

Oct 09, 2023

Colony PCR

DOI

<https://dx.doi.org/10.17504/protocols.io.5jyl8pex8g2w/v1>

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Protocol Citation: NUS iGEM 2023. Colony PCR. protocols.io <https://dx.doi.org/10.17504/protocols.io.5jyl8pex8g2w/v1>

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Protocol status: Working

We use this protocol and it's working

Created: September 28, 2023

Last Modified: October 09, 2023



Protocol Integer ID: 88552

Keywords: Colony PCR, PCR, Polymerase Chain Reaction, Sequencing, Gibson Assembly, preliminary screening of plasmid, plasmid extraction the next day, colony pcr, plasmid extraction, results of the colony pcr, assembled plasmid, correct assembly of dna fragment, plasmid, target dna fragment, presence of target dna fragment, dna fragment, singapore igem team, overnight cell culture, pcr, dna, preliminary screening, sequencing, containing colony, gibson assembly, correct assembly, screening, specific colony

Abstract

2023 NUS-Singapore iGEM team followed this protocol to perform preliminary screening of plasmid-containing colonies without the need for overnight cell culture. This screening aimed to confirm the correct assembly of DNA fragments during Gibson Assembly or the presence of target DNA fragments in the plasmid. After obtaining the results of the colony PCR, the team could then decide whether specific colonies required overnight culturing. This would allow them to perform plasmid extraction the next day, obtain the fully assembled plasmid, and send it out for sequencing to confirm the final structure.

Materials

KOD One™ PCR Master Mix (Blue)

Safety warnings

- !
 - Proper lab PPE must be worn at all times.
 - Since cells are used in this protocol, a Biosafety Cabinet (BSC) is required to ensure safety.

- 1 Circle the colony of interest.



- 2 Prepare the mixture below in a PCR tube, the final volume is  50 μL :

Item	Volume
KOD One™ PCR Master Mix (Blue)	25 μL
Forward Primer	1.5 μL
Reverse Primer	1.5 μL
DI Water	22 μL

- 3 Mix the solution well by vortexing the tubes and then split the solution equally into 5 new PCR tubes (each PCR tube would contain  10 μL of the solution).
- 4 Use an inoculation loop to inoculate some cells from the colony of interest and dunk the inoculation loop into the PCR tube according to the labels.
- 5 Run a PCR with the setting below:

Temperature	Duration
98°C	10s
55°C	5s
68°C	1 minute



	Temperature	Duration
	Go to step 1, repeat the cycle 25 times	

- 6 After the PCR is complete, proceed to run gel electrophoresis for the samples.
- 7 After gel electrophoresis is finished, put the agarose gel onto a UV Sample Tray, next, put it into the Gel Doc EZ System, and then run the gel imaging program on the desktop.
- 8 Check if the expected band occurs.
- 9 Save the gel image and discard the gel into the biohazard bin.