2-step PCR mixture and conditions (Barcoded-head primers for seqs pooling) V.2

Version 1 is forked from 2-step PCR mixture and conditions (Barcoded-head primers for seqs pooling)

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VERSION 2

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ABSTRACT

PCR mixture and condition (2X SUPERGREEN PCR MASTER MIX)

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

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PROTOCOL integer ID:
83100

1 Wear glove, clean up the working bench w. 1% bleach

For 1' PCR head-primers

2 Prepare 1' PCR master mixutre for **head-primers** (**prepare 1.2X of solutions for pipetting error if needed**)

PCR mixture for head-primers for each reaction

	A	B	C	D
	Component	Volume	Volume (1.2X)	Final conc.
	Forward Primer (10 µM)	0.5 µl	1.2 µl	0.2 µM
	Reverse Primer (10 µM)	0.5 µl	1.2 µl	0.2 µM
	PowerPol 2X PCR Master Mix	12.5 µl	15 µl	-
	ddH20	10.25 µl	11.1 µl	-
	Total volume	23.75 µl	28.5 µl	-

Note

Negative control ALWAYS NEEDED! For example, if you have 5 PCR reactions to run, prepare master mixture for 6 reactions (5 DNA template + 1 negative control).

3 Mix the 1' PCR master mixture gently by pippeting. Quick spin the tube.

4 Transfer 23.75 µL 1' PCR master mixutre in 8-strip PCR tubes.

5 Add 1.25 µL DNA template in 8-strip PCR tubes, resulting in a 25 µL reaction mixture for 1' PCR.

Note

Negative control contains only 23.75 µL master mixture but not DNA template

6 Mix the reaction mixture gently by tapping the tubes. Quick spin the tubes.

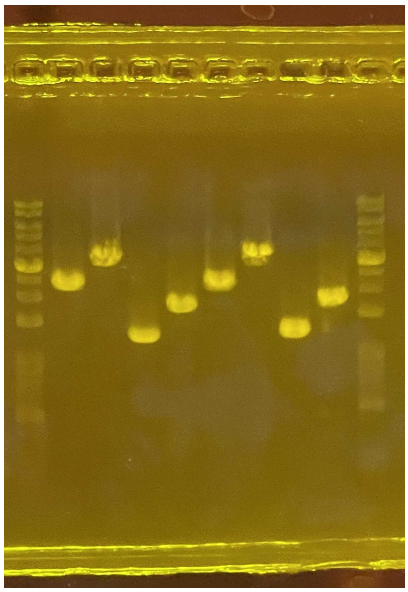
7 Carry out PCR using the following condition:

1' PCR condition for **head-primers**

	A	B	C	D
	Step	Temp	Sec	Cycle
	Initial denaturation	95 °C	180	
	Denaturation	95 °C	30	35 cycles
	Annealing	60-66 °C varied (b)	30	
	Extension	72 °C	180	
	Final extension	72 °C	420	
	Preservation	Preservation	4 °C	∞

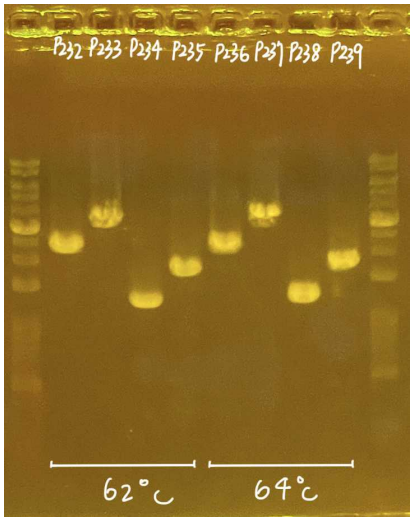
b. Annealing varied, **60-66C** is working; Refer to 1' PCR primers for annealing temperature
c. 1kb ~ 1min extension; enough time allow full extension of sequence

8 Carry out **electrophoresis** for inspection of DNA products



Gel before markdown

9 Markdown wells and upload the pictures to the Lab Google drive



Marked gel picture go to the Lab Google drive

For 2' PCR barcoded-head primers

10 Prepare 2' PCR master mixutre for **barcoded-primers** (**prepare 1.2X of solutions for pipetting error if needed**)

PCR mixture for barcoded-primers for each reaction **(NO PRIMERs at this point!!)**

	A	B	C	D
	Component	Volume	Volume (1.2X)	Final conc.
	ZEJU PCR Master Mix	7.5 µL	9 µL	-
	ddH20	5.55 µL	6.66 µL	-
	Total volume	13.05 µL	15.66 µL	-

Note

Negative control ALWAYS NEEDED! For example, if you have 5 PCR reactions to run, prepare master mixture for 6 reactions (5 DNA template + 1 negative control).

11 Mix the 2' PCR master mixture gently by pippeting. Quick spin the tube.

12 Transfer 13.05 µL of the 2' PCR master mixture to 8-strip PCR tubes.

13 Add 1.2 µL **pre-mixed barcoded-head primers** (Forward + Reverse) to each PCR tubes.

14 Add 0.75 µL of **1' PCR product as template**, resulting in 15 µL reaction mixture for 2' PCR.

Negative control contains only 14.25 µL master mixture and premixed barcoded-head primers but not DNA template

15 Mix gently by tapping the tubes. Quick spin the tubes.

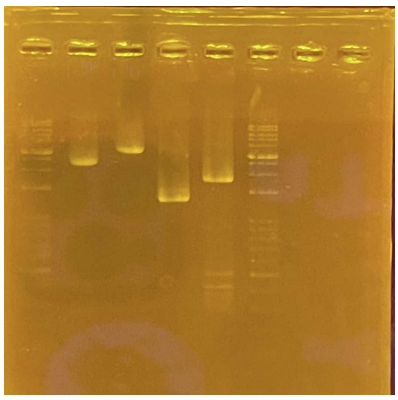
16 Carry out 2' PCR using the following condition:

2' PCR condition for **barcoded-head primers**

	A	B	C	D
	Step	Temp	Sec	Cycle
	Initial denaturation	98 ºC	30	
	Denaturation	98 ºC	15	15 cycles
	Annealing	64-68 ºC varied (a)	15	
	Extension	72 ºC	20 (b)	
	Final extension	72 ºC	210	
	Preservation	Preservation	4 ºC	∞

a. Annealing varied, **65 C** is working based on test on 220531; Refer 2' PCR primers for annealing temperature
b. 1kb ~ 1min extension; enough time allow full extension of sequence

17 Carry out **electrophoresis** for inspection of DNA products



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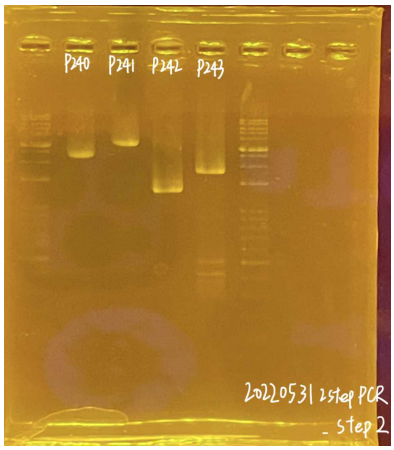

Marked gel picture go to the Lab Google drive