

## **GFP Cloning**

- 1] Grow up pMAD21 (contains PBC KS+ with GFP in Hind-Pst Fragment)
- 2] Plasmid Prep
- 3] Cut with Sac I overnight
- 4] Gel purify cut fragment
- 5] Mung Bean Nuclease
- 6] Clean up with qiagen kit
- 7] Blunt end ligate
- 8] Transform E. coli
- 9] Plasmid prep
- 10] Cut with Kpn 1
- 11] Gel purify
- 12] Cut with Xho 1
- 13] Gel purify
- 14] Grow up C. albicans K1
- 15] Isolate genomic DNA
- 16] Use DNA as template – 2 pcr reactions = primer 1+2 and 3+4
- 17] Gel purify each reaction
- 18] Kinase 2 reactions
- 19] Gel purify
- 20] Ligate cut plasmid with act1p fragment
- 21] Transform E. coli
- 22] Plasmid prep
- 23] Cut with Sac I to open plasmid within act1p
- 24] Transform C. albicans (2 strains)
- 25] Pick colonies based on fluorescence