

PCR for Ycia-sfp-CAR amph part 3

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

- › PCR Machine
- › Primers P018 & P032
- › Sterile water
- › NEB's Q5 2x Mastermix
- › Template DNA (YciA-sfp-CAR part 3 from IDT)
 - › Amplicon's length 1510 bp
 - ›

Procedure

1. 25µl reaction
 - 12,5µl 2x Mastermix
 - 1,25µl 10µM P018
 - 1,25µl 10µM P032
 - 0,1pg-1,0ng template DNA (YciA-sfp-CAR part 3 from IDT)
 - Adjust final volume to 25µl with sterile H₂O
2. Mix the tube gently by tapping or pipetting up and down (DO NOT VORTEX)
3. Quickly spin everything to the bottom of the tube
4. PCR program
 1. 98°C - 30 sec
 2. 98°C - 10 sec
 3. 67°C - 20 sec
 4. 72°C - 40 sec
 5. 72°C - 2 min
 6. 4°C - Forever
5. Run 5µl of the reaction on gel (add 1µl of 6x Loading dye) to check the results.
6. If the reaction was successful, clean with PCR purification kit
7. Store purified product in -20°C
8. -> Ready for Gibson Assembly to create amph YciA+sfp+CAR