Preparation of Adaptor / Insertion into Vector:

1. Purify linkers from Column and add purity if greater than 25 μg using 18% PAGE 0.5X TBE isolating full length linker by UV Scanning.

2. Incubate Crushed gel slice in 0.3M NaCl, 60 μl at 37°C. Spin out at R.T. temp 15 mins.

3. Phenol / chloroform extract agarose layer and EtOH precip linkers. Wash in 70% EtOH and dry. Resuspend in 10μl sterile H2O.


5. Add linker to a 1.5mls appendix tube to a final Concentration of 50ng/μl in 50μl to 100μl total volume.

6. Add 5μl NaCl 1μl for 50μl 2μl for 100μl Volume.

7. Heat at 95°C - 100°C for 15 mins.

8. Allow to cool Slowly to Room temperature in the bath.

9. Adaptor can be Store at -20°C for future use.

Insertion:

1. Add 100ng linker to 500μg DNA.

2. Ligate at 16°C 0/h.

3. Heat to 70°C 5-10 mins.

4. Ppt DNA in PEG, NAcl (20% PEG/2.5M Nacl)
   (0.10 X Volume of PEG Mixture)

5. Incubate 10 min at 37°C. Mix well 15 mins at R.T. Temp

6. Wash in 70% EtOH twice.

7. Resuspend in 10μl H2O. Heat at 70°C 5-10 mins.

8. Anneal at 37°C for 1 hour to allow Slow annealing