





CBM3a-mRFP Gene Block Amplification




Introduction

This protocol allows to amplify the CBM3a-mRFP gene block using primers with BsaI restriction enzyme sites designed in Geneious.

Reagents

-  CBM3a-mRFP gene block
-  CBM3a forward primer
-  CBM3a reverse primer
-  Milli-Q H₂O

Equipment

-  PCR tubes
-  Thermocycler
-  Pipette and tips

Procedure

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

Reagent	Amount
CBM3a-mRFP gene block (10 ng/μL)	1 μL
CBM3a forward primer (10 μM)	1.25 μL
CBM3a reverse primer (10 μM)	1.25 μL
Q5 Hi-Fi 2X master mix	12.5 μL
Milli-Q H ₂ O	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	66°C	20 seconds
Elongation	72°C	33 seconds
Final Elongation	72°C	2 minutes
Hold	4°C	∞