pET-28a(+) Backbone Amplification

Introduction

This protocol allows to amplify the pET-28a(+) backbone using primers with BsaI restriction enzyme sites designed in Geneious.

Reagents

- pET-28a(+) backbone
- pET-28a(+) backbone forward primer
- pET-28a(+) backbone reverse primer
- Q5 Hi-Fidelity 2X Master Mix
- Milli-Q H_20

Equipment

- PCR tubes
- Thermocycler
- Pipette and tips

Procedure

1. Add the following amounts of reagents to a sterile PCR tube:

Reagent	Amount
pET-28a(+) backbone (10 ng/µl)	1 µL
pET-28a(+) forward primer (10 µM)	1.25 µL
pET-28a(+) reverse primer (10 µM)	1.25 µL
Q5 Hi-Fi 2X master mix	12.5 µL
Milli-Q H_20	9 µL

2. Use the following PCR thermocycler program:

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
Repeat the following 3 steps for 27 cycles		
Denaturation	98°C	7 seconds
Annealing	72°C	15 seconds
Elongation	72°C	2 min 50 sec
Final Elongation	72°C	2 minutes
Hold	4°C	∞