# MAT KO PCR

## Introduction

The primers contain homologous sequences upstream (F) and downstream (R) of MAT and bind to the Universal F1 or Universal R1 region flanking His3 on OLP45. The PCR product is used to delete MAT locus with His3.

## Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM OLPr008 MAT -33 UKO F
- 10 µM OLPr012 MAT +34 SEY UKO R
- Template: OLP045

## Procedure

### PCR

1. Chose the number of 50 µL reactions (include one additional reaction for no-template control):

**Settings**

| | # of reactions |
|---|---|
| 1 | 37 |

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies):

**Master mix**

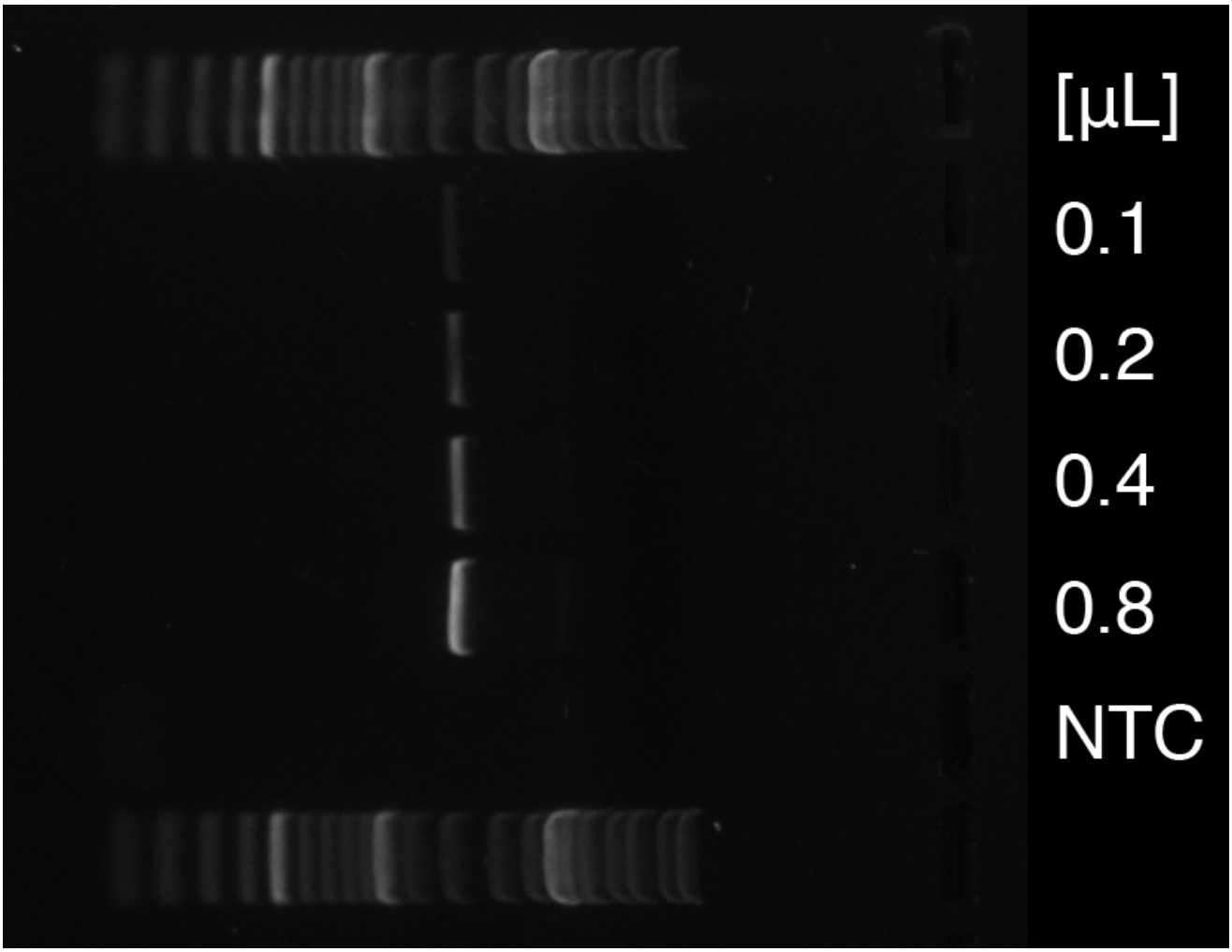
| | A | B |
|---|---|---|
| 1 | | **37.5 X [µL]** |
| 2 | ddH2O | 1068.75 |
| 3 | 10X LA PCR Buffer | 187.5 |
| 4 | 25 mM MgCl2 | 187.5 |
| 5 | dNTP Mix (2.5 mM each) | 300 |
| 6 | 10 µM OLPr008 MAT -33 UKO F | 37.5 |
| 7 | 10 µM OLPr012 MAT +34 SEY UKO R | 37.5 |
| 8 | LA Taq Polymerase (5 U/µL) | 18.75 |
| 9 | OLP045 (pFA6a-His3MX6) (5 ng/µL) | 37.5 |
| 10 | **Total** | **1875** |

- [x] 3. PCR program: 94°C/2 min—[94°C/0.5 min—61°C/0.5 min—68°C/1.5 min]x30—68°C/5 min—10°C/∞

## Gel

- [x] 4. Gel: sample volume see below/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

    Load 0.1, 0.2, 0.4 and 0.8 µL of the PCR product (i.e. by diluting PCR product 1:10 by mixing 2 µL product with 18 µL $H_2O$, then load 1, 2, 4, and 8 µL of the diluted product).
    Load 10 µL of no-template control.
    Expected product size: 1447 bp.
    Estimated concentration of PCR product: 60 ng/0.6 µL = 100 ng/µL.




## Additional Resources

## Primers and template

| | A | B |
|---|---|---|
| 1 | Forward Primer | OLPr008 MAT -33 UKO F |
| 2 | Reverse Primer | OLPr012 MAT +34 SEY UKO R |
| 3 | Template | OLP045 (pFA6a-His3MX6) |

## PCR Volume and reagent concentrations

| | A | B |
|---|---|---|
| 1 | PCR Volume [µL] | 50 |
| 2 | Starting buffer conc [X] | 10 |
| 3 | Final buffer conc [X] | 1 |
| 4 | Starting  Mg2+ conc [mM] | 25 |
| 5 | Final Mg2+ conc [mM] | 2.5 |
| 6 | Starting dNTP conc each [mM] | 2.5 |
| 7 | Final dNTP conc each [mM] | 0.4 |
| 8 | Starting Primer F conc [µM] | 10 |
| 9 | Final Primer F conc [µM] | 0.2 |
| 10 | Starting Primer R conc [µM] | 10 |
| 11 | Final Primer R conc [µM] | 0.2 |
| 12 | Starting Polymerase conc [U/µL] | 5 |
| 13 | Final Polymerase conc [U/µL] | 0.05 |
| 14 | Starting Template conc [ng/µL] | 5 |
| 15 | Final Template conc [ng/µL] | 0.1 |

## Single PCR reaction

| | A | B |
|---|---|---|
| 1 | | [µL] |
| 2 | ddH2O | 28.5 |
| 3 | 10X PCR Buffer | 5 |
| 4 | 25 mM MgCl2 | 5 |
| 5 | dNTP Mix (2.5 mM each) | 8 |
| 6 | 10 µM Primer F | 1 |
| 7 | 10 µM Primer R | 1 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.5 |
| 9 | Template (5 ng/µL) | 1 |
| 10 | Total | 50 |