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RPA DNA Amplification using Agdia AmplifyRP® Acceler8® Discovery Kit

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1 Works for me dx.doi.org/10.17504/protocols.io.8ephtdn

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ABSTRACT

This protocol describes the procedure to perform an RPA DNA Amplification using the Agdia AmplifyRP® Acceler8® Discovery Kit

MATERIALS TEXT

Rehydration Buffer	5.9 µl/sample
Primer A	0.42 µl/sample
Primer B	0.42 µl/sample
Template (DNA)	1.0 µl/sample
MgOAc (280 mM)	0.50 µl/sample
dH2O	1.76 µl/sample

Quantities for preparation of a 10 µl RPA reaction using Agdia AmplifyRP® Acceler8® Discovery Kit

1 Prepare the RPA pellet rehydration solution following the table in the Materials section.
If possible do a Master Mix that will then be divided.

2 For each sample, transfer the entire volume (10 µl) to a reaction pellet. Pipette up and down until the full pellet has been resuspended.

3 Cap the reaction tubes. Vortex and spin briefly two times.

4 Transfer the reaction tubes to a heat block. Incubate at 39°C for 20 min.

5 After the incubation, purify the sample if you want to run the RPA product on an agarose gel.

PCR purification kits work well, but since RPA primers can be very long (60+ bp), a high cutoff is recommended.