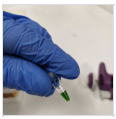

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PCR

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1 Works for me dx.doi.org/10.17504/protocols.io.6h9hb96

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ABSTRACT

Our aim with this protocol is to amplify DNA. This protocol has been optimized has a general amplification

As the quantity of DNA is exponentially increased during the performance of the selection, further modification in the numbers of cycle will be needed to be implemented.

GUIDELINES

We sure to have all the surfaces and materials clean before the start.
All the procedures must be done in an sterile environment to avoid contamination.

MATERIALS

NAME	CATALOG #	VENDOR
Speedy Supreme Green Master Mix	MB39102	NZYtech
Agarose (LM-ultrapure grade)	MB123	NYZtech

MATERIALS TEXT

- Aptamer library (order to IDT)
  5`- G TTG CTC GTA TTT AGG GAA TG $N_{40}$ ACA CCA GTC TTC ATC CGC $TTT_6$ – 3`

- Forward primer (order to IDT):
  G TAG GCG AAA_6 – Cy3 – 5`

- Reserve primer (order to IDT):
  5`- BiodTG TTG CTC GTA TTT AGG GAA TG

- Thermocycler
- TAE buffer

1 Prepare the PCR reaction mixture following the specifications below:

Component	Positive control (V; ul)	Negative control (V; ul)
Template	5	0
Fwd primer	1.25	1.25
Rev primer	1.25	1.25
dH2O	15	20
DMSO	2.5	2.5
Master Mix	25	25

2 Perform the amplification in a general thermocycler in the following conditions. Adjust the annealing temperature according to the primers used, and the hotstart to the specifications of your polymerase:

Hot start	95 º	5 min	
Amplification cycles			
Denaturing	95 º	30s	
Annealing	52 º	30s	X 15 cycles
Extension	72 º	30s	
Final extension	72 º	3 min	
Hold	4º		

3 Prepare a 3% agarose gel. Load the samples and perform the electrophoresis at 90V for 50 min.

4 Remove the gel and observe the bands under UV light.