Cleaning genotype data for diversity outbred mice

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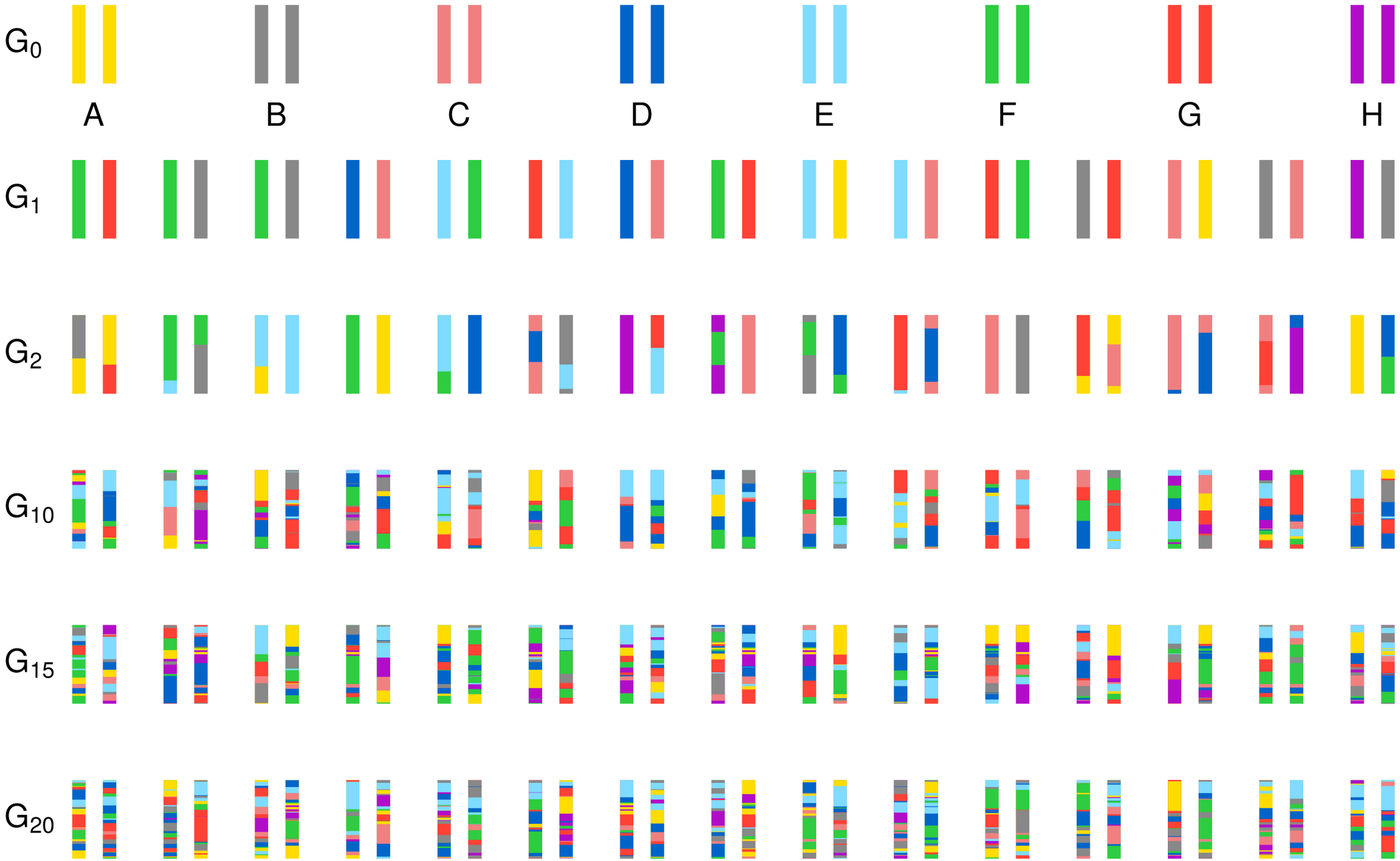
Multi-parent advanced intercross



Diversity outbred mouse data

- 500 DO mice
- GigaMUGA SNP arrays (114k SNPs)
- RNA-seq data on pancreatic islets
- Microbiome data (16S and shotgun sequencing)
- protein and lipid measurements by mass spec
- Collaboration with Alan Attie, Gary Churchill, Brian Yandell, Josh Coon, Federico Rey, and many others

Principles

What might have gone wrong?

How could it be revealed?

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Also, just make a bunch of graphs.

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What might have gone wrong?

How could it be revealed?

Also, just make a bunch of graphs.

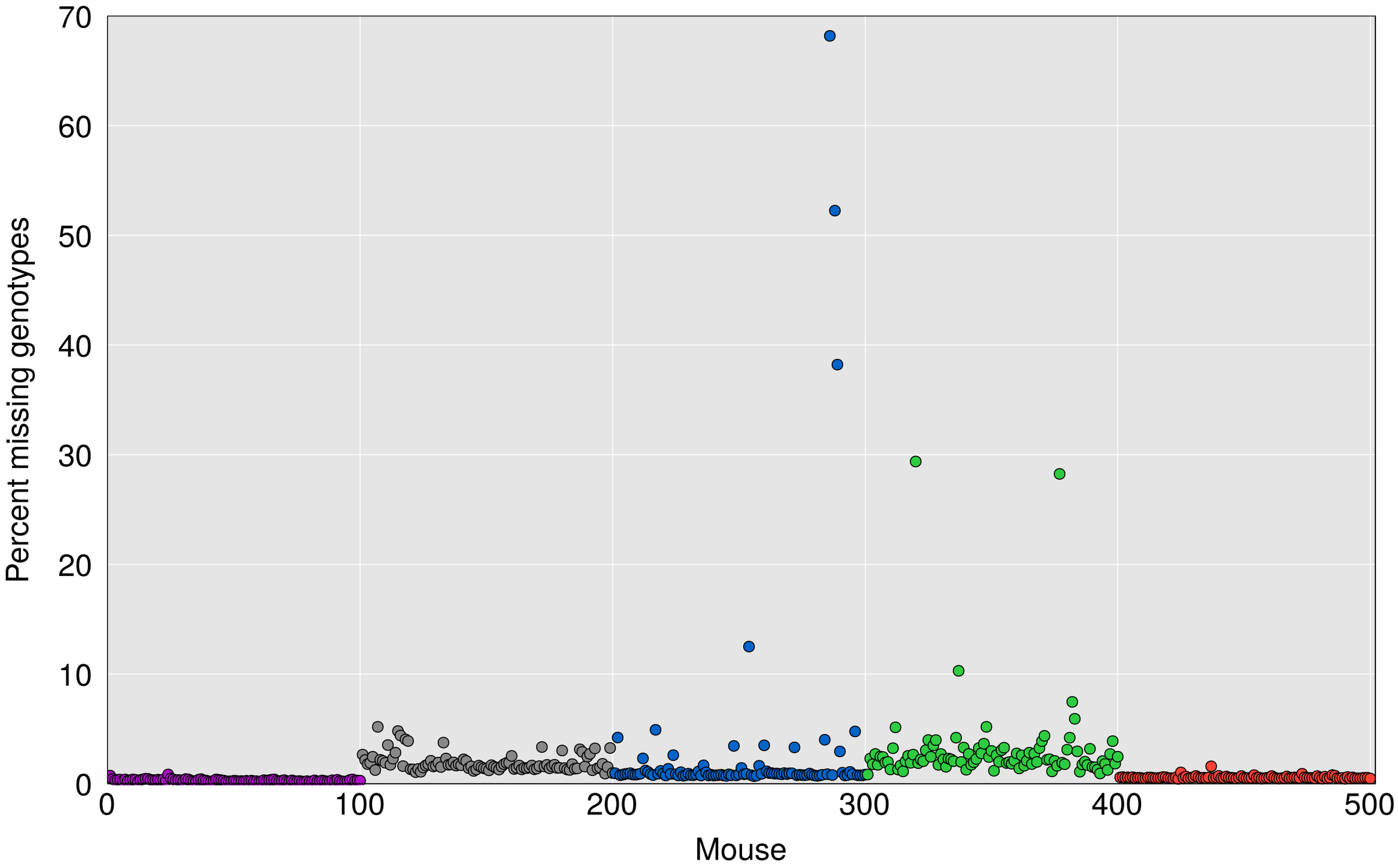
If you see something weird, try to figure it out.

Possible problems

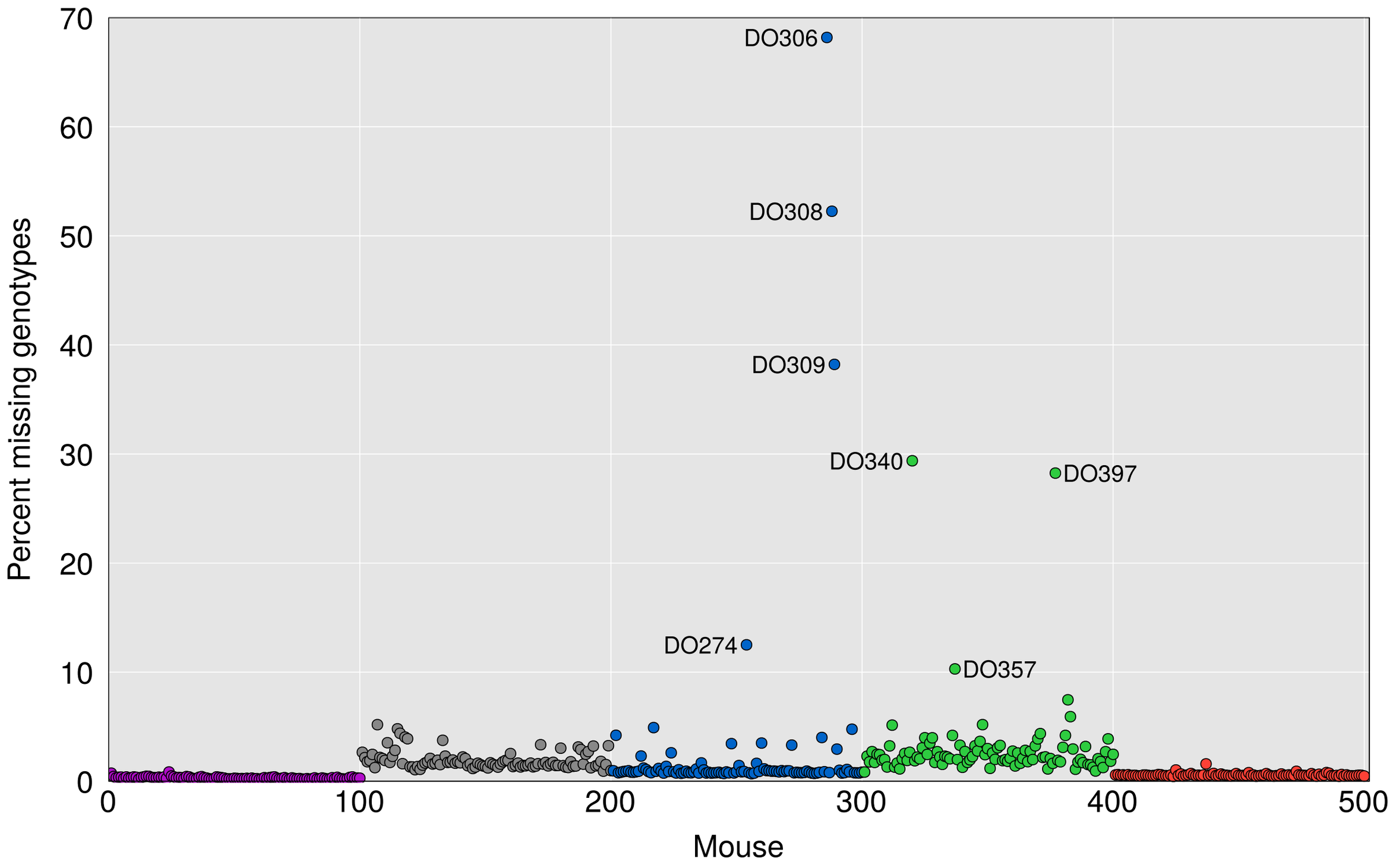
- Sample duplicates
- Sample mix-ups
- Bad samples
- Bad markers
- Genotyping errors in founders

What to look at first?

Missing data per sample

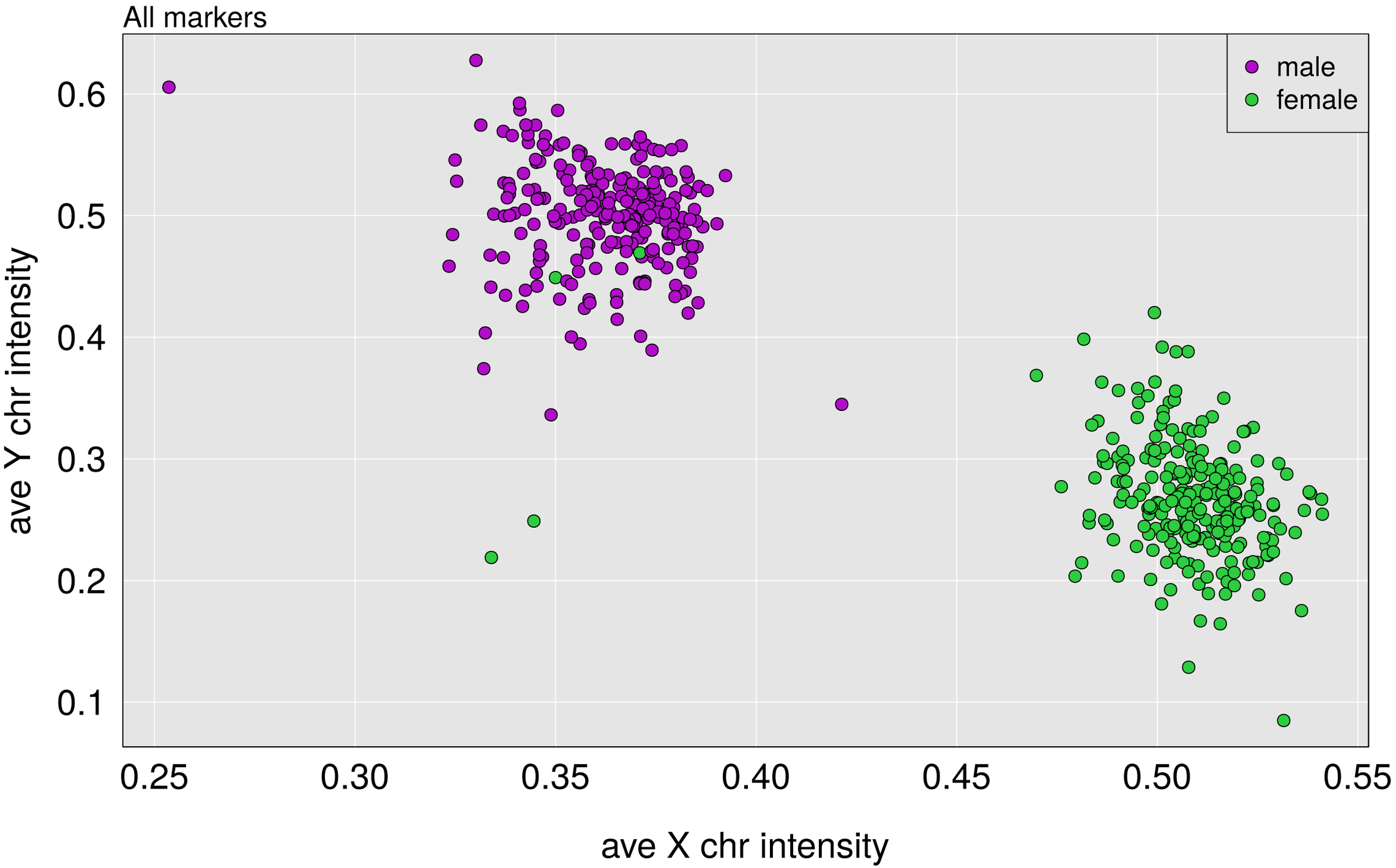


Missing data per sample

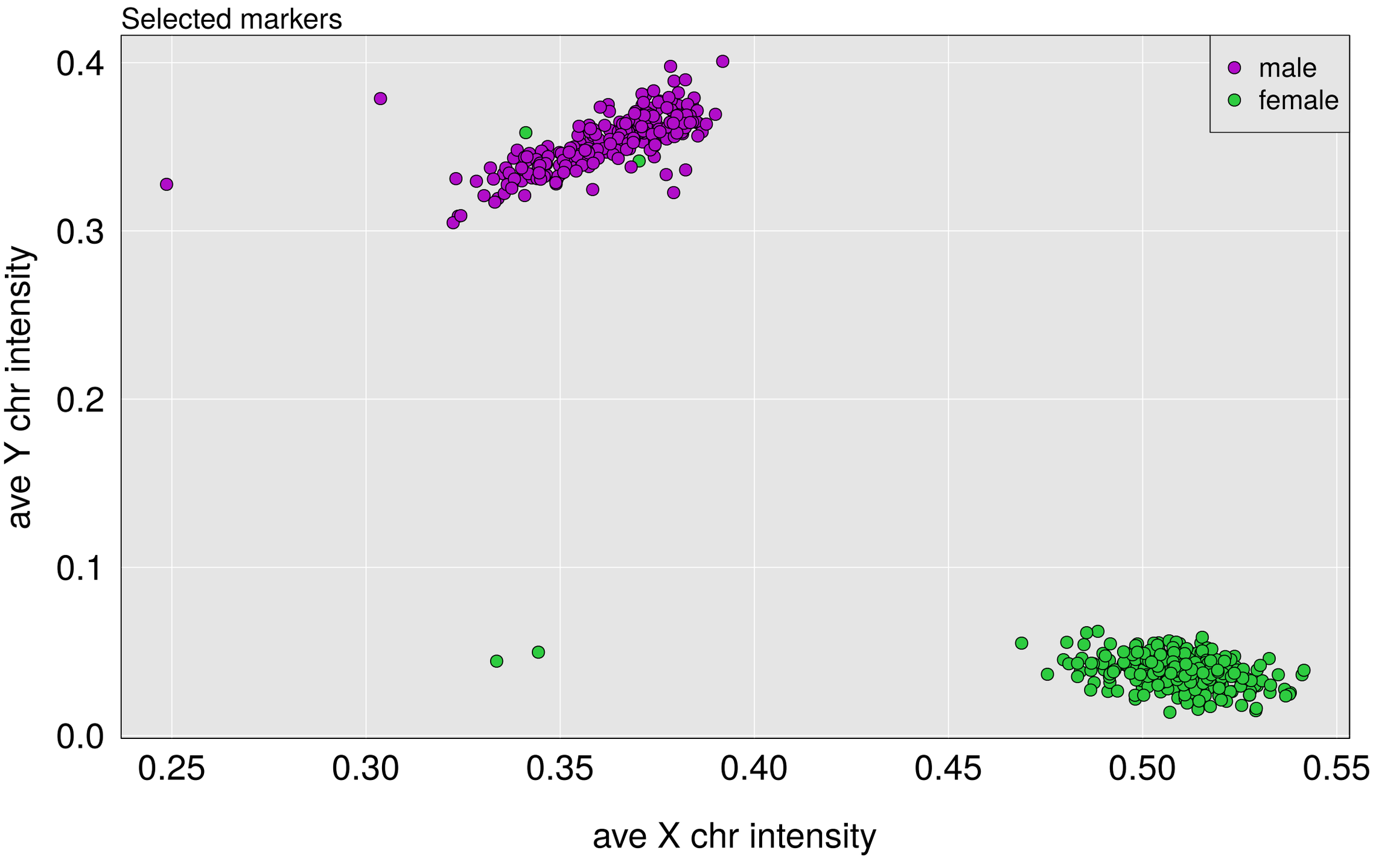


Swapped sex labels

Average SNP intensity on X and Y chr



Average SNP intensity on X and Y chr



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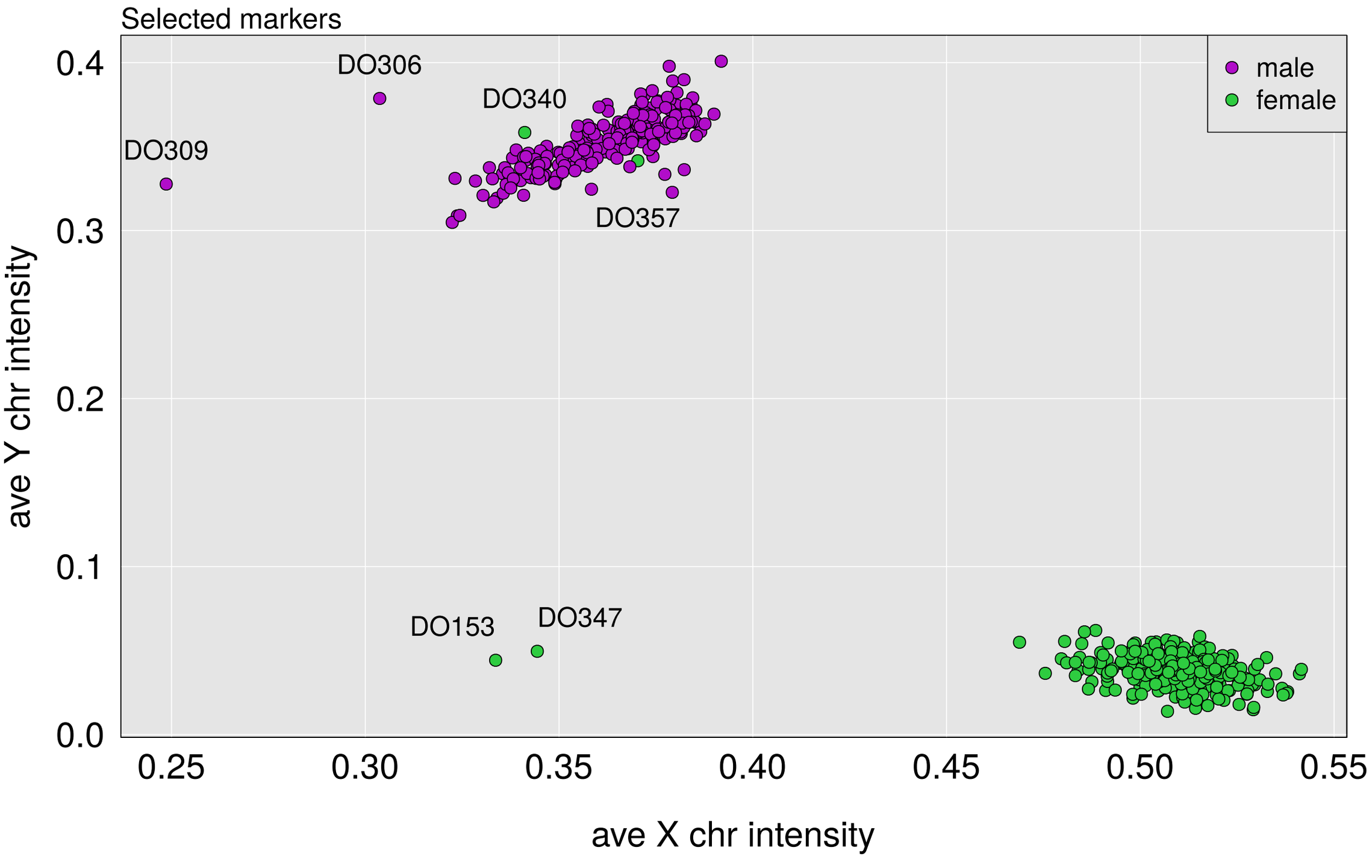
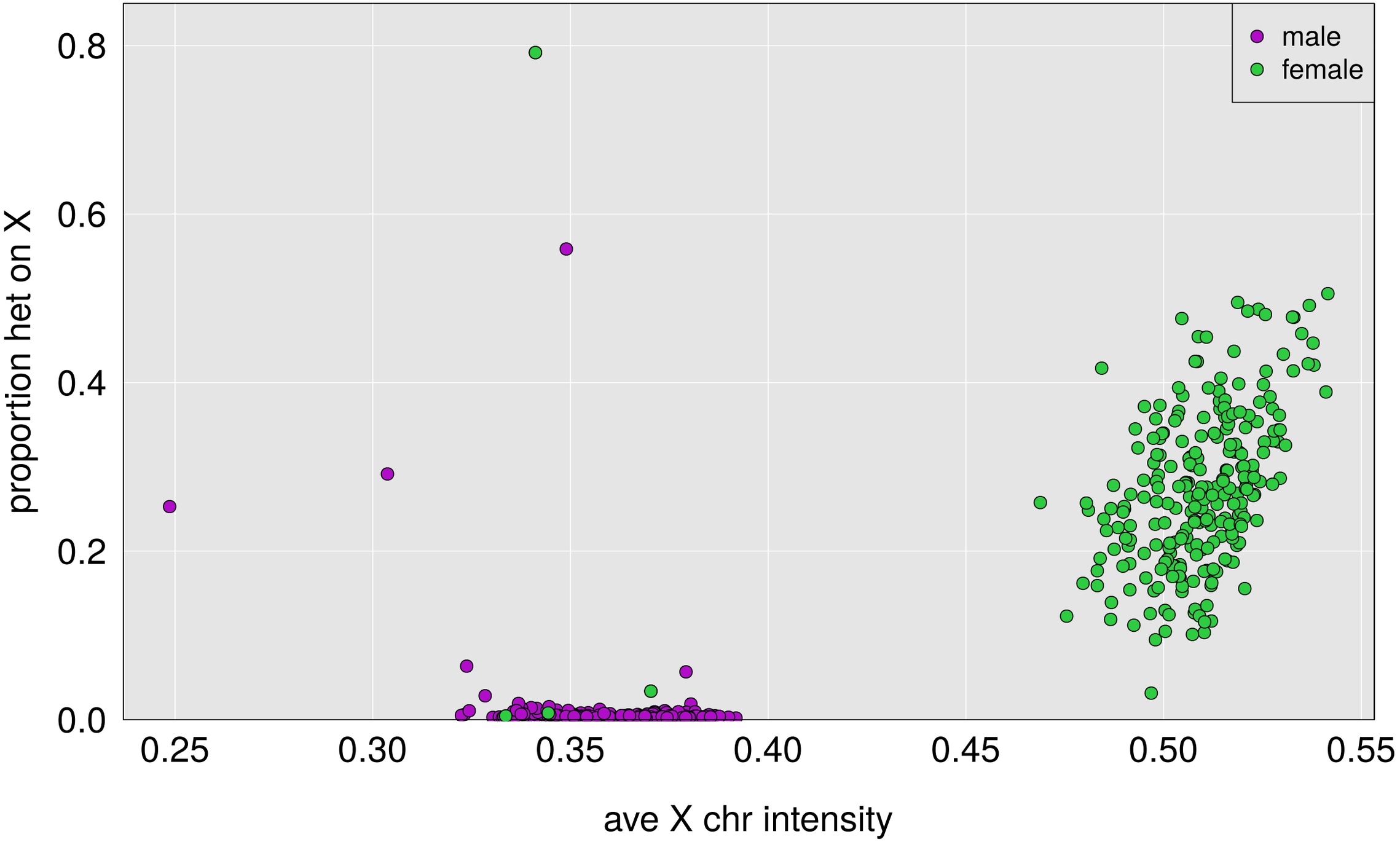
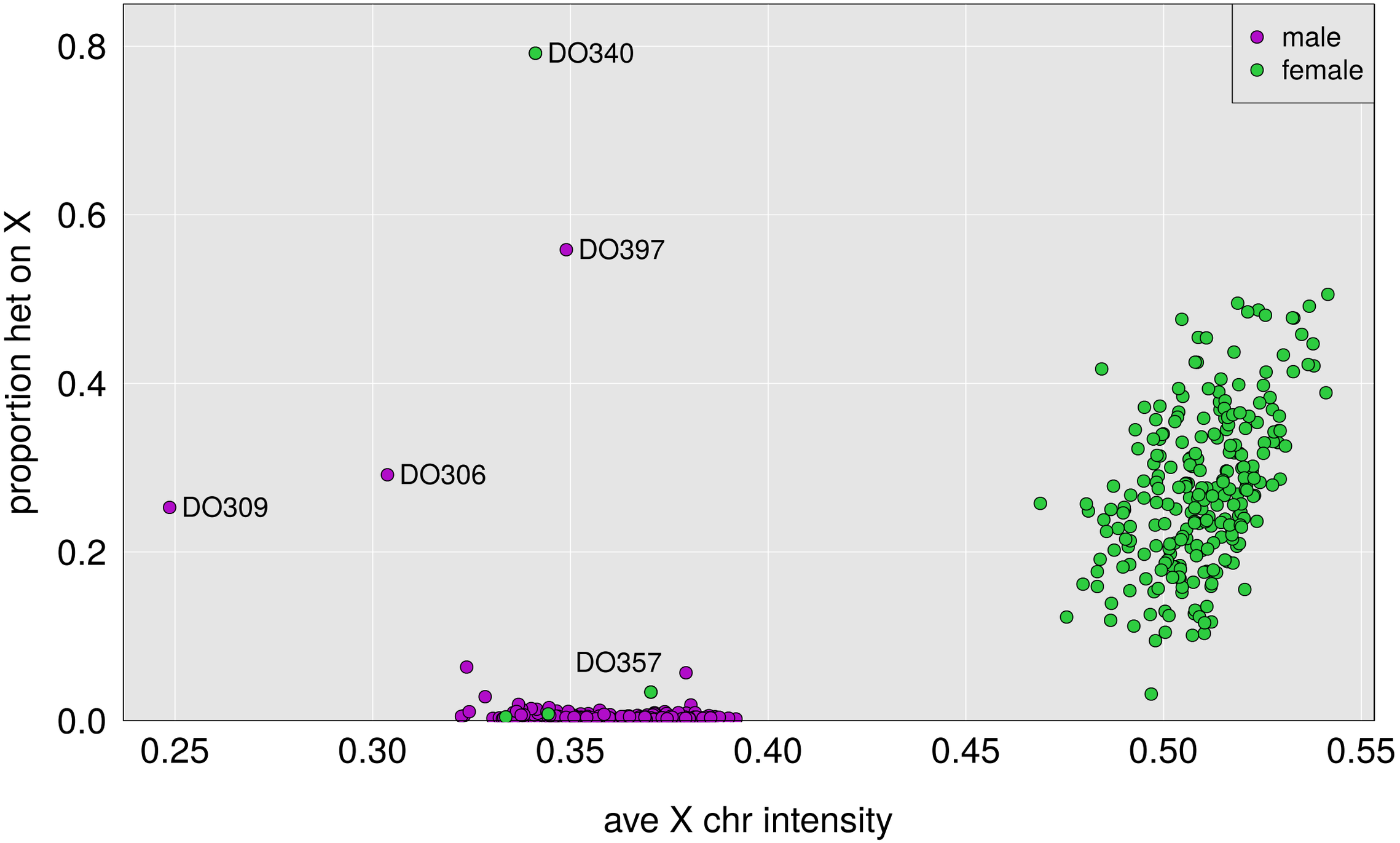


Heterozygosity vs SNP intensity on X chr

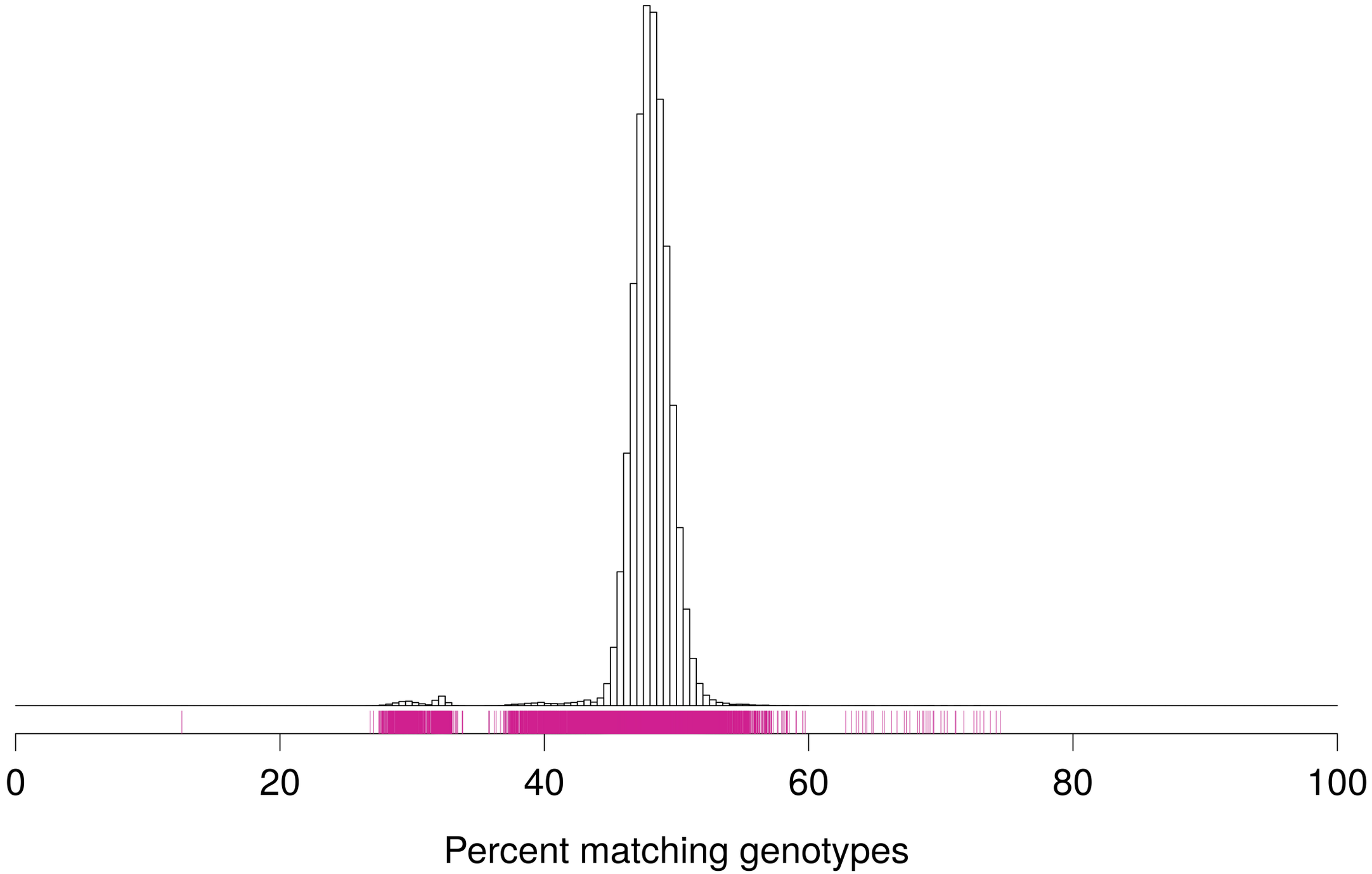


Heterozygosity vs SNP intensity on X chr

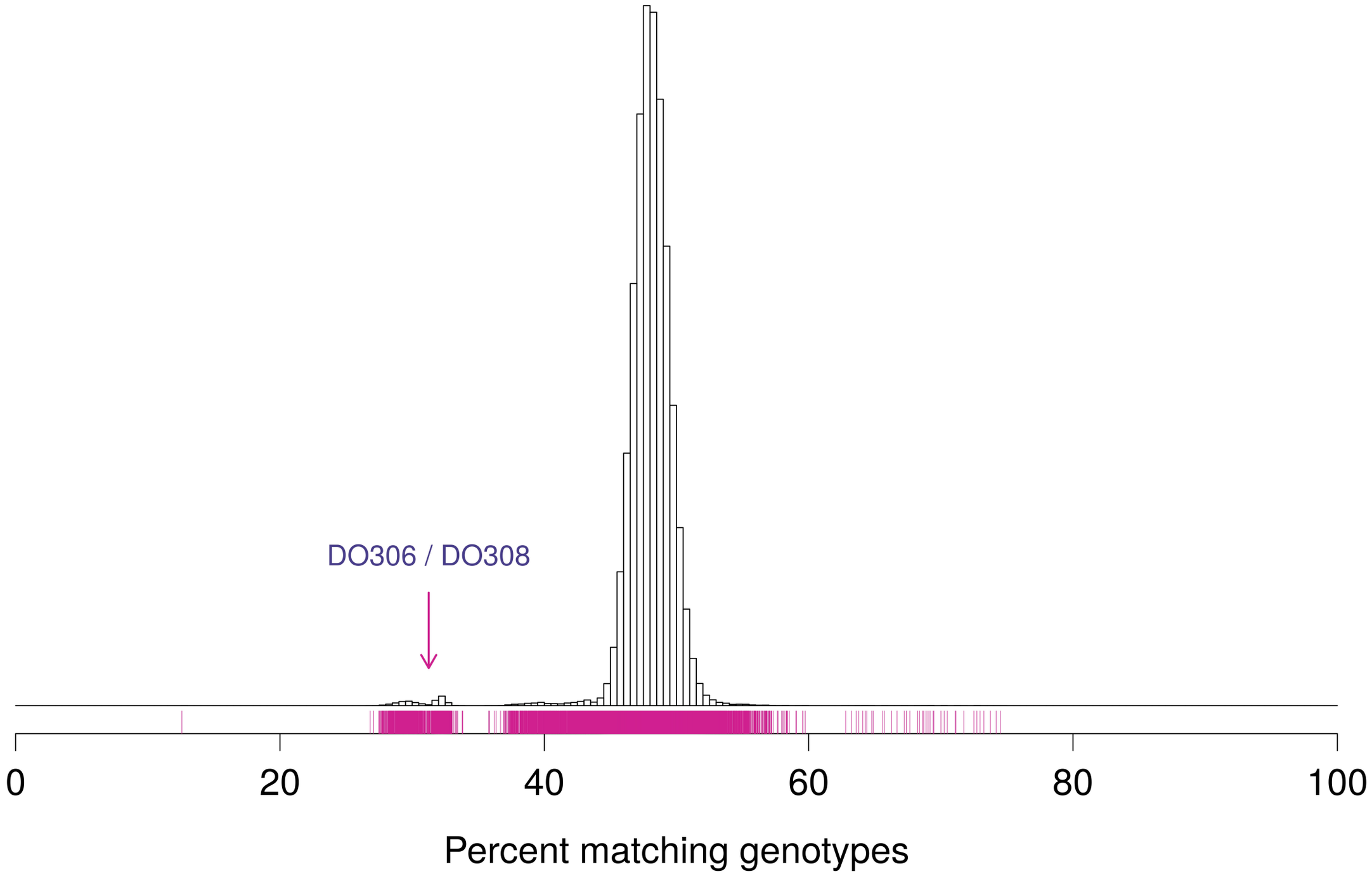


Sample duplicates

Percent matching genotypes



Percent matching genotypes



Sample mix-ups

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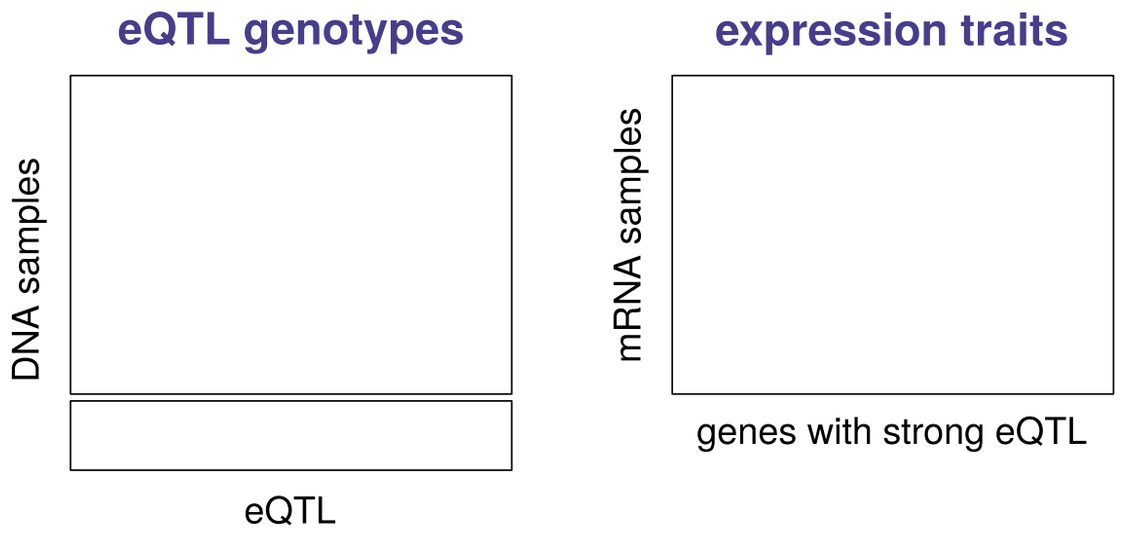

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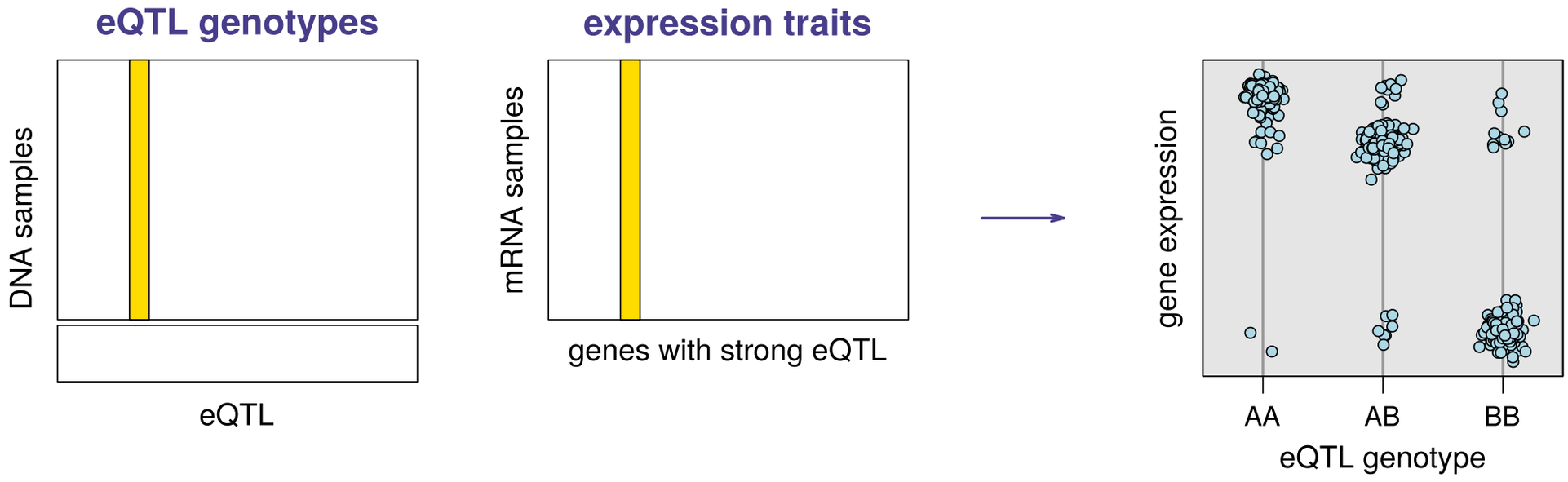

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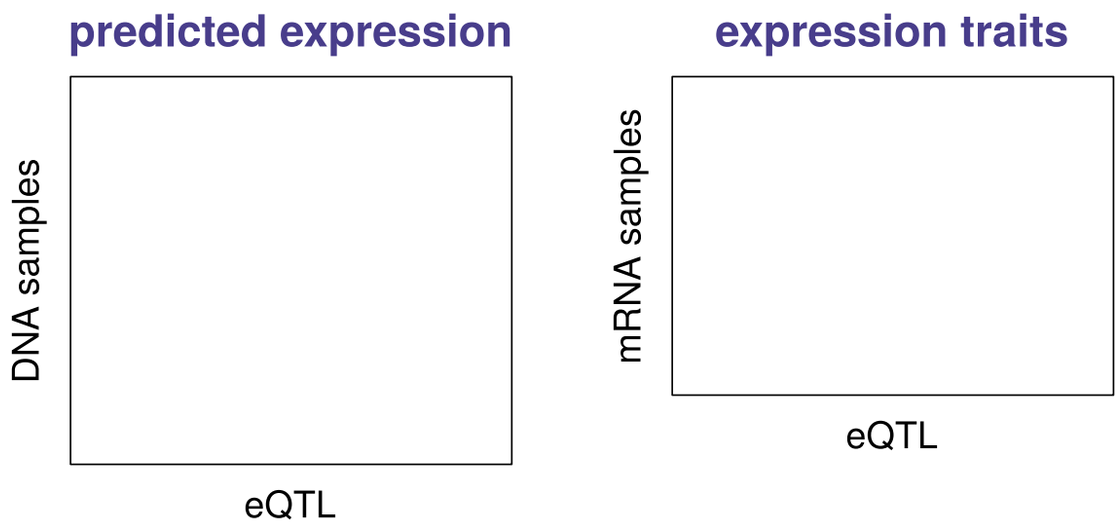

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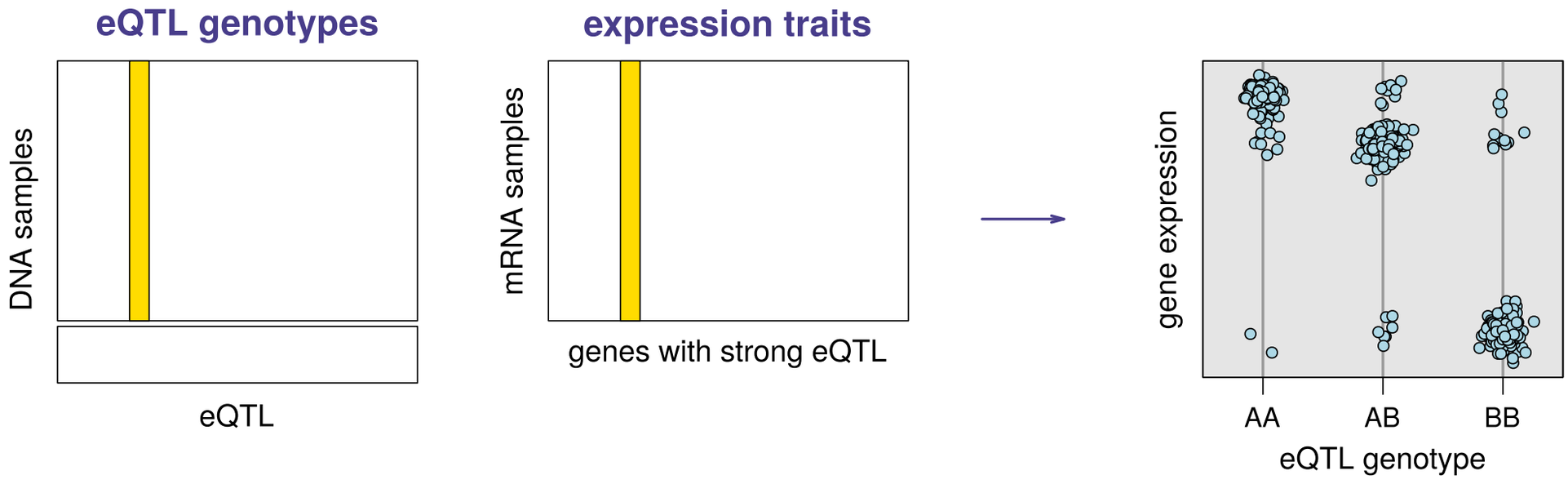
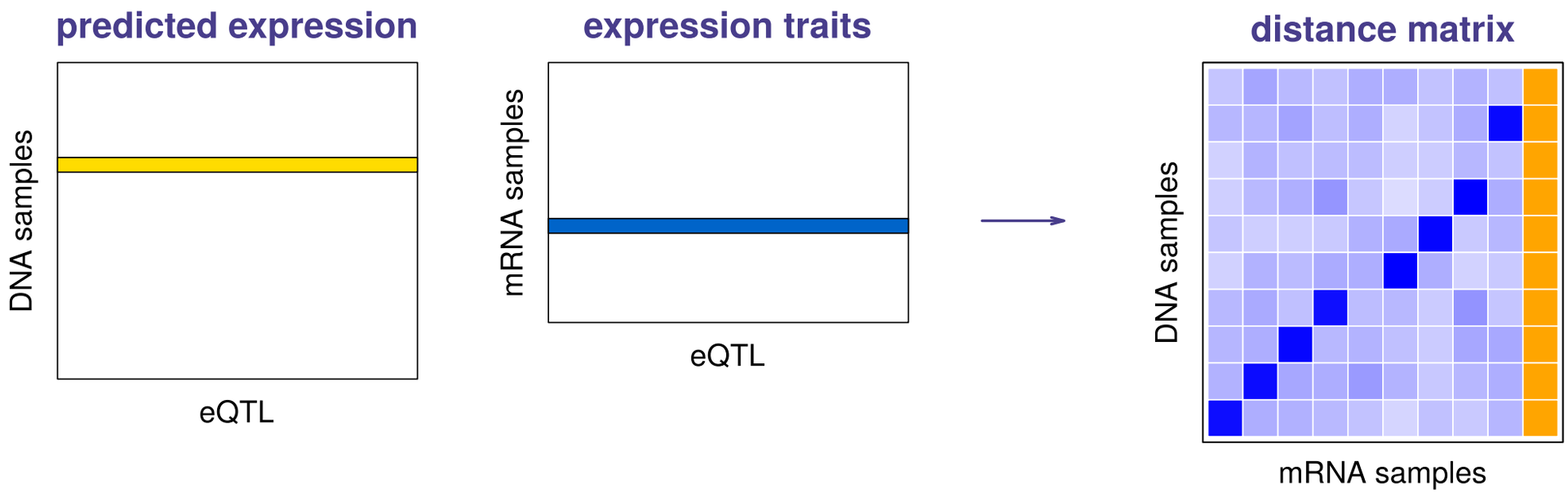
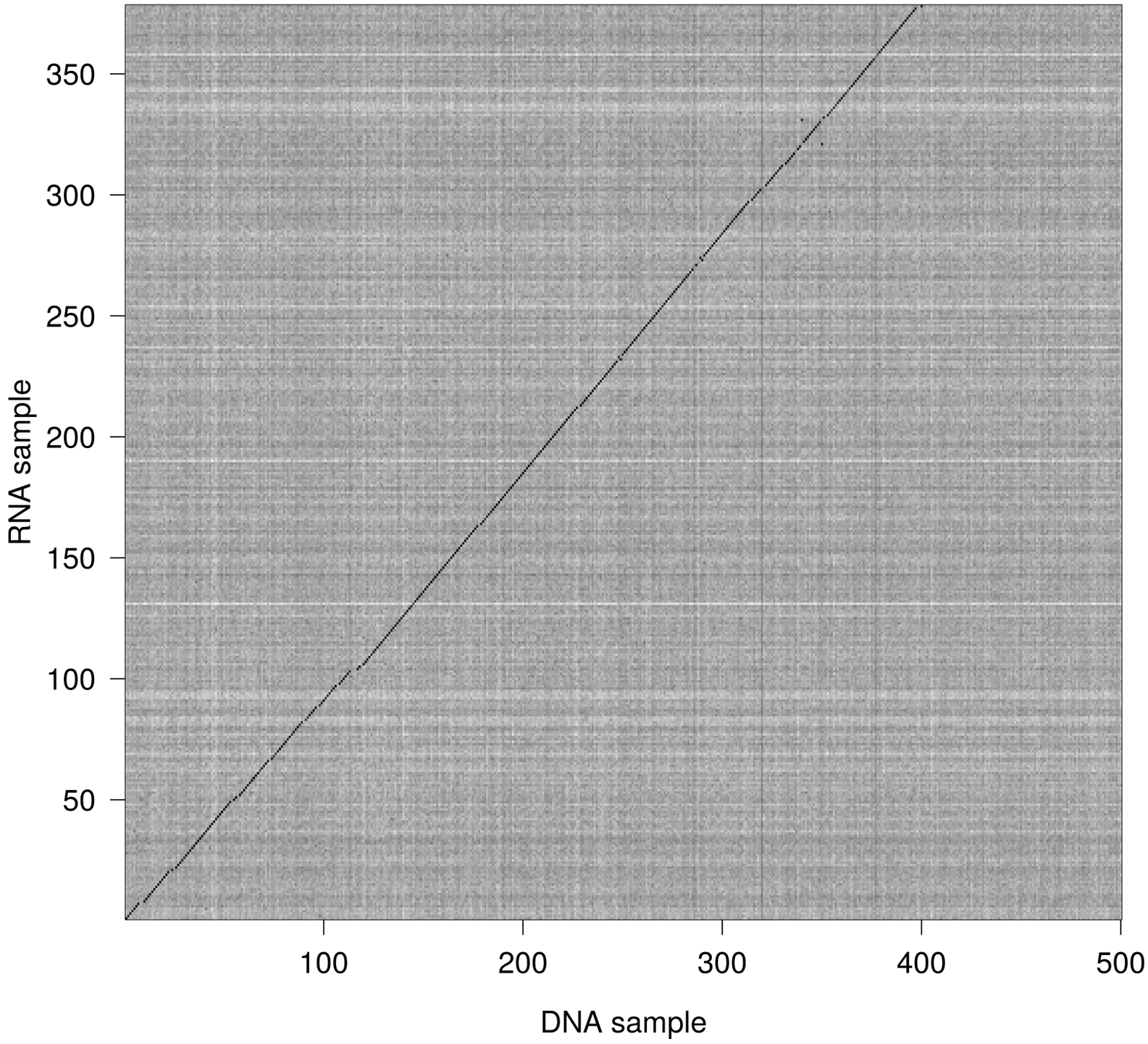
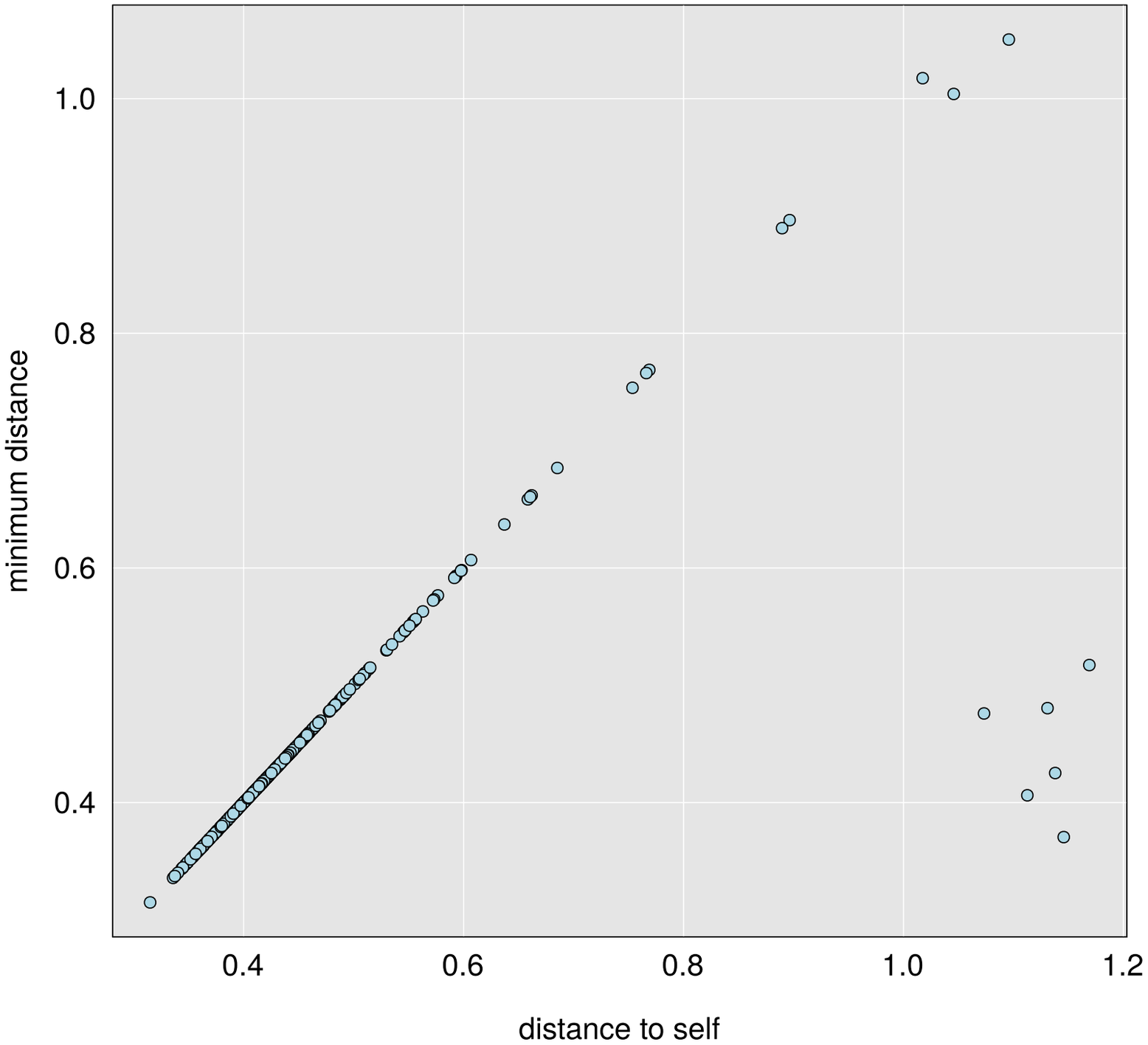




RNA-seq sample mix-ups: distance matrix



RNA-seq sample mix-ups: min vs self distance



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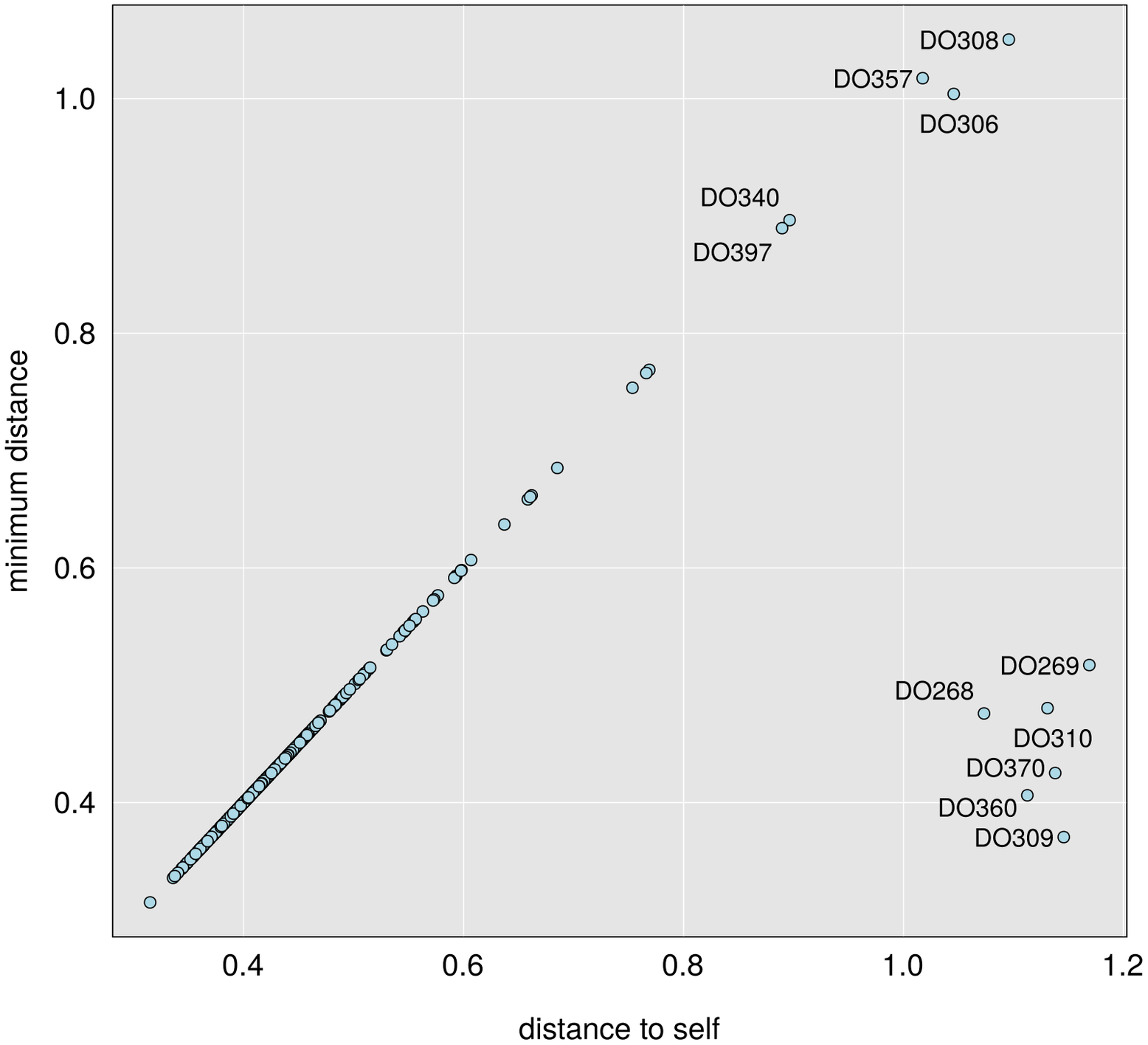
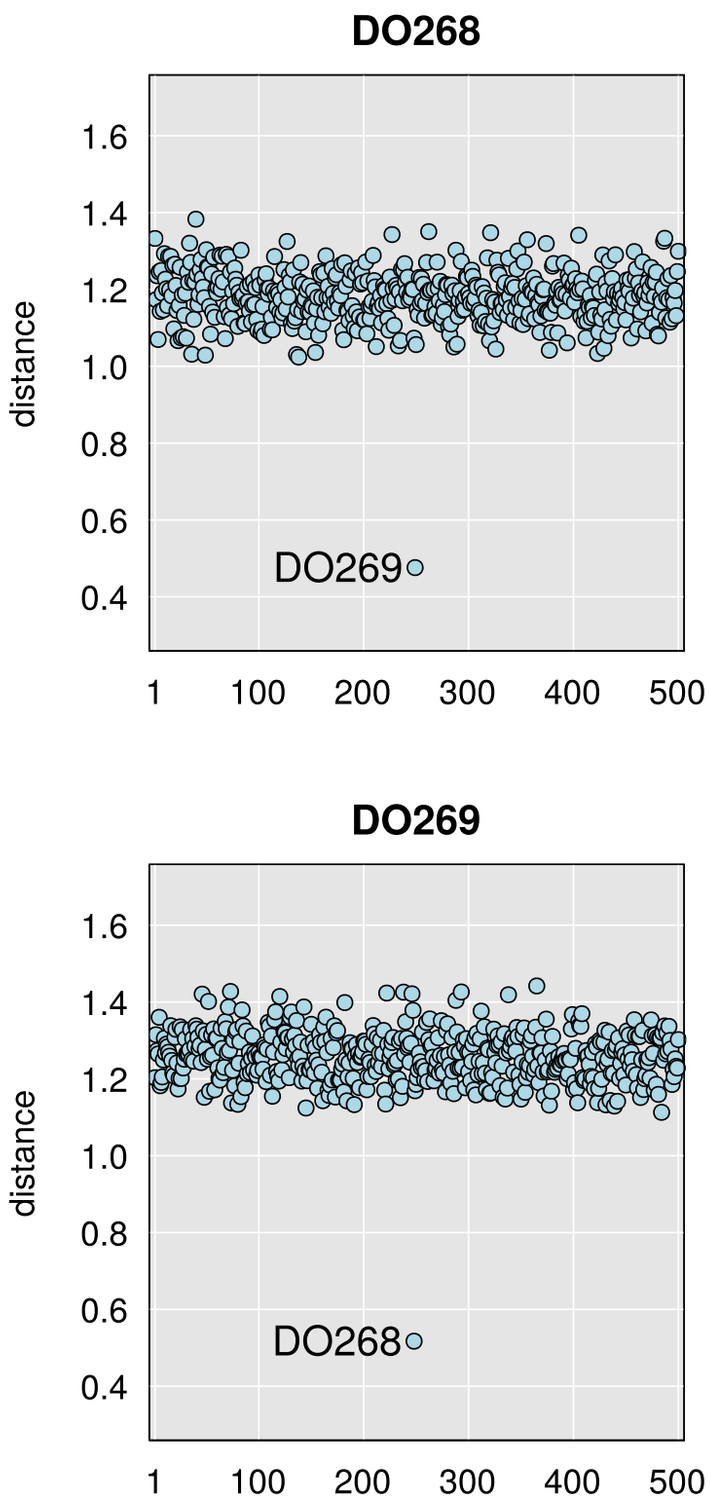
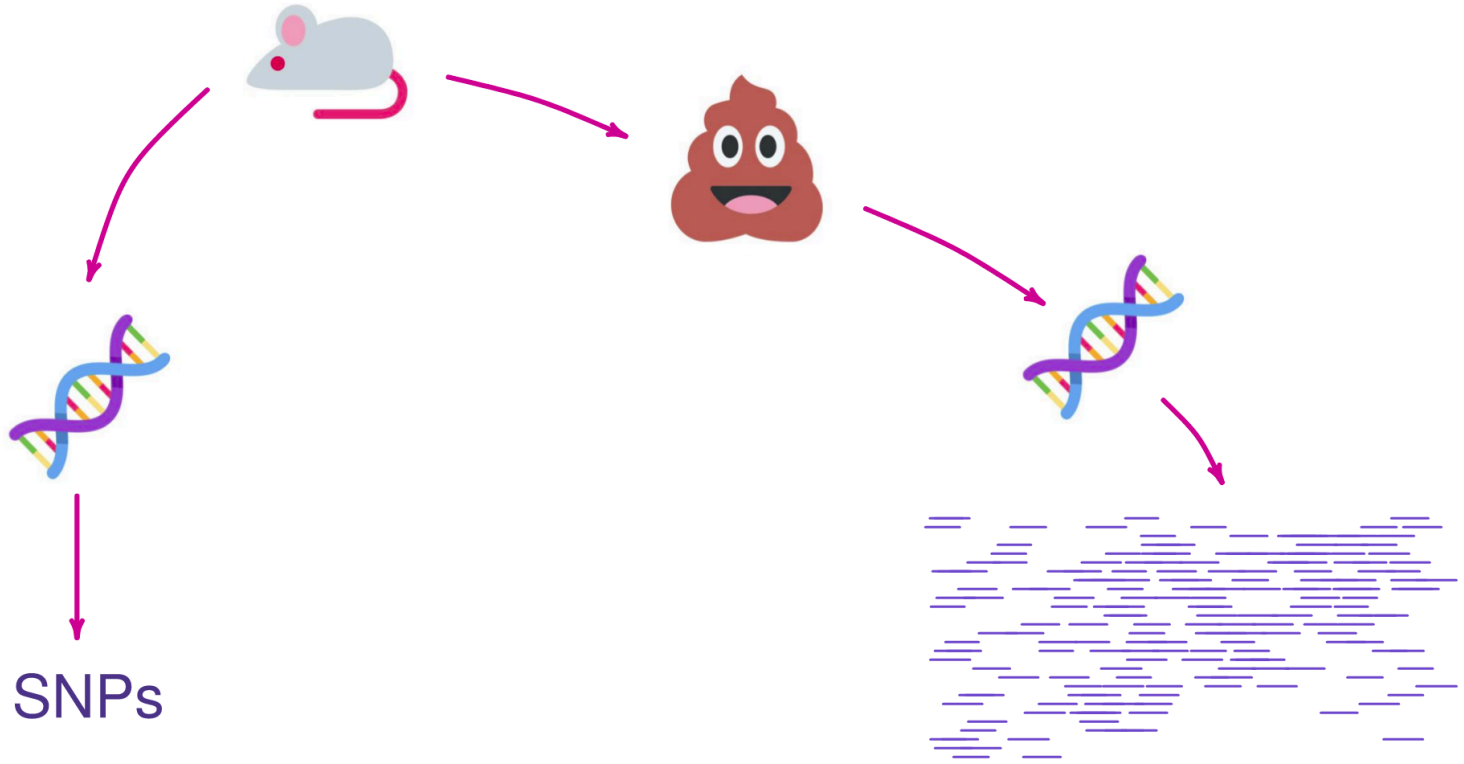


RNA-seq sample mix-ups: detail



Microbiome data



Sample mix-ups: Microbiome data

- Impute genotypes at all SNPs in DNA samples
- Map microbiome reads to mouse genome; find reads overlapping a SNP
- For each pair of samples (DNA + microbiome):
  - Focus on reads that overlap a SNP where that DNA sample is homozygous
  - Distance = proportion of reads where SNP allele doesn't match DNA sample's genotype

Genomic DO361 vs Microbiome DO361

	A	B
AA	939,918	2,998
BB	1,044	125,962

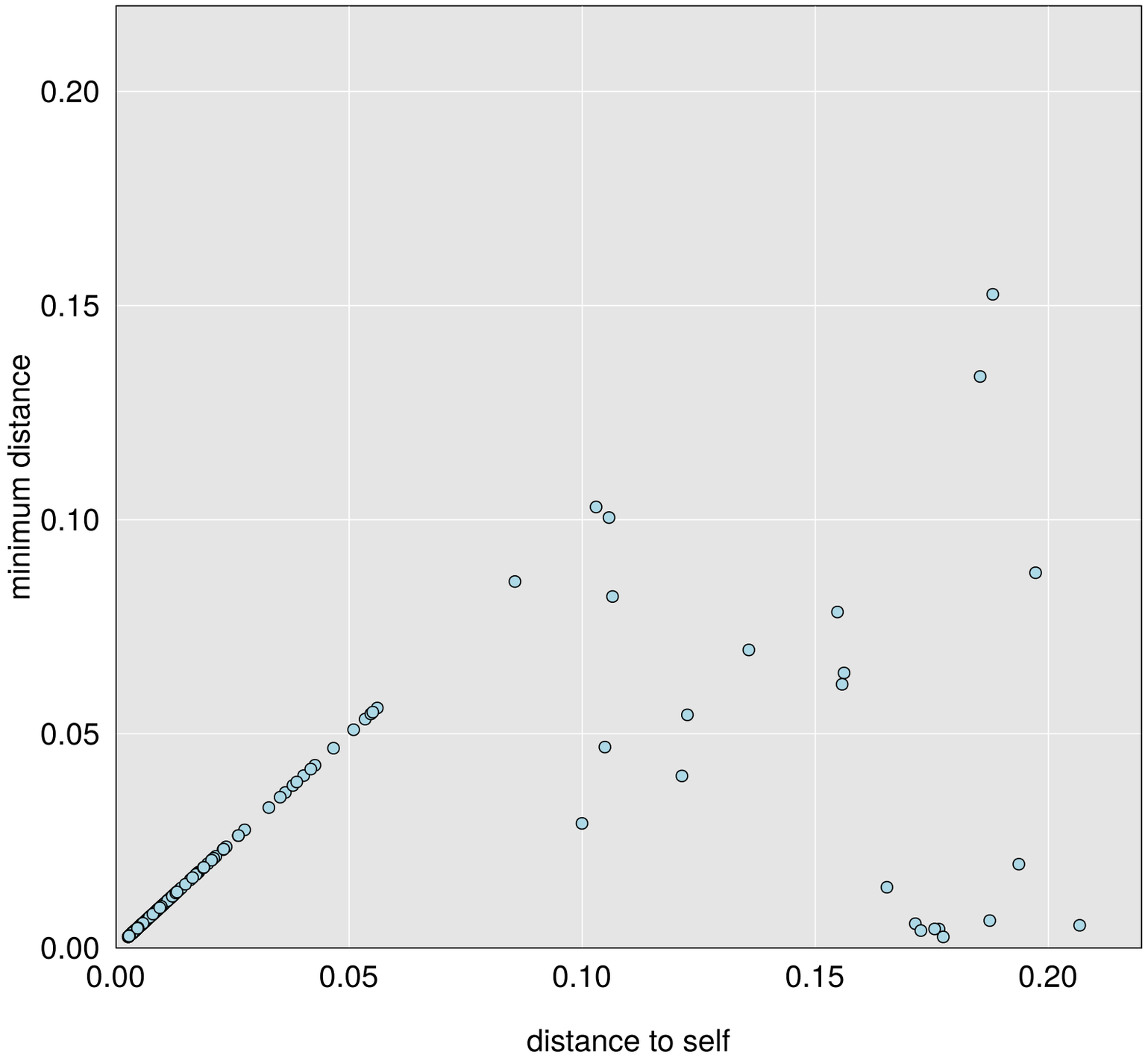
Genomic DO360 vs Microbiome DO360

	A	B
AA	2,661,645	427,685
BB	190,188	202,335

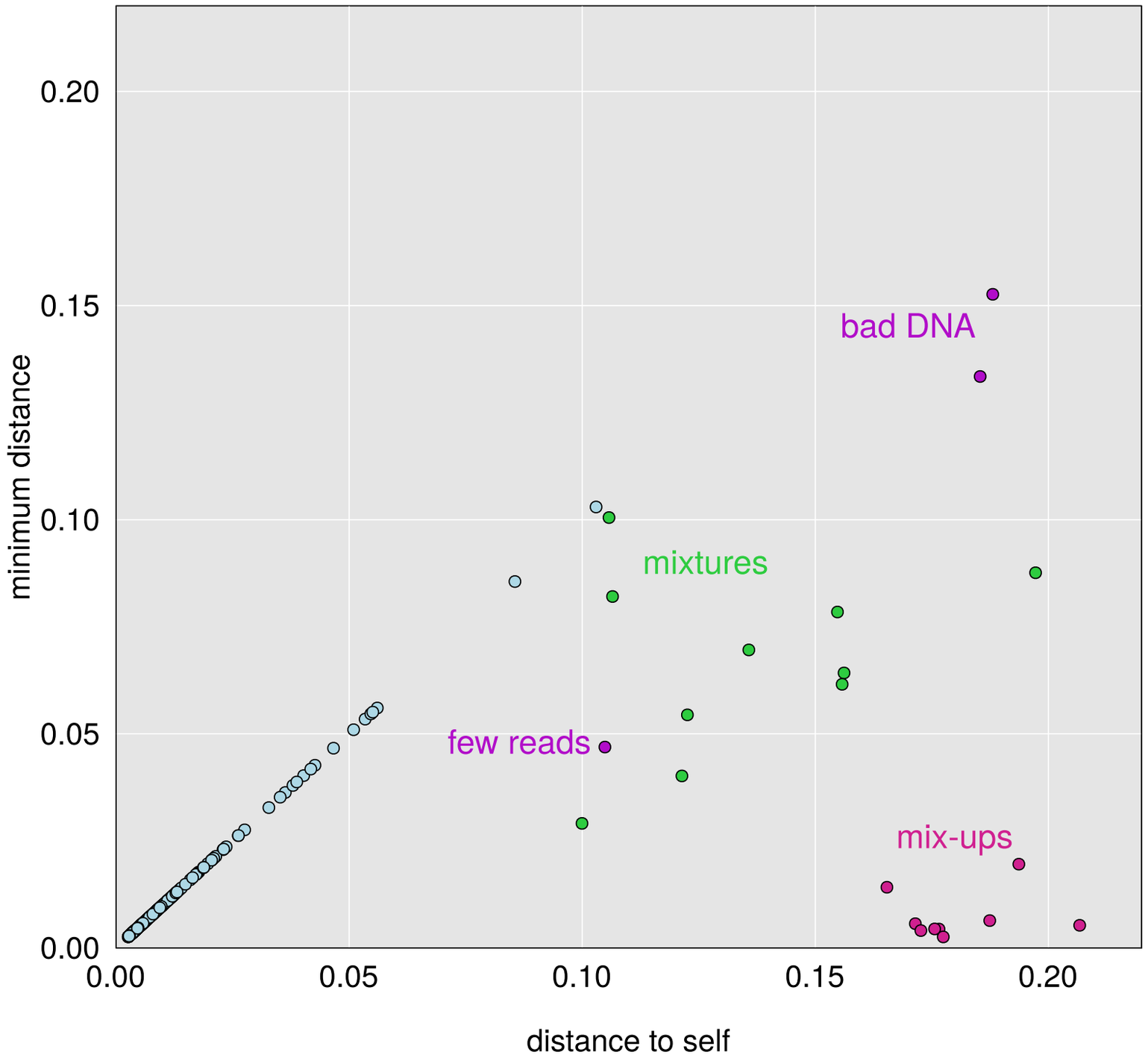
Genomic DO370 vs Microbiome DO360

	A	B
AA	3,137,751	7,461
BB	1,475	310,369

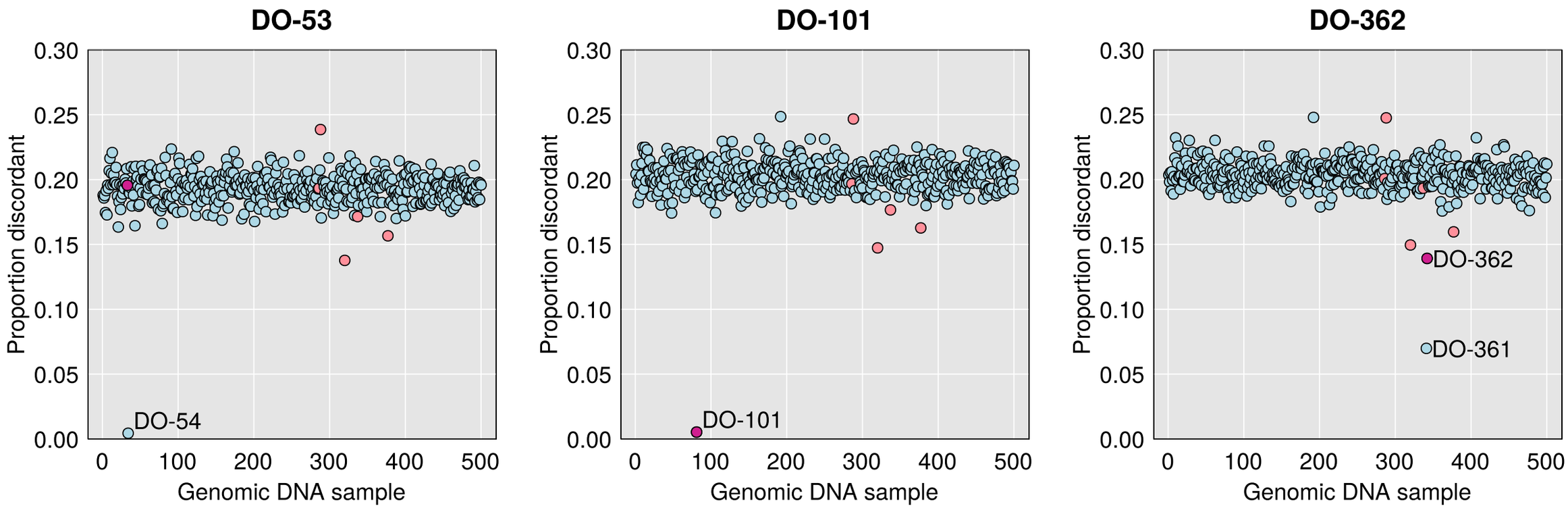
Microbiome mix-ups: min vs self distance

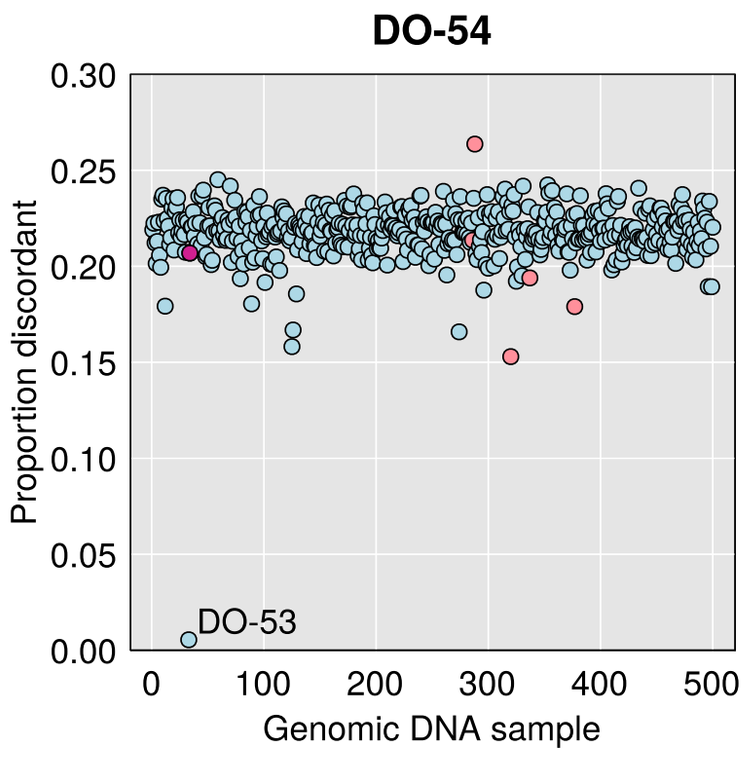


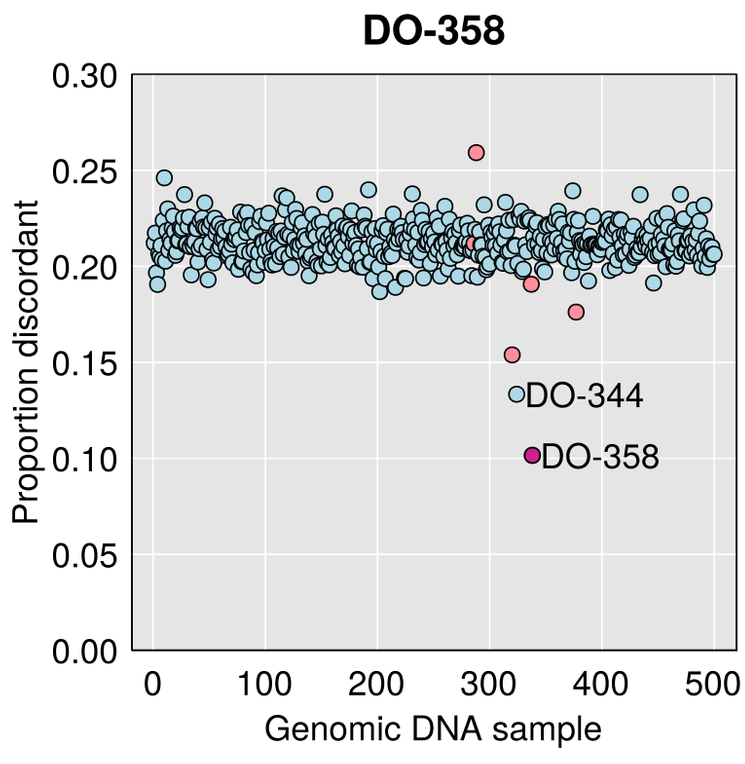
Microbiome mix-ups: min vs self distance

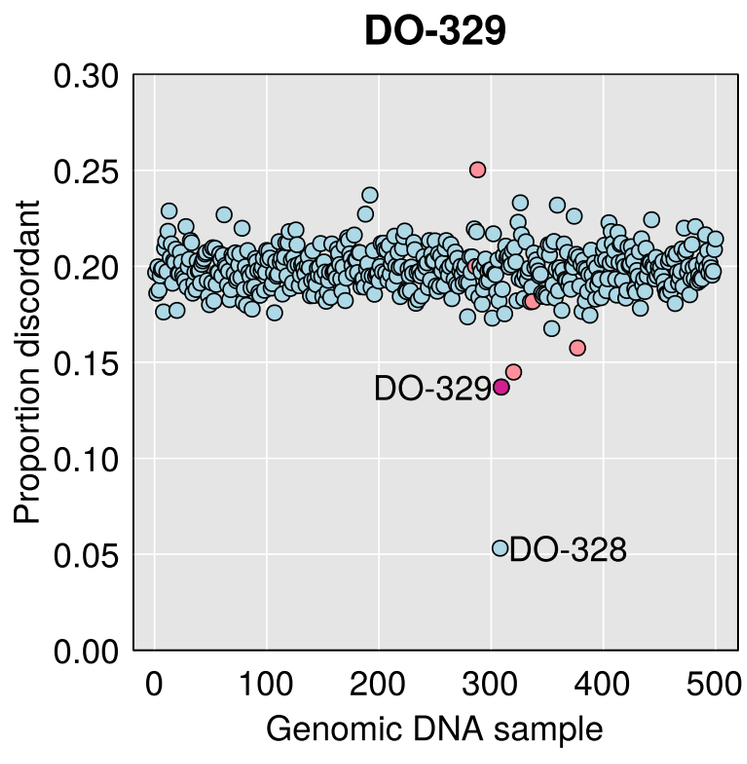


Microbiome mix-ups and mixtures: detail









Gen DO101 & DO102 vs Mic DO101

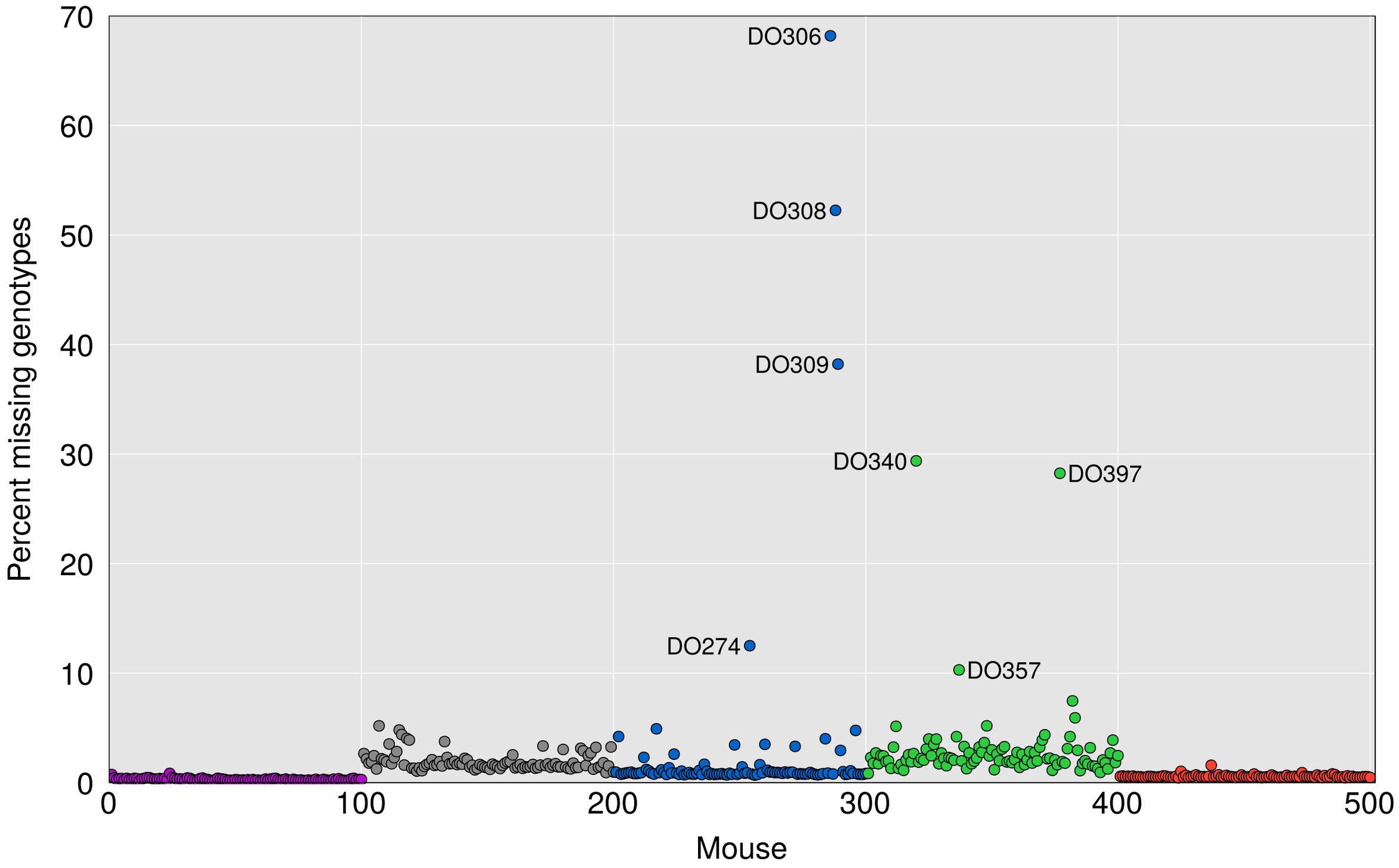
		allele in DO-102 microbiome			
DO-101 genotype	DO-102 genotype	A (%)		B (%)	
AA	AA	3,664,076	(99.6%)	14,305	(0.4%)
AA	AB	1,161,383	(99.5%)	6,187	(0.5%)
AA	BB	153,501	(99.3%)	1,067	(0.7%)
AB	AA	651,287	(52.0%)	600,434	(48.0%)
AB	AB	378,800	(51.8%)	352,828	(48.2%)
AB	BB	155,967	(51.2%)	148,703	(48.8%)
BB	AA	3,088	(1.6%)	185,825	(98.4%)
BB	AB	3,210	(1.3%)	240,712	(98.7%)
BB	BB	2,162	(1.0%)	217,882	(99.0%)

Gen DO358 & DO344 vs Mic DO358

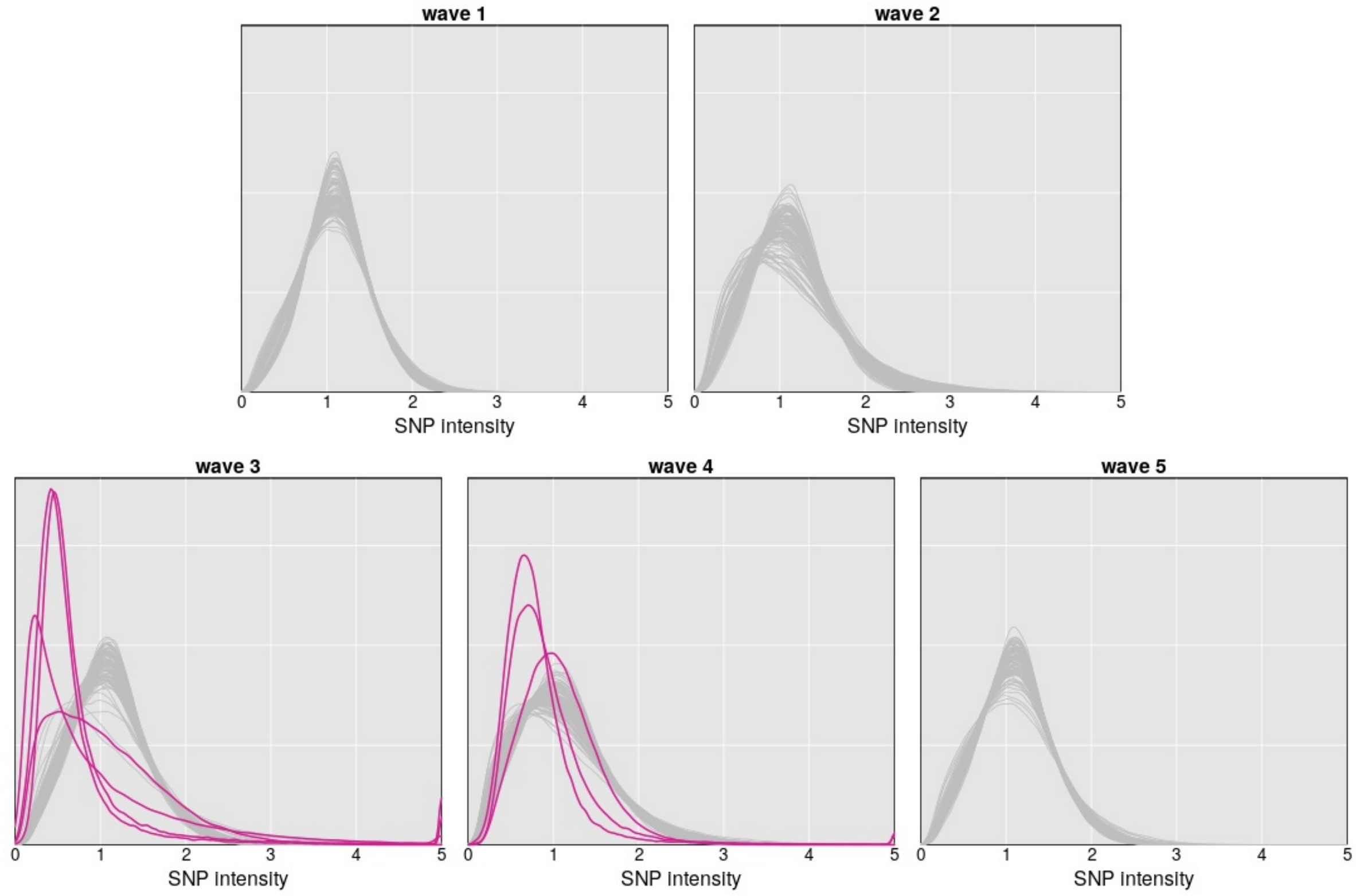
		allele in DO-358 microbiome			
DO-358 genotype	DO-344 genotype	A (%)		B (%)	
AA	AA	2,394,215	(99.7%)	6,050	(0.3%)
AA	AB	869,613	(79.5%)	224,483	(20.5%)
AA	BB	103,036	(59.1%)	71,332	(40.9%)
AB	AA	686,970	(71.8%)	269,447	(28.2%)
AB	AB	297,500	(51.4%)	280,958	(48.6%)
AB	BB	55,982	(29.9%)	131,111	(70.1%)
BB	AA	73,727	(42.9%)	98,257	(57.1%)
BB	AB	47,000	(21.9%)	167,513	(78.1%)
BB	BB	542	(0.5%)	117,802	(99.5%)

Sample quality

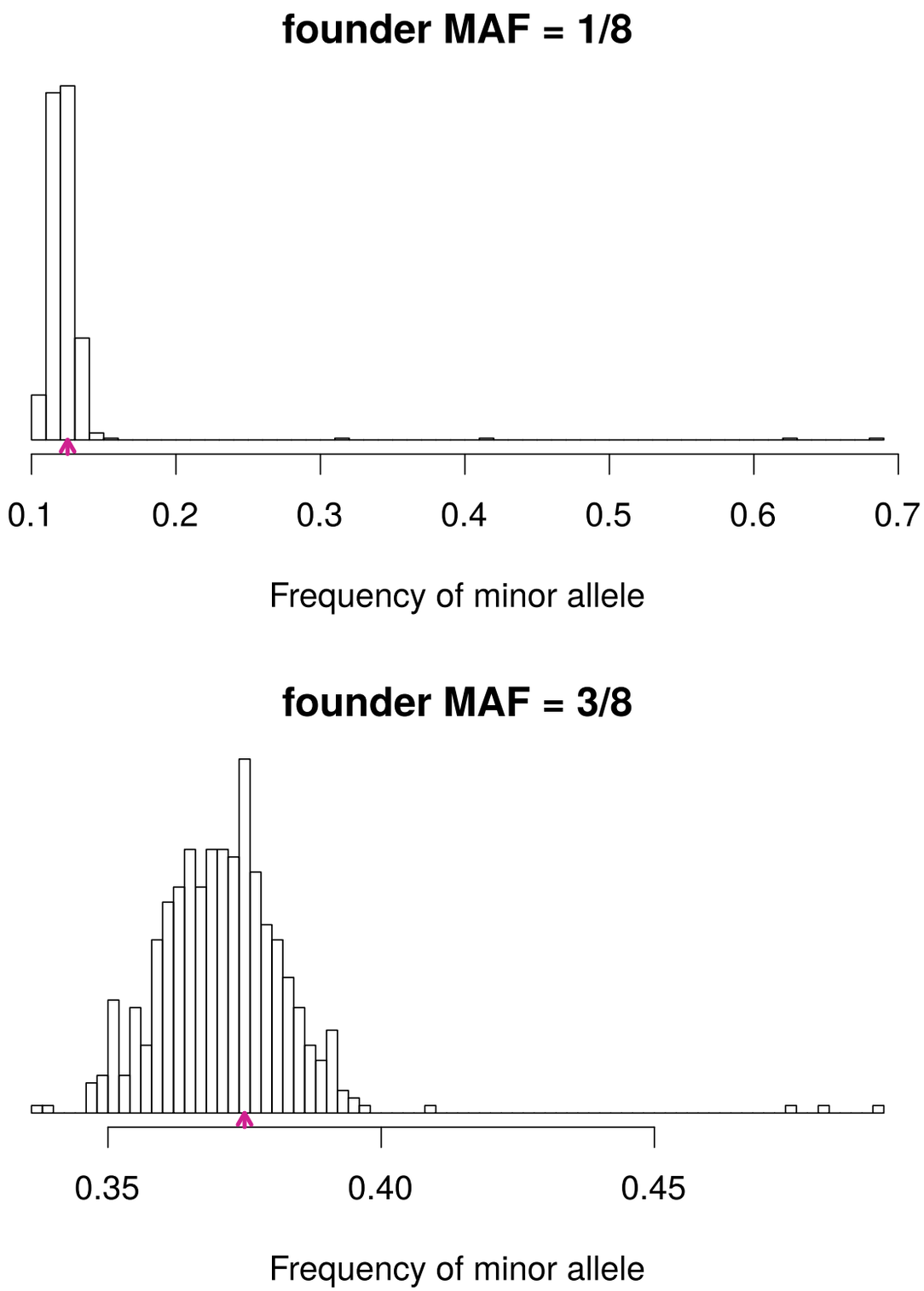
Missing data per sample

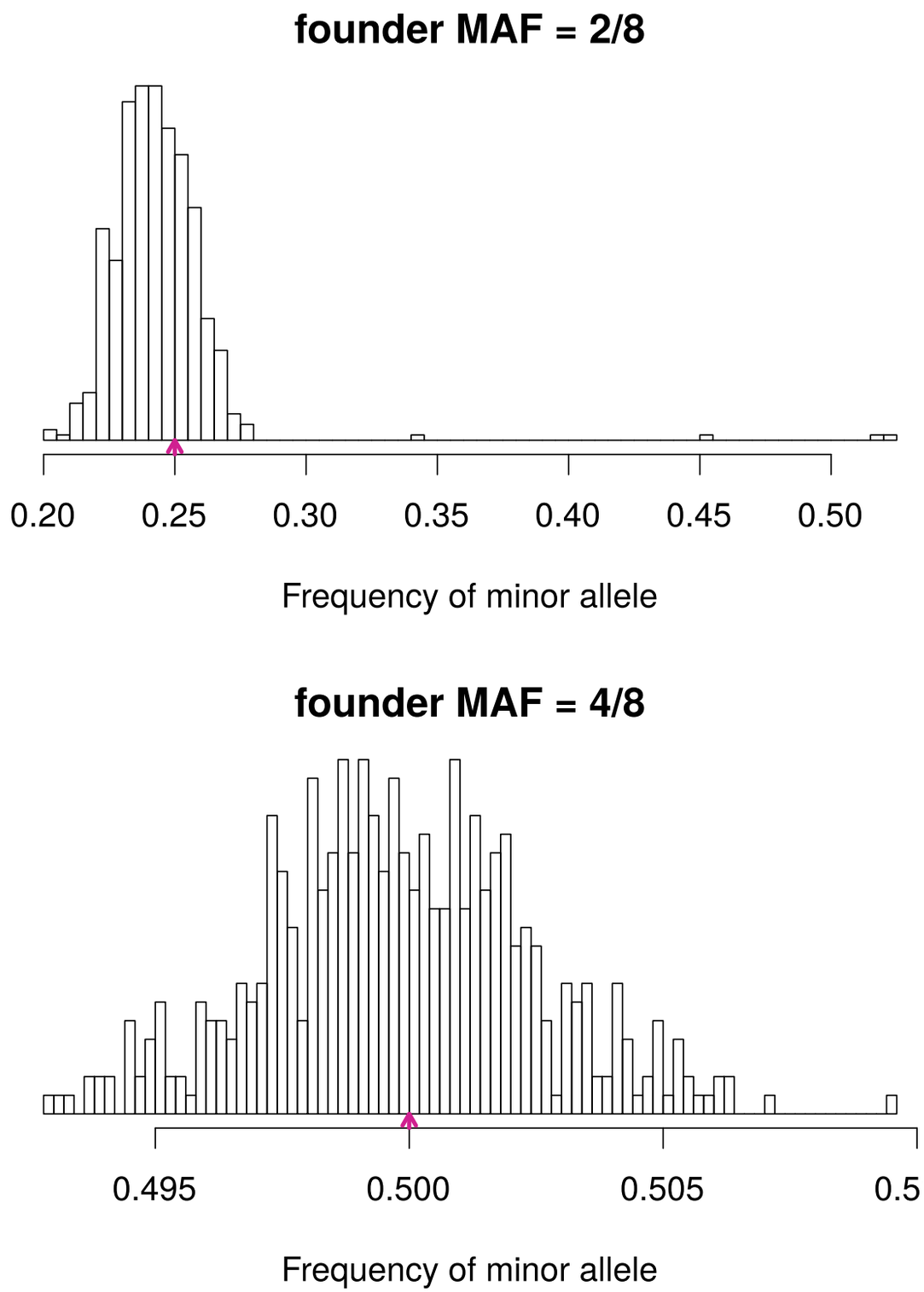


Array intensities



Allele frequencies, by individual





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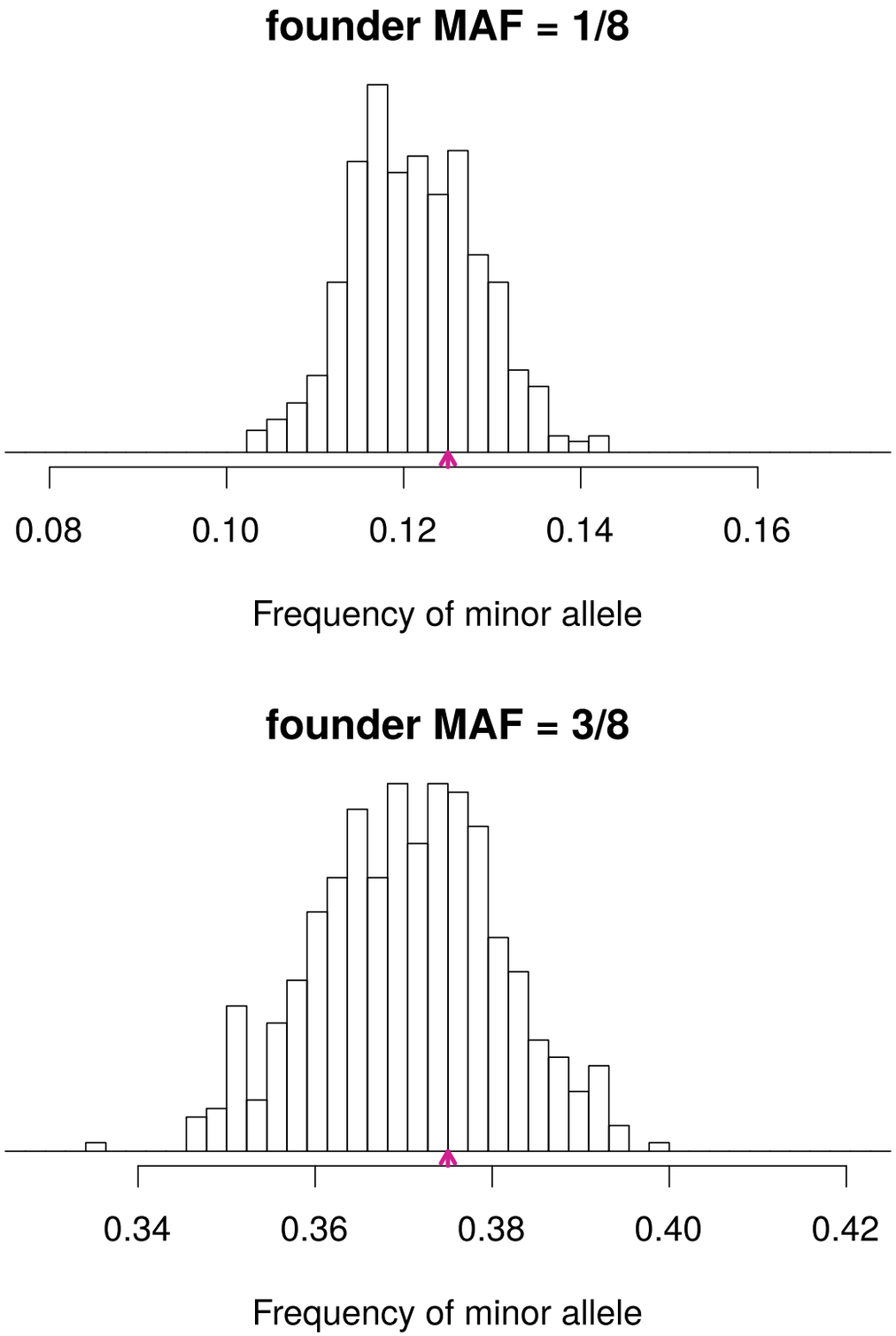

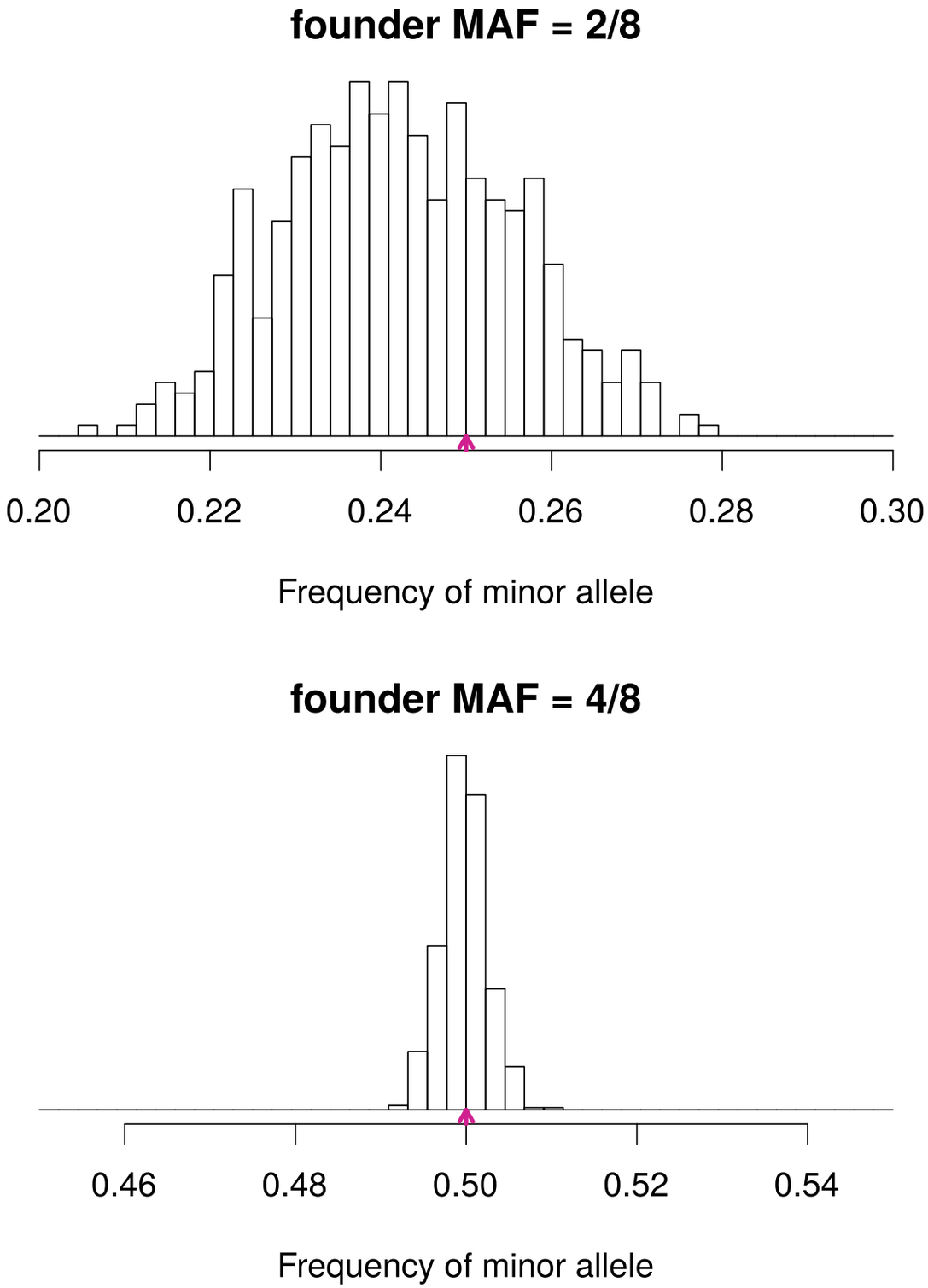

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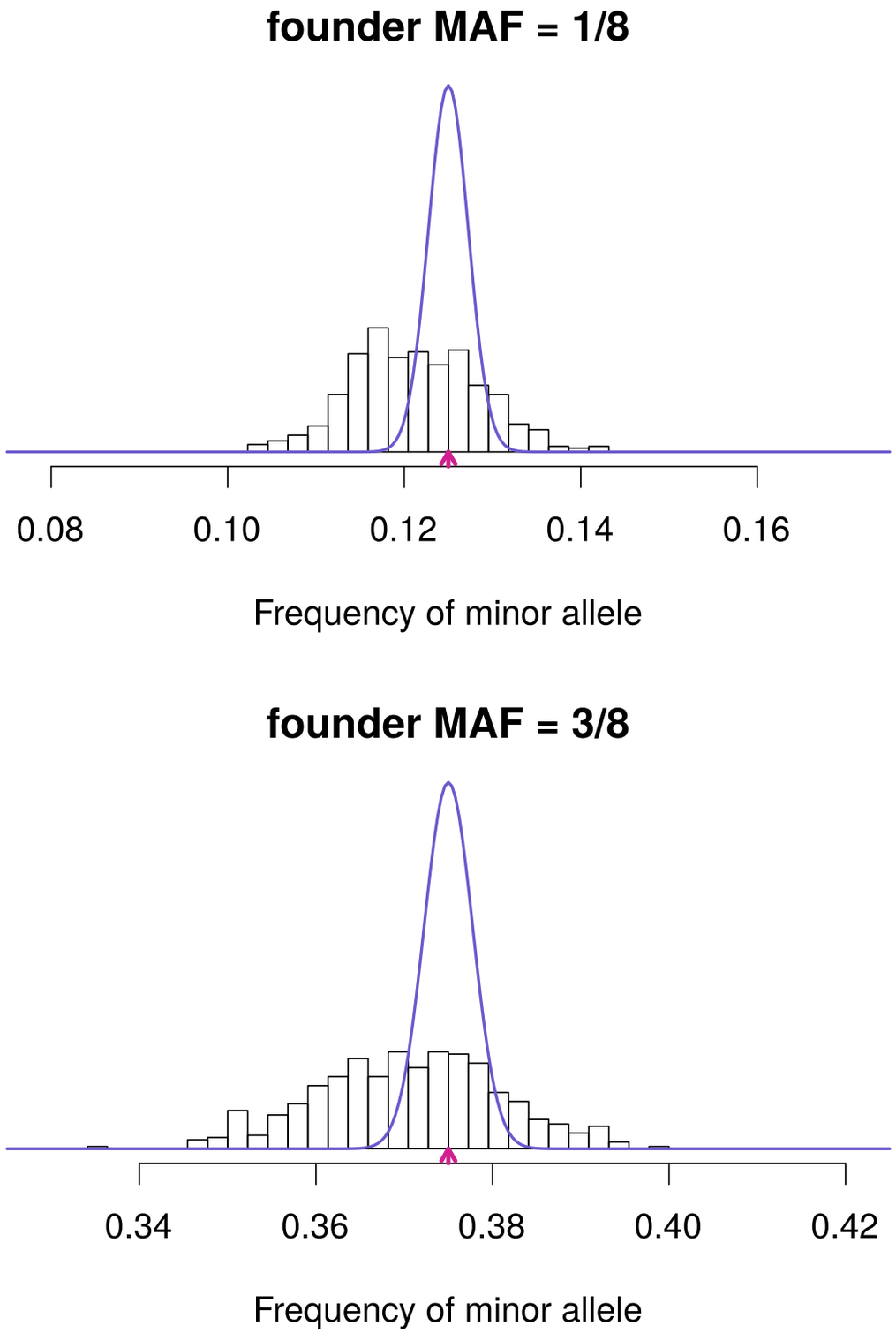
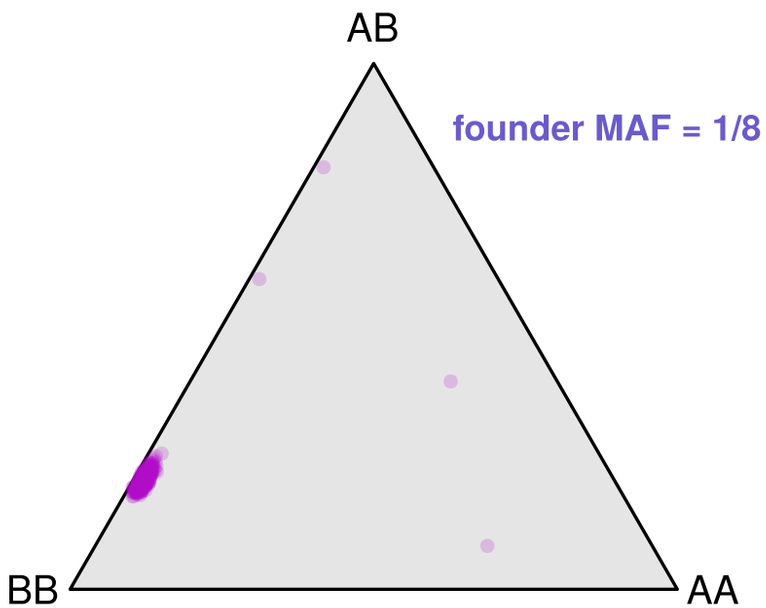
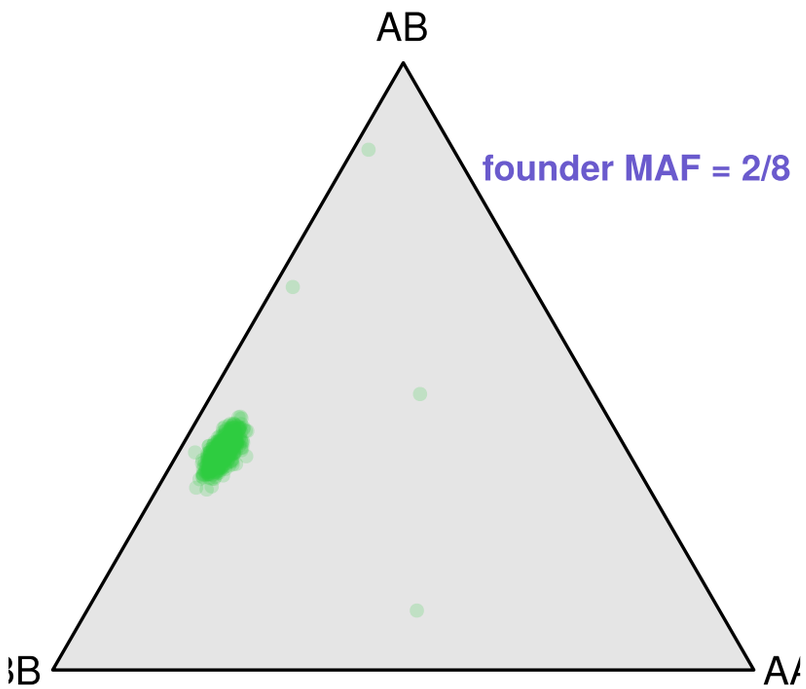
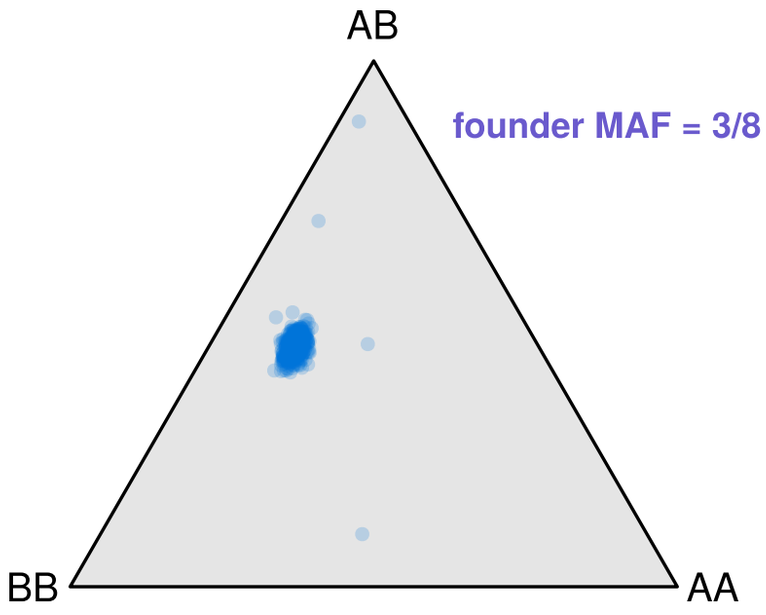
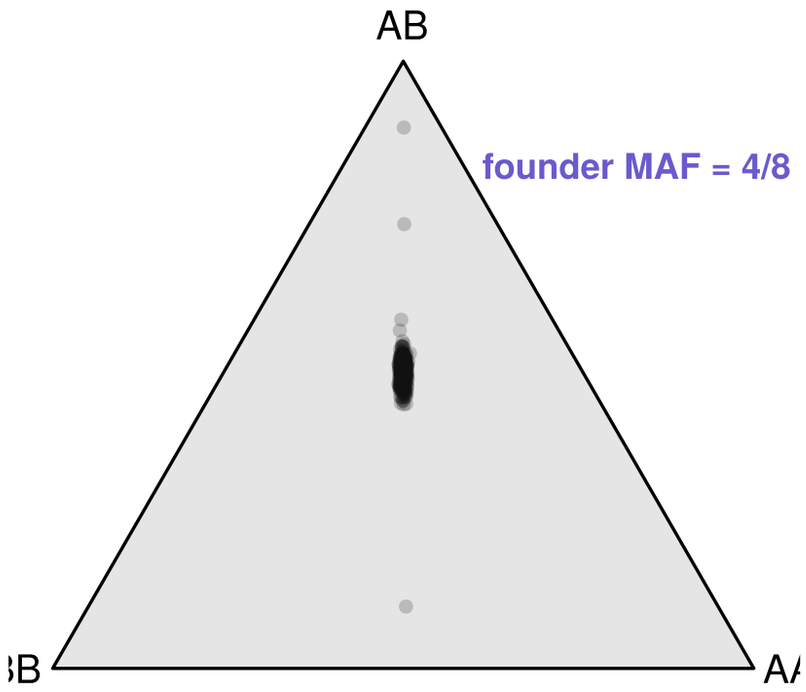


Genotype frequencies, by individual









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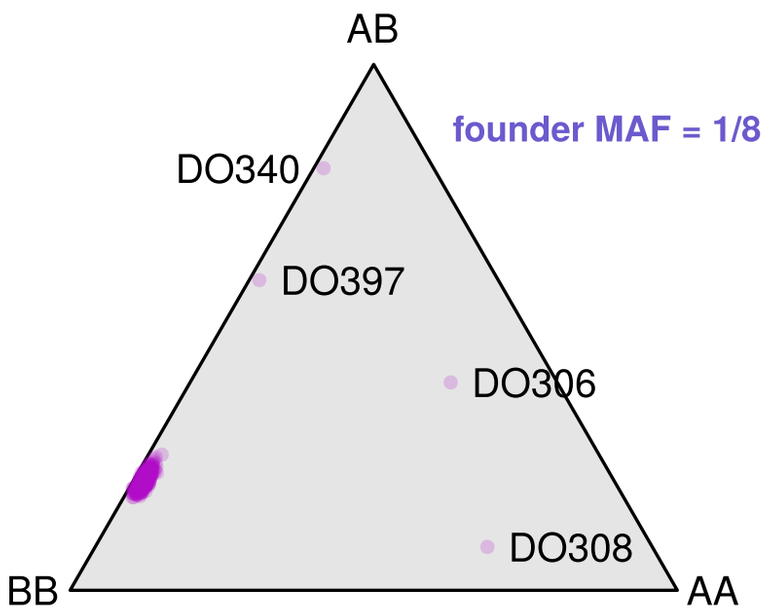
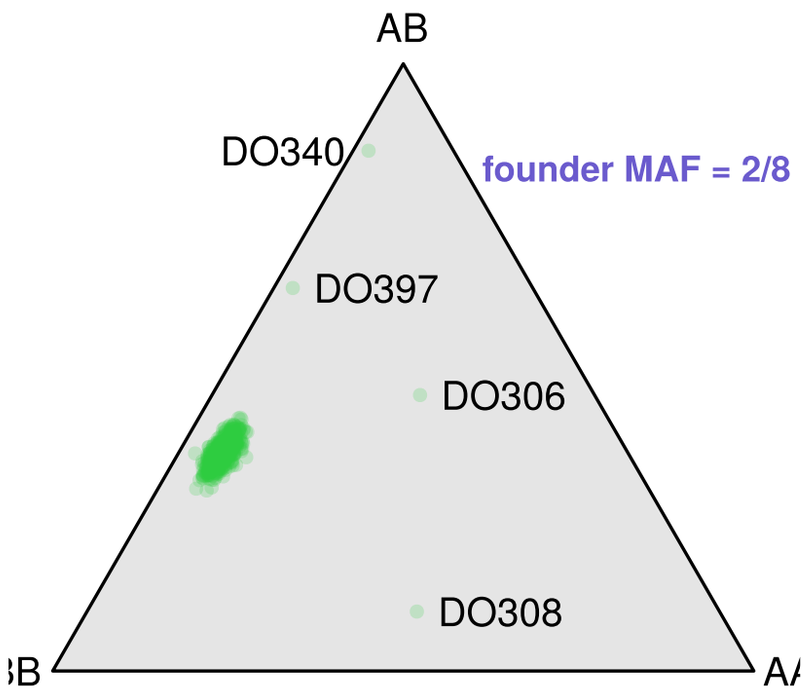
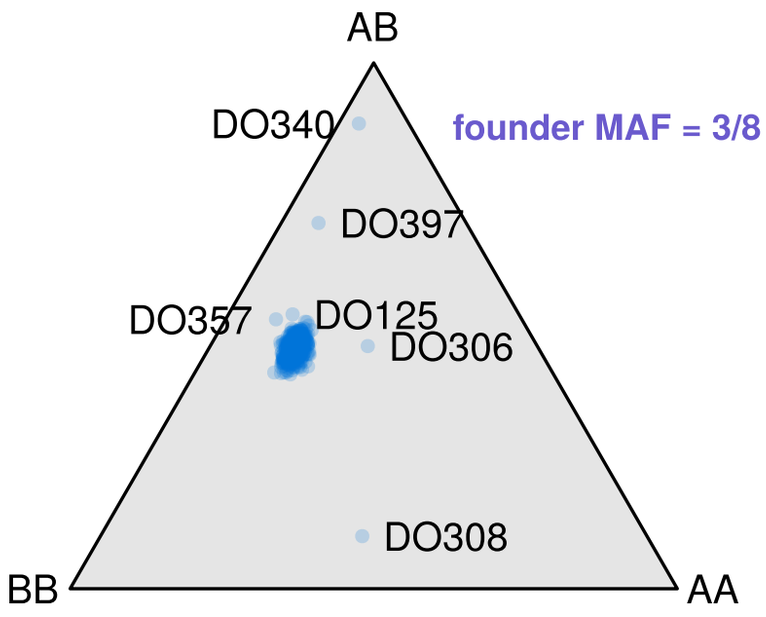
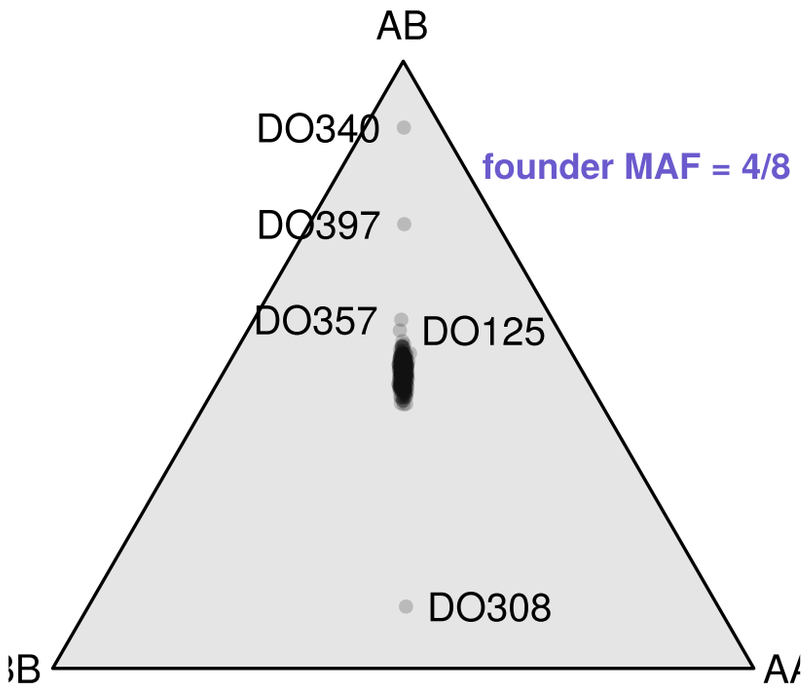
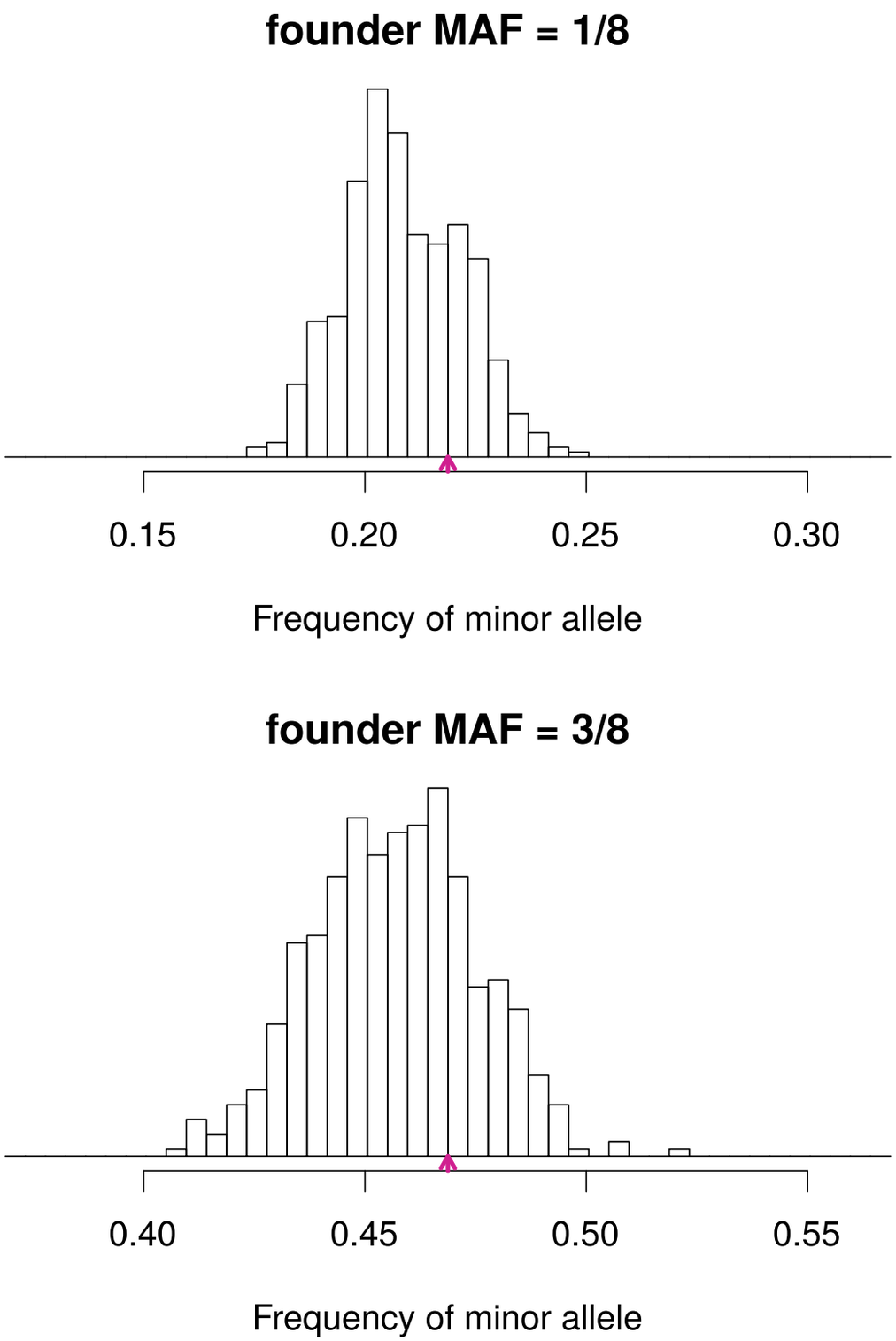


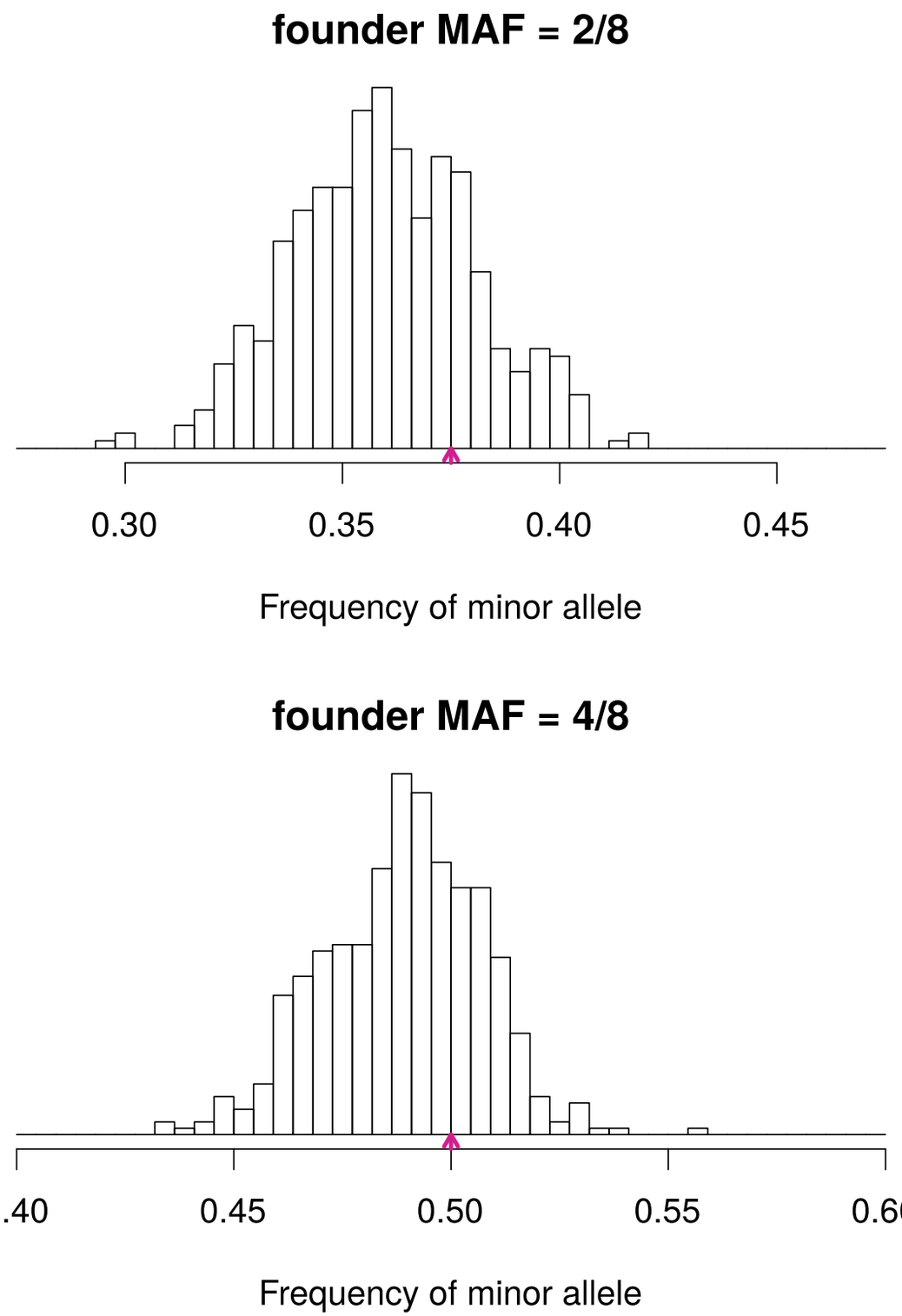




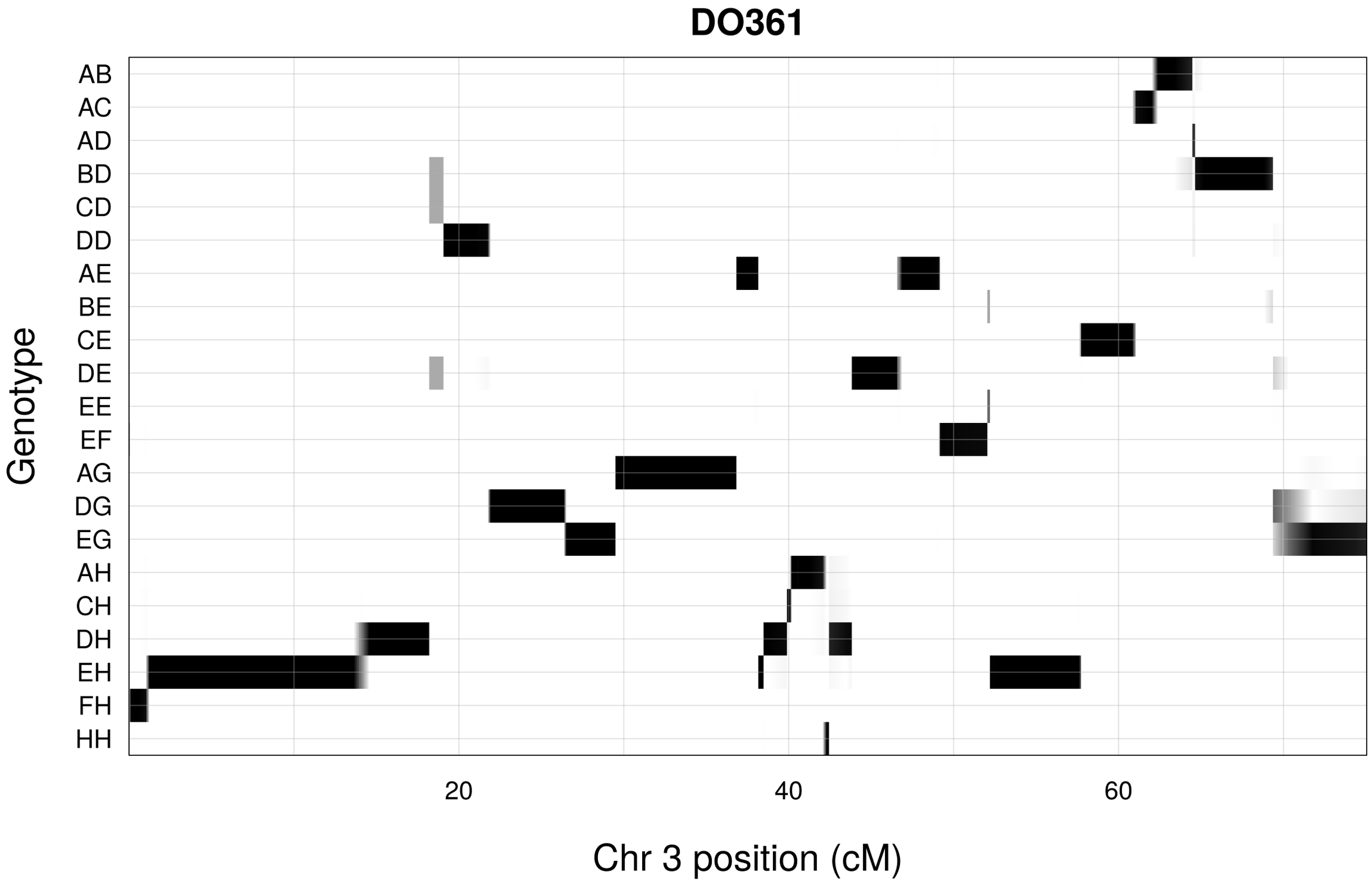


Heterozygosities, by individual

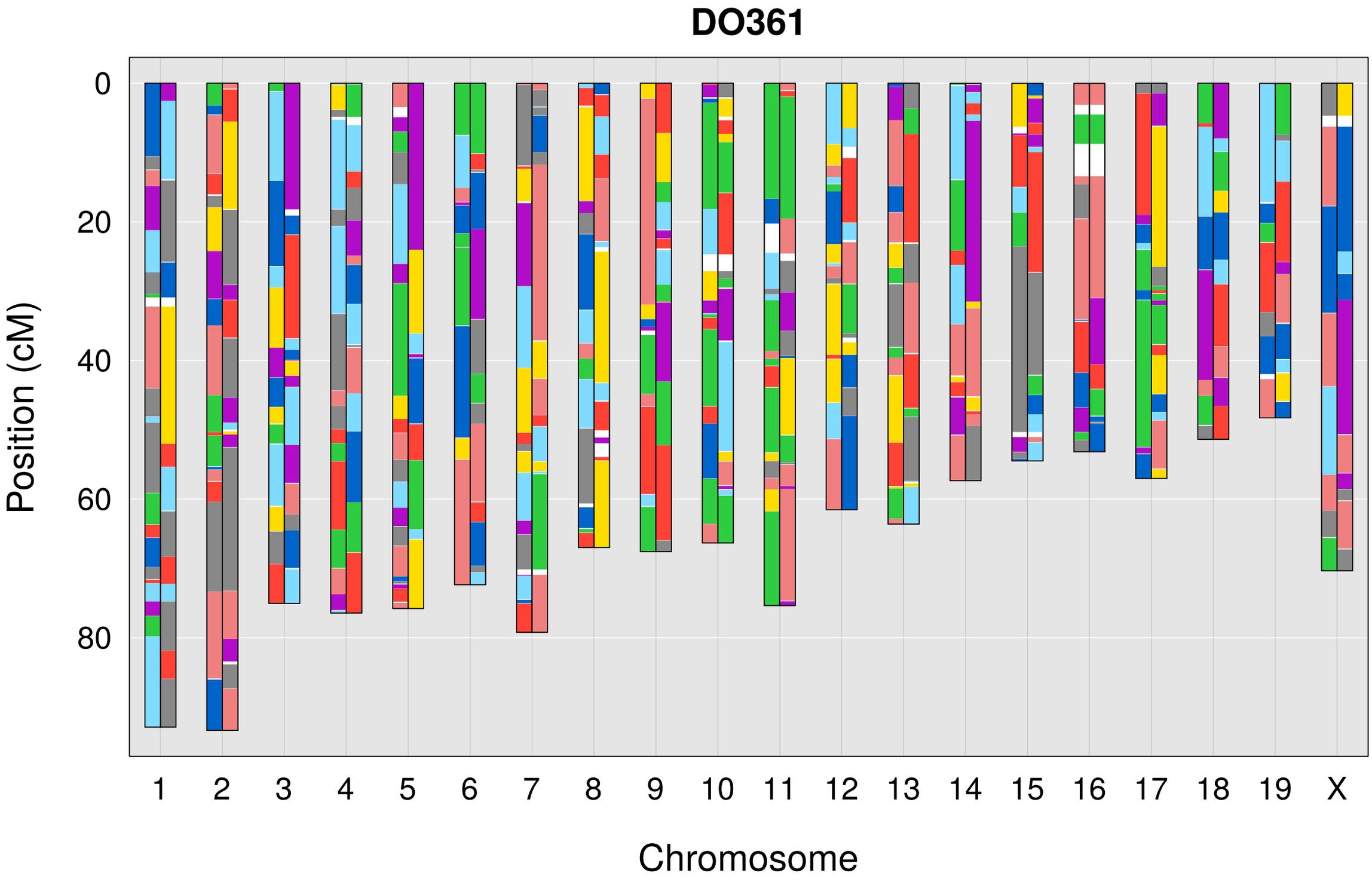




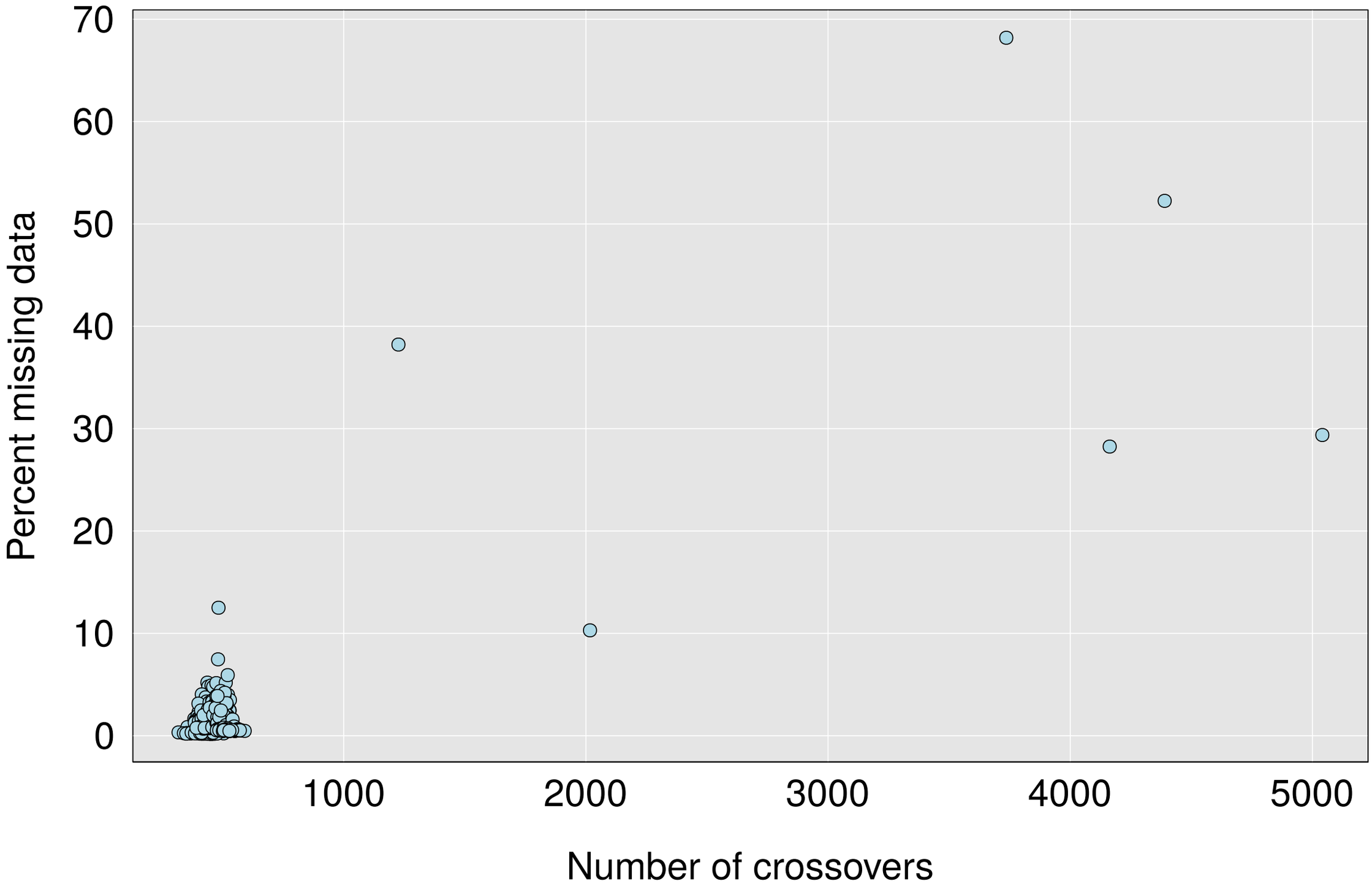
Genotype probabilities (one mouse on one chr)



Genome reconstruction (one mouse)



Percent missing vs number of crossovers



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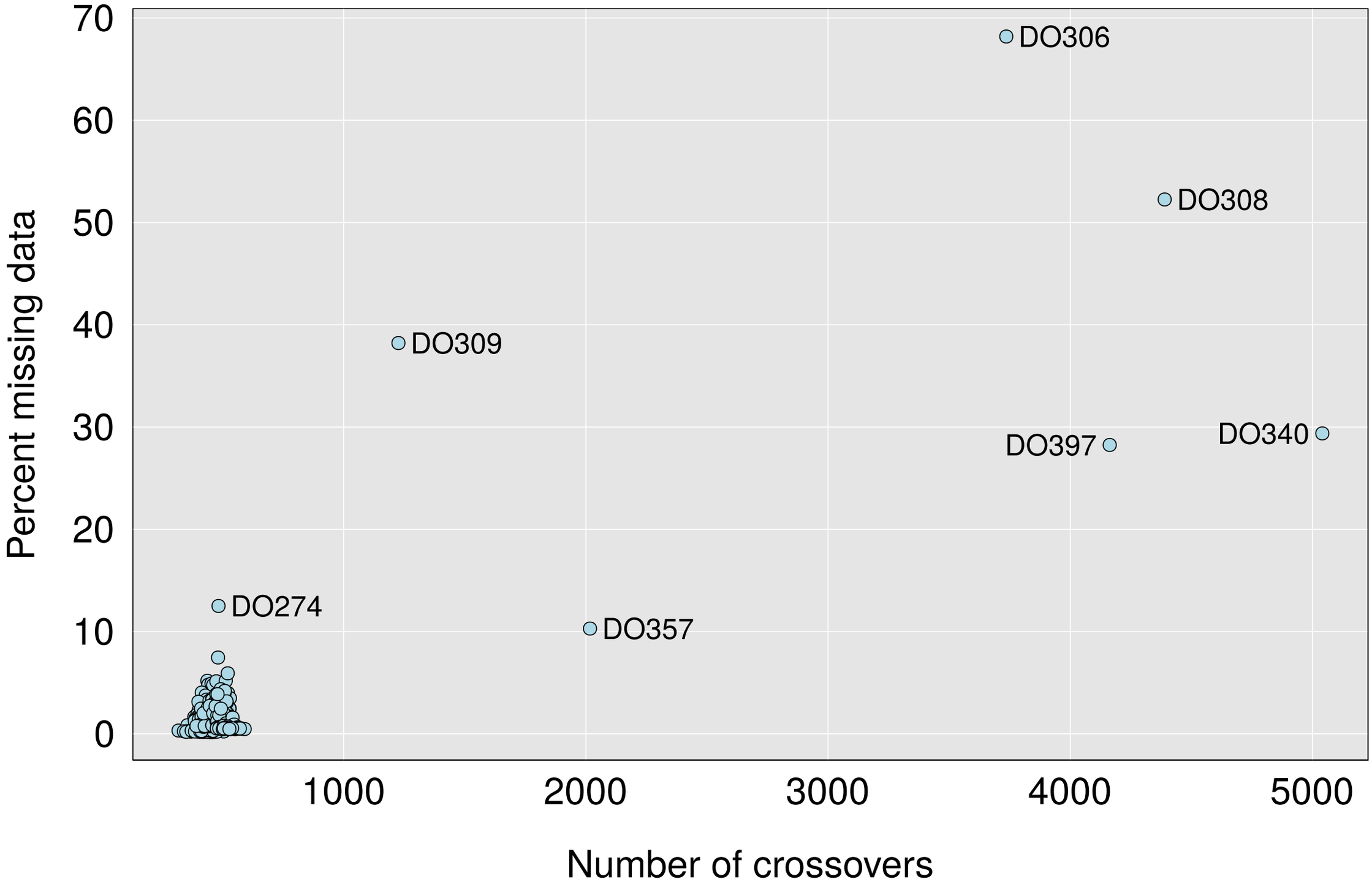

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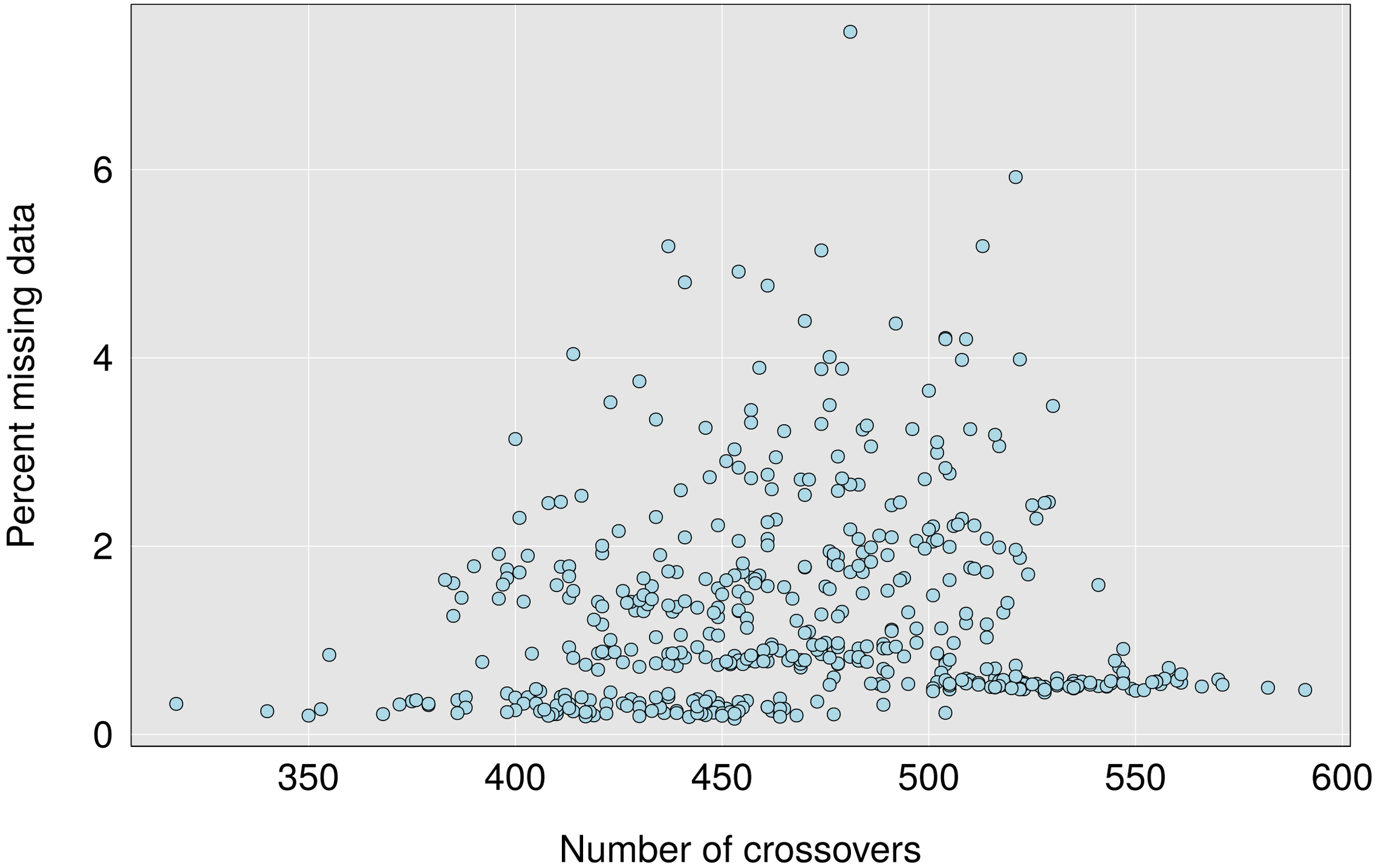

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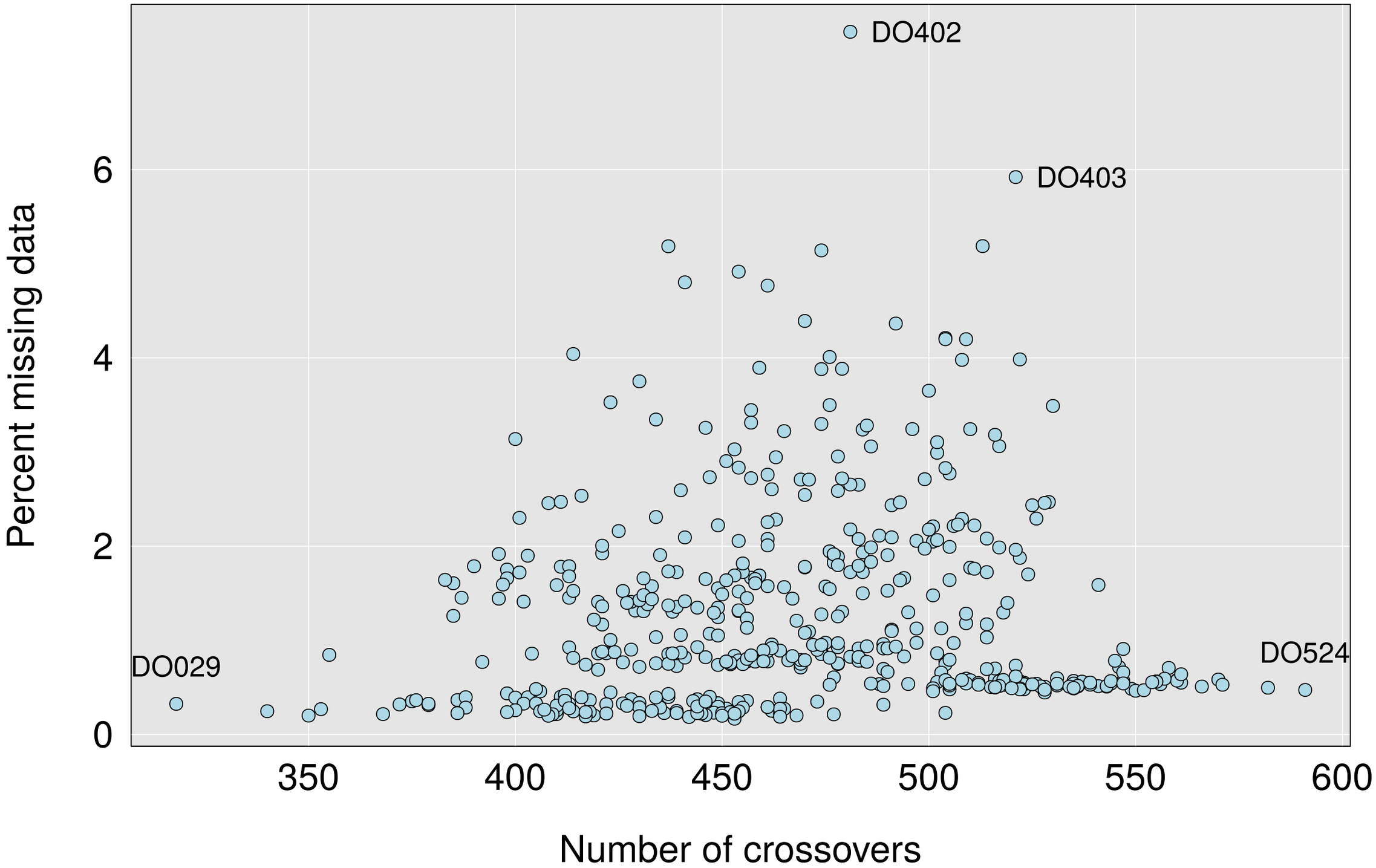
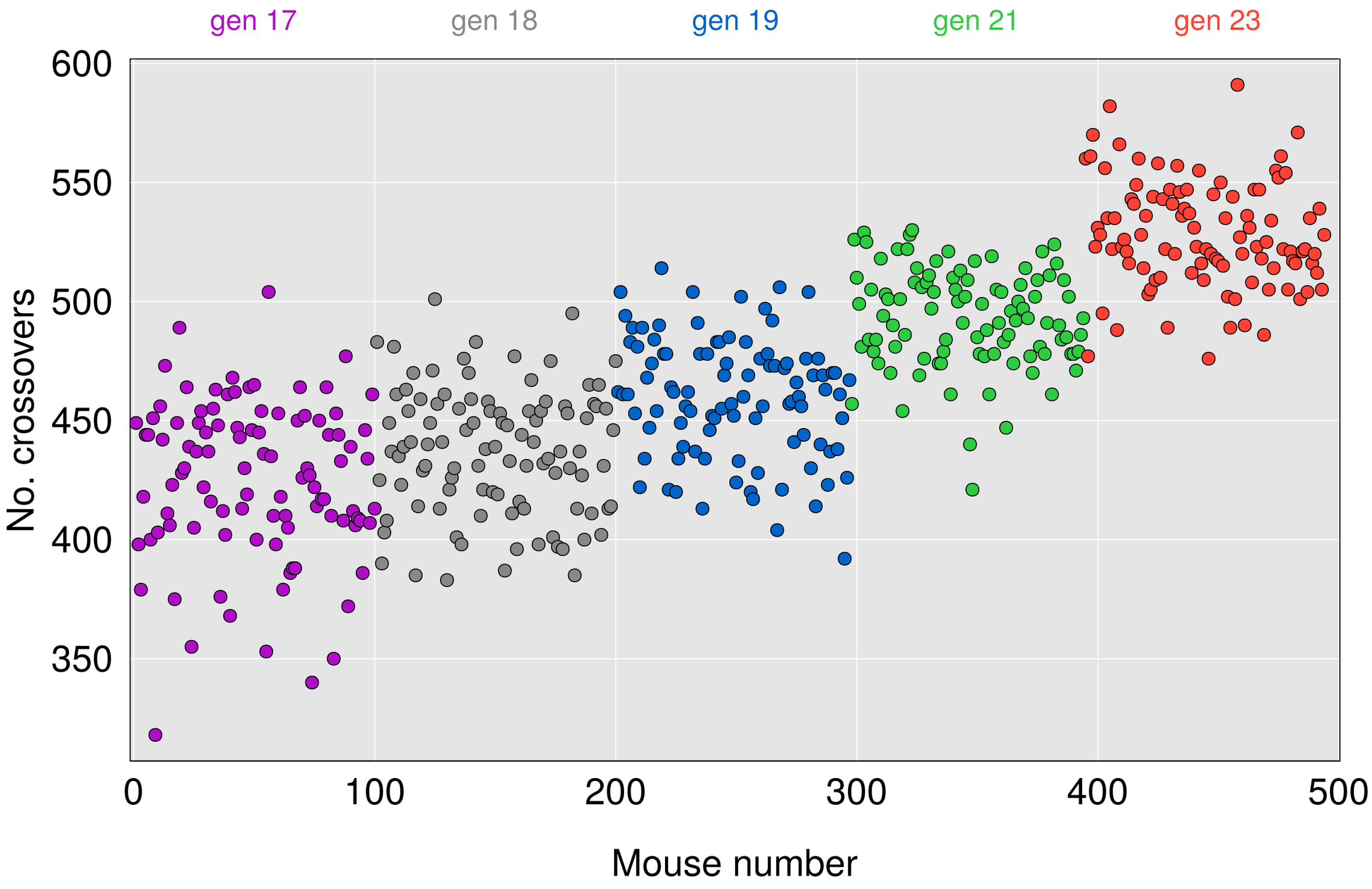
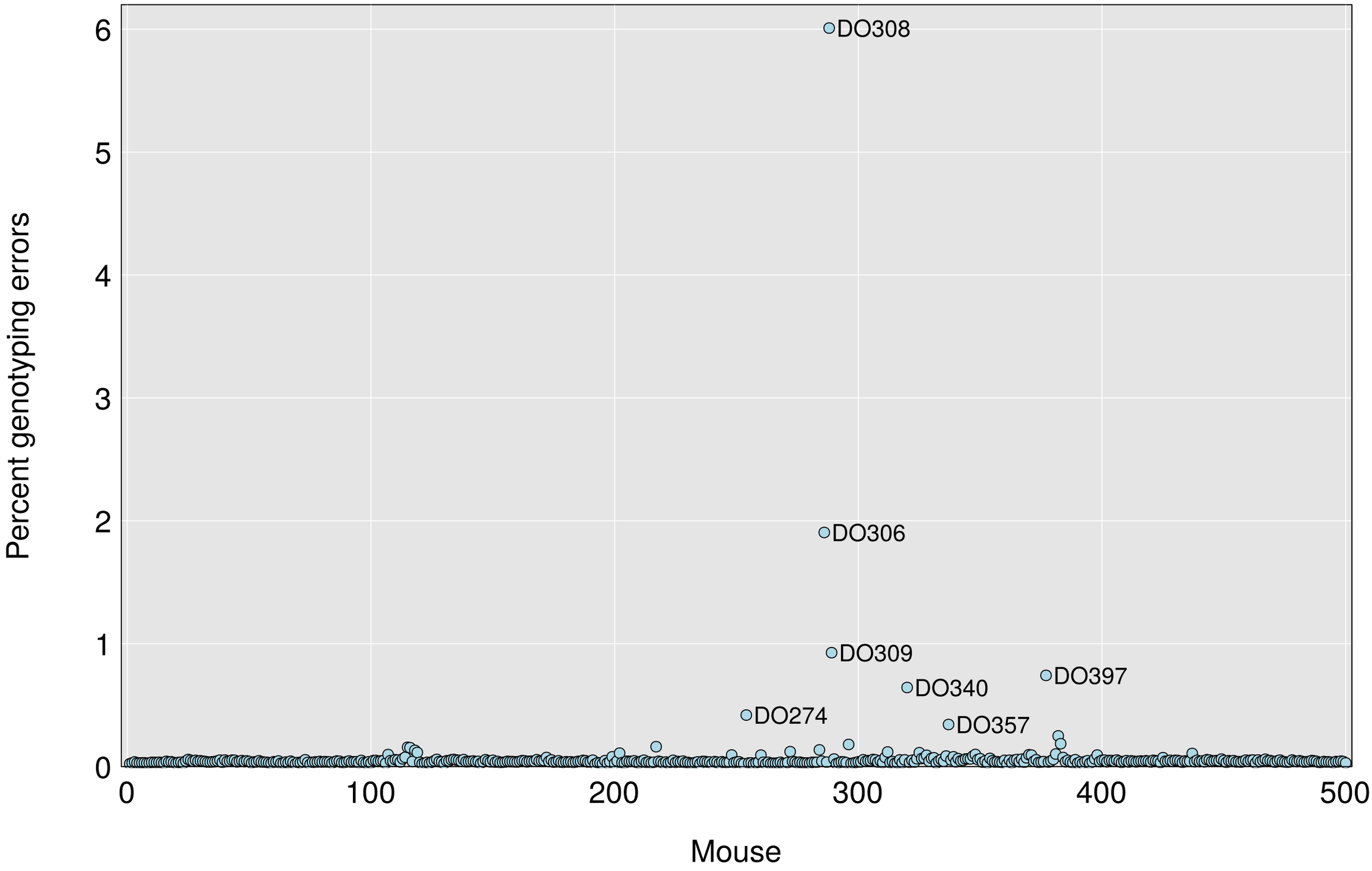


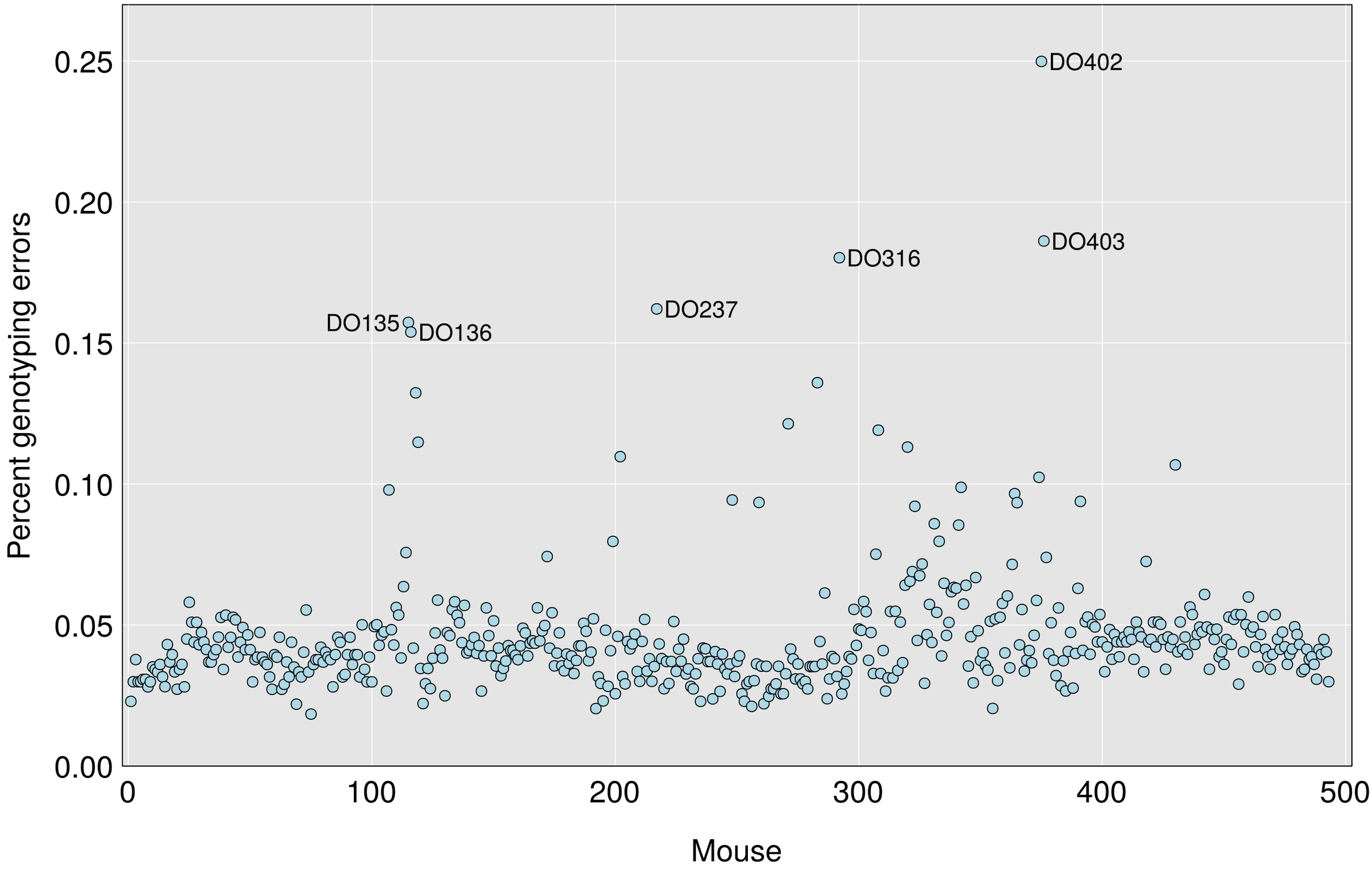
No. crossovers by generation



Estimated percent of genotyping errors

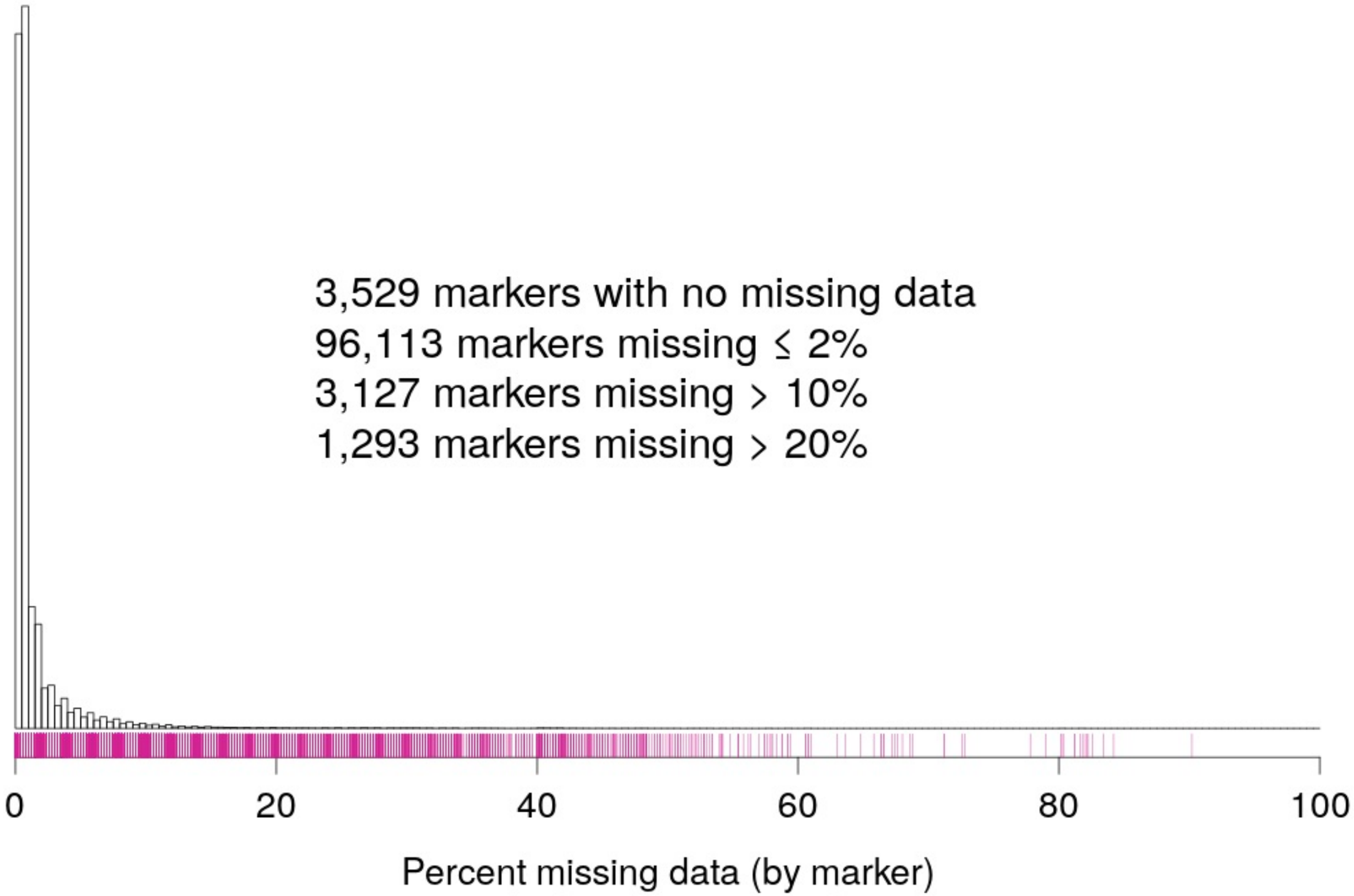


Estimated percent of genotyping errors

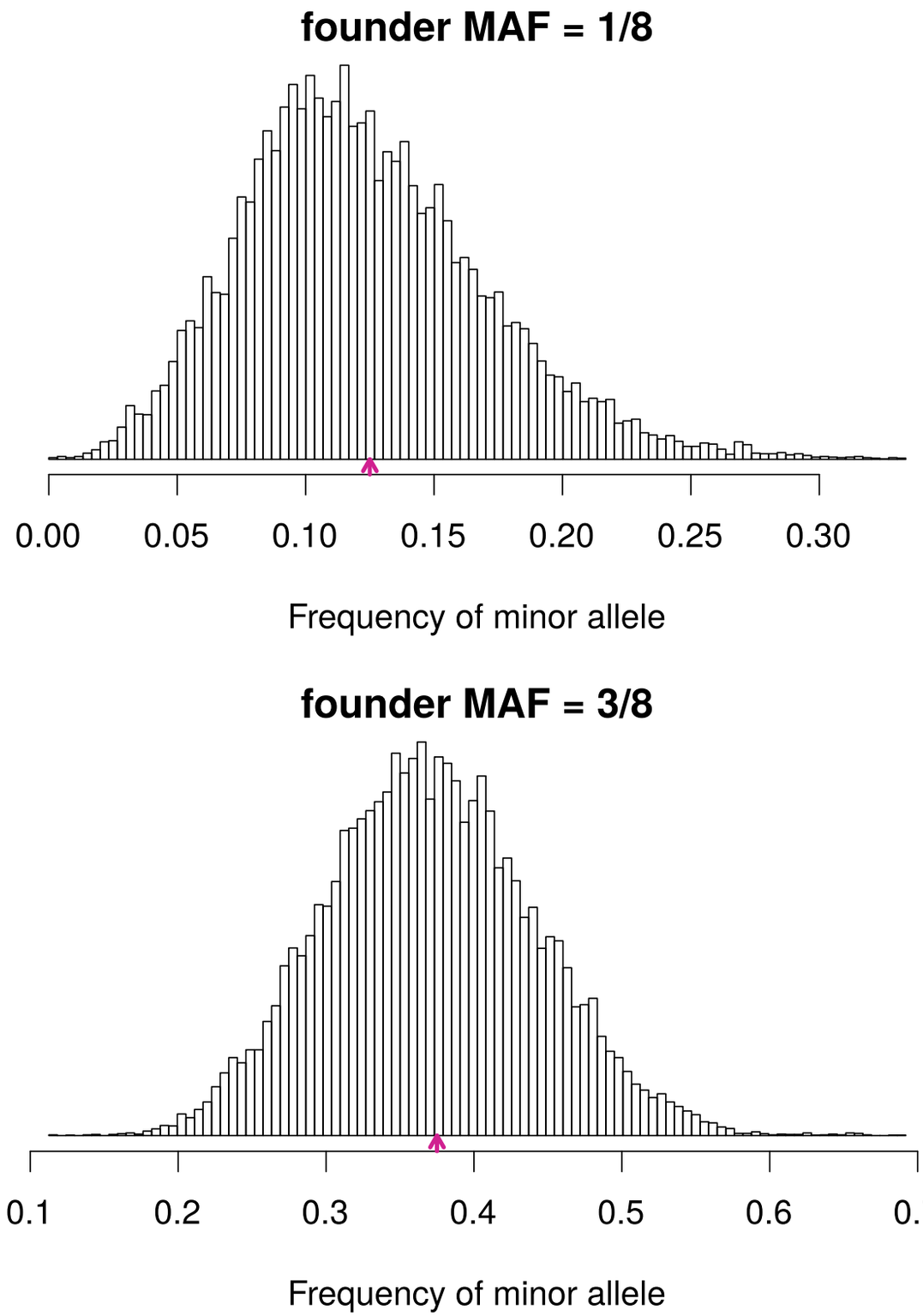


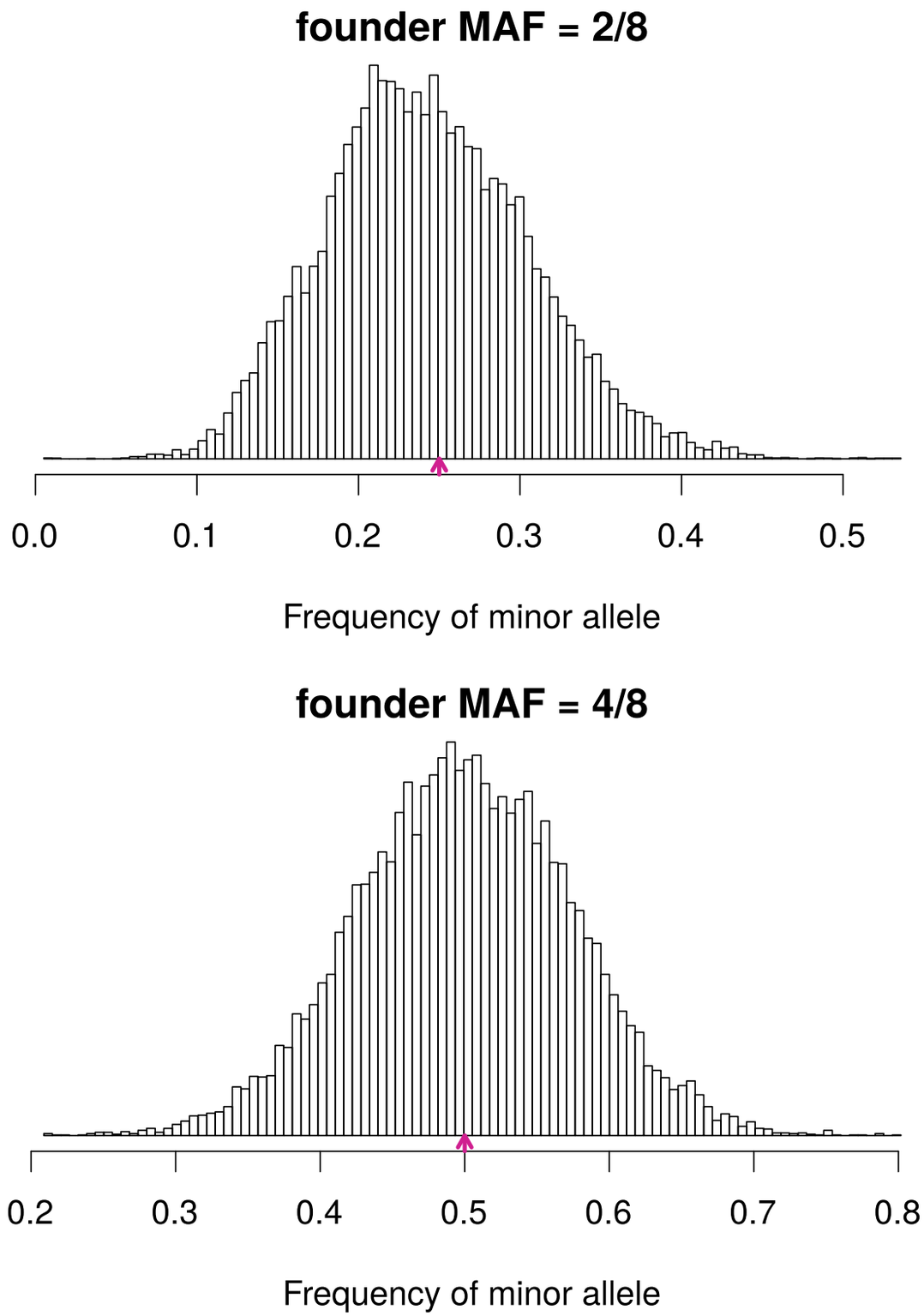
Marker quality

Proportion missing data

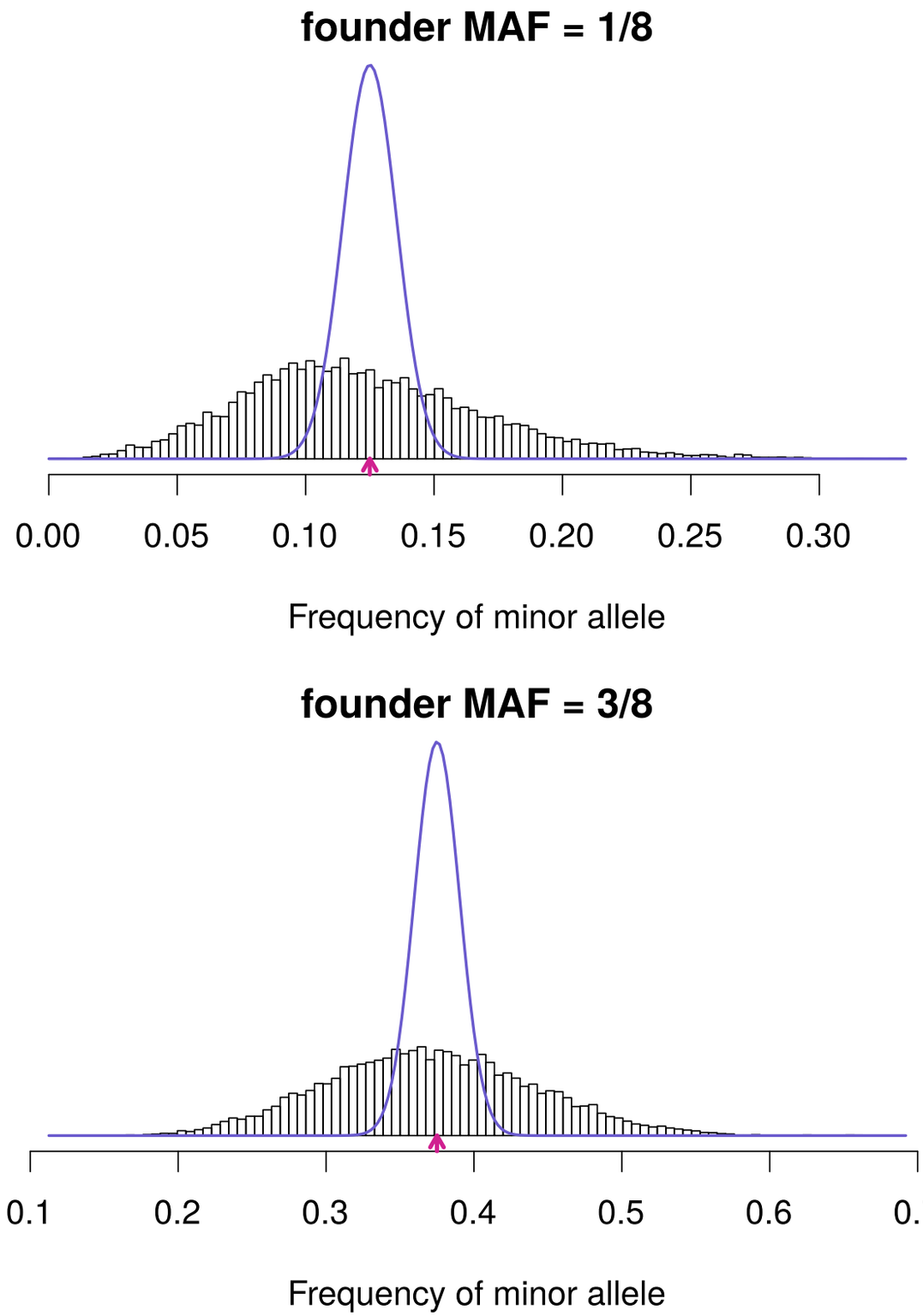


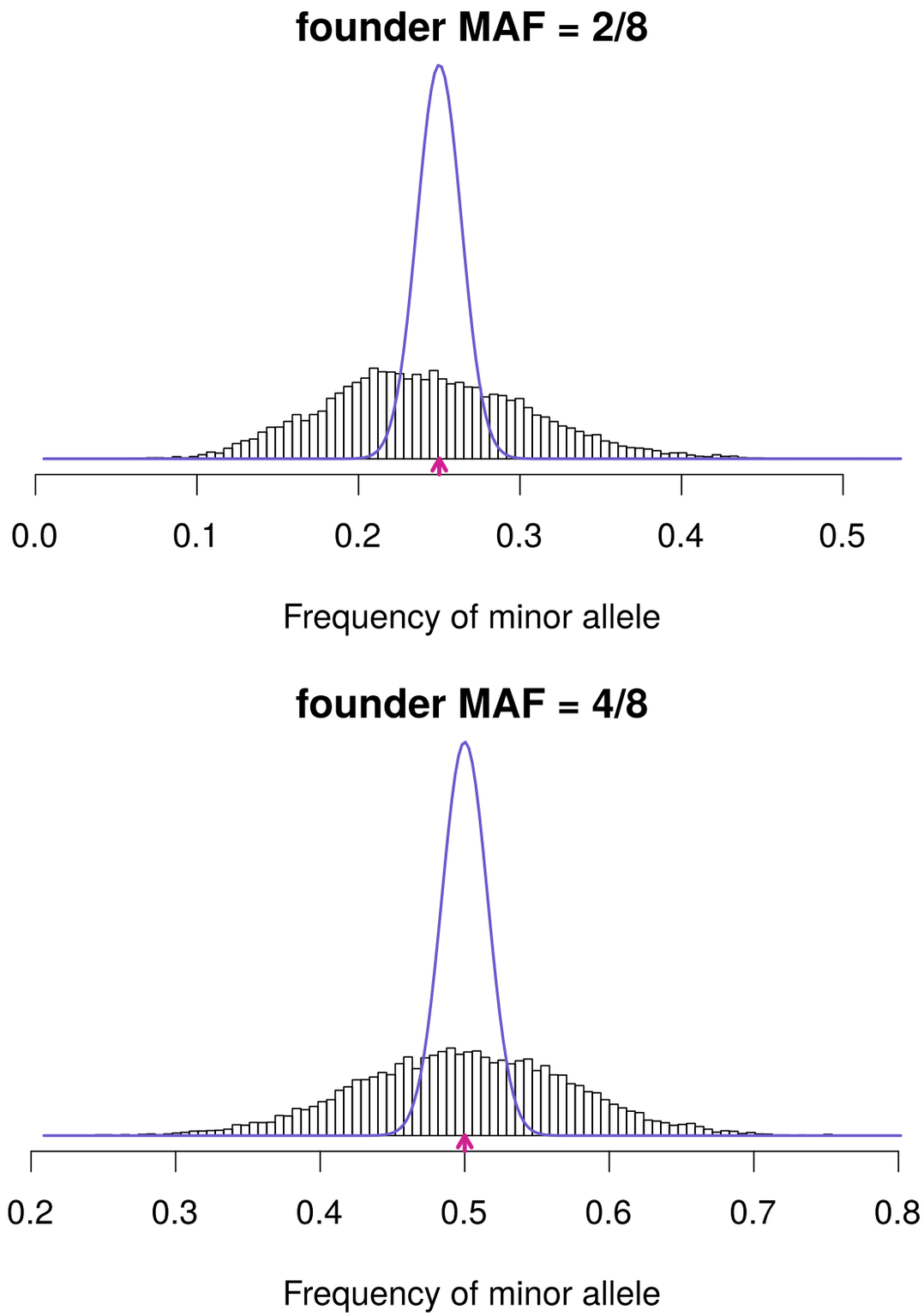
Allele frequencies, by marker



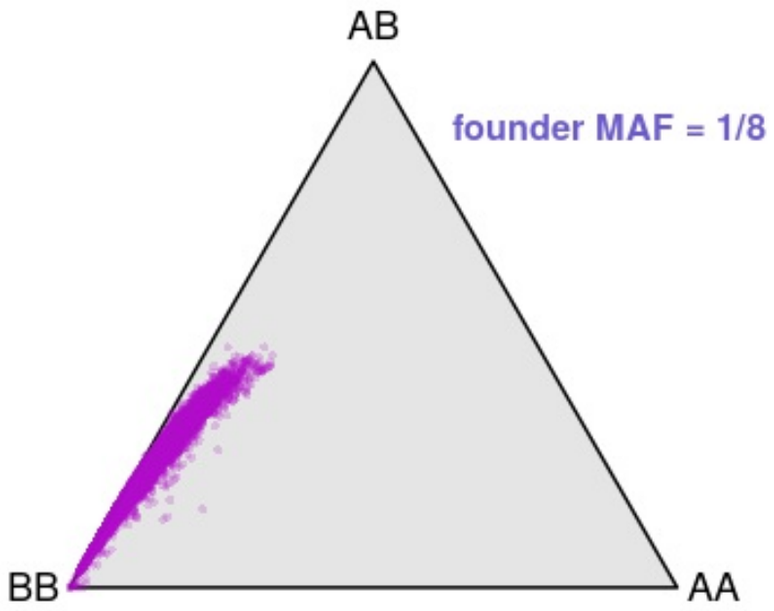


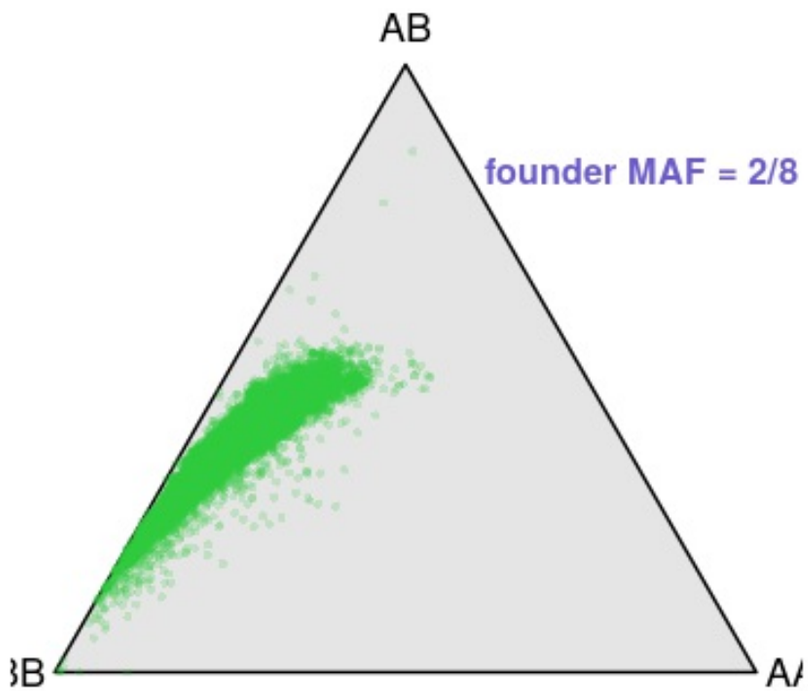
Allele frequencies, by marker

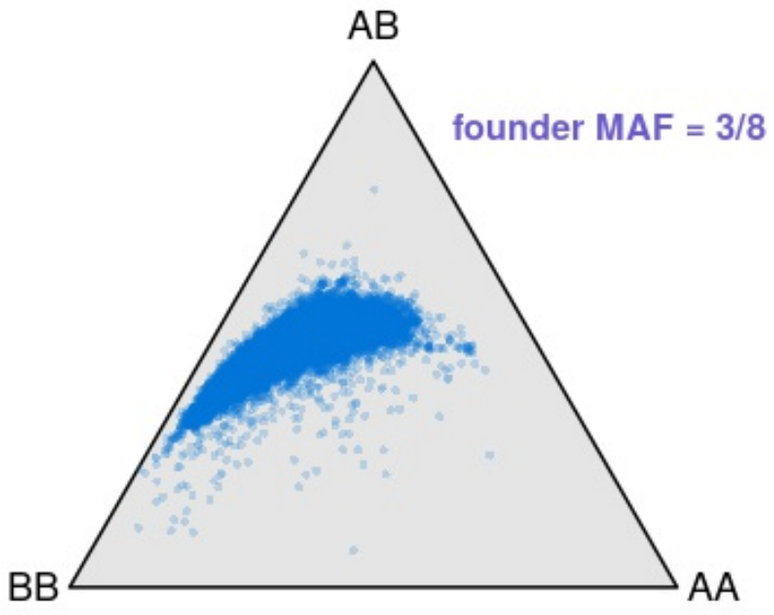


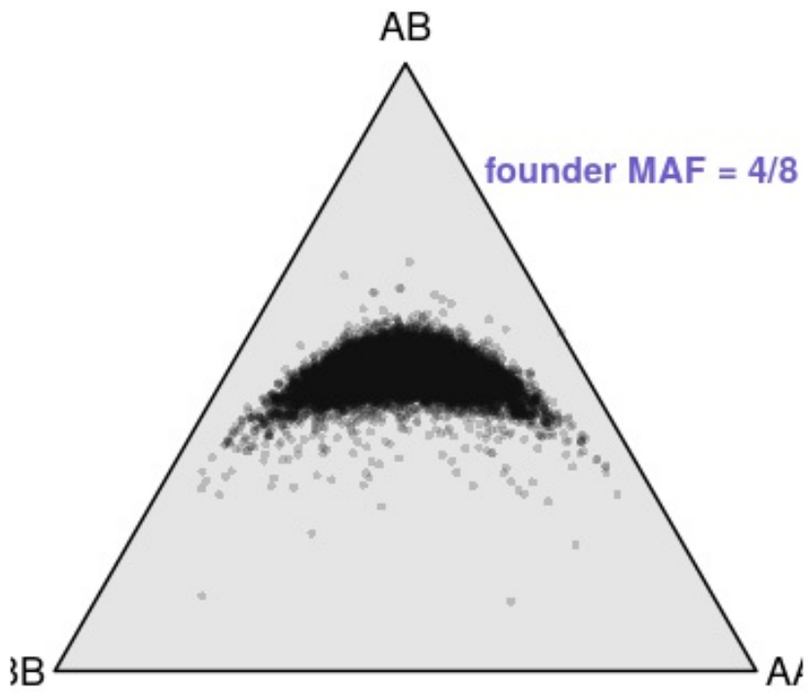


Genotype frequencies, by marker

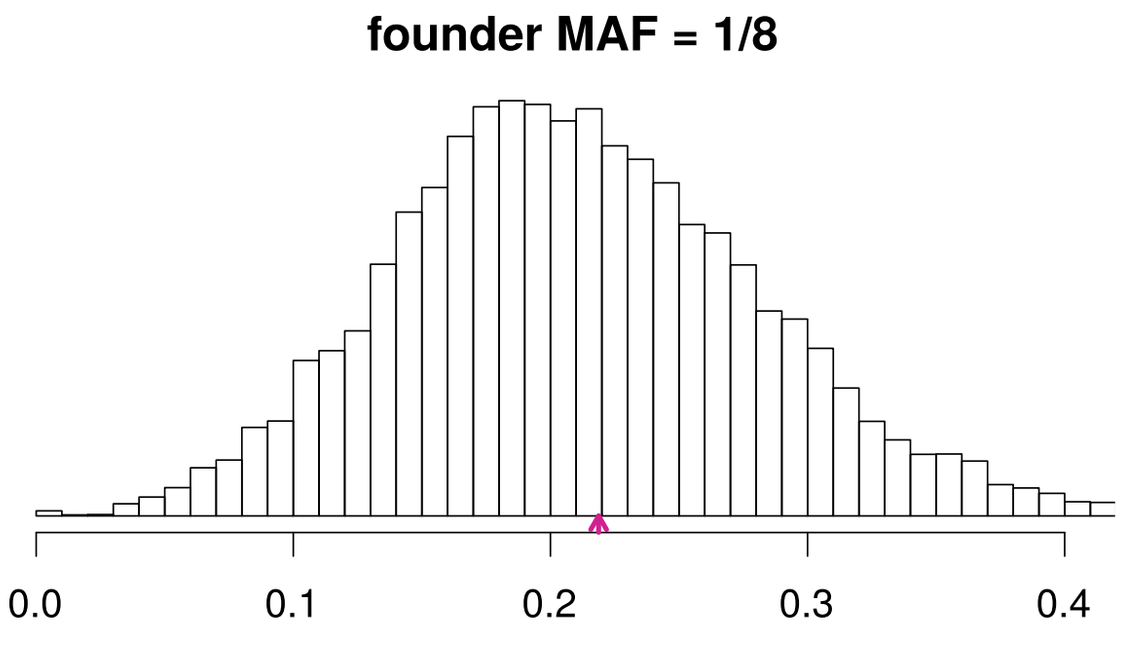


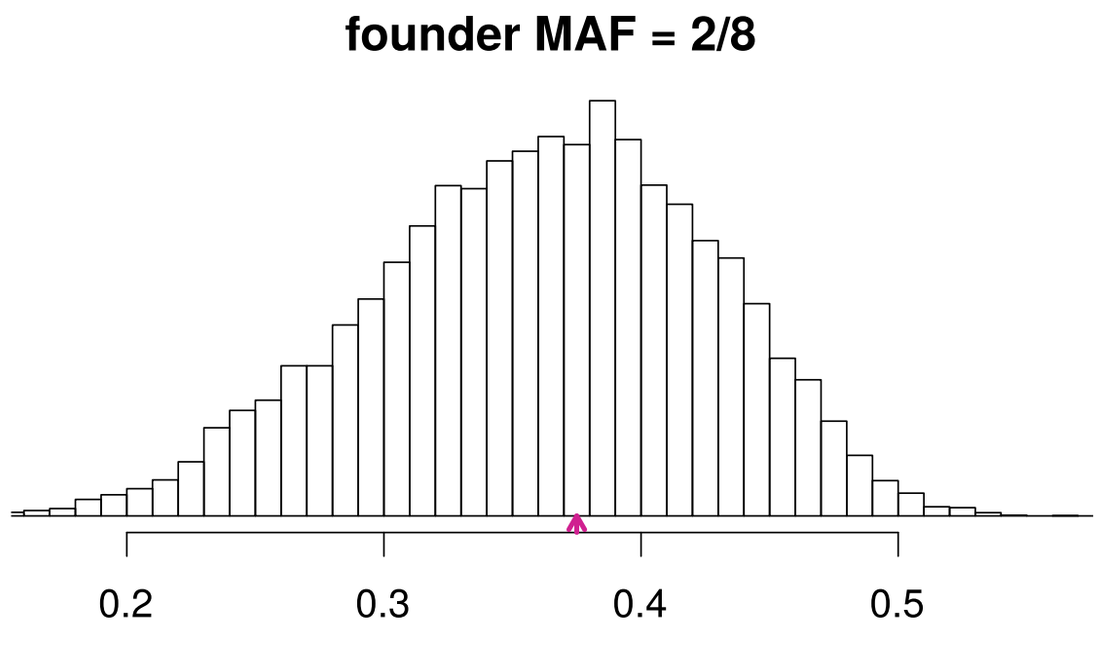


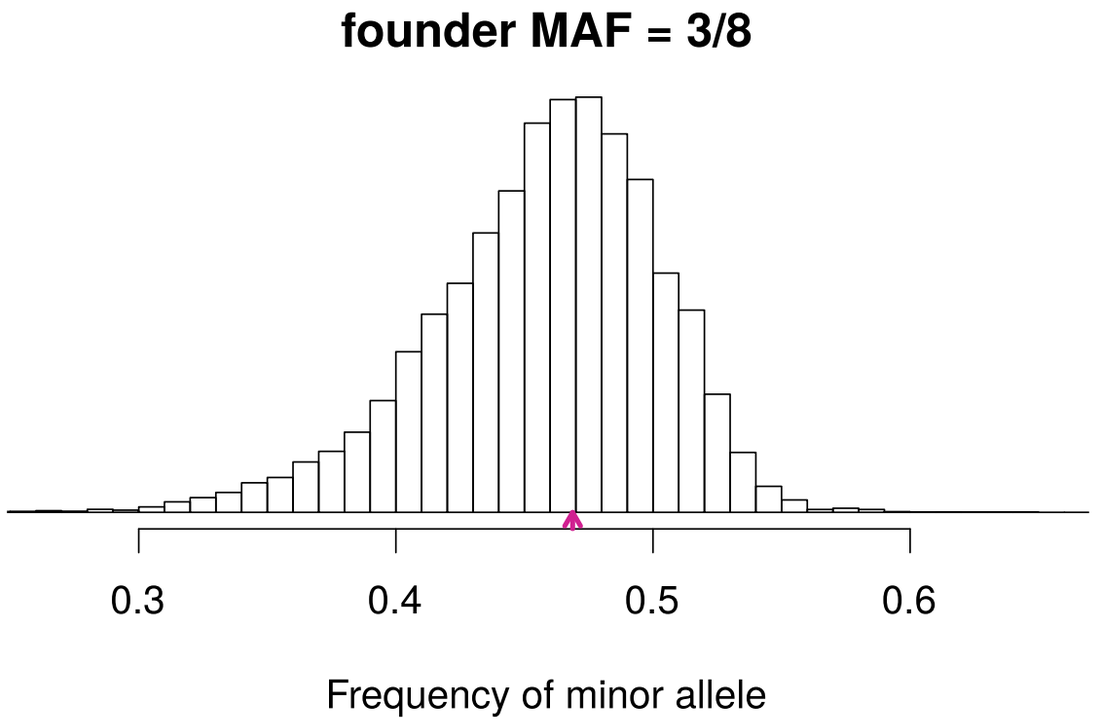


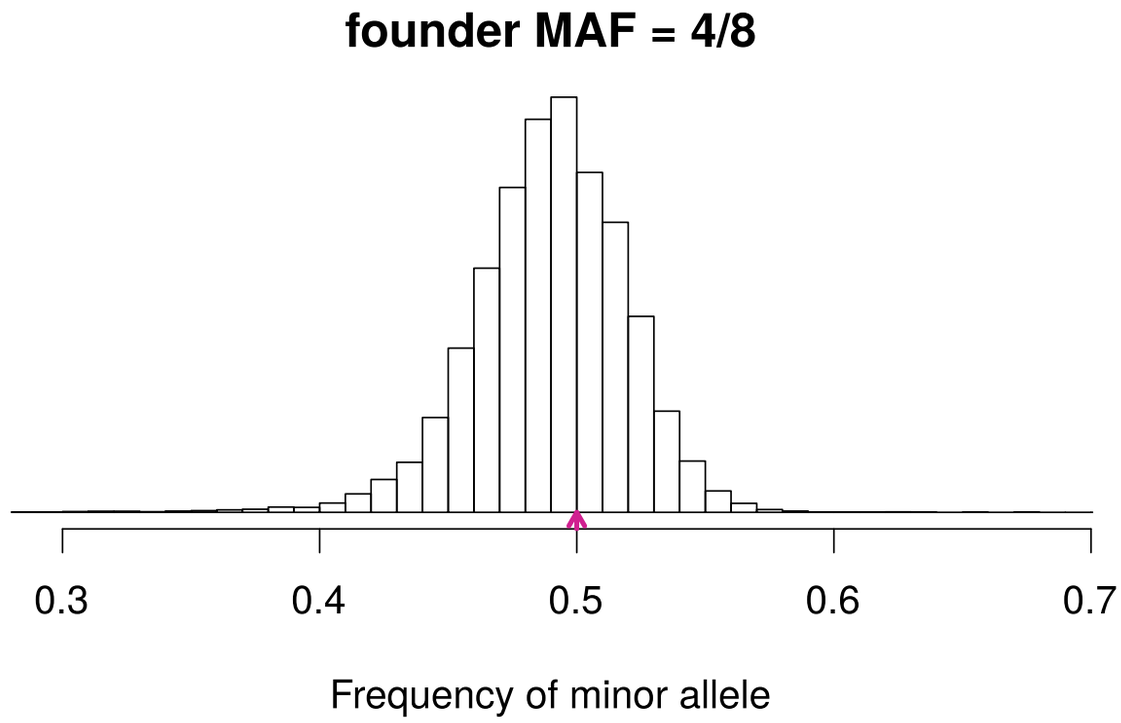


Heterozygosities, by marker

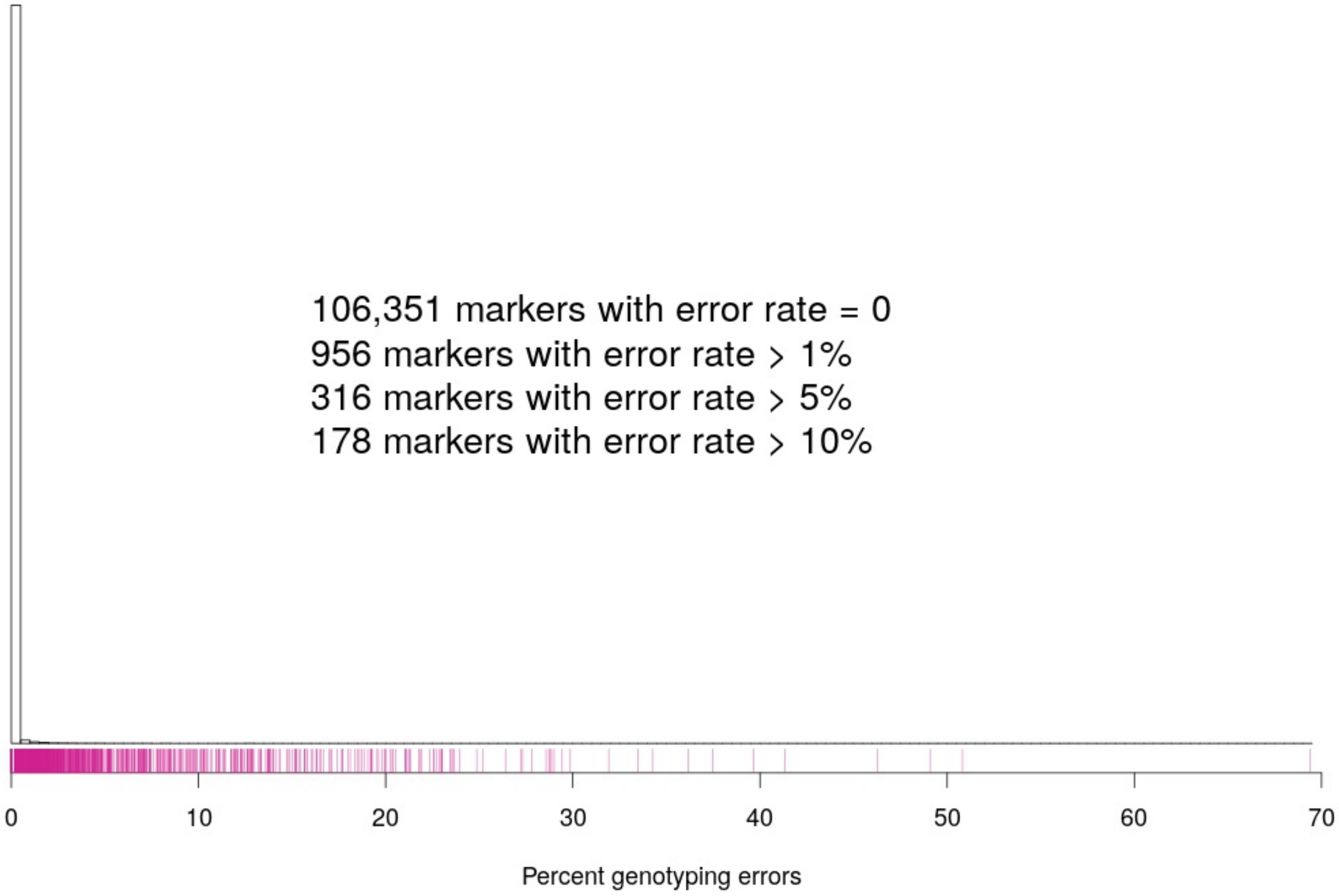




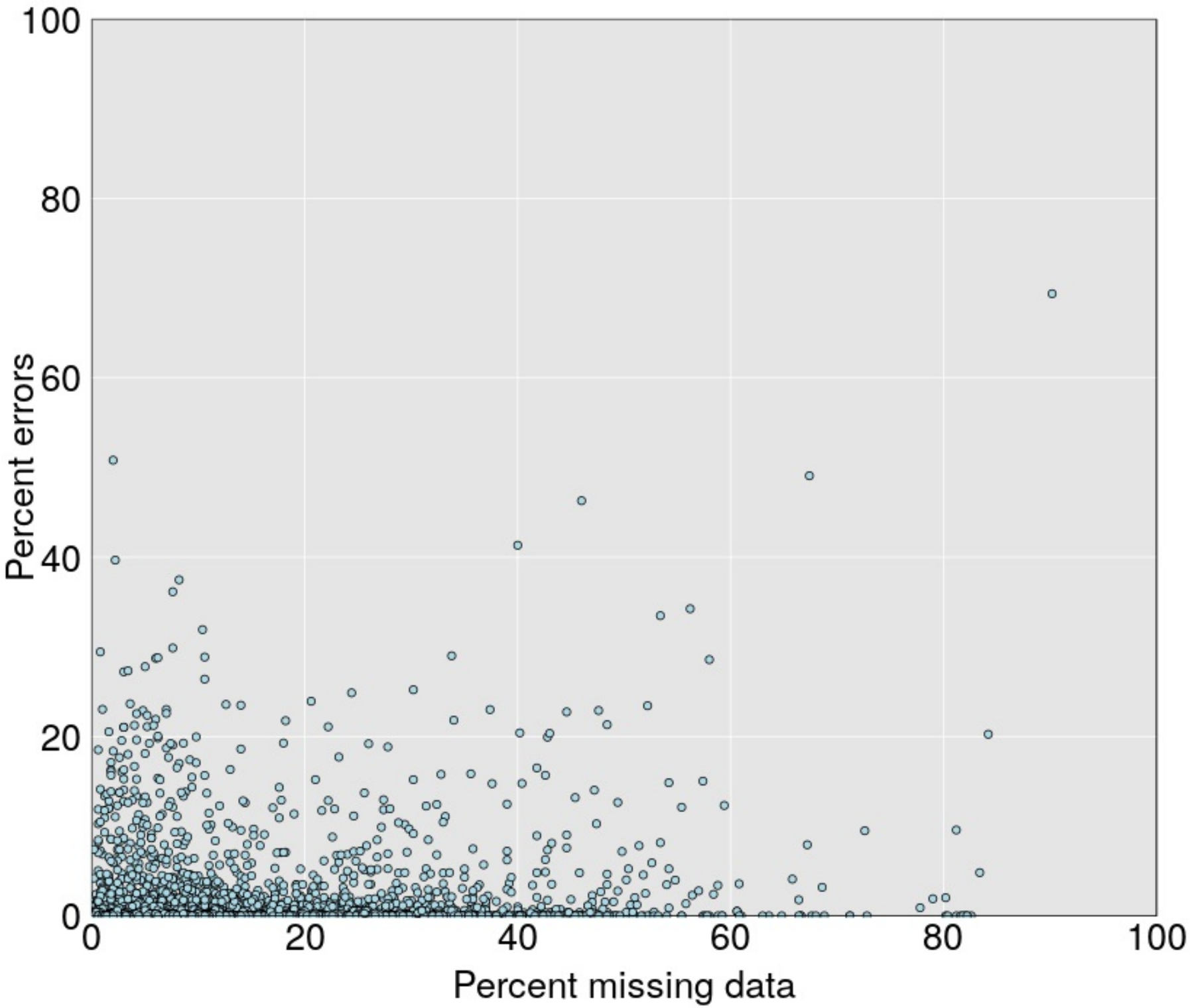




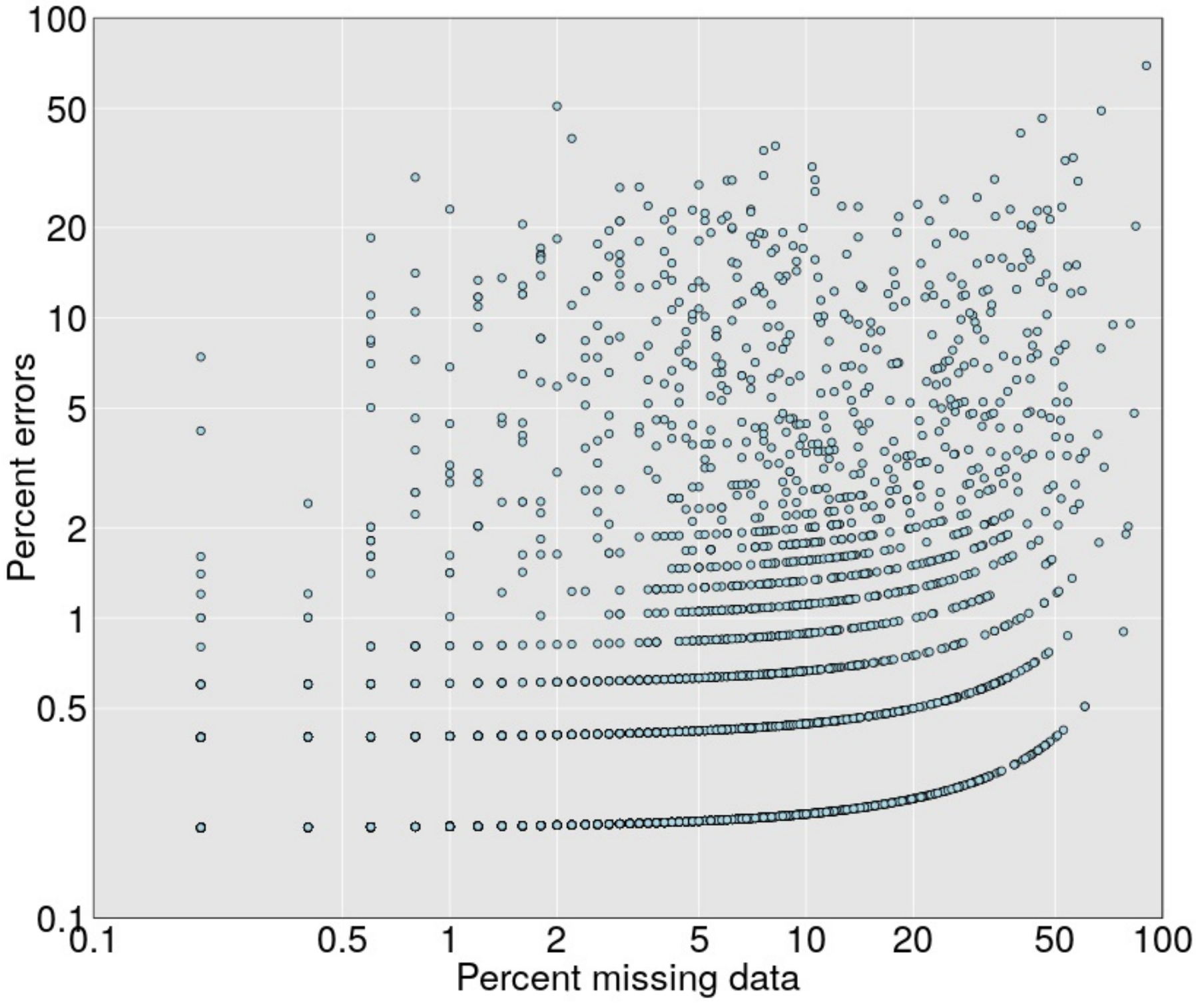
Genotyping error rates



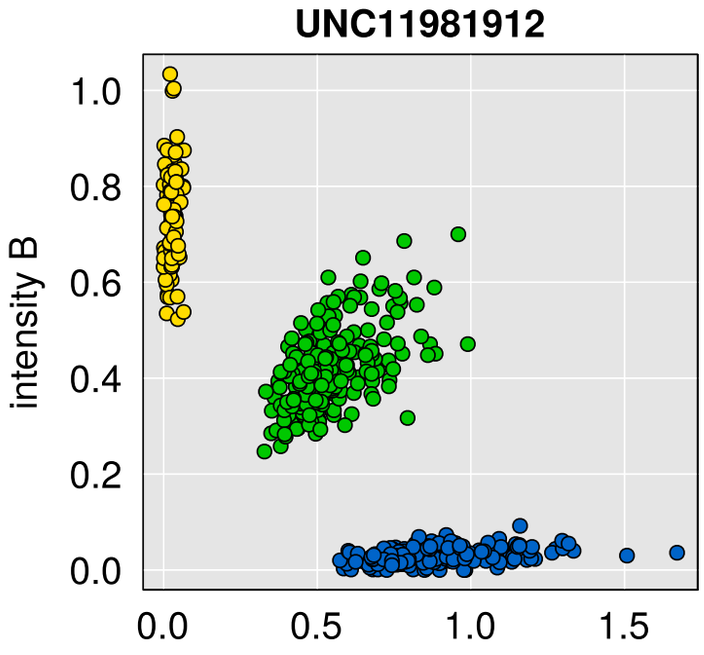
Genotyping error rate vs percent missing

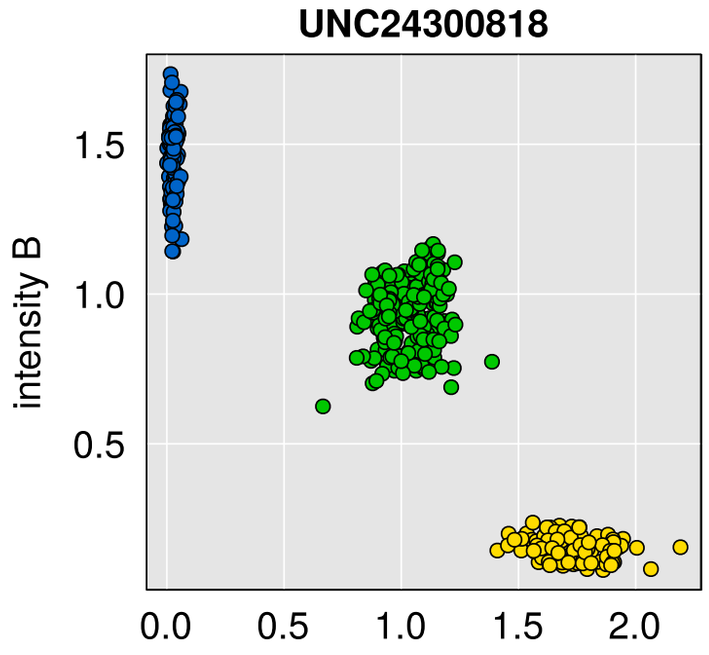


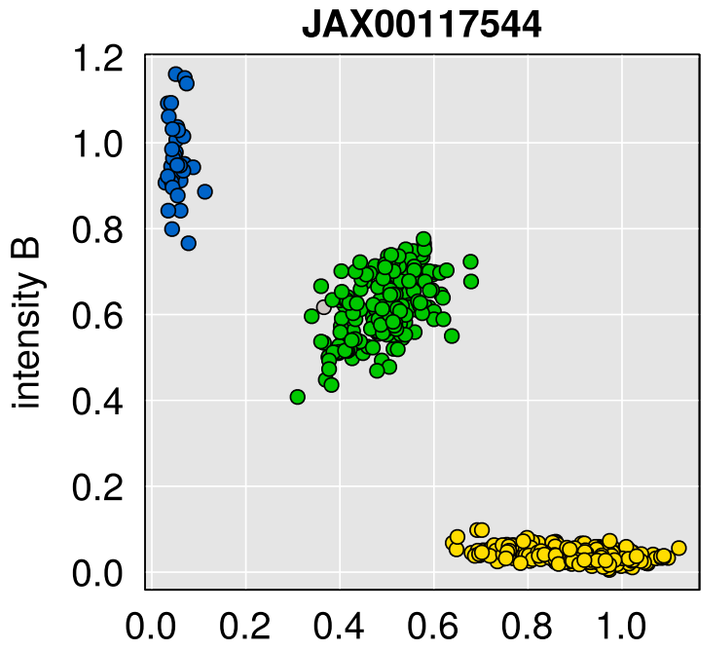
Genotyping error rate vs percent missing

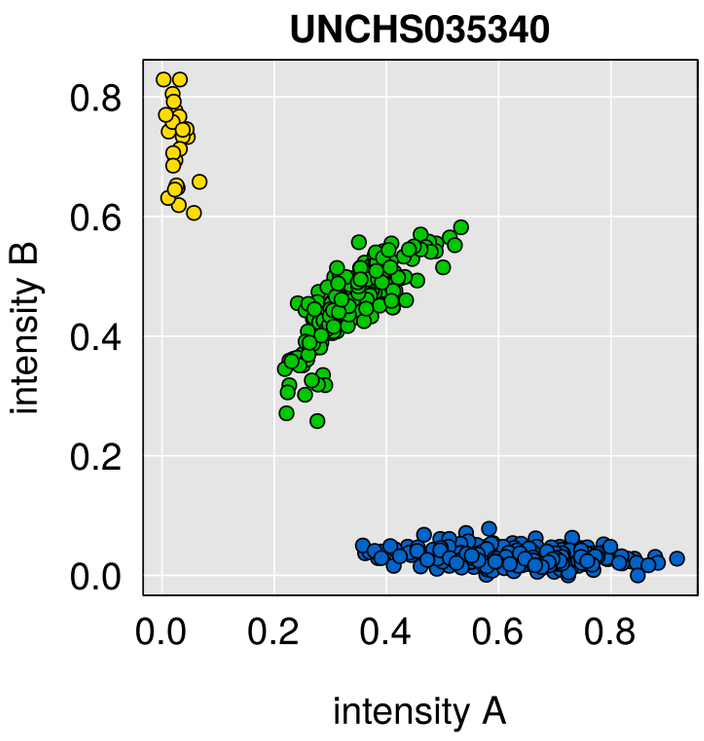


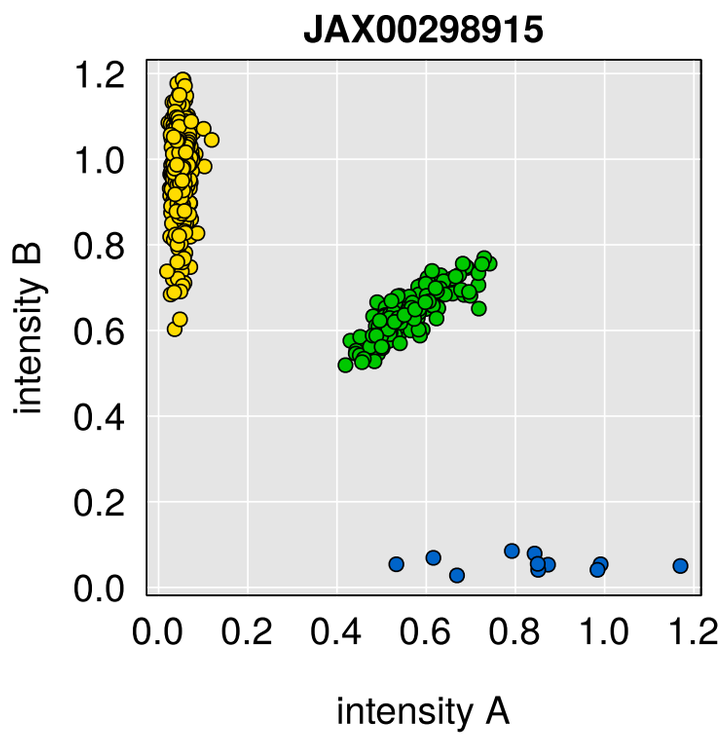
Nice markers

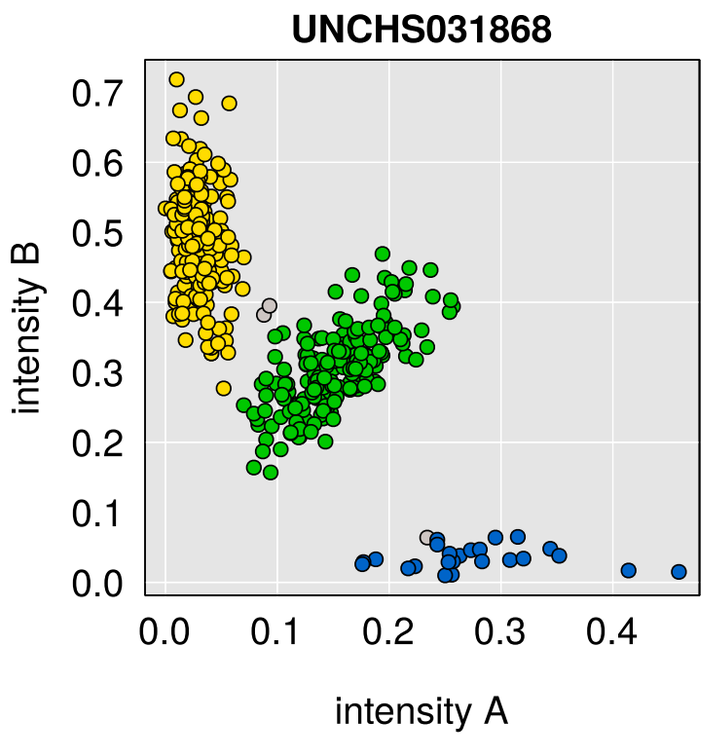




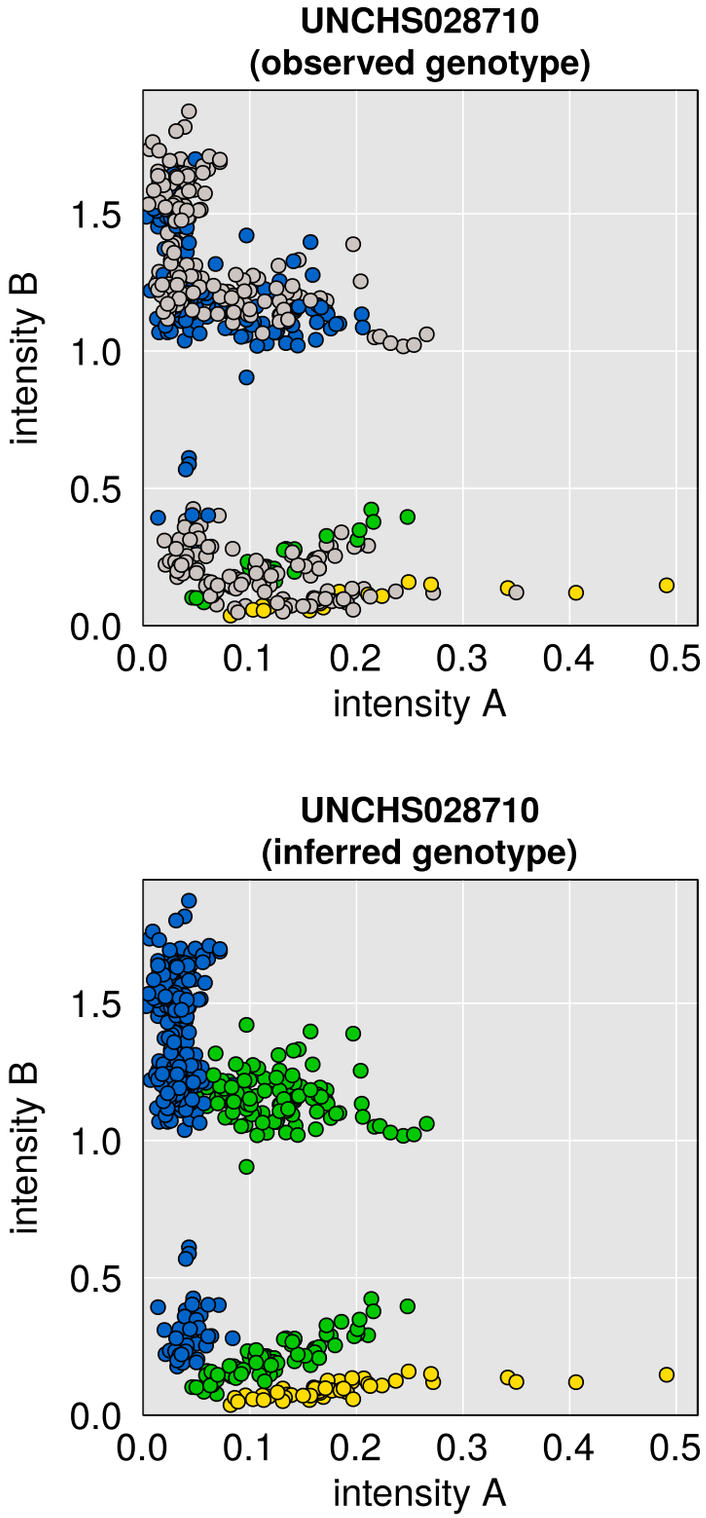


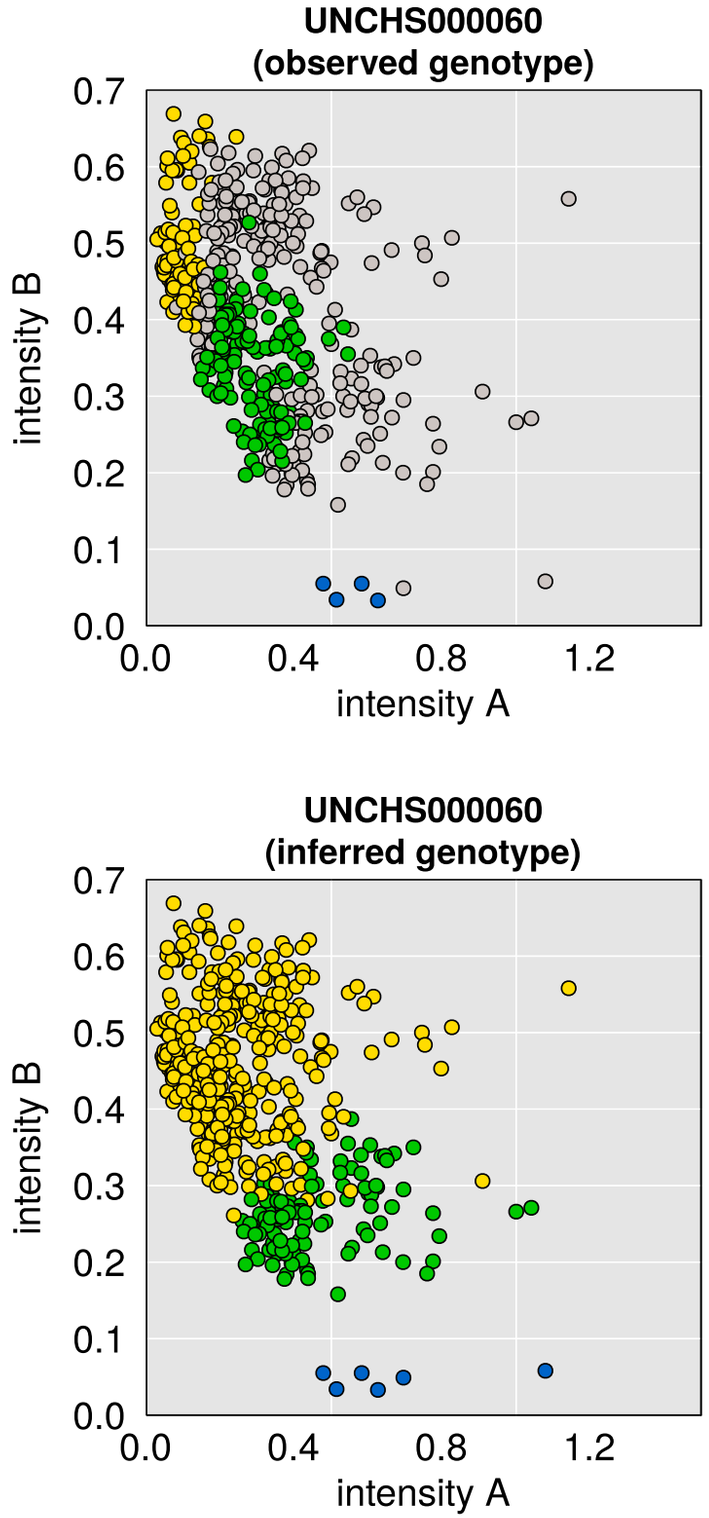


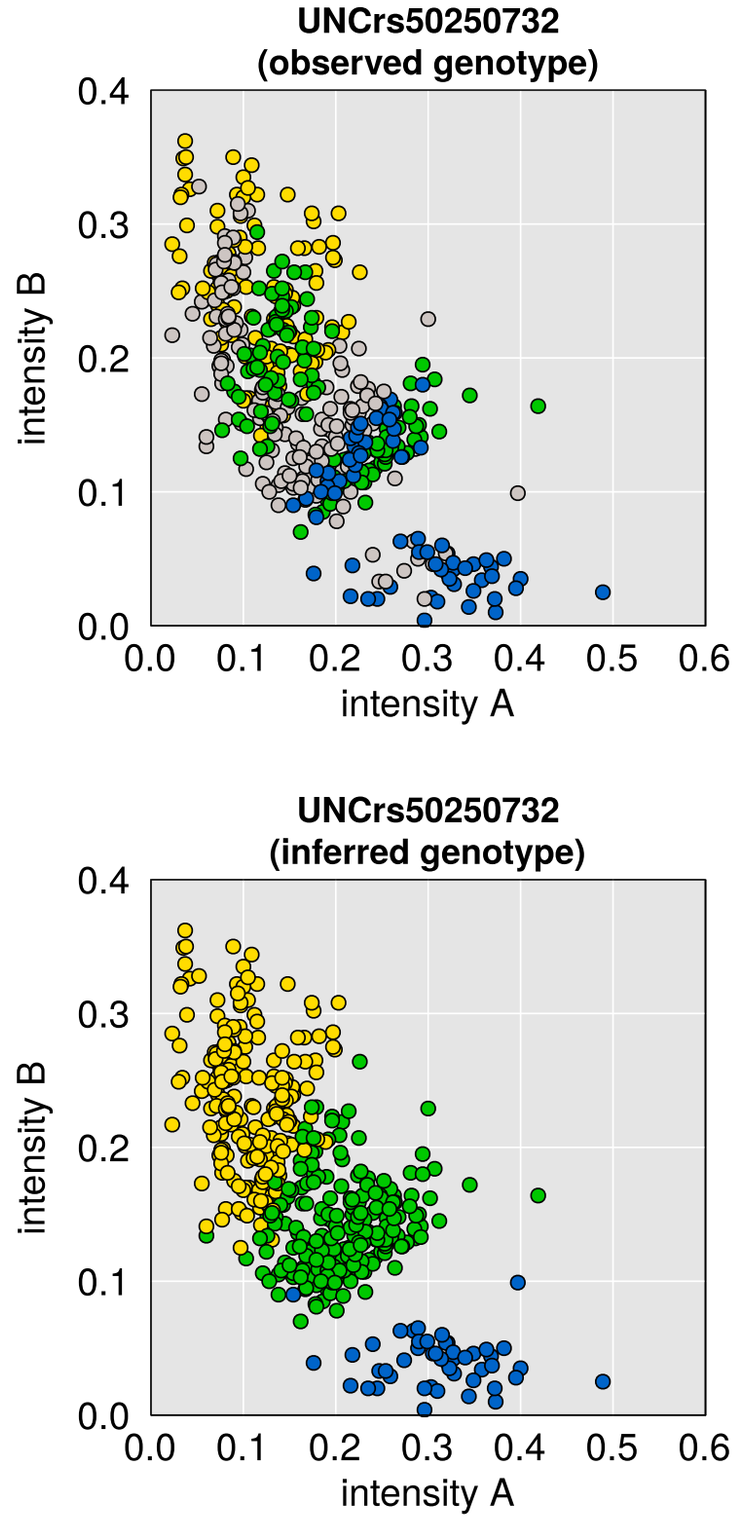




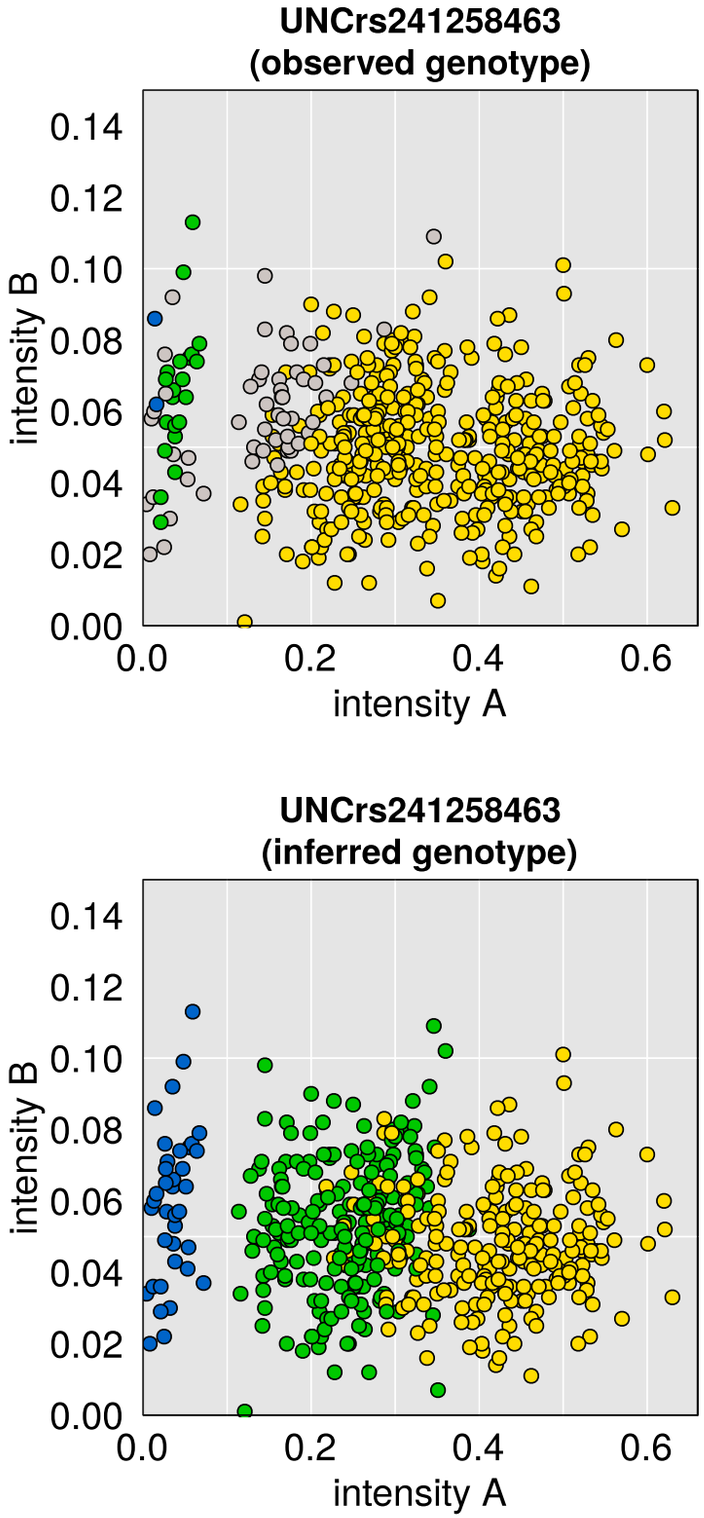
Crap markers

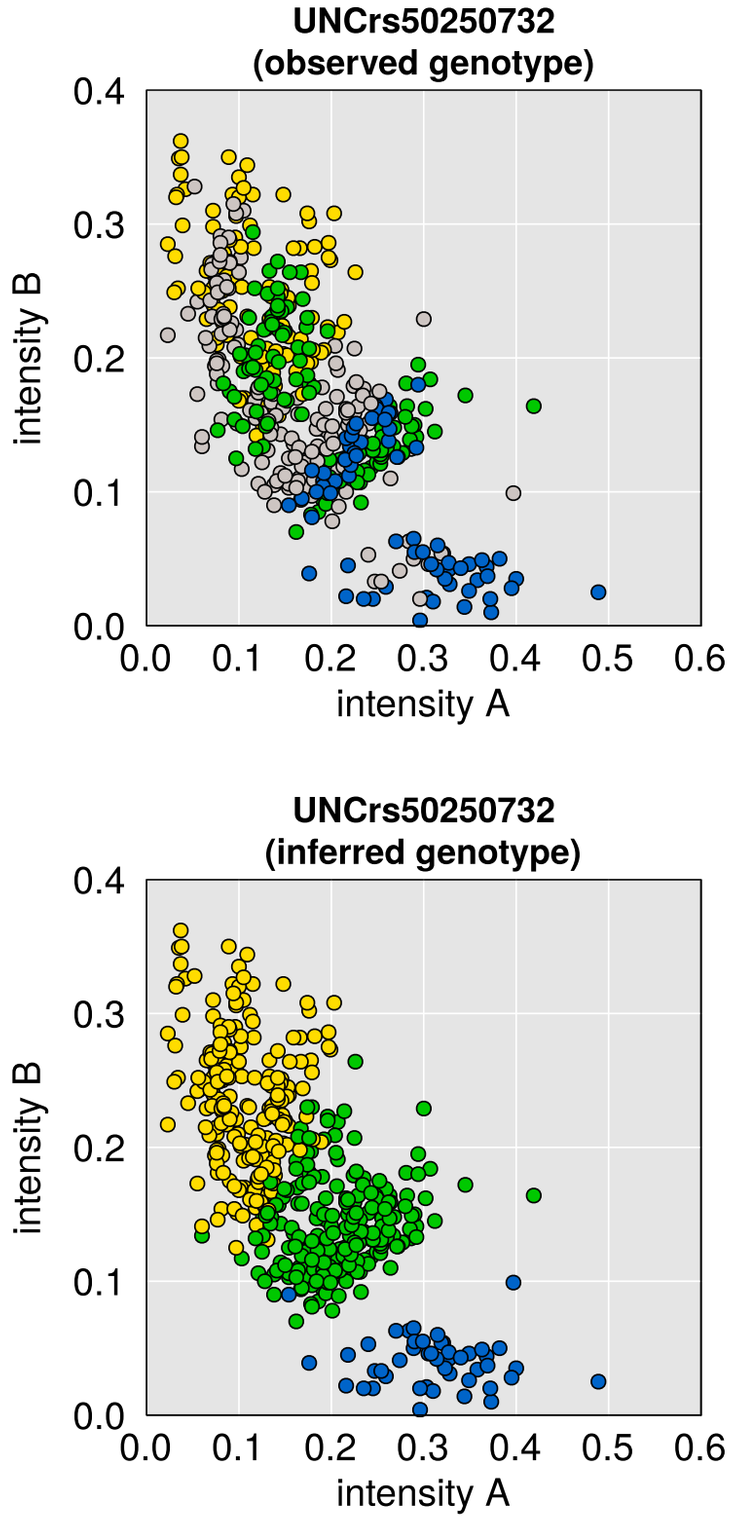


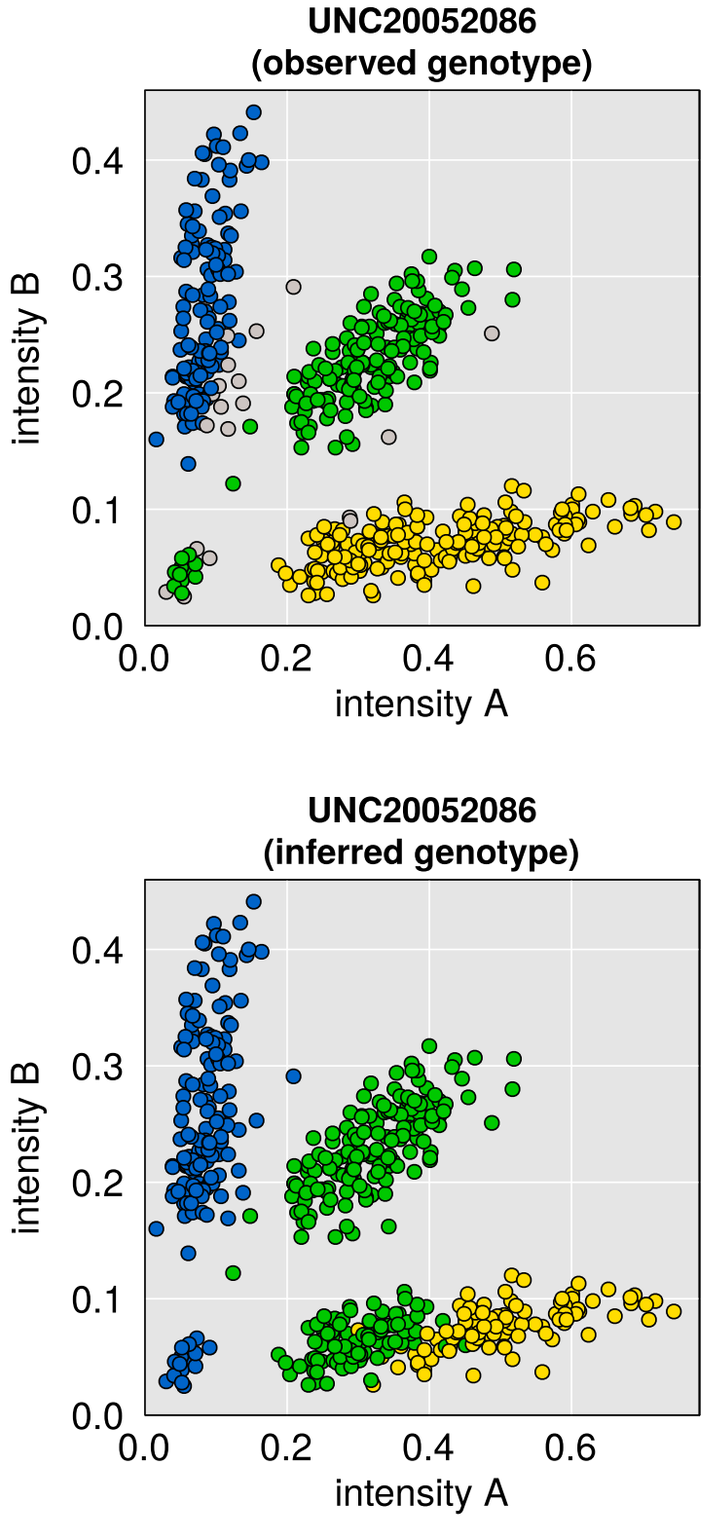




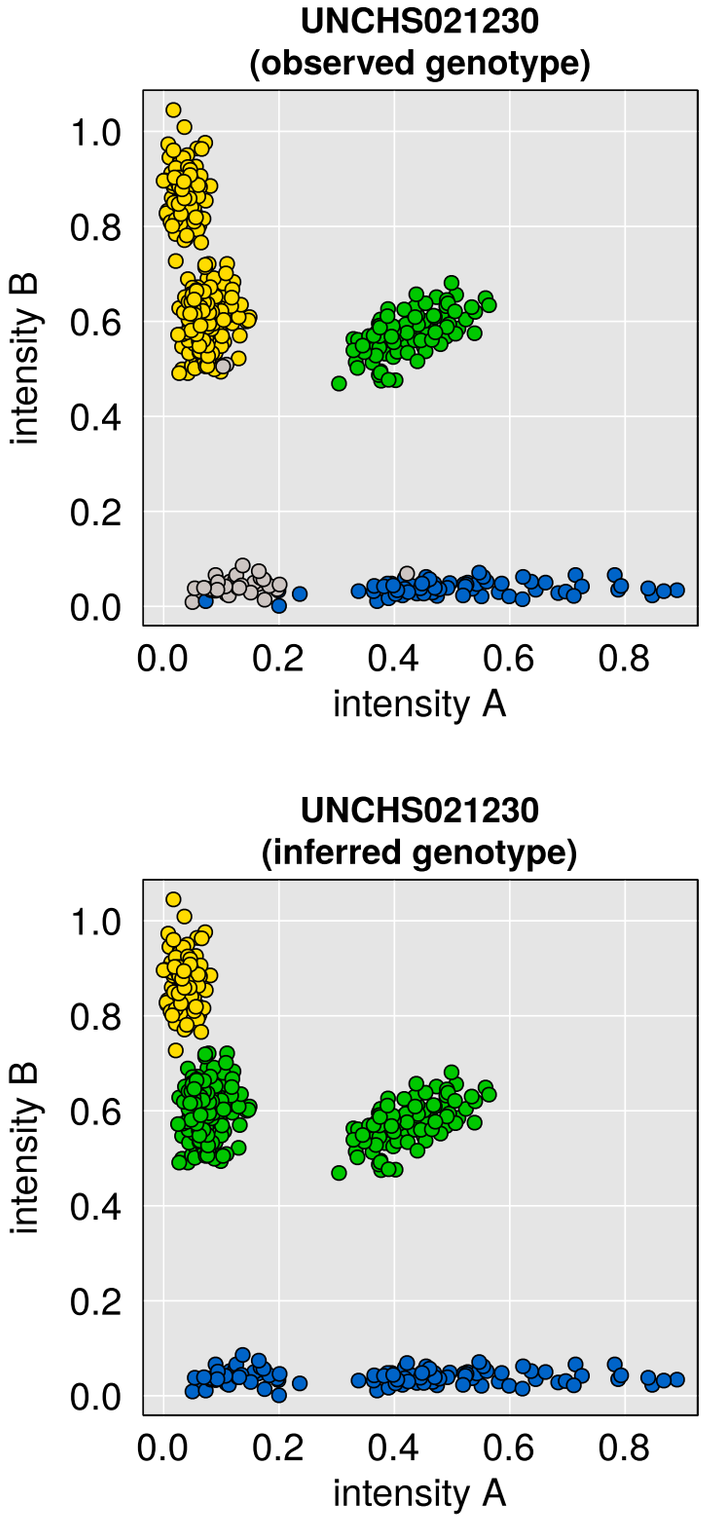
More crap markers

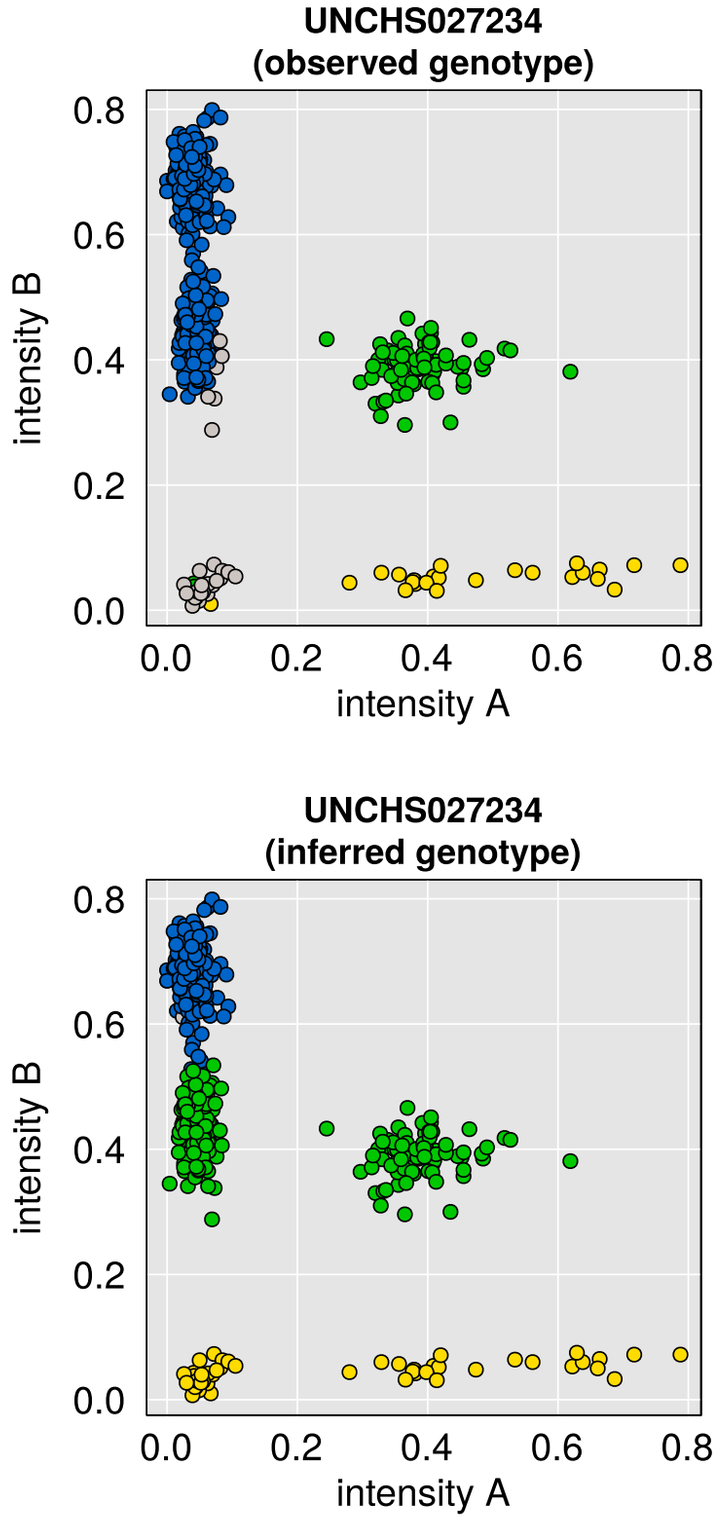






One bad blob


UNCHS021230
(observed genotype)
intensity B
intensity A
0.0
0.2
0.4
0.6
0.8
1.0


UNCHS027234
(observed genotype)
intensity B
intensity A
0.0
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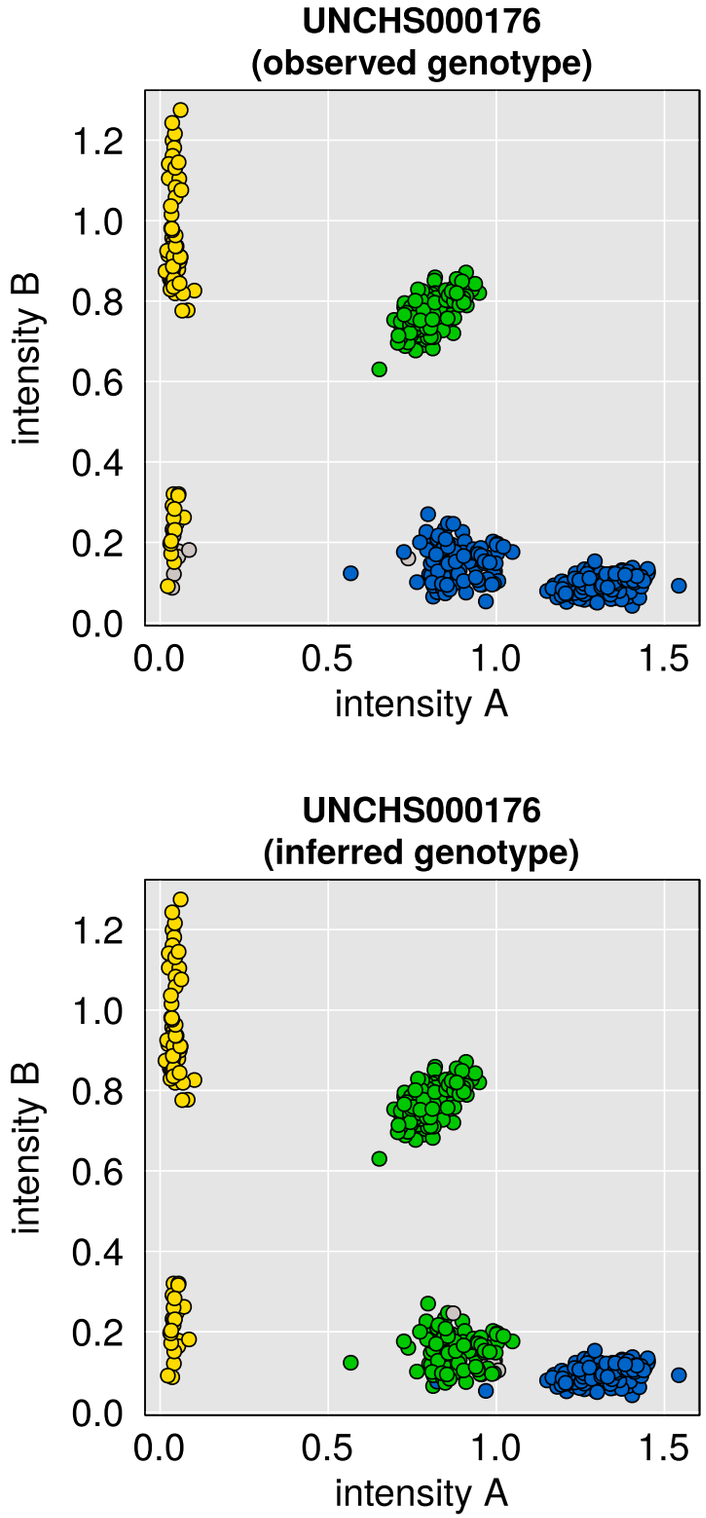
0.4
0.6
0.8


UNCHS000176
(observed genotype)
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intensity A
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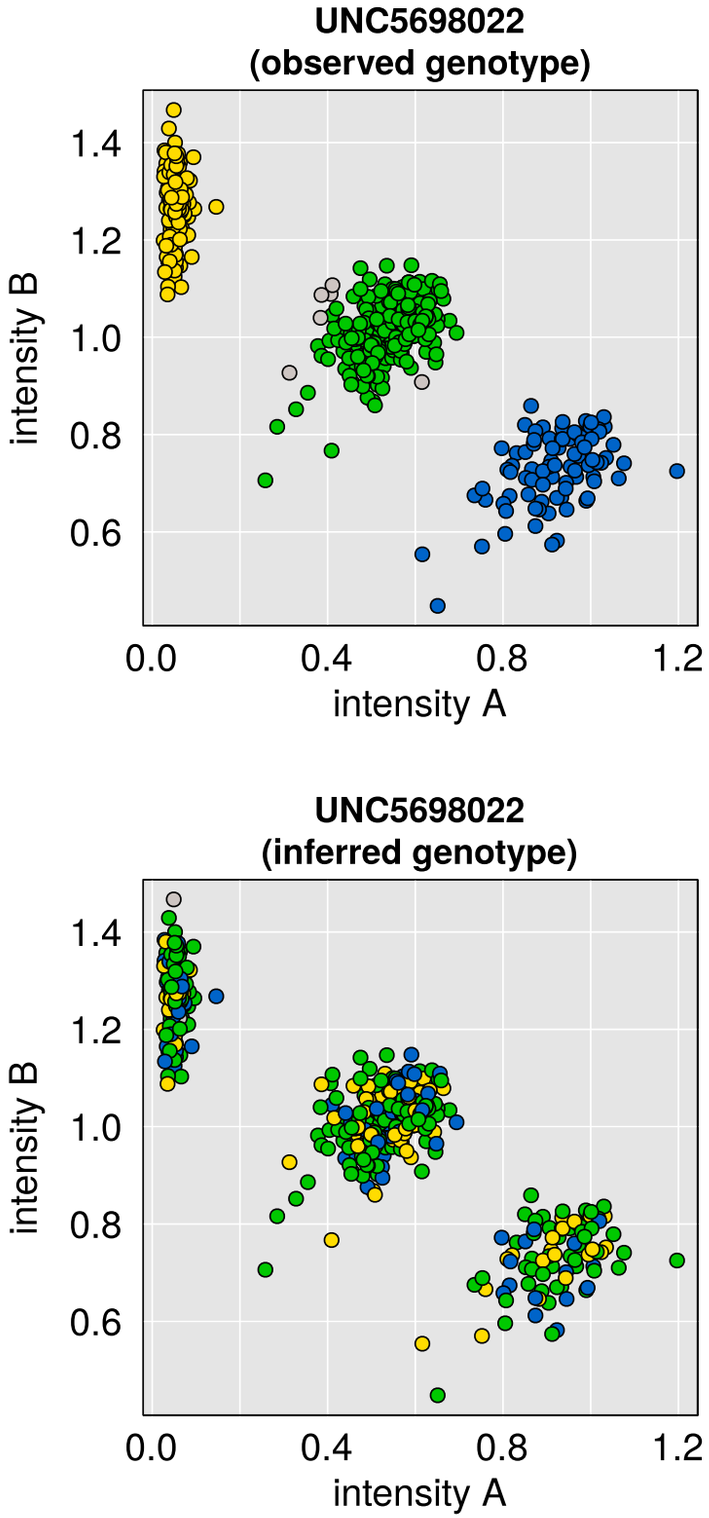
1.0
1.5
0.2
0.4
0.6
0.8
1.0
1.2

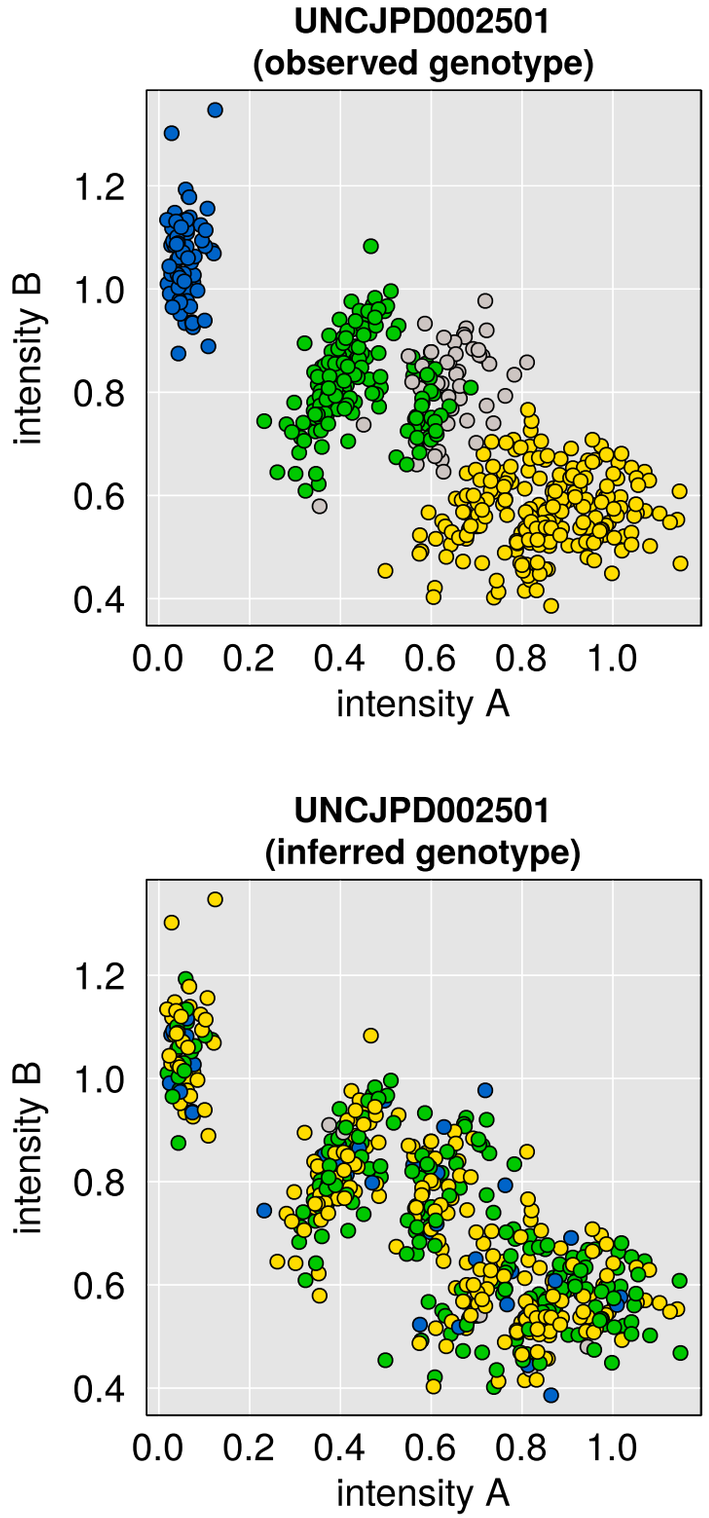
UNCHS021230
(inferred genotype)
intensity B
intensity A
0.0
0.2
0.4
0.6
0.8
1.0

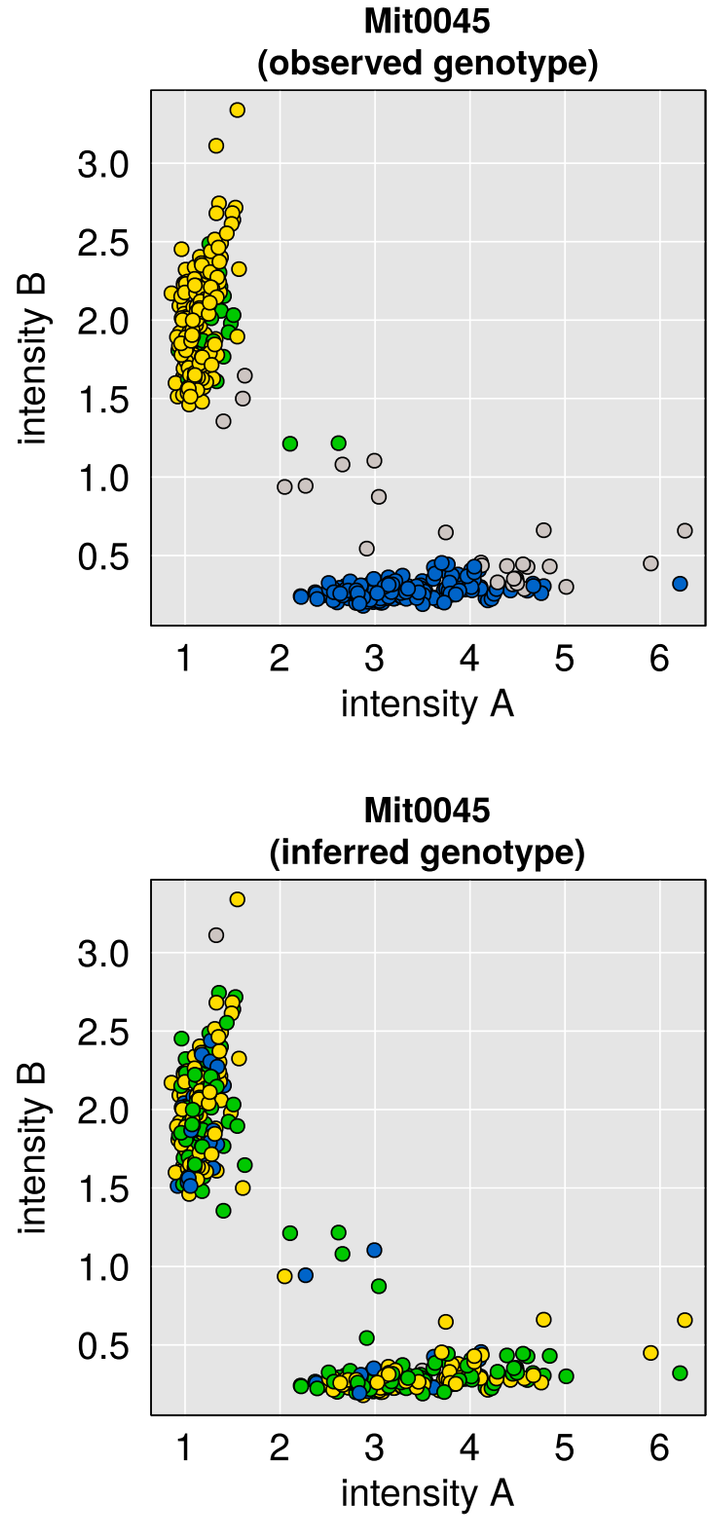
UNCHS027234
(inferred genotype)
intensity B
intensity A
0.0
0.2
0.4
0.6
0.8

UNCHS000176
(inferred genotype)
intensity B
intensity A
0.0
0.5
1.0
1.5
0.2
0.4
0.6
0.8
1.0
1.2

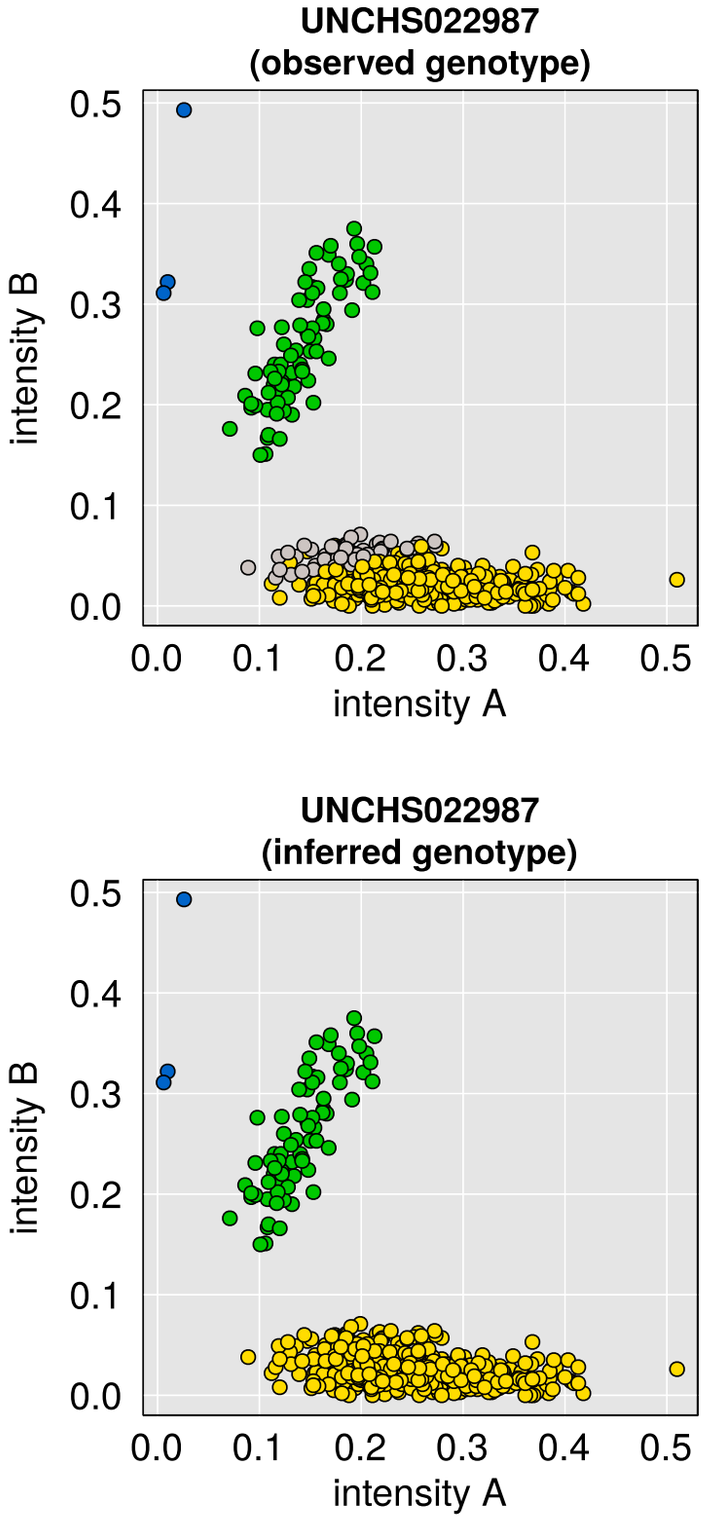
Wrong genomic coordinates

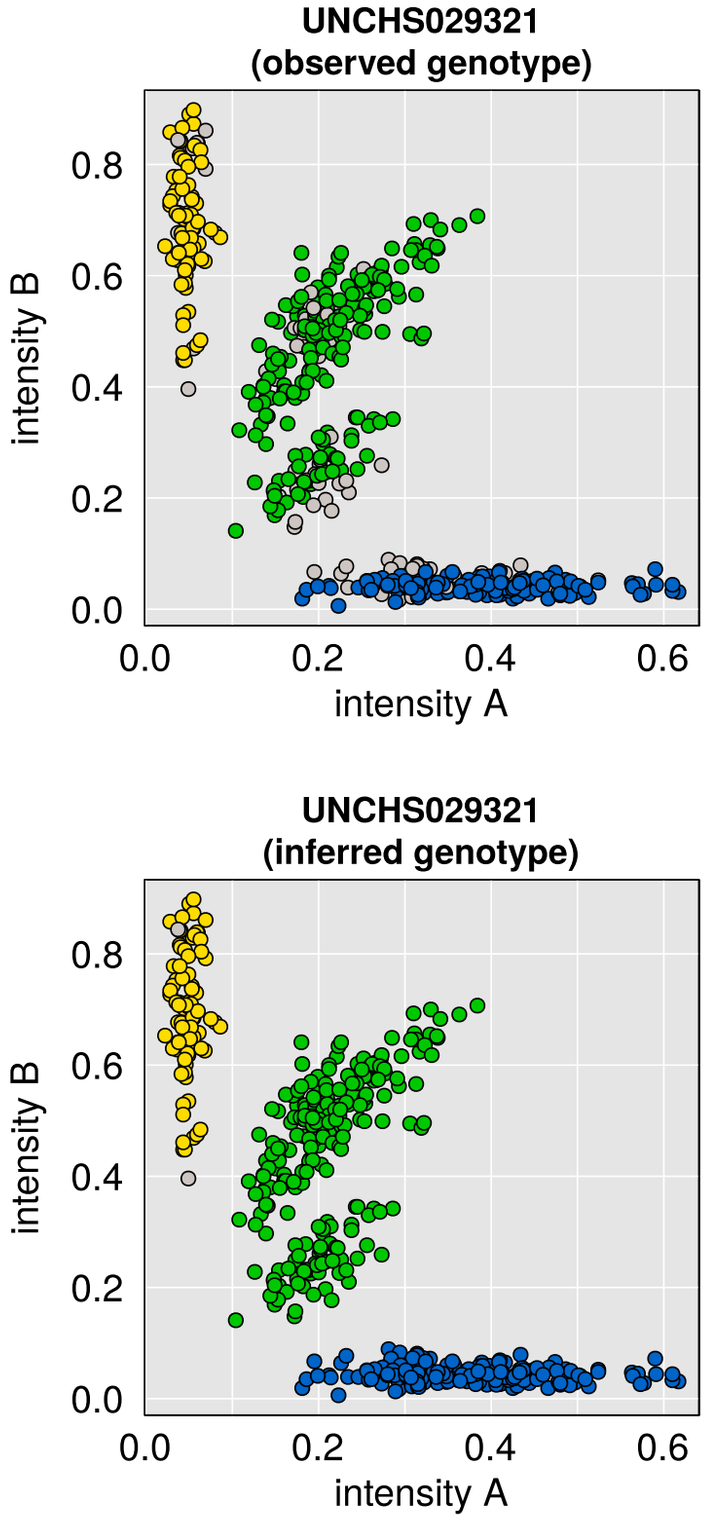


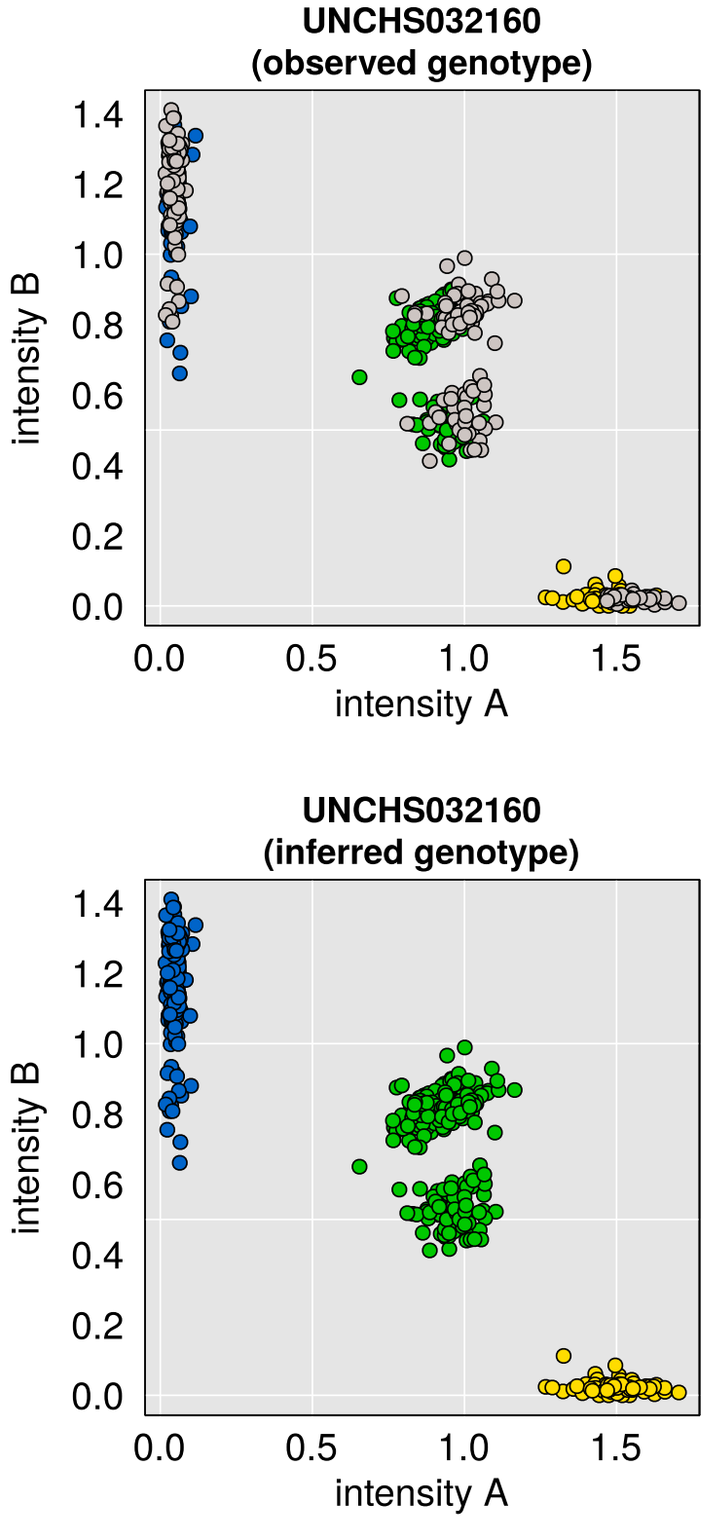




Puzzling no calls

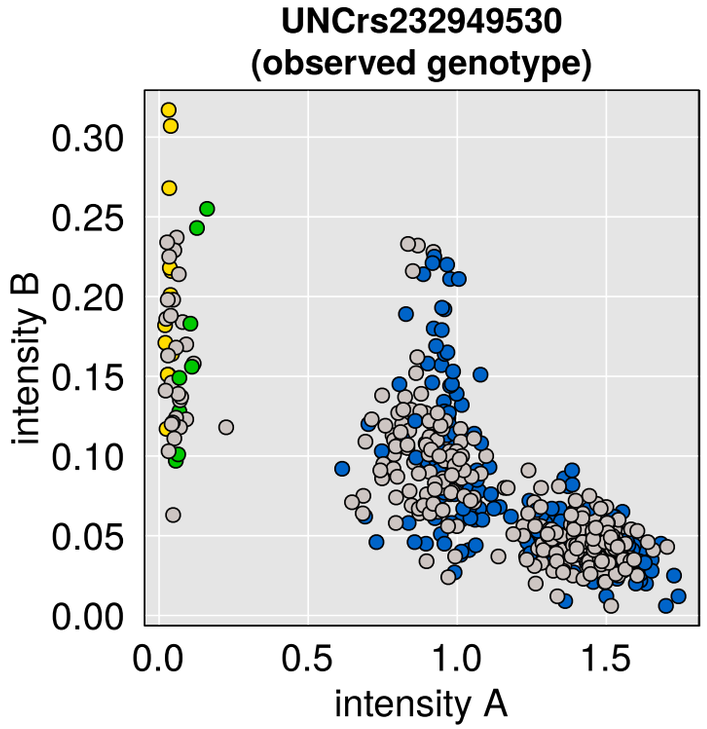


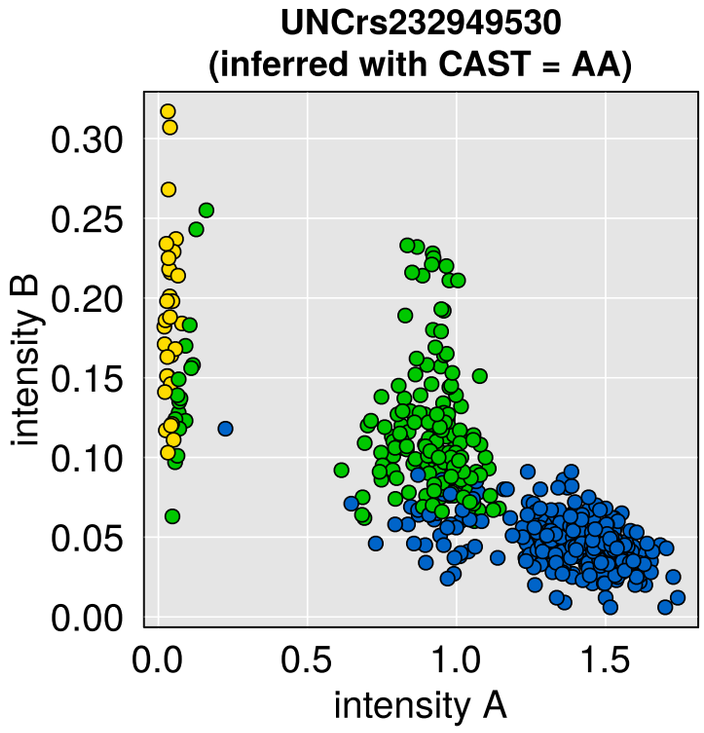


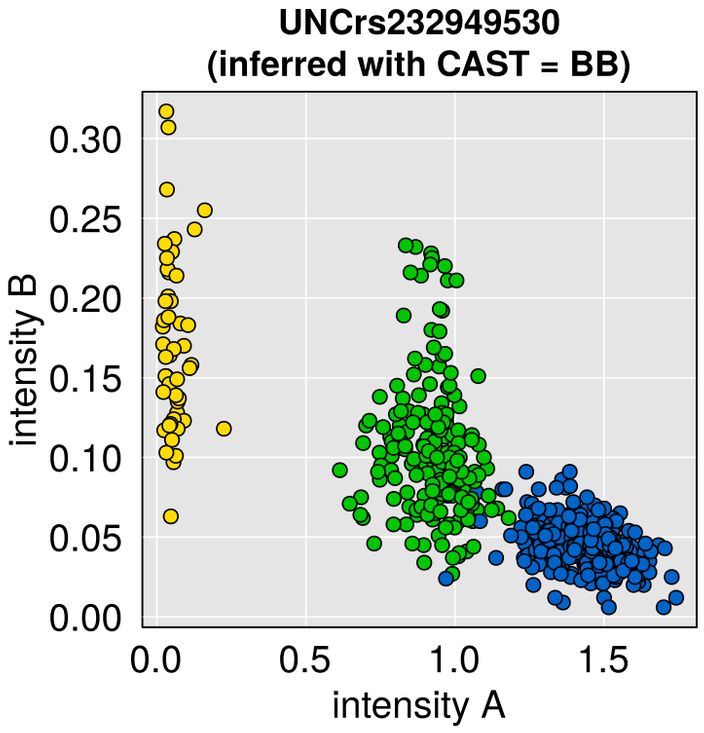


Founder genotyping errors

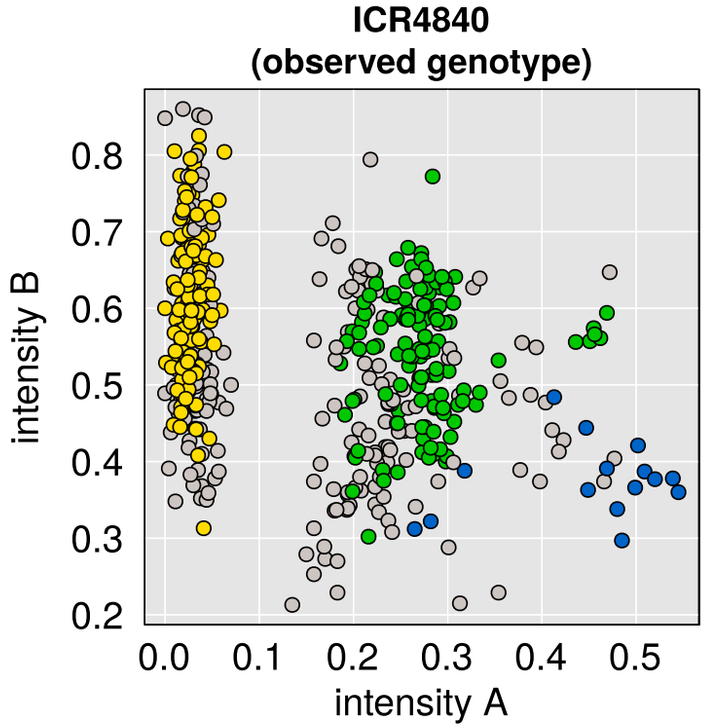
One founder missing

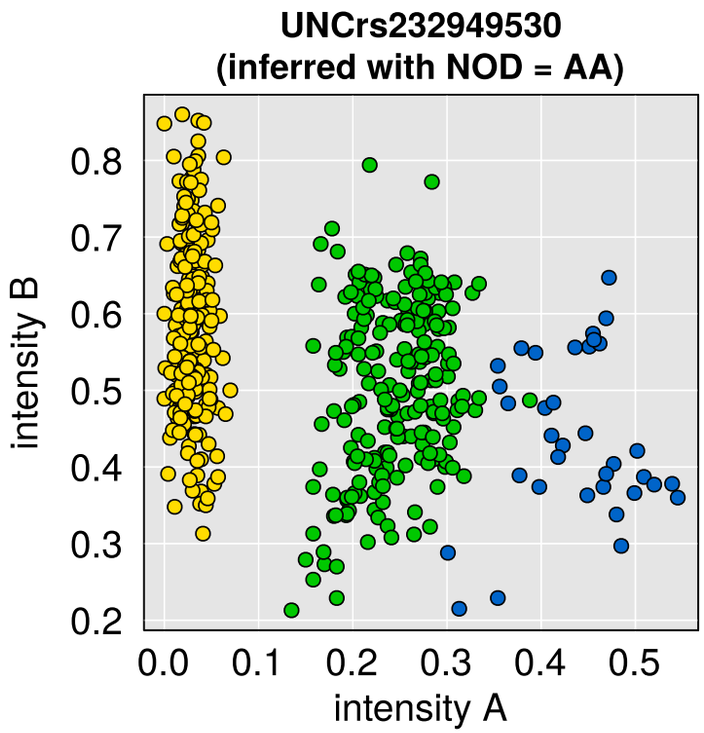


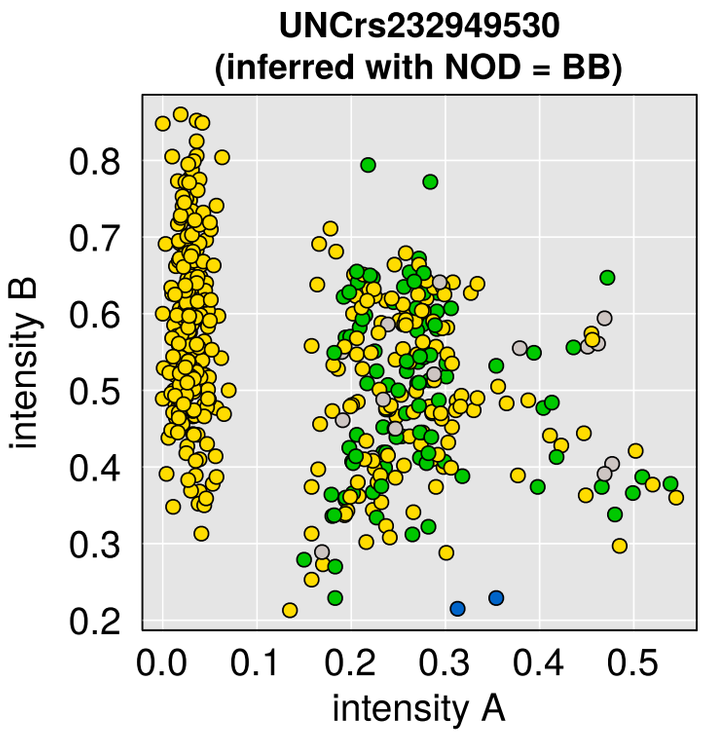




Another case with one founder missing







Summary

- Quality of results depends on quality of data
- Think about what might have gone wrong, and how it might be revealed
- Pulling out the bad samples is the most important thing
- Sex swaps: look at array intensities
- Look for sample duplicates, and if possible sample mix-ups
- Samples: missing data, array intensities, crossovers, errors
- Markers: lots of reasons for the bad ones

Acknowledgments

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