

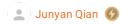
Toehold switch assembly

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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: H₂O -> Q5 MasterMix -> primers)

Nl f	-				
Number of	5				
reactions per					
Master mix:					
Reaction Volume:	25				
	Reference (25 uL)	Master mix1	Master mix2	Master mix3	Master mix4
DNA	1				
template(~60ng/u L)					
Common primer	1.25	6.25	6.25	6.25	6.25
(10µM)					
Toehold_primer_1(1.25	6.25			
10μΜ)					
Toehold_primer_2(6.25		
10μΜ)					
Toehold_primer_3(6.25	
10μM)					
Toehold_primer_4(6.25
10μM)					
Q5 MasterMix	12.5	62.5	62.5	62.5	62.5
H20 (DNAse	9	45	45	45	45
RNAse free distill)					
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 μ l 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.

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