PCR amplification

1. Design primers to amplify the desired DNA sequence from template. Add restriction recognition sites of the enzymes that will be used in cloning at 5' ends of the primers, with a few extra nucleotides prior to the restriction site to increase restriction efficiency. Use a Tm calculator tool (e.g. https://www.thermofisher.com/us/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/tm-calculator.html) to design primer pair such that the Tm values differ no more than 5°C.

   More tips: (https://www.thermofisher.com/fi/en/home/products-and-services/product-types/primers-oligos-nucleotides/invitrogen-custom-dna-oligos/primer-design-tools.html)

2. Prepare the mixture for PCR reaction:

2X Phusion Master Mix	25µL
Template DNA	0.6µL
Primer Fw*	0.5µL
Primer Rv*	0.5µL
H_2O	23.4µL
Total	50µL

*if 50µM

Phusion High-Fidelity PCR Master Mix (2X) contains Phusion DNA Polymerase, nucleotides and optimized reaction buffer including $MgCl_2$.

Forward primer (used to amplify Smt3) : TATCATATGGGATCGGACTCAGAAGTC
Reverse primer (used to amplify Smt3): TGATCTCGAGTTAGGATCCACCAATCTGTTC

3. Run the PCR reaction with a suitable program, e.g.:

	Temperature	Time
1.	98°C	30s
2.	98°C	10s
3.	46°C	30s
4.	72°C	5s
5.	repeat steps ii-iV 3 times	
6.	98°C	5s
7.	55°C	20s
8.	72°C	10s
9.	repeat steps vi-viii 21 times	
10.	72°C	2min
11.	4°C	Forever

Preparation of an agarose gel (1%) & electrophoresis

1. Weigh 0.6g of agarose in an Erlenmeyer flask.
2. Add 60mL of 1X TAE buffer and heat up the mixture in the microwave oven so that the agarose has completely dissolved.
3. Cool down the solution and add 1 drop of ethidium bromide or 6µL of SYBR Safe DNA Gel Stain (ThermoFisher Scientific, no. S33102).
4. Pour the liquid into a casting tray, place a comb (or two combs) in the tray and let the gel solidify.
5. Once solidified, place the gel in a gel electrophoresis box, remove the combs and cover the gel with 1X TAE buffer.

6. Load a DNA ladder (used: ThermoFisher Scientific GeneRuler DNA Ladder Mix, ready-to-use, no. SM0333) and the samples (see: sample preparation*) into the wells.
7. Connect the electrodes to a power supply (negative electrode is the end where the samples are loaded and positive electrode is the other end of the gel towards which the samples run).
8. Run the gel electrophoresis at 110V.

Sample preparation:

1. Add 1μL of 6X DNA Loading Dye (used: ThermoFisher Scientific DNA Gel Loading Dye (6X), no. R0611) in 5μL of DNA sample.

PCR product clean-up

1. Follow the PCR clean-up protocol in the Macherey-Nagel NucleoSpin Gel and PCR Clean-up kit.

DNA extraction from an agarose gel

1. Cut the DNA band of the correct size from the agarose gel under UV light.
2. Weigh the gel piece and extract DNA according to the protocol in the Macherey-Nagel NucleoSpin Gel and PCR Clean-up kit.