PCR (Error-prone PCR)

Zhujun Wei[1]
[1]2020 iGEM NEFU China

Oct 25, 2020

1 Works for me dx.doi.org/10.17504/protocols.io.bnxrmfm6

2020 iGEM NEFU China

Zhujun Wei

DOI
dx.doi.org/10.17504/protocols.io.bnxrmfm6

PROTOCOL CITATION
Zhujun Wei 2020. PCR (Error-prone PCR). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnxrmfm6

CREATED
Oct 25, 2020

LAST MODIFIED
Oct 25, 2020

PROTOCOL INTEGER ID
43729

SAFETY WARNINGS

Please wear gloves during experiments.Don't touch the lid after PCR program initiation.
Synthesize primers in advance before starting.

1 Set up a small box with ice, put the tubes of DNA, 2 x Mut Random System, Mut Enhancer and ddH_2O into it before loading them into the Bio-rad S1000TM Thermo Cycler.

2 Add the following reagents to a PCR tube (20 µL).

Ingredient	Volume
Template Plasmid(1-10 ng/µL)	0.4 µL
2 x Mut Random System	10 µL
Forward Primer (10 µM)	0.4 µL
Reverse Primer (10 µM)	0.4 µL
Mut Enhancer	0-20 µL
ddH2O	To 20 µL

3 Program the thermocycler as follows:

Temperature	Time
95°C	2 min
94°C	30 s
55°C	1 min
72°C	1 min/kilo basepairs
72°C	7 min
4°C	∞

Note: Higher initial template concentrationscan lead to lower mutation rates. More amplification cycles may cause higher mutation rates.

4 Build a mutant library, expand the strains, and add the corresponding inducers, collect data using a Microplate reader operating procedure V.1, and use the GraphPad Prism for data processing and analysis.

5 Select the positive results for DNA sequencing to determine the mutated nucleotides in the DNA promoter or coding sequence.

Citation: Zhujun Wei (10/25/2020). PCR (Error-prone PCR). https://dx.doi.org/10.17504/protocols.io.bnxrmfm6