DYS SEE

RPA.

iGEM Thessaly

Protocols

RPA Protocol (Kit: TwistAmp Basic from TwistDX)

- This protocol is destined for single-plex use only.

Sample Set-Up (single-plex)

1. Prepare reaction mix in 1.5ml tube:
Primer A (10µM) 2,4µl
Primer B (10µM) 2,4µl
Primer Free Rehydration buffer 29,5µl
Template and water to 13,2µl
(Total volume 47,5µl).
Vortex and spin briefly.

2. Add reaction mix to a TwistAmp basic reaction. Pipette to mix.
3. Add 2,5µlof 280mM Magnesium Acetate (MgOAc) (supplied in the kit) and mix well to start reaction.
- Note: RPA reaction starts as soon as MgOAc is added.

4. Incubate at 39 degrees Celsius for 20 minutes. For low template copy number, remove strip after 4 minutes, vortex and spin briefly, replace in heating device.
5. After 20 minutes, clean amplicons before running on agarose gels.
- Note: If tubes are opened after amplification, there is a great risk of contamination of work surfaces with amplicon. Ensure that appropriate avoidance measures are taken.

Kit Positive Control Set-up (single-plex)

1. Prepare reaction mix in 1,5ml tube:
Positive control primer mix 8µl
Primer Free Rehydration buffer 29,5µl
Positive control DNA template 1µl
Water 9µl
(Total volume 47,5µl)
Vortex and spin briefly

2. Add reaction mix to a TwistAmp Basic reaction. Pipette to mix.

3. Add 2,5mM of Magnesium Acetate (MgOAc) (supplied in the kit) and mix well to start reaction.

- Note: RPA reaction starts as soon as MgOAc is added.

4. Incubate at 39 degrees Celsius for 20 minutes. For low template copy number, remove strip after 4 minutes, vortex and spin briefly, replace in heating device.
5. After 20 minutes, clean amplicons before running on agarose gels.

- Note: If tubes are opened after amplification, there is a great risk of contamination of work surfaces with amplicon. Ensure that appropriate avoidance measures are taken.

Modified protocol to reduce reaction volume

To create 5 µl final reaction volumes, single 50 µl reactions should be prepared as described above, while being store on ice to prevent amplification. Aliquots (portions) of 5µl are then transferred into different tubes (@paper 200µl PCR tubes) and then placed into the incubator. (Lillis, et al. , 2016)

Protocols

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