Preparation of Adaptor & Insertion into Vector.

1. Purify Linkers from Column and Gel purify if greater than 25 mer using 18% PAGE 0.5X TBE isolating full length linker by UV Shawdowing.
2. Incubate Crushed Gel Slice in 0.3M $NaCH_3COO$ O/N at 37°C. Spin out at R.temp 15 mins
3. Phenol/CHCl$_3$/IAA extract aqueous layer and ETOH ppt Linkers. Wash in 70% and dry. Resuspend in 100µl sterile H_2O.
4. Take O.D reading. Calculate Concentration.
5. Add linker to a 1.5mls eppendorf tube to a final concentration of 500ng/µl in 50µl to 100µl total Volume.
   1µl = 500ng.
6. Add 5M 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 H_2O bath.
9. Adaptor can be store at -20°C for future use.

INSERTION:

1. Add 500ng linker to 500ng DNA.
2. Ligate at 16°C O/N
3. Heat to 70°C 5-10 mins.
4. PPt DNA w PEG, NaCl (20% PEG/2.5M NaCl)
   (0.6 x Volume of PEG Mixture)
5. Incubate 10 mins at 37°C. Microfuge 15 mins at R.temp
6. Wash in 70% ETOH twice.
7. Resuspend in 10µl H_2O. Heat at 70°C 5-10 mins.
8. Anneal at 39°C for 1 hour to allow Slow annealing
9. Transform Bacteria.