



Pipetting V.2

Carlos Goller¹, Carly Sjogren¹

¹North Carolina State University

Delftia and SCoOP

Tech. support phone: +91 95134-135 email: ccgoller@ncsu.edu



nrgrover

VERSION 2

FEB 27, 2024

OPEN  ACCESS



DOI:

dx.doi.org/10.17504/protocols.io.n2bj8x35gk5/v2

Protocol Citation: Carlos Goller, Carly Sjogren 2024. Pipetting .

protocols.io

<https://dx.doi.org/10.17504/protocols.io.n2bj8x35gk5/v2> Version created by [Carlos Goller](#)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development
We are still developing and optimizing this protocol

ABSTRACT

Overview and Goals

Lab micropipettes allow us to accurately transfer small volumes of liquids. 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.

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

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

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. For this activity, to reduce unnecessary waste, we will reuse tips when only pipetting water.

Created: Feb 12, 2023

Last Modified: Feb 27, 2024

PROTOCOL integer ID: 76858

Keywords: Pipetting

Funders Acknowledgement:

Biotechnology Program

MATERIALS

- One 2-20 µl micropipette (p20)
- Micropipette tips for p20
- One Pipette Practice Card from miniPCR bio
- Chem 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

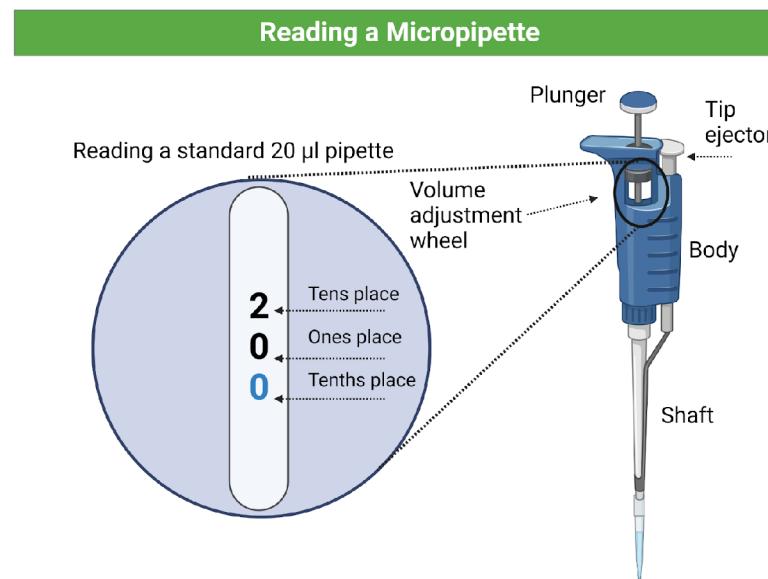
Reference resources

In the lab, we will use [Micropipetting Practice Cards](#) from the company miniPCR bio. Our procedure has been adapted from the following [Micropipette Mastery Activity](#).

BEFORE START INSTRUCTIONS

How to use a micropipette - We will practice pipetting small volumes of colorful liquids using micropipettes. We will work together practicing this new skill to improve our technique. Before coming to the lab watch this 5-minute video, [How to Micropipette](#), that demonstrates 3 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 a desired volume of a reagent



Using a pipette

1 To draw up liquid



1.1 Set volume using the volume adjustment wheel

1.2 Press a new tip onto the shaft

1.3 Press plunger **T0** 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

- 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.

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.

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.

This is what your card should look like:



Note

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

Activity 2

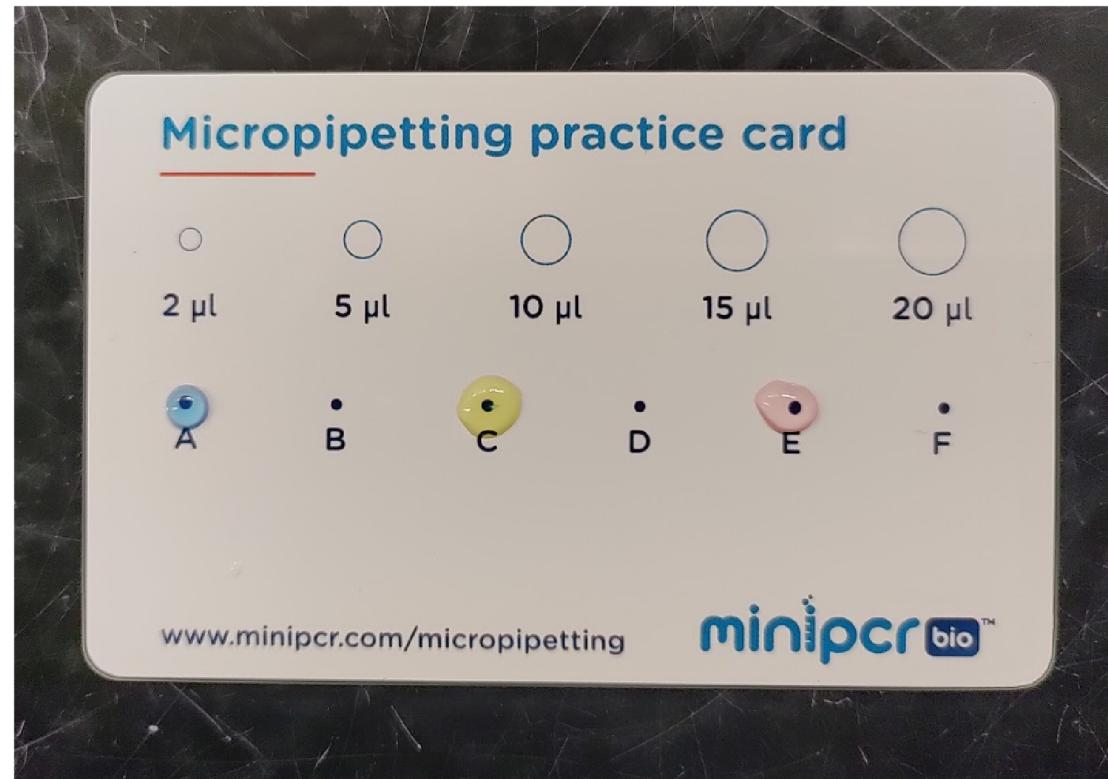
7 Preparation of cards and dyes

7.1 Add  13.5 μL of blue dye to dot A

7.2 Add $\text{17.5 } \mu\text{L}$ of yellow dye to dot C

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

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

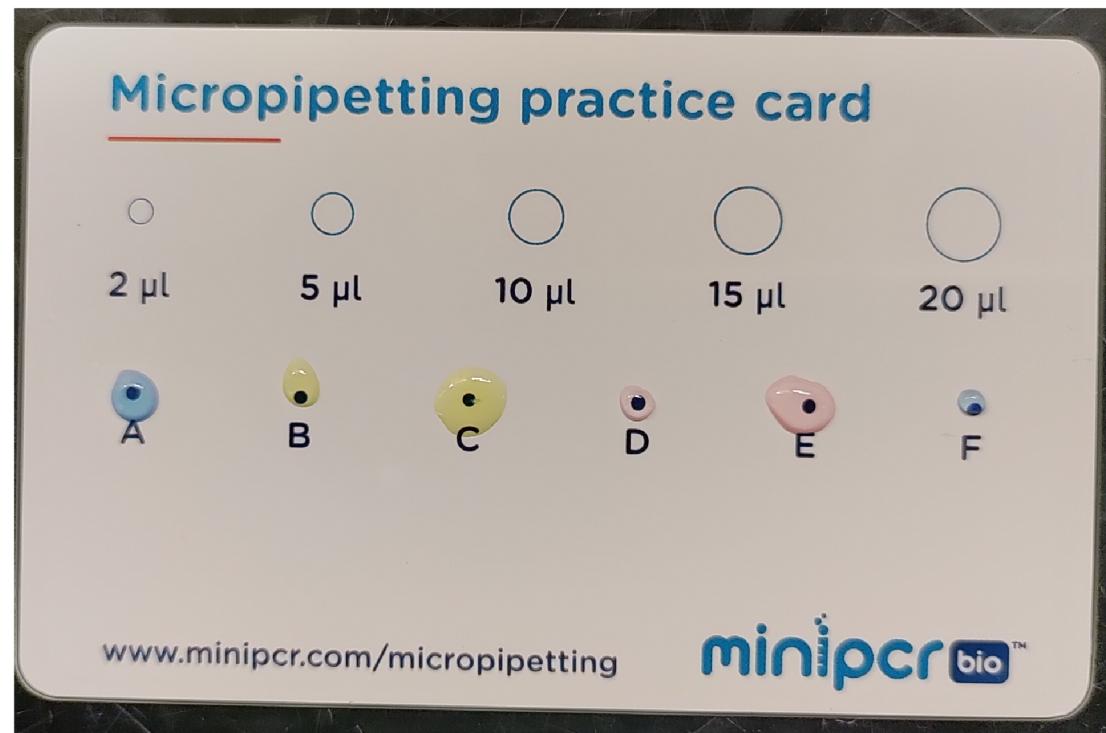


7.4 From dot A, pick up $\text{2 } \mu\text{L}$ and place it on dot F

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

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

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



Note

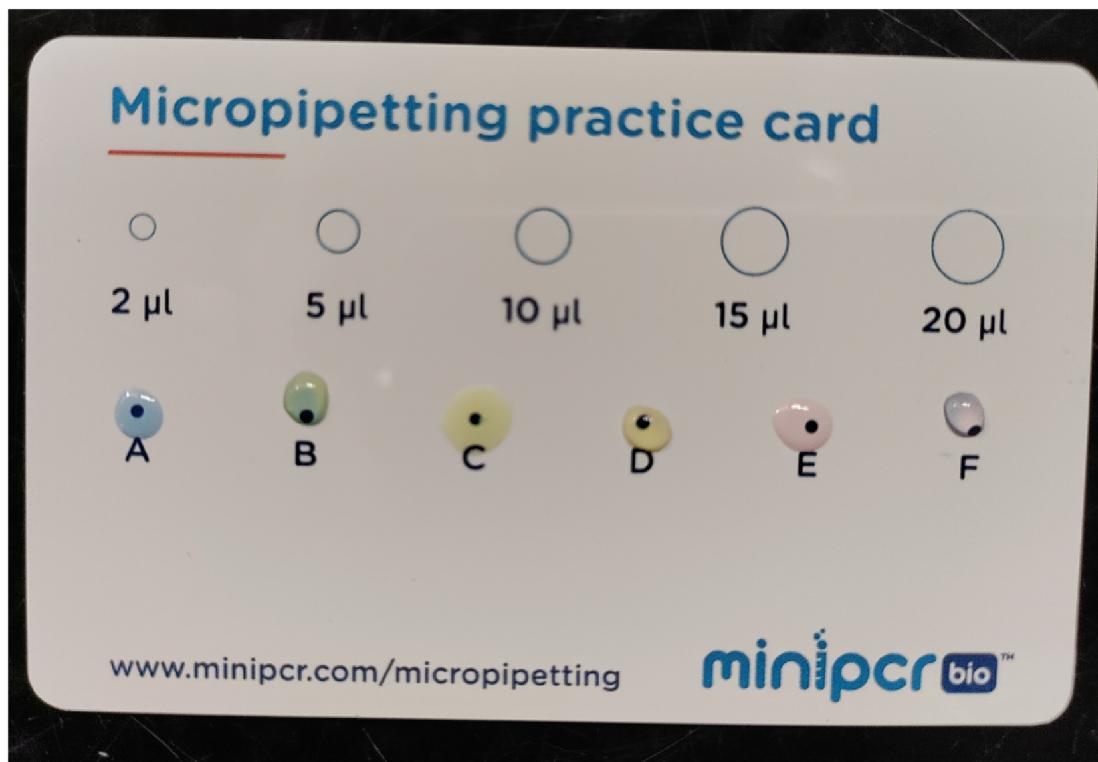
Now that you have set up your card, your goal is to mix these dyes with great care and precision. To mix, you will gently pipette up and down to the micropipette's first stop 2-3 times. Only pipette through to your micropipette's second stop at the end to remove all liquid from your tip to avoid making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

- 8 From dot E, pick up $\text{6 } \mu\text{L}$ and mix it with the volume already present on dot F
- 9 From dot A, pick up $\text{3.5 } \mu\text{L}$ and mix this volume onto dot B

10

From dot C, pick up  $5 \mu\text{L}$ 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						

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).

Note

Critical thinking Questions

1. When using a micropipette, what are some benefits to checking levels of liquid in your pipet tip?
2. When using a micropipette, did drops repeatedly get measured accurately (was there liquid left over on the card or was there empty 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!