

Fill in Reactions (Klenow and T4 DNA polymerase)

Klenow Fill in (for 5' Protruding Ends)

10 X Nick Translation buffer: (200 uL)

100 uL	1 M Tris (pH 7.2)	(0.5 M Tris, pH 7.2)
20 uL	1 M MgSO ₄	(0.1 M MgSO ₄)
0.4 uL	0.5 M DTT	(1 mM DTT)
100 ug	BSA	(500 ug / mL BSA)

1. Mix up the reaction:

5 uL	DNA (0.5 – 2 ug total)
2 uL	10 X Nick Translation Buffer
1 uL	2 mM dATP
1 uL	2 mM dCTP
1 uL	2 mM dTTP
1 uL	2 mM dGTP
1 uL	Klenow (5 U)
2 uL	BSA (500 ug / mL)
6 uL	DI H ₂ O

2. Incubate at Room Temp. for 1 hour.

3. Phenol / Chloroform extraction.

4. Chloroform Extraction.

5. Adjust salt, add tRNA.

6. Ethanol Precipitation.

T4 Polymerase Fill in (for 3' Protruding Ends)

10 X T4 Polymerase Buffer (200 uL)

10 uL	1 M DTT
140 uL	1 M Tris (pH 7.4)
20 uL	1 M MgCl ₂
30 uL	DI H ₂ O

1. Mix up the reaction:

9 uL	DNA (0.5 – 2 ug total)	
2 uL	10 X T4 Polymerase Buffer	
3 uL	DI H ₂ O	
1 uL	2 mM dATP	
1 uL	2 mM dCTP	
1 uL	2 mM dTTP	
1 uL	2 mM dGTP	
2 uL	T4 DNA Polymerase (1 U / uL)	(use 2 – 5 U)

2. Incubate reaction for 15 minutes at 37 C.

3. Incubate reaction for 10 minutes at 70C.

4. Phenol / Chloroform Extraction.

5. Chloroform Extraction.

6. Adjust salt, add tRNA.

7. Ethanol Precipitation.