PCR using Q5 High Fidelity

Introduction

We used this protocol in order to increase the amount of our Toehold Switches with PCR. For this procedure we used NEB's Q5 High-Fidelity 2x Master Mix.

For more information about this protocol check the following link: [https://international.neb.com/protocols/2012/12/07/protocol-for-q5-high-fidelity-2x-master-mix-m0492]

Materials

- Materials
  - Q5® High-Fidelity 2X Master Mix
  - DNA template
  - Primers
  - Nuclease-free H_2O
  - Mineral oil
- Equipment
  - PCR thermocycler
  - Centrifuge
  - Microcentrifuge tubes
  - Pipette & tips

Procedure

Preparation of the reagents

1. Assemble the following reactions on ice:

Reagents for a PCR reaction

	Component	25 ul Reaction
1	Q5 High-Fidelity 2X Master Mix	7.5 ul
2	10 µM Forward Primer	0.75 ul
3	10 µM Reverse Primer	0.75 ul
4	Mg 2+	0.6 ul
5	Template DNA	15 ng
6	Nuclease-Free Water	till 15 ul

According to the manufacturer's protocol assembling the reagents on ice and then transfering the reactions to a preheated to the denaturation temperature thermocycler is recommended.
The Master Mix contains Mg^{2+} and when the recommended quantities are used, the concentration of Mg^{2+} is 2 mM. If the DNA template contains EDTA, you can add more Mg^{2+} to compensate for the Mg^{2+} that is chelated. Since our g-blocks were resuspended in TE that contains EDTA, we added 0.6 ul of a 25 mM Mg2+ solution.

2. Spin the tubes shortly, in order to collect the mixture at the bottom.

3. Apply a thin layer of mineral oil, if the thermocycler you are using does not have a heated lid, in order to avoid evaporation of the reaction components. We added 20 ul of mineral oil for a 15 ul reaction.

4. Short spin again.

Instructions for PCR conditions

5. Insert the tubes into the thermocycler and choose the appropriate settings. The settings that seemed to work for our experiments are the following:

General instructions for PCR conditions

	Step	Temperature	Time
1	Initial Denaturation	98 °C	2 min
2	35 cycles	98 °C	20 s
3		71 °C	35 s
4		72 °C	35 s
5	Final Extension	72 °C	2 min
6	Hold	4 °C	