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Functional Epitopes at the Ribosome Subunit Interface

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Supplementary Methods

Measuring Enrichments from Single Nucleotide Mutants

To determine the enrichment of wild-type bases from libraries that randomize the nucleotide identity of a single position we used a method common for quantifying single nucleotide polymorphisms that exploits the linear relationship between the signal arising from fluorescent dideoxynucleotide terminators in a sequencing reaction and the abundance of a DNA polymorphism[1, 2]. The peak volume corresponding to the mutated nucleotide in the pool (Vp) and a reference nucleotide within 10 bases in primary sequence (Vp-ref) were measured. The peak volume for the same two positions were measured on the selected pool(Vs, Vs-ref) and on a homogeneous wild type sequence (Vwt, Vwt-ref). Each volume was determined from 3 to 5 independent chromatograms. The percentage of wild-type ribosomes before and after selection and their enrichment was calculated as follows.

% wild-type in pool= [(Vp/Vp-ref)/(Vwt/Vwt-ref)]x100

%wild-type selected=[(Vs/Vs-ref)/(Vwt/Vwt-ref)]x100

enrichment= %wild-type selected/% wild-type in pool

Measuring Nucleotide covariation.

Fisher's exact test, implemented in StatXact (Cytel Studio), was used to test the null hypothesis that nucleotide positions are independent. Exact p values are reported.

REFERENCES

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