

FLAG-tagging (3 copies) yeast proteins by PCR

Ref: Gelbart et al., MCB 21:2098-2106, 2001(2001)

1. Plasmid: p3FLAG-KanMX

Oligonucleotides encoding three copies of FLAG epitope sequence followed by a termination codon, **CGATTATAAAGATGACGATGACAAGGATTATAAAGATGACGA** **TGACAAGGATTATAAAGATGACGATGACAAGTAACTGCA** and **GTTACTTGTTCATCGTCATCTTTATAATCCTTGTTCATCGTCATCTTTATAATCCT** **TGTCATCGTCATCTTTATAATCGAGCT**, were annealed, generating overhanging ends compatible with *SacI* at one end and *PstI* at the other. The annealed fragment was then ligated into *SacI/PstI*-digested pBluescript SK(-) to create pBS-3FLAG. Subsequently the *NdeI* - *SpeI* fragment of pUG6 (Guldener et al., NAR 24:2519-2524, 1996) containing the KanMX marker flanked by *loxP* sites, was ligated into the *EcoRI-XhoI* sites of pBS-3FLAG (downstream of the FLAG sequence) to create p3FLAG-KanMX.

2. Primer design

- Primer 1 (+strand): [40-50 b of sequence just upstream of the termination codon of the gene of interest- AGG GAA CAA AAG CTG GAG]
- **** The underlined bases indicate the sequences that anneal to p3FLAG-KanMX during PCR. Make sure that the region of the homology to the gene of interest and the underlined sequence are fused **in frame**.*****
- Primer 2 (- strand): [40-50b sequence just 3'end of the gene of interest- CTA TAG GGC GAA TTG GGT]
- It should be noted that p3FLAG-KanMX was designed such that oligonucleotides used for FLAG tagging are also compatible with the pMPY vectors described previously (Schneider et al., Yeast 11:1265-1274, 1995), and thus can also be used for 3XMyC and 3XHA tagging, using these vectors as templates for PCR.

3. PCR and transformation

- We use 100-200 µl PCR reaction / transformation. Taq polymerase works fine.
- Typical condition using Robocycler (Stratagene): 95 °C 2', [95 °C 20", 54 °C 40", 72 °C 2' 30"] x15 cycles, 72 °C 5'. Expect ~1.8 kb PCR fragment.
- After transforming PCR reaction using normal LiAc method, plate cells on YPD. Incubate @ 30 °C overnight, then replica on YDP+G418 (500 µg / ml for W303).
- Check integration by PCR, Southern and western.
- *****For purification of proteins containing 3XFLAG, elution with triple FLAG peptide, rather than single copy FLAG, is recommended. *****

- **Triple FLAG peptide: Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys**
Use this at 1 mg / ml.