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

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Error-prone PCR (Random Mutagenesis)

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ABSTRACT

2023 NUS-Singapore iGEM Team followed this protocol to introduce random mutation in DNA fragments.

PROTOCOL MATERIALS

GeneMorph II Random Mutagenesis Kits **Agilent Technologies Catalog #200550**

Step 1

SAFETY WARNINGS

Proper lab PPE must be worn at all times.

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88974

Keywords: Error-prone PCR, PCR, Polymerase Chain Reaction, Mutation, Mutagenesis, Random Mutagenesis

Error-prone PCR (Mutagenesis)

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1 Add the primers, the DNA template, and the following reagents from the GeneMorph II Random Mutagenesis Kits **Agilent Technologies Catalog #200550** into a PCR tube to make a 50 µL PCR sample:

	Item	Volume
	10x Mutazyme II (10x Reaction Buffer)	5µL
	40mM dNTP Mix	1µL
	DI water	41.5µL
	Each Primer (both forward & reverse)	0.5µL
	Mutazyme II	1µL
	DNA Template	0.25µL

2 Mix the solution well.

3 Place the sample into the Thermal Cycler and set it with the following conditions:

	Purpose	Temperature	Duration
	Initial Denaturation	95°C	2 minutes
	Denaturation	95°C	1 minute
	Annealing	55°C	1 minute
	Extension	72°C	1 minute
	Go to step 2, repeat the cycle 44 times		
	Extension	72°C	10 minutes

	Purpose	Temperature	Duration
	Finish	12°C	Infinite Loop

4 Upon finishing the PCR steps, add 10 µL of DNA loading dye.

5 Proceeds to the gel electrophoresis to isolate the gene fragment of interest.