### ADVANCED BIOTECHNOLOGY

## Pipetting skills

<table>
<thead>
<tr>
<th>Focus question</th>
<th>How can we develop and practice micropipetting skills? Why is micropipetting important?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learning target</td>
<td>Students practice micropipetting and check their accuracy.</td>
</tr>
<tr>
<td>Vocabulary</td>
<td>Micropipette</td>
</tr>
</tbody>
</table>

Adapted from "Pipetting by Design" [cpet.uf1.edu](http://cpet.uf1.edu)

### LS3: Heredity: Inheritance and Variation of Traits

<table>
<thead>
<tr>
<th>Performance expectation</th>
<th>Classroom connection: This activity helps build lab skills to be successful using other biotech techniques.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HS-LS1-1</td>
<td></td>
</tr>
<tr>
<td>Performance expectation</td>
<td>Classroom connection: Students practice micropipetting small amounts of liquids, a necessary skill in a biotechnology lab.</td>
</tr>
<tr>
<td>HS-LS3-1</td>
<td></td>
</tr>
</tbody>
</table>

### Science and engineering practices

<table>
<thead>
<tr>
<th>Asking Questions and Defining Problems</th>
<th>Classroom connection: Students will micropipette colored water into cell well plates to make patterns. The patterns will encourage them to ask questions and define problems.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constructing Explanations and Designing Solutions</td>
<td>Classroom connection: Students will create the pattern assigned to them and construct an explanation to connect the pattern to biotechnology.</td>
</tr>
</tbody>
</table>

### Disciplinary core ideas

<table>
<thead>
<tr>
<th>LS3.A: Inheritance of Traits</th>
<th>Classroom connection: Students will practice moving small amounts of liquids to develop skills for performing other biotech lab activities.</th>
</tr>
</thead>
</table>

learn more at [nourishthefuture.org](http://nourishthefuture.org)
Cross-cutting concepts

<table>
<thead>
<tr>
<th>Structure and Function</th>
<th>Classroom connection: Students will compare the accuracy of transferring small amounts of liquid using micropipettors and 1 mL disposable pipettes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause and Effect</td>
<td>Classroom connection: Using micropipettors instead of disposable pipettes results in greater accuracy for these activities.</td>
</tr>
<tr>
<td>Science is a Human Endeavor</td>
<td>Classroom connection: Micropipettors are a technological advance that has influenced the progress of science.</td>
</tr>
</tbody>
</table>

Prior knowledge
Students may have used a disposable 1mL pipette before. It is difficult to control accuracy when using these pipettes. This activity shows students that micropipettors can transfer much smaller amounts of liquid and are much more accurate if the proper technique is used. It is possible to do the lab with the sterile pipettes that are included in the kit from BioRad. The ability to use micropipettes is a valuable skill for employment in any biotech lab.

Teacher preparation
In order to develop a skill with accuracy, students need to practice. This activity allows students to practice with micropipettors and check their accuracy by measuring the mass of their results. This also gives students the opportunity to determine a percent error measurement.
1. Copy protocol instructions.
2. Organize students in groups of 3–4, with two pipettes each of different sizes, to work together to create the pattern. From the protocols (attached on additional pages) there are multiple amounts called for and each student will have ample opportunity to pipette. If a check for accuracy is desired after each student adds their assigned color/amounts, they can determine the mass at that point and measure to see where they are in terms of accuracy.

<table>
<thead>
<tr>
<th>Final totals for each protocol (adjustable volume pipettes)</th>
<th>Final totals for each protocol (fixed volume pipettes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = DNA = 5045µl = 5.045g</td>
<td>A = DNA = 2655µl = 2.655g</td>
</tr>
<tr>
<td>B = corn = 5841µl = 5.841g</td>
<td>B = corn = 2760µl = 2.76g</td>
</tr>
<tr>
<td>C = pGLO = 5217µl = 5.217g</td>
<td>C = soil = 2930µl = 2.93g</td>
</tr>
<tr>
<td>D = a plasmid = 1580µl = 1.58g</td>
<td>D = a plasmid = 1910µl = 1.91g</td>
</tr>
<tr>
<td>E = GMO = 4621µl = 4.621g</td>
<td>E = GMO = 2340µl = 2.34g</td>
</tr>
<tr>
<td>F = soy = 5003µl = 5.003g</td>
<td>F = soy = 3560µl = 3.56g</td>
</tr>
</tbody>
</table>
Differentiation
Other ways to connect with students with various needs:

• **Local community**: Students may do a search to see what genetic modification resources are available in their community. Medical labs are using genetic modification techniques to target specific diseases, and agriculture companies (Corteva, Bayer, Syngenta, BASF) may have education and outreach departments that would send a speaker to your class. Lab techs in hospitals and veterinary clinics often use micropipetting skills and may be interested in sharing experiences with your class.

• **Students with special needs (language/reading/auditory/visual)**: This lesson requires fine motor coordination and may result in frustration among students who are not detail-oriented or able to be precise. They may be employed as accuracy assessors. They could travel around the class to check progress and measure the mass of the plates to see how accurate each group is being.

• **Extra support**: Watch Basic Pipetting at: [youtu.be/bex0itUMxmI](https://youtu.be/bex0itUMxmI)

• **Extensions**: Students may want to do more than one protocol, or work on their own to check their accuracy.

Assessments
Have students determine their percent error.

**Rubric for self-assessment**

<table>
<thead>
<tr>
<th>Skill</th>
<th>Yes</th>
<th>No</th>
<th>Unsure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our group pipetted the correct amounts into the cell well plate and we calculated our percent error.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Our pattern helped me to ask questions and define problems related to biotechnology.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>We were able to connect our pattern to a biotechnology-related topic.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Protocols for adjustable volume pipettes

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

**Using the red water**
- 20 µl: B1, B2, B3, B11
- 26 µl: D1, D3, D10, D11, D12
- 27 µl: E1, E3, E10, E12
- 28 µl: F1, F2, F10, F12
- 29 µl: C1, C3, C10, C12

**Using the blue water**
- 38 µl: B5, B8
- 36 µl: D5, D7, D8
- 48 µl: E5, E7, E8
- 59 µl: F5, F8
- 67 µl: C5, C6, C8

**Using the red water**
- 90 µl: B1, B2, B3, B11
- 132 µl: D1, D3, D10, D11, D12
- 127 µl: E1, E3, E10, E12
- 103 µl: F1, F2, F10, F12
- 112 µl: C1, C3, C10, C12

**Using the blue water**
- 126 µl: B5, B8
- 102 µl: D5, D7, D8
- 96 µl: E5, E7, E8
- 138 µl: F5, F8
- 114 µl: C5, C6, C8

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

**Using the yellow water**
- 20 µl: E5, E6, E9, E12
- 27 µl: F4, F7, F9, F10, F12
- 45 µl: G4, G7, G9, G11, G12
- 38 µl: H5, H6, H9, H12

**Using the green water**
- 119 µl: A2, A3, A7, A8
- 48 µl: B1, B4, B7, B9
- 67 µl: C1, C7, C8
- 76 µl: D2, D3, D4, D7, D9
- 55 µl: E2, E3, E4

**Using the yellow water**
- 70 µl: E5, E6, E9, E12
- 110 µl: F4, F7, F9, F10, F12
- 116 µl: G4, G7, G9, G11, G12
- 93 µl: H5, H6, H9, H12

**Using the green water**
- 129 µl: A2, A3, A7, A8
- 118 µl: B1, B4, B7, B9
- 107 µl: C1, C7, C8
- 96 µl: D2, D3, D4, D7, D9
- 88 µl: E2, E3, E4
**Protocol C**
Micropipette the indicated volumes into designated wells on the 96 well plate.

**Using green water**
- 20µl: B7, C7
- 47µl: D1, D2, D7
- 28µl: E1, E3, E7
- 59µl: F1, F2, F7, F8, F9
- 35µl: G1, H1

**Using blue water**
- 28µl: B5, B11
- 62µl: C4, C6, C10, C12
- 51µl: D4, D10, D12
- 90µl: E4, E5, E6, E10, E12
- 79µl: F5, F11

**Using green water**
- 70µl: B7, C7
- 116µl: D1, D2, D7
- 110µl: E1, E3, E7
- 85µl: F1, F2, F7, F8, F9
- 93µl: G1, H1

**Using blue water**
- 118µl: B5, B11
- 96µl: C4, C6, C10, C12
- 88µl: D4, D10, D12
- 199µl: E4, E5, E6, E10, E12
- 107µl: F5, F11

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

**Using blue water**
- 20µl: A6, A7
- 39µl: B5
- 28µl: C4, D4, E4
- 42µl: F5
- 51µl: G6, G7

**Using red water**
- 100µl: A8
- 111µl: B9
- 120µl: C10, D10

**Using green water**
- 140µl: E10
- 176µl: F9
- 115µl: G8

**Using blue water**
- 70µl: A6, A7
- 81µl: B5
- 76µl: C4, D4, E4
- 92µl: F5
- 101µl: G6, G7
Protocol E
Micropipette the indicated volumes into designated wells on the 96 well plate.

Using red water
- 20µl: B2, B3, B4, B11
- 35µl: C1, C10, C12
- 47µl: D1, D3, D4, D10, D12
- 28µl: E1, E4, E10, E12
- 59µl: F2, F3, F11

Using yellow water
- 88µl: B6, B8
- 26µl: C6, C7, C8
- 45µl: D6, D8
- 79µl: E6, E8
- 27µl: F6, F8

Using red water
- 70µl: B2, B3, B4, B11
- 116µl: C1, C10, C12
- 110µl: D1, D3, D4, D10, D12
- 135µl: E1, E4, E10, E12
- 179µl: F2, F3, F11

Using yellow water
- 118µl: B6, B8
- 96µl: C6, C7, C8
- 89µl: D6, D8
- 129µl: E6, E8
- 107µl: F6, F8

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

Using blue water
- 119µl: B1, B2, B10, B11, B12
- 102µl: C1, C3, C9
- 127µl: D1, D3, D9, D11, D12
- 115µl: E1, E3, E9, E12
- 132µl: F1, F2, F10, F11

Using red water
- 20µl: B5, B6
- 34µl: C5, C7
- 89µl: D5, D7
- 44µl: E5, E7
- 100µl: F5, F6

Using blue water
- 22µl: B1, B2, B10, B11, B12
- 31µl: C1, C3, C9
- 29µl: D1, D3, D9, D11, D12
- 22µl: E1, E3, E9, E12
- 40µl: F1, F2, F10, F11

Using red water
- 74µl: B5, B6
- 158µl: C5, C7
- 37µl: D5, D7
- 196µl: E5, E7
- 88µl: F5, F6
Protocols for fixed volume pipettes

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

Using the red water
- 20 μl: B1, B2, B3, B11
- 205 μl: C1, C3, C10, C12
- 30 μl: D1, D3, D10, D11, D12
- 50 μl: E1, E3, E10, E12
- 100 μl: F1, F2, F10, F12

Using the blue water
- 40 μl: B5, B8
- 55 μl: C5, C6, C8
- 20 μl: D5, D7, D8
- 100 μl: E5, E7, E8
- 200 μl: F5, F8

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

Using the yellow water
- 20 μl: E5, E6, E9, E12
- 30 μl: F4, F7, F9, F10, F12
- 50 μl: G4, G7, G9, G11, G12
- 100 μl: H5, H6, H9, H12

Using the green water
- 200 μl: A2, A3, A7, A8
- 55 μl: B1, B4, B7, B9
- 100 μl: C1, C7, C8
- 40 μl: D2, D3, D4, D7, D9
- 120 μl: E2, E3, E4

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

Using green water
- 50 μl: B1, B2, B3, B4, B10
- 100 μl: C1
- 70 μl: D1, D2, D3, D4, D10
- 200 μl: E4, E10
- 60 μl: F1, F2, F3, F4, F10

Using blue water
- 70 μl: B12, C12
- 100 μl: D6, D7, D8, D12
- 210 μl: E6, E8, E12
- 90 μl: F6, F7, F8, F12

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

Using blue water
- 110 μl: A6, A7
- 50 μl: B5
- 90 μl: C4, D4, E4
- 100 μl: F5
- 200 μl: G6, G7

Using red water
- 100 μl: A8
- 200 μl: B9
- 120 μl: C10, D10

Using green water
- 120 μl: E10
- 100 μl: F9
- 110 μl: G8
**Protocol E**
Micropipette the indicated volumes into designated wells on the 96 well plate.

**Using red water**
- 20μl: B2, B3, B4, B11
- 50μl: C1, C10, C12
- 100μl: D1, D3, D4, D10, D12
- 30μl: E1, E4, E10, E12
- 60μl: F2, F3, F11

**Using yellow water**
- 100μl: B6, B8
- 150μl: C6, C7, C8
- 70μl: D6, D8
- 200μl: E6, E8
- 60μl: F6, F8

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

**Using blue water**
- 100μl: A1, A2, A3, A4, A8, A12
- 110μl: B1, B9, B11
- 50μl: C1, C10
- 30μl: D1, D2, D3, D4, D10
- 200μl: E4, E10, F4, F10
- 70μl: G1, G2, G3, G4, G10

**Using red water**
- 20μl: B5, B6, B7, B8
- 30μl: C5, C8, D5, D8
- 60μl: E5, E8, F5, F8
- 200μl: G5, G6, G7, G8