

PRIMERS FOR QCB 301

Purposes:

1. Primers for *FAR1-wt* from genome to *GAL4pr* plasmid.
2. Primers for creating *FAR1-22* mutation (to be used with 1.).
3. Primers for transferring *FAR1-wt* and *FAR1-22* from plasmid to genome.
4. Primers for transferring *ZEVpr* to genome.
5. Primers for knocking out *TRP1* with *CORE*.

Primers:

1. Forward pairs with *HindIII* and backward pairs with *BamHI*:

These are the old primers. Sites can be found in FAR1 for each enzyme.

5' *HindIII* site ... Start of *FAR1* 3'

5' **AATAAAGCTTATGAAGACACCAACAAGAGTTTC** 3'

5' *BamHI* site ... RC end of *FAR1* 3'

5' **AATAGGATCCCTAGAGGTTGGGAACTTCC** 3'

These are the new primers. Only one site each in pMM86 and none in FAR

5' *XhoI* site ... Start of *FAR1* 3'

5' **AATACTCGAGATGAAGACACCAACAAGAGTTTC** 3'

5' *NotI* site ... RC end of *FAR1* 3'

5' **AATAGCGGCCGCTAGAGGTTGGGAACTTCC** 3'

These are the new primers for recombination. The first matches the end of GAL. Note that it is best to cut with NotI or a site after XhoI but before NotI

5' End of pGAL ... Start of *FAR1* 3'

5' **TATACTTTAACGTCAAGGAGAAAAACTATACTCGAGATGAAGACACCAACAAGAGTTTC** 3'

5' RC After *NotI* site ... RC end of *FAR1* 3'

5' **CGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCTAGAGGTTGGGAACTTCC** 3'

2. First will be used with second from **1.**, and second will be used with first from **2.**:

5' **CAAATCTTGGCCTAATGATCCACCCACCAAGTTTGAAGAAAAC** 3'
 5' **GTTTTCTTCAAACCTTGGTGGGTGGATCATTAGGCCAAGATTTG** 3'

3. Connect ends of *FAR1* to ZEVpr (front) and pCORE (back)

5' *ZEVpr end ... Start of FAR1* 3'
 5' **CGTCAAGGAGAAAAAACTATAGGTACCCTAGTATGGACGTATGAAGACACCAACAAGAGTTTC** 3'
 5' *RC of after Trp1 ... RC of end of FAR1* 3'
 5' **GTGCACAAACAATACTTAAATAAATACTACTCAGTAATAACCTAGAGGTTGGGAACTTCC** 3'

4. Connect ZEVpr to *FAR1* (back) and pCORE (back)

5' *before Trp1 ... Start of ZEVpr* 3'
 5' **GTGAGTATACGTGATTAAGCACACAAAGGCAGCTTGGAGTTTATATTGAATTTTCAAAAATTCTTA** 3'
 5' *RC of start of FAR1 ... RC of end of ZEVpr* 3'
 5' **TGTATTTTTTTTTTCAAACGAACTCTTGTTGGTGTCTTCATACGTCCATACTAGTGGTAC** 3'

5. pCORE to TRP1 (Genomic)

5' *before TRP1 ... Start of CORE* 3'
 5' **GTGAGTATACGTGATTAAGCACACAAAGGCAGCTTGGAGTTCCTTACCATTAAGTTGATC** 3'
 5' *RC of after TRP1 ... RC of end of CORE* 3'
 5' **GTGCACAAACAATACTTAAATAAATACTACTCAGTAATAACGAGCTCGTTTTTCGACACTGG** 3'