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

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Overlap PCR

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ABSTRACT

2023 NUS-Singapore iGEM team followed this protocol to combine DNA fragments with overlapping regions, thereby reducing the number of fragments used in the Gibson Assembly and thus, improving the success rate of the Gibson Assembly. These overlapping regions are created during the PCR procedures by designing forward and reverse primers for each fragment to contain overlapping sequences.

GUIDELINES

This protocol outlines a general method for combining two DNA fragments with overlapping regions through overlap PCR. The number of fragments to be overlapped can be increased by adjusting the volume ratio between each DNA fragment.

MATERIALS

- KOD OneTM PCR Master Mix (Blue)
- DNA fragments
- DI Water

SAFETY WARNINGS

Proper lab PPE must be worn at all times.

PROTOCOL integer ID:
88524

Keywords: PCR, Overlap PCR, Polymerase Chain Reactions, DNA, DNA Fragments

1 Determine the volume ratio for each DNA fragment required in the reaction to calculate the appropriate volume for each DNA fragment. The final sample volume is 47 µL, with 25 µL being the KOD OneTM PCR Master Mix (Blue). Therefore, the remaining volume (22 µL) will be allocated to the gene fragments and DI Water for topping it up.

*This calculation requires the fragment size and the concentration of the target DNA fragments.

1.1 Divide the bigger fragment size by the smaller fragment size to get the volume ratio of the DNA fragment with the bigger fragment size.

1.2 Divide the concentration of the bigger fragment by the answer obtained in the previous step.

1.3 Divide the number obtained by the concentration of the smaller fragment to get the volume ratio of the DNA fragment with the smaller fragment size.

1.4 The volume ratio of each fragment may be adjusted to reach the final sample volume of 47 µL (DI water will be used to top up the remaining volume).

2 Make the 47 µL PCR sample by adding the following into a PCR tube:

	Item	Volume
	KOD OneTM PCR Master Mix (Blue)	25µL
	DNA Fragment 1	From Calculation
	DNA Fragment 2	From Calculation
	DI Water	Top up the solution to 47µL

3 Place the PCR tube into the Thermal Cycler and set the conditions to:

	Purpose	Temperature	Duration	Remarks
	Denaturation	98°C	10s	
	Annealing	65°C	5s	
	Extension	68°C	10s	10s per every 1kb
	Go to Step 1, repeat the cycle 15 / 20 times			
	Extension	68°C	5 minutes	Time to add primers
	Finish	12°C	Infinite Loop	

4 In Step 5 of the above condition, the "Extension" step, quickly add 1.5 µL of each primer into the PCR tube before the 5-minute duration is completed.

Note

After Step 3, the 2 DNA fragments should have already been combined together. To amplify this combined gene, 2 primers are required. One of the primers should bind to the 5' end of the combined gene, while another primer should bind to the 3' end of the combined gene.

5 After adding the primers, cancel the previous run protocol immediately and reset the conditions of the Thermal Cycler to:

	Purpose	Temperature	Duration	Remarks
	Denaturation	98°C	10s	
	Annealing	65°C	5s	
	Extension	68°C	10s	10s per every 1kb
	Go to Step 1, repeat the cycle 20 / 25 times			
	Extension	68°C	5 minutes	Time to add primers
	Finish	12°C	Infinite Loop	

6 Proceeds to the gel electrophoresis to isolate the combined gene fragment.