ste4 KO PCR

Introduction

The primers contain homologous sequences upstream (F) and downstream (R) of ste4 and bind to the Universal F1 or Universal R1 region flanking TRP1 on OLP44. The PCR product is used to delete ste4 with TRP1.

Materials

- TaKaRa LA Taq® DNA Polymerase (Mg2+ free buffer) RR002A
- 10 µM Primer F: OLPr010 STE4 -37 UKO F
- 10 µM Primer R: OLPr011 STE4 1309 UKO R
- Template: OLP044

Procedure

PCR

1. Chose the number of 50 µL reactions:

Settings

	# of reactions
1	31

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		**31.5 X [µL]**
2	ddH2O	897.75
3	10X LA PCR Buffer	157.5
4	25 mM MgCl2	157.5
5	dNTP Mix (2.5 mM each)	252
6	10 µM OLPr010 STE4 -37 UKO F	31.5
7	10 µM OLPr011 STE4 1309 UKO R	31.5
8	LA Taq Polymerase (5 U/µL)	15.75
9	OLP044 (pFA6a-TRP1) (5 ng/µL)	31.5
10	**Total**	**1575**

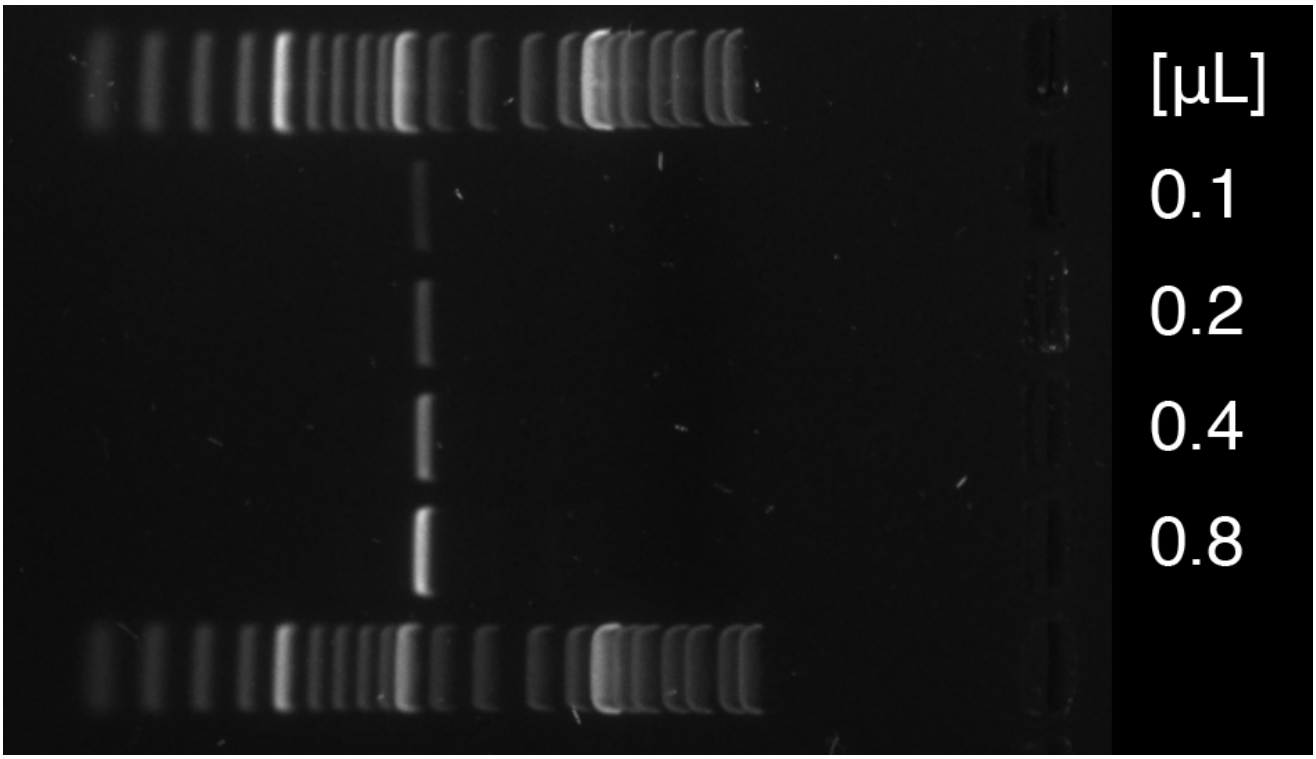
- [x] 3. PCR program: 94°C/2 min—[94°C/0.5 min—61°C/0.5 min—68°C/1 min]x30—68°C/5 min—10°C/∞

Gel

- [x] 4. Pool all PCR reactions.

- [x] 5. 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).
    Expected product size: 1043 bp.
    Estimated concentration of PCR product: 60 ng/0.6 µL = 100 ng/µL



Additional Resources

Primers and template

	A	B
1	Forward Primer	OLPr010 STE4 -37 UKO F
2	Reverse Primer	OLPr011 STE4 1309 UKO R
3	Template	OLP044 (pFA6a-TRP1)

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