Oct 09, 2023

# Error-prone PCR (Random Mutagenesis)

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzx57xgx1/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzx57xgx1/v1

**Protocol Citation:** NUS iGEM 2023. Error-prone PCR (Random Mutagenesis). **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzx57xgx1/v1



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

**Created:** October 08, 2023

**Last Modified:** October 09, 2023

**Protocol Integer ID:** 88974

**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) | 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 |
| | 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.