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RPA DNA Amplification using TwistAmp™ Basic

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We use this protocol and it's working

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Abstract

This protocol describes the procedure to perform an RPA DNA Amplification using the TwistAmp^{TM} Basic

Materials

	Primer-free rehydration buffer	29.5 µl
	Primer A (10µM)	2.4 µl
	Primer B (10µM)	2.4 µl
	Template (DNA)*	5 µl
	Nuclease-free water	8.2 µl
	Total	47.5 µl

Quantities for preparation of a 50 µl RPA reaction using TwistAmp™ Basic

* TwistAmp™ Basic RPA reactions are very sensitive, it is recommended to use low copy numbers. Solutions with a concentration over 10 000 copies/µl should not be opened in the area you set up your RPA as they might contaminate all your reactions.

Troubleshooting

1 Prepare the RPA pellet rehydration solution in a 1.5 ml tube following the table in the Materials section.
If possible do a Master Mix that will then be divided.

2 Vortex and spin briefly.

3 For each sample, transfer the entire volume (47.5 µl) to a reaction pellet. Pipette up and down until the pellet is fully resuspended.

4 Add 2.5 µl of 280mM MgOAc to the cap of each reaction tube. MgOAc will make the reaction start, it is added to the cap so that all reactions start simultaneously when spun down.

5 Do a short spin to make the MgOAc drop fall down into the tube, vortex briefly and do another quick spin.

6 Incubate at 39°C for 20 min
For low copy numbers, incubate 4 minutes, vortex and spin briefly and incubate again for 16 minutes.

7 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.

Do not open post-amplification RPA reactions in the same workspace as you set up pre - amplification RPA, or take appropriate measures to limit DNA contamination.