2016\_01\_26 MM1574 (Glycine Synthase) Knockout

## Design/Order Primers (01/25)

Designed 2 sets of primers with NotI sites on the outsides and AscI sites on the insides. The plan is to amplify both sets, then cut them both with AscI and ligate them together. Then we’ll cut with NotI and ligate them with cut plasmid. Primer sequences are as follows:

KOMmp1574upfwdNot (MR1)

5’ – AAG CGG CCG CAG GTC GTT TGA AAT TTC ATC G – 3’

KOMMP1574uprevAsc (MR2)

5’ – AAG GCG CGC CCA TAA AGA CAC CTA ATA AAC AAT C – 3’

KOMMP1574dnfwdAsc (MR3)

5’ – AAG GCG CGC CAT GAT TTA AAC GCT ATT TGT AAC G – 3’

KOMMP1574dnrevNot (MR4)

5’ – AAG CGG CCG CTT GAT AAT AAT TAT ATA TAC CC – 3’

## PCR Amplification (01/26)

Received primers and diluted them with TRIS-E (TRIS+EDTA to scavenge magnesium and keep DNA from reacting) to 200 uM stocks, then diluted those to get 20 uM stocks of each primer. Measured genomic DNA at 5136 ng/uL, diluted it to 30 ng/uL as follows:

2 uL DNA + 48 uL sterile water 🡪 205.44 ng/uL (dilution 1)

7.3 uL DNA + 42.7 uL sterile water 🡪 30 ng/uL (dilution 2)

Created PCR reaction mixes as follows:

|  |  |  |
| --- | --- | --- |
| Reagent | Rxn 1 Volume (μL) | Rxn 2 Volume (μL) |
| Water | 71.0 | 71.0 |
| 5X iProof HF Buffer | 20.0 | 20.0 |
| 10mM dNTPs | 2.0 | 2.0 |
| MR1 | 2.0 | 0 |
| MR2 | 2.0 | 0 |
| MR3 | 0 | 2.0 |
| MR4 | 0 | 2.0 |
| Template | 2.0 | 2.0 |
| iProof polymerase | 1.0 | 1.0 |
| Total Volume: | 100.0 | 100.0 |

From each reaction type, created two 30 uL PCR reactions (tubes 1 and 2 are Rxn. 1; tubes 3 and 4 are Rxn. 3). Reaction program was as follows:

Cycle Temperature Time # Cycles

initial denaturation 98ºC 2’ 1

denaturation 98ºC 15”

annealing **53ºC** 15” 30

extension 72ºC 30”

final extension 72ºC 1’ 1

## More PCR, Restriction Digest and Ligation (01/27)

My first PCR was done with very little overall volume (60 uL per reaction). Tom wants more, so I redid it with 300 uL per reaction, 3 tubes each. Thus, I made a reaction mix with 4 reactions worth for each as follows:

|  |  |  |
| --- | --- | --- |
| Reagent | Rxn 1 Volume (μL) | Rxn 2 Volume (μL) |
| Water | 284.0 | 284.0 |
| 5X iProof HF Buffer | 80.0 | 80.0 |
| 10mM dNTPs | 8.0 | 8.0 |
| MR1 | 8.0 | 0 |
| MR2 | 8.0 | 0 |
| MR3 | 0 | 8.0 |
| MR4 | 0 | 8.0 |
| Template | 8.0 | 8.0 |
| iProof polymerase | 4.0 | 4.0 |
| Total Volume: | 400.0 | 400.0 |

Then ran PCR with very slight change in program (on lengthening time):

Cycle Temperature Time # Cycles

initial denaturation 98ºC 2’ 1

denaturation 98ºC 15”

annealing **53ºC** 15” 30

extension 72ºC 20”

final extension 72ºC 1’ 1

Using PCR products, did digest with AscI, but accidentally used 3:1 buffer at first. Tried to balance with CutSmart, so final amounts of each component are as follows:

Meanwhile, ran a 2nd gel to see if both upstream and downstream were amplified; gel showed 2 bands at 1 kb, which suggests I got both products I wanted.