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Polymerase Chain Reaction (PCR)

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Protocol status: Working

We use this protocol and it's working

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Abstract

This is the common Polymerase Chain Reaction (PCR) protocol followed by the 2023 NUS-Singapore iGEM team.

Materials

1. KOD One™ PCR Master Mix (Blue)
2. DNA Template
3. Primer(s)
4. DI Water

Safety warnings

! Proper lab PPE must be worn.

- 1 Prepare a PCR tube.
- 2 Thaw the KOD One™ PCR Master Mix (Blue) from the -20°C fridge.
- 3 For a 50µL PCR sample, add the following into a PCR tube (follow the sequence):

Item	Volume
KOD One™ PCR Master Mix (Blue)	25µL
Each Primer (2 primers in total)	1.5µL
DNA Template	1µL
DI Water	21µL

- 4 Vortex the PCR tubes to mix well.
- 5 Place the PCR tube into the Thermal Cycler with the following conditions:

**Lid Temperature: 105°C; Volume: 50µL

A	B	C
98°C	10s	
68°C	5s	Edit: -1°C per cycle
68°C	1 minute	
Go to step 1, repeat the cycle 13 times		
98°C	10s	
55°C	5s	
68°C	1 minute	
Go to step 5, repeat the cycle 25 times		
68°C	5 minutes	
12°C	Infinite Loop (∞)	



- 6 Upon finishing, cancel the current protocol and take out the PCR sample from the Thermal Cycler. Keep the sample in the 4°C fridge for storage. Otherwise, gel electrophoresis may be carried out to isolate the DNA fragment of interest.