

1. Wash the cDNA pellet with 200  $\mu\text{L}$  of 70% (v/v) ethanol and microcentrifuge for 2 min.
3. Carefully remove the ethanol wash and vacuum evaporate until the cDNA pellet is dry.
4. Each fraction can contain 0–250 cps. If the pellet contains 0–10 cps, resuspend the cDNA in 5.0  $\mu\text{L}$  of sterile water. If the pellet contains >10 cps, resuspend the cDNA in 12.0  $\mu\text{L}$  of sterile water.
5. Quantitate the cDNA by UV visualization of samples spotted on ethidium bromide agarose plates (*see Note 7*). The cDNA can be stored at  $-20^{\circ}\text{C}$ .

### 3.9. Ligating cDNA to Prepared Vector

The cloning vector should be double-digested with *Xho*I and an enzyme which leaves ends compatible with the adaptors. The vector should also be dephosphorylated to prevent vector-to-vector ligations. The final ligation reaction volume is 5  $\mu\text{L}$ .

1. To a 0.5-mL microcentrifuge tube, add in order:  $X \mu\text{L}$  water, 0.5  $\mu\text{L}$  10X ligation buffer, 0.5  $\mu\text{L}$  10 mM rATP, 1  $\mu\text{g}$  prepared  $\lambda$  arms, 100 ng cDNA, 0.5  $\mu\text{L}$  T4 DNA ligase (4 Weiss U/ $\mu\text{L}$ ).
2. Incubate overnight at  $4^{\circ}\text{C}$ .

### 3.10. Packaging and Plating

The ligation is packaged and transfected into an appropriate *E. coli* host strain.

#### 3.10.1. Preparation of Plating Cells

1. Inoculate 50 mL of NZY medium with a single colony of the appropriate *E. coli* host. Do not add antibiotic.
2. Grow at  $30^{\circ}\text{C}$  with gentle shaking (1000g) overnight (*see Note 9*).
3. Spin the culture at 1000g for 10 min.
4. Gently resuspend the cells in 20 mL sterile 10 mM  $\text{MgSO}_4$ .
5. Determine the concentration of the cells by reading  $\text{OD}_{600}$  on a spectrophotometer. Store this cell stock at  $4^{\circ}\text{C}$  for no more than 1 wk. To use, dilute cells to  $\text{OD}_{600} = 1.0$  in 10 mM  $\text{MgSO}_4$ .

#### 3.10.2. Packaging

Package the ligation reaction following manufacturer's instructions. Stop the reaction by adding 500  $\mu\text{L}$  SM buffer and 20  $\mu\text{L}$  chloroform.

#### 3.10.3. Plating

1. Mix the following components in a Falcon 2059 polypropylene tube: 200  $\mu\text{L}$  appropriate diluted host cells (*see Subheading 3.10.1.*), 1  $\mu\text{L}$  final packaged reaction.