

## PCR primers for KO, tagging, detection

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*amplification of pRS400a (KanMX) or pRS403 (HIS3) for KO:*

5'- X<sub>40</sub> 5' end YFG- GAT TGT ACT GAG AGT GCA CC -3'

5'-X<sub>40</sub> 3' end YFG- CTG TGC GGT ATT TCA CAC CG -3'

-> ~1.6 kb KanMX PCR product, ~2.1 kb *HIS3* PCR product

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*KanMX (Reverse Primer) internal detection oligo (use with YFG 3' UTR oligo):*

5'- TAC GGG CGA CAG TCA CAT CAT G -3'

*KanC3 (Forward Primer) internal detection oligo (use with YFG 5' UTR oligo):*

5'- CCT CGA CAT CAT CTG CCC AGA T-3'

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*HIS3 internal detection oligo (sense strand; use with YFG 3' UTR oligo):*

5'- AGT GCG TTC AAG GCT CTT GC -3'

*HIS3 detection Forward oligo (sense strand; use with YFG 3' UTR oligo):*

5'- CGA GGC AAG AAT GAT CAT CAC C-3'

*HIS3 detection Reverse oligo (antisense strand; use with YFG 5' UTR oligo?):*

5'- GGG AAG ATC GAG TGC TCT ATC G-3'

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*amplification of pAG25 (natMX) for KO:*

5'- X<sub>40</sub> 5' end YFG- CCA GCT GAA GCT TCG TAC GC -3'

5'-X<sub>40</sub> 3' end YFG- GCA TAG GCC ACT AGT GGA TCT G -3'

-> *natMX* PCR product ~ 1.3 kb

*natMX* internal detection oligo (antisense strand; use with YFG 5' UTR oligo):

5'- CAA GAC TGT CAA GGA GGG TA -3'  
(will amplify ~140 bp natMX plus YFG 5' sequences)

*JS519 natMX internal detection oligo (antisense strand; use with YFG 5' UTR oligo):*

5'- GTA AGC CGT GTC GTC AAG AGT GGT-3'

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*amplification of URA3 for C-terminal TAP-tag*

F: 5' – 43-48 upstream of stop codon of YFG – TCC ATG GAA AAG AGA AG

R: 5' - 43-48 downstream of stop codon of YFG –TAC GAC TCA CTA TAG GG

-> ~2.2 kb PCR product

*TAP Tag detection oligo -TAP3CONF(antisense strand; use with YFG coding sense oligo made near the 3' end of the coding sequence):*

5'-CGCCGAAAGTAGACGCGAATTCCGCG-3'

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*amplification of pFA6a-13 myc KAN MX, pFA6a-13 myc TRP1, and pFA6-13 myc HIS3 MX for C-terminal myc-tag:*

**F:** 5'-X40 upstream of stop codon of YFG – CGG ATC CCC GGG TTA ATT AA – 3'

**R:** 5'-X40 downstream of stop codon of YFG –GAA TTC GAG CTC GTT TAA AC –3'

\*NOTE: don't include stop codon and make sure forward primer (F) is in frame with tagging sequence

- ➔ *KAN MX-myc PCR product ~2.3 kb*
- ➔ *TRP1-myc PCR product ~1.8 kb*
- ➔ *HIS3 MX-myc PCR product ~ 2.2 kb*

*Myc-tagged internal detection oligos*

**pTEF-R:** 5'- GGG CTA AAT GTA CGG GCG AC –3'

**pTEF-F:** 5'- GTT CTC ACA TCA CAT CCG AAC -3'

\*NOTE: design other detection oligos just upstream of “**F**” myc-tagging primer and just downstream of “**R**” myc-tagging primer

➔ will amplify ~1.0 kb

\*NOTE: Can use the same primer sequence to tag with a 3HA sequence on YFG just have to use pFA6aHA KANMX, TRP1, and HIS3 for C-terminal HA-tag.

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*TRP1 detection Forward oligo (sense strand; use with YFG 3' UTR oligo):*

5'- GTG ATG CGC TTA GAT TAA ATG G-3'

*TRP1 detection Reverse oligo (antisense strand; use with YFG 5' UTR oligo?):*

5'- CAT CGG AAT CTA GAG CAC ATT C-3'

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