dCTP, reducing the probability of methylating the second strand, since the *Xho*I restriction site in the linker-primer must be susceptible to restriction enzyme digestion for subsequent ligation into the vector.

The uneven termini of the double-stranded cDNA must be polished with cloned *Pfu* DNA polymerase, to allow efficient ligation of adaptors ***(22,23)***. Adaptors are complementary oligonucleotides that, when annealed, create a phosphorylated blunt end and a dephosphorylated cohesive end. This double-stranded adaptor will ligate to other blunt termini on the cDNA fragments, and to other adaptors. Since the cohesive end is dephosphorylated, ligation to other cohesive ends is prevented. After the adaptor ligation reaction is complete and the ligase has been inactivated, the molecules are phosphorylated to allow ligation to the dephosphorylated vector.

An *Xho*I digestion releases the adaptor and protective sequence on the linker-primer from the 3' end of the cDNA. These fragments are separated from the cDNA on a size-fractionation column. The purified cDNA is then precipitated and ligated to the vector. This strategy is illustrated in **Fig. 2**.

2. Materials

2.1. First-Strand Synthesis

1. 10X first-strand buffer: 500 m*M* Tris-HCl, pH 7.6, 700 m*M* KCl, 100 m*M* $MgCl_2$.
2. First-strand methyl-nucleotide mixture (10 m*M* dATP, dGTP, dTTP, and 5 m*M* 5-methyl dCTP).
3. Linker-primer (3.0 μg at 1.5 μg/L).
4. Diethylpyrocarbonate (DEPC)-treated water.
5. Ribonuclease inhibitor (40 U).
6. Poly(A)$^+$ mRNA (5.0 μg in ≤36 μL DEPC-treated water; *see* **Notes 1** and **2**).
7. [α-^{32}P]-labeled deoxynucleotide (800 Ci/mmol) [α-^{32}P]dATP, [α-^{32}P]dGTP, or [α-^{32}P]dTTP. Do not use [α-^{32}P]dCTP (*see* **Note 3**).
8. Reverse transcriptase (250 U) (RNase H-deficient is recommended ***[20,21]***).

2.2. Second-Strand Synthesis

1. 10X second-strand buffer: 700 m*M* Tris-HCl, pH 7.4, 100 m*M* $(NH_4)_2SO_4$; 50 m*M* $MgCl_2$.
2. Second-strand dNTP mixture (10 m*M* dATP, dGTP, dTTP, and 26 m*M* dCTP).
3. *E. coli* RNase H (4.0 U).
4. *E. coli* DNA polymerase I (100 U).

2.3. Blunting the cDNA Termini

1. Blunting dNTP mixture (2.5 m*M* dATP, dGTP, dTTP, and dCTP).
2. Cloned *Pfu* DNA polymerase (5 U).
3. Phenol-chloroform (1:1 [v/v], pH 7.0–8.0) (*see* **Note 5**).
4. Chloroform.