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Error-prone PCR (Random Mutagenesis)

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Protocol status: Working

We use this protocol and it's working

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Keywords: Error-prone PCR, PCR, Polymerase Chain Reaction, Mutation, Mutagenesis, Random Mutagenesis, random mutation in dna fragment, random mutation, random mutagenesi, dna fragment, dna, pcr, prone pcr, singapore igem team

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**

Safety warnings

 Proper lab PPE must be worn at all times.



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

- 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
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.