







## CBM2a Assembly





### Introduction

This protocol creates the CMB2a protein that is used extensively throughout our project.

### Reagents

-  CBM2a gene block from IDT
-  mRFP gene block from IDT
-  pET28a plasmid
-  Forward and Reverse primers for CBM2a and mRFP gene block.
-  Q5 Hi-Fidelity 2X Master Mix
-  Milli-Q H<sub>2</sub>O

### Equipment

-  PCR tubes
-  Thermocycler
-  Pipette and tips
-  Ice bucket

### Procedure

1. In an ice bucket to prevent premature amplification, place multiple PCR tubes and add 1  $\mu$ L each of the mRFP gene block, CBM2a gene block, and pET28a plasmid into three separate tubes.
2. For 25  $\mu$ L PCR reactions, add 1.25  $\mu$ L of forward and reverse primers AND Q5 Hi-Fidelity 2X Master Mix into the tubes.
3. For 50  $\mu$ L PCR reactions, add 2.5  $\mu$ L of forward and reverse primers AND Q5 Hi-Fidelity 2X Master Mix into the tubes.
4. Backfill the tubes with Milli-Q H<sub>2</sub>O until reaching a final volume of either 25 or 50  $\mu$ L.
5. Use the following PCR thermocycler program to amplify the mRFP gene block:

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
<b>Repeat the following 3 steps for 25 cycles</b>		
Denaturation	98°C	10 seconds
Annealing	69°C	15 seconds
Elongation	72°C	21 seconds
Final Elongation	72°C	2 minutes
Hold	4°C	$\infty$

## CBM2a Assembly Continued

6. Use the following PCR thermocycler program to amplify the pET28a plasmid:

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
<b>Repeat the following 3 steps for 27 cycles</b>		
Denaturation	98°C	10 seconds
Annealing	70°C	15 seconds
Elongation	72°C	170 seconds
Final Elongation	72°C	2 minutes
Hold	4°C	∞

7. Use the following PCR thermocycler program to amplify the CBM2a gene block:

Step	Temperature	Time
Initial Denaturation	98°C	30 seconds
<b>Repeat the following 3 steps for 27 cycles</b>		
Denaturation	98°C	10 seconds
Annealing	67°C	15 seconds
Elongation	72°C	51 seconds
Final Elongation	72°C	2 minutes
Hold	4°C	∞