VNTR Analysis
The Science Behind DNA Fingerprinting
Teacher Materials

In this lab, students will analyze a single VNTR locus (Variable Number of Tandem Repeats) in several different subjects. The VNTR they will be studying, known as D1S80, is a 16 bp segment that is repeated 14 to 41 times in the genome. They will use agarose gel electrophoresis to separate the PCR products and compare the bands on the gel to a DNA ladder in order to determine each subject’s genotype.

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VNTR Analysis

Learning Goals

Student Learning Goals:

• Students will understand what short tandem repeats (STRs) and variable number of tandem repeats (VNTRs) are and how they are used in science.
• Students will understand how genetic information is passed from parents to children.
• Students will understand the process of agarose gel electrophoresis.
• Students will understand the process of polymerase chain reaction (PCR).
• Students will understand how both PCR and gel electrophoresis is used for DNA profiling.

Student Learning Objectives:

• Students will perform the technique of agarose gel electrophoresis.
• Students will estimate the size of DNA fragment from agarose gel data.
• Students will analyze the results of the molecule separation by gel electrophoresis.
• Students will identify VNTR alleles based on PCR product size using gel data.

Scientific Inquiry Skills:

• Students will pose questions and form hypotheses.
• Students will design and conduct scientific investigations.
• Students will use experimental data to make conclusions about the initial question and to support or refute the stated hypothesis.
• Students will follow laboratory safety rules and regulations.

Laboratory Technical Skills:

• Students will demonstrate proper use of micropipettes.
• Students will consider safety considerations when working with an electric current.
• Students will demonstrate proper use of gel electrophoresis equipment.
• Students will prepare and pour agarose gels.
Experimental Timing:

From start to finish this lab takes a single 45-50 minute class period. Additional time may be required if you choose to have students prepare their own gels.

Notes:

- Time required for electrophoresis is approximately 20-25 minutes, but may vary depending on the type of electrophoresis equipment and voltage.
- If this is the first agarose gel students have experienced, you should expect the students to need extra time to load the gel.

Specialized Equipment:

- p20 micropipettes
- gel electrophoresis units with power supplies (as written, each student group will run four samples plus a ladder. However, you can adjust this as desired)
- Blue light or UV transilluminator
- centrifuge (optional)

Ordering Information:

The NoLimits™ DNA fragments used in this lab can be ordered through Fisher Scientific using the product numbers provided in the materials section of this guide. Each tube of NoLimits™ DNA contains 10 μg of DNA in 20 μL (at a concentration 500 ng/μL). If you follow the set-up procedures described here, a single set of NoLimits™ DNA fragments (400, 600, 700, 800) will provide enough DNA for 65 groups to perform this lab, assuming that each group is provided with four Subject DNA samples.

*The reagents from New England BioLabs can be ordered (at no cost) by going to their website (https://www.neb.com). A calculation tool for ordering NEB Reagents for this lab can be found on the final page of this document.

Procedure Tips:

1. Before starting the experiment, ask students to check their materials list to make sure they have everything.

2. Demonstrate how to pipet very small volumes of liquid and load a gel. If your students have not had the opportunity to run many gels, you may want to have them practice loading gels before you begin this experiment. Refer to “A Guide to Agarose Gel Electrophoresis” on the website (https://www.massbioed.org/educators/curriculum), for instructions on how to make practice gels.
3. Remind students to use a fresh pipette tip with each sample and to record the location of each of
the samples as they load them into the gel.

4. If your gel units have a blue light to visualize the DNA, remind students to turn off the light while
they run the gel. DNA stains are light sensitive and it is possible to bleach the stain during a run
making it difficult to visualize the DNA. If this accidently happens, you can soak the gel after running
in buffer with 2X GelGreen™ for 30 minutes and then visualize the gel.

Teaching Tips:

1. The protocol for preparing the electrophoresis gels is not included in this version of the lab. You can
download “A Guide to Agarose Gel Electrophoresis” document from the website
(https://www.massbioed.org/educators/curriculum), adapt it to your equipment and insert it in the
lab.

2. This lab provides an opportunity to discuss PCR without actually doing a PCR reaction. Consider
using the BioTeach video “The Ups and Downs of PCR” to reinforce the concepts behind PCR.

3. Although we have chosen not to provide a crime scene or other scenario for this lab, you could
easily use this protocol to create your own crime scene analysis, paternity test, or other heredity
study.

Safety Considerations:

- Gloves, lab coats and eye protection should be used whenever possible, as a part of good
  laboratory practice.

- Practice sterile techniques whenever possible to avoid contamination of reagents.

- Exercise caution when heating and/or melting reagents during gel preparation.

- Exercise caution when working with electrical equipment.

- UV protective shields and/or glasses must be used if visualizing gels with a UV
  transilluminator.

- Always wash hands thoroughly after handling biological materials or reagents.

- Obtain the Material Safety Data Sheets (MSDS) available from the suppliers and follow all
  safety precautions and disposal directions as described in the MSDS.

- Check with your school’s lab safety coordinator about proper disposal of all reagents and
gels containing DNA stains.
VNTR Analysis
Instructor Preparation Guide

Materials: This guide assumes 30 students, working in groups of two, for a total of 15 groups.

Materials for Teacher Advanced Preparation:

<table>
<thead>
<tr>
<th>1 tube</th>
<th>NoLimits™ DNA Fragment 400 bp (Fisher# FERSM1631) Each tube contains 10 μg of DNA in 20 μL at a concentration 500 ng/μL ▲</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 tube</td>
<td>NoLimits™ DNA Fragment 600 bp (Fisher# FERSM1461) Each tube contains 10 μg of DNA in 20 μL at a concentration 500 ng/μL ▲</td>
</tr>
<tr>
<td>1 tube</td>
<td>NoLimits™ DNA Fragment 700 bp (Fisher# FERSM1651) Each tube contains 10 μg of DNA in 20 μL at a concentration 500 ng/μL ▲</td>
</tr>
<tr>
<td>1 tube</td>
<td>NoLimits™ DNA Fragment 800 bp (Fisher# FERSM1481) Each tube contains 10 μg of DNA in 20 μL at a concentration 500 ng/μL ▲</td>
</tr>
<tr>
<td>1 tube</td>
<td>100bp Quick Load DNA ladder (NEB# N0467S) ▲</td>
</tr>
<tr>
<td>1 mL</td>
<td>6X loading Dye (NEB# B7024S)</td>
</tr>
<tr>
<td>2 mL</td>
<td>1X TE (Tris-EDTA) or sterile distilled water</td>
</tr>
<tr>
<td>100</td>
<td>microcentrifuge tubes (1.5 mL or 0.5 mL)</td>
</tr>
<tr>
<td>1</td>
<td>p20 micropipette and tips</td>
</tr>
<tr>
<td>1</td>
<td>p200 micropipette and tips</td>
</tr>
<tr>
<td>1</td>
<td>p1000 micropipette and tips</td>
</tr>
<tr>
<td>2-3</td>
<td>microcentrifuge tube racks</td>
</tr>
<tr>
<td>1</td>
<td>centrifuge (optional)</td>
</tr>
<tr>
<td>1</td>
<td>ice bucket with crushed ice</td>
</tr>
<tr>
<td>1</td>
<td>ultrafine point permanent marker</td>
</tr>
</tbody>
</table>

▲ Caution: Ladders and NoLimits™ DNA samples are heat sensitive. Keep on ice at all times while working. Store in freezer long-term.

Materials for each Student Workstation:

| 4 tubes | subject DNA with 12 μL of subject DNA * |
| 1 tube | DNA Ladder with 12 μL 100bp Quick Load DNA ladder |
| 1 | agarose gel (2.0%) with DNA stain |
| 1 | p20 micropipette with tips |
| 1 | microcentrifuge rack |
| 1 | waste container |
| 1 | gel electrophoresis unit with power supply |

Common Workstation:

| 1X electrophoresis buffer |
| UV or blue light source |
| centrifuge (optional) |

*specific labels will vary based on your assigned subjects
Easy Substitutions:

- Sterile distilled water can be used instead of TE to prepare the DNA samples.
- If you do not have a centrifuge, have students gently tap the PCR tubes on the lab bench to collect all the reagents at the bottom of the tube.

Set-up Calendar:

2 weeks before lab:

- Check supplies. Order any needed materials.
- If making any substitutions to the supply list, edit the student protocol accordingly.
- Mix “Subject” DNA samples using NoLimits™ DNA and the information in Table 1. Alternatively, you can create your own “ Subjects”

Table 1. Alleles and NoLimits™ DNA Lengths.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Actual Allele Length (bp)</th>
<th>NoLimits™ DNA Fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D14</td>
<td>370</td>
<td>NONE</td>
</tr>
<tr>
<td>D16</td>
<td>402</td>
<td>400</td>
</tr>
<tr>
<td>D29</td>
<td>610</td>
<td>600</td>
</tr>
<tr>
<td>D35</td>
<td>706</td>
<td>700</td>
</tr>
<tr>
<td>D41</td>
<td>802</td>
<td>800</td>
</tr>
</tbody>
</table>

Note: D14 is given as example for student calculations only

1. Dilute 6X loading dye to 2X – You will need 200 µL of 2X loading for each Subject that you are preparing. As written, this lab directs you to prepare 4 subjects.

Prepare 1000 µL of 2X loading dye: Mix 333 µL of 6X loading dye with 667 µL distilled H₂O.

2. Each Subject DNA sample will be a combination of 2 fragments, so you will need to mix and dilute stock DNA to create the Subject DNA samples.

To make Subject A DNA sample with genotype (D16, D41) you will mix:
4 µL of 400 bp DNA + 4 µL 800 bp DNA + 192 µL 1X TE (or sterile distilled water).
Add 200 µL of the 2X loading dye you prepared in step 1. You will have 400 µL of DNA.

To make Subject B DNA sample with genotype (D16, D35) you will mix:
4 µL of 400 bp DNA + 4 µL 700 bp DNA + 192 µL 1X TE (or sterile distilled water).
Add 200 µL of the 2X loading dye you prepared in step 1. You will have 400 µL of DNA.

To make Subject C DNA sample with genotype (D29, D29) you will mix:
8 µL of 600 bp DNA + 192 µL 1X TE (or sterile distilled water).
Add 200 µL of the 2X loading dye you prepared in step 1. You will have 400 µL of DNA.

To make Subject D DNA sample with genotype (D35, D41) you will mix:
4 µL of 700 bp DNA + 4 µL 800 bp DNA + 192 µL 1X TE (or sterile distilled water).
Add 200 µL of the 2X loading dye you prepared in step 1. You will have 400 µL of DNA.
3. The final volume of each Subject DNA sample will be 400 µL at a concentration of 5ng/µL of DNA or enough DNA for 33 lanes.

4. You should have 12 µL remaining in each of the stock NoLimits™ DNA tubes. Keep this DNA frozen. It will be stable in the freezer indefinitely.

- Aliquot out DNA samples, ladder DNA. Keep all tubes in the freezer.
  1. Aliquot 12 µL of each Subject DNA into microfuge tubes labeled Sub A, Sub B...etc. As the lab is written, each student group will need one tube each of the 4 Subject DNA samples, but you can adapt as needed.
  2. Aliquot 12 µL of the 100 bp Quick Load DNA ladder into microfuge tubes labeled Ladder. Prepare one tube per lab group.

⚠️ Caution: Store all DNA samples and ladder in the freezer until the morning of the lab.

Note: The genotypes for Subjects A, B, C, and D were suggested for several reasons. In subjects A and B, the size difference between the 400 bp and 700 or 800 bp fragments will allow a quick and clear separation. Subject C represents a homozygote. Subject D offers an extra challenge because the 700 bp and the 800 bp fragments will only separate if the gel is run long enough.

The suggested subjects are optional. You can create your own by using the NoLimits™ DNA sizes recommended or by purchasing different sized NoLimits™ DNA fragments. We have included the table below for you to use if you are creating different Subject DNA samples.

Table 2 (Optional.). Alternative Subject Genotypes – Don’t forget to include homozygous individuals.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Genotype</th>
<th>NoLimits™ DNA Fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 day before lab:
- Set up student lab stations with all durable materials according to the materials list above.
- Prepare TAE or buffer of your choice
- Prepare 2.0 % agarose gels with DNA Stain. (Each group will run four samples plus a ladder.)

⚠️Tip: Gels can be prepared ahead of time. If you pour the gels several days before the lab, they should be stored in a plastic container/bag with a damp paper towel to keep them from drying out. Gels should be stored in a cool location.

⚠️ Caution: DNA stains such as GelGreen® are light sensitive. Gels should be stored in an opaque container in the dark.

Morning of lab: (Just before Lab)
- Set out DNA samples, ladder and gels at each student lab station.
VNTR Analysis
Answers to Student Questions

Pre-Lab:

1. STRs (short tandem repeats) consist of short (2-6 bp) sequences that are repeated in a tandem or head-to-tail fashion. VNTRs (variable number of tandem repeats) have longer (10-100 bp) repeated sequences.

2. Every individual has two copies of the D1S80 locus. One allele is on the chromosome #1 that was inherited from their mother, and another allele is on the homologous chromosome #1 that was inherited from their father.

3. You must perform PCR on DNA samples before you analyze a VNTR. The amount of DNA extracted from a single tissue sample is usually so small that it can’t be visualized on a gel without some sort of manipulation. PCR generates enough copies of the DNA that it can be visualized on a gel. The primers used during PCR are also designed to specifically copy only the VNTR regions not the entire genome.

4. c. Lane 4 represents a homozygous individual.
   
d. Lane 4 represents a homozygous individual. However, the father whose DNA is shown in Lane 2 does not carry a copy of the only allele present in the child whose DNA is in lane 4. Thus, the child represented in Lane 4 must have a different father.

Embedded Questions:

Table 3

<table>
<thead>
<tr>
<th>Allele</th>
<th>Number of Repeats</th>
<th>Size of PCR Product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D14</td>
<td>14</td>
<td>370</td>
</tr>
<tr>
<td>D16</td>
<td>16</td>
<td>402</td>
</tr>
<tr>
<td>D29</td>
<td>29</td>
<td>610</td>
</tr>
<tr>
<td>D35</td>
<td>35</td>
<td>706</td>
</tr>
<tr>
<td>D38</td>
<td>38</td>
<td>754</td>
</tr>
<tr>
<td>D41</td>
<td>41</td>
<td>802</td>
</tr>
</tbody>
</table>
**Data Collection:**

1. **Sample Gel:**

   ![Sample Gel Image]

   Lane 1: DNA Ladder
   Lane 2: 800, 400
   Lane 3: 750, 750
   Lane 4: 800, 700
   Lane 5: 700, 400
   Lane 6: 800, 800
   Lane 7: 800, 600
   Lane 8: 750, 400
   Lane 9: 800, 750

2. **Answers may vary based on how you have set up the lab. As written:**
   - Subject A: 800, 400
   - Subject B: 700, 400
   - Subject C: 600, 600
   - Subject D: 800, 700

   If you have prepared your own Subject DNA, refer to Table 2 in the Teacher Preparation Guide.

**Post-Lab:**

1. **PCR and gel electrophoresis were used to create DNA profiles of the subjects.** PCR (polymerase chain reaction) was used to amplify the D1S80 locus of each Subject’s DNA. During the PCR process, millions of copies of the VNTR were made. This large amount of DNA is enough for us to be able to more easily visualize the DNA using gel electrophoresis. During gel electrophoresis, we used an agarose gel matrix and an electrical current to separate the PCR products based on the size of the fragments. By comparing the bands in the gel to a standard, we can estimate their size.

2. A DNA ladder (also referred to as a DNA standard) is a mixture of DNA fragments of known sizes. The DNA ladder is needed to identify the size of the PCR products and determine the genotype or DNA profile of each subject.
3. Answers will vary depending upon how you set up the experiment. However, you will not see all 29 alleles within the class. Students may attribute the results to the small number of subjects studied and/or the fact that some alleles are not as common as others. In fact, allele frequencies vary according to the population being studied. For example, another common STR used in forensics - TH01 – has been shown to vary greatly between different ethnic groups. See the figure below.

![TH01 allele distribution](Image Source: Bio-Rad)

4. Suspects 1 and 3 could be ruled out of the investigation because their genotypes do not match the DNA found at the crime scene. Suspect 2 has the same DNA profile as the DNA found at the crime scene and shares one allele in common with the victim. Therefore, Suspect 2 should be included in the investigation. The standard established by the FBI using the Combined DNA Index System (CODIS) includes 20 STR loci and the Interpol set of STR include nine loci. (To learn more about the CODIS go to [http://www.biology.arizona.edu/human_bio/activities/blackett2/str_codis.html](http://www.biology.arizona.edu/human_bio/activities/blackett2/str_codis.html)).

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CS</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>S1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>S2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>S3</td>
<td></td>
</tr>
</tbody>
</table>

**Lane** | **Sample** | **Genotype**
--- | --- | ---
CS | Crime Scene | 7,3
V | Victim | 10,3
S1 | Suspect 1 | 5,2
S2 | Suspect 2 | 7,3
S3 | Suspect 3 | 10,2
VNTR Analysis
Standards Alignments

MA Science and Technology/Engineering Standards – High School (2016)

Biology

**HS-LS1-1.** Construct a model of transcription and translation to explain the roles of DNA and RNA that code for proteins that regulate and carry out essential functions of life.

**HS-LS3-1.** Develop and use a model to show how DNA in the form of chromosomes is passed from parents to offspring through the processes of meiosis and fertilization in sexual reproduction.

**HS-LS3-2.** Make and defend a claim based on evidence that genetic variations (alleles) may result from (a) new genetic combinations via the processes of crossing over and random segregation of chromosomes during meiosis, (b) mutations that occur during replication, and/or (c) mutations caused by environmental factors. Recognize that mutations that occur in gametes can be passed to offspring.

**HS-LS3-3.** Apply concepts of probability to represent possible genotype and phenotype combinations in offspring caused by different types of Mendelian inheritance patterns.

**HS-LS3-4(MA).** Use scientific information to illustrate that many traits of individuals, and the presence of specific alleles in a population, are due to interactions of genetic factors and environmental factors.

Chemistry

**HS-PS1-3.** Cite evidence to relate physical properties of substances at the bulk scale to spatial arrangements, movement, and strength of electrostatic forces among ions, small molecules, or regions of large molecules in the substances. Make arguments to account for how compositional and structural differences in molecules result in different types of intermolecular or intramolecular interactions.

**HS-PS1-11(MA).** Design strategies to identify and separate the components of a mixture based on relevant chemical and physical properties.

**HS-PS2-6.** Communicate scientific and technical information about the molecular-level structures of polymers, ionic compounds, acids and bases, and metals to justify why these are useful in the functioning of designed materials.

Physics

**HS-PS3-5.** Develop and use a model of magnetic or electric fields to illustrate the forces and changes in energy between two magnetically or electrically charged objects changing relative position in a magnetic or electric field, respectively.

NRC Practices

- Asking questions and defining problems
- Planning and carrying out investigations
- Analyzing data
- Mathematical and computational thinking
- Constructing explanations and designing solutions
- Engaging in argument from evidence
• Obtaining, evaluating, and communicating information


Life Sciences

**HS-LS1-1.** Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

**HS-LS3-1.** Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.

**HS-LS3-2.** Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors.

**HS-LS3-3.** Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population.

Chemistry

**HS-PS1-3.** Cite evidence to relate physical properties of substances at the bulk scale to spatial arrangements, movement, and strength of electrostatic forces among ions, small molecules, or regions of large molecules in the substances. Make arguments to account for how compositional and structural differences in molecules result in different types of intermolecular or intramolecular interactions.

**HS-PS2-6.** Communicate scientific and technical information about the molecular-level structures of polymers, ionic compounds, acids and bases, and metals to justify why these are useful in the functioning of designed materials.

Physics

**HS-PS3-5.** Develop and use a model of magnetic or electric fields to illustrate the forces and changes in energy between two magnetically or electrically charged objects changing relative position in a magnetic or electric field, respectively.

Common Core State Standards Connections:

**ELA/Literacy**

**RST.9-10.7** Translate quantitative or technical information expressed in words in a text into visual form (e.g., a table or chart) and translate information expressed visually or mathematically (e.g., in an equation) into words.

**RST.9-10.8** Assess the extent to which the reasoning and evidence in a text support the author’s claim or a recommendation for solving a scientific or technical problem.

**RST.11-12.1** Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.

**RST.11-12.7** Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.
RST.11-12.8 Evaluate the hypotheses, data, analysis, and conclusions in a science or technical text, verifying the data when possible and corroborating or challenging conclusions with other sources of information.

RST.11-12.9 Synthesize information from a range of sources (e.g., texts, experiments, simulations) into a coherent understanding of a process, phenomenon, or concept, resolving conflicting information when possible.

WHST.9-12.1 Write arguments focused on discipline-specific content.

WHST.9-12.2 Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.

WHST.9-12.5 Develop and strengthen writing as needed by planning, revising, editing, rewriting, or trying a new approach, focusing on addressing what is most significant for a specific purpose and audience.

WHST.9-12.7 Conduct short as well as more sustained research projects to answer a question (including a self-generated question) or solve a problem; narrow or broaden the inquiry when appropriate; synthesize multiple sources on the subject, demonstrating understanding of the subject under investigation.

WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research.

SL.11-12.5 Make strategic use of digital media (e.g., textual, graphical, audio, visual, and interactive elements) in presentations to enhance understanding of findings, reasoning, and evidence and to add interest.

Mathematics -

MP.2 Reason abstractly and quantitatively.

MP.4 Model with mathematics.

HSF-BF.A.1 Write a function that describes a relationship between two quantities.

HSF-IF.C.7 Graph functions expressed symbolically and show key features of the graph, by hand in simple cases and using technology for more complicated cases.

HSN.Q.A.1 Use units as a way to understand problems and to guide the solution of multi-step problems; choose and interpret units consistently in formulas; choose and interpret the scale and the origin in graphs and data displays.

HSN.Q.A.2 Define appropriate quantities for the purpose of descriptive modeling.

HSN.Q.A.3 Choose a level of accuracy appropriate to limitations on measurement when reporting quantities.

HSS-IC.A.1 Understand statistics as a process for making inferences about population parameters based on a random sample from that population.

HSS-IC.B.6 Evaluate reports based on data.
Calculation tool for ordering NEB Reagents for: VNTR: The Science Behind DNA Fingerprinting

Please keep in mind that NEB is a fantastic and generous partner and will provide up to $1000 of reagents for each school. Please check with your colleagues to coordinate your ordering to ensure that your school plans ahead for ALL of the planned labs requiring NEB reagents, and please, only order as much as you need. The calculation tool below will help you determine how much of each reagent to order. *Importantly, the amount needed per group shown below includes the extra needed in case of mistakes or when aliquots are provided for each group.*

Fill out the chart below to determine how many tubes of each of the ladder you need to order.

**Calculation tool:**

<table>
<thead>
<tr>
<th>NEB Reagent</th>
<th>NEB Catalog #</th>
<th>Amount of Reagent In NEB Tube</th>
<th>Amount Needed per Group</th>
<th>Total Number of Groups Doing the Lab</th>
<th>Total Amount You Will Need</th>
<th># Tubes Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent X</td>
<td>X0000</td>
<td>40 µL</td>
<td>4 µL</td>
<td>8</td>
<td>32 µL</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>You fill this in</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 µL X (# groups)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32 µL &lt; 40 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NEB Reagent</th>
<th>NEB Catalog #</th>
<th>Amount of Reagent In NEB Tube</th>
<th>Amount Needed per Group</th>
<th>Total Number of Groups Doing the Lab</th>
<th>Total Amount You Will Need</th>
<th>NED Tubes Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quick-Load® 100 bp DNA Ladder</td>
<td>N0551S</td>
<td>1250 µL</td>
<td>12 µL*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gel Loading Dye, Purple (6X)</td>
<td>B7024S</td>
<td>4 mL</td>
<td>&lt; 10 µL</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

*This is per gel. Each student will need to run four samples so it is likely that a pair of students will need one gel.

Once completed, you can submit your order here: [https://www.neb.com/forms/BioTeach](https://www.neb.com/forms/BioTeach)