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Pipetting V.4

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Protocol status: Working

We use this protocol and it's working

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Disclaimer

This protocol is used in teaching labs.

Abstract

Overview and Goals

Lab micropipettes allow us to transfer small volumes of liquids accurately. Units to measure small volumes with micropipettes are microliter (μL) and milliliter (mL). There are 1000 μL in 1 mL). Effectively using lab micropipettes is a valuable skill that takes practice! We will work together to learn and improve our pipetting to improve accuracy.

Learning Objectives

After completing this lab, you will gain the following lab skills:

- Lab safety and proper personal protective equipment (**PPE**)
- Pipetting with **micropipettes**

Image Attribution

Pipette by **Andi Nur Abdillah**

Purchased from The Noun Project



Guidelines

Reducing waste - micropipette tips - Pipetting best practices dictate that a new micropipette tip should be used every time you pipette a new volume of liquid. This can be very important in avoiding contamination in molecular biology. Unfortunately, this also creates a considerable amount of plastic waste. To reduce unnecessary waste, we will reuse tips for this activity when only pipetting water.

Gloves will still go into the biohazard bins.

Materials

- One 2-20 µl micropipette (p20)
- Micropipette tips for p20
- One Pipette Practice Card from miniPCR bio
- Wipes
- 200 µl water (tap water is fine)
- 80 µl blue micropipetting practice fluid
- 80 µl yellow micropipetting practice fluid
- 80 µl red micropipetting practice fluid
- tip disposal container

Reference Resources

We will use **Micropipetting Practice Cards** from the company miniPCR bio in the lab. Our procedure has been adapted from the following **Micropipette Mastery Activity**.

Safety warnings

- ❗ Please be careful when handling micropipettes. We want to ensure that they are calibrated and working correctly.

Ethics statement

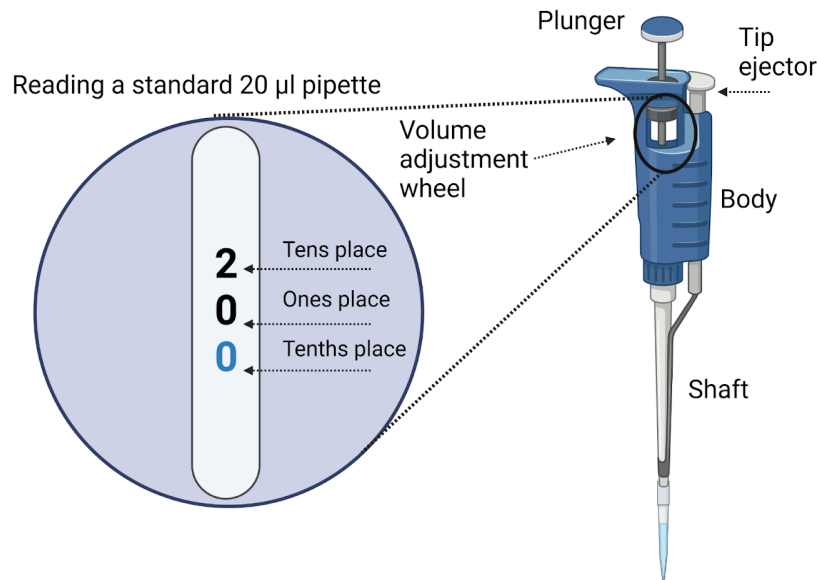
This protocol does not involve animals.

Before start

How to use a micropipette - We will practice pipetting small volumes of colorful liquids using micropipettes. We will work together to practice this new skill to improve our technique. Before coming to the lab, watch this 5-minute video, [How to Micropipette](#), that demonstrates three key pipetting skills:

1. How to hold a micropipette
2. How to set a micropipette to a desired volume
3. How to use the plunger and ejector of the micropipette to measure the desired volume of a reagent

Reading a Micropipette



Pipettor with labeled parts: plunger, tip ejector, volume adjustment wheel, body, shaft



Using a pipette

10m

1 To draw up liquid

10m



1.1 Set volume using the volume adjustment wheel

1.2 Press a new tip onto the shaft

1.3 Press plunger **TO** the FIRST STOP



1.4 Dip tip into liquid

1.5 Slowly release the plunger to collect liquid into the tip.

2 To dispense sample

2.1 Touch tip to dispensing container

2.2 Press plunger **THROUGH** to the SECOND STOP



2.3 Remove tip from liquid then release plunger

2.4 Dispose of tip using the tip ejector

Activity 1

6m



- 3 Read the volumes specified on the Micropipetting Practice Card and use your micropipette to add the correct amount of liquid to each circle.

Safety information

- Try to be precise so that the liquid stays in the circle.
- If you have trouble, dry the spot with a paper towel and try again.
- If you have trouble, try using both hands. Dr. Sjogren has shaky hands and uses her non-dominant hand to stabilize the micropipette being held in her dominant hand.

- 4 Try to pick up all of the liquid without leaving any behind.

2m

Safety information

- Set your micropipette to the same volume of liquid that is specified for each of your drops.
- Check to see if you can pick up all of the liquid without leaving any behind. There should be no space at the end of your pipette tip.

- 5 In the blank space at the bottom of the card, pipette  5 μL of water three times.

2m

Note

- How similar do the drops look in size?
- If they do not look exactly the same what could be some sources of error?

- 6 On a dry place on your card place  4 μL water. Add  4 μL to the same place three more times.

2m

This is what your card should look like:



Micropipetting practice card from MiniPCR. Liquid spots on the 2, 5, 10, 15, and 20 μl circles with yellow, green, blue, red, and yellow food coloring, respectively. Photo taken by BIT 295 Staff.

Note

- How much volume should you now have? _____
- Set your pipette to the calculated volume and try to pick up the entire drop.
- Was there any liquid left on the card or space left in the tip of your pipette? _____

Activity 2

5m

7 Preparation of cards and dyes

7.1 Add  13.5 μL of blue dye to dot A

7.2 Add  17.5 μL of yellow dye to dot C

7.3 Add $17.5\ \mu\text{L}$ of red dye to dot E

After this step, this is what your card should look like:



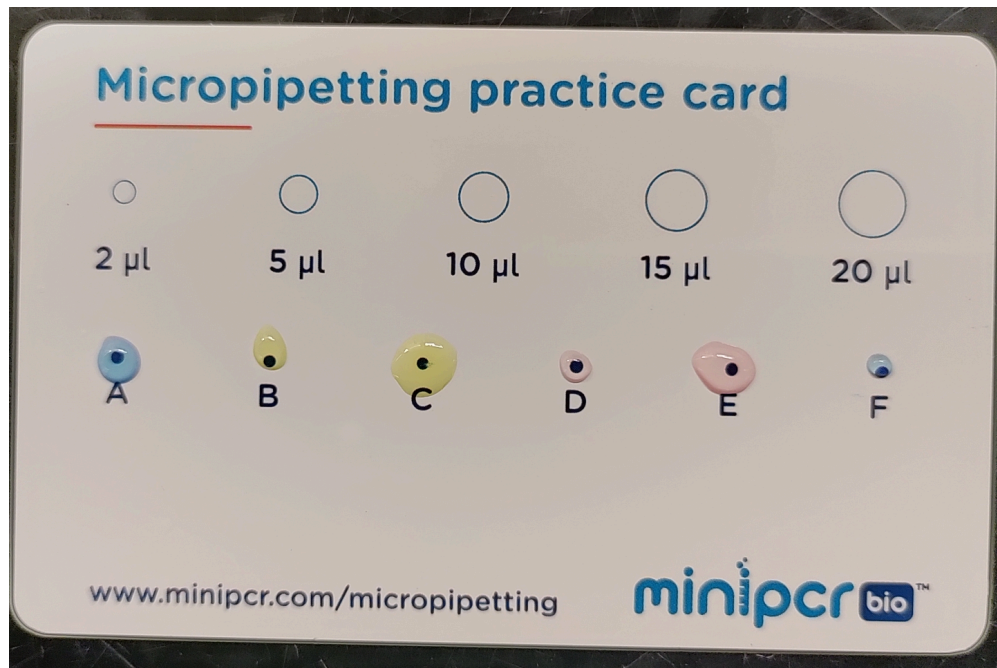
Micropipetting practice card from MiniPCR. Liquid spots on the A, C, and E circles with blue, yellow, and red food coloring, respectively. Photo taken by BIT 295 Staff.

7.4 From dot A, pick up $2\ \mu\text{L}$ and place it on dot F

7.5 From dot C, pick up $4.5\ \mu\text{L}$ and place it on dot B

7.6 From dot E, pick up $3\ \mu\text{L}$ and place it on dot D

After this step, this is what your card should look like:

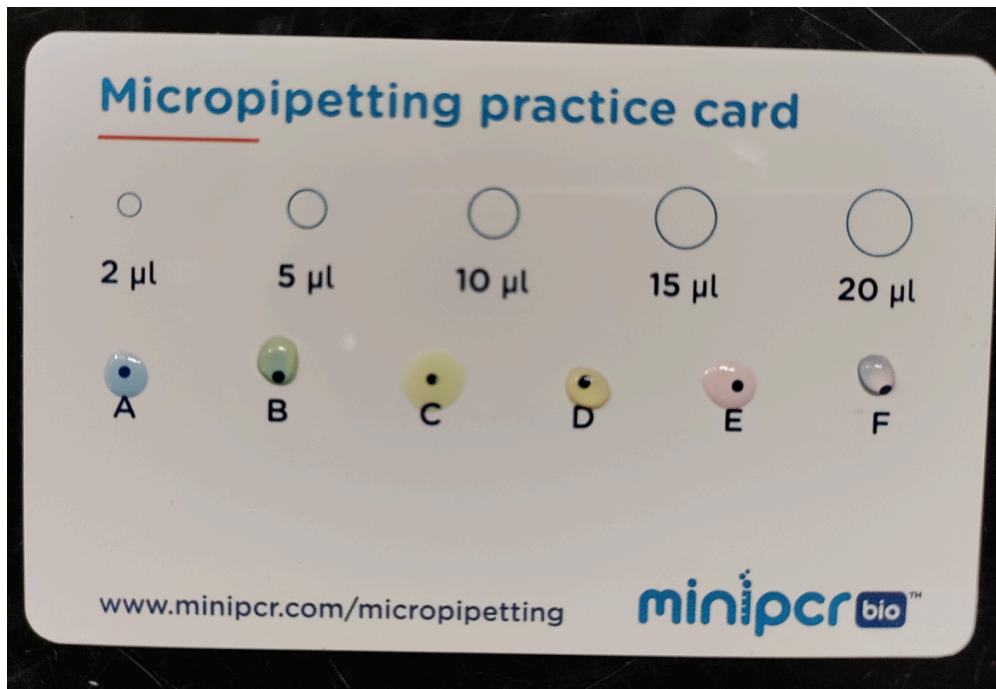


Micropipetting practice card from MiniPCR. Liquid spots on the A, B, C, D, E, and F circles with blue, yellow, yellow, red, red, and blue food coloring, respectively. Photo taken by BIT 295 Staff.

Note

Now that you have set up your card, your goal is to mix these dyes with great care and precision. To mix, you will gently pipette up and down to the micropipette's first stop 2-3 times. Only pipette through to your micropipette's second stop at the end to remove all liquid from your tip to avoid making any air bubbles. Follow the guidance below.

- 8 From dot E, pick up  6 μL and mix it with the volume already present on dot F
 - 9 From dot A, pick up  3.5 μL and mix this volume onto dot B
 - 10 From dot C, pick up  5 μL and mix this volume onto dot D
- After this step, this is how your card should look like:



- 11 Calculate how much volume (in units of microliters) should now be on each dot:

Dot	A	B	C	D	E	F
Volume (in microliters)						
Notes						

Pipetting Exercise Table. Use this table to record your observations.

- 12 Set your micropipette to that volume and pick up each drop one by one and move to the open space at the bottom of your card. Each time you pick up a drop, note if liquid was left behind (code: LB), or if there is any space at the end of your pipette tip (code: S).

5m



Note

Critical thinking Questions

1. When using a micropipette, what are some benefits to checking liquid levels in your pipet tip?
2. When using a micropipette, did drops repeatedly get measured accurately (was liquid left over on the card, or was there space in the bottom of the pipette tip)? What could be some sources of error?
3. Based on your experience today, how would you rate your pipetting skill?
 - Total Amateur
 - Intermediate
 - Pro
 - Pipette Master!

Protocol references

Our procedure has been adapted from the MiniPCR [Micropipette Mastery Activity](#).

Acknowledgements

We appreciate the support from the NC State Biotechnology Program and students who have helped improve this and other protocols.