2. Incubate the phage and the bacteria at 37°C for 15 min to allow the phage to attach to the cells.
3. Add 2–3 mL of NZY top agarose (48°C) containing IPTG and X-gal (*see* **Note 10**). Plate onto NZY agar plates and place them upside down in a 37°C incubator.
4. Plaques should be visible after 6–8 h. Background plaques are blue, recombinant plaques are clear and should be 10- to 100-fold above the background.
5. Count the plaques and calculate the titer. Primary libraries can be unstable. Immediate amplification of at least a portion of the library is recommended to produce a large, stable quantity of a high-titer stock of the library.

3.11. Amplification of the Primary Library

After amplification, the library is suitable for screening by a variety of techniques *(2)*. More than one round of amplification is not recommended, since slower growing clones may be significantly underrepresented.

1. Prepare the host strains (*see* **Subheading 3.10.1.**).
2. Mix aliquots of the packaged library containing ~50,000 plaque-forming units (PFU) (≤300 µL vol) with 600 µL of host cells in Falcon 2059 polypropylene tubes. Usually, 1×10^6 PFU are amplified (20 tubes).
3. Incubate the tubes containing the phage and host cells for 15 min at 37°C.
4. Mix 8.0 mL of melted NZY top agarose (48°C) with each aliquot of infected bacteria and spread evenly onto a freshly poured 150-mm NZY plate.
5. Incubate the plates at 37°C for 6–8 h. Do not allow the plaques to grow larger than 1–2 mm.
6. Overlay the plates with 8–10 mL of SM buffer. Store the plates at 4°C overnight with gentle rocking. The phage will diffuse into the SM buffer.
7. Recover the SM buffer containing the bacteriophage from each plate and pool it in a sterile polypropylene container. Add chloroform to a 5% final concentration and mix well.
8. Incubate for 15 min at room temperature.
9. Remove the cell debris by centrifugation for 10 min at 500*g*.
10. Recover the supernatant and transfer it to a sterile polypropylene container. Add chloroform to a 0.3% final concentration and store at 4°C.
11. Check the titer of the amplified library by making serial dilutions in SM buffer and plating on host cells (*see* **Subheading 3.10.3.**). The average titer is usually 10^9–10^{12} PFU/mL.
12. Frozen stocks can be made by adding dimethylsulfoxide (DMSO) to a final concentration of 7%, mixing well, and freezing at –80°C.

4. Notes

1. The mRNA sample must be highly purified for efficient cDNA synthesis. The mRNA sample may contain inhibitors that can be removed by phenol:chloroform extractions. The presence of DNA or rRNA will give an inaccurate concentration of mRNA, leading to an insufficient amount of sample used. Treat the mRNA