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

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Yin-Tse Huang

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

DOI

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protocol

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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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	-

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.

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:

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

A	B	C	D
Step	**Temp**	**Sec**	**Cycle**
Initial denaturation	95 ºC	30-180 (a)	
Denaturation	98 ºC	15	20-25 cycles
Annealing	64-68 ºC varied (b)	15	
Extension	72 ºC	60-180 (c)	
Final extension	72 ºC	210	
Preservation	Preservation	4 ºC	∞

a. Varied depend on template complexity
b. Annealing varied, **62-68C** 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
ITS4ngs_H1	GCTATGCGCGAGCTGCtcctscgcttattgatatgc	69	55.6
LR5_H1	GCTATGCGCGAGCTGCtcctgagggaaacttcg	70.2	60.6
EF1-526F_H1	GCTATGCGCGAGCTGCgtcgtygtyatygghcaygt	71	59.3
EF1-1567R_H1	GCTATGCGCGAGCTGCachgtrccrataccaccratctt	70.6	56
EF1-2218R_H1	GCTATGCGCGAGCTGCatgacaccracrgcracrgtytg	72.2	60.3
Ben2f_H1	GCTATGCGCGAGCTGCtccagactggtcagtgtgtaa	70.5	56.8
Bt2b_H1	GCTATGCGCGAGCTGCaccctcagtgtagtgacccttggc	74.5	62.5
T22_H1	GCTATGCGCGAGCTGCtctggatgttgttgggaatcc	70.3	56.8
RPB2-3bF_H1	GCTATGCGCGAGCTGCggwggwtayttyatyatyaatgg	65.6	48.7
RPB2-7cR_H1	GCTATGCGCGAGCTGCcccatrgcttgyttrcccat	72.3	59.7
fRPB2-11aR_H1	GCTATGCGCGAGCTGCgcrtggatcttrtcrtcsacc	71.7	60.8

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

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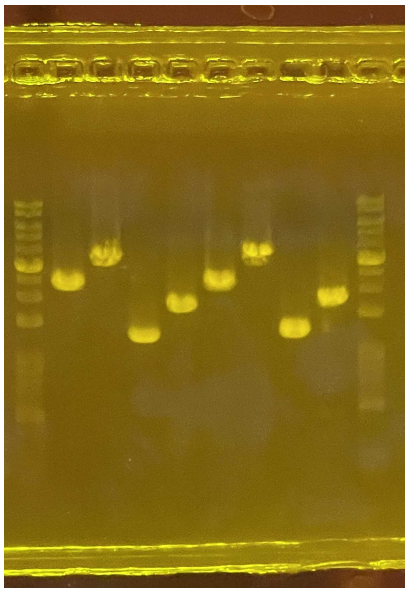
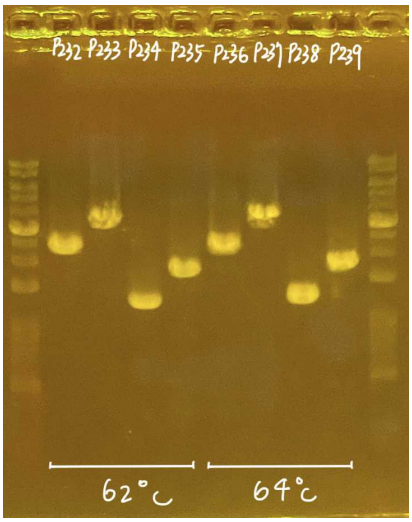


Gel before markdown

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!!)**

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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	-

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**

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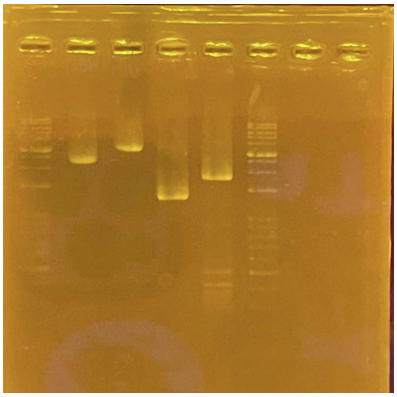
A	B	C	D
Step	**Temp**	**Sec**	**Cycle**
Initial denaturation	98 ºC	30	
Denaturation	98 ºC	15	10-15 cycles
Annealing	64-68 ºC varied (a)	15	
Extension	72 ºC	60 (b)	
Final extension	72 ºC	210	
Preservation	Preservation	4 ºC	∞

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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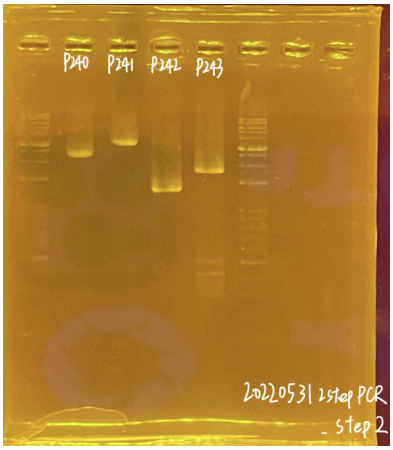

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