TITLE: Pipetting by Design

KEY QUESTION(S):

- What is a micropipette?
- How do you properly use a micropipette?
- Why is a micropipette necessary in biotechnology laboratories?

OVERALL TIME ESTIMATE:

- Advanced Preparation: 10 Minutes
- Student Procedure: 30 Minutes

LESSON SUMMARY: In this lesson, students learn and practice the proper technique for measuring small volumes of liquid using a micropipette. The students will a follow a micropipetting protocol to create an image by pipetting the proper color and volume of water into their well plate.

STUDENT LEARNING OBJECTIVES:

The student will be able to:

1. Properly operate a micropipette.
2. Determine the appropriate micropipette to use according the volume of liquid being measured.
3. Correctly read the volume indicator on the micropipette.
4. Measure volume in microliters (µl) using a micropipette.
5. Convert volume into mass.
6. Use a scale to determine accuracy of pipetting.

Next Generation Sunshine State Standards

SC.912.L.16.11: Discuss the technologies associated with forensic medicine and DNA identification, including restriction fragment length polymorphism (RFLP) analysis.

SC.912.L.16.12: Describe how basic DNA technology (restriction digestion by endonucleases, gel electrophoresis, polymerase chain reaction, ligation, and transformation) is used to construct recombinant DNA molecules (DNA cloning).

MATERIALS:

- (1) Multicolor Food Coloring Package (Red, Blue, Yellow, Green)

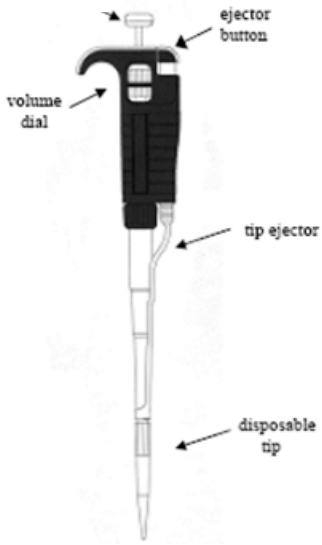
Needed For Each Student Pair:

- (2) 10mL aliquots of Colored Water (Color of water needed is dependent upon which protocol each group is using)
    - A- DNA: Red and Blue water
    - B- GATC: Blue and Green water
    - C- Gator: Green and Brown water
    - D- UF: Orange and Blue water
    - E- Virus : Purple and Red water
    - F – Atom; Red, Orange and Blue water
    - G – Flask; Blue, Orange and Red water
    - H – DNA Strand; Blue and Red water
    - I – Insect; Green and Blue water
    - J – PCR; Green, Orange and Blue water

- (1) 96 Well Plate
- (1) P20 Micropipette
- (1) P200 Micropipette
- (1) 2-200ul Tip Box

BACKGROUND INFORMATION:

Image 1



Micropipettes are precise instruments used to accurately measure very small quantities of liquids in science laboratories. Image 1 shows a micropipette and the main components of the instrument. They are available in a variety of sizes to best match your measurement needs. The size of the micropipette is indicated directly on the instrument. The most commonly used micropipettes are the P10, P20, P200, and P1000. The number following the "P" refers to the maximum volume in microliters (µl) that can be measured using the instrument.

In this activity, P20 and P200 micropipettes will be used. The proper method for reading the volume indicator and directions on how to use the P20 and P200 micropipettes are listed below:

Reading the volume on the micropipette:

- **P20 Micropipettes:** The volume indicator consists of three number dials and is read from top to bottom. Black digits indicate tens of microliters and microliters; red digits indicate tenths of microliters. A P20 is used to measure volumes up to 20µl. **NOTE: Do not dial past 20µl.**

0	2
7	0
3	0
7.3 µl	20.0 µl

- **P200 Micropipettes:** The volume indicator consists of three number dials and is read from top to bottom. Black digits indicate hundreds and tens of microliters; red digits indicate microliters. A P200 is used to measure volumes between 20µl and 200µl. **NOTE: Do not dial past 200µl.**

0	2
7	0
3	0
73 µl	200.0 µl

Directions on how to use a micropipette:

- Hold micropipette in one hand. With the other hand turn the black volume adjustment dial 1/3 of a revolution above the desired setting then slowly down until the required volume shows on the digital indicator. *This prevents mechanical backlash from affecting accuracy*.
- Press disposable tips firmly onto the shaft to ensure an airtight seal. Do this by tapping the micropipette in the tip (tapping the tip on).

- Depress plunger to the **first stop**. Holding the micropipette vertically, immerse the tip approximately two mm into the sample liquid. **Allow the pushbutton to return slowly to the up position!**
- Withdraw the tip from the liquid. Touch the tip end against the side wall of the receiving vessel and depress the plunger slowly to the first stop.
- Wait one second then press the plunger to the second stop, expelling any residual liquid in the tip.
- With the plunger fully depressed, withdraw micropipette and allow the plunger to slowly return to the up position.
- Discard the tip by depressing the ejector button. **Use a fresh tip for the next sample to avoid contamination.**

Important information to note:

- Do not use the micropipette without a disposable tip in place. Moisture can damage the piston and reduce accuracy.
- Do not lay a liquid loaded micropipette down. Moisture can run back inside causing damage to the micropipette.
- Do not allow the button to snap back after pushing the plunger. Allow it to return gradually.

ADVANCE PREPARATION:

1. (10 Minutes)Prepare colored water solution.
    - Each pair of students needs 10mL of each colored water, according to the protocol each pair is completing. The water colors needed for each protocol are:
        - A- DNA: Red and Blue
        - B- GATC: Blue and Green
        - C- Gator: Green and Brown
        - D- UF: Orange and Blue
        - E- Virus : Purple and Red
        - F – Atom; Red, Orange and Blue
        - G – Flask; Blue, Orange and Red
        - H – DNA Strand; Blue and Red
        - I – Insect; Green and Blue
        - J – PCR; Green, Orange and Blue

2. (5 Minutes) Prepare student protocols
    - Copy protocol for each student pair or group. For prolonged use, consider laminating.

PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:

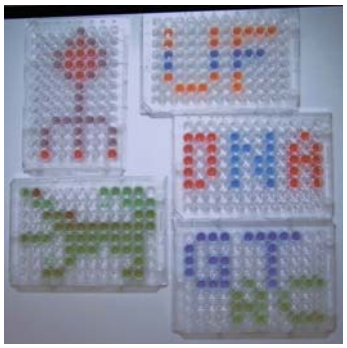
A. Introduction (10 Minutes)

1. Teach students the parts and proper method for handling the micropipette.
2. Model using the micropipette to measure the volume of several samples of water.
    - Explicitly instruct students on how to set the volume using the adjustment knob, properly read the volume indicator, how to eject the tip and when each micropipette should be used.
3. Separate students into pairs.
4. Distribute a micropipette protocol sheet and all the necessary materials to each pair of students or have student collect items from a common workstation.

B. Micropipetting Activity (20 Minutes)

1. Students work with their partner to complete the micropipette protocol activity.

- Activity results are dependent upon which protocol the students followed.
    - A- DNA: Red and Blue
    - B- GATC: Blue and Green
    - C- Gator: Green and Brown
    - D- UF: Orange and Blue
    - E- Virus : Purple and Red
    - F – Atom; Red, Orange and Blue
    - G – Flask; Blue, Orange and Red
    - H – DNA Strand; Blue and Red
    - I – Insect; Green and Blue
    - J – PCR; Green, Orange and Blue



C. Check for Accuracy (20 minutes)

1. As an extension, students can check for accuracy by determining the mass of their design. To do so, the students should do the following:
    - Determine the volume of colored water added to their plate
    - Convert volume into mass (1000µl = 1ml; 1ml = 1g)
    - Zero a scale with an empty 96-well plate
    - Calculate the mass of their completed plate

D. Create own design

1. Have students create their own designs and protocols.

ASSESSMENT SUGGESTIONS:

- Ensure all students have successfully completed the activity.
- Students can determine the mass

RESOURCES/REFERENCES:

Background Information Modified From: "Biotechnology Laboratory: Micropipet Technique." Biotechnology In The Classroom- University of California Davis. N.p., 2002. Web. <http://ceprap.ucdavis.edu>.

Many thanks to the CPET undergraduates who contributed designs!

Protocol A:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the RED dye,

20 µL: B1, B2, B3, B11,
16 µL: D1, D3, D10, D11, D12
17 µL: E1, E3, E10, E12
18 µL: F1, F2, F10, F12
19 µL: C1, C3, C10, C12

Using the BLUE dye,

8 µL: B5, B8
6 µL: D5, D7, D8
8 µL: E5, E7, E8
9 µL: F5, F8
7 µL: C5, C6, C8

Using the RED dye,

70 µL: B1, B2, B3, B11,
116 µL: D1, D3, D10, D11, D12
110 µL: E1, E3, E10, E12
85 µL: F1, F2, F10, F12
93 µL: C1, C3, C10, C12

Using the BLUE dye,

118 µL: B5, B8
96 µL: D5, D7, D8
88 µL: E5, E7, E8
129 µL: F5, F8
107 µL: C5, C6, C8

Protocol B:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the GREEN dye,

20 µL: E6, E10, E11, E12
16 µL: G5, G6, G7, G9
17 µL: F5, F7, F9
18 µL: H5, H7, H10, H11, H12

Using the BLUE dye,

8 µL: B1, B8
6 µL: D1, D4, D8
8 µL: E2, E3, E4, E8
9 µL: A2, A3, A4, A6, A7, A8, A9, A10
7 µL: C1, C3, C4, C8

Using the GREEN dye,

70 µL: E6, E10, E11, E12
116 µL: G5, G6, G7, G9
110 µL: F5, F7, F9
93 µL: H5, H7, H10, H11, H12

Using the BLUE dye,

118 µL: B1, B8
96 µL: D1, D4, D8
88 µL: E2, E3, E4, E8
129 µL: A2, A3, A4, A6, A7, A8, A9, A10
107 µL: C1, C3, C4, C8

Protocol C:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the GREEN dye:

20 µL: A1, A 9, A10, A 11
19.5 µL: B2, B3, B8, B10, B11, B12
18.2 µL: D9, D10, D11, D12
17.7 µL: D4, D5, D6, D7, D8
89 µL: D10, D11, D12, B2, B3
95 µL: B8, B10, B11, B12
100 µL: H3, H4, H10, H11
111 µL: A1, A 9, A 10, A 11, C3, C4, C11, C12
120 µL: C3, C4, C11, C12
135 µL: D4, D5, D6, D7, D8, D9
15.7 µL: H3, H4, H10, H11
13 µL: G5, G11
12 µL: F5, F6, F7, F9, F10, F11, A2, C5
11 µL: G5, G11
140 µL: D4, D5, D6, D7, D8, D9, D10, D11, D12
160µL: H3, H4, H10, H11,
180 µL: G5, G11
177 µL: F5, F6, F7, F9, F10, F11
188 µL: E1, E2, E3, E5, E6
190 µL: E7, E8, E9, E10, E11

Using the BROWN dye:

200 µL Brown: A2, C5

Protocol D:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the ORANGE dye:

20 µL: B2, C2
19 µL: B6, C6
18.6 µL: B8, C8
17.3 µL: F2, F6, F8
15.9 µL: G3, G4, G5, G8

Using the BLUE dye:

13 µL: D2, E2
11.5 µL: D6, E6, E8
10 µL: D8, D9, D10

NEXT,

Using the ORANGE dye:

179 µL: B9, B10, B11
164 µL: B8, C8
159 µL: B6, C6
143 µL: B2, C2
133 µL: F2, F6, F8
127 µL: G3, G4, G5, G8

Using the BLUE dye:

111 µL: D2, E2
100 µL: D6, E6, E8
120 µL: D8, D9, D10

Protocol E:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the RED dye,

20 µL: A12

16 µL: C3, C4

17 µL: D2, D3, D4, D5, D12

18 µL: E3, E4

19 µL: G12

Using the PURPLE dye,

8 µL: A10, A11, B3, B4, B9

6 µL: D1, D6, D7, D8, D9, D10, D11

8 µL: E2, E5, E9

9 µL: F3, F4, F9

7 µL: C2, C5, C9, G10, G11

Using the RED dye,

70 µL: A12

116 µL: C3, C4

110 µL: D2, D3, D4, D5, D12

93 µL: E3, E4

85 µL: G12

Using the PURPLE dye,

118 µL: A10, A11, B3, B4, B9

133 µL: D1, D6, D7, D8, D9, D10, D11

122 µL: E2, E5, E9

129 µL: F3, F4, F9

141 µL: C2, C5, C9, G10, G11

Protocol F:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the RED dye,

20 µL: A2, A3, E4

14 µL: B2, B4, C2, C5, D3

15 µL: G9, F8, H7, H8

Using the ORANGE dye,

20 µL: A8, A9, A10

18 µL: B7, B10, C10, D4, D9

17 µL: E3, E8, F3, F7

19 µL: G3, G6, H4, H5

Using the BLUE dye,

18 µL: C6, D5, D6, D7

19 µL: E5, E6, E7, F6

Using the RED dye,

85 µL: A2, A3, E4

90 µL: B2, B4, C2, C5, D3

70 µL: G9, F8, H7, H8

Using the ORANGE dye,

90 µL: A8, A9, A10

85 µL: B7, B10, C10, D4, D9

75 µL: E3, E8, F3, F7

102 µL: G3, G6, H4, H5

Using the BLUE dye,

93 µL: C6, D5, D6, D7

97 µL: E5, E6, E7, F6

Protocol G:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the BLUE dye,
- 17 µL: A7, A8, A9, C5, C9
- 19 µL: B6, B9, E2, E9
- 21 µL: D2, D3, D4, D9
- 18 µL: F2, F3, F4, F9, G5, G9
- 15 µL: H6, H7, H8, H9

Using the ORANGE dye,
- 18 µL: B7, B8, D7, D8
- 14 µL: C7, C8, E7, E8
- 16 µL: F7, F8, G7, G8

Using the RED dye,
- 19 µL: C11, D10, D11, D12
- 20 µL: E10, E11, E12, F11

Using the BLUE dye,
- 89 µL: A7, A8, A9, C5, C9
- 72 µL: B6, B9, E2, E9
- 84 µL: D2, D3, D4, D9
- 91 µL: F2, F3, F4, F9, G5, G9
- 105 µL: H6, H7, H8, H9

Using the ORANGE dye,
- 108 µL: B7, B8, D7, D8
- 104 µL: C7, C8, E7, E8
- 79 µL: F7, F8, G7, G8

Using the RED dye,
- 95 µL: C11, D10, D11, D12
- 100 µL: E10, E11, E12, F11

Protocol H:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the BLUE dye,
- 17 µL: C1, C10, C11, E8
- 19 µL: D2, D9, D12
- 18 µL: F4, F7, G5, G6

Using the RED dye,
- 16 µL: G1, G10, G11
- 19 µL: F2, F9, F12, E3
- 22 µL: D4, D7
- 14 µL: C5, C6

Using the BLUE dye,
- 101 µL: C1, C10, C11, E8
- 74 µL: D2, D9, D12
- 96 µL: F4, F7, G5, G6

Using the RED dye,
- 72 µL: G1, G10, G11
- 108 µL: F2, F9, F12, E3
- 105 µL: D4, D7
- 102 µL: C5, C6

Protocol I:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the GREEN dye,

9 µL: A5, A7, C4, C6, C8

12 µL: B3, B4, B6, B8

8 µL: D4, D5, D6, D7, D8, D9

14 µL: E4, E5, E6, E7, E8, E9

16 µL: F4, F6, F8, H5, H7

13 µL: G3, G4, G6, G8

Using the BLUE dye,

14 µL: A11, B11, G11

12 µL: C10, F10, H10

Using the GREEN dye,

99 µL: A5, A7, C4, C6, C8

102 µL: B3, B4, B6, B8

85 µL: D4, D5, D6, D7, D8, D9

114 µL: E4, E5, E6, E7, E8, E9

106 µL: F4, F6, F8, H5, H7

131 µL: G3, G4, G6, G8

Using the BLUE dye,

104 µL: A11, B11, G11

112 µL: C10, F10, H10

Protocol J:

Micropipette the indicated volumes into designated wells on the 96 well plate.

Using the GREEN dye,

9 µL: B1, B2, E1, D1, D2

19 µL: C1, C3, F1

Using the ORANGE dye,

18 µL: B5, B6, C4, C7

16 µL: D4, F5, F6

14 µL: E4, E7

Using the BLUE dye,

7 µL: B9, B10, B11

9 µL: C9, C11, D9, D10

13 µL: E9, E11, F9, F12

Using the GREEN dye,

95 µL: B1, B2, E1, D1, D2

109 µL: C1, C3, F1

Using the ORANGE dye,

118 µL: B5, B6, C4, C7

67 µL: D4, F5, F6

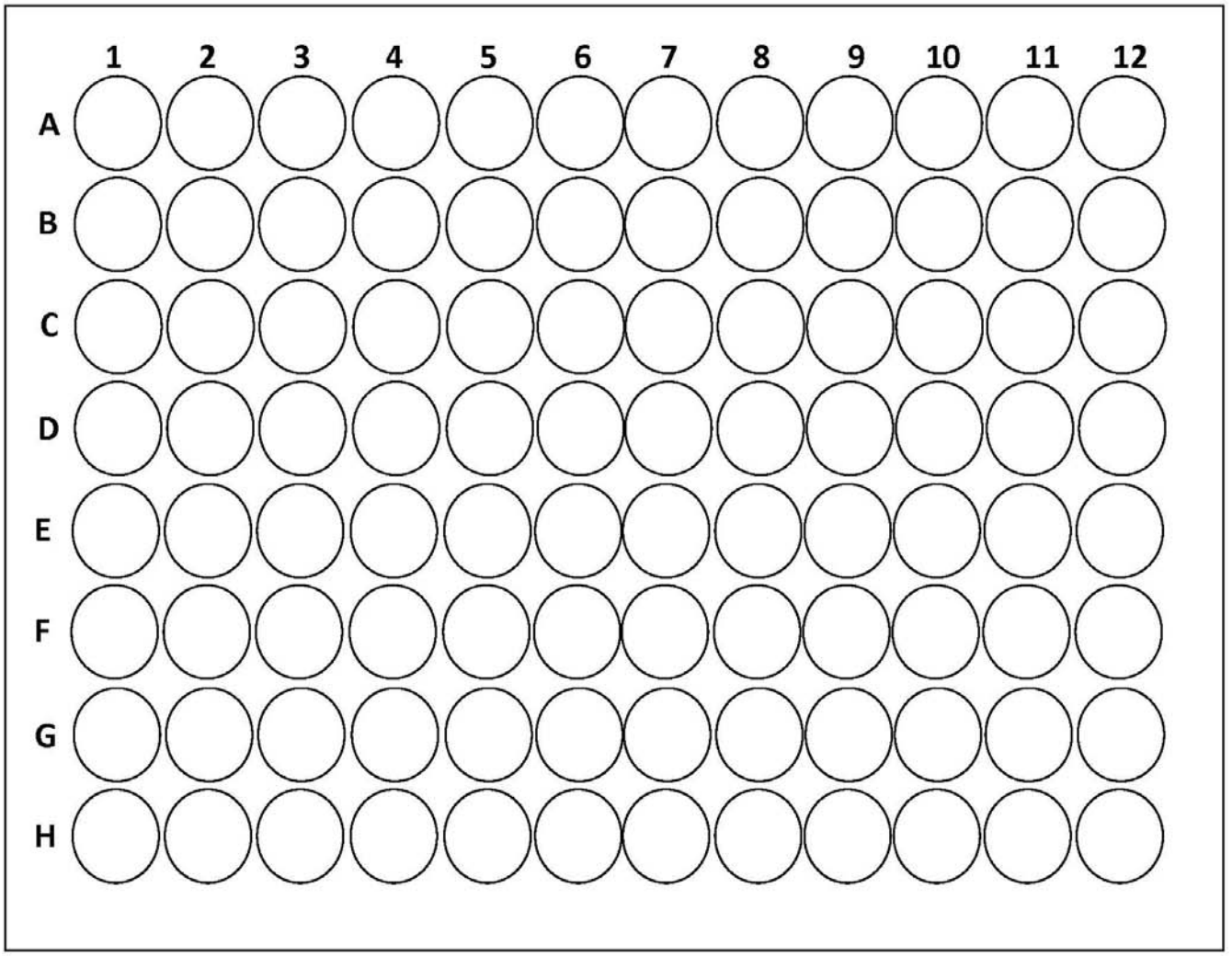
104 µL: E4, E7

Using the BLUE dye,

78 µL: B9, B10, B11

99 µL: C9, C11, D9, D10

130 µL: E9, E11, F9, F12


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H