

## **Protocol DNA Amplification Using TempliPhi Amplification Kit**

(For use with GeoChip)

Amplification should be used for samples with low concentrations of DNA.

*Try to amplify all samples within a group at the same time. If there are too many samples, make sure you amplify a random selection of samples to minimize the impact of day-to-day variation.*

*Success of amplification and subsequent steps greatly increases with the use of high quality DNA.*

### **Materials**

Purified DNA

TempliPhi Amplification Kit

Sample buffer: contains random hexamers and is used for re-suspending DNA

Reaction buffer: contains salts and deoxynucleotides and is adjusted to a pH that supports DNA synthesis

Enzyme mixture: contains phi29 DNA polymerase and random primers in 50 % glycerol

Additives (added to increase amplification representativeness, see Wu et al., 2006 for more details)

Single-Stranded DNA binding Protein (SSB) (*stored at -20 °C*)

Spermidine: 2.4 mM stock solution

### **Precautions to limit background DNA**

- **Use the dedicated amplification hood and the dedicated pipettes (stored in the hood)**
- **UV irradiate all tubes and water in the Stratalinker for 10 min**
- **Use new, unopened boxes of filtered tips for each experiment**
- **Place all items needed in the hood (tubes, tips, gloves, ice bucket, etc.) and turn on UV light for 20-30 min prior to start**
- **Remember to clean hood and UV it for 10-15 min after each experiment**

### **Procedure:**

**IMPORTANT:** keep all reagents on ice, and keep all components at 0-4 °C until ready for amplification

- (1) Transfer 10 µl aliquots of sample buffer to an appropriate reaction tube or micro-well plate.
- (2) Transfer DNA (up to 5 µl, add water if using less DNA) to the sample buffer. Use the same amount of DNA for all samples. Ideally, 100 ng of DNA should be used, but lower amounts can be used. For samples with lower amounts of DNA, 50 ng of DNA is commonly used. If the original concentration of a sample is very low, the DNA can be concentrated.
- (3) Mix DNA and buffer thoroughly and incubate at RT 10 min.

- (4) While DNA and buffer are incubating, prepare Templiphi premix: (mix and spin all reagents twice before use)
- In a separate tube, for each Templiphi reaction combine:
- a) 10  $\mu$ l of reaction buffer
  - b) 0.6  $\mu$ l enzyme mixture
  - c) 1.25  $\mu$ l SSB (final concentration:  $\sim$ 260 ng/ $\mu$ l)
    1. *Current SSB stocks are 5  $\mu$ g/ $\mu$ L*
    2. *If the SSB stock is a lower concentration, increase the volume to reach  $\sim$ 260 ng/ $\mu$ L. **Do not add more than 2  $\mu$ L because this dilutes the remaining components.***
  - d) 1  $\mu$ l spermidine (2.4 mM stock) (final concentration: 0.1  $\mu$ M)
- (5) Mix the Templiphi premix well and transfer 12.85  $\mu$ l to the DNA/buffer mixture.
- (6) Mix the reaction and spin twice (Remember to run a negative control to check for background DNA. Using the steps outlined above the negative control should be much lower than the samples).
- (7) Incubate the reaction at 30 °C for 6 hours.
- (8) Heat-inactivate the enzyme by incubating the reaction at 65 °C for 10 min and then cool at 4 °C.
- (9) You can run a gel of the amplified samples (no more than 2  $\mu$ l) to make sure all the samples amplified. *The gel should have a slight “smear” of DNA at the top of the gel.*
- (10) Measure the amount of amplified DNA product using the PicoGreen protocol. *The presence of primers and dNTPs in sample results in inaccurate measurements when using the 260/280 ratio.*

#### Troubleshooting:

- If no or poor amplification occurs, try decreasing the amount of DNA template used to dilute out any inhibitors that may be present.
- Concentration and serial dilution (2-3 dilution steps) of the sample can be used to ‘wash’ the DNA. Serial dilution can also be used if the DNA quality is above the recommended thresholds, but the amplification results are poor.

#### To cite this method, use:

Wu, L., X. Liu, C. W. Schadt, and J.-Z. Zhou. 2006. Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. *Appl. Environ. Microbiol.* 72:4931-4941