



# Baylor College of Medicine Microarray Core Facility

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## **PCR Preparation:**

10 ng/ul DNA  
2mM MgCl<sub>2</sub>  
Buffer 1x  
0.4mM DNTP's at 80%T and 20%U  
0.5 units/25 ul Taq  
0.5uM of Primer

## **Clean up:**

1. Use Microcon kit from Millipore to concentrate products that are greater than 140 BP. Follow protocol.
2. Then use Edge kit to clean the products. Edge kit is from Biosystems. Follow Protocol.

## **Degradation Reaction:**

This step degrades the incorporated U's and makes the DNA fragment smaller for the APEX reaction.

20ul purified DNA  
4ul Thermo Sequenase Reaction Buffer  
14 ul dH<sub>2</sub>O  
1ul of UNG  
1ul of SAP  
total volume of 40ul

1. Incubate at 37°C for 2.5 hrs. in heat block .
2. After incubation, place tubes in a 95°C heat block for 15 min. This will inactivate the enzymes.
3. Then store at -20°C or on ice if your going to do the APEX experiment.

### **APEX (Arrayed Primer Extension):**

40uL of degraded DNA

5ul of Thermo sequenase Reaction Buffer.

1. Place on heat block to denature DNA for 10 minutes.
2. Labeling:
3. Add the dyes and enzyme to your reation.
  - 1ul Cy5 ddGTP at 50 uM concentration
  - 1ulCy3 ddCTP at 50 uM concentration
  - 1ul Cy2 ddUTP at 50 uM concentration
  - 1ul TRA ddATP at 50 uM concentration
4. Add 1ul of Thermo Sequenase Enzyme at 1:17 dilution. Dilute with Thermo sequenase Reaction Buffer. Total volume of 50ul
5. Place slide on 48°C heat block. Pipette up and down to mix the reaction and then gently apply to slide with out touching the array. Form a bubble with pipette and distribute to cover area of interst.
6. Let reaction take place for 2min. covered. Check to see that the reaction mixture does not dry. Remove the chip from the heat block after about 4 min.
7. Then wash slide in 95°C dH2O for 5 min. Dry and Scan.

### **CHIP Preparation:**

Before hybridization wash the slide in 95°C dH2O for 5min.