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PCR (Error-prone PCR)

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

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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_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℃	2 min
	94℃	30 s
	55℃	1 min
	72℃	1 min/kilo base pairs
	72℃	7 min
	4℃	∞

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.