Edvo-Kit #858

Lighting Up Life: Expression of GFP in *C. elegans*

Experiment Objective:

This experiment explores molecular methods used by scientists to create and identify transgenic animals. Students will use fluorescent microscopy and PCR to analyze *C. elegans* that have been engineered to express the Green Fluorescent Protein.

See page 3 for storage instructions.
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Safety Data Sheets can be found on our website: [www.edvotek.com/safety-data-sheets](http://www.edvotek.com/safety-data-sheets)
## Experiment Components

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<thead>
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<tr>
<td>B GFP Primer Mix Concentrate</td>
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<td>C EdvoQuick™ DNA Ladder</td>
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<td>D Control DNA Concentrate</td>
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<tr>
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<td>F Sterile Water</td>
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<td>G Wild-type C. elegans</td>
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</tr>
<tr>
<td>H Transgenic C. elegans</td>
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<td>• NGM Salts</td>
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#### Reagents & Supplies *(included with this experiment)*

Store all components below at room temperature.

<table>
<thead>
<tr>
<th>Component</th>
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<tr>
<td>• Petri Dishes</td>
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</tr>
<tr>
<td>• Sterile individually wrapped transfer pipets</td>
<td>☐</td>
</tr>
<tr>
<td>• Small Transfer Pipets</td>
<td>☐</td>
</tr>
<tr>
<td>• 10 ml Pipet</td>
<td>☐</td>
</tr>
<tr>
<td>• Screw-cap conical tube (15 ml)</td>
<td>☐</td>
</tr>
<tr>
<td>• UltraSpec-Agarose™</td>
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<tr>
<td>• Electrophoresis Buffer (50x)</td>
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<tr>
<td>• 10x Gel Loading Solution</td>
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<tr>
<td>• InstaStain® Ethidium Bromide <em>(preferred method)</em></td>
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</tr>
<tr>
<td>• FlashBlue™ Liquid Stain</td>
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<tr>
<td>• Screw-top Microcentrifuge Tubes (1.5 ml)</td>
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</tr>
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<td>• Snap-top Microcentrifuge Tubes (1.5 ml)</td>
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<td>• Sterile loops</td>
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<tr>
<td>• Wax beads</td>
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Experiment Requirements *(NOT included with this experiment)*

- Fluorescent and Bright Field Microscopes (For fluorescent microscope specifications see page 25.)
- Thermal cycler (EDVOTEK Cat. # 541 highly recommended) or three water baths*
- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Balance
- Microcentrifuge
- Waterbath (55°C and boiling) (EDVOTEK Cat. # 539 highly recommended)
- UV Transilluminator or UV Photodocumentation system (use if staining with InstaStain® Ethidium Bromide)
- UV safety goggles
- White light visualization system (optional - use if staining with FlashBlue™)
- Adjustable micropipets (5-50 μl, 20-200 μl 100-1000 μl) with tips
- Microwave or hot plate
- Pipet pump
- Covered box or incubator
- Timers
- Permanent Markers
- 250 ml flasks or beakers
- Hot gloves
- Disposable laboratory gloves
- Distilled or deionized water
- Sterile water
- Ice buckets and ice
- Bleach solution or laboratory disinfectant

*If you do not have a thermal cycler, PCR experiments can be conducted, with proper care, using three water baths. However, a thermal cycler assures a significantly higher rate of success. See Appendix C for detailed instructions.
**Background Information**

**WHY STUDY C. elegans?**

A model organism is any plant, animal or microorganism that allows us to study fundamental questions in biology that may be hard to study directly in complex organisms like humans. In the 1970’s, Dr. Sydney Brenner established the nematode *Caenorhabditis elegans* (see-no-rab-DITE-iss el-leh-GANS) as a model system because they have a simple genome, a fast generation time, and are easy and inexpensive to maintain. While characterizing the worm, Brenner, along with Drs. John Sulston and Robert Horvitz, discovered that the developmental fate of every cell in the worm is invariable between animals. They also discovered key genes involved in organ development and programmed cell death. For this work, Brenner, Sulston and Horvitz were awarded the Nobel Prize in Physiology or Medicine in 2002.

Today, *C. elegans* continues to be an important model organism for the study of embryogenesis, morphogenesis, development, nerve function, behavior and aging, and genetics. The entire *C. elegans* genome has been completely sequenced and several thousand genetic mutants are available for study. This allows scientists to correlate changes at the DNA level with changes in phenotype. Notably, by comparing their DNA sequences, it was determined that over 35% of worm genes have human homologs. Many of these genes are important for human health and development.

**GROWTH AND DEVELOPMENT OF C. elegans**

*C. elegans* is a free-living, non-parasitic nematode that lives in temperate soil, where it feeds on microbes that are found in decaying organic matter. Adult worms measure approximately one millimeter (mm) in length. The outer cuticle of *C. elegans* is transparent, making it easy to visualize growth and development of internal structures like the pharynx, the intestine, the gonads and the muscles (Figure 1). The worm also has an extensive nervous system – in fact, the nervous system comprises almost 1/3 of the worm’s 959 somatic cells! This makes *C. elegans* a valuable model system for neuroscientists.

There are two naturally occurring sexes in *C. elegans*. The vast majority of worms are self-fertile hermaphrodites, meaning that they produce both the sperm and the eggs used for reproduction. Free-living males represent <1% of the total nematode population. However, free-living males plus a hermaphrodite can produce over 1000 offspring in a generation; in contrast, self-fertilized hermaphrodite worms will produce about 300. Because their sperm will preferentially fertilize a hermaphrodite’s eggs and produce more offspring, free-living males are often used to introduce specific genetic mutations into a worm population to be studied.

Figure 1: *C. elegans* anatomy
C. elegans develop from embryo to adult in four days, allowing for rapid studies in the laboratory (Figure 2). The worms are grown on agar plates or in liquid culture and they feed on E. coli. After being laid, the worm embryo will develop for approximately 14 hours before hatching. Juvenile worms progress through four larval stages (L1-L4) over the next two days, increasing in size with each stage. After the fourth larval molt (L4), the worms are reproductively mature, meaning that they can be used for further genetic studies. Adults will live for 2-3 weeks, over which time they gradually age and lose vigor.

**GENETIC ENGINEERING IN C. elegans**

Changes to an organism’s DNA sequence, called mutations, can affect the way an organism interacts with its environment. Most mutations produce negative effects because the DNA sequence directs an organism’s growth, development and maintenance. However, an advantageous mutation may promote an organism’s survival. Today, genetic engineering allows scientists to directly manipulate an organism’s genome to produce a phenotype. These engineered genes, called transgenes, can be inserted, deleted or mutated in a matter of weeks. Transgenes that express a wild-type protein can be used to repair – or “rescue” – a related mutant phenotype. Other transgenes include “tagged” proteins to simplify its purification. These tags also allow scientists to determine the sub-cellular location of a protein or its interacting partners. Furthermore, by combining regulatory DNA sequences with a reporter gene, scientists can observe patterns of when and where genes are turned on and off, revealing the role those DNA sequences might normally play in a cell.
Transgenes are assembled in vitro using recombinant DNA technology. In order to be repeated properly expressed in vivo, a transgene must include the following elements (summarized in Figure 3):

- The **promoter** – a DNA sequence that recruits RNA polymerase to the gene for transcription. Promoters are typically located just before or just after (“upstream” or “downstream” of) the beginning of a gene.
- The **5' Untranslated Region (UTR)** – a sequence that begins at the transcriptional start site and ends at the start codon. This region may contain special sequences that regulate translation.
- **Start codon** – a sequence (ATG) that signals the start of translation.
- The **protein coding sequence** for the gene of interest. This DNA sequence will be transcribed into the mRNA that, in turn, will be translated into protein.
- **Stop codon** – a sequence (TAG, TAA, TGA) that signals the end of translation.
- An **intron** – a short sequence that is removed (or “spliced”) from the RNA after transcription. For many transcripts, splicing regulates nuclear export, translation, and mRNA stability.
- The **3' Untranslated Region (UTR)** – a sequence that continues after the stop codon and ends with a polyadenylation signal. This region features elements that modulate the stability and translation of the mRNA.

The transgene DNA is then mixed with a dominant genetic marker such as *rol-6*, which encodes a mutant collagen protein that induces the “roller” phenotype (animals travel in circles, rather than in a line). To introduce the transgene and its marker, scientists use a technique known as ‘microinjection’. Simply, the DNA is injected using a fine needle directly into a hermaphrodite’s gonad, where it is incorporated into the developing oocyte (Figure 4). After injection, researchers screen the progeny for the dominant marker to identify any worms that have successfully incorporated the transgene DNA. Microinjection is fast and efficient; many transgenic animals can be produced in less than two weeks.

One disadvantage of microinjection is that the transgene DNA is maintained as an extrachromosomal array and may be lost after successive rounds of cell division. As a result, the temporary nature of a transgene may result in variable gene expression between animals. To alleviate this problem, the extrachromosomal array can be integrated into the worm’s genome. To achieve this, scientists expose the worms gamma radiation to induce double-stranded DNA breaks throughout the chromosomal DNA. The worm’s DNA repair system will attempt to repair the breaks by ligating DNA back together and will occasionally incorporate the transgene DNA into its genome.
Figure 5: Polymerase Chain Reaction

Target Sequence

Cycle 1

Separation of two DNA strands

3' = Primer 1
5' = Primer 2

Denature 94°C

Anneal 2 primers 40°C - 65°C

Extension 72°C

Cycle 2

Cycle 3

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Another method used to generate transgenic worms is the biolistic (or “gene gun”) method using microbombardment. The transgene and marker DNAs are bound to tiny gold beads, which are then “shot” through the worm’s cuticle and into developing oocytes. With microbombardment, the transgene is integrated directly into the worm’s genome. However, this technique produces fewer transgenic animals than microinjection and requires extensive preparation and reagent cost.

**USING PCR TO IDENTIFY TRANSGENIC ORGANISMS**

In 1984, Dr. Kary Mullis revolutionized the field of molecular biology when he devised a simple and elegant method to copy specific pieces of DNA. Mullis discovered that he could replicate DNA in vitro using short, synthetic DNA primers and DNA Polymerase I. Furthermore, this method allowed for the rapid amplification of a selected DNA sequence in vitro because the primers are engineered to target a specific gene. For the development of this technique, known today as the Polymerase Chain Reaction (or PCR), Mullis was awarded the Nobel Prize in Chemistry in 1993.

To perform PCR, purified double-stranded DNA is mixed with primers (short synthetic DNA molecules that target DNA for amplification), a thermostable DNA polymerase (Taq) and nucleotides. The mixture is heated to 94° C to denature (i.e., unzip into single strands) the DNA duplex. Next, the sample is cooled to 45-60° C, allowing the primers to base pair with the target DNA sequence (a process called “annealing”). Lastly, the temperature is raised to 72° C, the optimal temperature at which Taq polymerase will extend the primer to synthesize a new strand of DNA. Each “PCR cycle” (denaturation, annealing, extension) doubles the amount of the target DNA in less than five minutes (Figure 5). In order to produce enough DNA for analysis, 20-40 cycles may be required. To simplify this process, a specialized machine, called a “thermal cycler” (or “PCR machine”), was created to rapidly heat and cool the samples to the designated temperature for each of the three steps.

Because of its ease of use and its ability to rapidly amplify DNA, PCR has become the method of choice to identify and analyze transgenic organisms. For example, commercially available foods are monitored for the presence of transgenic corn or soy; transgenic mice are often genotyped using PCR before complex genetic experiments are performed.

In this experiment, PCR is used to differentiate between (1) wild-type *C. elegans* and (2) *C. elegans* that have been genetically engineered to express the Green Fluorescent Protein (GFP) under the control of a muscle-specific promoter (Figure 6). GFP possesses the ability to absorb blue light and emit green light in response, without any special substrates or cofactors. Because it can function within living organisms, scientists regularly use GFP, together with fluorescent microscopy, to observe biological processes as they occur. You will first cultivate populations of both strains and observe each under a fluorescent microscope. Next, you will extract DNA from both populations. Finally, you will amplify two PCR products - a 670 bp DNA fragment derived from the GFP transgene and a 150 bp fragment from a common metabolic gene found in all *C. elegans*. Amplified DNA will be visualized using gel electrophoresis.

*Figure 6: GFP expression in *C. elegans*.*
**EXPERIMENT OBJECTIVE:**

This experiment explores molecular methods used by scientists to create and identify transgenic animals. Students will use fluorescent microscopy and PCR to analyze *C. elegans* that have been engineered to express the Green Fluorescent Protein.

**BEFORE YOU START THE EXPERIMENT**

1. Read all instructions before starting the experiment.
2. Culture *C. elegans* for several days to increase the number of animals for the experiment.
3. Write a hypothesis that reflects the experiment and predict experimental outcomes.

**LABORATORY NOTEBOOKS:**

Address and record the following in your laboratory notebook or on a separate worksheet.

**Before starting the Experiment:**
- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

**During the Experiment:**
- Record (draw) your observations, or photograph the results.

**After the Experiment:**
- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.
Laboratory Safety

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

1. Wear gloves and goggles while working in the laboratory.

2. Exercise caution when working in the laboratory – you will be using equipment that can be dangerous if used incorrectly.

3. Neither the E.coli nor the C. elegans used in this experiment are considered pathogenic. Regardless, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with worms.

A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.

B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with worms should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:

   • Autoclave at 121° C for 20 minutes.
     Tape several petri plates together and close tube caps before disposal.
     Collect all contaminated materials in an autoclavable, disposable bag.
     Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.

   • Soak in 10% bleach solution.
     Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.

C. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.
Module I: Preparation of *C. elegans* Food Source ("Seeding" the Plates)

1. **OBTAIN** two Nematode Growth Medium (NGM) plates, the OP50 culture, a transfer pipet, and a sterile inoculating loop from your instructor. **LABEL** the bottom of both plates with your group number or names.

2. While maintaining sterile technique, **ADD** two drops (100 μl) of OP50 culture to each plate.

3. Using the loop, **SPREAD** the culture over the entire surface of the NGM plates. **COVER.**

4. **INCUBATE** the plates inverted and at room temperature for 24 hours.

**NOTE:** Be careful to avoid gouging or scratching the agar surface as this can affect visibility as well as worm movement.
Module II: Cultivation of *C. elegans* ("Chunking" the Plates)

1. **LABEL** the bottom of the seeded NGM plates (from Module I) with “Wild-type” or “Transgenic”.

2. Your instructor will have NGM plates containing wild-type or transgenic *C. elegans*. Using a sterile loop, **CUT OUT** a small portion of the NGM plate containing the wild-type strain (~1 cm square). Make sure to completely cut the agar by pushing the loop all the way to the bottom plate.

3. **REMOVE** the “chunk” from the plate. **PLACE** the chunk, *worm side down*, in the center of the Module I Wild-type plate.

4. With a new sterile loop, **REPEAT** step 2 and 3 for the transgenic strain.

5. After 5-10 minutes, use a microscope to **CONFIRM** the presence of *C. elegans* on the “chunked” plates.

**NOTE:** Occasionally, a “chunk” is transferred nematode side up. Worms in this position will eventually migrate to the plate. If the "chunk" is incorrectly positioned, keep the plate but wait one hour before confirming the presence of *C. elegans* and continuing on to step 6.

6. **COVER** and **PLACE** the plates into a cardboard box. **INCUBATE** at room temperature for 3 days.

**OPTIONAL STOPPING POINT**

Plates may be stored for additional days but need extra OP50 to avoid drying and to feed the growing population. See Appendix A.

7. **CHECK** growth of *C. elegans* under a microscope. If the plate contains 50 or more worms, proceed with Module III. If the plate contains fewer than 50 worms, continue incubating at room temperature.

**STEP 7 HINT:**

To quickly confirm worm numbers, divide the plate into quarters. If you see 12 or more in the first quarter, the plate is ready.
Module III: Observation of GFP Expression

This module requires a fluorescent microscope to visualize GFP, which requires excitation between 450 – 490 nm. Please refer to your microscope instruction manual for specific instructions.

1. **PLACE** the plate of wild-type *C. elegans* on the microscope stage. **VISUALIZE** the worms using bright-field microscopy. We recommend using 20-40X magnification. **RECORD** your observations.
2. **TURN OFF** the white light source. **VISUALIZE** the wild-type worms using the fluorescent light source. **RECORD** your observations.
3. **REPEAT** steps 1 and 2 with the transgenic *C. elegans*. **RECORD** your observations.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bright Field Microscopy</th>
<th>Fluorescence Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type worms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transgenic worms</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1**: Observations of *C. elegans* using different microscopy techniques.
Module IV: Isolation of DNA from *C. elegans*

1. **LABEL** one 1.5 ml screw-cap tube with “WT” (wild-type) and a second screw-cap tube with “TG” (transgenic). **LABEL** two 15 ml tubes of S-buffer, two large transfer pipets, and two snap-top microcentrifuge tubes in the same way.

2. Using the large transfer pipet labeled “WT”, **TRANSFER** 2.5 ml of extraction buffer to the Petri dish containing the wild-type worms.

3. **DISLODGE** worms by rinsing the dish several times. Rinse the dish by either (a) swirling the plate or (b) holding the plate at a slight angle and allowing the buffer to collect near the bottom. Next, suck up the buffer using the transfer pipet and then expel the buffer near the top so that it runs down the plate.

4. Using the same transfer pipet, **TRANSFER** the worms to 15 ml conical tube labeled “WT”.

5. **REPEAT** steps 2-4 with the transgenic worms using the transfer pipet and tube labeled “TG”.

6. **ALLOW** the worms to settle to the bottom of the tubes (~10 minutes).

7. Using the appropriate transfer pipet, **REMOVE** ~2 ml of the cleared extraction buffer without disturbing the worms that have settled to the bottom of the tube. Between 300 and 500 μl of buffer with worms should remain at the bottom of the tube.

8. **RESUSPEND** the worms using the appropriate transfer pipet. **TRANSFER** 300 μl of the worm suspension to the labeled screw-cap tube.

9. **INCUBATE** the samples in a 99° C water bath for 5 minutes.

10. **CENTRIFUGE** the samples for one minute at low speed (~6000 rpm).

11. **TRANSFER** 100 μl of the supernatant to the clean, labeled snap-top microcentrifuge tube. **PROCEED** to Module V: Amplification of the GFP Transgene.

**OPTIONAL STOPPING POINT**
The supernatant may be stored at -20° C for amplification at a later time.

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**Warning!**

**STEP 9:** Students should use screw-cap tubes when boiling samples.
Module V: Amplification of the GFP Transgene

1. **LABEL** two 0.2 ml PCR tubes “WT”, and “TG”.

2. **ADD** one PCR EdvoBead™, 20 μL GFP primer mix, and 5 μL extracted DNA (either wild-type or transgenic from Module IV).

3. **MIX** the PCR samples gently. Make sure the PCR EdvoBead™ is completely dissolved.

4. **CENTRIFUGE** to collect the samples at the bottom of the tubes.

5. **AMPLIFY** DNA using PCR:

   **PCR Cycling Conditions**
   - Initial denaturation 94° C for 5 minutes
   - 94° C for 30 seconds
   - 55° C for 30 seconds
   - 72° C for 30 seconds
   - 35 cycles
   - Final Extension 72° C for 5 minutes

6. **ADD** 5 μL of 10x gel loading solution to each tube. **PROCEED** to Module VI.

**OPTIONAL STOPPING POINT**
The PCR samples may be stored at -20° C for electrophoresis at a later time.
Module VI: Separation of PCR Products by Electrophoresis

1. **DILUTE** concentrated (50X) buffer with distilled water to create 1X buffer (see Table A).
2. **MIX** agarose powder with 1X buffer in a 250 ml flask (see Table A).
3. **DISSOLVE** agarose powder by boiling the solution. **MICROWAVE** the solution on high for 1 minute. Carefully **REMOVE** the flask from the microwave and **MIX** by swirling the flask. Continue to **HEAT** the solution in 15-second bursts until the agarose is completely dissolved (the solution should be clear like water).
4. **COOL** agarose to 60° C with careful swirling to promote even dissipation of heat.
5. While agarose is cooling, **SEAL** the ends of the gel-casting tray with the rubber end caps. **PLACE** the well template (comb) in the appropriate notch.
6. **POUR** the cooled agarose solution into the prepared gel-casting tray. The gel should thoroughly solidify within 20 minutes. The gel will stiffen and become less transparent as it solidifies.
7. **REMOVE** end caps and comb. Take particular care when removing the comb to prevent damage to the wells.

### Table A

<table>
<thead>
<tr>
<th>Size of Gel</th>
<th>Concentrated Buffer (50X)</th>
<th>Distilled Water</th>
<th>Amount of Agarose</th>
<th>TOTAL Volume</th>
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<tbody>
<tr>
<td>7 x 14 cm</td>
<td>1.0 ml</td>
<td>49.0 ml</td>
<td>0.75 g</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

**NOTES:**
- 7 x 14 cm gels are recommended. Each gel can be shared by 2 groups. Place well-former template (comb) in the first set of notches.
- QuickGuide instructions and guidelines for casting various agarose gels can be found on our website. www.edvotek.com/quick-guides
- If you are unfamiliar with agarose gel prep and electrophoresis, detailed instructions and helpful resources are available at www.edvotek.com

**Wear gloves and safety goggles**
Module VI: Separation of PCR Products by Electrophoresis

8. **PLACE** gel (on the tray) into electrophoresis chamber. **COVER** the gel with 1X electrophoresis buffer (See Table B for recommended volumes). The gel should be completely submerged.

9. **LOAD** the entire sample (30 μl) into the well. **RECORD** the position of the samples in Table 2, at right. We recommend running a ladder and one control sample per gel to confirm that PCR and staining were successful.

10. **PLACE** safety cover. **CHECK** that the gel is properly oriented. Remember, the DNA samples will migrate toward the positive (red) electrode.

11. **CONNECT** leads to the power source and **PERFORM** electrophoresis (See Table C for time and voltage guidelines).

12. After electrophoresis is complete, **REMOVE** the gel and casting tray from the electrophoresis chamber and proceed to **STAINING** the agarose gel.

### Table B: 1x Electrophoresis Buffer (Chamber Buffer)

<table>
<thead>
<tr>
<th>EDVOTEK Model #</th>
<th>Total Volume Required</th>
<th>Dilution</th>
<th>5X Conc. Buffer</th>
<th>Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>M6+</td>
<td>300 ml</td>
<td>6 ml</td>
<td>294 ml</td>
<td></td>
</tr>
<tr>
<td>M12</td>
<td>400 ml</td>
<td>8 ml</td>
<td>392 ml</td>
<td></td>
</tr>
<tr>
<td>M36</td>
<td>1000 ml</td>
<td>20 ml</td>
<td>980 ml</td>
<td></td>
</tr>
</tbody>
</table>

### Table C: Time and Voltage Guidelines (1.5% - 7 x 14 cm Agarose Gel)

<table>
<thead>
<tr>
<th>Volts</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>45 min.</td>
<td>60 min.</td>
</tr>
<tr>
<td>125</td>
<td>55 min.</td>
<td>1 hour 15 min.</td>
</tr>
<tr>
<td>70</td>
<td>2 hours 15 min.</td>
<td>3 hours</td>
</tr>
</tbody>
</table>

Table 2: Student Sample Table

### Reminder:

Before loading the samples, make sure the gel is properly oriented in the apparatus chamber.

Includes EDVOTEK’s All-NEW EdvoQuick™ DNA Ladder
- Better separation
- Easier band measurements
- No unused bands

EdvoQuick™ DNA ladder sizes:
- 1728, 1400, 1190, 990, 700, 600, 400, 200

Wear gloves and safety goggles
Module VII-A: Staining with InstaStain® Ethidium Bromide

1. Carefully REMOVE the agarose gel and casting tray from the electrophoresis chamber. SLIDE the gel off of the casting tray on to a piece of plastic wrap on a flat surface. DO NOT STAIN GELS IN THE ELECTROPHORESIS APPARATUS.

2. MOISTEN the gel with a few drops of electrophoresis buffer.

3. Wearing gloves, REMOVE and DISCARD the clear plastic protective sheet from the unprinted side of the InstaStain® card(s). PLACE the unprinted side of the InstaStain® Ethidium Bromide card(s) on the gel. You will need 2 cards to stain a 7 x 14 cm gel.

4. With a gloved hand, REMOVE air bubbles between the card and the gel by firmly running your fingers over the entire surface. Otherwise, those regions will not stain.

5. PLACE the casting tray on top of the gel/card stack. PLACE a small weight (i.e. an empty glass beaker) on top of the casting tray. This ensures that the InstaStain® Ethidium Bromide card is in direct contact with the gel surface. STAIN the gel for 8-10 minutes.

6. REMOVE the InstaStain® Ethidium Bromide card(s). VISUALIZE the gel using a long wavelength ultraviolet transilluminator (300 nm). DNA should appear as bright orange bands on a dark background.

BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!
Module VII-B: Staining with FlashBlue™

1. **DILUTE** 10 ml of 10x concentrated FlashBlue™ with 90 ml of water in a flask and **MIX** well.
2. **REMOVE** the agarose gel and casting tray from the electrophoresis chamber. **SLIDE** the gel off of the casting tray into a small, clean gel-staining tray.
3. **COVER** the gel with the 1x FlashBlue™ stain solution. **STAIN** the gel for 5 minutes. For best results, use an orbital shaker to gently agitate the gel while staining. **STAINING THE GEL FOR LONGER THAN 5 MINUTES WILL REQUIRE EXTRA DESTAINING TIME.**
4. **TRANSFER** the gel to a second small tray. **COVER** the gel with water. **DESTAIN** for at least 20 minutes with gentle shaking (longer periods will yield better results). Frequent changes of the water will accelerate destaining.
5. Carefully **REMOVE** the gel from the destaining liquid. **VISUALIZE** results using a white light visualization system. DNA will appear as dark blue bands on a light blue background.

**Alternate Protocol:**

1. **DILUTE** one ml of concentrated FlashBlue™ stain with 149 ml dH₂O.
2. **COVER** the gel with diluted FlashBlue™ stain.
3. **SOAK** the gel in the staining liquid for at least three hours. For best results, stain gels overnight.
Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. What is a model organism?

2. What are some advantages to using *C. elegans* as a model system?

3. List the important elements of a transgene. In your own words, explain the purpose of each of these elements.

4. You are growing two plates of *C. elegans* in a box on your bench. One plate contains wild-type worms and the other has a transgene that expresses the Red Fluorescent Protein (RFP). Unfortunately, the labels fell off of the plates! Describe the experiment(s) you could perform to distinguish between the worms on the two plates.
# Instructor's Guide

## OVERVIEW OF INSTRUCTOR’S PRELAB PREPARATION:

<table>
<thead>
<tr>
<th>Preparation For:</th>
<th>What to do:</th>
<th>When:</th>
<th>Time Required:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E-Z elegans™ Preparation</strong></td>
<td>Prepare NGM Agar Plates</td>
<td>7 days before Module I</td>
<td>45 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare OP50 culture</td>
<td>6 days before Module I</td>
<td>15 min., (plus 20+ min. incubation)</td>
</tr>
<tr>
<td></td>
<td>Reserve microscope with 20-40X magnification</td>
<td>Before E-Z elegans™ rehydration</td>
<td>Varies</td>
</tr>
<tr>
<td></td>
<td>Rehydrate E-Z elegans™ and Seeding NGM Plates</td>
<td>6 days before Module I</td>
<td>30 min., (plus 24-48 hour incubation)</td>
</tr>
<tr>
<td></td>
<td>Monitor E-Z elegans™ growth</td>
<td>5 days before Module I</td>
<td>15 min., (plus 3-5 day incubation)</td>
</tr>
<tr>
<td><strong>Module I: Preparation of C. elegans Food Source</strong></td>
<td>Prepare classroom OP50 culture</td>
<td>Up to a week before the class period of Module I</td>
<td>15 min.</td>
</tr>
<tr>
<td></td>
<td>Distribute plastics and reagents</td>
<td>Up to a week before the class period of Module I</td>
<td>10 min.</td>
</tr>
<tr>
<td><strong>Module II: Cultivation of C. elegans</strong></td>
<td>Procure &amp; set up microscopes</td>
<td>Varies</td>
<td>Varies</td>
</tr>
<tr>
<td></td>
<td>Distribute plastics and reagents</td>
<td>Before the class period of Module II</td>
<td>10 min.</td>
</tr>
<tr>
<td><strong>Module III: Observation of GFP Expression</strong></td>
<td>Prepare bright light and fluorescent microscopes</td>
<td>One hour before performing the experiment.</td>
<td>10 min.</td>
</tr>
<tr>
<td><strong>Module IV: Isolation of DNA from C. elegans</strong></td>
<td>Prepare and aliquot extraction buffer</td>
<td>Up to one day before performing the experiment.</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>Equilibrate waterbath at 99 °C</td>
<td>One hour before performing the experiment.</td>
<td>15 min.</td>
</tr>
<tr>
<td><strong>Module V: Amplification of the GFP Transgene</strong></td>
<td>Prepare and aliquot various reagents (Primer, DNA template, ladder, etc.)</td>
<td>One day to 30 min. before performing the experiment.</td>
<td>30 min.</td>
</tr>
<tr>
<td></td>
<td>Program Thermal Cycler</td>
<td>One hour before performing the experiment.</td>
<td>15 min.</td>
</tr>
<tr>
<td><strong>Module VI: Separation of PCR Product by Electrophoresis</strong></td>
<td>Prepare diluted TAE buffer</td>
<td>Up to one hour before performing the experiment.</td>
<td>45 min.</td>
</tr>
<tr>
<td></td>
<td>Prepare molten agarose and pour gel</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Module VII: Staining Agarose Gels</strong></td>
<td>Prepare staining components</td>
<td>The class period or overnight after the class period.</td>
<td>10 min.</td>
</tr>
</tbody>
</table>
Pre-Lab Preparations

POURING NGM PLATES

*NOTE:* One bottle of ReadyPour™ NGM Agar should make 25 plates.

1. **BREAK** solid ReadyPour™ medium into small chunks by vigorously squeezing and shaking the plastic bottle.
2. **LOOSEN**, but DO NOT REMOVE, the cap on the ReadyPour™ medium bottle. This allows the steam to vent during heating.
   
   **CAUTION:** Failure to loosen the cap prior to heating may cause the bottle to break or explode.

3. **MICROWAVE** the ReadyPour™ medium on high for 60 seconds to melt the agar. Carefully **REMOVE** the bottle from the microwave and **MIX** by swirling the bottle. Continue to **HEAT** the solution in 30-second intervals until the agar is completely dissolved (the solution should be free of small particles).
4. **COOL** the ReadyPour™ to 60°C with careful swirling to promote even dissipation of heat.
5. **ADD** 12 ml of NGM Medium Salts. **RECAP** the bottle and **SWIRL** to mix the reagents.
   
   **ONLY ADD REAGENTS TO COOLED MEDIUM.**

6. Using a fresh 10 ml pipet, **POUR** 8 ml of the medium into 24 small petri plates. **COVER**.
7. **WAIT** at least twenty minutes for the agar to solidify. For optimal results, leave plates at room temperature overnight.
8. **KEEP** four plates at room temperature, to rehydrate the E-Z elegans™, and **STORE** the remaining plates in the refrigerator (4°C). To prevent drying, keep plates inverted and in a sealable plastic bag.
Pre-Lab Preparations

PREPARING REHYDRATION BUFFER AND OP50 CULTURE FOR E-Z ELEGANS™

1. **INCUBATE** Rehydration Buffer (Component E) overnight at room temperature. This solution should be between 15-25° C when adding to *C. elegans* vials.
2. **TRANSFER** 1 ml of Sterile Water (Component F) to a 1.5 ml snap-top tube. **LABEL** it as “OP50”.
3. **REMOVE** one OP50 BactoBead™ from the vial using a sterile loop and **ADD** it to the OP50 tube.
4. **MIX** and **INCUBATE** tube at room temperature for 20 minutes.

SEEDING NGM PLATES

1. **ADD** 100 μl of OP50 culture to four NGM plates using a pipet tip or transfer pipet.
2. Using a sterile loop, **SPREAD** the liquid over the entire surface of each plate.
3. **INCUBATE** the plates at room temperature for 24-48 hours.
4. **SAVE** the remaining OP50 culture in the fridge. This additional OP50 can be used if *C. elegans* petri plates need to be stored for additional days.

REHYDRATION OF E-Z ELEGANS™

*E-Z elegans™* takes advantage of *C. elegans*’ ability to arrest development and go into an alternative larval state when rapidly dehydrated. These dauer larvae are stable in the refrigerator for up to two weeks. To prepare the *E-Z elegans™*, you will gently rehydrate and then incubate for one week. Due to the stress of drying and rehydrating, we expect a mixture of live and dead nematodes for several days, but this extended incubation time allows the dauers to return to normal development and lay eggs. The student experiments will be carried out on this second generation of worms.

**NOTE:** The *C. elegans* are fragile during rehydration and resuspension. Be very gentle during steps 2 through 6 particularly when pipetting.

1. This kit contains four vials of *C. elegans*, two wild-type and two transgenic strain. Before rehydrating the *E-Z elegans™*, **LABEL** the bottom of the four seeded NGM plates with either “wild-type” (2 plates) or “transgenic” (2 plates).
2. Carefully open the vials of *E-Z elegans™* and **ADD** 250 μl of Rehydration Buffer (Component E) to each tube.
3. **INCUBATE** the *E-Z elegans™* for 1 hour at room temperature.
4. **RESUSPEND** the wild-type nematodes by gently pipetting the solution up and down 3-4 times in both vials using a large sterile transfer pipet.
5. **REPEAT** this process for the transgenic strain nematodes using a new large sterile transfer pipet.
6. **TRANSFER** the solution in each vial of rehydrated nematodes to an appropriately labeled NGM plates. Each vial should contain ~250 μl of solution which is enough to inoculate a single plate.
7. **OBSERVE** the 4 plates under a microscope. You should see many nematodes spread evenly around the plate – it is not necessary to see any movement at this point!
8. **COVER** and **INCUBATE** the plates at room temperature for 3 days.

**NOTE:** Not all worms will survive the rehydration process. However, you should observe movement from many living *C. elegans* within 3-6 days of recovery in at least one wild-type plate and at least one transgenic strain plate. 

continued
Pre-Lab Preparations

REHYDRATION OF E-Z ELEGANS™, CONTINUED

8. OBSERVE the worms under a microscope after 72 hours for mobility. Often the first signs of mobility will be worm trails (Figure 7). Refer to Appendix A “Troubleshooting” if you do not observe a moving worm or a trail.

9. Continue to INCUBATE the worms at room temperature for an additional 3 days. This will allow a second (and non dauer) generation of worms to grow.

10. OBSERVE the worms under a microscope daily. Plates are ready when they contain approximately 50 live worms and will be used in Module II.

MICROSCOPE REQUIREMENTS

The fluorescence generated by the transgenic *C. elegans* in this experiment can only be visualized under a specialized fluorescent stereo microscope or a dissecting microscope equipped with fluorescent optics. We have found that the following range of conditions are excellent for GFP visualization:

- Excitation Laser (Argon): 488 nm
- Excitation Filter (CWL/BW): 470 nm / 30-50 nm
- Dichromatic Mirror Cut-On: 480-495LP nm
- Barrier Filter (CWL/BW): 510-520 nm / 30 – 50 nm
- Relative Brightness: 48-125% of EGFP

The rehydration of E-Z elegans™, Module I, and Module II can be carried out with standard bright field microscopes. All microscopes should have magnifications between 20-40x.

PREPARATIONS FOR MODULE I

**NOTE:** The OP50 culture can be prepared ahead of time and stored in the refrigerator for several days.

1. REMOVE NGM plates from the refrigerator and let sit for at least 30 minutes at room temperature.
2. PREPARE a classroom OP50 culture by combining 3 ml of Sterile Water (Component F) with three OP50 BactoBeads™ in a 15 ml conical tube.
3. MIX and INCUBATE at room temperature for 20 minutes.
4. LABEL ten snap top tubes “OP50 culture” and aliquot 250 μl of the classroom OP50 culture to each group.
5. DISTRIBUTE an OP50 tube, two NGM plates, a sterile loops, and a small transfer pipet or fixed volume pipet to each group.
Pre-Lab Preparations

PREPARATIONS FOR MODULE II

1. **PROCURE** and set up microscopes. We suggest top illumination or bright field microscopes with 20-40x magnification for this module.
2. **CONFIRM** that at least one wild-type and one transgenic strain E-Z elegans™ plate has approximately 50 moving worms.
3. **PREPARE** incubation oven or box(es) for student’s plates.
4. **DISTRIBUTE** two sterile loops to each group.

PREPARATIONS FOR MODULE III & IV

1. Set up fluorescent microscope. Please note, a fluorescent microscope is required to visualize the GFP signal.
2. Aliquot 2.5 ml of Extraction Buffer into twenty 15 ml conical tubes. These tubes will also be used to hold collected *C. elegans* so remind students not to dispose of them once the Extraction Buffer is used.
3. Distribute to each group 2 Extraction Buffer tubes, 2 large sterile pipets, 2 small pipets, 2 snap-top tubes, and 2 screw-top tubes.

PREPARATIONS FOR MODULE V

**Preparation of the GFP Primer Solution**

1. Thaw the GFP Primer Mix Concentrate (Component B) on ice.
2. Add 1 ml of TE buffer to the tube of Primer Mix Concentrate. Cap tube and mix.
3. Aliquot 50 μl of the diluted Primer Mix into 10 labeled microcentrifuge tubes.
4. Save the remaining diluted Primer Mix for control reactions and keep all tubes on ice or in the fridge until Module V.

**Preparation of the Control DNA**

1. Thaw the tube of Control DNA Concentrate (Component D) on ice.
2. Add 20 μl of TE buffer to the tube containing the Control DNA Concentrate. Pipet up and down to mix.
3. Keep tube on ice or in the fridge.

**PCR Amplification**

The thermal cycler should be programmed for the following cycles.

- Initial Denaturation: 94° C for 5 min.
- 35 cycles: 94° C for 30 seconds, 55° C for 30 seconds, 72° C for 30 seconds
- Final Extension: 72° C for 5 min.

At the completion of the cycling, the thermal cycler can be programmed to hold the samples at 4° C overnight.
Pre-Lab Preparations

Preparation of Control Reactions

Prepare up to five control reactions during or immediately before Module V.

1. Label five PCR tubes “C”.
2. Add one PCR EdvoBead™ (Component A), 20 μl GFP Primer Mix, and 5 μl Control DNA.
3. Mix until the PCR Edvobead™ is completely dissolved.
4. Centrifuge to collect the samples at the bottom of the tubes.
5. Keep these tubes on ice until student samples are ready.
6. Amplify all PCR reactions (controls and student samples) during the same thermacycler run.
7. Add 5 μl of 10x Gel Loading Solution to each control tube.

During Module VI, two groups will share a control reaction. (These two groups will also be sharing a ladder and a gel.)

PREPARATIONS FOR MODULE VI

Preparation of Agarose Gels

This experiment requires one 1.5% agarose gel per two student groups. For best results, we recommend using a 7 x 14 cm gel. You can choose whether to prepare the gels in advance or have the students prepare their own. Allow approximately 30-40 minutes for this procedure.

Individual Gel Preparation:
Student groups can be responsible for casting their own individual gel prior to conducting the experiment. See Module VI in the Student’s Experimental Procedure. Students will need 50x concentrated buffer, distilled water and agarose powder.

Batch Gel Preparation:
To save time, a larger quantity of agarose solution can be prepared for sharing by the class. See Appendix D.

Preparing Gels in Advance:

Gels may be prepared ahead and stored for later use. Solidified gels can be store under buffer in the refrigerator for up to 2 weeks.

Do not freeze gels at -20º C as freezing will destroy the gels.

Gels that have been removed from their trays for storage should be “anchored” back to the tray with a few drops of molten agarose before being placed into the tray. This will prevent the gels from sliding around in the trays and the chambers.

Additional Materials:
Each 1.5% gel should be loaded with the EdvoQuick™ DNA ladder and PCR reactions from one or two student groups.

- Aliquot 30 μl of the EdvoQuick™ DNA ladder (Component C) into labeled microcentrifuge tubes and distribute one tube of EdvoQuick™ DNA ladder per gel.
Pre-Lab Preparations

PREPARATIONS FOR MODULE VII

**InstaStain® Ethidium Bromide (PREFERRED METHOD)**

InstaStain® Ethidium Bromide provides the sensitivity of ethidium bromide while minimizing the volume of liquid waste generated by staining and destaining a gel. An agarose gel stained with InstaStain® Ethidium Bromide is ready for visualization in as little as 3 minutes! Each InstaStain® card will stain 49 cm² of gel (7 x 7 cm). You will need 2 cards to stain a 7 x 14 cm gel.

Use a mid-range ultraviolet transilluminator (Cat. #558) to visualize gels stained with InstaStain® Ethidium Bromide. **BE SURE TO WEAR UV-PROTECTIVE EYEWEAR!**

- Standard DNA markers should be visible after staining even if other DNA samples are faint or absent. If bands appear faint, repeat staining with a fresh InstaStain card for an additional 3-5 min. If markers are not visible, troubleshoot for problems with electrophoretic separation.
- Ethidium bromide is a listed mutagen. Wear gloves and protective eyewear when using this product. UV protective eyewear is required for visualization with a UV transilluminator.
- InstaStain® Ethidium Bromide cards and stained gels should be discarded using institutional guidelines for solid chemical waste.

**FlashBlue™**

FlashBlue™ can be used as an alternative to Ethidium Bromide in this experiment. However, FlashBlue™ is less sensitive than InstaStain® Ethidium Bromide and will take a longer time to obtain results.

FlashBlue™ stain, however, is optimized to shorten the time required for both staining and destaining steps. Agarose gels can be stained with diluted FlashBlue™ for 5 minutes and destained for only 20 minutes. For the best results, leave the gel in liquid overnight. This will allow the stained gel to “equilibrate” in the destaining solution, resulting in dark blue DNA bands contrasting against a uniformly light blue background. A white light box (Cat. #552) is recommended for visualizing gels stained with FlashBlue™.

- Stained gels may be stored in destaining liquid for several weeks with refrigeration, although the bands may fade with time. If this happens, re-stain the gel.
- Destained gels can be discarded in solid waste disposal. Destaining solutions can be disposed of down the drain.

**Photodocumentation of DNA (Optional)**

Once gels are stained, you may wish to photograph your results. There are many different photodocumentation systems available, including digital systems that are interfaced directly with computers. Specific instructions will vary depending upon the type of photodocumentation system you are using.
Expected Results

![DNA gel image with bands at 2640 bp, 1400 bp, 1100 bp, 700 bp, 600 bp, 400 bp, 200 bp, 670 bp, and 150 bp.]

<table>
<thead>
<tr>
<th>Lane</th>
<th>Sample</th>
<th>Result</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EdvoQuick™ DNA Ladder</td>
<td>670 bp</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Wild-type <em>C. elegans</em></td>
<td>Control Gene</td>
<td>150</td>
</tr>
<tr>
<td>3</td>
<td>Transgenic <em>C. elegans</em></td>
<td>Control Gene, GFP</td>
<td>150, 670</td>
</tr>
</tbody>
</table>

**NOTE:**

For reference, the control DNA provided with this kit will produce the same results as the DNA extracted from the transgenic *C. elegans*.
Please refer to the kit insert for the Answers to Study Questions
Appendices

A EDVOTEK® Troubleshooting Guide
B Preparation and Handling of PCR Samples With Wax
C Performing the PCR Experiment Using Three Waterbaths
D Bulk Preparation of Agarose Gels

Safety Data Sheets can be found on our website: www.edvotek.com/safety-data-sheets
### Appendix A
**EDVOTEK® Troubleshooting Guides**

#### C. elegans CARE AND CULTIVATION

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Z <em>elegans™</em> not visible in vials.</td>
<td><em>E-Z elegans™</em> are small and translucent so difficult to see.</td>
<td>A faint ring may be seen around the circumference of the vial. Even if no ring is seen continue with rehydration steps.</td>
</tr>
<tr>
<td>Nematodes on E-Z <em>elegans™</em> plates do not move when observed through a microscope.</td>
<td>Nematodes are still dormant.</td>
<td>The majority of E-Z <em>elegans™</em> are shipped in a dauer phase and will take several days to recover. Keep plate covered to prevent drying. If there are no signs of movement after 3 days of rehydration contact Edvotek customer service for assistance.</td>
</tr>
<tr>
<td>Nematodes are dead.</td>
<td></td>
<td>While many nematodes will not survive the rehydration process even a few nematodes can quickly repopulate a plate. If there are any moving nematodes or any nematode trails allow the plate to incubate for additional days. If there are no signs of movement check the alternative E-Z <em>elegans™</em> plate or contact Edvotek customer service for assistance.</td>
</tr>
<tr>
<td>While transferring the nematodes to a new plate the agar was flipped in the wrong orientation.</td>
<td>It is difficult to transfer the small piece of agar.</td>
<td>Nematodes will leave the chunk and move towards their food regardless of orientation, but allow extra time for migration.</td>
</tr>
<tr>
<td>No nematodes are visible on chunked plates when observed through a microscope.</td>
<td>Nematodes haven’t migrated from the chunk onto plate.</td>
<td>Wait for an additional 5-10 minutes and observe under the microscope again.</td>
</tr>
<tr>
<td>Chunk did not contain enough live nematodes.</td>
<td>Make new chunk of agar from the source plate and repeat the transfer process.</td>
<td></td>
</tr>
<tr>
<td>Nematodes on chunked plates do not move when observed through a microscope.</td>
<td>Nematodes have become dormant.</td>
<td>Add 200 µl of additional OP50 solution to plate.</td>
</tr>
<tr>
<td>Nematodes are dead.</td>
<td>Examine initial E-Z <em>elegans™</em> plates or a classmate’s plates for live nematodes and re-chunk.</td>
<td></td>
</tr>
<tr>
<td>Nematode population is growing slowly.</td>
<td>In hospitable conditions are slowing nematode development.</td>
<td>This is most likely a food supply issue. Add 200 µl of additional OP50 culture. Also check that classroom temperatures are between 20°C and 25°C.</td>
</tr>
<tr>
<td>OP50 growth is not visible on a plate after incubation.</td>
<td>An OP50 lawn on agar can be hard to see.</td>
<td>Examine under the plate under the microscope OR use a sterile loop to gently scrape the top of the agar for growth OR smell plate for bacterial odor. If no growth is still observed re-seed the plate with new OP50 liquid culture.</td>
</tr>
<tr>
<td>The plates containing the nematodes show a white/pink growth, white threads, or brown/green/yellow spots.</td>
<td>The plates are contaminated with bacteria, yeast, or mold.</td>
<td>Most contamination will not harm the nematodes but can make observation more difficult. If necessary, prepare a new NGM plate and transfer a chunk from a clean region of the contaminated plate.</td>
</tr>
</tbody>
</table>
## Appendix A

### EDVOTEK® Troubleshooting Guides

#### C. elegans CARE AND CULTIVATION

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGM agar is too thin and does not cover the full plate.</td>
<td>Incorrect volume was poured into the plates.</td>
<td>It is important to have the correct thickness of agar in the plates. Be sure to pour the amount recommended by the protocol.</td>
</tr>
<tr>
<td></td>
<td>The plates are dry.</td>
<td>Keep plates sealed and inside of a box or bag. If they are in a high temperature environment cover the edges with parafilm or saran wrap. Dry plates can be partially rehydrated with 200 µl of OP50 solution.</td>
</tr>
<tr>
<td>More OP50 liquid culture is needed.</td>
<td>Experiment involves extra steps that require OP50.</td>
<td>Large (&gt;10ml) OP50 solution can be created from a single BactoBead™ or from a small amount of old solution. Incubate the new solution at room temperature overnight, ideally on a shaking incubator. Alternatively, order new OP50 Bactobeads.</td>
</tr>
<tr>
<td>Need to delay the experiment after rehydrating E-Z elegans™ or after chunking student plates.</td>
<td>Life happens.</td>
<td>Nematode populations can be maintained for a week or more. Feed these populations with 200 µl of OP50 liquid culture every 3-4 days. If a longer delay is expected prepare additional NGM plates and chunk the nematodes onto a new plate every two weeks. Alternatively incubate plates at 16°C for up to a month with weekly feeding.</td>
</tr>
</tbody>
</table>
## Appendix A

**EDVOTEK® Troubleshooting Guides**

### DNA EXTRACTION

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor DNA Extraction</td>
<td>Samples not mixed well enough during extraction</td>
<td>In addition to flicking the tube, vortex or pipet up and down to mix the sample.</td>
</tr>
<tr>
<td></td>
<td>Water bath not at proper temperature</td>
<td>Use a thermometer to confirm water bath set point.</td>
</tr>
<tr>
<td></td>
<td>Not enough DNA</td>
<td>Repeat extraction. Try using more worms.</td>
</tr>
<tr>
<td>The extracted DNA is very cloudy.</td>
<td>Cellular debris from pellet transferred to tube</td>
<td>Centrifuge sample again and move supernatant to a fresh tube. Take care to avoid pellet.</td>
</tr>
<tr>
<td></td>
<td>Cellular debris not separated from supernatant</td>
<td>Centrifuge sample again. If possible, centrifuge at a higher speed. Move cleared supernatant to a fresh tube.</td>
</tr>
</tbody>
</table>
### Appendix A
EDVOTEK® Troubleshooting Guides

#### PCR AND ELECTROPHORESIS

<table>
<thead>
<tr>
<th>PROBLEM:</th>
<th>CAUSE:</th>
<th>ANSWER:</th>
</tr>
</thead>
<tbody>
<tr>
<td>There is very little liquid left in tube after PCR</td>
<td>Sample has evaporated</td>
<td>Make sure the heated lid reaches the appropriate temperature.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If your thermal cycler does not have a heated lid, overlay the PCR reaction with wax. (See Appendix B for details.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Make sure students close the lid of the PCR tube properly.</td>
</tr>
<tr>
<td></td>
<td>Pipetting error</td>
<td>Make sure students pipet 20 µL primer mix and 5 µL extracted DNA into the 0.2 mL tube.</td>
</tr>
<tr>
<td>The ladder, control DNA, and C. elegans PCR products are not visible on the gel.</td>
<td>The gel was not prepared properly.</td>
<td>Ensure that the electrophoresis buffer was correctly diluted.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gels of higher concentration (&gt; 0.8%) require special attention when melting the agarose. Make sure that the solution is completely clear of “clumps” and glassy granules before pouring gels.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The proper buffer was not used for gel preparation. Make sure to use 1x Electrophoresis Buffer.</td>
</tr>
<tr>
<td>After staining the gel, the DNA bands are faint.</td>
<td>The gel was not stained for a sufficient period of time.</td>
<td>Repeat staining protocol.</td>
</tr>
<tr>
<td>After staining, the ladder and control PCR products are visible on the gel but some C. elegans samples are not present.</td>
<td>C. elegans DNA sample was not concentrated enough.</td>
<td>Poor DNA extraction. Repeat Module IV (Isolation of C. elegans DNA).</td>
</tr>
<tr>
<td></td>
<td>C. elegans DNA sample was degraded.</td>
<td>If DNA is not used right after extraction, store sample at -20°C.</td>
</tr>
<tr>
<td></td>
<td>Wrong volumes of DNA and primer added to PCR reaction.</td>
<td>Practice using pipettes.</td>
</tr>
<tr>
<td>Some C. elegans samples have more/less amplification than others.</td>
<td>Concentration of DNA varies by sample.</td>
<td>There is an inherent variability in the extraction process.</td>
</tr>
<tr>
<td>Low molecular weight band in PCR samples</td>
<td>Primer dimer</td>
<td>Low concentration of extracted DNA in PCR reaction.</td>
</tr>
<tr>
<td>DNA bands were not resolved.</td>
<td>Tracking dye should migrate at least 6 cm from the wells to ensure adequate separation.</td>
<td>Be sure to run the gel at least 6 cm before staining and visualizing the DNA (approximately one hour at 125 V).</td>
</tr>
<tr>
<td>DNA bands fade when gels are kept at 4°C.</td>
<td>DNA stained with FlashBlue™ may fade with time.</td>
<td>Re-stain the gel with FlashBlue™.</td>
</tr>
</tbody>
</table>
Appendix B
Preparation and Handling of PCR Samples with Wax

Preparation and Handling of PCR Samples With Wax

ONLY For Thermal Cyclers WITHOUT Heated Lids, or Manual PCR Using Three Waterbaths

Using a wax overlay on reaction components prevents evaporation during the PCR process.

HOW TO PREPARE A WAX OVERLAY

1. Add PCR components to the 0.2 ml PCR Tube as outlined in Module V.
2. Centrifuge at full speed for five seconds to collect sample at bottom of the tube.
3. Using clean forceps, add one wax bead to the PCR tube.
4. Place samples in PCR machine and proceed with Module V.

PREPARING PCR SAMPLES FOR ELECTROPHORESIS

1. After PCR is completed, melt the wax overlay by heating the sample at 94° C for three minutes or until the wax melts.
2. Using a clean pipet, remove as much overlay wax as possible.
3. Allow the remaining wax to solidify.
4. Use a pipet tip to puncture the thin layer of remaining wax. Using a fresh pipet tip, remove the PCR product and transfer to a new tube.
5. Add 5 μl of 10x Gel Loading Buffer to the sample. Proceed to Module VI to perform electrophoresis.
Appendix C
Performing the PCR Experiment Using Three Water Baths

This experiment can be modified to use three water baths in place of a thermal cycler. In this method, the PCR samples are cycled through three water baths, each maintained at a different temperature, for a specified period. The sequential placement of the reaction sample through the three water baths constitutes one PCR cycle. Please note that results obtained when using three water baths are often variable. A thermal cycler assures a significantly higher rate of success.

We recommend EDVOTEK’s Digital PCR Waterbath (Cat. #544) if you do not have access to a thermal cycler.

Set up the samples as outlined in Module V, page 16. Before cycling the samples, be sure to:

• Allow at least 15 minutes for the water baths to reach the temperatures specified in Module I (94° C, 55° C, 72° C).
• Cover the water baths when not in use to maintain the proper temperature and to prevent water evaporation.
• The volume of the PCR sample is small and can evaporate easily. To prevent this, transfer one wax bead to each PCR sample. The melted wax bead forms a barrier over the PCR sample to prevent its evaporation during heating.
• Make sure that the sample remains undisturbed at the bottom of the tube. If necessary, centrifuge or shake the tube to get the sample to the bottom of the tube.
• Place the PCR samples in a water bath float before placing them in the water bath.

Continue with Step 5 (PCR cycling):

Initial denaturation 94° C for 5 minutes
94° C for 30 seconds
55° C for 30 seconds } 35 cycles
72° C for 30 seconds
Final Extension 72° C for 5 minutes

Handle the samples carefully when shifting between water baths. Use forceps to carefully raise/lower the float into the water baths.

Remove samples at time points specified by Module V. Take care to avoid the liquid wax layer when removing the sample. We recommend placing the tube on ice for a few seconds to solidify the wax. Use a clean pipet tip to gently break through the wax layer, making enough room to fit a clean pipet tip. Using a fresh, clean pipet tip, remove the PCR product and transfer to the appropriate tube.
Appendix D

Bulk Preparation of Agarose Gels

To save time, the electrophoresis buffer and agarose gel solution can be prepared in larger quantities for sharing by the class. Unused diluted buffer can be used at a later time and solidified agarose gel solution can be remelted.

Bulk Electrophoresis Buffer

Quantity (bulk) preparation for 3 liters of 1x electrophoresis buffer is outlined in Table D.

<table>
<thead>
<tr>
<th>50x Conc. Buffer</th>
<th>Distilled Water</th>
<th>Total Volume Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 ml</td>
<td>2,940 ml</td>
<td>3000 ml (3 L)</td>
</tr>
</tbody>
</table>

Batch Agarose Gels (1.5%)

For quantity (batch) preparation of 1.5% agarose gels, reference Table E.

1. Use a 500 ml flask to prepare the 1X Diluted gel buffer.
2. Pour the measured UltraSpec-Agarose™ into the prepared buffer. Refer to Table E for the mass. Swirl to disperse clumps.
3. With a marking pen, indicate the level of solution volume on the outside of the flask.
4. Heat the agarose solution as outlined previously for individual gel preparation. The heating time will require adjustment due to the larger total volume of gel buffer solution.
5. Cool the agarose solution to 60°C with swirling to promote even dissipation of heat. If evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask in step 3.
6. Dispense the required volume of cooled agarose solution for casting each gel. Measure 25 ml for each 7 x 7 cm gel, 50 ml for each 7 x 14 cm gel. For this experiment, 7 x 14 cm gels are recommended.
7. Allow the gel to completely solidify. It will become firm and cool to the touch after approximately 20 minutes. Then proceed with preparing the gel for electrophoresis.