Pipetting for Molecular Biology

Dr. Louise Judd
Centre for Pathogen Genomics- Innovation Hub
Lead Laboratory Scientist and Genomics Trainer

CENTRE FOR PATHOGEN GENOMICS

Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

LAB RULES & SAFETY REQUIREMENTS

General Lab Rules

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This is a PC2 laboratory - the following rules must be strictly adhered to

1. Wear appropriate PPE (lab coat, safety glasses, gloves, closed-toed shoes).
2. No food and drink in the lab
3. Tie long hair back
4. Do not touch face, hair, or safety glasses with gloves on
5. No mobile phone use in laboratory spaces, especially with gloves on
6. Handwash thoroughly before leaving the lab
7. Do not remove lab reagents/consumables from the lab space
8. You will be handling potentially infectious micro-organisms, please follow appropriate infection prevention practices
9. Please keep notebooks/pens or other clean items in the designated clean areas only
10. In the event of a spill, please immediately inform closest trainer

Bench Rules

- Please show each other respect.
- Please ask questions if you are ever unsure of what to do – we are here to help! No question is silly – we promise.
- Centrifuges and thermocyclers are shared between a bench group. Please wait until all trainees from your bench have loaded their samples before starting runs.
- We cannot separate pre- and post- PCR work in this lab space
  - Ensure your benches and equipment are thoroughly cleaned before you perform any work
  - Ideally you would have dedicated spaces and PPE for pre- and post-PCR work to prevent amplicon contamination

Waste Discard

Benchtop Bins

- All disposable labware (e.g. tips, tubes, etc.)
- Liquid waste <1 mL
- Do not overfill
- Tie off and place in biohazard bin when done

Biohazard Bins (Yellow Bag)

- Used gloves to go into biohazard waste bins

General Waste

- Paper towel from handwashing
- Clean paper waste

Image Source: https://www.suedlabor.de/item/40-TS30020, https://www.pinpak.com.au/product/biohazard-bags/, https://www.stanleypackaging.com.au/product/80-litre-heavy-duty-black-bin-liners-garbage-bags-250pcs/

Pipetting is challenging!

Laboratory staff are confronted with numerous variables:

- What pipette brand is best to use/buy?
- Type of pipette tip to use – what size? Filtered?
- What size pipette should I use for my task?

Technique choices on:

- holding the pipette?
- what size pipette should i use?
- what "stop" do I go to?
- can I lay the pipette down?
- should I pre-wet the pipette tip?

Types of Pipettes in Molecular Biology

Pipette usage for Molecular Biology

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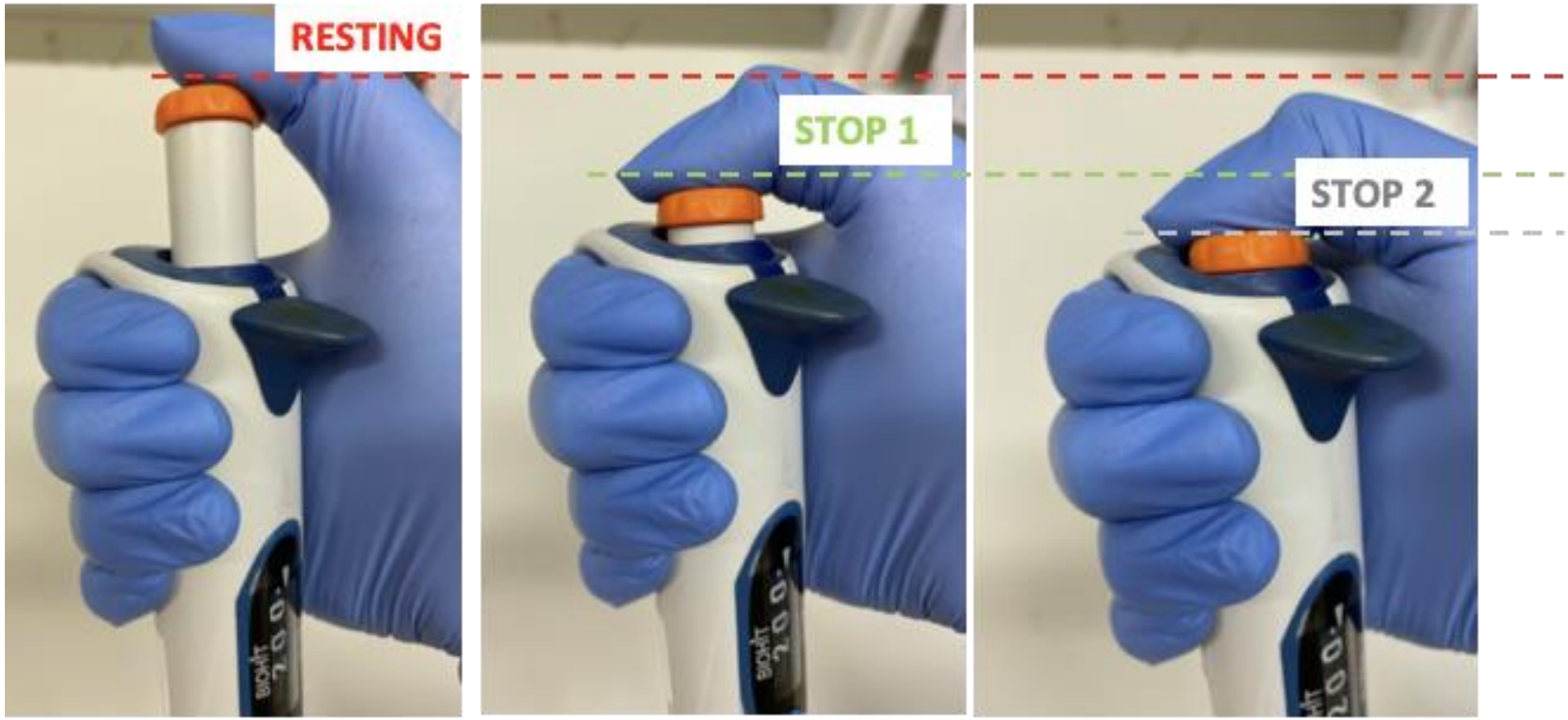
1. Always use the correct tips for your pipettes and ensure a tight fit
2. Use filter or barrier tips for ALL molecular biology tasks
3. Where possible the following is best practice
    - Keep the pipettes of different sizes together as a single set
    - Sets of pipettes are used by a single scientist or for a particular task
    - Do not move pipettes around from different labs or parts of the labs
4. Pipetting consist of two steps
    i. Aspirate- sucking liquid up into the tip
    ii. Dispense- pushing the liquid out of the tip into another container

Pipette usage for Molecular Biology- continued

5. Become familiar with the first and second stop on your pipettes
   - First stop is used to aspirate and the first part of dispense
   - Second stop is the final part of dispense when a small amount to air is eject though tip to ensure full volume is dispensed
6. Always store pipettes upright with no tips on them
7. Be conscious of ergonomics when setting up workspace to reduce risk of repetitive strain injury
8. When available use at multichannel pipette for large scale tasks

Pipette plunger positions



Rest

No pressure on the plunger

Desired measurement
Stop 1

Feel for a light stopping pressure

Full discharge
Stop 2

Press down until liquid is discharged

Selecting appropriate pipette for different volumes

Standard set of pipettes should include 4 pipettes to be used for different volume ranges

For accurate pipetting it is important to select to most appropriate volume pipette

1. P1000
   201 ul – 1000 ul (1 ml)
2. P200
   21 ul – 200 ul
3. P20
   2 ul – 20 ul
4. P2
   0.5 ul – 2 ul

Some pipettes may have P100 or P10 sizes as alternatives

Pipette Measurement Dials

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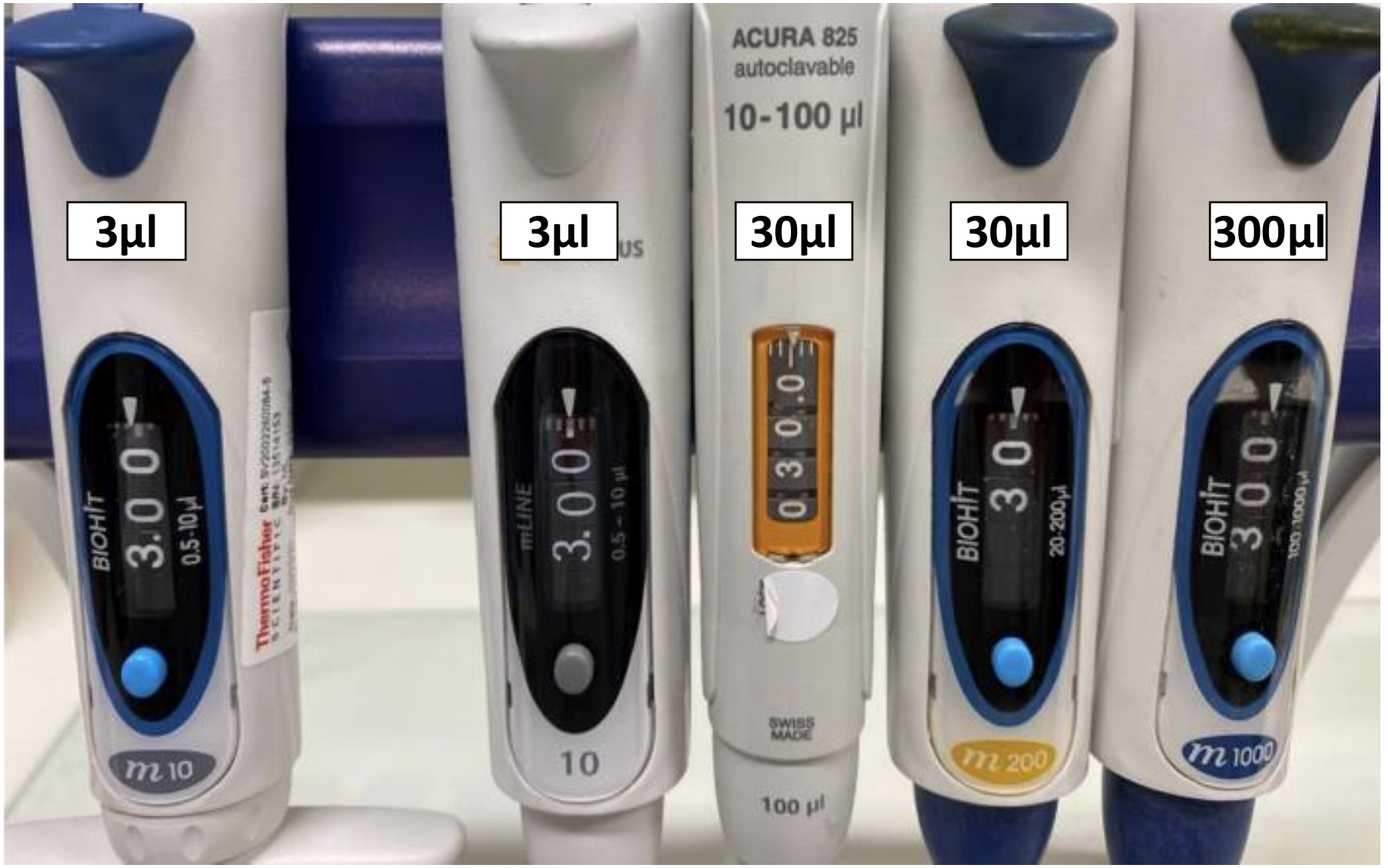
VOLUME SET



Pipette Measurement Dials

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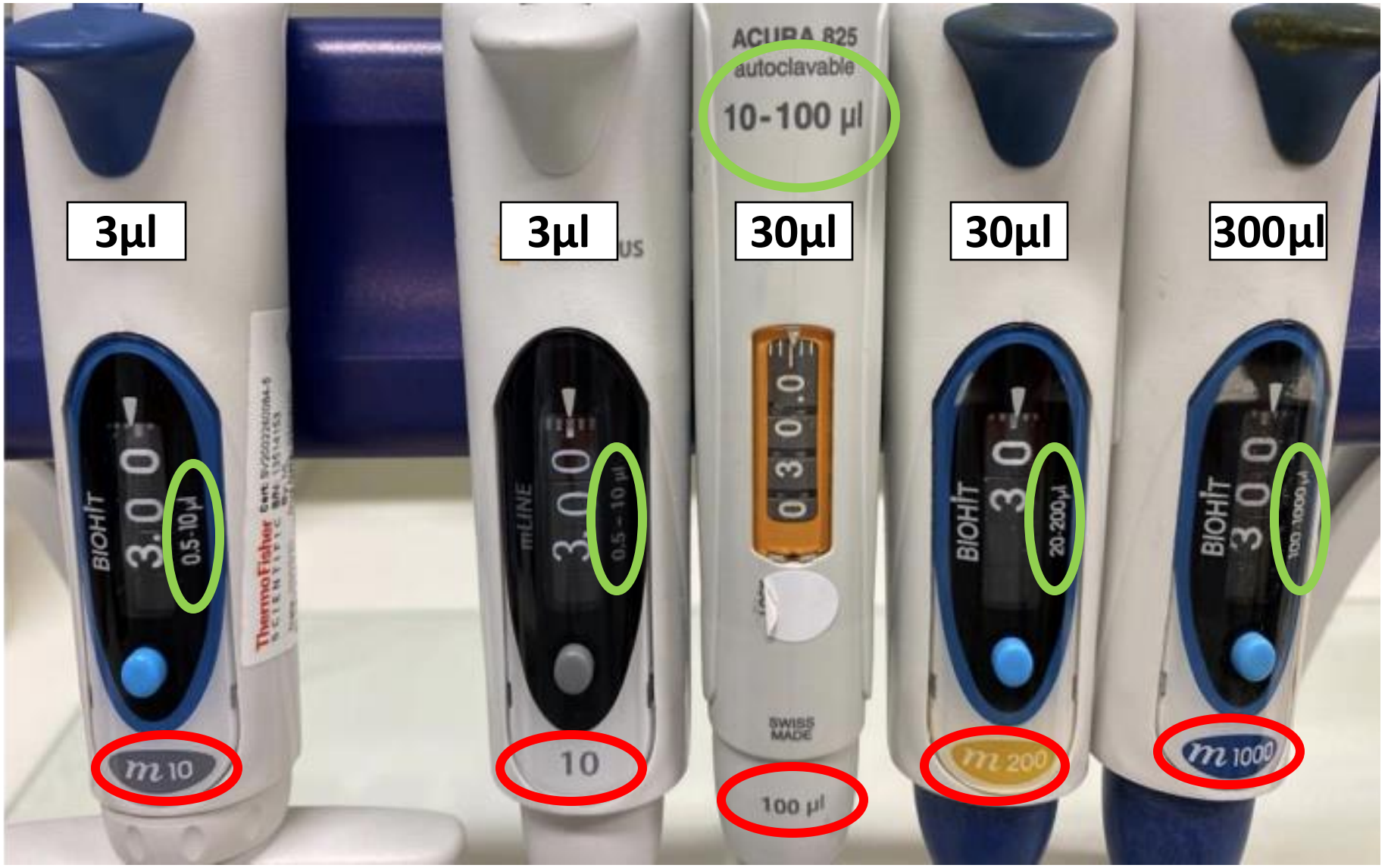
VOLUME SET

VOLUME RANGE

PIPETTE SIZE



Steps to accurate pipetting

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1. Set the pipette to the correct volume you wish to pipette
    - Become familiar with the way your pipette displays volumes
2. Load tip onto the pipette using gentle pressure only
3. Open the lid of the container you wish to aspirate from
4. Depress plunger of pipette to the **first stop** only
5. Place the end of the tip ONLY into the solution you wish to **aspirate** and slowly release the plunger
6. Remove the tip from the container. Try not to touch sides of container as you do this
7. Close the lid of the container

Steps to accurate pipetting- continued

8. Visually inspect the volume in the tip.
   - Does it seem like the correct volume?
   - Are there any bubbles present?
9. Move the tip to the bottom of the new container and depress the plunger to the **second stop** to **dispense** all the liquid
   - Try not to touch sides of container as you do this
10. Remove the pipette and tip from the container and close the lid
11. Eject the tip into waste receptacle

Accurate pipetting examples

bubbles in liquid

Plunger lifted too quickly, inconsistent liquid uptake.

incorrect volume

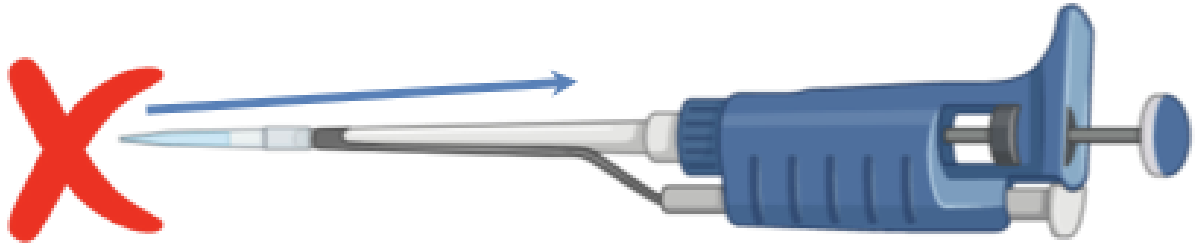
Pipette lifted out of tube before full amount of liquid has been measured

correct volume

No bubbles

Keep your pipette upright

Keep upright when in use.
The liquid stays in the tip and doesn't touch the filter



Laying the pipette down – liquids can move inside the tip through the filter and into the shaft of the pipette.
Causing cross contamination and damaging pipette.

Pipette maintenance for Molecular Biology

Set up a 6 monthly pipette check schedule including the following

- Visual inspection of pipettes to make sure they are intact and clean, with no broken parts
- Repair and clean exterior of pipettes as required
- Check tip supplies to ensure sufficient stocks are available

Pipette maintenance for Molecular Biology- continued

- Check accuracy of pipettes by pipetting and weighing a known volume of water
  - The density of water is 1 g/mL
  - Measure in triplicate and average result
  - 100 ul of water should weight 0.1 g (+/- 5%)
- Recalibrate pipettes as necessary

THANK YOU

centre-pathogen@unimelb.edu.au

CENTRE FOR PATHOGEN GENOMICS

Schedule Day 1

MONDAY	ACTIVITY	PRESENTER
8:45 – 9.00	Registration	Lisa
9.00 – 9.15	Overview of the Doherty Institute/CPG/VIDRL/MDU	Lisa
9.15 – 9:45	Welcome and introductions	
9:45 –10.00	Training overview	Jean
10.00– 10.30	LAB: Pipetting exercise	Louise
10.30 – 11.00	**Morning tea**	
11.00 – 11.30	**LECTURE:** Introduction to MPXV genomics at VIDRL	Jean
11.30 – 12.30	**LECTURE:** Tiled amplicon MPXV and viral theory	Jean
12.30 – 13.30	Lunch	
13.30 – 15.30	**LAB:** Tiled amplicon PCR	Louise
15.30 – 16:00	Afternoon tea	
16:00 – 16.30	**LECTURE:** Introduction to ONT sequencing viruses	Louise
16.30 – 17:00	Group discussion: Opportunity for Q&A and further discussion	Nicole