# ste4 Colony PCR

## Introduction

For genotyping of ste4 locus: the primers bind upstream (F) and downstream (R) of ste4, and PCR is used to determine if TRP1 has replaced ste4 by homologous recombination.

## Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr022 STE4 -133 F
- 10 µM Primer R: OLPr023 STE4 1402 R
- SC-Trp plates
- Template: yeast colonies transformed with ste4 KO PCR product

## Procedure

### PCR

1. Chose the number of 10 µL reactions (include 3 additional reactions to account for wild-type, mutant, and no-template control):

**Settings**

| | # of reactions |
|---|---|
| 1 | 0.5 |

2. Combine the following reagents to prepare the master mix (contains 0.5 additional reaction to account for pipetting discrepancies), and aliquot 10 µL master mix/pcr tube:

**Master mix**

| | A | B |
|---|---|---|
| 1 | | **1 X [µL]** |
| 2 | ddH2O | 5.9 |
| 3 | 10X LA PCR Buffer | 1 |
| 4 | 25 mM MgCl2 | 1 |
| 5 | dNTP Mix (2.5 mM each) | 1.6 |
| 6 | 10 µM OLPr022 STE4 -133 F | 0.2 |
| 7 | 10 µM OLPr023 STE4 1402 R | 0.2 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.1 |
| 9 | **Total** | **10** |

3. In sterile work environement (e.g. laminar flow), gently touch a yeast colony with a 10 µL pipette tip so a small number of cells stick to it, touch the tip to a fresh reference SC-Trp plate, and resuspend cells in PCR reaction.

   Do not use too many cells as they might inhibit the PCR reaction. The PCR reaction should only look slightly opaque from the resuspended cells. If the reaction looks milky then you might have used too many cells.
   The reference plate is incubated 30°C/2–3 days.

4. PCR program "STE4COLO" in folder "STEPHAN":

   94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/1.5 min]x35—68°C/5 min—10°C/∞

## Gel

5. Gel: 10 µL/0.5 µg O'GeneRuler DNA Ladder Mix/1% TAE/120 V/35 min

   00:35:00

   Expected product sizes:
   ste4 1535 bp
   ste4Δ::TRP1 1232 bp
   Stop processing any yeast strains with the wrong genotype (i.e. discard any corresponding overnight cultures and patches on reference plate).

## Additional resources

**Primers and template**

| | A | B |
|---|---|---|
| 1 | Forward Primer | OLPr022 STE4 -133 F |
| 2 | Reverse Primer | OLPr023 STE4 1402 R |
| 3 | Template | yeast colonies |

**PCR Volume and reagent concentrations**

| | A | B |
|---|---|---|
| 1 | PCR Volume [µL] | 10 |
| 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 |

**Single PCR reaction**

| | A | B |
|---|---|---|
| 1 | | [µL] |
| 2 | ddH2O | 5.9 |
| 3 | 10X PCR Buffer | 1 |
| 4 | 25 mM MgCl2 | 1 |
| 5 | dNTP Mix (2.5 mM each) | 1.6 |
| 6 | 10 µM Primer F | 0.2 |
| 7 | 10 µM Primer R | 0.2 |
| 8 | LA Taq Polymerase (5 U/µL) | 0.1 |
| 9 | Template (5 ng/µL) | 0 |
| 10 | Total | 10 |