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

[1]Kaohsiung Medical University

Tsu-Chun Hung



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

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**Keywords:** pcr mixture, step pcr mixture, 2x supergreen pcr master mix, head primers for seq, pcr, head primer, primer, seq

## Abstract

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

## Troubleshooting

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.

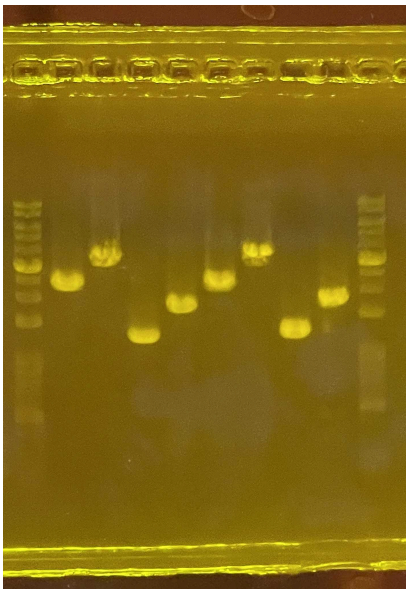
7 Carry out PCR using the following condition:

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

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

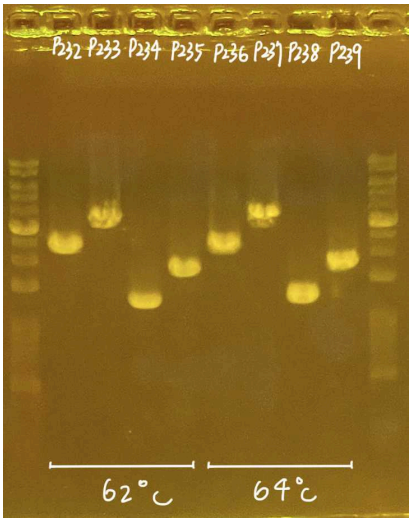
b. Annealing varied, **60-66C** is working; Refer to 1' PCR primers for annealing temperature
c. 1kb ~ 1min extension; enough time allow full extension of sequence

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.

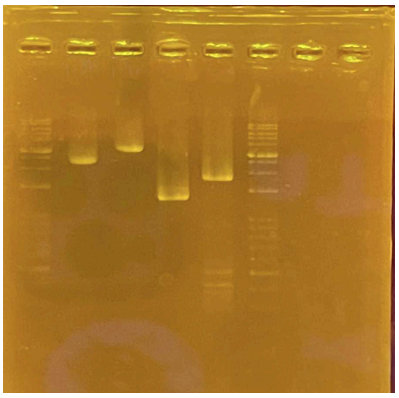
16 Carry out 2' PCR using the following condition:

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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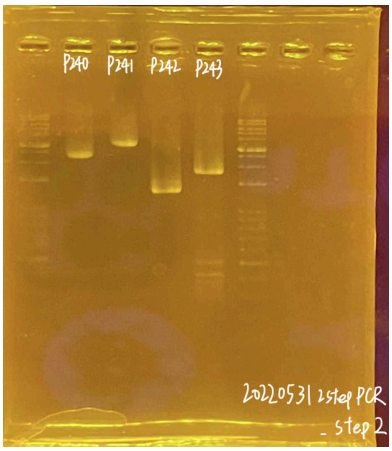


Marked gel picture go to the Lab Google drive