5. The gel can be transferred onto Whatman paper at this point and dried under vacuum at 80°C, with a trap attached for 2 h, or it can be fixed first. Fixing the gel should reduce its drying time to 30-45 min by removing the hygroscopic urea in the gel. Place the gel and back plate in a large tray, pour the fix over the gel, and make sure the gel is covered for 15–20 min. Carefully pour off the fix, and transfer to Whatman paper and dry in the usual way.
6. Expose the dried gel to X-ray film overnight. Develop and dry the autorad to read the sequence (*see* **Notes 8–10** for problem-shooting on the appearance of the sequence).

3.3. Single-Stranded DNA Sequencing

3.3.1. Preparation of Single-Stranded DNA

pGEM-T (Promega) and Bluescript (Stratagene) contain F1 origins of replication enabling the export of a single-stranded molecule, including insert sequences. Single-stranded sequencing offers a speedier protocol, longer reads (up to 700 bp), and better clarity and resolution than double-stranded sequencing.

1. Add the following to a 50 mL sterile screw-top plastic tube: 5 mL LB broth; 25 μL M13KO7; 24 μL Ampicillin (25 mg/mL); 100 μL fresh overnight culture of pGEM-T or Bluescript clone.
2. Incubate at 37°C overnight in a shaking incubator.
3. Spin the whole 5-mL culture at 12,000*g* in a benchtop centrifuge for 5 min to pellet the cells.
4. Split the supernatant into three microcentrifuge tubes, placing 1.5 mL in each, and microcentrifuge for 5 min at 12,000*g*.
5. Remove 1 mL of supernatant from each of the tubes to fresh Eppendorfs containing 200 mL of 20% PEG 6000/2.5*M* NaCl, and mix by inverting.
6. Leave at room temperature for 20 min.
7. Microcentrifuge for 10 min at 12,000*g* and pour off most of the supernatant.
8. Recentrifuge for a further 3 min and remove the remaining PEG mixture with a drawn-out Pasteur pipet. The white pellet of phage should be visible at this stage.
9. Add 100 mL sterile distilled water to each pellet, resuspend, and add 50 mL of phenol. Vortex for 10 s.
10. Microcentrifuge for 3 min at 12,000*g* and transfer upper aqueous layer to a fresh Eppendorf, bulking up the contents of the three tubes into one.
11. Extract once more with an equal volume of chloroform.
12. Add 0.25 vol of 10*M* ammonium acetate and 2 vol of absolute ethanol. Mix and store at –20°C until required.
13. Precipitate the ssDNA by centrifugation for 10 min at 12,000*g*. Wash the pellet in 1 mL 70% ethanol and vacuum dry.
14. Resuspend the pellet in 40 μL of water. Check the quality of the ssDNA by spectrophotometer analysis or on a normal 1% agarose gel (*see* **Note 11**).