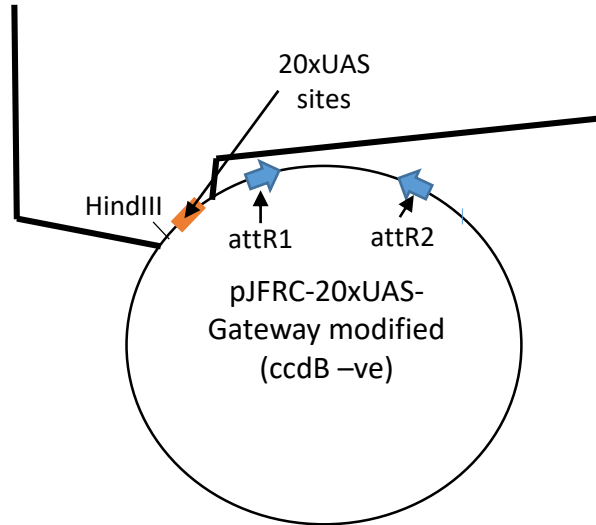


Forward Primer



5' GTG ACC TGT TCG GAG TGA TTA GCG TTA CAA 3'
3' CAC TGG ACA AGC CTC ACT AAT CGC AAT GTT 5'



5' CT AGC ACG TCG AGC GCC GGA GTA TAA ATA GAG 3'
3' GA TCG TGC AGC TCG CGG CCT CAT ATT TAT CTC 5'



Reverse Primer

Forward Primer:

5' GTG ACC TGT TCG GAG TGA TTA GCG 3'

Reverse Primer:

5' AAG CTT CTC TAT TTA TAC TCC GGC GCT CG 3'
HindIII

PCR

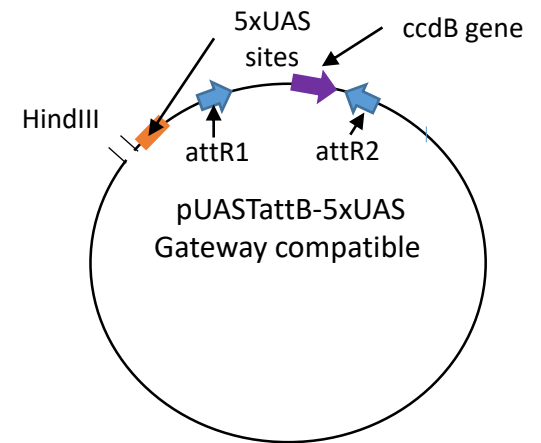
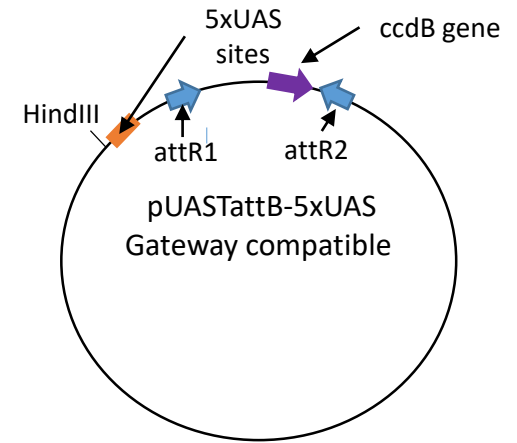


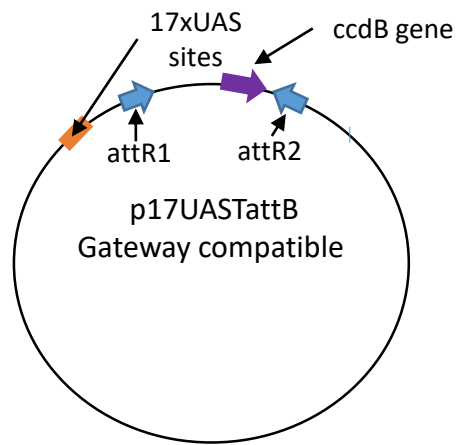


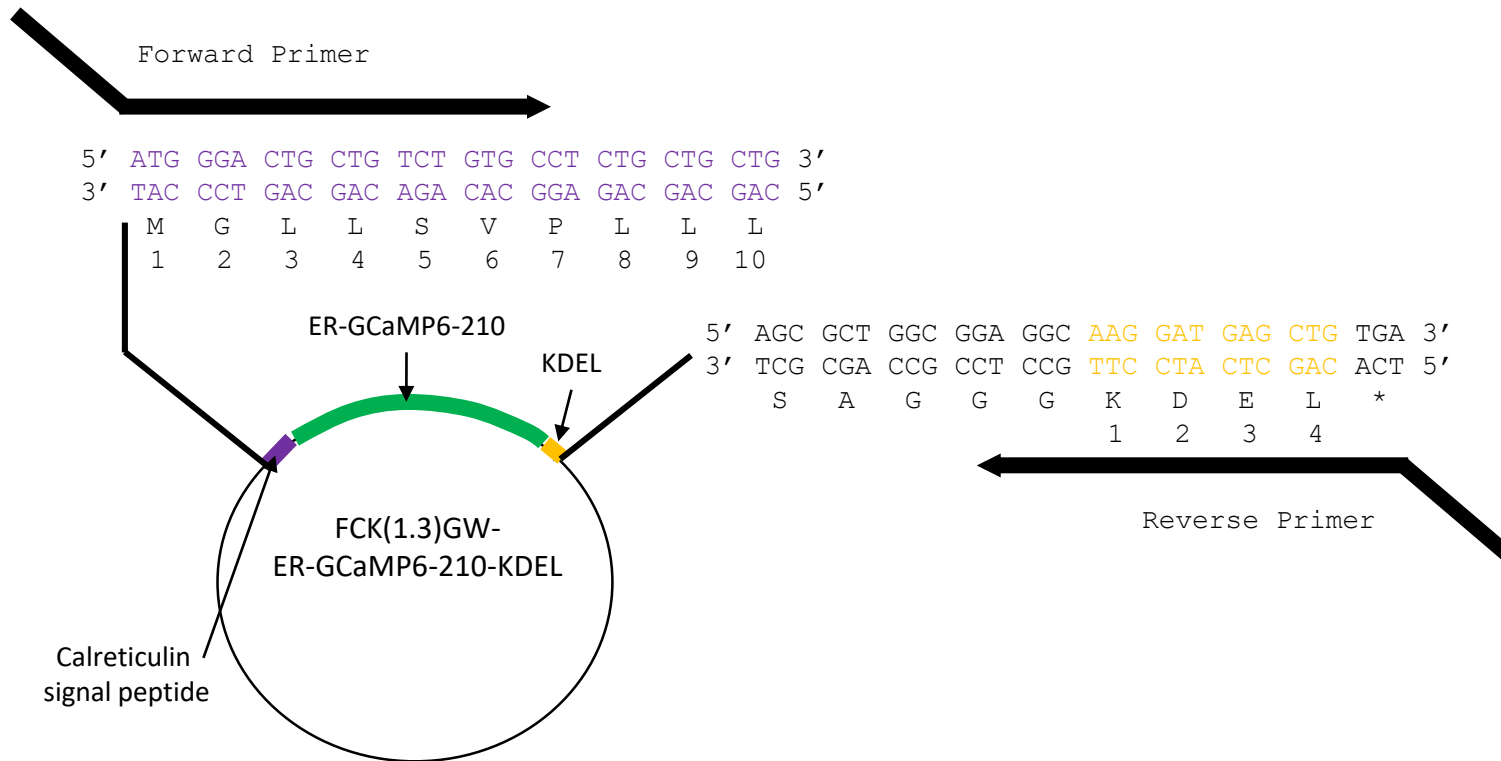
HindIII digestion ↓



Ligation ↓







Forward Primer:

5' GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTA CAAAC ATG GGA CTG CTG TCT GTG C 3'

attB1 **Kozak** M G L L S V

1 2 3 4 5 6

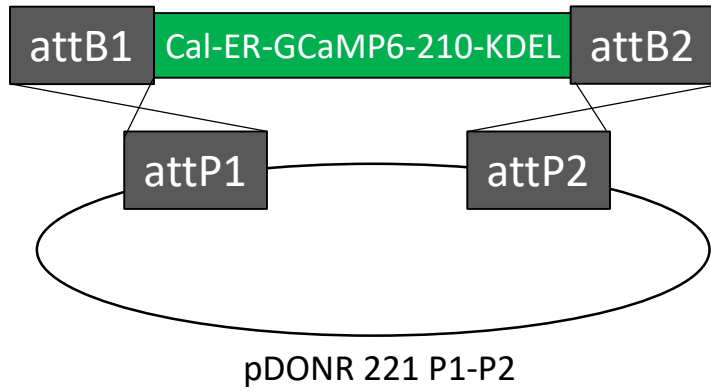
Reverse Primer:

5' GGGG AC CAC TTT GTA CAA GAA AC TGG GTT TCA CAG CTC ATC CTT GCC T 3'

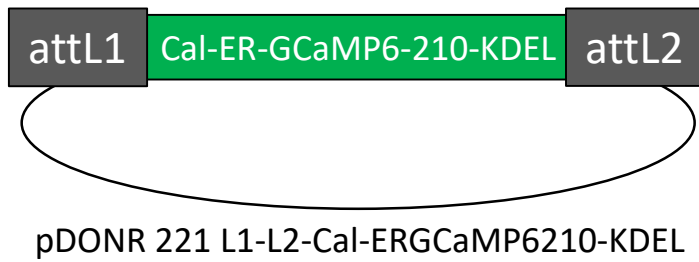
attB2 * L E D K G

4 3 2 1

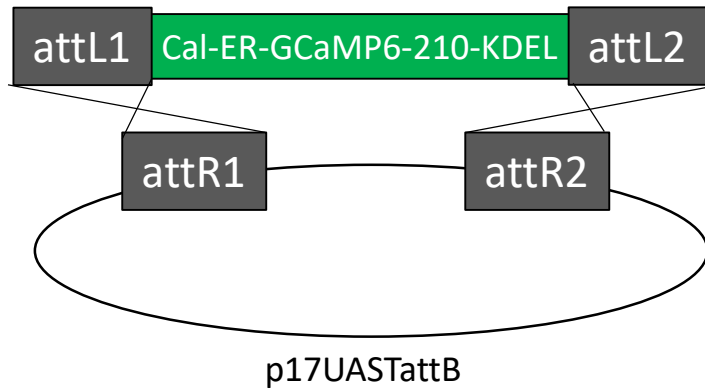
PCR



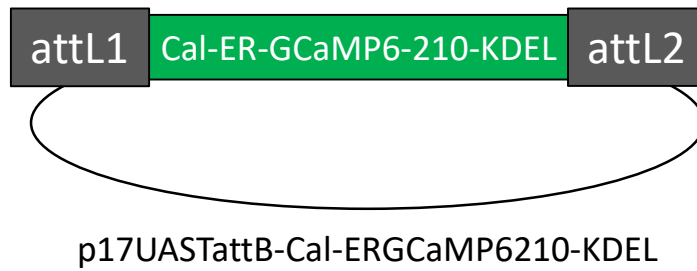
BP reaction

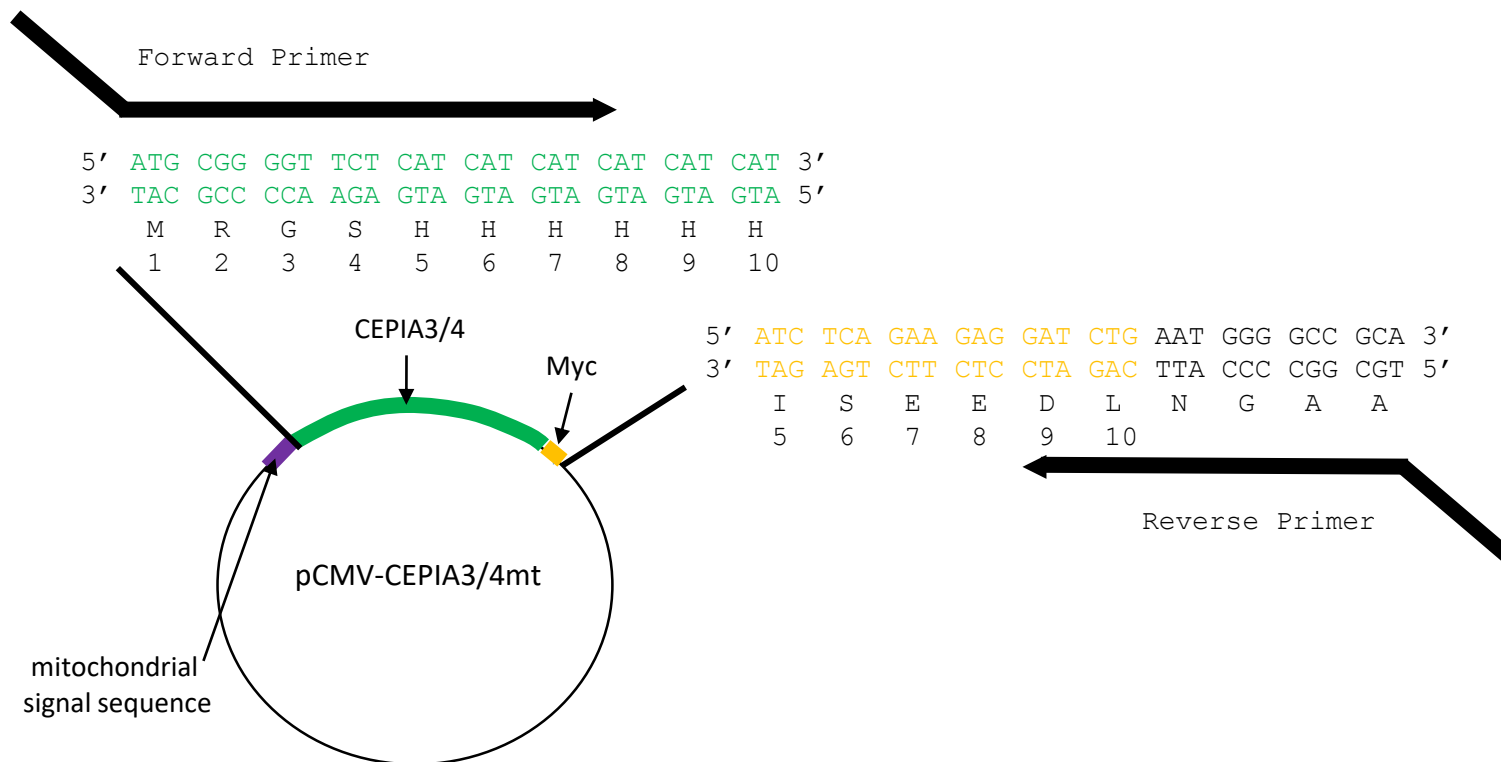


LR reaction



Expression
clone





Forward Primer:

5' GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTA CAAAC ATG AAG TTA TGC ATA TTA CTG GCC GTC GTG GCC TTT GTT GGC CTC 3'

attB1 Kozak M K L C I L L A V V A F V G L

TCG CTC GGG ACC GGT GGC GGA ATG CGG GGT TCT CAT CAT CAT C 3'

S L G T G G G M R G S H H H

1 2 3 4 5 6 7

BiP signal sequence

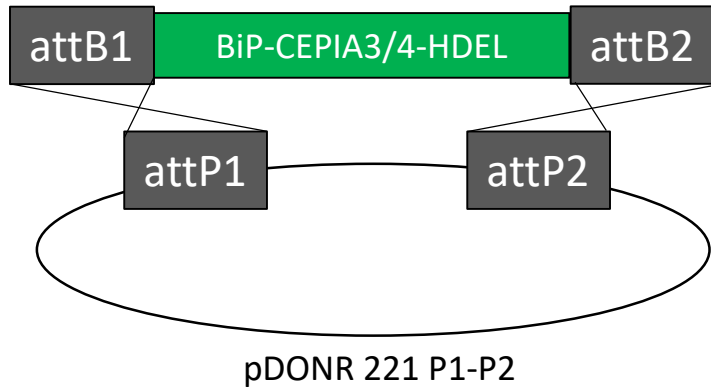
Reverse Primer:

5' GGGG AC CAC TTT GTA CAA GAA AC TGG GTT TCA CAA TTC GTC GTG TGC GGC CCC ATT CAG ATC 3'

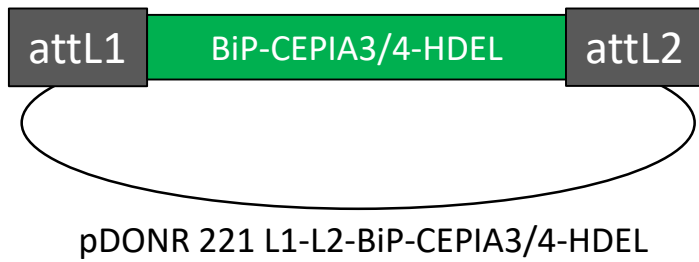
attB2 * L E D H A A G N L D

HDEL 10 9

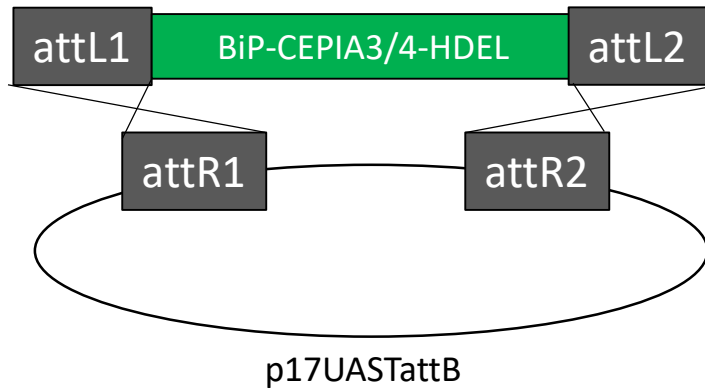
PCR



BP reaction



LR reaction



Expression
clone

