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# PCR (Error-prone PCR)

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Works for me

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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<sub>2</sub>O 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
ddH <sub>2</sub> O	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 base pairs
72°C	7 min
4°C	∞

Note: Higher initial template concentrations can 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.