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PCR mixture and condition (2X SUPERGREEN PCR MASTER MIX) V.3

Yin-Tse Huang¹¹Kaohsiung Medical University

protocol .



Yin-Tse Huang

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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-b755rq86>

Yin-Tse Huang



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For generic primers

1 PCR mixture PCR condition 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 | - |
| ddH ₂ O | up to 25 µL | - |
| Total volume | 25 µL | - |

2 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 | ∞ | |

For barcoded primers

3 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 | - |

4 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 | 15 | |
| Extension | 72 °C | 210 | |
| Final extension | 72 °C | 210 | |
| Preservation | Preservation | 4 °C | ∞ |