

Leia Jiang 8/8

MONDAY, 8/8/2022

62 C for both PCRs

PCR gblock 3a and 3b, 1 rxn each:

- 7.5 uL forward primers
- 7.5 uL reverse primers
- 30 uL Phusion buffer
- 3 uL dNTPs
- 1.5 uL Phusion polymerase
- 6 uL DMSO
- 91.5 uL diH₂O

GGA insertion rxns (2049 Up + Pd 3a; CymR + Pd 3b + 2049 Down):

- 2 uL T4 buffer
- 2 uL BsaI T7 mix
- $100/76.3 = 1.31$ uL 2049 U + 2 uL 3a; 2 uL CymR + 2 uL 3b + $100/33.8 = 2.96$ uL 2049 D
- 12.7 uL dH₂O; 9.04 uL dH₂O

TUESDAY, 8/9/2022

Clean and concentrate #2 (3.69 ng/uL) and CymR (2.22 ng/uL)

WEDNESDAY, 8/10/2022

PCRs:

64 and 1 (2049 UF)

65 and 4 (2049 DR)

CymR: 61 and 62

2 min extension time