Tsai Lab SOP – General Instructions for preparing media/Good Lab Practice

1. Wear gloves, lab coat. Take an appropriate size beaker and a stir bar, rinse both with deionized water.
2. Begin with water, ½ to ¾ of the final volume. Turn on the stirrer to help uniform mixing of the components.
3. Refer to the media prep checklist. Gather all the media components you need to add pre-autoclaving (some heat-sensitive components can only be added after autoclaving at the laminar flow).

   We have many different medium types, double check the catalog number to make sure you use the correct one.
   Do not open autoclaved media, boxes, and filter-sterilized stock solutions outside of the laminar flow.

4. Weigh and add media components in the order as they appear on the media prep checklist. Make sure that you let all the added components dissolve.

   Use clean weighing paper and spatulas. Do not cross contaminate media source by using the same spatulas, or weighing paper.

5. Adjust the media to the final volume using a cylinder. Pour the media back to the beaker/bottle, adjust the pH, add MES, then **again** check/adjust pH. **NEVER add MES buffer before adjusting pH.** MES has a buffering range of pH 5.5-6.7. Therefore, it is important that you adjust pH to the desired level (5.8) **BEFORE** adding MES, or you would need to add a lot more acid or base solution to change the pH!

   Before and after use, rinse pH electrode with deionized water and gently pat dry the electrode using Kimwipe. NEVER rub the electrode. Make sure there is enough electrolyte solution in the electrode and in the electrode dipping bottle.

6. Transfer (and divide) the media into autoclavable bottles or conical flasks. Select the size of the container so that there is at least 2/3 empty space above media to avoid boiling over during autoclave cycle.
7. Add gellan gum to each container (0.3% final). **Add a stir bar** to each container and cover with aluminum foil (for flasks) or screw caps for bottles (close all the way then backward a bit to allow for venting).

   Wipe clean thoroughly media prep station and keep all the media components back to their storage location immediately.
   Wash weighing accessories and rinse thoroughly.

8. Autoclave for 30 minutes. After autoclaving, let media cool while stirring on a stirrer until the temperature is optimum (~60°C) to add heat sensitive components.

   DO NOT leave media in autoclave longer than necessary or it will cause browning (Maillard reaction).
   The medium temperature is optimum when it is 'bearable" to touch with the inside of your arm.

9. Use a laminar flow, add heat-sensitive, filter-sterilized plant growth regulators and/or antibiotics to the media using autoclaved tips that have **NEVER** been open outside the laminar flow. Let media stir for 30 sec for uniform distribution of added components. Pour into petri dishes/cups as appropriate.

   Organize boxes and petri plates in hood so that you do not reach over any open sterile items and as you might unknowingly shed particles leading to contamination.

10. After media gel, close lids properly, and label with date and initials. Bag solidified plates in original plastic sleeve. Store media at 4°C if containing antibiotics. Regular media can be stored in B309. **The plates should sit upside/topside up to avoid contamination.** Label media and your initial on the bag as well.
11. Rinse all plasticware and glassware with **HOT water** until remaining media is washed off. Check containers against light as the gelled media is hard to visualize. Dump any remaining agar debris in regular trash can (or in autoclave waste bag if containing antibiotics) and **NEVER in sink**. Place rinsed glassware in wash tubs for washing (don't leave them inside the sink).