Oct 09, 2019

Toehold switch assembly

Junyan Qian[1]

[1]EPFL

1 Works for me dx.doi.org/10.17504/protocols.io.72bhqan

iGEM EPFL

Junyan Qian

ABSTRACT

General toehold assemble method using NEB Q5 High-Fidelity 2x Master Mix. Here we took Boir Noir toeholds as an example, it has four candidates we wanted to test. In order to have a higher yield, run 5 reactions for each candidates.

MATERIALS

NAME	CATALOG #	VENDOR
Q5 High-Fidelity 2X Master Mix - 500 rxns	M0492L	New England Biolabs
UltraPure™ DNase/RNase-Free Distilled Water	10977015	Thermo Fisher Scientific
Toehold primer	View	IDT
Commin primer	View	IDT
DNA template	View	

PCR preparation

1 Prepare 4 effendorfs label them, each one will contain the master mix of a certain toehold primer.
Attention: Keep the polymerase on ice avoiding denaturation.
(Order of pipetting: H_2O -> Q5 MasterMix -> primers)

Number of reactions per Master mix:	5				
Reaction Volume:	25				
	Reference (25 uL)	Master mix1	Master mix2	Master mix3	Master mix4
DNA template(~60ng/uL)	1				
Common primer (10µM)	1.25	6.25	6.25	6.25	6.25
Toehold_primer_1(10µM)	1.25	6.25			
Toehold_primer_2(10µM)			6.25		
Toehold_primer_3(10µM)				6.25	
Toehold_primer_4(10µM)					6.25
Q5 MasterMix	12.5	62.5	62.5	62.5	62.5
H2O (DNAse RNAse free distill)	9	45	45	45	45
Total	25	120	120	120	120

2 Pipette up and down mix the master mix thoroughly.

3 Take 20 PCR tubes label them, pipette 24 µl of the master mix into the tubes accordingly.

4 Add 1 µl of DNA template into each PCR tubes

PCR

5 PCR machine setting: Calculate the reaction temperature according to the primer sequence using NEB online Tm calculator: http://tmcalculator.neb.com/#!/main

Template	Fw Primer	Rev Primer	Length	Elongation time	Tm - Q5	Tm used
DNA template	Toehold primer	Common primer	900-1000	30s	T1/T2	T = Average (T1,T2)

6 Thermo cycle setting:

Initial Denaturation	98°C	30 seconds
25–35 Cycles	98°C	5–10 seconds
	T°C	10–30 seconds
	72°C	20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4°C	

Check detail :https://international.neb.com/protocols/2012/12/07/protocol-for-q5-high-fidelity-2x-master-mix-m0492

7 Start the PCR reaction.