

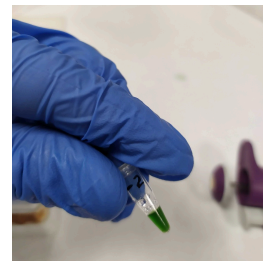
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Version 1

PCR V.1

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Protocol status: Working

We use this protocol and it's working

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Protocol Integer ID: 26913

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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

MATERIALS

⊗ Speedy Supreme Green Master Mix **NZYTEch Catalog #MB39102**

⊗ Agarose (LM-ultrapure grade) **NZYTEch Catalog #MB123**

- Aptamer library (order to IDT)

5` - G TTG CTC GTA TTT AGG GAA TG N₄₀ ACA CCA GTC TTC ATC CGC TTT₆ - 3`

- Forward primer (order to IDT):

G TAG GCG AAA₆ - Cy3 - 5`

- Reserve primer (order to IDT):

5` - BiodTG TTG CTC GTA TTT AGG GAA TG

- Thermocycler
- TAE buffer

Troubleshooting



- 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 |
| dH ₂ O | 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 ° | 5 min | |
| Amplification cycles | | | |
| Denaturing | 95 ° | 30s | |
| Annealing | 52 ° | 30s | X 15 cycles |
| Extension | 72 ° | 30s | |
| Final extension | 72 ° | 3 min | |
| Hold | 4° | | |

- 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.