Perturbing proteomes at single residue resolution using base editing

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Highlights

1) A genome-wide base editing screen identifies sensitive sites in yeast essential genes

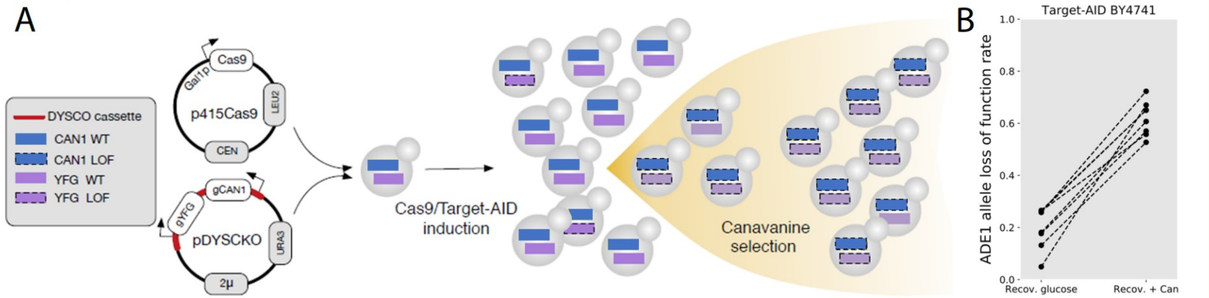
2) gRNA base editing outcomes are predicted with a model validated by deep sequencing data

3) Screen hits are enriched in mutations predicted to be deleterious

Takeaway: Base editing allows efficient screening of many mutations in many genes in a single tube

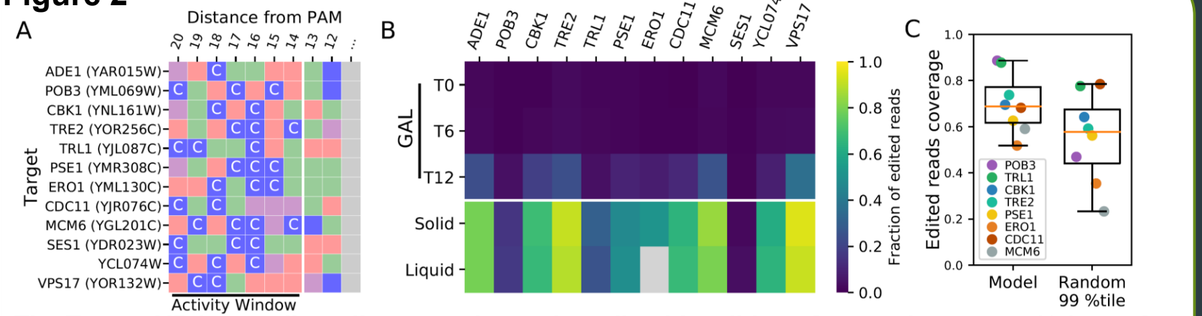
Background

Base editors derived from CRISPR-Cas9 such as Target-AID[1] allow targeted mutagenesis without DNA segment replacement. They offer new opportunities for genome-wide perturbation studies and systems biology.



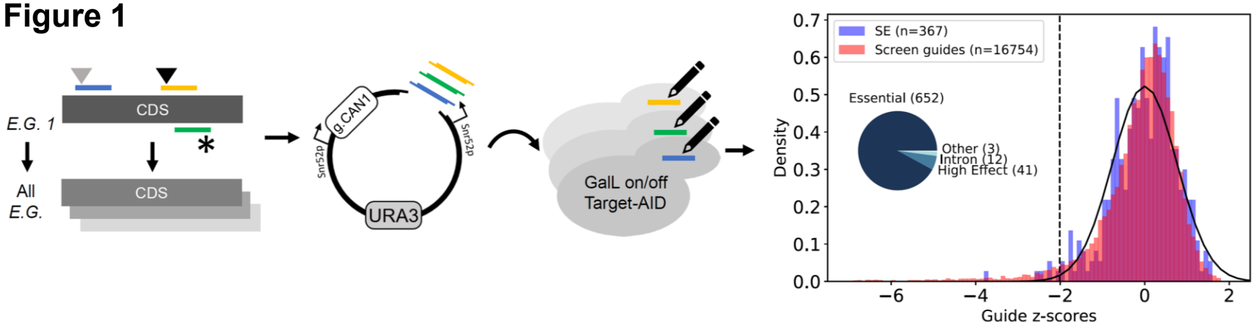
Base editing using co-selection in yeast. A) Target-AID base editing (Adapted from Nishida et al[1] and Wikipedia). Mutation outcome depends on DNA repair pathways. B) Co-selection for high efficiency base editing in yeast[2]. Screening for edited cells using canavanine can increase two to three-fold mutagenesis efficiency of the target of interest (YFG).

Figure 2



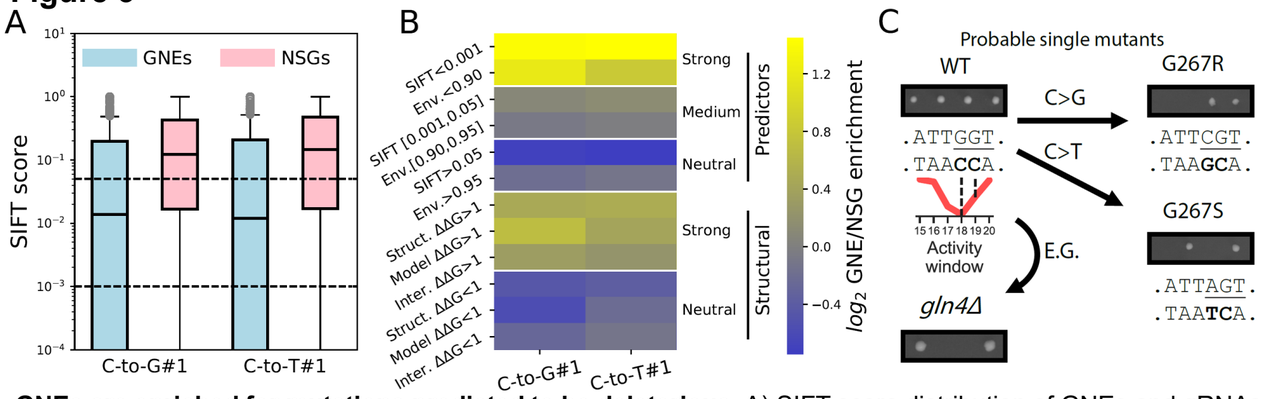
The Target-AID base editor allows precise and predictable editing of genomic targets. A) A set of gRNAs targeting different yeast protein coding genes. B) Editing frequency of targets through the editing process as measured by next-generation sequencing. C) A parsimonious model based on previous studies[1,2] identifies the most likely outcomes of Target-AID mutagenesis in yeast.

Figure 1



Systematic perturbation of essential genes identify sensitive sites across the genome. Essential genes (E.G.) were scanned for sites that could be edited by Target-AID. 708 gRNAs with negative effects (GNEs) were identified using gRNAs with synthesis errors (SE) as controls.

Figure 3



GNEs are enriched for mutations predicted to be deleterious. A) SIFT score distribution of GNEs and gRNAs with non-significant effect (NSGs) mutation outcomes. B) Enrichment folds of GNEs over NSGs for different variant effect prediction measurements. Envision scores (Env.) from Gray et al[3], SIFT and structural data from Wagih et al[4]. C) Validation study using classical genetics of a GNE targeting Glycine 267 from the tRNA-synthetase *GLN4*.

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References

1. Nishida et al, 2016. Science (80-) 353:553–563.
2. Després et al, 2018. G3 200461.2018
3. Gray VE et al 2018, Cell Syst. 6,116–124
4. Wagih et al, 2018. Mol Syst Biol, 14(12):e8430.

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