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Apr 24, 2022

PCR mixture and condition (2X SUPERGREEN PCR MASTER MIX) V.1

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protocol .



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Yin-Tse Huang 2022. PCR mixture and condition (2X SUPERGREEN PCR MASTER MIX). **protocols.io**

<https://protocols.io/view/pcr-mixture-and-condition-2x-supergreen-pcr-master-b745rqy6>



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1 PCR mixture for **generic primers**

A	B	C
Component	Volume	Final conc.
Template (< 1 µg)	X µL	-
Forward Primer (10 µM)	1 µL	0.4 µM
Reverse Primer (10 µM)	1 µL	0.4 µM
2x Master Mix	12.5 µL	-
ddH2O	up to 25 µL	-
Total volume	25 µL	-

1.1 PCR condition for **generic primers**

A	B	C	D
Step	Temp	Sec	Cycle
Initial denaturation	95 °C	180	
Denaturation	95 °C	30	35 cycles
Annealing	53-58 °C	30	
Extension	72 °C	60	
Final extension	72 °C	210	
Preservation	4 °C	∞	

2 PCR mixture for **barcoded primers**

A	B	C
Component	Volume	Final conc.
Template (< 1 µg)	X µL	-
Forward Primer (10 µM)	5 µL	2 µM
Reverse Primer (10 µM)	5 µL	2 µM
2x Master Mix	12.5 µL	-
ddH2O	up to 25 µL	-
Total volume	25 µL	-

2.1 PCR condition for **barcoded primers**

A	B	C	D
Step	Temp	Sec	Cycle
Initial denaturation	95 °C	180	
Denaturation	98 °C	20	35 cycles
Annealing	64 °C		
Extension	72 °C		
Final extension	72 °C	210	
Preservation	Preservation	4 °C	∞