Polyacrylamide Gel Electrophoresis (PAGE):

For a basic PA gel, the necessary components are:

- Acrylamide/bisacrylamide stock solution
- Ammonium persulfate (APS)
- Tetramethylethylenediamine (TEMED)

The acrylamide/bis-acrylamide solution is what will polymerize, and the degree of crosslinking is set by the ratio of acrylamide to bis-acrylamide. For most nucleic acid gels, a 19:1 ratio is generally used, usually with a stock solution of 40%. The density of the gel and pore sizes are controlled both by the ratio of monomer to dimer, as well as the amount of acrylamide in the final gel mixture- the "percent" of a gel. APS and TEMED are added just before the gel is molded, and act to initiate the acrylamide polymerization.

Buffer selection is important- denaturing gels add a denaturing agent (i.e., SDS or Urea) to the buffer, and non-denaturing gels do not. Borate and EDTA are common additives to help chelate metals, and Tris is a common basal buffering agent. TBE (Tris-Borate-EDTA) and TAE (Tris-Acetate-EDTA) are commonly used gel buffers, and the pH can be adjusted depending on the species to be separated. A straight Tris buffer can also be used.

A typical recipe for a simple 12% gel with a non-denaturing tris buffer is shown below.

- 3 mL 40% 19:1 acrylamide/bis-acrylamide stock
- 2.5 mL 4x Tris buffer
- 4.5 mL DI water

To which we add:

- 50 uL of an APS stock (0.05 g APS / 500 uL H_2O)
- 10 uL TEMED

This solution is mixed, and quickly added to a gel mold, taking care not to create bubbles, followed by the addition of a comb to the top of the gel. The gel is allowed to polymerize, and the process can be followed by the gel stock remaining in the mixing vessel.

The gel is moved to the running container, the inner buffer tank is filled with 1x Tris buffer (diluted from the same 4x TBE buffer used in the gel), followed by careful removal of the comb (the buffer helps lubricate it to slide out without tearing).

The outer tank is filled with buffer (enough to at least cover the bottom of the gel, ~200 mL), and the wells are washed with 1x Tris buffer using a syringe and needle.

Samples should be mixed to a 20 uL total volume with 5 uL of a 1% bromophenol blue loading dye, and mixed thoroughly. Each sample is carefully loaded into a well using a long gel-loading tip, and should settle at the bottom of the well without floating up.

For nucleic acids, we run the gel at 180 V, following the movement of the bromophenol blue dye, which runs at about the same pace as a 10-12mer oligonucleotide, and should be stopped when the dye has run sufficiently down the gel.

To image the gel, staining is effected with a 0.02% methylene blue dye, where the gel is placed into the staining container for 15-20 minutes, until the entire gel has developed a dark blue color.

The gel is then destained in DI water for another 20-30 minutes, until the background has been removed and the bands corresponding to the nucleic acids remain.

Methylene blue can be imaged visually under ambient light, and a picture taken for documentation.

Determining the amount of nucleic acid to load is not an exact science, although between 4-7 ug of nucleic acid is recommended, although lower loads can also work. High loads lead to better staining for visualization, but can cause broad and smeared bands.

If you choose to run a fluorescein-tagged synthetic oligonucleotide alongside your extracted RNA, you can use a hand-helped UV lamp to visualize the green fluorescence from the conjugated fluorescein in the gel.

Some excellent video resources for remembering the procedure for molding and running a gel:

How to Run an SDS-PAGE Gel (LabTricks): https://youtu.be/XUjLO-ek2C8

How to Make an SDS-PAGE Gel (LabTricks): https://youtu.be/EDi_n_0NiF4