Aurora Protocol

Prep time: Roughly 2-3 hours

Materials needed:

* Agarose
* 10x TBE Buffer diluted to 0.25x
* UltraPure Distilled Water
* Aurora Reusable Cartridge
* Cartridge dams
* 10% concentration bleach

**Additional Steps**

* Begin to heat water in beaker for agarose bath. Heat to ~95 C
* Turn on Thermocube and press “start”

**Preparing the Cartridge**

Each run uses approximately 4 ml of agarose. Preparing 5 ml is preferential in case extra agarose is needed. Measure 0.05 g of the dry agarose powder in a falcon tube. To prepare the 0.25x TBE Buffer, add 1.25 ml of the 10x TBE Buffer to approximately 48.75 ml of UltraPure Distilled water for a total volume of 50 ml. Sterilize 0.25x TBE Buffer in UV flask in UV hood for at least 1 hour. In the falcon tube, combine 5 ml of the 0.25x TBE Buffer and measured dry agarose powder, and vortex to ensure no agarose is clumped together. (Mixture will not homogenize until boiled and set in hot water bath.) Microwave approximately 9-11 seconds with lid slightly loosened, until mix is about to boil. When hot water bath has reached right below boiling point, tighten the lid of the falcon tube, vortex, and set in the bath for at least 1 hour.

While the agarose is in the bath, begin cartridge sterilization. **Do not use ethanol to clean cartridge; it will cause the cartridge to crack.** Soak the cartridge in 10% bleach for 30 minutes, and soak the dams for 20 minutes. **Do not soak any items longer than 30 minutes; this can damage the equipment.** After soaking process is complete, set both cartridge and dams in the UV hood for 30 minutes, or until dry. Leave in hood to avoid contamination until agarose is ready.

Once agarose is ready, insert dams into their proper locations (see manual) and pour molten agarose into mold. Be sure to check for, and correct, any abnormalities (such as bubbles) in the molten gel. Place cartridge with molten agarose back in the UV hood and sterilize for 20-30 minutes until agarose sets.

Once gel is ready, gently remove dams by slightly pulling out, and then up. Check extraction well to ensure that no agarose is present, which could affect DNA yield and concentration. Fill each buffer chamber with 5 ml of 0.25x TBE Buffer, except for the chamber to the left of the extraction well, which will be filled with 4 ml of buffer. Place lid on cartridge. Pipette 60 ul of 0.25x TBE buffer into the extraction well, and immediately seal with PCR tape, using the extraction well sealing tool to ensure a strong seal. Place approximately 1 ml of distilled water on the cold plate of the Aurora to ensure a good connection between the cartridge and machine.

Dilute the sample with ~5 ml of UltraPure water and check salinity levels. For the DNA Clean-Up protocol, the sample conductivity needs to be ≦100 uS/cm. Ideal salinity for the sample is ~70 uS/cm. Once ideal conductivity has been reached, carefully pour into the sample chamber in the cartridge. Close the drawer and select the desired protocol.

**Tips**

* If salinity is too high, use Amicon columns. Be sure to keep track of DNA concentration, as a significant amount of DNA can be lost during this process.
* If working with a large amount of sample to extract a low biomass of DNA, multiple runs can be created and paused before the focus step, and in place of the 60 ul of blank 0.25x TBE buffer, insert the previously cleaned sample in buffer to add to the total concentration.
* Work with the 0.25x TBE buffer carefully, and do not store for too long. Various methods of autoclaving, filtering, and UV sterilization are strongly suggested to minimize possible contamination.
* DNA fragments of less than 300 bases will be mistaken for contaminates, so shearing prior to cleaning is not recommended.
* When run is complete, extract purified DNA as soon as possible. DNA will begin to be reabsorbed by the gel if left too long. If you’re late to extract the sample, a block of refocusing can be done to try and recover any DNA loss that may have occurred. Normal focus block is done for about an hour and a half, but the Focus Block protocol can be adjusted to desired length. The longer the focus, the (theoretically) higher DNA yield.
* A larger amount of agarose can be prepared at one time, but should only be stored up to 2-3 days. Old agarose can be used, but is not ideal for an optimal run.
* The Thermocube can take a while to set. Make sure the “\*” on the cube turns into a “+” or “-” when the start button is pushed. The asterisk means the thermocube temperature control is not active.
* The initial conductivity check done by the Aurora may also takes a while, in which errors can occur. It is ideal to wait around for a short amount of time to make sure the run continues properly.
* When microwaving the agarose, be sure to loosen lid, and not to microwave for too long! Overheating can cause the agarose to boil over, and not loosening the lid enough can cause the boiling agarose to spew when the pressure is released. You will lose agarose, you will burn yourself, and you will have to restart the agarose procedure.