FLAG-tagging (3 copies) yeast proteins by PCR

9/13 by Toshi

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, CGATTATAAAGATGACGATGACAAGGATTATAAAGATGACGATGACAAGGATTATAAAGATGACGATGACAAGTAACTGCA and GTTACTTGTCATCGTCATCTTTATAATCCTTGTCATCGTCATCTTTATAATCCTTGTCATCGTCATCTTTATAATCGAGCT, 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): [~55 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): [~55 b 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.

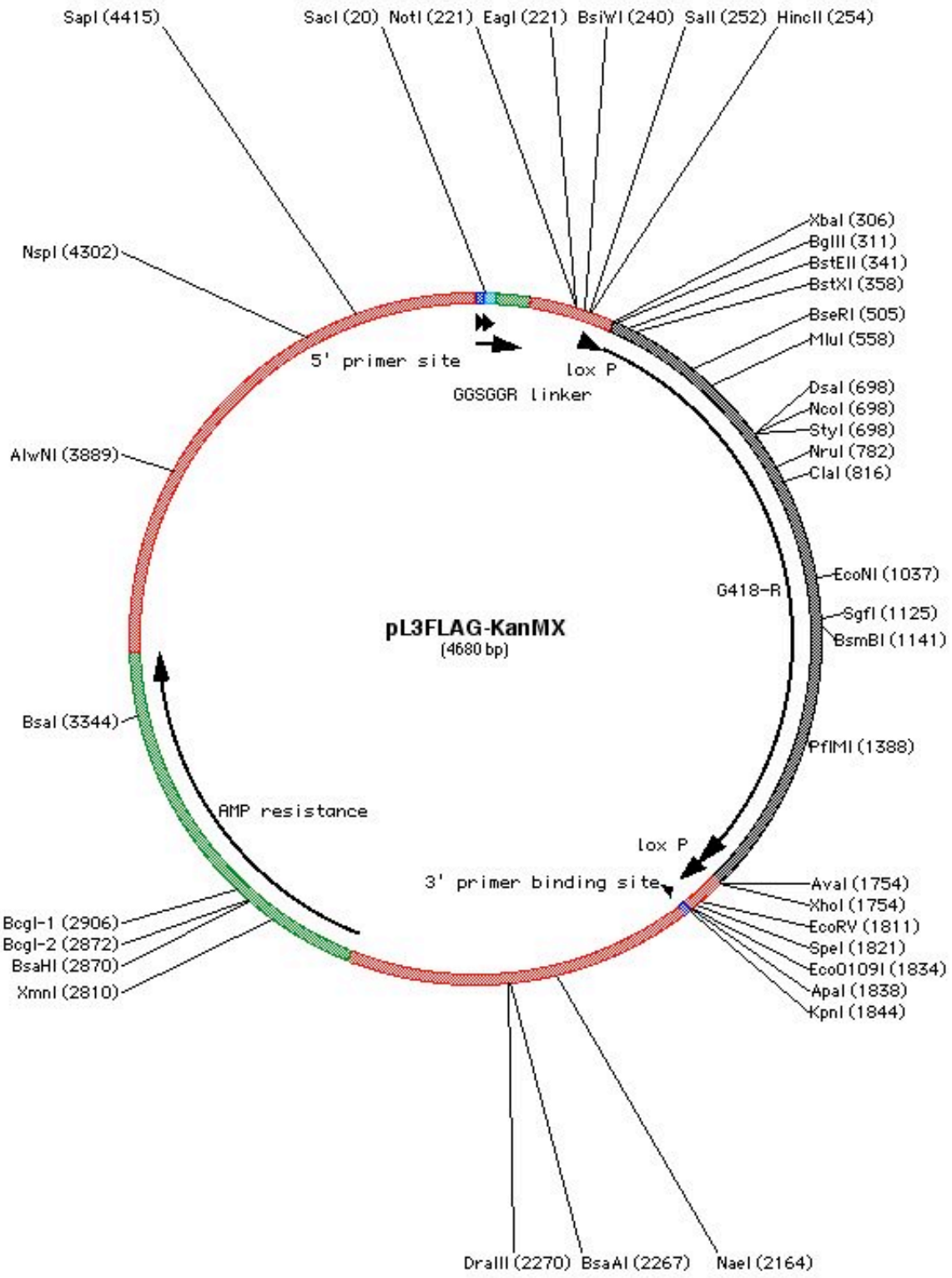
3. PCR and transformation

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.



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