

# 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 (include one additional reaction for 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):

Master mix		
	A	B
1		1 X [µL]
2	ddH2O	28.5
3	10X LA PCR Buffer	5
4	25 mM MgCl2	5
5	dNTP Mix (2.5 mM each)	8
6	10 µM OLPr010 STE4 -37 UKO F	1
7	10 µM OLPr011 STE4 1309 UKO R	1
8	LA Taq Polymerase (5 U/µL)	0.5
9	OLP044 (pFA6a-TRP1) (5 ng/µL)	1
10	Total	50

3. PCR program "STE4KO" in folder "STEPHAN":

## Gel

4. Pool all PCR reactions except for controls.

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<sub>2</sub>O, then load 1, 2, 4, and 8 µL of the diluted product).

Load 10 µL of no-template control.

Expected product size: 1043 bp

## Additional Resources

### Primers and template

	A	B
1	Forward Primer	<a href="#">OLPr010 STE4 -37 UKO F</a>
2	Reverse Primer	<a href="#">OLPr011 STE4 1309 UKO R</a>
3	Template	<a href="#">OLP044 (pFA6a-TRP1)</a>



### 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 Mg <sup>2+</sup> conc [mM]	25
5	Final Mg <sup>2+</sup> 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

