Standard gel electrophoresis of nucleic acids or PCR products – for other applications with other agarose types, check specific literature

Reagents / equipment:

Standard agarose 1-1,5 %. Dissolve 1-1,5 gram in 100 ml 1xTAE buffer (diluted with Aqua dest from a 100 x stock solution). Heat in micro oven. Pour the gel and select appropriate combs to produce wells of desired size. TAE-Puffer (100x): 4 M Tris, 1 M Natriumacetate, 0,1 M EDTA. Mix with Aqua dsest. Adjust pH to 8,0 with acetic acid. The standard agarose can be exchanged to agaroses of higher qualities or other properties (e.g. low melting agarose).

DNA ladder: Select a ladder with appropriate sizes. For example, for 16S rRNA PCR, select a 1 kb DNA ladder (e.g. from Biolabs or Promega). Be sure to explore:

a) which product and brand you are using.
b) if the ladder needs to be diluted with a loading dye (check the protocol from the company).

Example of receipt for own production of loading dye (for 1:1 mix with the PCR product):

25 % (w/v) Ficoll
0,5 % (w/v) Bromphenolblue
0.5 % (w/v) Xylencyanol
50 mM EDTA (sterile)

} Add a magnetic bar and stir for several hours at RT.

Ethidiumbromide-solution: 10 mg EtBr/ml H_2O_{dest} Must be produced at least once a week. Store at RT. Note, Ethidiumbromide is hazardous! Ethidum bromide can be replaced with other nucleic acid stains like SYBR Green or SYBR Gold.

Procedure:

1) Place the agarose gel in the electrophoresis tray, with 1x TAE buffer.
2) Pipette 5 to 20 µl (depending on the concentration of your PCR product) of your PCR product into each well. If you used a PCR buffer without dye, then mix the PCR product with a loading dye (e.g. on a parafilm or in tubes), following the protocols recommendations.
3) Pipette a DNA ladder on one or more wells.
4) Turn on the electricity. Conditions: 130 mA (maximum180 mA, maximum 150º C). Operation time ~ 0,5-1 h.
5) Turn off the electricity, remove the gel from the tray and place the gel into a tray with nucleic acid stain (e.g. EtBr). Stain for 20-30 minutes, wash in Aqua dest for 10 min.
6) Record image with a UV and corresponding digital image software.
7) Where appropriate, bands can be excised with a razor blade or other similar devices for subsequent extraction of specific PCR products.