8-24-17 Site directed mutagenesis of mamba in BB1C3 → Change codon to remove 2nd EcoRI restriction site.

purpose: use site directed mutagenesis to change a base in the middle of EcoRI site upstream of mamba.

Preparation

✓ Dilute mutagen primers to 125 ng/μL

✓ Dilute DNA samples to 50 ng/μL.

- Ligation A #4
  1.2 μL sample + 8.8 μL dH$_2$O
- Ligation A #6
  1.2 μL sample + 8.8 μL dH$_2$O
- Ligation A #7
  1.3 μL sample + 8.7 μL dH$_2$O
- Ligation A #9
  2.4 μL + 7.6 μL dH$_2$O

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1 Control Rxn and 4 experimental Rxns.

Control Rxn volumes

5.0 µL 10x Rxn Buffer
2.0 µL pWhitescript DNA
1.25 µL control primer 1
1.25 µL control primer 2
1.0 µL dNTP
38.5 µL di H_2O
1.0 µL PfuUltra HF DNA polymerase

Experimental Rxn volumes

mastermix

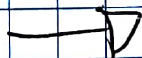
20.0 µL 10x Rxn Buffer
4.0 µL Antisense primer
4.0 µL forward sense primer
4.0 µL DNTP mix
164.0 µL di H_2O.

Samples
4, 6, 7, 9
from ligation A

41.0 µL of mastermix was added to each sample tube.

1.0 µL of each DNA experimental sample was added to corresponding numbered tubes.

1.0 µL of Pfu Ultra HF DNA polymerase was added to each tube.



8-24-17 Mamba mutagenesis.

Thermal cycler settings

	1. 95°C	30 sec
12x cycles	2. 95°C	30 sec
	3. 55°C	1.0 min
	4. 68°C	3.0 min

1.0 μL of DpnI was added to the control and experimental Rxns.

- centrifused for 1.0 min.
- Incubated @ 37°C for 60 min.

DNA samples were stored in freezer overnight.

PCR Clean up of mutant samples

- used Qiagen Qiaquick PCR clean up kit
  40.0 μL of each sample.
- kept 10.0 μL of each sample unclean.