

$$\text{Normalized frequency of pure parental alleles} = \frac{\left(\begin{array}{c} \# \text{ pure parental alleles at each mutant strain variant position} \\ \text{(per bin for pooled sequenced mutant)} \end{array} \right)^2}{\left(\begin{array}{c} \# \text{ heterozygous and} \\ \text{homozygous variants in} \\ \text{pooled mutant (per bin)} \end{array} \right) - \left(\begin{array}{c} \# \text{ pure parental alleles} \\ \text{at each mutant strain} \\ \text{variant position (per bin for} \\ \text{pooled sequenced mutant)} \end{array} \right)} \times \text{Average pure parental alleles at} \\
 \text{each mutant strain variant position} \\
 \text{(per bin, per chromosome, for} \\
 \text{pooled sequenced mutant)}$$

Figure S4 *Variant Discovery Mapping* normalization equation. Pure parental alleles are defined as those positions in the pooled sequenced mutant where variant reads/total reads = 1 (after the appropriate variant subtraction strategy has been applied). Normalization is applied by default although users have the option of turning it off.