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🌐 Toehold switch assembly

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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

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- 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 Contributed by users

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 (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 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 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>

Templat e	Fw Prime r	Rev Prime r	Lengt h	Elong ation time	Tm - Q5	Tm used
DNA template	Toeh old prime r	Com mon prime r	900-1000	30s	T1/T2	T = Avera ge (T1,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.