MAT Colony PCR

Introduction

For genotyping of MAT locus: the primers bind upstream (F) and downstream (R) of MAT locus, and PCR is used to determine if His3 has replaced MAT by homologous recombination.

Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr014 MAT -283 F
- 10 µM Primer R: OLPr015 MAT +295 R
- SC-His-Trp plates
- Template: yeast colonies from transformation of STE4Δ::TRP1 strains with MAT 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	20

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		**20.5 X [µL]**
2	ddH2O	120.95
3	10X LA PCR Buffer	20.5
4	25 mM MgCl2	20.5
5	dNTP Mix (2.5 mM each)	32.8
6	10 µM OLPr014 MAT -283 F	4.1
7	10 µM OLPr015 MAT +295 R	4.1
8	LA Taq Polymerase (5 U/µL)	2.05
9	**Total**	**205**

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-His-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 "MATCOLON" in folder "STEPHAN":

   94°C/5 min—[94°C/0.5 min—61°C/0.5 min—68°C/3 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:

MATa	2974 bp
MATα	3085 bp
MATΔ::His3	1958 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	OLPr014 MAT -283 F
2	Reverse Primer	OLPr015 MAT +295 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