

## 2.6. *Xho*I Digestion

1. *Xho*I reaction buffer: 200 mM NaCl, 15 mM MgCl<sub>2</sub>.
2. *Xho*I restriction endonuclease (120 U).
3. 10X STE buffer: 1M NaCl, 100 mM Tris-HCl, pH 8.0, 10 mM EDTA.

## 2.7. Size Fractionation

1. 1X STE buffer: 100 mM NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
2. Sephacryl S-500 column filtration medium (Pharmacia).
3. 5% Nondenaturing acrylamide gel.
4. Phenol:chloroform (1:1 [v/v], pH 7.0–8.0) (*see Note 5*).
5. Chloroform.
6. 100% (v/v) Ethanol.

## 2.8. Quantitating the cDNA

1. 70% (v/v) Ethanol.
2. TE buffer: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
3. 0.8% Agarose.
4. Ethidium bromide (10 mg/mL).

## 2.9. Ligating the cDNA to Prepared Vector

1.  $\lambda$  Vector (such as Lambda ZAP, ZAP Express, SeqZAP) double digested and dephosphorylated. Vectors are digested with *Xho*I and a second restriction enzyme, which leaves ends compatible with the adaptors.
2. 10X Ligation buffer (*see Subheading 2.4., item 4*).
3. 10 mM rATP.
4. T4 DNA ligase (4 Weiss U).

## 2.10. Packaging and Plating

1. NZY medium, plates and top agarose: 5 g NaCl, 2 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 5 g yeast extract, 10 g NZ amine (casein hydrolysate) per liter. Add 15 g agar for plates or add 0.7% (w/v) agarose for top agarose. Adjust the pH to 7.5 with NaOH and sterilize by autoclaving.
2. Appropriate *E. coli* host strains (such as XL1-Blue MRF<sup>+</sup> or DH5 $\alpha$ MCR) freshly streaked on an LB agar plate containing the appropriate antibiotic (*see Note 8*).
3. 10 mM MgSO<sub>4</sub>.
4. Packaging extract (such as Gigapack<sup>®</sup> II  $\lambda$  packaging extract [Stratagene] [23,24]).
5. SM buffer: 5.8 g NaCl, 2.0 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 50.0 mL 1M Tris-HCl, pH 7.5, 5.0 mL 2% (w/v) gelatin per liter. Autoclave.
6. Chloroform.
7. LB agar plates: 10 g NaCl, 10 g bacto-tryptone, 5 g bacto-yeast extract, 15 g agar per liter. Adjust the pH to 7.5 with NaOH and sterilize by autoclaving.
8. Isopropyl- $\beta$ -D-thio-galactopyranoside (IPTG), 0.5M in water and 5-bromo-4-chloro-3-indoyl- $\beta$ -D-galactopyranoside (X-gal), 250 mg/mL in dimethylformamide (*see Note 10*).