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RPA from banana DNA

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iGEM EPFL

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MATERIALS TEXT

- nuclease-free water/ double-distilled autoclaved water

TE buffer

- 10 mM Tris pH 8.0
- 1 mM EDTA

DNA extraction :

- Banana
- Dish soap
- 1g table salt
- 25 ml pre-chilled (4°C) 90% isopropyl alcohol (rubbing alcohol)
- 70% ethanol

Primers :

- Fwd : 5' CATTCTAATACGACTCACTATAGGGATTATCTGCAAAA AACTACGG 3'
- Rev : 5' TAAAAAGTGCTTCGGTGCAAAATAAGAAACGAT 3'

Rehydration solution

- 1.18 µl rehydration buffer
- 0.84 µl 10µM forward primer
- 0.84 µl 10µM reverse primer
- 1 µl 280 mM magnesium acetate

- 2 reaction pellets (included in the kit)
- DNA ladder
- Loading buffer
- 2% agarose gel

Banana DNA extraction

1 Put chopped banana in a closed ziploc-like bag

2 Add diluted 1:10 dish soap solution and 1g table salt to fruits

3 Mash it until homogeneous mixture is obtained

4 Strain the mixture through a household coffee filter

5 Add 25 ml isopropyl alcohol to strained liquid

6 Let it sit for 5 min until phase separation

7 Collect upper white layer (precipitated DNA) with a toothpick, place it on a new coffee filter and wash it with 70% ethanol

8 Pat it dry with paper towels

9 Dissolve DNA in 1 ml TE buffer

Nanodrop

10 Perform a NanoDrop analysis

Storage

11 Set 1 µl aside; store rest at 4°C

Prepare amplification mix

12 Prepare the rehydration solution for the RPA

	experiment	RPA control
Rehydration buffer	5.9 µl	5.9 µl
10µM forward primer	0.42 µl	0.42 µl
10µM reverse primer	0.42 µl	0.42 µl
280 mM magnesium acetate	0.5 µl	0.5 µl
Sample extract	1 µl	0 µl
Water	1.76 µl	2.76 µl
Total	10 µl	10 µl

13 Vortex and spin briefly

14 Rehydrate 1 RPA reaction pellet in each one of the rehydration solutions

15 Pipette up and down to resuspend pellet

16 Vortex and spin briefly

RPA reaction

17 Heat the reaction at 39°C for 20 minutes.

DNA purification step

18 should do a standard DNA purif kit (which we don't have at the moment) so we'll try the same purif as we did for the banana DNA

Gel electrophoresis

19 Run a gel electrophoresis:

Lane	1	2	3
Sample	DNA ladder	Sample	PCR control