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August 24 2017 : *PCR T7 terminator to toehold LacZalpha*

1. Aim:

Adding the T7 terminator to the LacZalpha toehold construct.

2. Materials:

- MilliQ water
- 5X HF buffer
- dNTPs
- DMSO
- Forward (elongation T7 LBNC forward 2) / reverse (3'ext2 from LBNC lab) primers
- DNA : already existing Toehold LacZalpha
- Phusion polymerase
- PCR machine

3. Procedure

Prepare the below PCR master mix of 100ul, then split it into two small PCR tubes.

DNA : LacZalpha toehold						
Component	**Concentration**	**Units**	**Desired concentration**	**Units**	**Volume to add (uL)**	**Total volume (uL)**
MilliQ water					71,1	100
5x HF buffer	5	x	1	x	20	
dNTPs	10	mM	0,2	mM	2	
DMSO	100	%	5	%	5	
Forward primer	100	uM	0,5	uM	0,5	
Reverse primer	100	uM	0,5	uM	0,5	
DNA	2	ng/uL	1	ng	0,5	
Phusion polymerase	2000	U/ml	0,75	U/50uL	0,375	
					(in each two tubes)	

Set the PCR machine to the following program and run it.

Standard PCR	
Temperature [°C]	Time [s]
98ºC	30s
98ºC	10s
52ºC, 30x	20s
72ºC	120s
72ºC	420s
4ºC	∞

4. Results:

The gel showed no bands for the samples, the PCR did not work.

5. Conclusion:

The PCR was repeated twice and twice the gel showed no bands. Variations on the temperature could be tested. Else this program failed.