TECHNIQUES IN MOLECULAR BIOLOGY – BASIC TECHNIQUES: PIPETING AND STERILE CULTURE

Mastery of micropipetting and sterile technique is essential for reliable success in molecular biology. Take these exercises and our discussions about pipetting seriously.

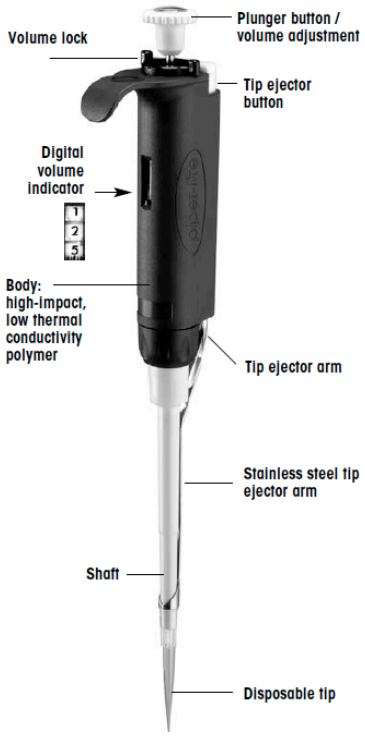
Digital micropipettors A digital micropipettor is essentially a precision pump fitted with a disposable tip. The volume of air space in the barrel is adjusted by screwing the plunger in or out of the piston, and the volume is displayed on a digital readout. Depressing the plunger displaces the specified volume of air from the piston; releasing the plunger creates a vacuum which draws an equal volume of fluid into the tip. The fluid in the tip is expelled by depressing the plunger again, but to a further, second 'blow-out' stop.

The volume range of digital micropipettors varies with the manufacturer, but most have fairly similar ranges. The Biology Department labs, use mainly the **Finnpipette** (or Fisher brand equivalent) micropipettors with the following ranges:

Color*	Nickname**	Range^	Tip^^
Blue	"P1000"	100 µl – 1000 µl	blue
Yellow	"P200"	20 µl – 200 µl	yellow
Orange	"P50"	5 µl – 50 µl	yellow
Grey	"P10"	0.5 µl – 10 µl	white/clear

* Color coded at the top of the plunger /volume adjustment knob.
** The nickname is originally derived from a different pipettor brand ('Pipetman'), but still widely used by molecular biologists for all micropipettors.
^ Important conversions: 1 ml = 1000 µl, 1 µl = 0.001 ml = 10^{-3} ml = 10^{-6} liter
^^ Traditional color for tip (can be other colors, most commonly white/clear)

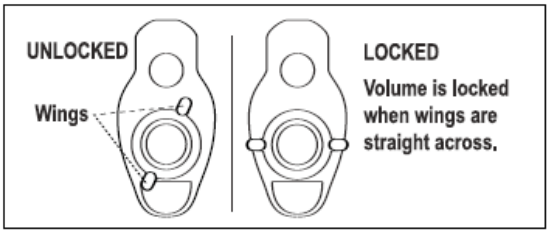
The biochemistry labs use the **Pipet-Lite** (LTS) style from Rainin. Pipet-Lite (LTS) is a brand of micro-pipettor used in the laboratory. Pipet-Lite is an air-displacement pipette which uses a magnetic assist and is slightly different than the Gilson pipets used in other labs. Become familiar with this pipet and it's components. These pippetors use a completely different style of tip than the rannin or finnipipettes. Do NOT try to use the wrong tip. If they don't fit they will not give you the correct volumes! These pipettes are used in the same range as shown above but are labeled L1000, L200, … ext.



Take the following precautions when using the pipettor:

- Never rotate the volume adjustor beyond the upper or lower range indicated on the pipettor.
- Never use without a tip in place.
- Never invert or lay down a pipettor with a filled tip (fluid can run into the barrel).
- Never let the plunger snap back after withdrawing or expelling fluid (could damage the piston or cause fluid to enter the barrel, and contact the piston – aka 'fountaining').
- Never immerse the barrel into fluid.
- Never reuse a tip that has been used to measure a different reagent, or that has entered a tube containing a different reagent. When in doubt, use a fresh tip.

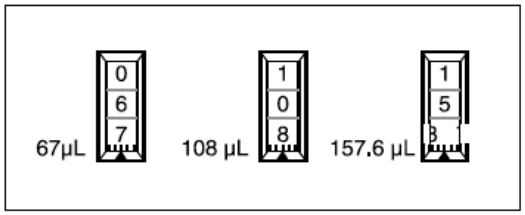
Pipetting: Pipet-Lite (LTS) is a brand of micro-pipettor used in the laboratory. Pipet-Lite is an air-displacement pipette which uses a magnetic assist and is slightly different than the Gilson pipets used in other labs. Become familiar with this pipet and its components.



Setting Volume:

- Turn the volume lock counter-clockwise to **unlock the pipet**. The position shown at left below so the volume setting mechanism is unlocked and free to turn.

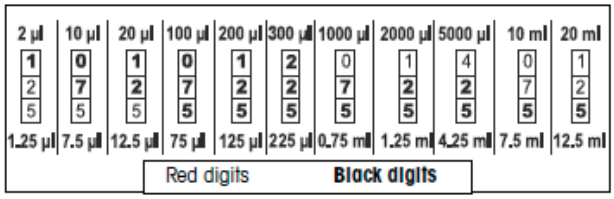
Example volumes for the 200 µL model are shown below (note the intermediate setting at the right).



- With the mechanism unlocked, then rotate the plunger button to **change volume** – counter-clockwise to increase, and clockwise to decrease volume.
- When setting the desired volume, first turn the knob slowly clockwise until the desired volume is displayed. **Always dial down** to the desired volume.
- **Lock the pipet** by turning the volume lock clockwise (see figure for locked position)

Operation:

- Press the plunger button tot the **first stop**, and **hold** it in this position. The magnetic latch will help you sense and hold this position.
- Holding the pipet **vertically**, place the tip into the sample at the proper depth (see tip emersion chart) and relax your thumb pressure on the plunger. Do not let go of the plunger button, or the piston may snap up quickly, resulting in inaccurate measurement.
- **Pause briefly** to ensure the full volume of sample is drawn into the tip.



2–20 µL: Black – µL. Red – tenths, hundredths of µL.
100–300 µL: All digits black – whole µL.
1000–5000 µL: Red – mL. Black – tenths, hundredths of mL.
10 mL: Red – mL. Black – tenths of mL
20 mL: Red – mL. Black – tenths of mL.

- **Withdraw the tip** from the sample. If any liquid remains on the outside surface of the tip, wipe it carefully with a lint-free tissue, taking care NOT to touch the tip orifice.
- **Touch the tip end against the side wall of the receiving vessel** and press the plunger slowly, past the first stop. Wait one to two seconds.
- **Still holding the plunger down, with draw the tip. Then release the plunger.**

Simple pipetting tips

1. Always keep an eye on the tip to see if all of the liquid was drawn into the tip.
2. If you have picked up a significant amount of liquid with the tip touch it against a tube or a tissue, but do not wipe the tip. Capillary action will draw out some of the liquid.
3. Always add appropriate amounts of a single reagent first to reduce contamination
4. Release the liquid onto a new location in the tube or just into the liquid. DO not just shoot small volumes into the tube. This will lead to a very inaccurate pipetting.
5. The tip should be just into the liquid. Too far and you are likely to leave additional liquid on the outside of the tip and this can lead to a significant error. Slowly release the plunger. Never snap the plunger up. Pause for a second or two. Then place the tip in the receiving vessel, and depress the plunger all of the way down past the first stop to the blow out region of the plungerUse a fresh tip when switching to a new reagent
6. If the tip becomes contaminated, switch to a new one.
7. Do not contaminate the stock reagent by using a used tip from one of your tubes
8. Pipettes can be used to mix samples but be very careful in that the solution does not get into the barrel of the pipettor
9. Do not lay the pipettor down or place the tip higher than the barrel while liquid is in the tip

Sterile Use of a 10 ml Serological Pipet

Sterile plastic serological pipets

Pre-sterilized, disposable pipets are a convenient option for bacterial and other molecular biological work, supplied in bulk pack or individually wrapped. We will use individually wrapped sterile pipets. They provide the most reliable and convenient form of pipet for our purposes.

To Flame or not to Flame Some scientists flame pipets and the mouths of tubes with a Bunsen burner as a part of sterile technique. Flaming is not typically done when working with plastic pipets and tubes. We will *not* flame tubes and pipets in lab because we will use disposable plastic exclusively.

The key to successful sterile technique at the bench is to work quickly and efficiently. Before beginning, clear off the lab bench and arrange tubes, pipets and culture media within easy reach. You may want to simulate the sequence of events of taking off and replacing caps *without setting them down* before you proceed. If you believe at any time that you may have compromised sterility, *assume that you have* and replace any plastic or media with reliably clean items.

As a general rule of thumb, use sterile technique if live bacteria are needed at the end of the manipulation (e.g., general culturing, transformation). Sterility is not required if bacteria are being destroyed in the experiment (e.g., *after* an overnight culture, isolating bacteria for DNA isolation). Cleanliness, of course, is always desirable. When in doubt, use sterile technique.

1. Loosen caps on any tube or media container (sterile culture tube, container of sterile LB broth) so they can be easily lifted off.
2. Open the end of the pipet sleeve and attach the pipet bulb. Squeeze the bulb so that it is prepared to suck. Remove the sleeve from the pipet.
3. Lift the lid from the LB broth container (*do not set down*), insert the pipet and withdraw broth into the pipet.
4. Replace the LB broth container lid loosely.
5. Remove the lid from the culture tube (*do not set down*), and transfer the LB broth to the tube. Replace the lid, with the lid on the 'fingers.'

Basic Techniques – Bacterial Culture

I. Isolation of Individual Colonies by Streaking

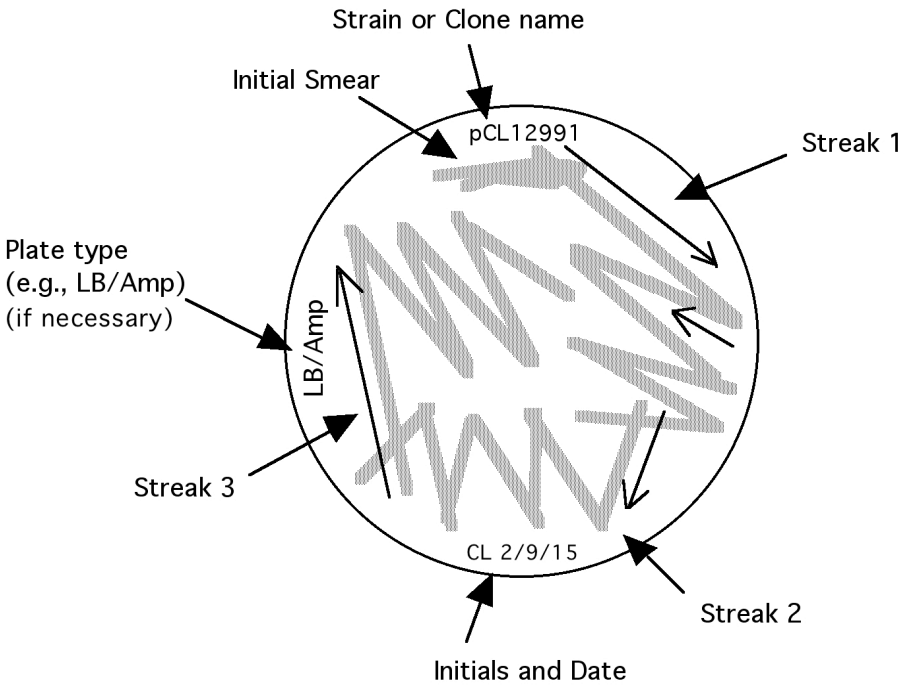
Good bacteriological technique dictates that any culture is begun with a single well-isolated bacterial colony, which represents a clone – the progeny of a single bacterium. This way one is reasonably certain that the culture grown contains a single, isogenic entity (all the same genotype), and not more than one kind of bacteria or other microbe.

In general, an unmarked plate (for this lab) is considered to contain LB agar without antibiotic. Plates with antibiotic (or other components) are typically striped on the side with a color code indicating additions to the plate. It is important that the media portion of the plate is marked and not only the lid. Otherwise you should mark the plate on the bottom.

Ideally culture plates are removed from the refrigerator where they are stored long-term and allowed to warm to room temperature before use (incubating at 37°C). Too rapid warming (transfer directly from 4°C to 37°C) can cause bubbles to appear in or under the plate making it difficult to see colonies on the plate. If the plate is still cool, condensation may need to be wiped off immediately prior to writing with a marker.

Note that plates should be stored with the lid down/media side up (unless wet) – this is **bacteriologically 'right side up'** – this way any airborne contaminants are more likely to land on the lid than on the media in the plate.

Figure: Anatomy of a Bacterial Streak Plate



Notes: Each new streak begins in the final pass of the previous streak. (The number of back & forth spreading is actually much more than illustrated.) When labeling the plate, write small, near the edges, on the media-containing part of the plate (not the lid). Write the plate type if there is any potential for confusion.

In general, overnight cultures should be incubated closer to **16 hrs or less** rather than 24 hrs or more (set up evening before, harvested the next morning). For example, a culture set up at 5 PM and harvested at 9 AM the next day would have grown for 16 hours. As the culture goes to 24 hrs and beyond, more death of bacterial cells occurs, compromising the quality of the culture for a number of purposes. If you cannot use the culture in the morning, it is best to remove it from the 37°C incubator and leave at room temperature or even refrigerate.

Why do shorter cultures? See the bacterial growth curve at right – after the cells run out of nutrients and accumulate waste products, entering 'stationary phase' in which no further cell division occurs, eventually cells begin to die. The actual course of the growth curve depends on the bacterial strain and the precise growth conditions.

Figure modified from image found at http://pathmicro.med.sc.edu/fox/growth_c.jpg