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PCR (Error-prone PCR)

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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.

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Protocol status: Working
We use this protocol and it's working

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- 1 Set up a small box with ice, put the tubes of DNA, 2 x Mut Random System, Mut Enhancer and ddH₂O into it before loading them into the Bio-rad S1000TM Thermo Cycler.

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

Ingredient	Volume
Template Plasmid(1-10 ng/ μ L)	0.4 μ L
2 x Mut Random System	10 μ L
Forward Primer (10 μ M)	0.4 μ L
Reverse Primer (10 μ M)	0.4 μ L
Mut Enhancer	0-20 μ L
ddH ₂ O	To 20 μ L

3 Program the thermocycler as follows:

Temperature	Time
95°C	2 min
94°C	30 s
55°C	1 min
72°C	1 min/kilobase 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.