

1. PRINCIPLE

- 1.1. Ultra-pure water is recommended for preparation of 1x TBE running buffer used in polyacrylamide gel electrophoresis of sequencing reactions.^{5,2} In the Visible Genetics Genotyping System, labeled oligonucleotides migrate past laser detectors and this data is used to generate the DNA sequence of the specimen. The use of ultra-pure or Type I Reagent Water, also referred to as double distilled water, ensures control of electrophoretic mobility and minimizes interference with the laser detection process. In our hands, Type I Reagent Water and TBE running buffer have been successfully stored in high-density polyethylene carboys or glass containers without effect on the assay.
- 1.2. The Milli-Q[®] A10 Biocel Synthesis Academic Gradient Water System provides Type I Reagent Water through a series of filtration and purification steps. This Millipore System pumps pre-treated deionized source water through the Q-Guard filter for an initial purification step. The water is then exposed to UV light at 185 and 251nm, oxidizing organic compounds and killing bacteria. The Quantum cartridge removes trace ions and oxidation products from the UV treatment. Purified water then passes through an Ultra filtration module, which retains colloids, particles and organic molecules with a molecular weight > 5000 Daltons. A final 0.22 µm filter prevents recontamination at the point of use.

2. STANDARD/CONTROL

- 2.1. De-ionized source water must be tested initially for silicates. If high concentrations are present, ensure that the water treatment process is adequate to bring the silicate content in range.
- 2.2. Specifications for Type I Reagent Water:
 - 2.2.1. Maximum Microbial Content: 10 CFU/ml
 - 2.2.2. Minimum resistivity, in-line: 10 megohm-cm
 - 2.2.3. Maximum silicate content: 0.05 mg/l SiO₂
 - 2.2.4. Particulate Matter: 0.22µm filter

3. EQUIPMENT

- 3.1. Millipore Water Purification System ZMQS60F0Y, Milli-Q[®] A10 Academic Gradient Biocel Water Filtration system
- 3.2. Sterile Containers

4. PROCEDURE

4.1. De-ionized source water

- 4.1.1. Maintenance: The UCHSC Facilities Department maintains the campus de-ionized water supply
- 4.1.2. Quality Control Testing: The UCHSC Facilities Department is responsible for all quality control and documentation related to the house supplied deionized water supply to the laboratories.
- 4.1.3. Corrective action: The UCHSC Facilities Department notifies the user when the supply of deionized water is interrupted or does not meet specifications.

4.2. Type I Reagent Water

- 4.2.1. Normal Operation: Press the OPERATE/STANDBY keypad until the display reads PRE-OPERATE. Open the valve with the control lever. Collect Type I Reagent Water when the resistance reads 18.2 megohm-cm and all quality warning lights are extinguished
- 4.2.2. Maintenance of the Milli-Q[®] A10 Academic Gradient Biocel Water Filtration System: the Fang Lab, Division Infectious Diseases, maintains this system for use by multiple laboratories in the Division. The system maintains an electronic record of maintenance activities and will display a warning light when maintenance is required. Although the unit is programmed to prompt the operator for replacement of the filters every 180 days, Millipore's policy indicates that filters should be changed annually, and performance validated every 180 days. If water quality is in range, the unit may be re-set by merely removing and replacing one of the existing filters, thereby turning off the warning light. The 180-day timer is hardwired and cannot be modified. Colorado Retrovirology Laboratory staff is not responsible for routine maintenance of this system, which includes a periodic overnight sanitization with sodium hydroxide, annual filter replacement, and corrective action whenever resistivity falls below 16 megohm-cm, as set by the manufacturer. Routine testing of water quality ensures that appropriate maintenance has been performed. Simple instructions for performance of these tasks are outlined in Milli-Q[®] Operating and Maintenance manual located by the unit. Contact Millipore Laboratory Water Division for technical service or on-site assistance, (800) 645-5476.
- 4.2.3. Quality Control Testing:
 - 4.2.3.1. Silicates: Collect an initial sample of 15 to 50 ml of Type I Reagent Water. Deliver to the University Hospital Clinical Laboratory for testing. Contact the manufacturer of the water purification system if results fall out of range.
 - 4.2.3.2. Microbial content: Each month, collect a sample of 15 to 50 ml of Type I Reagent Water in a sterile container. Deliver to the University Hospital Clinical Laboratory for testing.
 - 4.2.3.3. Resistivity: Each month, record the resistivity of the Type I Reagent Water as the specimen for microbial content is collected.

4.2.3.4. Documentation: Record the results of testing on the Water Quality Control Log.

4.2.3.5. Corrective action:

4.2.3.5.1. If the microbial growth is >10 CFU/ml, ensure that sanitization has been performed, and recollect. If the second culture fails and the resistance falls with specification, change the 0.22µm end filter, disinfect the shield at the dispensing line, and recollect. Use an alternate source of Type I Reagent Water until the problem can be resolved. Notify the maintaining laboratory and the other users. If the microbial content is still out of range, replace all the filters and repeat testing. Call for service if this does not resolve the problem.

4.2.3.5.2. If the resistance falls above or below acceptable levels, let the Milli-Q® operate a few minutes to force out any air in the resistivity cell. If the resistance is still out of range, notify the maintaining laboratory and use an alternate source of Type I Reagent Water until the problem can be resolved.

4.2.3.5.3. Refer to the troubleshooting section in the Milli-Q® Operating and Maintenance Manual for additional information.

5. REFERENCES

- 5.1. Sambrook, J., E.F. Fritsch, T. Maniatis. 1989. Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 6.2-6.7.
- 5.2. David Chan, Visible Genetics, personal communication 9/00.
- 5.3. NCCLS C3-A3, Volume 17, No.18, 1997. Preparation of Reagent Water in the Clinical Laboratory; Approved Guideline, Third Edition.
- 5.4. Millipore/Milli-Q® Operating and Maintenance Manual, 1997.

6. ATTACHMENTS

- 6.1. Water Quality Control Log