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Basic Rolling Circle Amplification (RCA) Protocol (Thermo phi29 polymerase)

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Aim: RCA is useful method to amplify circular DNA/RNA. By principle, RCA conducted using phi29 polymerase to obtain copies of DNA/RNA target in concatemeric form. phi29 works on isothermal reaction at 36-38^{0}C.

Materials :

gDNA

10U phi29 polymerase (Thermo Scientific)

10X reaction buffer (Thermo Scientific)
10mM dNTP mix (Thermo Scientific)
100µM n-hexamer primer (or specific primers to obtain specific target) (Thermo Scientific)
Nuclease-free water (Thermo Scientific)
Thermal cycler
Micropippetes
Tubes 0.2µL

Steps :

DNA Denaturation

- Prep denaturation mixture on 0.2µLtube :

Reagent	Volume (µL)
10X reaction buffer	0.5
100µM n-hexamer primer	1
Nuclease-free water	2-3
gDNA	0.5-1
Total	**5**
NOTE : you can change 100µM n-hexamer primer with specific primer to obtain more specific result	

- Incubate 95^{0}C for 3 minutes
- Put on ice immediately for 3-5 minutes

Rolling Circle Amplification

- Prep amplification mixture

Reagent	Volume (µL)
Denaturation mixture	5
10X reaction buffer	1.5
10mM dNTP mix	2
10U phi29 polymerase	1
Nuclease-free water	10.5
Total	**20**
NOTE : you can also add 100µM n-hexamer primer to increase RCA yield	

- Incubate 36^{0}C for 18 hours
- Inactivate reaction at 65^{0}C for 10 minutes

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