



OCT 09, 2023

🌐 Error-prone PCR (Random Mutagenesis)

NUS iGEM¹

¹National University of Singapore

NUS iGEM 2023



NUS iGEM

National University of Singapore

OPEN  ACCESS



DOI:
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>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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


Created: Oct 08, 2023

Last Modified: Oct 09, 2023

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



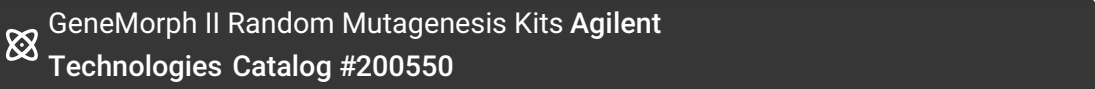
Proper lab PPE must be worn at all times.

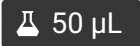
Keywords: Error-prone PCR,
 PCR, Polymerase Chain
 Reaction, Mutation,
 Mutagenesis, Random
 Mutagenesis

Error-prone PCR (Mutagenesis)

40m

- Add the primers, the DNA template, and the following reagents from the


 into 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

- Mix the solution well.
- 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.