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

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

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<https://dx.doi.org/10.17504/protocols.io.n92ld6rd7g5b/v1>

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Protocol status: Working

We use this protocol and it's working

Created: September 02, 2025

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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.
	PowerPol 2X PCR Master Mix	12.5 µL	15 µL	1X
	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
	ddH ₂ O	10.5 µL	11.1 µL	-
	Total volume	24 µL	28.5 µL	

	A	B	C	D
	Component	Volume	Volume (1.2X)	Final conc.
	2X KAPA HiFi HotStart ReadyMix	12.5 µL	15 µL	1X
	Forward Primer (10 µM)	0.75 µL	0.9 µL	0.3 µM
	Forward Primer (10 µM)	0.75 µL	0.9 µL	0.3 µM
	ddH ₂ O	10 µL	12 µL	-
	Total volume	24 µL	28.8 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).



- 3 Mix the 1' PCR master mixture gently by pipetting. Quick spin the tube.
- 4 Transfer  24 μL 1' PCR master mixutre in 8-strip PCR tubes.
- 5 Add  1 μL DNA template in 8-strip PCR tubes, resulting in a  25 μL reaction mixture for 1' PCR.



Note

Negative control contains only  24 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:

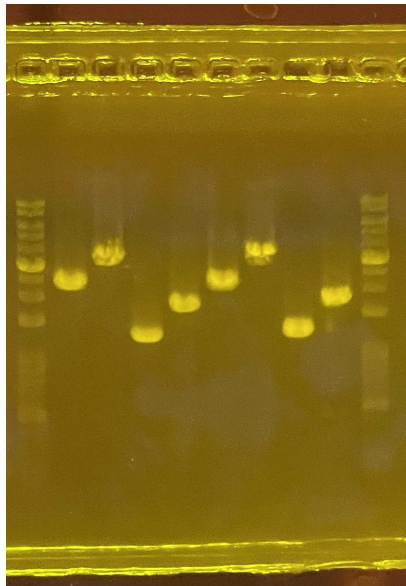
1' PCR condition for **head-primers**

	A	B	C	D
	Step	Temp	Sec	Cycle
	<i>Initial denaturation</i>	95 °C	180	
	<i>Denaturation</i>	95 °C	30	25 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

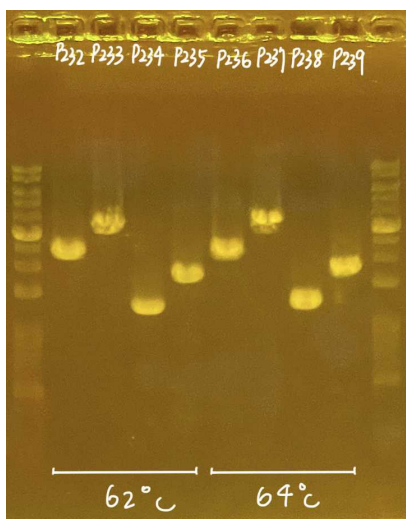
c. 1kb ~ 1min extension; enough time allow full extension of sequence

- 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:



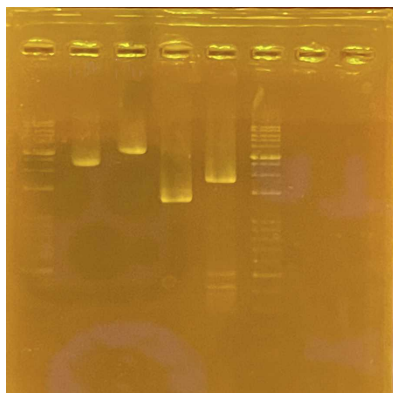
2' PCR condition for **barcoded-head primers**

	A	B	C	D
	Step	Temp	Sec	Cycle
	<i>Initial denaturation</i>	98 °C	30	
	<i>Denaturation</i>	98 °C	15	12 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

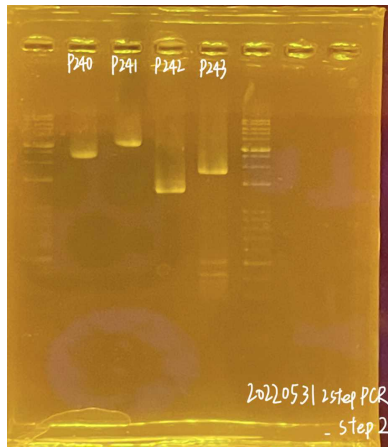
b. 1kb ~ 1min extension; enough time allow full extension of sequence

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



Gel before markdown

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