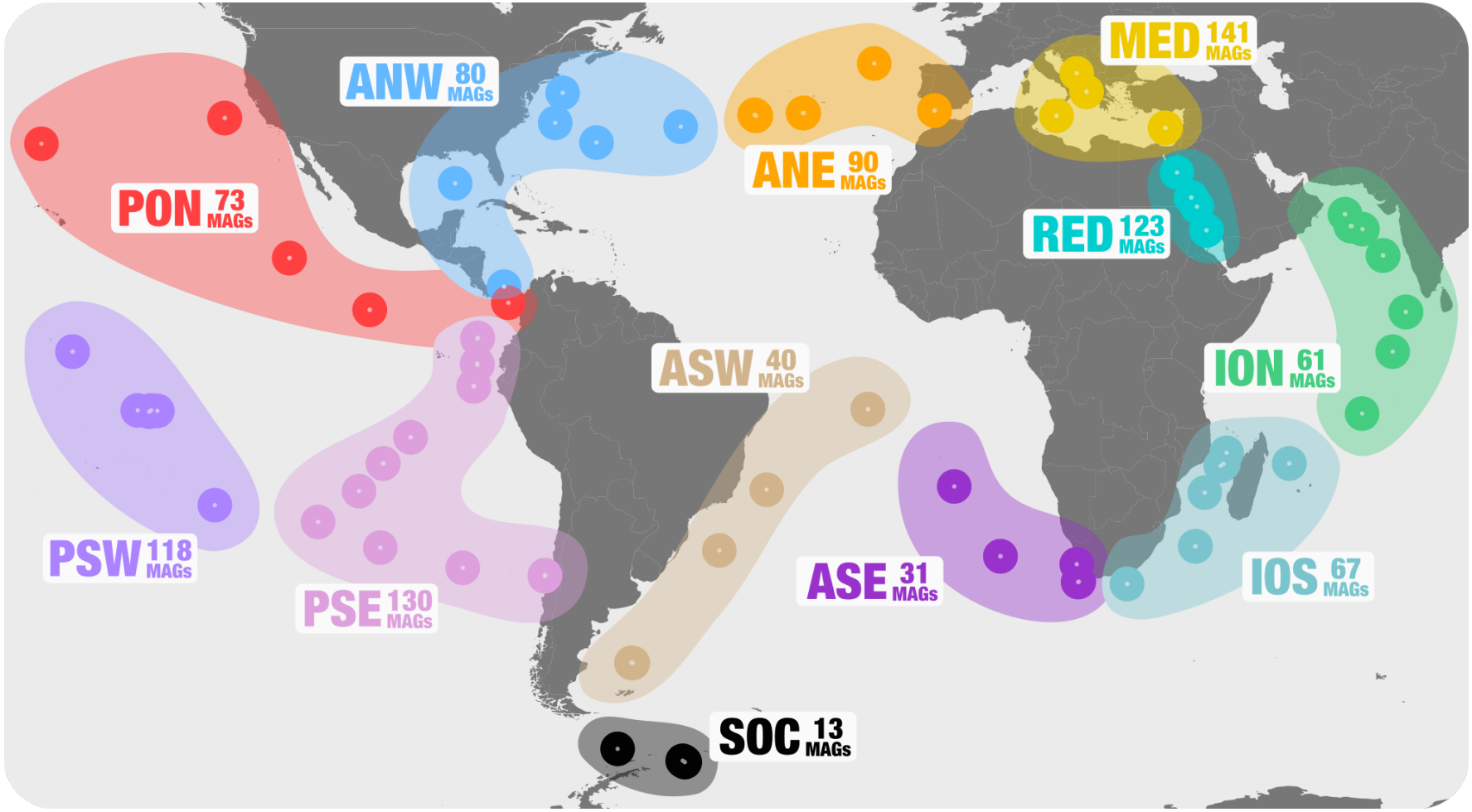


- Averaged over 67 detected haplotypes gene prediction accuracy was 94.9% (median 96.26%)
- Increased to 97.39% for the 39 haplotypes that we predicted with error rate < 1%

TARA Oceans sampling sites: Sunagawa et al. Science 2015



The 93 TARA Oceans metagenomes we analyzed represent the planktonic size fraction (0.2-3µm) of 61 surface samples and 32 samples from the deep chlorophyll maximum layer of the water column



93 metagenomes from the TARA Oceans project for which we performed a metagenomic co-assembly (n=12)

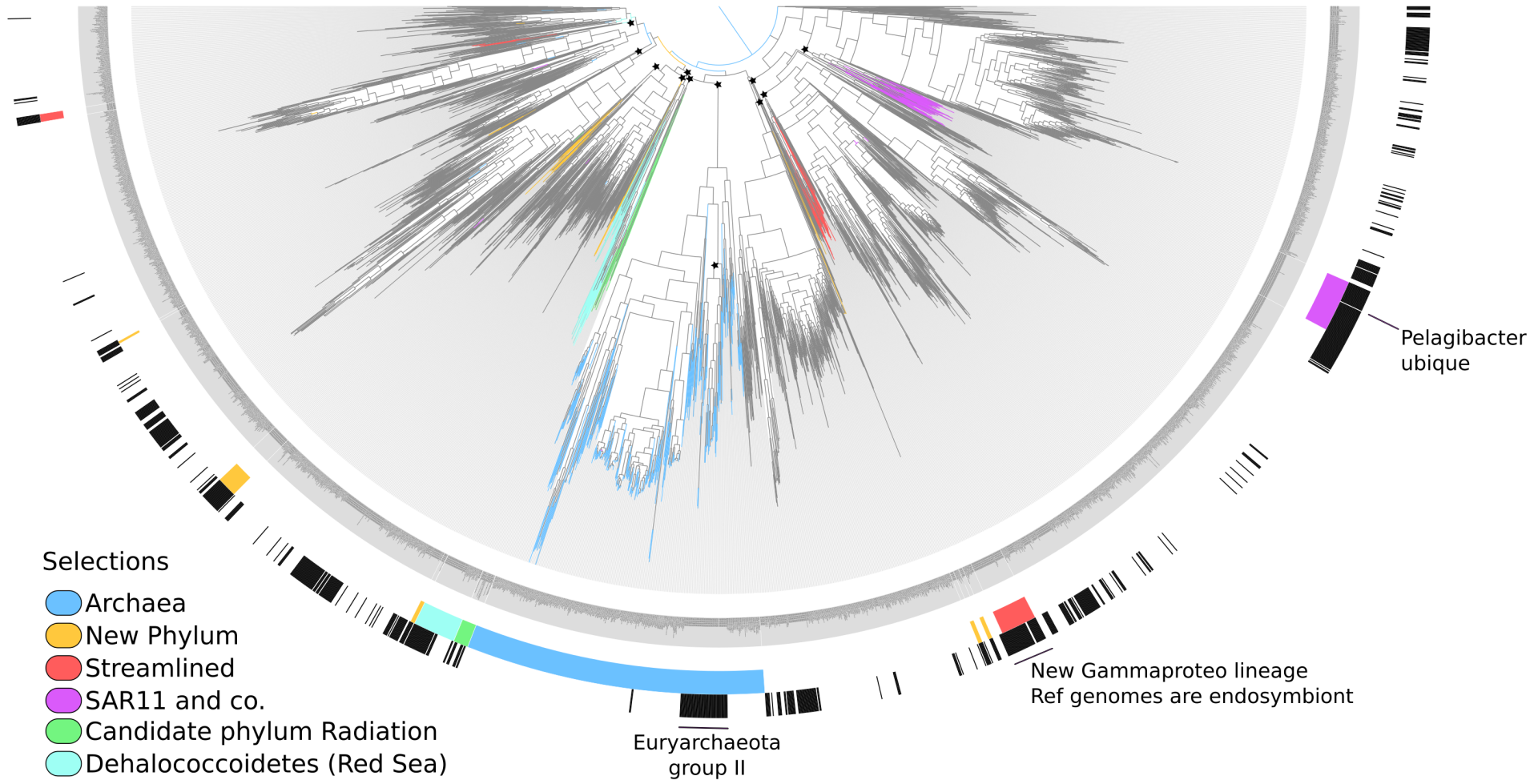
Generated 1,077 MAGS, varied from 13 to 141 after the removal of redundant MAGs, for a total of 957 non-redundant MAGs encompassing the three domains of life

Delmont et al Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in the surface ocean http://biorxiv.org/content/early/2017/04/23/129791

Phylogenetic analysis of 660 MAGs

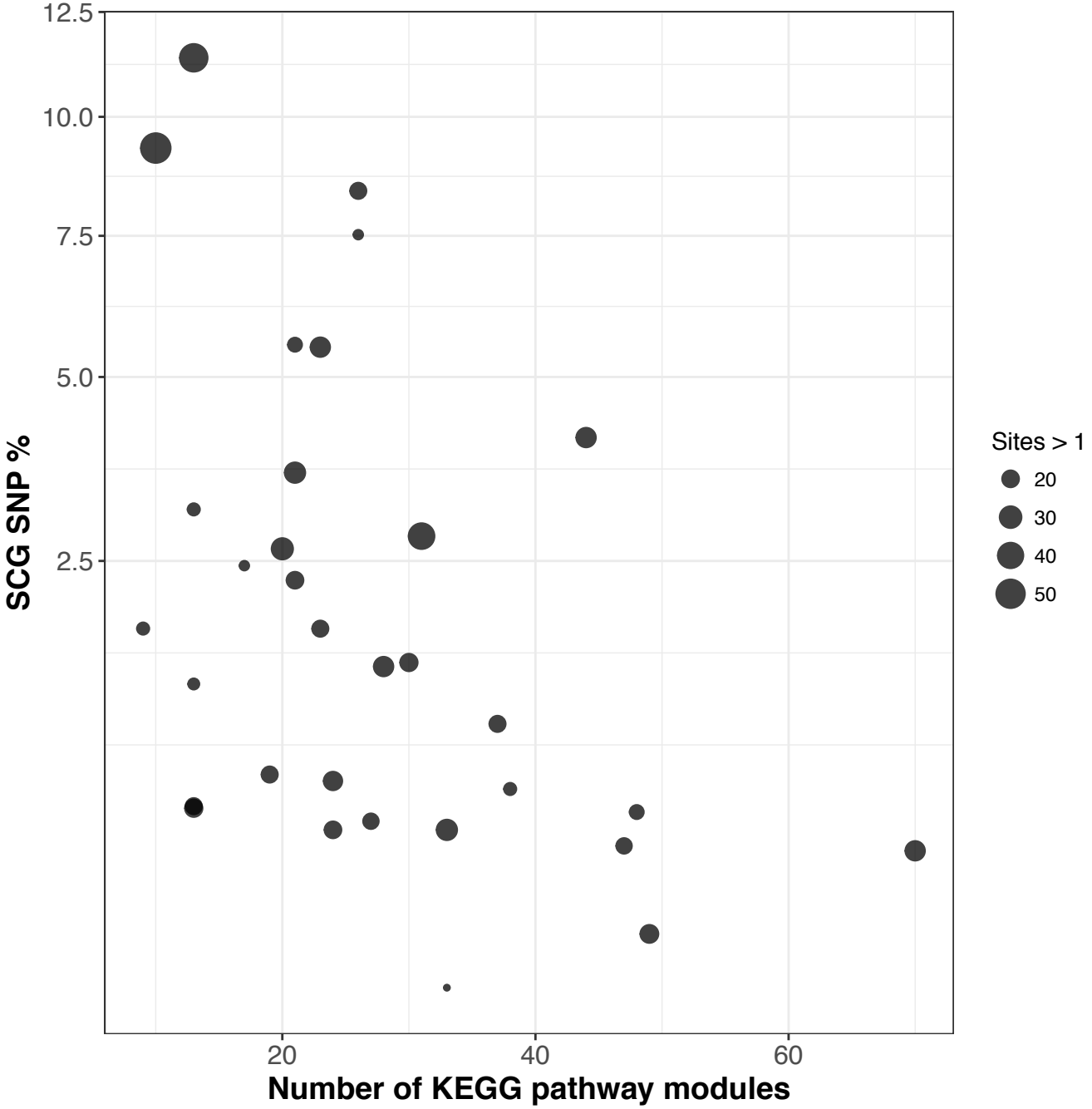
plus 1,755 ref genomes (1 genome per genus for bacteria)

→ Concatenated single copy genes



Delmont et al Nitrogen-fixing populations of Planctomycetes and Proteobacteria are abundant in the surface ocean http://biorxiv.org/content/early/2017/04/23/129791

Tara MAG variants



Consider 32 MAGs with cov. > 100.

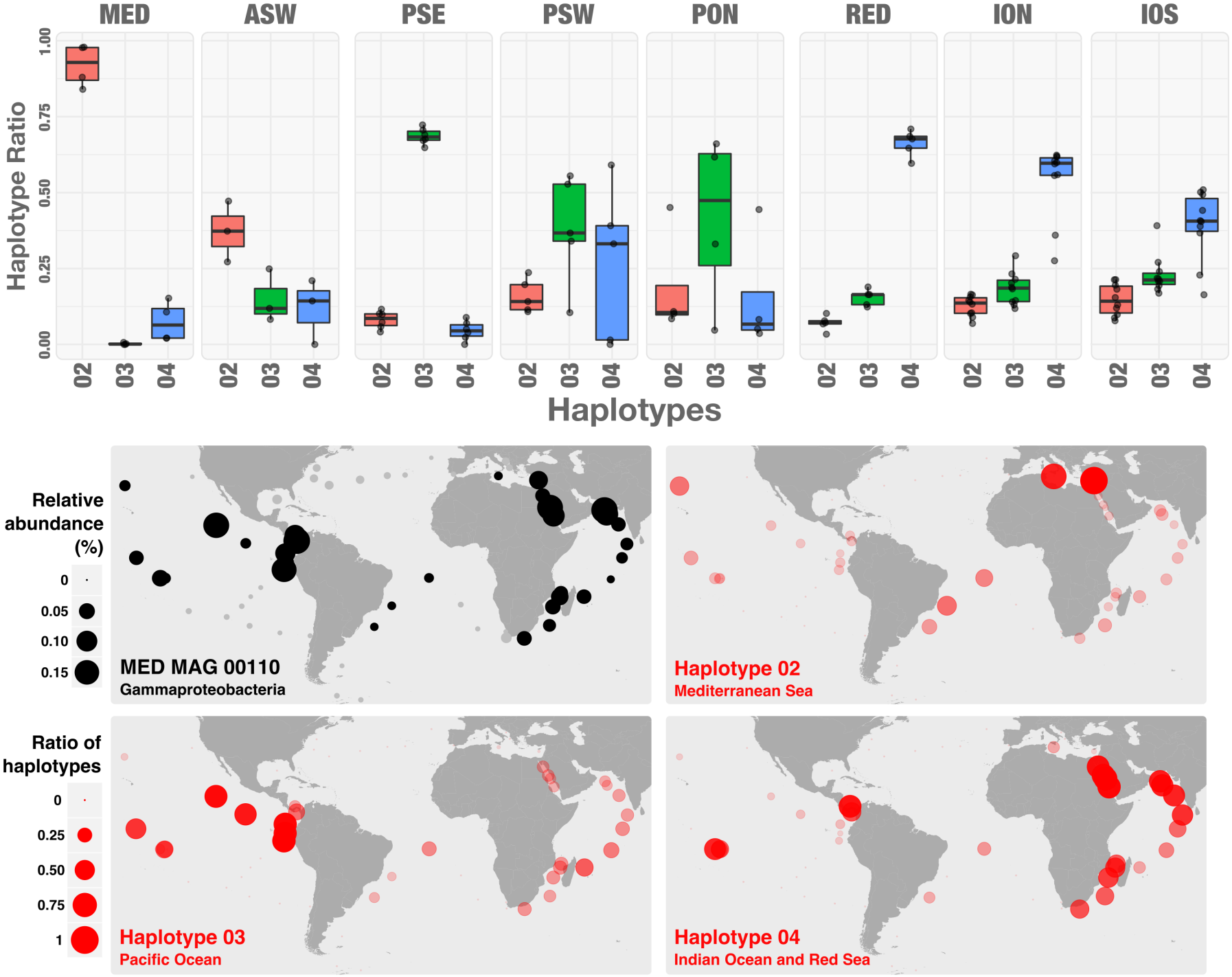
The SNP frequency was independent of MAG coverage

Observe a negative correlation with genome length (Spearman's p-value = 0.016)

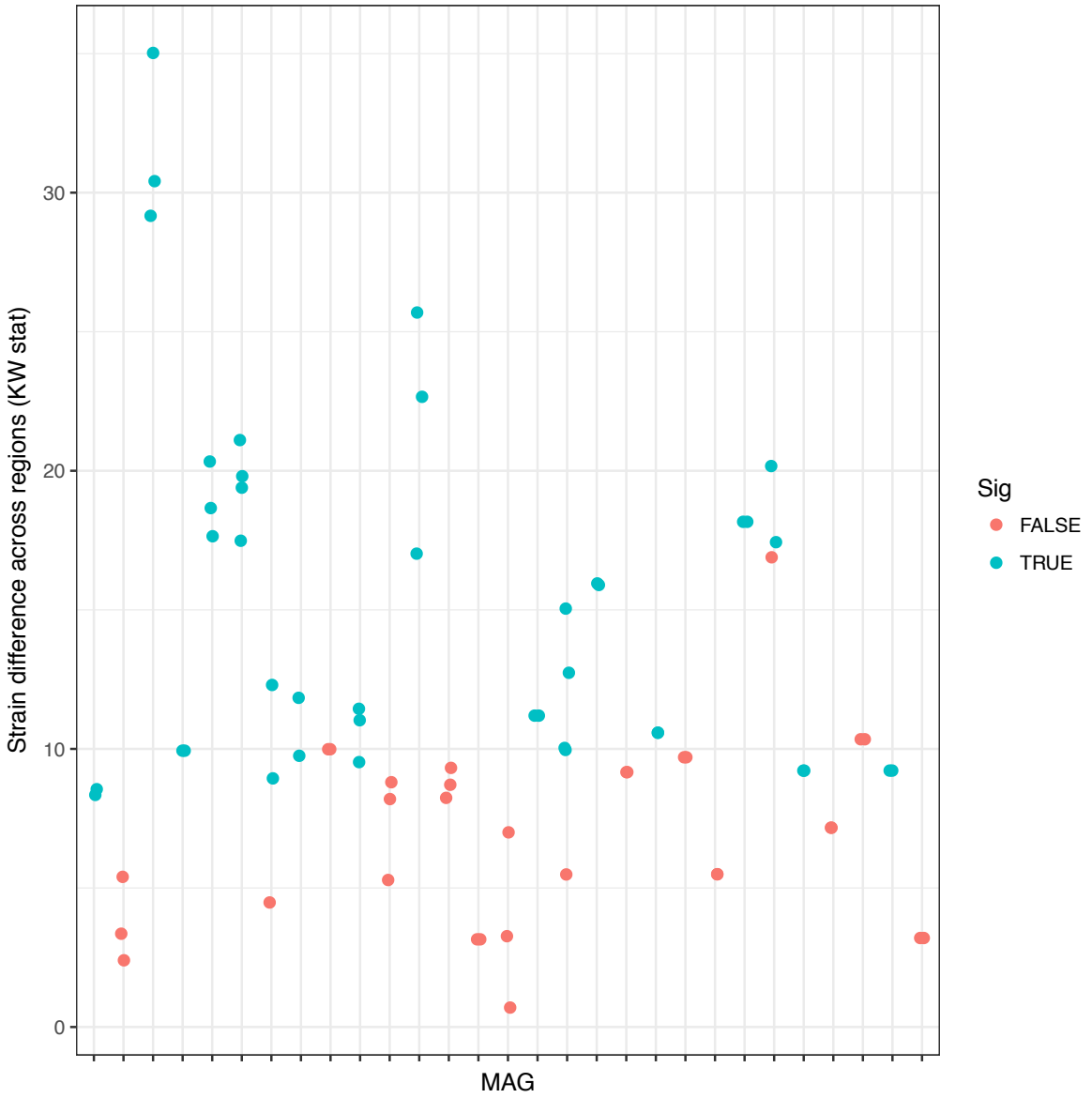
Stronger negative relationship with number of KEGG Pathway modules encoded in the genome (Spearman's p-value = 0.0045)

TARA DESMAN analysis

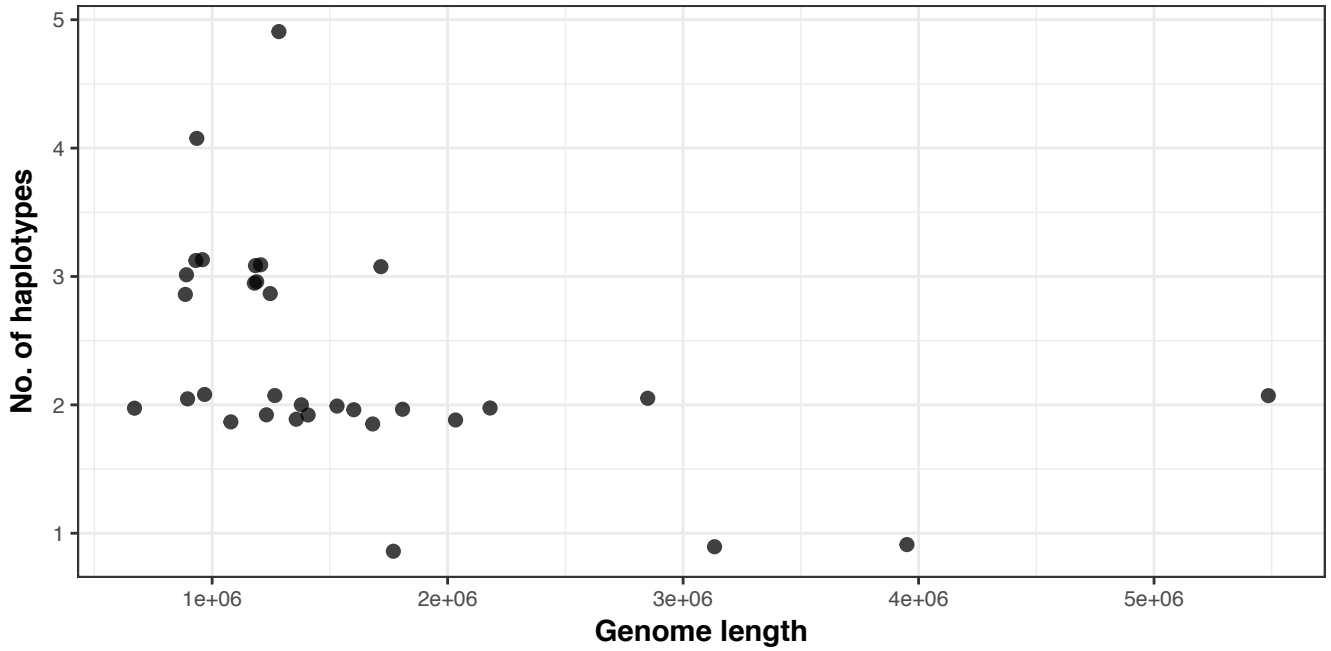
- Out of the 32 MAGs tested for haplotypes 29/32 had strain variation (1->3 2->17 3->10 4->1 5->1)
- The haplotypes were geographically localised e.g. TARA MAG 00110 a Proteobacteria with a highly streamlined 890,789 bp genome
  - Large group of uncultured organisms (relative *Candidatus Evansia muelleri* and *Riesia pediculicola*)
  - Three haplotypes that differed by around 2% ANI on core genes and between 13-21% of genes at 5% ANI clusters


MED
ASW
PSE
PSW
PON
RED
ION
IOS
Haplotype Ratio
0.00
0.25
0.50
0.75
1.00
02
03
04
Haplotypes
Relative abundance (%)
0
0.05
0.10
0.15
MED MAG 00110
Gammaproteobacteria
Haplotype 02
Mediterranean Sea
Ratio of haplotypes
0
0.25
0.50
0.75
1
Haplotype 03
Pacific Ocean
Haplotype 04
Indian Ocean and Red Sea

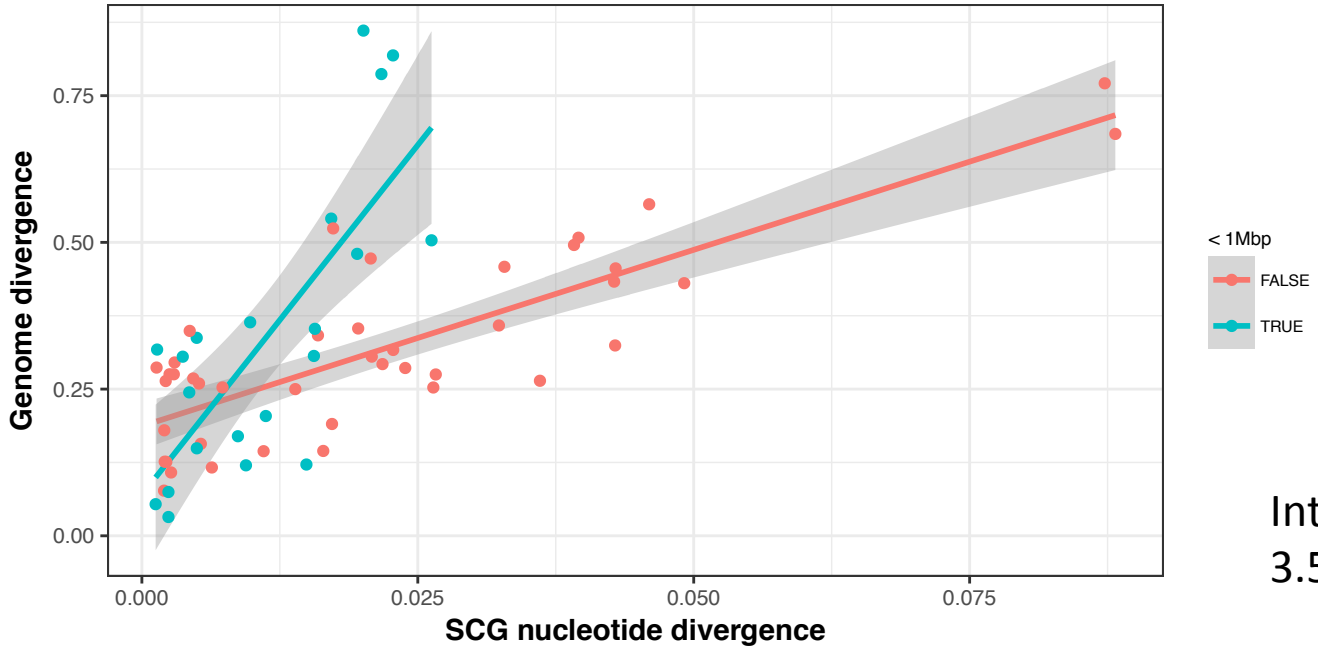
Majority of haplotypes across all MAGs showed correlation with geographic region



- 42/73 = 57% of inferred strains had a significant correlation with geographic region
- 29/73 = 40% correlated with mean sea temperature
- Ordering MAGs by length there is a trend to more geographic signal although not significant (p-value = 0.1753)



p-value = 0.000068



Interaction p-value = 3.51e-06

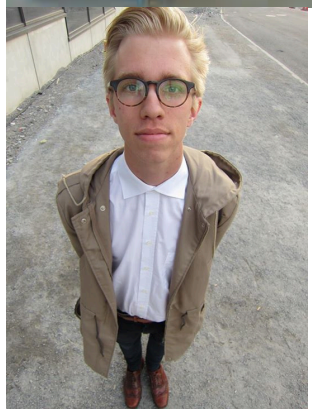
Summary

- DESMAN increases the amount of biologically relevant information that can be extracted from an assembly:
  - Resolve abundant strains *de novo* with high accuracy from core genome
  - Obtain accessory gene complement
  - https://github.com/chrisquince/DESMAN
- Tara Oceans:
  - Adaptive strain diversity endemic in the Oceans
  - Stream lined genomes associated with increased genetic diversity and strain diversity
  - Evidence of rapid relative genome divergence for very streamlined < 1Mbp organisms
- Future directions:
  - Strain resolution directly on the assembly graph
  - Long read technologies (Nanopore and Synthetic Long Reads)

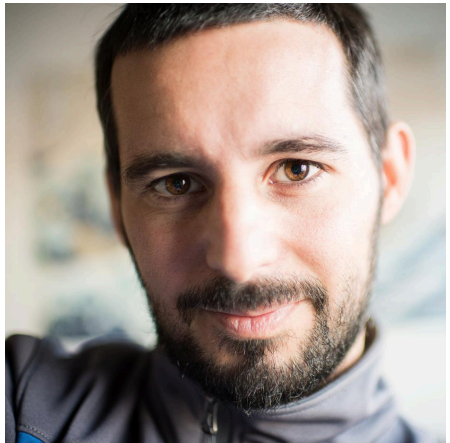
Stephanie Connelly + Gavin Collins + Seung Gu (U. of Glasgow)

Nick Loman + Joshua Quick (U. of Birmingham)

Brynjar Smári Bjarnason + Johannes Alneberg + Ino de Bruijn + Anders Andersson (KTH, Stockholm)



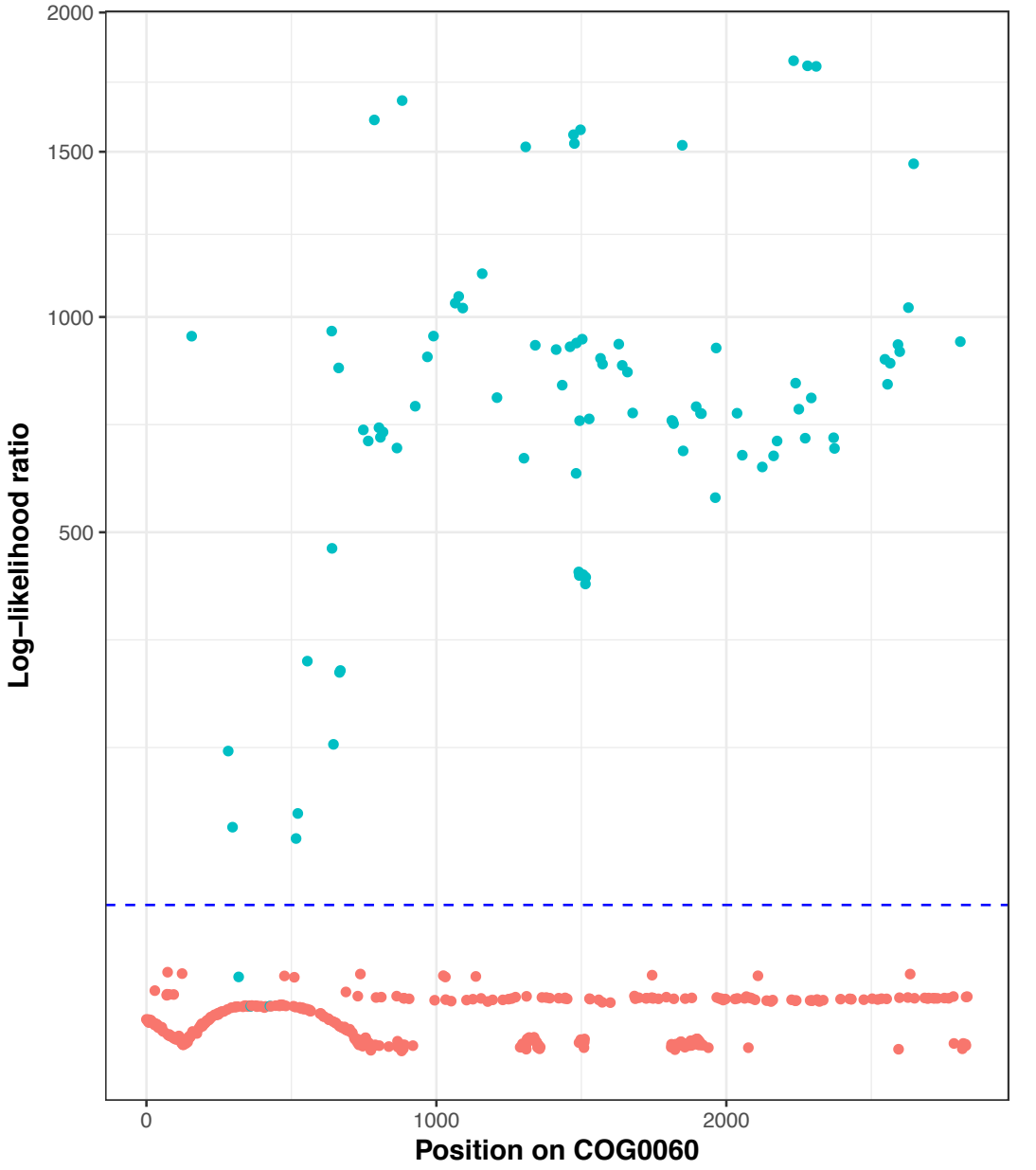
A. Murat Eren and Tom Delmont (Chicago)



STAMPS CONCOCT/DESMAN Tutorial

https://github.com/chrisquince/STAMPS_Tutorial2017

Variant prediction for Cluster 59 -> *Pseudomonas chlororaphis*



COG0060 predict 98 variant positions which includes 98 out of 99 true variants

Over all 34 SCGs and 23914 positions with q < 0.001

Predicted

	False	True
False	23595	29
True	16	274

Recall = 94%, Precision = 90.4%