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Dilutions and Pipetting Practice

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ABSTRACT

Nowadays, doing molecular biology frequently boils down to "mixing small volumes of clear liquids." The volumes we're working with are very small indeed: down to one or two microliters (a microliter, abbreviated µl, is one one-thousandth of a milliliter.)

To accurately transfer these small volumes from one container to another, we use tools called micropipettors. This protocol is intended to get you familiar with how to use a micropipettor. It is also an opportunity to practice computing dilutions, which is a skill you'll use over and over in this lab an others.

Adapted from Dilution and Pipetting Lesson Using Food Dyes, by Burnett et al (CourseSource)

EXTERNAL LINK

https://qubeshub.org/community/groups/coursesource/publications?id=2556&tab_active=about&v=1

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pipetting, food coloring, dilutions, practice

IMAGE ATTRIBUTION

James Burnette, Lisa Kanizay, Nikki Chester, Susan R Wessler, from "Dilution and Pipetting Lesson Using Food Dyes", https://qubeshub.org/community/groups/coursesource/publications?id=2556&tab_active=about&v=1

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PARENT PROTOCOLS

In steps of

Dilutions and Pipetting Practice (Instructor Protocol)

GUIDELINES

Pipetting tips:

DO NOT TWIST THE PIPETTOR BEYOND ITS MAXIMUM VOLUME. For example, do not try to set a P200 to 250 µl. **It will break the pipettor, and repairs are expensive.**

Do not re-use tips. Eject the tip after each transfer and use new one for the next transfer.

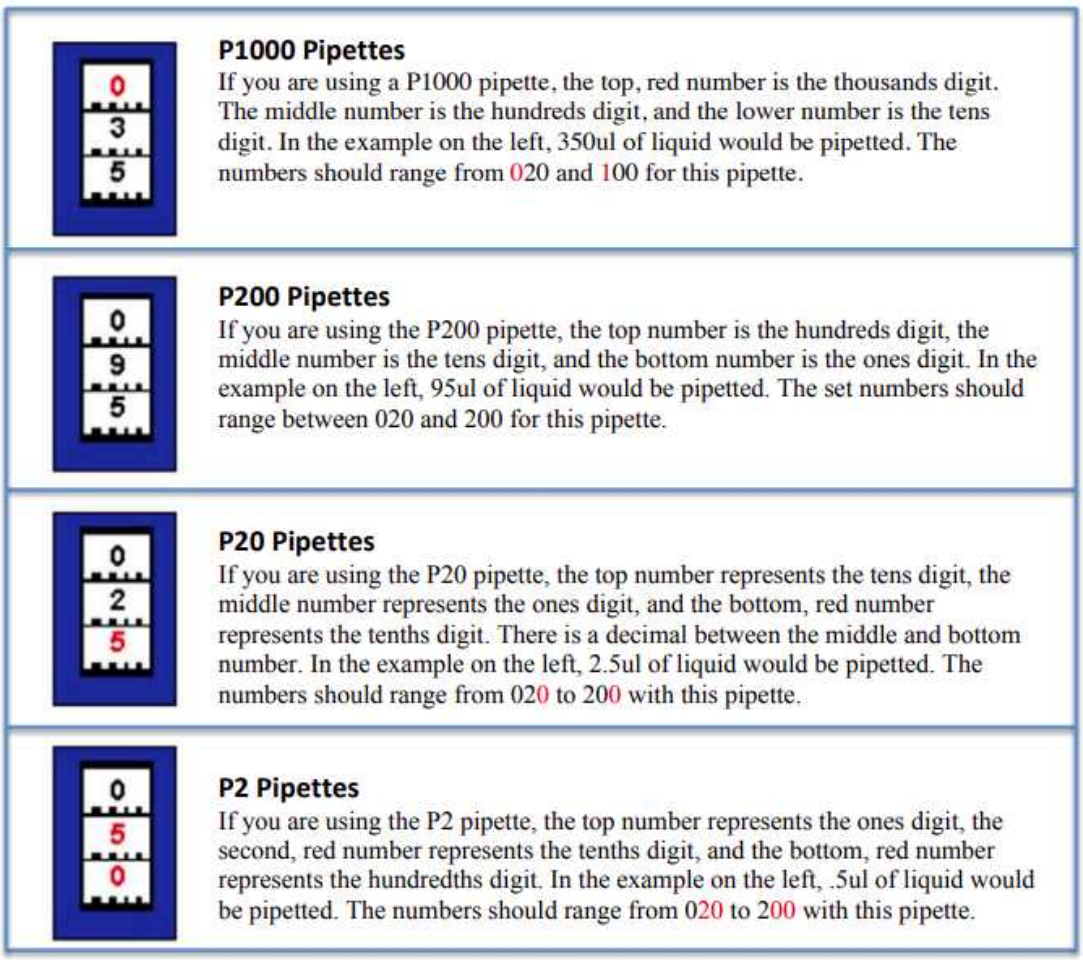
Use the smallest pipettor that will transfer the entire volume at once. For example, if you need to transfer 250 µl, set a P-1000 to 250 µl. Do NOT use a P-200 to transfer 200 µl, then 50 µl.

MATERIALS TEXT

- Micropipettor tips: 1000 µl, 200 µl, and 10 µl
- 10X stock food dye solutions: one tube each of red, blue, green and yellow
- Tap water (in a small beaker or flask)
- An empty small beaker or flask
- Eight empty microcentrifuge tubes
- Eight empty 200 µl PCR tubes

Pipetting Practice

1 Adjusting the volume of the micropipettor correctly is the most important part of the process. Refer to the image below for how to set each of the micropipettors.



(I adapted this figure from somewhere but I cannot remember or find where. If this is yours, please contact me so I can attribute you properly!)

You may have a P-10 instead of a P-2 -- adjust it like you would a P-20.

2 Grab the P-1000.

- Set it to transfer **400 µL** of liquid. **(DO NOT TRY TO MAKE IT READ 4-0-0 - YOU WILL BREAK THE MICROPIPETTOR. IF YOU HAVE QUESTIONS, ASK.)** Double-check with an instructor or a TA to make sure you have it set correctly.
- Press the barrel of the micropipettor firmly into a P-1000 tip. (These are often in *blue* tip boxes.)
- Press the plunger down to the *first stop*. (Do this out "in the air", not in the water.)
- Insert the tip into the beaker of water.
- *Smoothly* release the plunger. (Don't just take your finger off and let it "snap" back up.) This will draw water into the tip.
- Put the opening at the bottom of the tip against the side of the empty beaker.
- Press the plunger down *all the way to the bottom of its travel*. This will dispense the water into the beaker.

Don't dispense the water into the middle of the air; always dispense onto the side of the receiving container. This is especially important for very small volumes.

- Using the tip ejector, eject the tip into the sharps container or biohazard bag.
- If you'd like, repeat this process several times to get a feel for it.

Don't re-use tips! Use a single tip for a single transfer, then eject it and use a fresh one.

3 Repeat step 2 using the P-200. Set it to **175 µL** . Use 200 µl tips. (These are often in *green* tip boxes).

4 Repeat step 2 using the P-20. Set it to **12.5 µL** . Again, use the 200 µl tips.

5 Repeat step 2 using the P-2. (You may have a P-10 instead -- if so, you'll set it using the same instructions for the P-20.) Set it for **1.75 µL** . Use the 10 µl tips (they're often in red tip boxes.)

Here, it is especially important that you dispense the water into the liquid that's already in the container (preferred) or onto the side of the container.

Make working stock solutions

6 The food dye provided to you is at a *10X concentration*. You will need to make four working stocks at *1X concentration.* In the table below, you are given the final volume for a 1X solution of each dye. Use the formula $c_1 * v_1 = c_2 * v_2$ to compute the amount of dye and the amount of water you'll need to mix for a 1X solution and fill out the table. Have an instructor or TA double-

check your math.

A	B	C	D
Tube Label	**Amount of 10X dye (µl)**	**Amount of water (µl)**	**Total volume (µl)**
Red			500
Blue			1000
Green			750
Yellow			870

7 Label 4 tubes: Red (R), Blue (B), Green (G) and Yellow (Y)

8 Make 1X dilutions according to your calculations above in Table 1 by mixing the dye and water volumes you computed.

1. Add the volume of water to the first tube.
2. Add the dye to the first tube.
3. Snap the tube cap closed and flick or vortex briefly to mix.
4. Repeat with the rest of the tubes.

Make mixtures of the working stock solutions

9 Label four new tubes: 1, 2, 3 and 4

10 Using the table below, transfer the correct amount of 1X food dye to the new tubes

A	B	C	D	E	F
Tube	Green (µl)	Yellow (µl)	Red (µl)	Blue (µl)	Total volume (µl)
1	0	300	60	0	
2	0	0	30	16	
3	27	0	0	120	
4	27.5	50	57	12.5	

11 ⚠

Compare your results to the standards provided by the instructor.

Repeat at 1/10th scale

12 Starting at step 6, repeat the *entire protocol at 1/10th scale*.

- For example, if the volume says 500 µl, use 50 µl instead.
- Use PCR tubes instead of microcentrifuge tubes.
- Don't forget to compare your results to the standards provided by the instructor!