



Apr 20, 2023

Version 1

2-step PCR mixture and conditions (Barcoded-head primers for seqs pooling) V.1



Forked from [2-step PCR mixture and conditions \(Barcoded-head primers for seqs pooling\)](#).

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvm263og3p/v1>

Yin-Tse Huang¹, Tsu-Chun Hung¹

¹Kaohsiung Medical University



Tsu-Chun Hung

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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>

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Protocol status: Working

We use this protocol and it's working

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Abstract

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



- 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 μ M)	1 μ l	1.2 μ l	0.4 μ M
	Reverse Primer (10 μ M)	1 μ l	1.2 μ l	0.4 μ M
	ZEJU PCR Master Mix	12.5 μ l	15 μ l	-
	ddH2O	9.25 μ l	11.1 μ l	-
	Total volume	23.75 μ l	28.5 μ l	-

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 μ L 1' PCR master mixutre in 8-strip PCR tubes.
- 5 Add  1.25 μ L DNA template in 8-strip PCR tubes, resulting in a  25 μ L 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
	<i>Initial denaturation</i>	95 °C	180	
	<i>Denaturation</i>	95 °C	30	20-35 cycles
	<i>Annealing</i>	60-66 °C varied (b)	30	
	<i>Extension</i>	72 °C	180	
	<i>Final extension</i>	72 °C	420	
	<i>Preservation</i>	Preservation	4 °C	∞

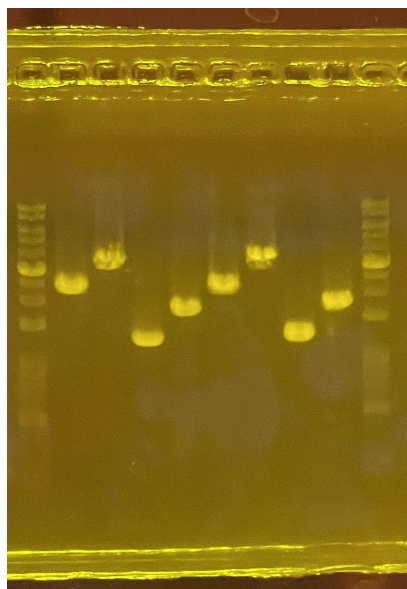
b. Annealing varied, **60-66C** is working; Refer to 1' PCR primers for annealing temperature
c. 1kb ~ 1min extension; enough time allow full extension of sequence

7.1 1' hear-primers used in Huang lab

	A	B	C	D
	Name	Sequence	Tm°C	CG%
	NS1B1ngs_H1	GCTATGCGCGAGCTGCcctngttgatyctg ccagt	71.7	60
	LR5_H1	GCTATGCGCGAGCTGCTcctgagggaaac ttcg	70.2	60.6
	EF1-526F_H1	GCTATGCGCGAGCTGCgtcgtgytyatygg hcaygt	71	59.3

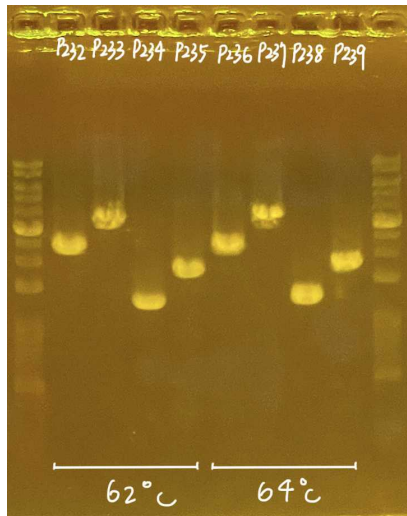
	A	B	C	D
	EF1-2218R_H1	GCTATGCGCGAGCTGCatgacaccracrg cracrgtytg	72.2	60.3

- 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.
ZEJU PCR Master Mix	7.5 µL	9 µL	-
ddH2O	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

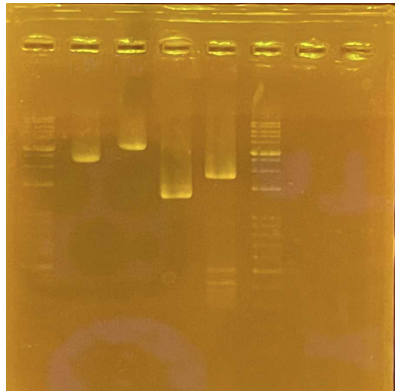
- 15 Mix gently by tapping the tubes. Quick spin the tubes.
- 16 Carry out 2' PCR using the following condition:

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

A	B	C	D
Step	Temp	Sec	Cycle
<i>Initial denaturation</i>	98 °C	30	
<i>Denaturation</i>	98 °C	15	10-15 cycles
<i>Annealing</i>	64-68 °C varied (a)	15	
<i>Extension</i>	72 °C	20 (b)	
<i>Final extension</i>	72 °C	210	
<i>Preservation</i>	Preservation	4 °C	∞

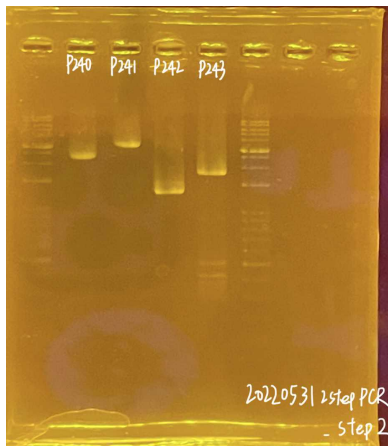
- a. Annealing varied, **65 C** is working based on test on 220531; Refer 2' PCR primers for annealing temperature
- b. 1kb ~ 1min extension; enough time allow full extension of sequence

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



Gel before markdown

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Marked gel picture go to the Lab Google drive