

Haplo-Insufficiency-Profiling of Yeast Essential Mutants with MUC7-12-mer and Other Peptides

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MUC7-12-mer (RKSYKCLHKRCR) is a cationic antimicrobial peptide (CAMP) derived from the human MUC7 mucin. To better understand its mechanisms of action, we have previously performed a fitness screen of 4741 homozygous, diploid non-essential deletion strains of *S. cerevisiae* with this peptide. The objective of this study was to extend this screen with Haplo-Insufficiency-Profiling of the *S. cerevisiae* essential gene mutant pool, using MUC7 and three other CAMPs, to identify possible targets of these peptides.

Yeast Knockout heterozygous essential mutant pool (~1100 strains) was grown in medium with or without a peptide (MUC7-12mer, cathelicidin KR20, lactoferricin1-11, or histatin fragment P113) for four consecutive 24h cultures. Cells were harvested, genomic DNA isolated and molecular barcodes (20nt sequences specific for each strain) amplified via PCR and universal, Cy3 or Cy5-labeled Uptag- and Downtag-specific primers. PCR products were hybridized to a custom Tag Array (Roche-NimbleGen) and scanned for data analysis.

Preliminary results indicate essential gene mutants showing strongest sensitivity across all four CAMPs were COG3 (oligomeric Golgi complex), RSC6 (RSC chromatin remodeling complex) and IMP4 (Component of the SSU processome). However, we also found a group of mutants showing differences in fitness among the four tested peptides, such as SRB4 and LCB1.

At least three deletions exhibiting the strongest fitness defects are shared between all four peptides suggesting the existence of important common features in modes of action and/or targets. Alternatively, identification of numerous mutations conferring different fitness for different peptides may help better understand those features that are specific to the particular peptides.