

1 Supplemental Figures

1.1 SUMO1

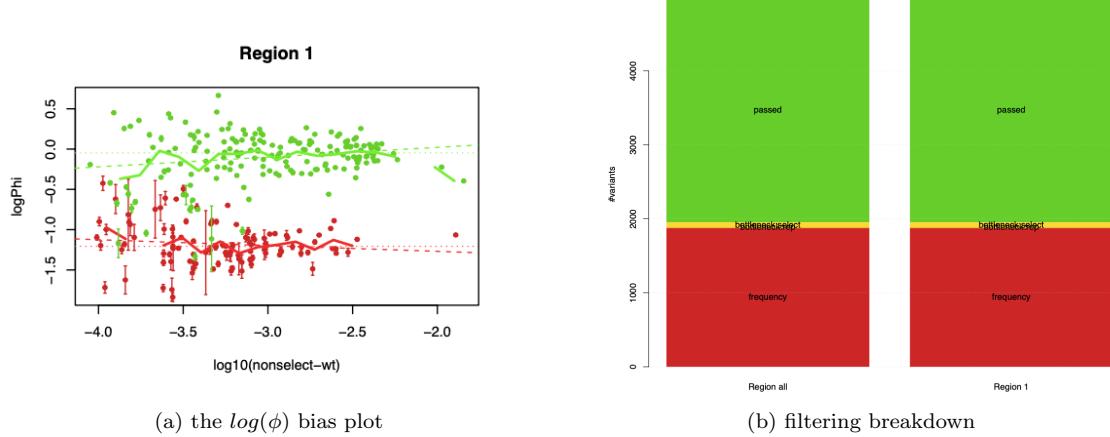


Figure 1: The $\log(\phi)$ bias plot and the filtering breakdown bar plot for SUMO1 map. The $\log(\phi)$ bias plot illustrates how the $\log(\phi)$ values of synonymous(green) and nonsense(red) variants changing as the read frequency cutoff increasing. The filtering breakdown plot show the number of SUMO1 variants in region 1 that were subjected to individual filters, including the frequency filter (excludes variants that have low marginal frequencies); the bottleneck:rep filter excludes variants have low correlation with their replicates; and the bottleneck:select filter excludes variants that have frequency drop-out in the selection condition.

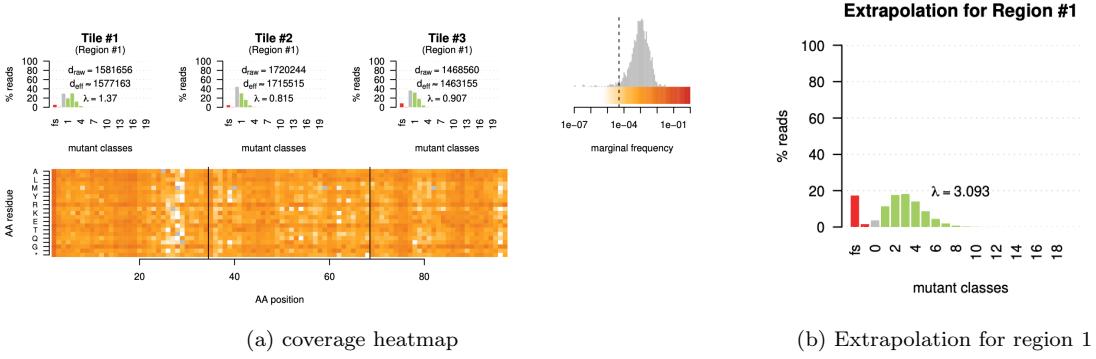


Figure 2: The coverage heatmap and an extrapolation of the number of variants per clone for Region 1 for the re-calculated SUMO1 map. The heatmap shows marginal frequencies for each amino acid change. λ refers to the mean of the best fitting Poisson distribution of the number of variants observed per read.

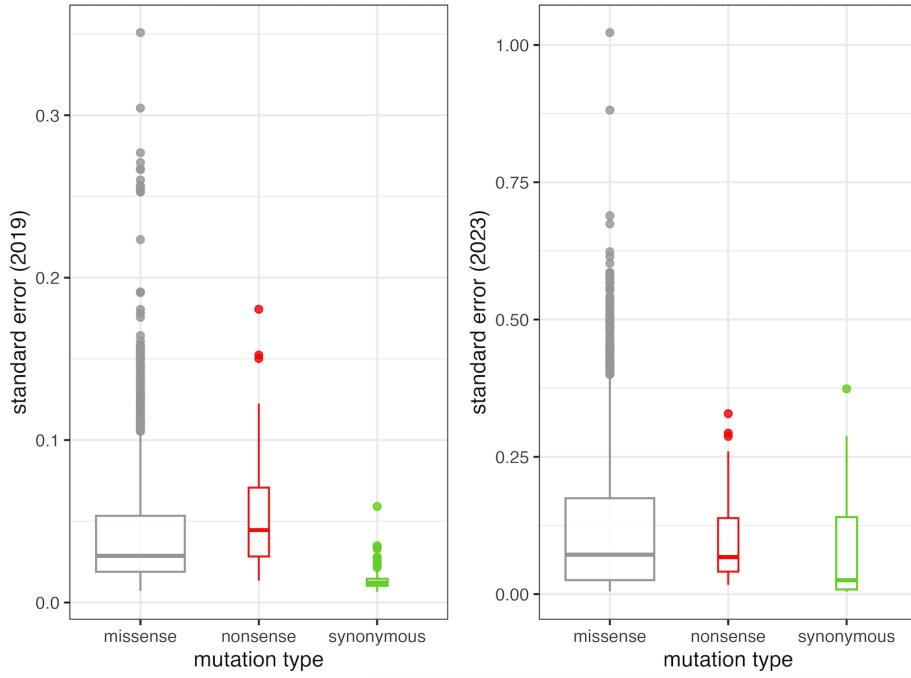
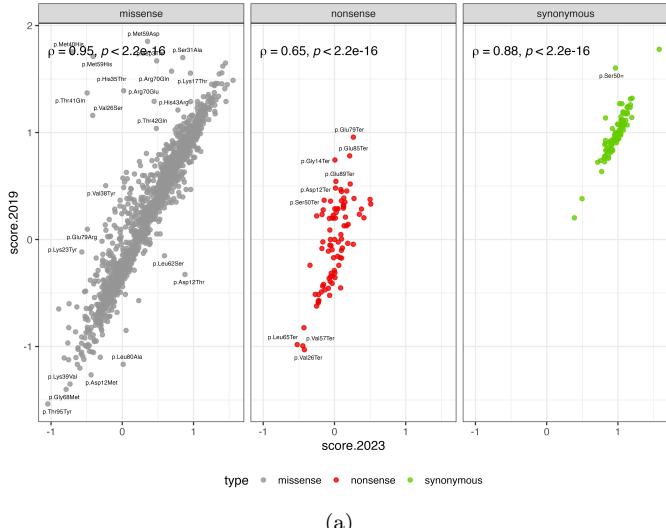
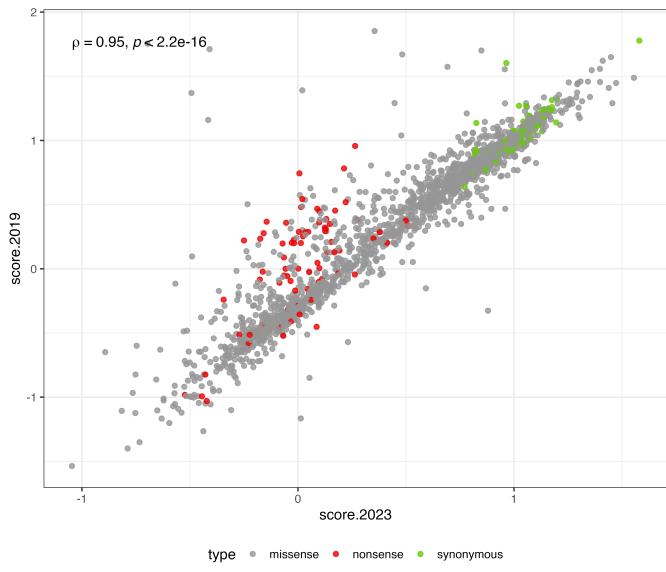


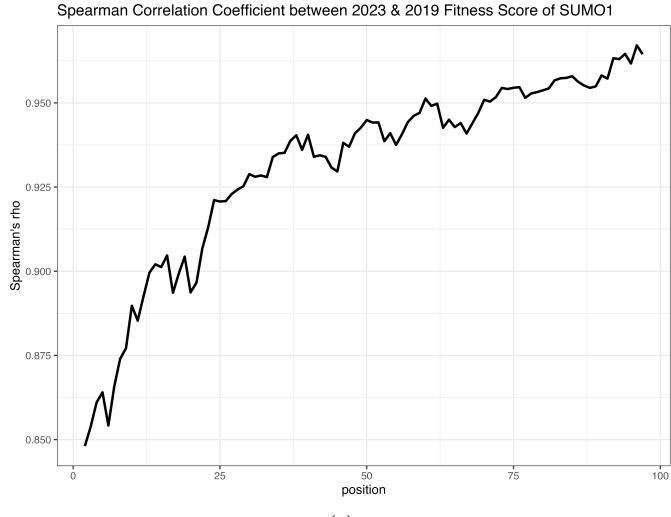
Figure 3: The distribution of standard error of fitness scores produced by the Legacy (2019) Tileseq pipelines and the TileseqPro (2023) pipelines.



(a)



(b)



(c)

Figure 4: Correlation between TileseqPro and Legacy scores. (a) and (b) show scatter plots for the two versions of fitness scores of SUMO1 with missense variants in gray, nonsense variants in red and synonymous variants in green, (c) Moving window analysis of Spearman's ρ between new and old scores along amino acid position.

1.2 CALM1

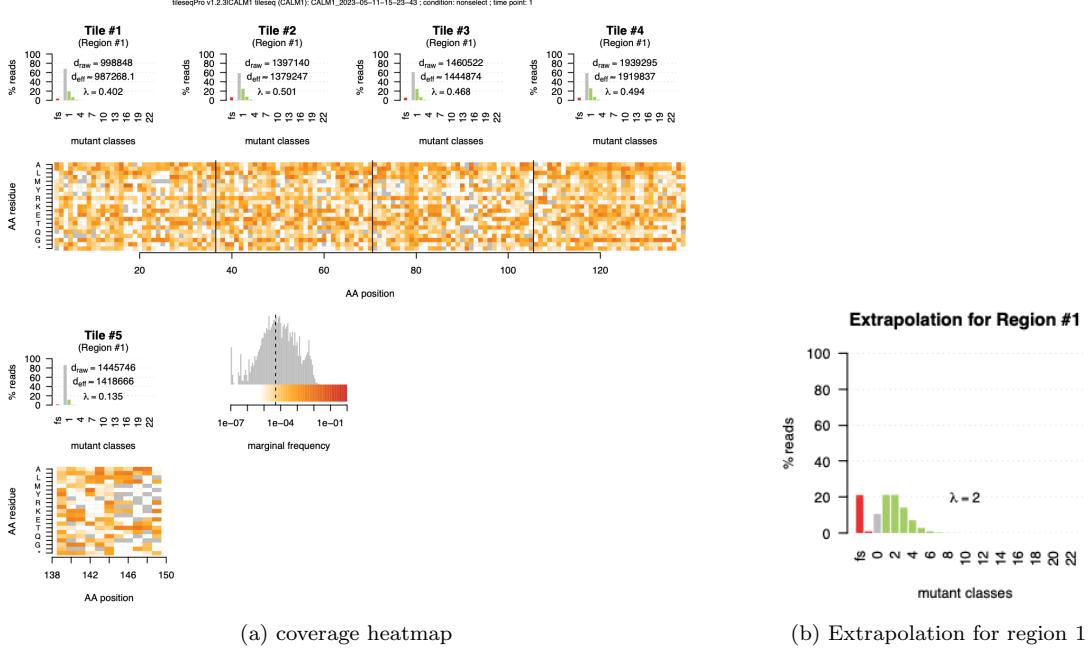


Figure 5: The coverage heatmap and an extrapolation of the number of variants per clone for Region 1 for the re-calculated CALM1 map. The heatmap shows marginal frequencies for each amino acid change. Lambda refers to the mean of the best fitting Poisson distribution of the number of variants observed per read.

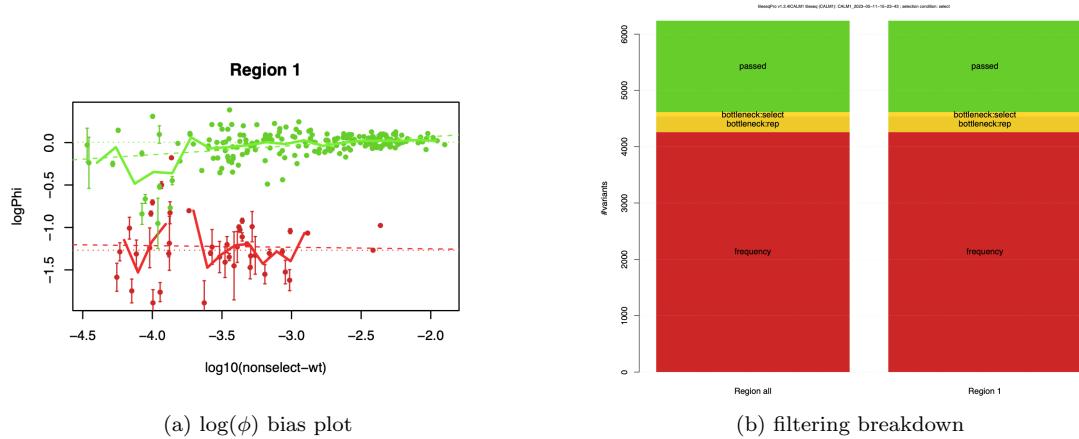


Figure 6: The $\log(\phi)$ bias plot and the filtering breakdown bar plot for CALM1 map. The $\log(\phi)$ bias plot illustrates how the $\log(\phi)$ values of synonymous(green) and nonsense(red) variants changing as the read frequency cutoff increasing. The filtering breakdown plot show the number of CALM1 variants in region 1 that were subjected to individual filters.

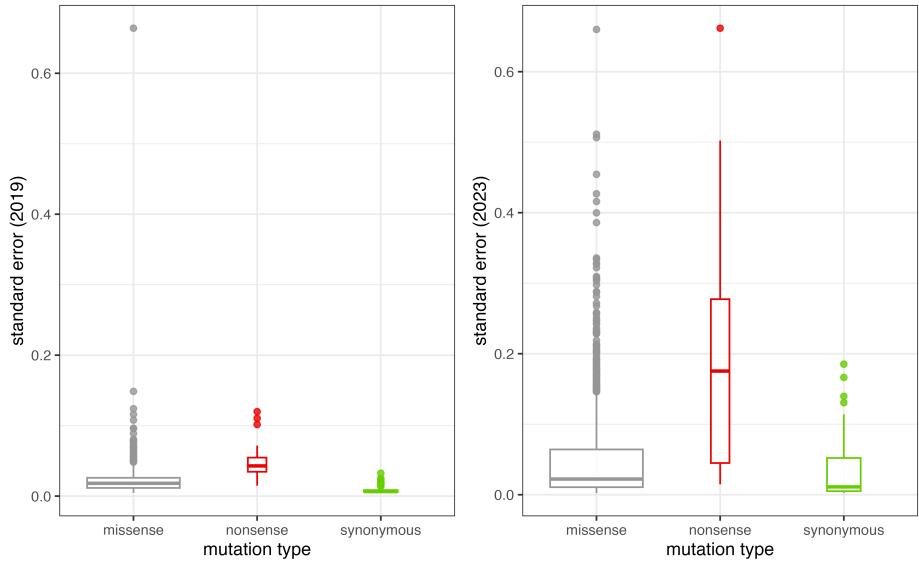
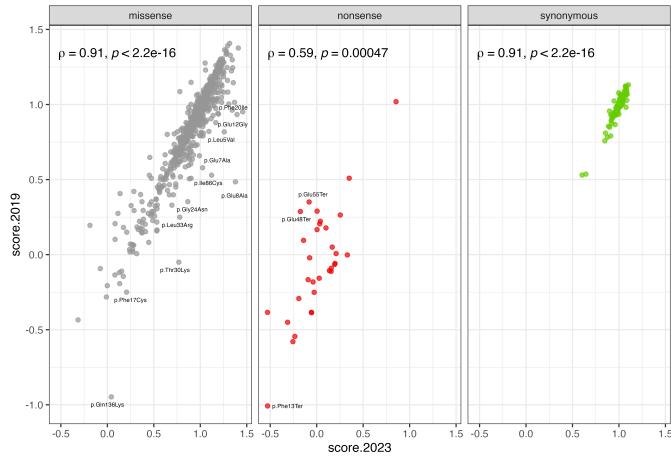
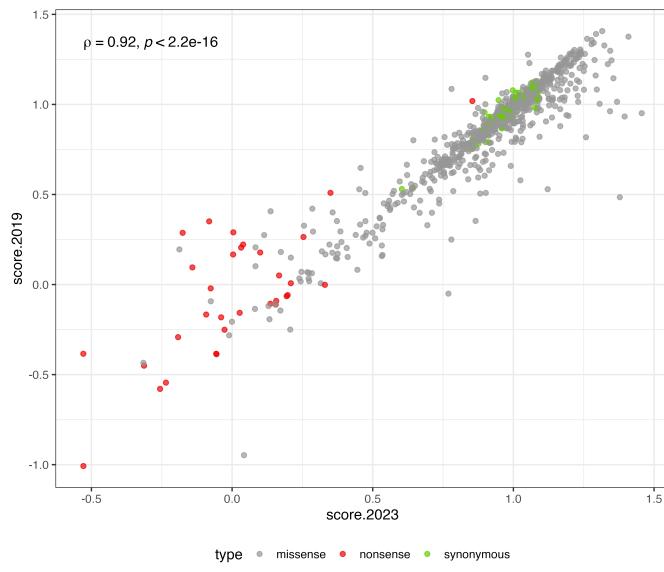


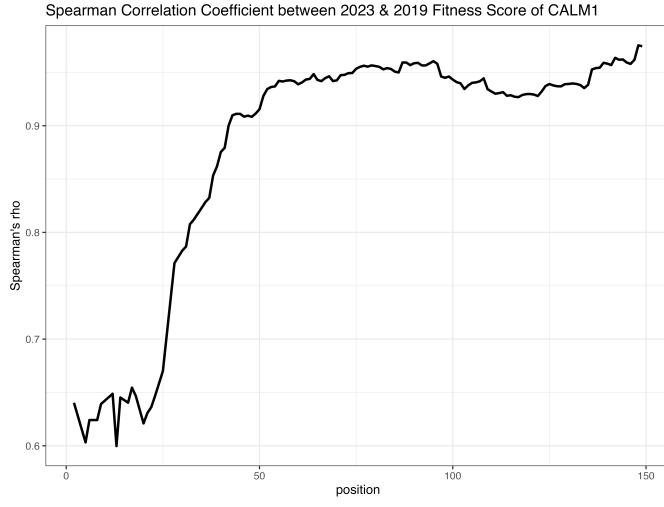
Figure 7: The distribution of standard error of new and old fitness scores (CALM1).



(a)



(b)



(c)

Figure 8: (a) and (b) show the scatter plot of fitness scores for CALM1 generated by TileseqPro and Legacy pipelines, (c) is a line graph describing the change of Spearman's correlation between fitness scores along amino acid positions.

1.3 MTHFR in A222V Background

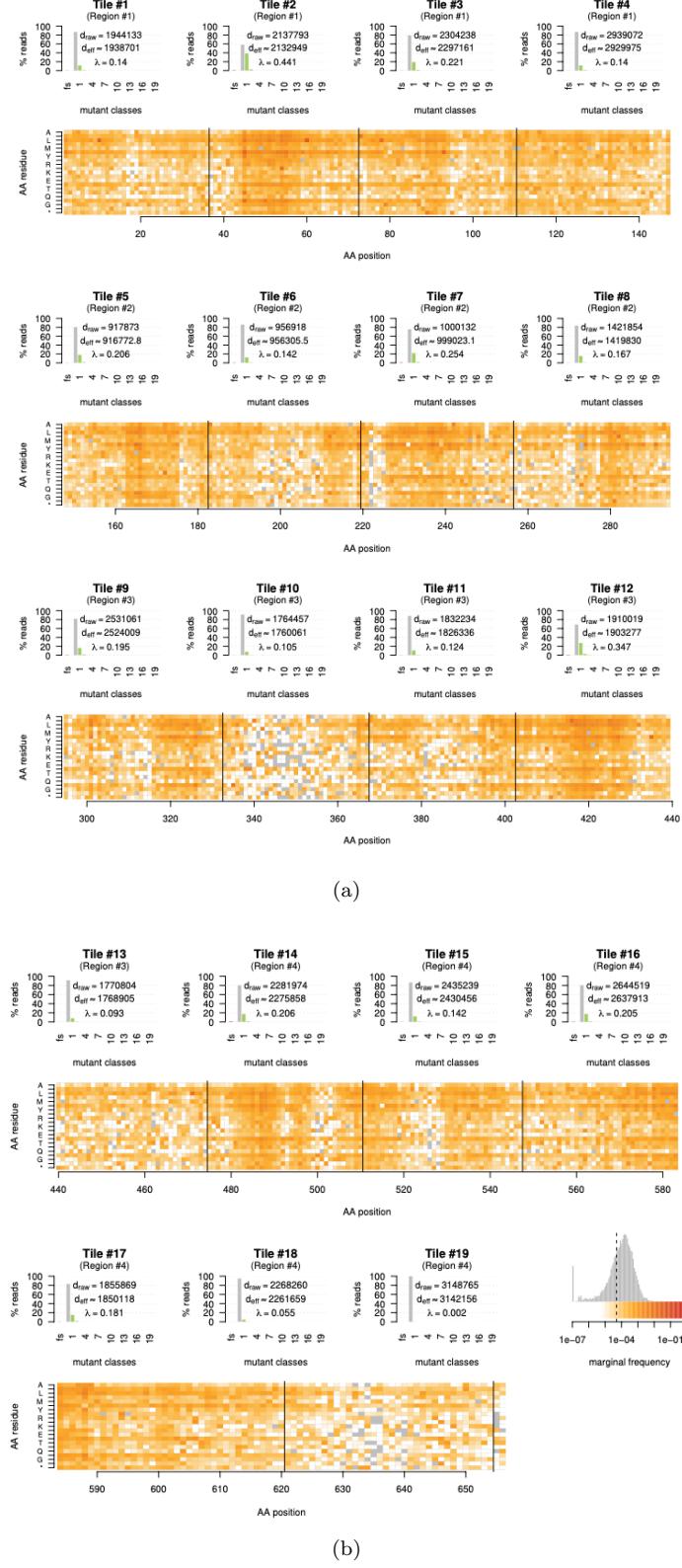


Figure 9: (a) and (b) show the coverage heatmaps for MTHFR in Ala222Val Background.

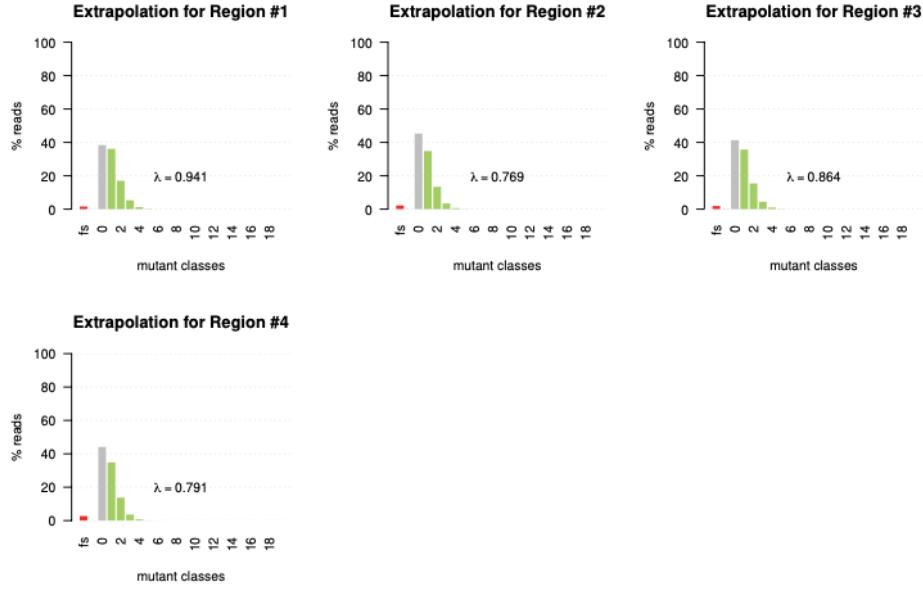


Figure 10: The extrapolation of the number of variants per clone (λ) for Region 1 to 4 for the re-calculated MTHFR in A222V background

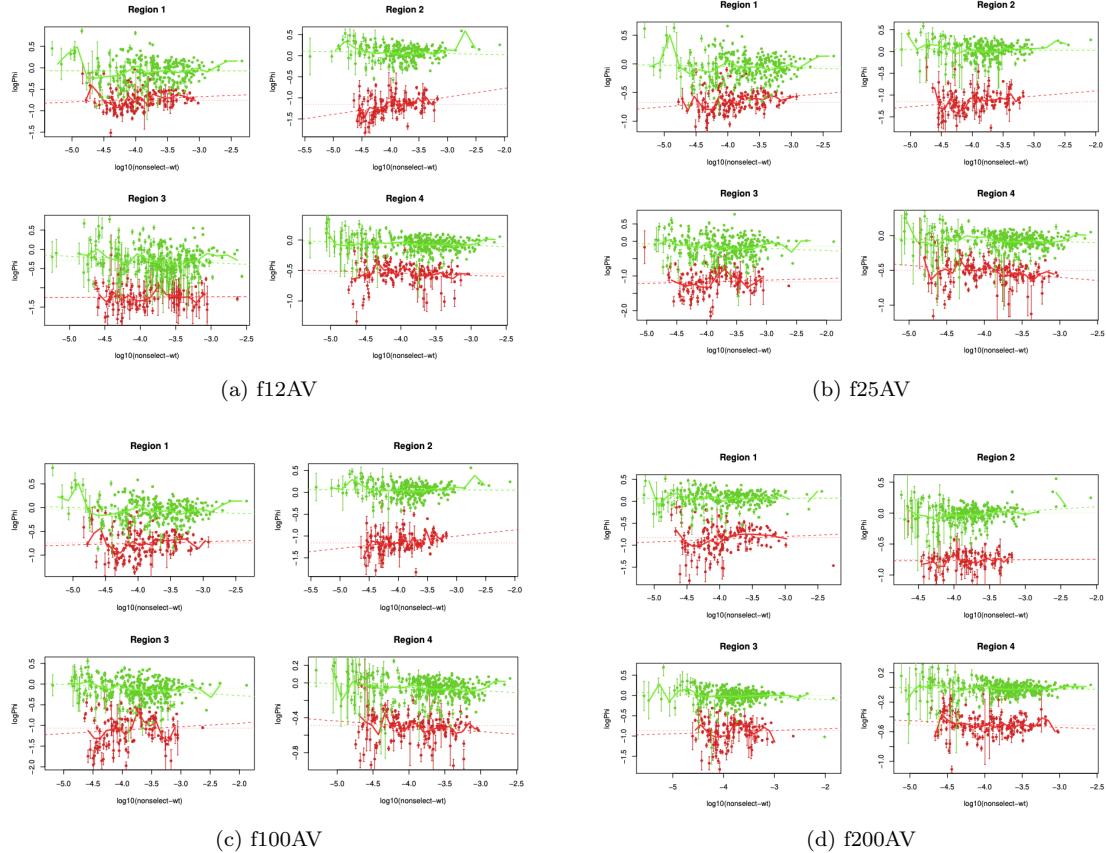


Figure 11: The distribution of nonsense and synonymous MTHFR variants enrichment ratio ($\log(\phi)$) relative to marginal frequency thresholds at different folate concentrations

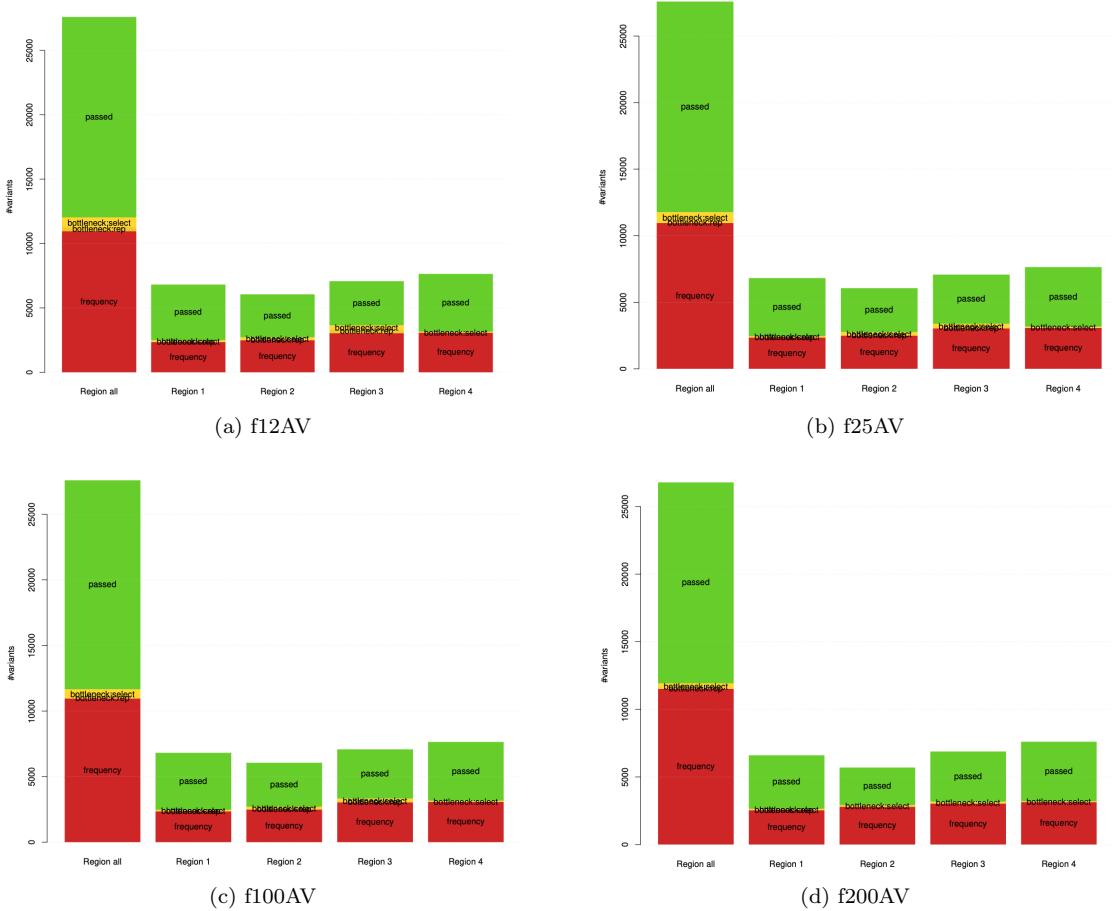
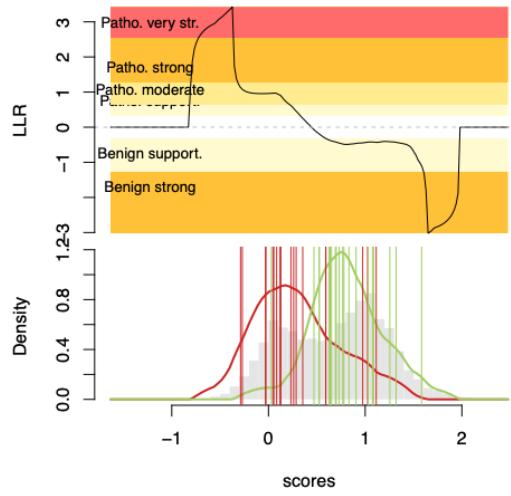
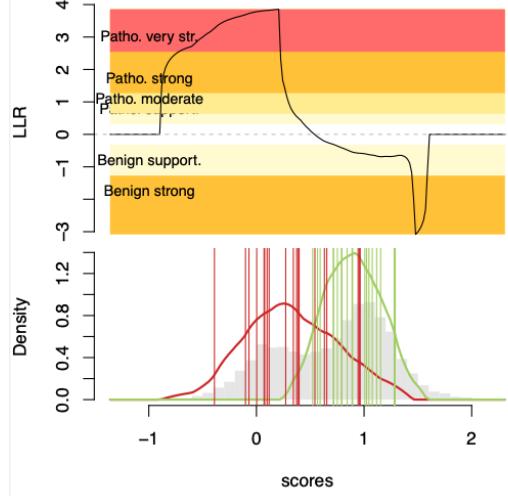


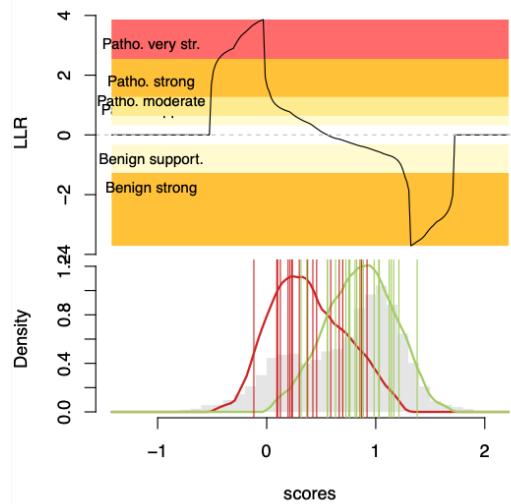
Figure 12: The filtering breakdown for MTHFR variants in A222V background at different folate concentrations.



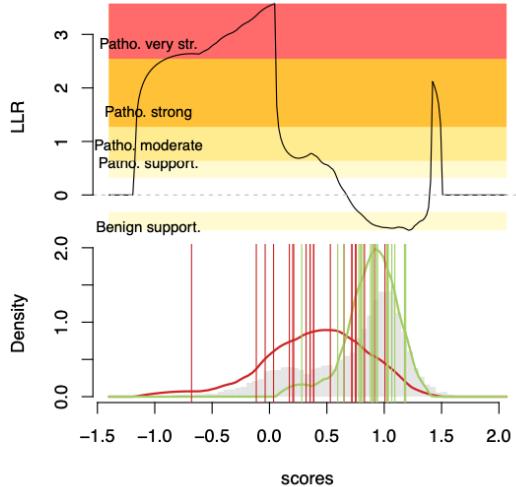
(a) f12AV



(b) f25AV



(c) f100AV



(d) f200AV

Figure 13: LLR of pathogenicity of MTHFR variants in A222V background, separated by different folate concentrations.