PCR for Removal of His6 Tag from DsbA::His6::saCas9

Introduction

This protocol outlines PCR for the removal of His6 Tag from DsbA::His6::saCas9 using Phusion HS Flex 2x Master Mix (MM). This will amplify the Region of the plasmid that is NOT the His6 and create a linear DNA fragment which can then be used in iPCR to insert a new His6

Materials

Materials (Per RXN)

- 2.5 uL 10 uM P6
- 2.5 uL 10 uM P7
- 1.5 uL DMSO
- 18.5 uL of 100 pg/uL pC34 (add 1 ng)
- 25 uL of Phusion HS Flex 2x MM
- PCR Tube

Procedure

PCR steps:

1. Add P6, P7, DMSO, pC34 in any order (Vortex briefly after all added)
2. Add MM (Vortex briefly and spin down for a second or two to get material out of lid)
3. Vortex final reaction briefly and spin quickly to collect in bottom of tube
4. Place in Thermocycler at the following conditions (HisRemoval):

   98 C for 30s
   98 C for 15s (Repeat Red 35x)
   61 C for 30s
   72 C for 2.5 minutes
   72 C for 10 min
   4 C for inf. Time

Gel Electrophoresis steps:

5. Heat 1% agarose in TAE and pour into mold
6. Add 3 uL Sybr Green and mix with pipette tip
7. Let harden and orient in the chamber
8. Pour 1x TAE over the gel to fully submerge

9. Mix 50 uL sample with 10 uL loading dye
10. Add 5 uL sample to each lane
11. Add ladder to lane 1
12. Apply 100 V for 45 min
13. Image gel to see if obtained **Correct Band 5 kb**
14. PCR purification