



Engineering Gcn5 Mutant

- Replace Arginine-164 with Alanine
 - Alanine is not basic
 - Point mutation

$$\begin{array}{c}
 \text{NH}_2^+ \\
 | \\
 \text{HN} \\
 | \\
 \text{CH}_2 \\
 | \\
 \text{CH}_2 \\
 | \\
 \text{CH}_2 \\
 | \\
 \text{H} - \text{C} - \text{NH}_3^+ \\
 | \\
 \text{COO}^-
 \end{array}$$

ARGININE Codon:GAA

$$\begin{array}{c}
 \text{H} \\
 | \\
 \text{CH}_2 \\
 | \\
 \text{H} - \text{C} - \text{NH}_2 \\
 | \\
 \text{COO}^-
 \end{array}$$

ALANINE Codon:CAA

QuickChange Site-directed Mutagenesis by Stratagene

1. Create E. Coli plasmid with WT Gcn5 gene and promoter (Template)
2. Denature template and anneal mutagenic primers containing desired mutation
3. Extend Primers. Repeat

One-Day Method

4. Digest template with Dpn1 (this enzyme digest methylated DNA)
5. Transform mutated DNA into special E.Coli

Culture Transformed Cells

- Cells will repair nicked DNA
- Transformed cells will appear blue when given IPTG and X-gal
- Cells will produce mutant protein