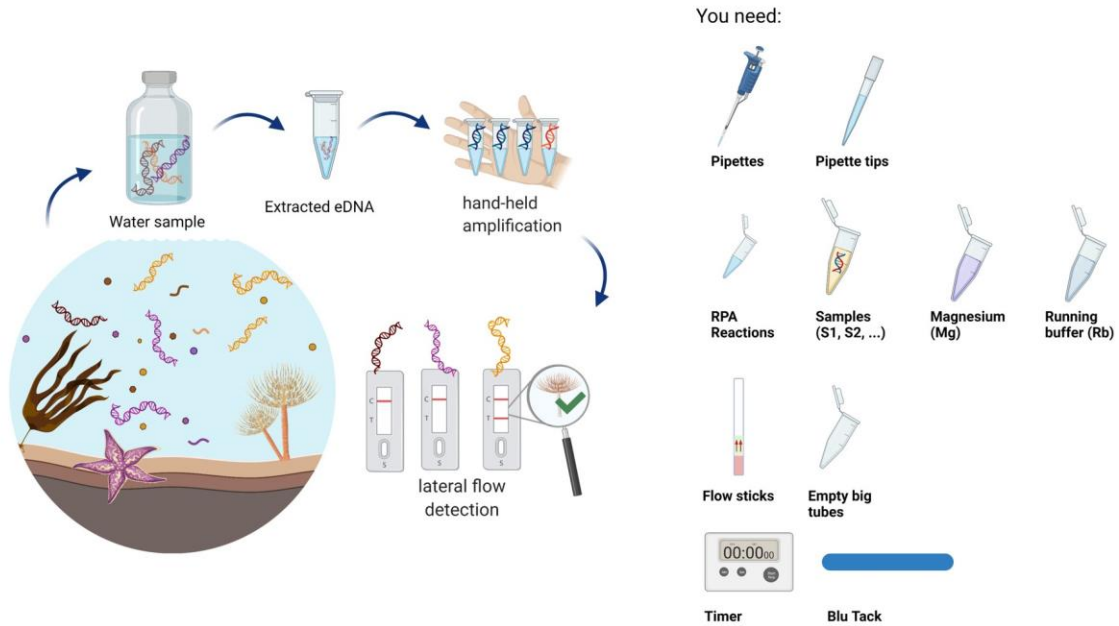


Simplified Recombinase Polymorphism Amplification-Lateral Flow (RPA-LF) protocol

RPA procedure



- 1 Grab reaction tubes and samples from ice**

Wait till defrosted

Small tube: RPA reaction
Big tube: samples

Grab Samples S1, S2, S3, S4, ... and same number of RPA reactions

Label RPA reaction tubes
-> R1, R2, R3, R4, ...
- 2 Pipet sample into reaction tube**

3 µL

Pipette 3 µL of Sample S1 (big tube) into RPA reaction R1 (small tube), S2 into R2, and so on

CHANGE TIP AFTER EACH SAMPLE
- 3 Add Magnesium into the tube**

3 µL

Magnesium

Add 3 µL Magnesium to each tube. Close the lid, and flick reaction tube with finger

CHANGE TIP AFTER EACH SAMPLE
- 4 Incubate Reaction in hand**

30:00:00

Hold RPA reaction tubes in hand or warm spot on body for **30 minutes** e.g. armpit

flick tube to mix after 5 min
- 5 Dilute RPA reaction**

80 µL

Running buffer

After 30 minutes:
Add 80 µL running buffer into each RPA reaction tube from step 4 (R1, R2, ...)

Close tubes and flick with finger to mix

set aside

CHANGE TIP AFTER EACH SAMPLE
- 6 Prepare running buffer tubes**

80 µL

Grab 4 empty big tubes

Pipette 80 µL running buffer into each big tube
- 7 Prepare flow sticks**

Sample area

Grab 4 flow sticks and label R1, R2, R3, R4

secure the flow sticks on a piece of blue tac sample area facing up (do not have sample area touch the blue tac)
- 8 Apply reaction to flowstick**

10 µL

Pipette 10 µL reaction from step 5 on flow stick -> pipette 10 µL of reaction R1 onto sample area of flow stick R1, Reaction R2 onto stick R2, ...
- 9 Run reaction on flow stick**

stick flow stick vertically into tube with 80 µL running buffer (tubes from step 6)

1 flow stick per tube
- 10 Read result**

10:00:00

positive negative

wait patiently **10 min** before reading results