QIAGEN Supplementary Protocol:

Polyacrylamide gel analysis of oligonucleotides

The quality of an oligonucleotide (i.e., how much is full-length product [length of n] versus incomplete products [n–1]) may be assessed by analysis of the oligo on a denaturing polyacrylamide gel.

This protocol has been optimized for the analysis of oligos 18–30 nucleotides in length, which is the typical size range for oligos used in PCR applications.

For further information on PCR, multiplex PCR, and RT-PCR please call your local QIAGEN Technical Service Department or visit our homepage at **www.qiagen.com**.

IMPORTANT: When working with hazardous chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Reagents and equipment required

- Polyacrylamide gel electrophoresis (PAGE) apparatus for 7–8 cm x 10 cm minigels; accessories (glass plates, 1 mm spacers, square-well or shark tooth combs); and power supply
- 40% acrylamide/bis-acrylamide (29:1) solution
- 10% ammonium persulfate (APS)
- TEMED (N,N,N',N'-Tetramethylethylenediamine)
- Urea
- 10x TBE buffer
- 1.25x formamide loading buffer
- 0.02% methylene blue in 0.1x TBE buffer

Preparation of reagents

- **40% acrylamide/bis-acrylamide (29:1) solution**

  Ready-to-use solutions of acrylamide/bis-acrylamide are commercially available, avoiding handling of harmful acrylamide powder. However, a 40% acrylamide/bis-acrylamide (29:1) solution can also be prepared using the following:

  38.62 g acrylamide
  1.38 g bis-acrylamide
  add water to obtain final volume of 100 ml

  This solution may be stored at 4°C in the dark and is stable for several weeks after filtering and degassing.

- **10% ammonium persulfate (APS)**

  Dissolve 1 g of ammonium persulfate (also known as ammonium peroxodisulfate) in 10 ml water. Store in aliquots at –20°C.

- **10x TBE buffer**

  Prepare a 10x stock solution of TBE in 1 liter of water:

  108 g Tris base
  55 g boric acid
  40 ml 0.5 M EDTA (pH 8.0)

  TBE buffer is normally used at 1x concentration (89 mM Tris-borate, 2 mM EDTA) for PAGE.

- **1.25x formamide loading buffer**

  900 µl formamide
  22.2 µl 0.5 M EDTA (pH 8.0)
  26.5 µl 7.5% orange G
  51.3 µl water

- **Methylene blue staining solution (0.02%)**

  10 mg methylene blue
  500 µl 10x TBE buffer
  49.5 ml water

  This staining solution may be reused several times.

Procedure

Preparation of 15% acrylamide/urea gel

This gel is optimal for resolving 18–30mer oligonucleotides.

The recipe below provides 15 ml gel solution, which is sufficient for two standard size gels (e.g., measuring 7 x 10 cm and 1 mm thick, and requiring 7 ml gel solution each).

1. **Combine the following in a beaker:**

   5.63 ml 40% acrylamide/bis-acrylamide (29:1) solution
   7.2 g urea
   1.5 ml 10x TBE buffer

   Warm the above mixture to 37°C to dissolve the urea.

   Note: Warming the above mixture can be done by heating the mixture in a microwave oven for a few seconds at full power; be careful not to overheat however, as this will destroy the urea.

2. **Add 1.9 ml water to make volume up to 15 ml. Mix by stirring.**

   Note: Placing the above mixture on ice for approximately 5 min prevents polymerization of the solution occurring too quickly after the subsequent addition of APS and TEMED. Extended cooling may result in precipitation of urea, which can be seen by the appearance of white crystals; should this happen, urea crystals can be redissolved by warming the solution.

3. **Add the following to the beaker containing the gel solution directly before pouring the gel:**

   75 µl 10% ammonium persulfate (APS)
   7.5 µl TEMED

   Swirl the beaker to mix the solution, then immediately pour the gel, avoiding the introduction of air bubbles. Place the appropriate comb into the gel and avoid moving the gel until polymerization is complete (after approximately 30–45 min).

Running the gel

1. **Pre-run the gel in 1x TBE buffer for 30 min at 200 V (for a minigel).**
   If using a minigel system, fill the outer buffer tank with 1x TBE buffer to approximately one third of the total volume.

2. **Prepare the samples by mixing approximately 200 pmol (100–300 pmol) of oligo with 1.25x formamide loading buffer.**
   For a quality assessment gel, use 200 pmol (2 µl of a 100 µM solution) of oligo, which is sufficient to identify n–1 products. Ensure that the final volume of the sample (including the loading buffer) is appropriate for the well size of the gel.

   If the required volume of your oligo solution is greater than 2 µl, reduce the volume by placing the sample in a 65°C heat block for several minutes. Do not overdry.

3. **Heat samples to 95°C for 2 min, then immediately chill by transferring samples onto ice.**
4. **Rinse the wells of the gel before loading, e.g. by gently aspirating buffer in the wells using either a Pasteur pipet or a syringe with a needle, until unpolymerized material has been removed.**
5. **Load the samples onto the gel. Also load a separate well with 1x formamide loading buffer containing xylene cyanol FF and bromophenol blue. On a 15% polyacrylamide gel, these marker dyes co-migrate with oligonucleotides with lengths of 30 and 9–10 bases respectively.**
6. **Run the gel for 1.5 h at 200 V (for a minigel).**
   When the dyes have migrated the desired distance, remove the gel and proceed with detection. (The gel may be run until the orange G dye has migrated out of the gel, typically until the bromophenol blue dye has reached the middle of the gel, approximately after 1.5 h).

Detection of oligonucleotides

Oligonucleotides in polyacrylamide gels can easily be detected by staining with 0.02% methylene blue staining solution.

1. **Remove the gel from the glass plates and put it into a plastic box (which should be slightly larger than the gel).**
2. **Add 0.02% methylene blue staining solution to the box.**
3. **Agitate the gel gently in the solution for 20–30 min.**
   Oligos should become visible after 10–15 min. If staining is weak after this time, add fresh methylene blue staining solution.

4. **Remove the staining solution (methylene blue staining solution may be stored for later reuse).**
5. **Destain the gel by adding distilled water to the box. Leave gel submerged in water for 2–3 min and replace with fresh water when it becomes deeply colored with the dye. Repeat 2–3 times until the blue background of the gel is sufficiently reduced.**
   Note: Silver staining, using commercially available reagents, can also be used for detection of oligonucleotides.
   Note: We do not recommend the use of ethidium bromide for staining of oligonucleotides, since staining intensity varies depending on the sequence of the oligonucleotide.

6. **Photograph the gel for documentation.**

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