

V. Preparation of Sequencing Template for Plasmids.

A. TempliPhi DNA Amplification (This protocol is from Amersham Biosciences TempliPhi DNA Sequencing Template Amplification Kit (25-6400-01))

1. Reaction mix

File name:

Chemicals	Per reaction	Total volume	8 reactions	Check
Denature buffer (410480)	5 μ l		40 μ l	
DNA template	2 μ l			
TempliPhi premix (410402)	5 μ l		40 μ l	
Bacteriophage phi 29 DNA polymerase (410478)	0.2 μ l		1.6 μ l	
Total	12.2 μ l			

2. Protocol

- Thaw the TempliPhi premix and denature buffer on ice. Vortex gently to ensure mixing.
- Dispense 5 μ l aliquots of denature buffer into an appropriate reaction tube.
- Transfer samples to the denature buffer. (1 μ l of glycerine stock or 1 toothpick colony)
- Heat the tubes at 95°C for 3 min and then cool on ice.
- Add 5 μ l of TempliPhi premix and 0.2 μ l polymerase to the cooled sample. Vortex briefly to mix.
- Incubate at 30°C for 16 hours in GeneAmp PCR system 9700 (Program F5-RCA).
Start: _____ finish: _____.
- Heat-inactivate the enzyme by incubating at 65°C for 10 min. Cool to 4°C.
- Store the amplified DNA at -20°C or 4°C.

3. Restriction Digestion of RCA products

Chemical	Standard reaction	8 reactions	Check
Rnase/Dnase free water	2.5 μ l	22.5	
BSA (10X) Promega R396E: 1mg/ml	1 μ l	9	
Reaction buffer H (10X) Promega R008A	1 μ l	9	
RCA product	5 μ l		
<i>EcoR</i> I (12unit/ μ l) Promega R601A	0.5 μ l	4.5	

Chemical	Standard reaction	8 reactions		Check
2.5-3 unit/ μg of DNA to be cut				
Total	10 μl			

- Add 10 μl water to RCA product.
- Mix the reactions by pipetting before and after enzyme is added.
- Incubate the reactions in water bath at 37°C for 1 hour.
- Store at -20°C

4. Sample record sheet

No	Sample #				Remark
.					
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
Conclusion:					