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Toehold switch assembly

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Protocol status: Working

We use this protocol and it's working

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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

MATERIALS

⊗ Q5 High-Fidelity 2X Master Mix - 500 rxns **New England Biolabs Catalog #M0492L**

⊗ UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Scientific Catalog #10977015**

⊗ Toehold primer **IDT**

⊗ Commin primer **IDT**

⊗ DNA template



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)

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 (10μ M)	1.25	6.25	6.25	6.25	6.25	
Toehold primer _1(10μ M)	1.25	6.25				
Toehold primer _2(10 μM)			6.25			
Toehold primer _3(10 μM)				6.25		

Toehold primer_4 (10 μ M)					6.25
Q5 MasterMix	12.5	62.5	62.5	62.5	62.5
H ₂ O (DNAse RNAs e 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 μ l 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 T_m calculator: <http://tmcalculator.neb.com/#!/main>

Template	Fw Primer	Rev Primer	Length	Elongation time	T _m - Q5	T _m used
DNA template	Toehold primer	Common primer	900-1000	30s	T ₁ /T ₂	T = Average (T ₁ , T ₂)

- 6 Thermo cycle setting:



	Initial Denaturation	98°C	30 seconds
		98°C	5–10 seconds
	25–35 Cycles	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.