2.11. Amplification of the Primary Library

1. Packaged and titered primary library.
2. Prepared, appropriate *E. coli* host strains.
3. NZY medium, plates, and top agarose: (*see* **Subheading 2.10., item 1**).
4. S*M* buffer: (*see* **Subheading 2.10., item 5**).
5. Chloroform.
6. Dimethylsulfoxide (DMSO).

3. Methods

3.1. First-Strand Synthesis

The final volume of the first strand synthesis reaction should be 50 µL. Take this into account when determining the volumes necessary.

1. In an RNase-free microcentrifuge tube, add the reagents in order: 5.0 µL 10X first-strand buffer, 3.0 µL methyl-nucleotide mixture, 2.0 µL linker-primer (1.5 µg/µL), *X* L DEPC-treated water, 40 U ribonuclease inhibitor.
2. Mix the reagents well. Add *X* µL of poly(A)$^+$ mRNA (5 µg) and gently vortex (*see* **Notes 1** and **2**).
3. Allow the mRNA template and linker-primer to anneal for 10 min at room temperature.
4. Add 0.5 µL of [α-^{32}P]-labeled deoxynucleotide (800 Ci/mmol). Do not use [α-^{32}P]dCTP (*see* **Note 3**).
5. Add 250 U of reverse transcriptase. The final volume of the reaction should now be 50 µL.
6. Gently mix the sample and briefly spin down the contents in a microcentrifuge.
7. Incubate at 37°C for 1 h.
8. After the 1-h incubation, place on ice.

3.2. Second-Strand Synthesis

The final volume of the second strand synthesis reaction should be 200 µL. Take this into account when determining the necessary volumes.

1. To the first-strand reaction (50 µL), add the following components in order: 20.0 µL 10X second-strand buffer, 6.0 µL second-strand dNTP mixture, *X* µL sterile distilled water (DEPC-treated water is not required), 4 U *E. coli* RNase H, 100 U *E. coli* DNA polymerase I.
2. The final volume of the reaction should now be 200 µL. Quickly vortex and spin down the reaction in a microcentrifuge. Incubate for 2.5 h at 16°C.
3. After the 2.5-h incubation, place on ice.

3.3. Blunting cDNA Termini

1. Add the following reagents to the synthesized cDNA: 23.0 µL blunting dNTP mixture, 2.0 µL cloned *Pfu* DNA polymerase (2.5 U/µL).