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

Yin-Tse Huang[1], Tsu-Chun Hung[1]

[1]Kaohsiung Medical University

Yin-Tse Huang

VERSION 2

MAR 23, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.j8nlkky3xl5r/v2

Protocol Citation: Yin-Tse Huang, Tsu-Chun Hung 2023. 2-step PCR mixture and conditions (Barcoded-head primers for seqs pooling). **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkky3xl5r/v2Version created by Yin-Tse Huang

MANUSCRIPT CITATION:
Herbold CW, Pelikan C, Kuzyk O, Hausmann B, Angel R, Berry D, Loy A. 2015. A flexible and economical barcoding approach for highly multiplexed amplicon sequencing of diverse target genes. Front. Microbiol. [Internet] 6:731. Available from: http://dx.doi.org/10.3389/fmicb.2015.00731

ABSTRACT

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

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

Created: Mar 23, 2023

Last Modified: Mar 23, 2023

PROTOCOL integer ID:
79299

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)	1.6 µl	1.9 µl	1 µM
	Reverse Primer (10 µM)	1.6 µl	1.9 µl	1 µM
	2X Supergreen PCR Master Mix	7.8 µl	9.4 µl	-
	ddH20	4.1 µl	4.9 µl	-
	Total volume	15 µl	18 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 15 µL 1' PCR master mixutre in 8-strip PCR tubes.

5 Add 0.6 µL DNA template in 8-strip PCR tubes, resulting in a 15.6 µL reaction mixture for 1' PCR.

Note

Negative control contains only 15 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**

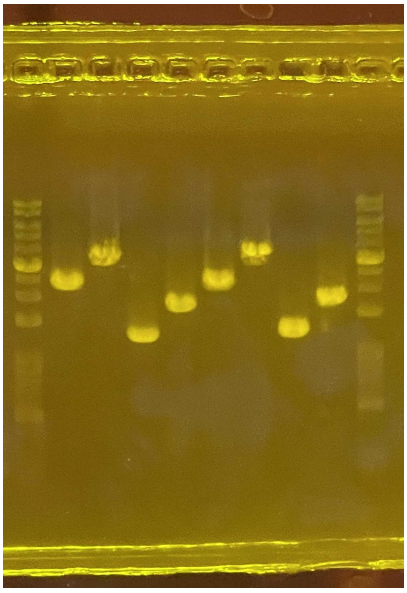
	A	B	C	D
	Step	Temp	Sec	Cycle
	Initial denaturation	95 °C	180	
	Denaturation	98 °C	30	20-25 cycles
	Annealing	60-66 °C varied (b)	30	
	Extension	72 °C	180	
	Final extension	72 °C	210	
	Preservation	Preservation	4 °C	∞

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

7.1 **1' hear-primers** used in Huang lab

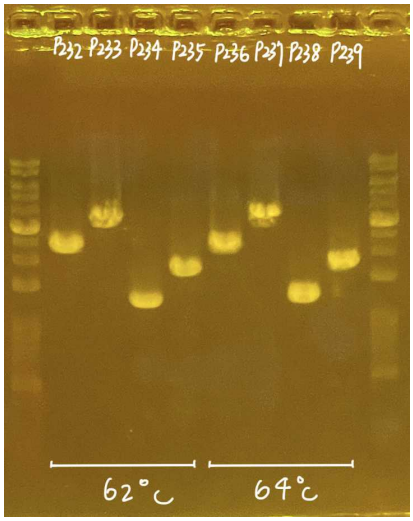
	A	B	C	D
	Name	Sequence	Tm°C	CG%
	NS1B1ngs_H1	GCTATGCGCGAGCTGCcctngttgatyctgccagt	71.7	60
	LR5_H1	GCTATGCGCGAGCTGCtcctgagggaaacttcg	70.2	60.6
	EF1-526F_H1	GCTATGCGCGAGCTGCgtcgtygtyatygghcaygt	71	59.3
	EF1-2218R_H1	GCTATGCGCGAGCTGCatgacaccracrgcracrgtytg	72.2	60.3

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.
	2X Supergreen PCR Master Mix	10.75 µL	12.9 µL	-
	ddH20	10.75 µL	12.9 µL	-
	Total volume	21.5 µL	25.8 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 21.5 µL of the 2' PCR master mixture to 8-strip PCR tubes.

13 Add 2.5 µL **pre-mixed barcoded-head primers** (Forward + Reverse) to each PCR tubes.

14 Add 1 µL of **1' PCR product as template**, resulting in 25 µL reaction mixture for 2' PCR.

Negative control contains only 24 µL master mixture and premixed barcoded-head primers but not DNA template

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

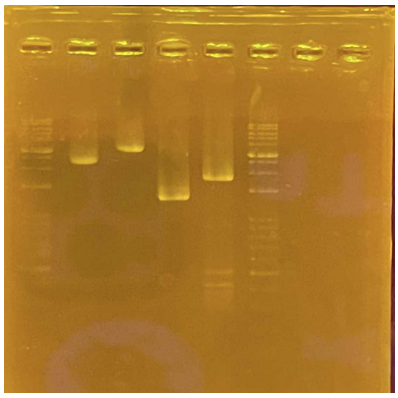
16 Carry out 2' PCR using the following condition:

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

	A	B	C	D
	Step	Temp	Sec	Cycle
	Initial denaturation	98 ºC	30	
	Denaturation	98 ºC	15	10-15 cycles
	Annealing	64-68 ºC varied (a)	15	
	Extension	72 ºC	30 (b)	
	Final extension	72 ºC	210	
	Preservation	Preservation	4 ºC	∞

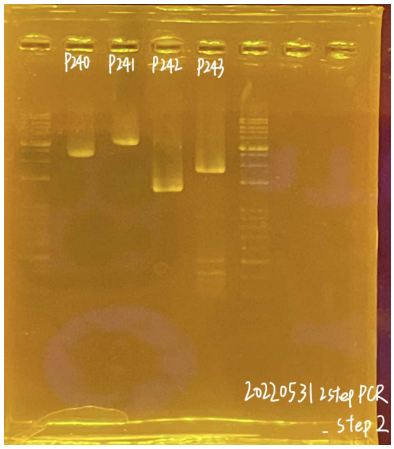
a. Annealing varied, **65 C** is working based on test on 220531; Refer 2' PCR primers for annealing temperature
b. 1kb ~ 1min extension; enough time allow full extension of sequence

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



Gel before markdown

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



Marked gel picture go to the Lab Google drive