

DNA Amplification Protocol of low yield DNA samples is based on the GenomiPhi™ DNA Amplification protocol.

Whole genome amplification (WGA) of small quantities of genomic DNA (yields <200ng) can be performed using the GenomiPhi™ DNA Amplification Kit (Amersham Biosciences). This method for representational WGA utilizes Phi29 DNA polymerase, producing microgram quantities of high molecular weight DNA from 10ng of starting material (Lage et al. 2003)

Materials

GenomiPhi™ DNA Amplification Kit (Amersham Biosciences 256600-01)

Mini Quick Spin Columns (Roche 1814419)

High molecular weight DNA ONLY

Procedure

1. Dilute aliquot of High molecular weight DNA to 10ng/μl to create template.
2. Mix 1 μl of template with 9 μl of sample buffer in 500μl tube.
3. Heat to 95°C for 3 min.
4. Cool on ice.
5. Add 10 μl of reaction mix (9 μl Reaction Buffer and 1 μl enzyme) to cooled sample on ice.
6. Note: Freeze; thawing of enzyme can limit the shelf life. Store reaction mix [9 μl Reaction Buffer and 1 μl enzyme] as 10 μl single use aliquots.
7. Incubate the sample at 30°C overnight (approx. 16-18 hrs.)
8. Heat inactivate the sample at 65°C for 10 minutes
9. Cool on ice.
10. Purification of Product: Vigorously invert the Mini Quick Spin Columns several times and remove the top cap, then snap off the bottom tip.
11. Remove excess buffer by centrifuging at 1000 x g for 1 min at RT into a 1.5 ml microtube.
12. Discard tube and place column into a clean, sterile 1.5 ml microtube.
13. Add 20 μl of sample to the center of the column bed and centrifuge at 100 x g for 4 min at RT.
14. Read A260 and A280 values on a spectrophotometer and store amplified DNA at 4°C. The typical yield from 10ng template DNA = 4 - 6μg.