

## Native gradient gel

### 3.5% gel

220  $\mu\text{L}$  40% acryl  
56  $\mu\text{L}$  2% bis  
250  $\mu\text{L}$  10x TG  
1.914 mL  $\text{H}_2\text{O}$   
----- 40% glycerol  
25  $\mu\text{L}$  10% APS  
2.5  $\mu\text{L}$  TEMED

### 15% gel

938  $\mu\text{L}$  40% acryl  
240  $\mu\text{L}$  2% bis  
250  $\mu\text{L}$  10x TG  
392  $\mu\text{L}$   $\text{H}_2\text{O}$   
605  $\mu\text{L}$  40% glycerol  
25  $\mu\text{L}$  10% APS  
2.5  $\mu\text{L}$  TEMED  
Bromphenol blue

### Stacker

100  $\mu\text{L}$  40% acryl  
28  $\mu\text{L}$  2% bis  
125  $\mu\text{L}$  10x TG (pH 6.8)  
957 mL  $\text{H}_2\text{O}$   
----- 40% glycerol  
25  $\mu\text{L}$  10% APS  
2.5  $\mu\text{L}$  TEMED

1. Add a 500  $\mu\text{L}$  “bumper” of 15% gel to bottom of gel caster.
2. To form the gradient in a disposable pipet, carefully suck up 1.5 mL 3.5% gel followed by 1.5 mL of 15% without mixing.
3. Mix by pipetting in 2 or 3 air bubbles and letting them rise through the gel solutions.
4. Carefully the pipet the gradient on top of the bumper, and add 3.5% gel to ~2 cm below the top of the gel. Add a thin layer of water-saturated butanol, let gel polymerize 30 min.
5. Pour off butanol and rinse well with water, add stacking gel and comb, polymerize for 30 min.
6. Load MCMs (1-2  $\mu\text{g}$ ) without loading dye, run for 3 hr at 170 V.

### Visualization

Silver stain normally, or Sypro stain O/N at room temp. If doing a Western, the transfer will take longer.