# 2.Agarose gel prep for 16S and 96 well gel

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ABSTRACT

Agarose gel preparation for 16S rRNA

GUIDELINES

Amplicon size: ~390 bp
Expected band size for 515F–806R is ~300–350 bp
Low-biomass samples may yield faint or no visible bands; alternative methods such as a Bioanalyzer could be used to verify presence of PCR product

MATERIALS TEXT

1. Agarose
2. Graduated cylinder
3. Erlenmeyer flask
4. Thermometer
5. Stir bars
6. Stir plate
7. 100 bp ladder
8. Nucleic acid stain (ex., gel red)
9. TBE or TAE at 1X
10. Casting tray
11. Well combs
12. Voltage source
13. Gel box
14. UV light source
15. Microwave
16. autoclave glove
17. Multichannel pippetter
18. Loading dye
19. Parafilm

SAFETY WARNINGS

- *Agarose must be cooled to 60C prior to setting on gel cast. If the gel is cast at hotter temperatures this can warp and crack the tray over time.*
- *Use autoclave glove when handling hot agarose*
- *Do not use Ethidium bromide*
- *Make sure to use the same buffer as the one in the gel box (do not mix different buffers and do not use water).*

## Pouring a Standard 1.5% Agarose Gel for 96 well gel:

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1. Measure 4.5 g of agarose.
2. Measure 300 mL of 1xTAE (or TBE) in graduated cylinder
3. Mix agarose powder with 300 mL of 1xTAE (or TBE) in a microwavable flask
4. Microwave for ~3 min in pulses and swirl flask in between pulses until the agarose is completely dissolved. If the solution has evaporated you can add DI water to bring it back to volume.
5. Let agarose solution cool down to about 60 °C . Do this by placing the flask on a plate spinner and placing a stir bar inside the flask. Monitor temperature with a thermometer.
6. Add 30ul of Gel Red or another nucelic acid stain.
7. Pour the agarose slowly into a gel tray with the well combs in place and remove any bubbles.
8. Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

9. Once solidified, place the agarose gel into the gel box
10. Fill gel box with 1xTAE (or TBE) until the gel is covered.

## Loading Samples and Running an Agarose Gel

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1. On a piece of parafilm add 1ul of loading dye for each sample.
2. Take 4ul of each PCR sample and mix it with a corresponding 1ul of loading dye.
3. Load a 100bp ladder to each row (I suggest to add it to the middle column).
4. Carefully load your samples into the additional wells of the gel. With multichannel every other well gets filled and some have suggested loading every other well is ideal for this protocol.
5. Run ~70V for about 30 min or until indicator band is 70% through the gel.

## Visualize gel

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1. View with gel imager
2. look for a band ~300–350 bp
3. Make sure negative is negative
4. Take picture
5. Note if there are any double bands.