2. Mix well and incubate at 70°C for 30 min. Do not exceed 30 min.
3. Phenol:chloroform/chloroform extract (*see* **Note 5**).
4. Precipitate the cDNA by adding the following: 20 µL 3*M* sodium acetate, 400 µL 100% (v/v) ethanol.
5. Mix by gently vortexing and incubate on ice for 10 min or overnight at –20°C.

3.4. Ligating Adaptors

1. Microcentrifuge the precipitated cDNA sample at maximum speed, 4°C for 1 h.
2. A large white pellet will form at the bottom of the microcentrifuge tube. Carefully remove the radioactive ethanol and properly discard. Counts left in this supernatant are unincorporated nucleotides.
3. Wash the pellet by gently adding 500 µL of 70% (v/v) ethanol and microcentrifuge for 2 min.
4. Aspirate the ethanol wash and lyophilize the pellet until dry.
5. Resuspend the pellet in 9.0 µL of adaptors (0.4 µg/µL) by gentle pipeting. Use a Geiger counter to confirm that the cDNA is in solution.
6. Remove 1.0 µL for analysis of cDNA synthesis on a 5% nondenaturing acrylamide gel. This aliquot may be frozen at –20°C (*see* **Notes 1–4**).
7. Add the following components to the tube containing the 8.0 µL of blunted DNA and adaptors: 1.0 µL 10X ligation buffer, 1.0 µL 10 m*M* rATP, 1.0 µL T4 DNA ligase (4 U/µL).
8. Mix well and briefly spin in a microcentrifuge. Incubate overnight at 8°C or for 2 d at 4°C.

3.5. Phosphorylating the Adaptors

The final volume of the phosphorylation reaction will be 25 µL. Take this into account when determining the necessary volumes.

1. After ligation, heat inactivate the ligase by incubating at 70°C for 30 min.
2. Spin down and allow the reaction to cool at room temperature for 5 min. Add 1.5 µL 10X ligation buffer, 2.0 µL 10 m*M* rATP, *X* µL sterile distilled water, 7 U T4 polynucleotide kinase.
3. Incubate at 37°C for 30 min.
4. Heat inactivate the kinase by incubating at 70°C for 30 min.
5. Spin down and allow the reaction to cool at room temperature for 5 min.

3.6. Xho*I* Digestion

The final volume of the digestion reaction will be 60 µL.

1. Add the following components to the phosphorylation reaction (25 µL): 30.0 µL *Xho*I reaction buffer, *X* µL sterile distilled water, 120 U *Xho*I restriction endonuclease. Be sure the volume of enzyme is ≤10% of the reaction volume.
2. Incubate for 1.5 h at 37°C.
3. Cool the reaction to room temperature and add 15 µL of 10X STE buffer, and 75 µL water.