

***Melitaea britomartis* population structure**

Intermediate report

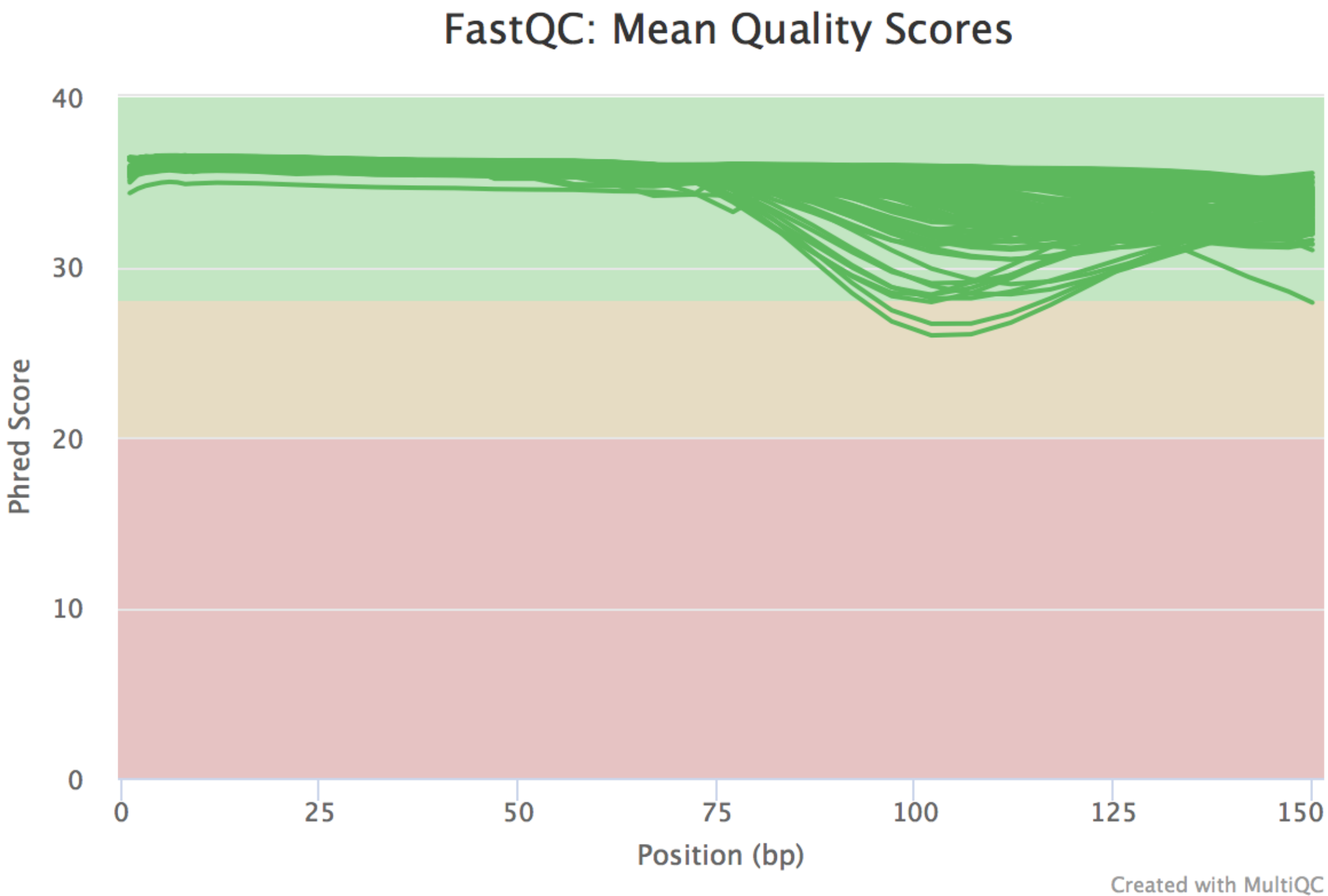
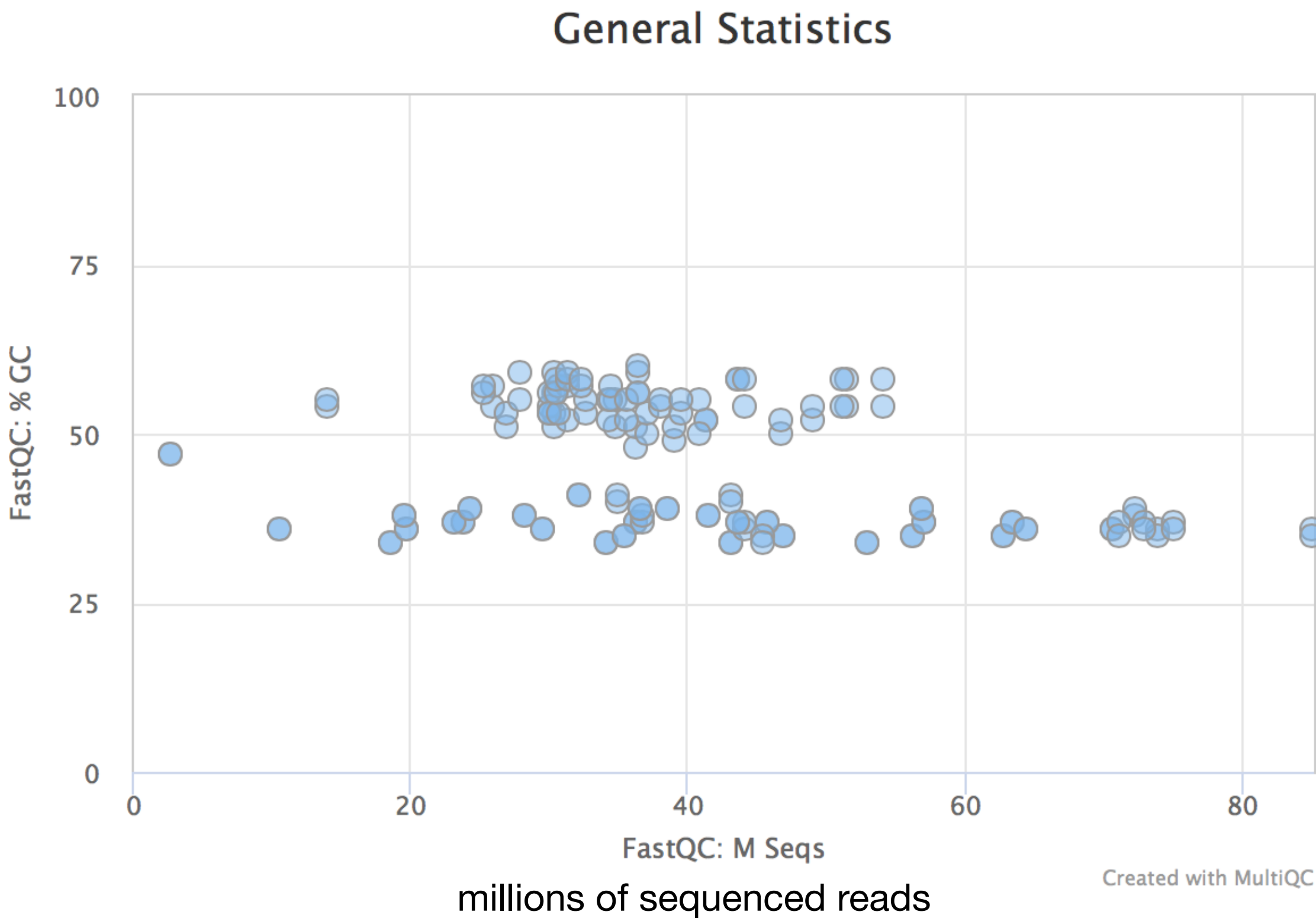
- Raw sequencing data QC
- Read mapping QC
- Variant calling summary
- Basic population structure (PCA)
- Mitochondrial tree (COI)



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Raw sequencing data QC

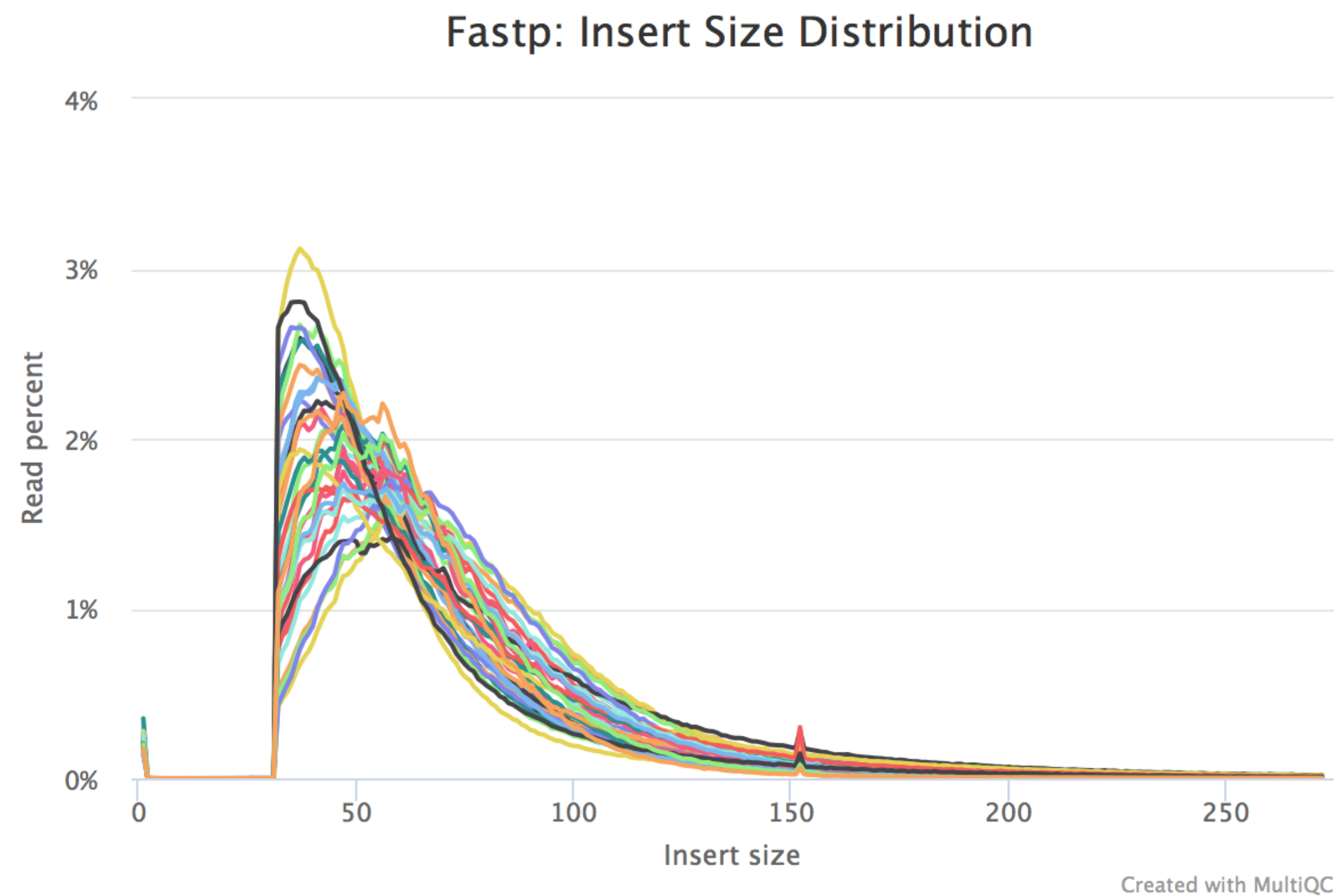
10-85M (single outlier 2.5M) of sequences reads obtained, most of the high quality
GC content in large proportion of the samples appeared abnormal (37% is expected)



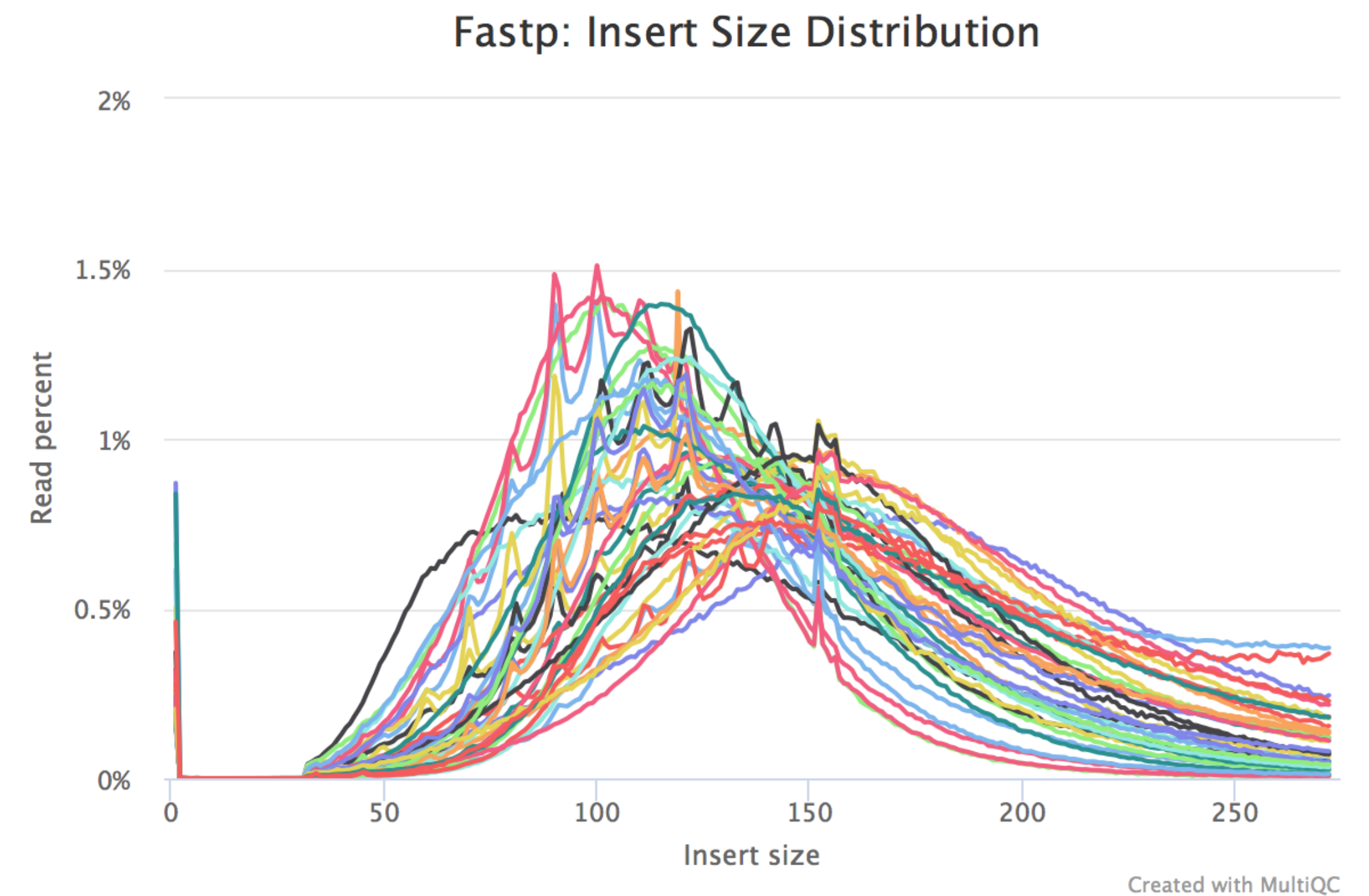
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Read mapping QC

Read mapping revealed difference in insert sizes (length of sequences fragments) between historical and contemporary samples



“Historical” samples
input GC > 50%

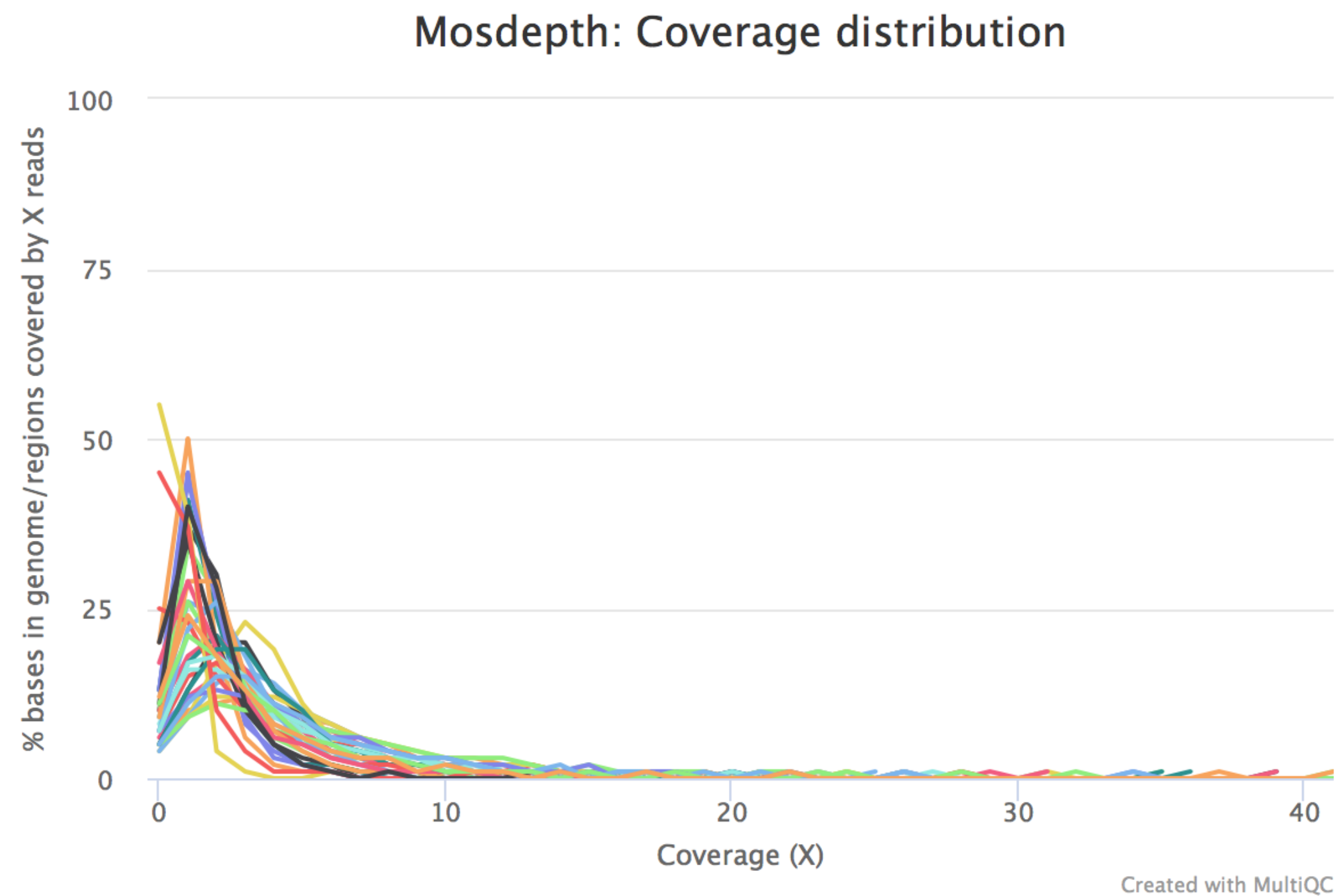


“Contemporary” samples
input GC < 50%

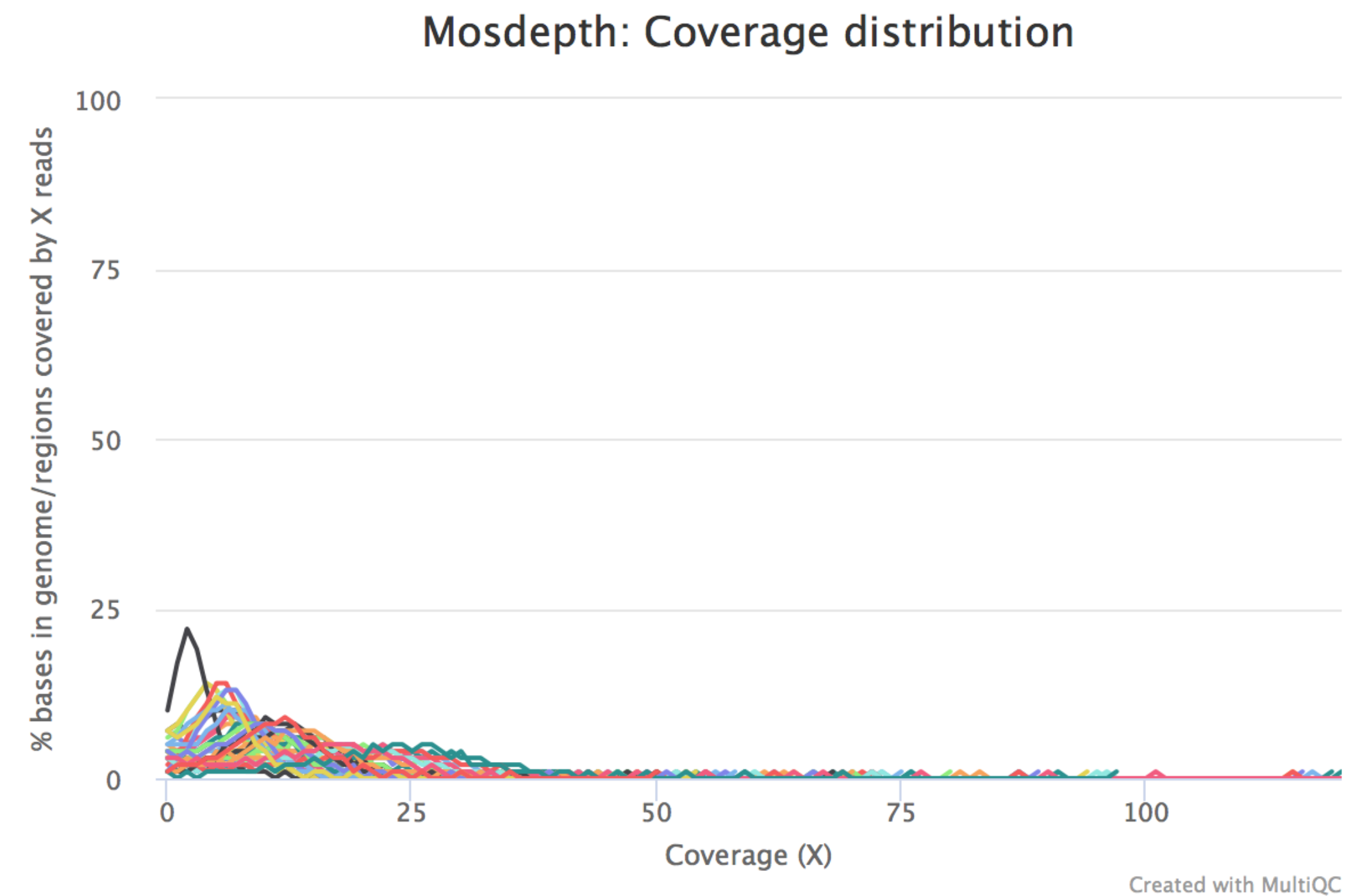
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Read mapping QC

Historical samples showed lower coverage / mapping depth, variant calling strategy adjusted to accommodate



“Historical” samples
input GC > 50%



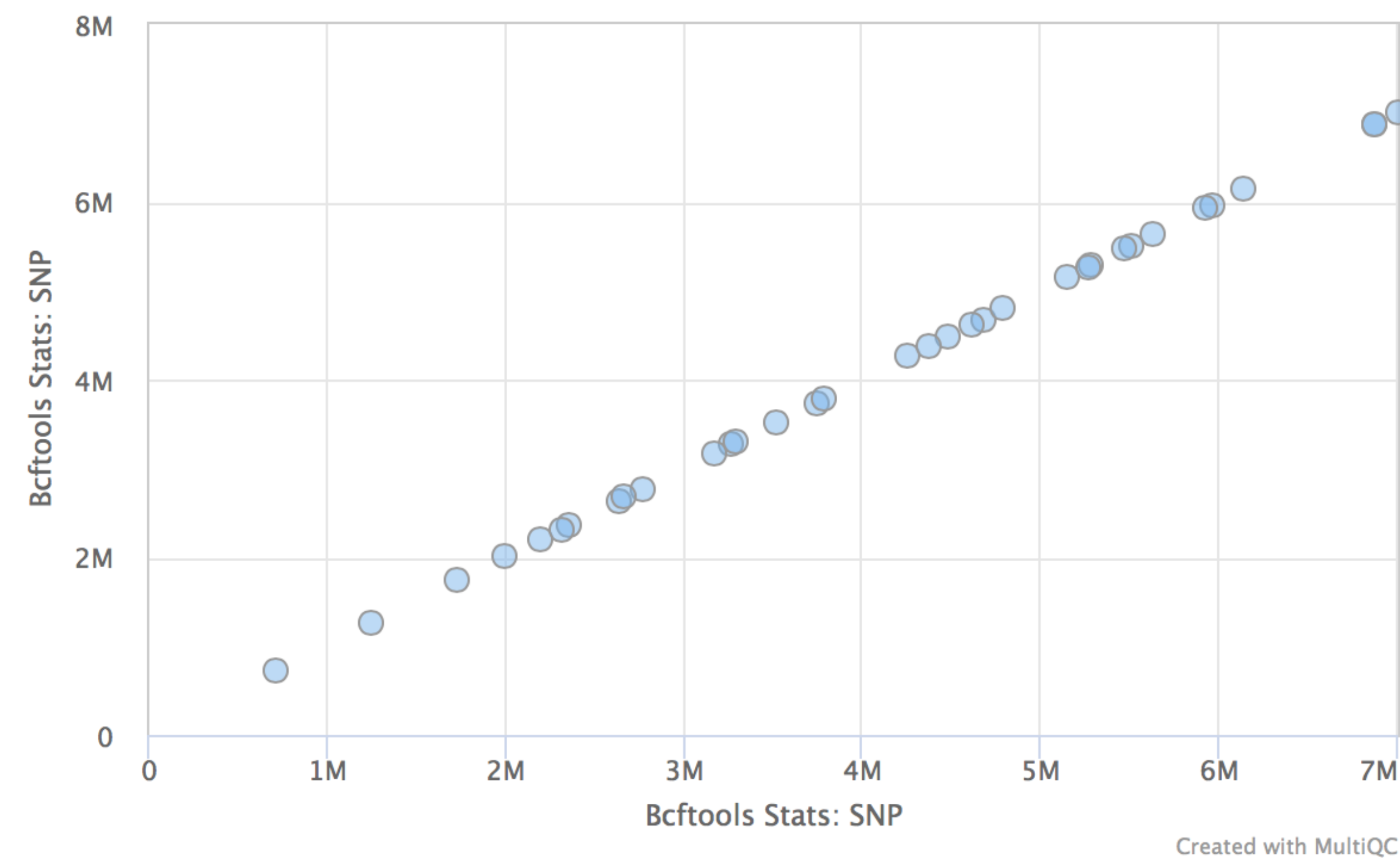
“Contemporary” samples
input GC < 50%

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Variant calling QC

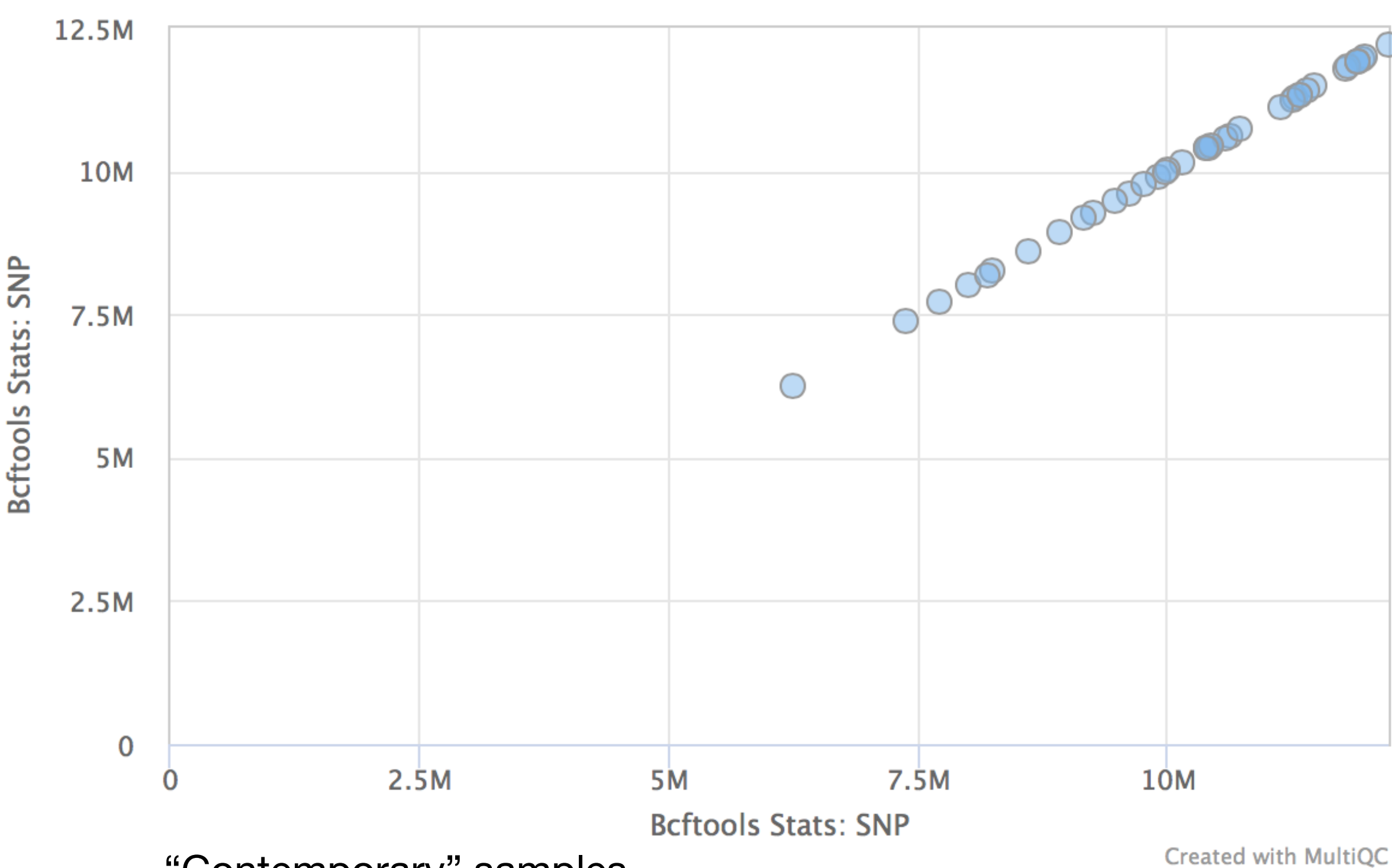
At the first step samples are called individually (freeBayes), sufficient number of SNPs recovered

General Statistics



“Historical” samples
input GC > 50%

General Statistics



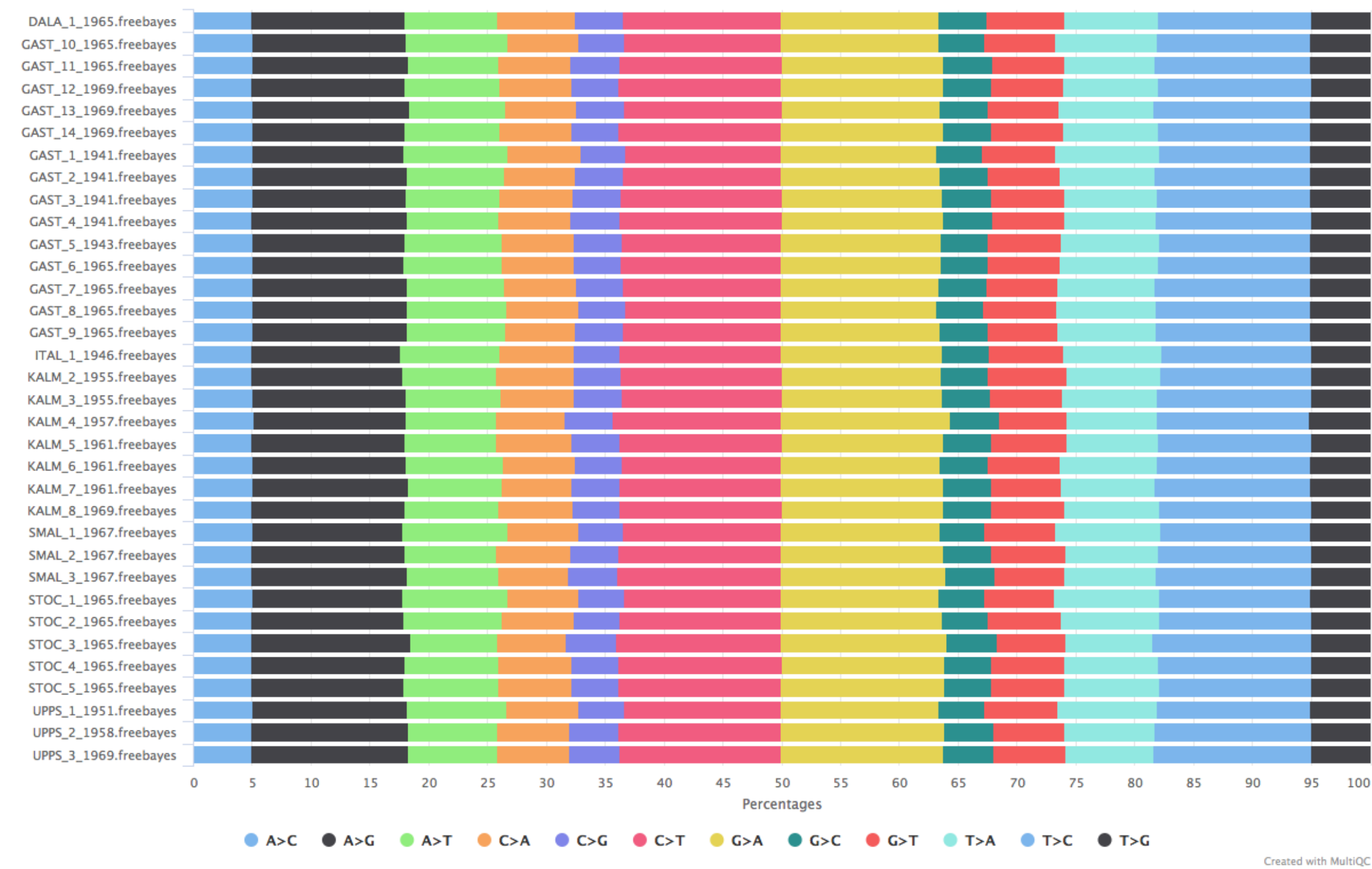
“Contemporary” samples
input GC < 50%

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Variant calling QC

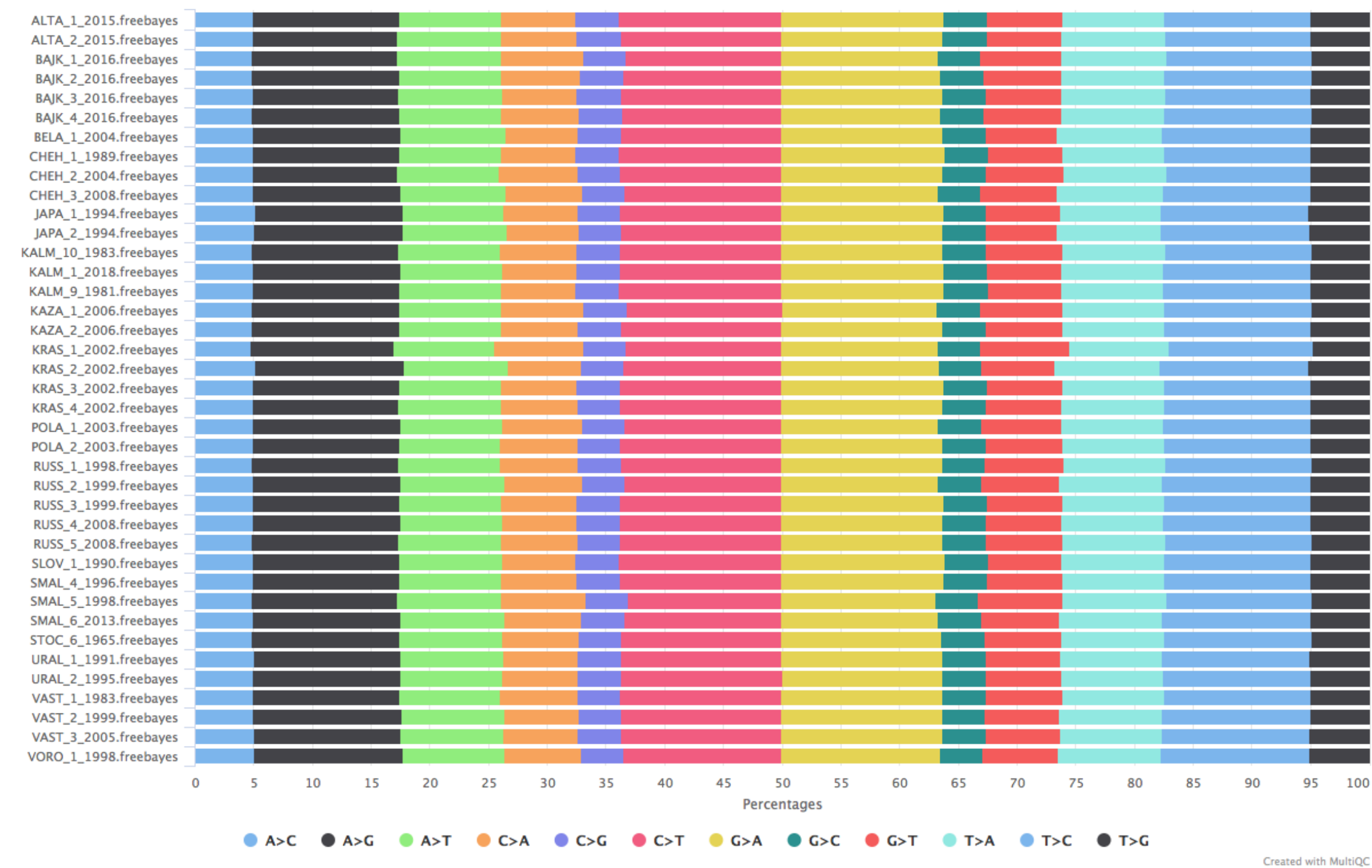
Conclusion: distribution of substitution types doesn't indicate strong signatures of deamination

Bcftools Stats: Substitutions



“Historical” samples
input GC > 50%

Bcftools Stats: Substitutions



“Contemporary” samples
input GC < 50%

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Variant calling QC

Joined variant calling performed with settings from GenErode* pipeline

Stringent filtering applied:

Quality filter: $MQ < 30$

Missingness filter: variants are required to be present in at least 70 individuals

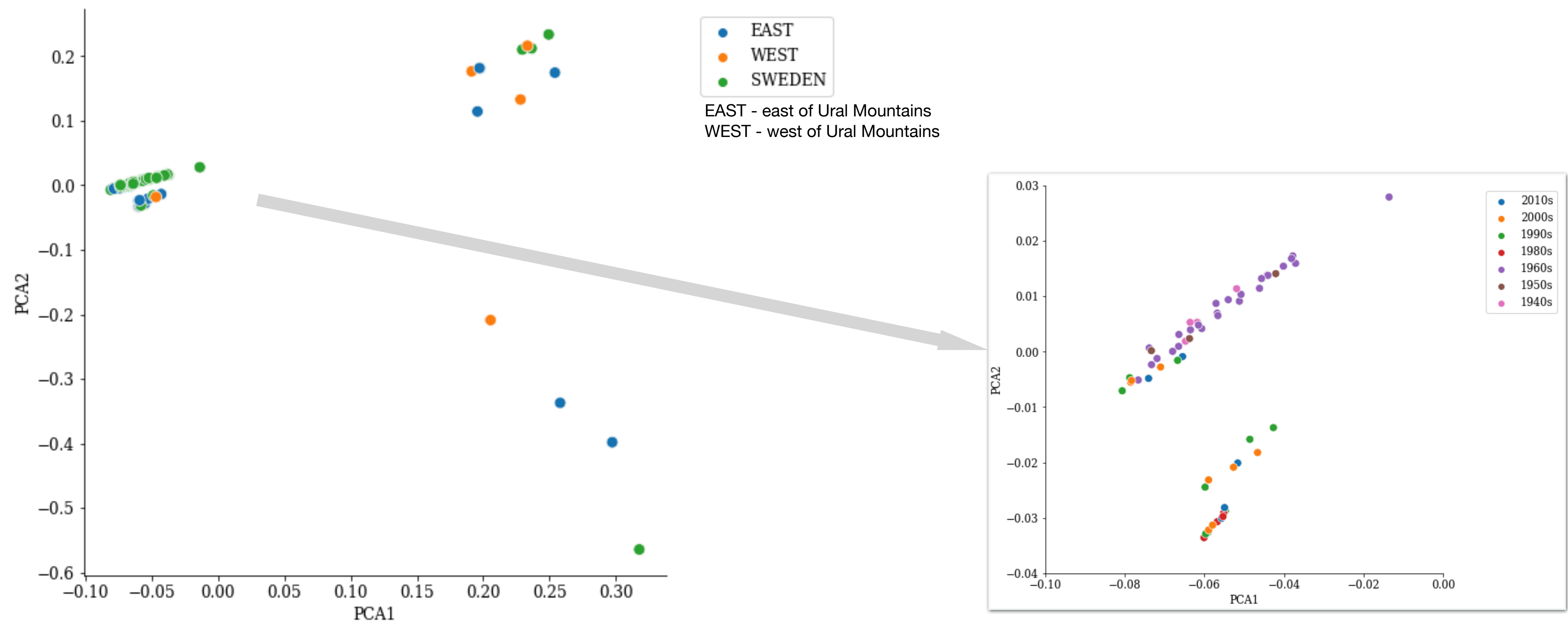
Total number of SNPs: **220,333**

*Kutschera, V.E., Kierczak, M., van der Valk, T. et al. GenErode: a bioinformatics pipeline to investigate genome erosion in endangered and extinct species. BMC Bioinformatics 23, 228 (2022). <https://doi.org/10.1186/s12859-022-04757-0>

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Population structure analysis

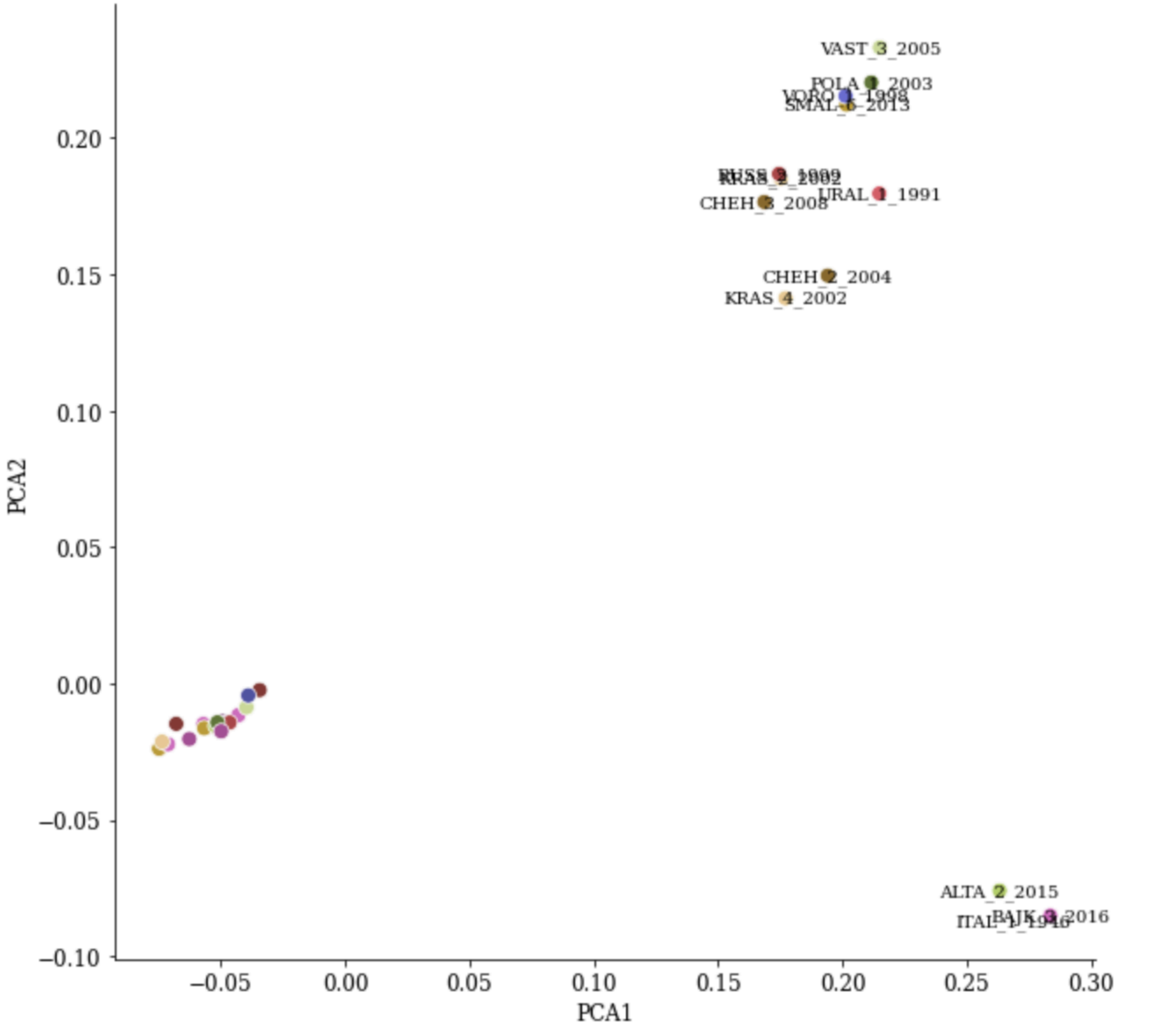
Basic principle component analysis (PCA) revealed unexpected grouping of samples based on geography and sampling year



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Population structure analysis

Excluding mtDNA, sex-chromosomes and lower quality (historical) samples did not change overall structure of the plot



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Mitochondrial tree (COI)

PCA outlier samples grouped with sister species

