2-step PCR mixture and conditions (Barcoded-head primers for seqs pooling) V.5

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May 31, 2022

protocol .

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

Yin-Tse Huang 2022. 2-step PCR mixture and conditions (Barcoded-head primers for seqs pooling). **protocols.io** https://protocols.io/view/2-step-pcr-mixture-and-conditions-barcoded-head-pr-cacisaue

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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.

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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:

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

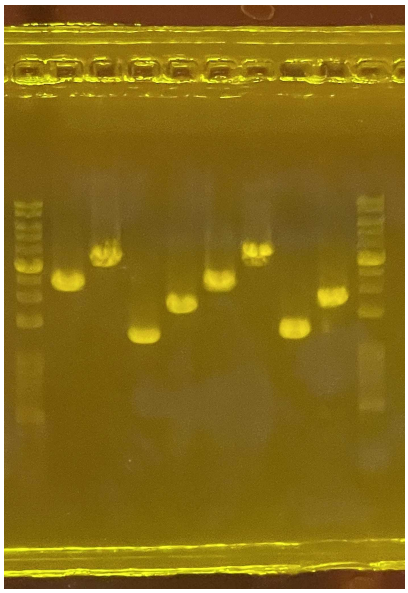
A	B	C	D
Step	**Temp**	**Sec**	**Cycle**
Initial denaturation	95 ºC	30-180 (a)	
Denaturation	98 ºC	15	20-25 cycles
Annealing	64-68 ºC varied (b)	15	
Extension	72 ºC	60-180 (c)	
Final extension	72 ºC	210	
Preservation	Preservation	4 ºC	∞

a. Varied depend on template complexity
b. Annealing varied, **62-65C** is working based on test on 220530; 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

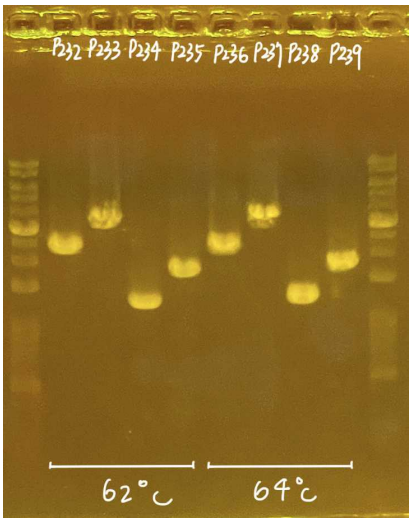
A	B	C	D
Name	**Sequence**	**Tm°C**	**CG%**
NS1B1ngs_H1	gctatgcgcgagctgccctngttgatyctgccagt	71.7	60
ITS4ngs_H1	gctatgcgcgagctgctcctscgcttattgatatgc	69	55.6
LR5_H1	gctatgcgcgagctgctcctgagggaaacttcg	70.2	60.6
EF1-526F_H1	gctatgcgcgagctgcgtcgtygtyatygghcaygt	71	59.3
EF1-1567R_H1	gctatgcgcgagctgcachgtrccrataccaccratctt	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



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	-

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 PCR tubes.

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

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**

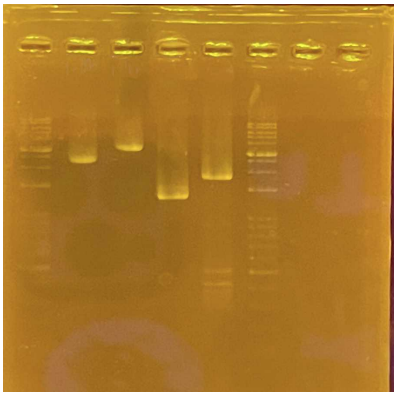
A	B	C	D
Step	**Temp**	**Sec**	**Cycle**
Initial denaturation	98 ºC	30	
Denaturation	98 ºC	15	10-15 cycles
Annealing	64-68 ºC varied (a)	15	
Extension	72 ºC	60 (b)	
Final extension	72 ºC	210	
Preservation	Preservation	4 ºC	∞

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

16.1 **2' barcoded-head primers** used in Huang lab

A	B	C	D
Name	**Sequence**	**Tm°C**	**CG%**
F1-1	aagaaagttgtcggtgtctttgtggctatgcgcgagctgc	70.3	52.5
F1-2	tcgattccgtttgtagtcgtctgtgctatgcgcgagctgc	70.9	55
F1-3	caggtagaaagaagcagaatcggagctatgcgcgagctgc	70	55
F1-4	ttcggattctatcgtgtttccctagctatgcgcgagctgc	69.2	52.5
F1-5	cttgtccagggtttgtgtaaccttgctatgcgcgagctgc	70.7	55
F1-6	ttctcgcaaaggcagaaagtagtcgctatgcgcgagctgc	71.3	55
F1-7	gtgttaccgtgggaatgaatccttgctatgcgcgagctgc	70.6	55
F1-8	ttcagggaacaaaccaagttacgtgctatgcgcgagctgc	70.2	52.5
R1-1	gattctgattactctattcgccaggctatgcgcgagctgc	68.5	52.5
R1-2	ggaataataccattgaagtagcacgctatgcgcgagctgc	67.5	50
R1-3	ttgctacggttgaccatgcagttagctatgcgcgagctgc	71.4	55
R1-4	aacttgaggtatcgtatattcaatgctatgcgcgagctgc	65.2	45
R1-5	gggtccctctactcatttagcatggctatgcgcgagctgc	71.4	57.5
R1-6	cagagctgaccctccagatatttggctatgcgcgagctgc	71.5	57.5
R1-7	atagctgaagcaatctacctatcggctatgcgcgagctgc	69.2	52.5
R1-8	cagagtaagggtataggttcggcagctatgcgcgagctgc	71.1	57.5
R1-9	caatcaacgaattagatgtcgggtgctatgcgcgagctgc	69.1	52.5
R1-10	gaccttagtcacatggtagtctaagctatgcgcgagctgc	68	52.5
R1-11	gttcggatgcaatatggttcactggctatgcgcgagctgc	70.7	55
R1-12	tagcagaagtccctgtaagaccatgctatgcgcgagctgc	70.7	55

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



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