The DNA Biotech Kit Manual

let's talk science

Version 8: 2011

Table of Contents

Symbols and Abbreviations used in the manual

New stuff or new explanations or seriously updated

SH Senior High school level – Grades 10-12. Usually Grade 12.

JH Junior High school level – Grades 6-9. Depth of content should vary from Gr 6 to Gr 9.

★ Disposables/Replacables: includes plastics, reagents and samples. Stock may be in the kit or will be available from LTS National.

♻ Materials or supplies are meant to be recycled or reused, thus are kept with the kit.

🛠 Something to make or reconstitute.

🌡 Temperature sensitive materials

❍ Use is optional. Items are included, but may not be needed based on options you choose about how to run your workshop.

◆ Presence in kit is optional. As the kits are sent around without necessarily going back to LTS for restocking, some items may no longer be in the kit and you can replace them with something easy to use/find.

× Not included in the kit materials

☠ **Safety issue**

🗐 MSDS in Appendix 3 or additional MSDS appendix.

DNA Deoxyribonucleic acid RNA Ribonucleic acid AA AminoAcids

TAE Tris Acetated EDTA buffer EDTA Na_4-Ethylene-diamine-tetra-acetic acid

dNTPs mixture of dATP, dTTP, dCTP and dGTP; the building blocks of DNA

2011 Version 8, November 2010

Send omissions/corrections to hutchins@ucalgary.ca

Funding for this project was provided by

Ontario

let's talk science
inspiring discovery

SGC

Structural Genomics Consortium, Toronto
Additional kit, 2010

The making of this kit was a collaboration of the following Partnership Programs

Laurentian University
Université Laurentienne

University of Calgary

University of Manitoba

uOttawa

UNB
University of New Brunswick

and

Ontario Genomics Institute
The Future is in Our Genes.

With
Science and education support
provided by the faculty from

University of Calgary
Faculty of Medicine

and their sponsors for these materials

Alberta Innovates Health Solutions
current

NSERC CRSNG
promo Science
past

Kit Stock Acknowledgements

Some of the supplies in this kit are provided to LTS by LTS at the University of Calgary. Many of these supplies are donated to them to support this and other outreach activities. Companies who have provided materials as donations or substantial student discount include:

Donations: VWR Supplier Partnerships for Customer Solutions and Fisher Scientific and some of their suppliers.

invitrogen by life technologies

SGC

BIO-RAD

Structural Genomics Consortium, Toronto

Retiring professor at UCalgary who donated the huge box of dyes

Discounts: InterScience Genuine Axygen Quality **ATK Technologies Inc.**

as well as the suppliers above

Financial Support: NSERC CRSNG promo Science Alberta Innovates Health Solutions University of Calgary Faculty of Medicine

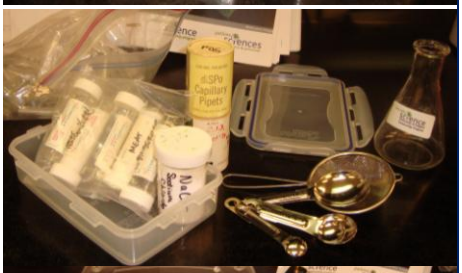
NEW in the kit. For demonstration: EasiCollect: Buccal cell DNA collection kit provided by Whatman, part of GE Healthcare

Contents of this Kit:

UPDATED!! CHECK IT OUT!
Be sure to read the WHOLE updated manual!

*****CHECK CONTENTS UPON RECEIPT. NOTE HOW THE KIT IS PACKED. GET FAMILIAR WITH ALL THE COMPONENTS 1-2 DAYS BEFORE YOUR EVENT.**
Please READ THE MANUAL – don't assume you know how to use everything.***
Some stuff just seems weird and complicated until you see how to use it.

Disposables (★): Contact LTS National office for new stocks of anything missing or short.
When forwarding the kit to another location or returning the kit to LTS home location, indicate missing disposables or broken equipment.



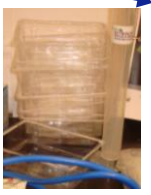
DNA Isolation materials (Module 4):

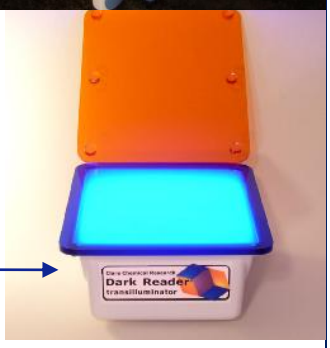
- ★Dish soap
- ★Salt
- Glass rods or glass pipettes
- Plastic test tubes without caps
- ★Meat tenderizer
- Measuring spoons and strainer
- × Clear plastic drinking glasses described in the protocol are not included in the kit.
- ☠× **NO ALCOHOL is included in the kit, nor should any be put in for shipping via commercial courier or bus.** ☠

Top and right: DNA isolation reagents, agarose and TAE, strainer and measuring spoons in the plastic boxes that can be used to store gels (see below).

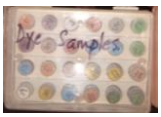
Gel Electrophoresis Materials (Module 5)

- VWR Gel Box, lid, tray, two rubber casting dams, two combs, and electrical cords (2 complete sets, see Appendix 1 for assembly of gel tray and casting gels.) →
- Power Supply and cord (1)
- Extension cord (1)
- Pipettes (usually 15-20)
- Green 10 uL fixed volume pipettes (5-8)
- Pipette Tips (8-10 boxes)
- ★Bag of extra tips, REFILL boxes as needed, stock at LTS National
- Practice Plastic Gel Trays (12) →
- Erlenmeyer Flask (200mL) (1)
- Cylinder (100mL) (1)
- Collapsible 2L bottle or LTS water bottle
- ★🗐50x TAE (1 bottle) 🗐For MSDS, see appendix 3.
- ★🗐Agarose (1 container)
- Water-tight clamp-lock containers (1 rectangular for TAE gels, 1 square for water practice gels. Shipped with supplies inside. Empty them if needed for carrying gels.)
- 🌡◆🗐TAE gels (1-2) large gels in TAE in large rectangular container
- 🌡◆Water or "practice" gels (16 or more) in water in smaller square container (🌡Store at 4°C until used. ◆May not be included in kit unless obtained from another site.)
- Blue Light Box/Transilluminator (Dark Reader), orange filter, and power supply (1) →
- ◆Gloves (1 box or so)
- ◆Tip Waste Buckets. If not included, you can use plastic or disposable cups.





Samples (Module 5, Appendix 2 for preparation)



- ★Practice Dye, single colour- ready to use (1 box)
- ★Stock 6X Practice Dye (1-2 bottles
- ★Dye Samples, mixed colours- ready to use (1 box)
- ★Dye Ladder (few 2.0 mL microfuge tubes, ready to use)

- ★DNA Ladder (few tubes)
- ★DNA samples dry (few tubes)
- ★6XPonceau S or 6XOrange G loading dye
- ★100X concentrated SYBR Safe DNA dye in 1xTAE in brown tube or black box

NEW: Sample of DNA collection kit NEW in the Biotech kit.

❍PCR materials (Module 5)

18 or so individual baggies with materials for two students:
PCR strip tubes and caps
Microfuge tubes with "DNA samples 1-6,
Positive control, water, Master mix"
Racks or foam blocks to hold samples

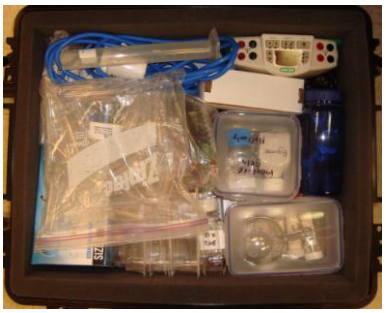
Copy of Manual and Scripts (Binder, CD, or check LTS portal for complete package of 2011 materials)

Packing the crate for shipping

Dry the gel boxes, practice trays, cups, cylinders, bottles, etc. Remove the cables from the gel box lid. Put all the components of the gel box back into the gel box (tray, combs, caster dams, cables) and attach the lid. Don't forget power cords!!!!

Please put everything back!!!…replacements are costly and another group may be counting on receiving them. Try to use every nook and cranny in the packing to prevent shifting and breakage. Once you have it packed, shake it to be sure that there is no or little movement of contents. If you are shipping gels on to another institution, put the materials that were in the gel storage boxes elsewhere in the kit or ship them separately.

Contact LTS National for next destination and shipping booking.
Shipping issues: Keep kit from freezing. Fragile. Heavy.



The DNA Biotech Kit

Please review this manual as many changes and additions have occurred since previous editions. Recycle old ones.

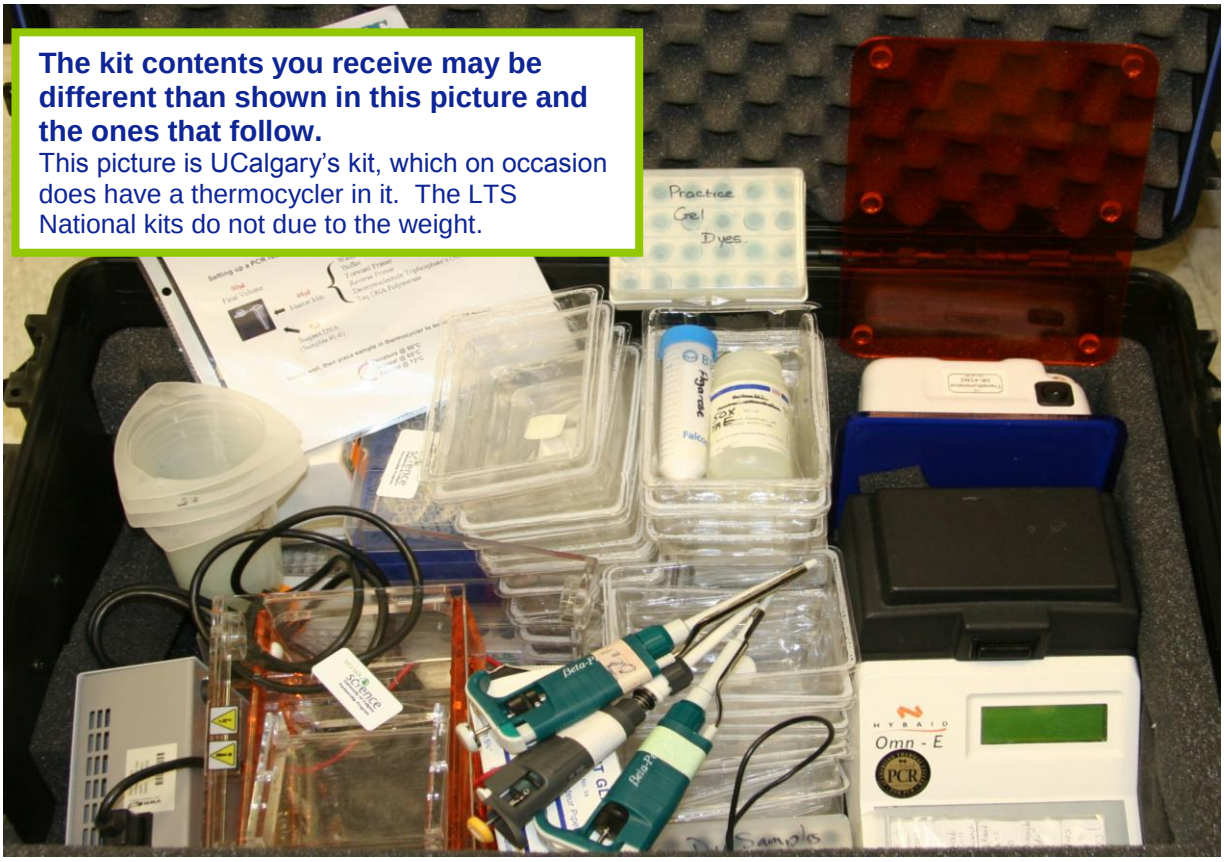
UPDATED!! CHECK IT OUT!

The DNA Biotech Kit contains several modules:

1) DNA 101 Elementary and Junior High Levels and Senior High Level
2) Paper DNA. Two levels.
3) Making a candy DNA molecule
4) Extracting DNA from an onion (or banana or strawberry). Two options to do it.
5) DNA WhoDunnit? Extra component for pipetting a PCR reaction
6) Special Report (DNA WhoDunnit? for the *Listeria* outbreak)
7) Gene Expression by DNA Arrays

How to use the kit?

1) Determine what modules you wish to use in an age appropriate way. **CHECK WITH THE TEACHER FOR PRIOR KNOWLEDGE.**
2) Use all the modules in a ½ day long workshop.
3) Use one or parts of the modules to make up an in class-time lesson.
   Suggestions included DNA 101 whole or part, isolate DNA, pipette lesson, load gels.



The kit contents you receive may be different than shown in this picture and the ones that follow.
This picture is UCalgary's kit, which on occasion does have a thermocycler in it. The LTS National kits do not due to the weight.

Module 1: DNA 101

KEYWORDS: nucleoside, nucleotide, nucleic acid

TIMING: 10 minutes or longer depending on age and subsections included

PRIOR KNOWLEDGE ASSUMED: the atom, molecules, carbon chemistry, organic chemistry, cell structures and the nucleus. Suitable for a Grade 6 class and above. Two versions available: one for junior high, one for senior high. CHECK WITH THE TEACHER FOR PRIOR KNOWLEDGE or areas that the teacher would like covered.

ACTIVITY
Discussion about the structure and function of DNA, and introduction to genomics, with an accompanying PowerPoint Presentation(s): Junior high/Elementary or Senior High levels. Senior high level also includes introduction to the central dogma, genetics (mutations), DNA sequencing as part of the introduction to genomics.

LEARNING OBJECTIVES:

1) **WHAT THEY ARE TO DO**?
   The students will learn what DNA is, the function of DNA as well as its structure.

2) **HOW THEY ARE TO DO IT?**
   The students will view a PowerPoint presentation on the structure of DNA.
   They may possibly be shown a model of DNA.
   The students may draw their own DNA molecule.
   The students may make a DNA molecule from candy (see Module 3) and/or extract DNA (see Module 4).

3) **HOW WE WILL KNOW THEY ARE SUCCESSFUL?**
   The participants will be successful if they participate in the discussion, answer questions, and/or draw and accurate DNA molecule.

MATERIALS
1. PowerPoint Presentation. Choose appropriate level. For Senior high in particular, check with teacher re: how many sub sections to include: DNA sequencing, genomics, central dogma and / or Genetics (translation/mutations).
2. Consider which other modules you would like to share with the students. For Senior High, Module 1, 6 and/or 7 make good lecture components to do alongside Module 5 to make a ½ day workshop.

METHODS:
1. Review the structure of DNA using the PowerPoint presentation.
2. Possibly ask them to draw a molecule of DNA

NOTES ON USE:

DNA 101 complete with genomics is easy enough that Grade 6 kids will be able to comment about the uses of genomics. DNA 101 is a good precursor to gene transformation, site directed mutagenesis and functional protein studies, and bioinformatics as well as adding to DNA discussions.

Module 2: Paper DNA

KEYWORDS: DNA Bases and base pairing. Genetic code

TIMING: 15 minutes

PRIOR KNOWLEDGE ASSUMED: CHECK WITH THE TEACHER re: prior knowledge
Suitable for just about any age from 9 years to adult. For younger grades, assume they have learned about the cell and discussed that one cell divides into two daughters.

ACTIVITY
Game requiring students to form lines and pair across the lines.

LEARNING OBJECTIVES:
1) WHAT THEY ARE TO DO?
Participants will learn DNA base letters, code and base pairing. If the additional activity is included, participants should also learn about RNA and protein translation.

2) HOW THEY ARE TO DO IT?
Role playing. See the activity instructions below.

3) HOW WE WILL KNOW THEY ARE SUCCESSFUL?
The participants will be successful if they participate in the discussion, name the bases and base-pairs and if they say they had fun.

MATERIAL TO BE INTRODUCED BY SESSION LEADER:
Discuss the 4 bases as GATC – use chemical names if older students are in the group. Talk about base pairing as the "BASE-PAIR RULE". Have them call out the base that pairs with each one as you call them out. (a reinforcing activity). Explain that the Base-Pairing rule is very important during cell division…because the DNA in a cell has to double before the cell can split into two daughter cells.

MATERIALS
1. PowerPoint Presentation (Module 1 – DNA 101)
2. ◆Coloured paper preprinted with GATC
3. ◆Optional: Sense sentence, antisense sentence, mRNA, tRNA/AminoAcids

METHODS:

Prior to class:
1. Review materials with teacher.
2. Review Module 2 PowerPoint for a picture view of this activity.
3. Print GATC one each on different coloured paper: G on one colour, T on another, etc.
4. Ensure you have equal numbers of G,A,T, and C in an appropriate number for the class.
5. Provide tape for the top of each paper so that the letter can be stuck onto the children's chest.

During class:
1. Review DNA Module 1, first part for elementary students.
2. Split the whole class into 4 groups by having them call off 1, 2, 3, 4.
3. Have all the students with the same number meet in one corner of the room.
4. Give each group 1 of the letters and sufficient tape to tape the paper letter to their chest.
5. If there is an odd man or two out, recruit that/those student(s) to be Polymerase!

6. Further split the groups into 2 more groups and have the first half of the group line up in any order in an area long enough for them to form one line (front of the classroom, hall way).
7. Have Polymerase put their left hand on the right shoulder of the person on their left.
8. Have them put their right hand out as if they were going to shake hands.
9. As you face the line, have the students who are waiting to call out the DNA sequence from left to right.
10. Have a student whose letter base-pairs with the right hand person in the front line go and shake hands with that student.
11. Now have the students start to call out who they should be base-pairing with, in order from right to left. As each student comes in, they shake hands with the person they are base-pairing with and then Polymerase again puts their left hand on the right shoulder.
12. Once complete, they can call out the sequence of the other strand in order.
13. Explain that DNA is just like them …all lined up and going in two directions: their left arms and shoulders are making up the DNA backbone and their hands are like the bases and make the rungs of the ladder.

Additional Activity for older students learning replication, transcription and translation

Preparation:

1. In order to explain a gene, print 1 colour of paper with the all caps words shown in the DNA Paper game PowerPoint (SAM THE BAD OLD CAT SAW THE BIG RED APE AND RAN AND BIT HIM AND HIT HIM). This is the DNA sense or sentence strand.
2. On the same colour of paper, print the "antisense" sentence (MAS EHT DAB DLO TAC, etc) DNA is always in capital letters, but in this case the words are all backwards.).
3. On another colour of paper, print the mRNA sentence (sam the bad old cat saw the big red ape and ran and bit him and hit him).
4. On yet another colour of paper, print the antisense tRNA sentence (mas eht, etc) and on the back of that, the sign language translation (translations indicated: **T**he =). These are the tRNAs. Once the mRNA has been assembled, a "ribosome" assembles the amino acid string (each tRNA turns their anticodon around to show the AA on the other side).
5. **Determine the number of words for ½ the class to form as sentence that makes sense.**
6. Sort out the words needed for your sentence from the DNA sense, reverse complement, mRNA and tRNA pages. Pair the DNA sense and mRNA pages back to back. These can be permanently glued or taped together. Pair the antisense DNA with the tRNA pages. Do not glue (top edge can be taped together).

Playing:

1. DNA: Have the participants with a DNA word line up in order to read the sentence. Discuss that like a sentence, there is a first word and a last word and we put the first word on the left, just like we when we are reading. Explain that DNA has only for letters in its alphabet and our alphabet has 26, so this example is a bit strange, but DNA encodes for genes much like our sentence.
2. The other ½ of the class then has to base pair (with antisense words) from last word to first (right to left) with the rest of the students in the class. This is the same type of thing that happens in DNA replication or DNA synthesis before the cell is ready to divide. You or extra students act as DNA polymerase to make the other DNA strand by putting right hand on the left shoulder of the student immediately next to them.
3. Have the sense strand DNA back away from the antisense strand and turn their DNA over to become mRNA. Now the mRNA has to base pair (individually and in order) with the sense to antisense from the first word (mas) to the last. Explain that this process is transcription of a gene and does not change the DNA of a cell. The gene is being transcribed so that a protein can be made. You or extra students act as RNA polymerase to make the mRNA strand by putting right hand on the left shoulder of the student immediately next to them.
4. Now have the antisense DNA step away from the mRNA. If you have a big enough room, have the sentence move to another location as the mRNA moves from the nucleus to the cytoplasm.

5. The antisense students turn their DNA into tRNAs. This time the cell uses tRNA and each tRNA has an amino acid attached to it. This time Ribosome can make the links, right hand to left shoulder. They match up in order first word to last, have the peptide bond formed (this time left hand on right shoulder), then turn their code words over to translate into Amino Acids. Let the mRNA line return to their seats.
6. In order for the protein to be functional, it must fold…have the protein now form into group hug and give a yell.
7. Gather all the papers back and reorder the sentence for the next time.

Conclusion:

If time remains, recap all materials.
If sufficient time is still available, introduce Genomics 101.

Consider which other modules you would like to share with the students. This activity can be combined with pipetting, loading practice gels and dye samples into a real gel. Include DNA in several lanes. While the gel is running, isolate DNA. Then show the final gel. This should take 1.5 hours.

Module 3: Making a Candy DNA Molecule

KEYWORDS: sugar, base, phosphate, nucleoside, nucleotide, nucleic acid

TIMING: 20 minutes

PRIOR KNOWLEDGE ASSUMED: the atom, molecules, carbon chemistry, organic chemistry, cell structures and the nucleus. Suitable for a Grade 6 class and above.

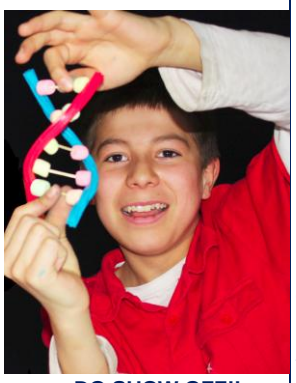
PLEASE, NO EATING THE CANDY DURING THE LAB ☺

ACTIVITY
Making a molecule of DNA with candy.

LEARNING OBJECTIVES:

1) WHAT THEY ARE TO DO?
The students will learn or review the structure of DNA. The students will make a DNA molecule out of candy.

2) HOW THEY ARE TO DO IT?
The students will view a Power Point presentation on the structure of DNA.
They may possibly be shown a model of DNA.
The students may draw their own DNA molecule.
The students will make a DNA molecule from candy.



DO SHOW OFF!!

Photo: LTS at UOttawa

3) HOW WE WILL KNOW THEY ARE SUCCESSFUL?
The participants will be successful if they participate in the discussion and create an accurate DNA molecule out of candy

Materials
1. Power Point presentation: DNA 101 and Module 3, but can be done without the Module 3 powerpoint.
2. ×Candy packs (in snack bags)
   - Toothpicks (round ones are stronger). Number depends on the candy type.
   - Two different colours of long candies such as licorice / student (Twizzlers make a pack with 8 colours, which can be cut into 8-10 pieces each. Pipe cleaners make a good material to string the Twizzlers onto and are able to twist. See pictures above.). Have extra toothpick for "stuck" Twizzlers. For even younger kids, simplify the activity by just give them two twizzlers.
   - 8 (or more) soft candies such as marshmallows, jujubes, wine gums, fruit gums, or gummy bears (4 different colours)
   - Consider food allergies: Chose gluten and peanut free candies and keep them separate from any food prep location where they can become contaminated. Wear gloves. Ensure clean knife or scissors.
3. ×Paper and pencil for each student. 11"x17" paper makes great place mats for this activity.

Instructions
1. The long candies are the "backbone" or "rails" or the sides of the DNA ladder – one candy is the phosphate, the other is the sugar group (deoxyribose). Link with pipe cleaners or toothpicks.
2. Connect the two sides of the "backbone" with soft candies using a toothpick
3. Remember only certain nucleosides can "stick" together in DNA
4. Make a key and indicate which candy represents which part of the DNA molecule: Phosphate, Deoxyribose/sugar, the Nucleosides A, C, G, T.

Module 4: Extracting DNA from an onion or fruit

KEYWORDS: DNA isolation, extraction

TIMING: 20 minutes

PRIOR KNOWLEDGE ASSUMED: the atom, molecules, carbon chemistry, organic chemistry, cell structures, the nucleus, cell membranes, lipids, proteins. Suitable for a Grade 6 class and above.

ACTIVITY
Extracting DNA from an onion (or other food: bananas and strawberries do not need meat tenderizer)

LEARNING OBJECTIVES:

1) WHAT THEY ARE TO DO?

The students will learn or review the structure of DNA. The students will extract DNA from an onion.

2) HOW THEY ARE TO DO IT?

The students will view a PowerPoint presentation on the structure of DNA.
They may possibly be shown a model of DNA.
The students will extract DNA from an onion.

3) HOW WE WILL KNOW THEY ARE SUCCESSFUL?

The participants will be successful if they participate in the discussion, answer the questions and successfully spin DNA on a glass rod.

Directions for Option 1: Demonstration

Materials

1. Power Point presentation
2. Supplies not provided in kit:
   - Besides onion, you can use most any fruit, but more successful ones included bananas (under-ripe is better due to polysaccharides in ripe ones, 3N), strawberries (weird 2N x up to 7 duplications = 56 chromosomes) and peaches (2N x 2 = 16). See below re: using meat.
   - 95% Rubbing alcohol. Chill on ice, icepacks, or in freezer, available by asking pharmacist or use lab-grade 95% ethanol. 70% Rubbing alcohol will work, but can take longer to get the DNA to spin.
   - Ice or ice packs, cold water, cups or tubes. Used 50 mL culture tubes are great for this.
3. Kit supplies for this module:
   - Dish soap
   - Salt
   - ♻ Glass rods or glass pipettes (♻ Recycle/Reuse)
   - ❍ Meat tenderizer (❍use is optional depending on food)
   - Measuring spoons and strainer

Method

It is best for you to try this lab beforehand so you know what you are doing and how to help the students. It also is nice to have a couple correctly extracted vials on hand for students whose lab does not work properly. The longer the mixture sits, the easier the DNA will be to see.

1. Coarsely chop onions and divide them in half.
2. Place half the onions in a "CONTROL" cup and the other in "SOAP AND ENZYMES" cup. Add 100 ml of water to each cup.
3. Add dishwashing soap to 1/10, ½ teaspoon of meat tenderizer and ½ teaspoon of salt to cup marked "SOAP AND ENZYMES"
4. Stir gently, wait 10 minutes
5. Separate the onions from the juice with a strainer and wait 10 minutes
6. SLOWLY pour alcohol into each juice cup and DO NOT stir!
7. Spin the DNA
8. Visually compare the control tube/cup to the experimental tube/cup. Ask students the following questions: What are the differences? What are the similarities? Why might we see some of these differences?
9. Prove that what you have isolated is DNA by re-dissolving one of the student's samples or one you have prepared ahead in water. Comment that DNA is soluble in water, but denatured proteins are not – think about the cooked egg white floating in the dishwater. Comment to use: "By the way, now you know how washing dishes works…"

Photo: Austin Children's Museum

Ask the students the following questions at the right steps or as a conclusion:

What did the detergent do?
Why did you add the meat tenderizer?
Why was the salt added?
Why did you add the alcohol?

Directions for Option 2: Class Participation

Materials

1. ❍ Power Point presentation or ❍method cards below or Appendix 4. Works just as well using verbal instructions alone.
2. Supplies not provided in kit:
   - × Onion, strawberry, banana, peaches. You can also use meat, but it is best to choose a very soft meat to make this work the easiest. Liver can work. Better is calf thymus (veal sweetbreads) because it is very soft. This meat is expensive as meat goes, but a piece can last for years. Cut in very tiny pieces and freeze. To use, it can be squished in a plastic baggie with the salt water. Tenderizer is not required. Usually you get too many cells to make the DNA isolation work, so have extra salt water handy.
   - × 95% Rubbing alcohol. Chill on ice, icepacks, or in freezer. Available by asking pharmacist or use lab-grade 95% ethanol. 70% Rubbing alcohol will work, but can take longer to get the DNA to spin. Have aliquoits ready to hand out to kids when they are ready for it.
   - × Ice or ice packs, cold water, cups or tubes.
   - × Used 50 mL culture tubes are great for this.
   - × If available, disposable transfer pipettes can make this activity a lot less messy.
   - × Wooden skewers
3. Kit supplies for this module:
   - Dish soap - Dilute 1 in 10 with water to use.
   - Salt - Make normal saline (0.9 gm% or 0.9 gm/100 mL)
   - ♻ Glass rods or glass pipettes (♻ Recycle/Reuse)
   - ♻ Plastic test tubes without caps
   - Meat tenderizer (❍use is optional)
   - Strainer

Method

It is best for you to try this lab beforehand so you know what you are doing and how to help the students. It also is nice to have a couple correctly extracted vials on hand for students whose lab does not work properly. The longer the mixture sits, the easier the DNA will be to see.

1. Give each student 1 test tube and 1 glass rod or wooden skewer (×).
2. For each pair of students, provide 1 tube of diluted soap solution, 1 tube of alcohol (chilling). Transfer pipettes for each solution if you have them.
3. Prepare while they are watching 1 piece of strawberry or banana by mushing them in a plastic bag. Add salt solution and mush some more. Get a volunteer to gently mush with you. Strain the solution into a cup or tube to get the chunks out. Dilute the cell suspension with more salt water if it is very thick – it should not be opaque.
4. Give each student 1 finger measure of cells in salt water in their test tube by tipping…transfer pipettes are great here. Have them put their tube and finger on the table side by side so you can measure to their finger.
5. Each student adds 1 finger measure of soap and mixes by gently finger flicking their tube. Have them make observations. An interesting thing happens with strawberries. Once you add the soap, the solution gets much pinker and the strawberry smell is much stronger. Kids can usually then figure out that the soap has made the strawberry cells pop open and all the good strawberry stuff has come out.
6. Each student pair is given an alcohol aliquoit and then carefully layers on two finger measures of alcohol onto their strawberry or other food soap.
7. Then they observe the DNA spinning out and can "poke" the DNA with their glass rod and see if they can catch it.
8. ♻ Collect glass rods and test tubes to wash and recycle/reuse in the kit – can be done in a lab or at home. To ensure the rod are clean of any DNA sticking to them, they should be left to soak in 2% bleach for ½ hour or so and then rinsed with lots of water. Let tubes and glass rods drain in a glass or dish with paper towel in the bottom.

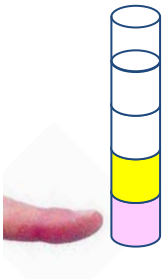
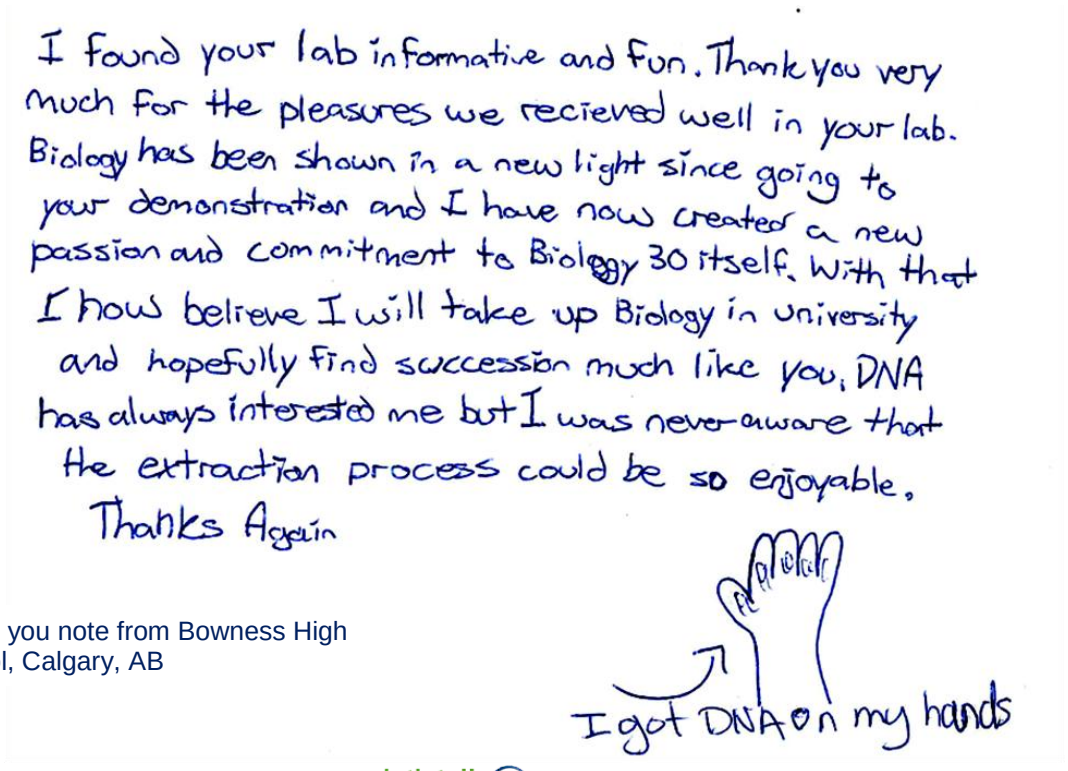


Photo: Mr. Pearson's math and science talk

Ask the students the same questions as Option 1.



Thank you note from Bowness High School, Calgary, AB

Take home DNA Isolation (with tubes not supplied – you may get them donated by suppliers or lab). Print 4 recipes/landscape page and cut to separate.

How to Isolate DNA at Home

1 cup (250 mL) of cold water with 1/2 tsp (2 mL) of salt
1/4 cup (50 mL) of dishwashing liquid diluted 1/10 with water (5 mL dish soap + 45 mL water)
Rubbing alcohol (on ice or in freezer)

1. Mush a bit of banana or 1 strawberry in some of the salt water. Let the pieces settle. Strain with cheesecloth. Get the solution cloudy, not so you can't see through it. You should be able to see this card through the solution, but not read the words.
2. Put 1 part (1 mL) in your test tube.
3. Add 1 part (1 mL) of dish soap solution. Mix very gently by finger flicking the tube. Avoid bubbles. The solution should clear.
4. Add 2 parts (2 mL) of ice cold rubbing alcohol as a layer over top of the soap/salt/fruit solution. The DNA is the white bits that float up into the soap layer. It can be covered in bubbles and look snotty. DNA floats. Proteins denatured by the soap sink.
5. Catch the DNA with a glass rod or wooden skewer. You can let it dry.
6. To prove it is DNA, as compared to fried egg white as a denatured protein, add your DNA to clean water in a clean test tube. If it dissolves, it is DNA. If it is denatured protein, like egg white, it will stay white and solid.

How to Isolate DNA at Home

1 cup (250 mL) of cold water with 1/2 tsp (2 mL) of salt
1/4 cup (50 mL) of dishwashing liquid diluted 1/10 with water (5 mL dish soap + 45 mL water)
Rubbing alcohol (on ice or in freezer)

1. Mush a bit of banana or 1 strawberry in some of the salt water. Let the pieces settle. Strain with cheesecloth. Get the solution cloudy, not so you can't see through it. You should be able to see this card through the solution, but not read the words.
2. Put 1 part (1 mL) in your test tube.
3. Add 1 part (1 mL) of dish soap solution. Mix very gently by finger flicking the tube. Avoid bubbles. The solution should clear.
4. Add 2 parts (2 mL) of ice cold rubbing alcohol as a layer over top of the soap/salt/fruit solution. The DNA is the white bits that float up into the soap layer. It can be covered in bubbles and look snotty. DNA floats. Proteins denatured by the soap sink.
5. Catch the DNA with a glass rod or wooden skewer. You can let it dry.
6. To prove it is DNA, as compared to fried egg white as a denatured protein, add your DNA to clean water in a clean test tube. If it dissolves, it is DNA. If it is denatured protein, like egg white, it will stay white and solid.

Module 5: DNA WhoDunnit? Gel Electrophoresis

This section is gel electrophoresis only. See the next section Page XX for adding the PCR set up activity. Adding PCR adds 30 min to a workshop.

KEYWORDS: Polymerase Chain Reaction (SH), gel electrophoresis, DNA Fingerprinting

TIMING: 60-90 minutes. Junior high (50-60 min). Senior high (90 min).

VOLUNTEER REQUIREMENT: The number of volunteers depends on size of the class and comfort of the presenter with materials. A well practiced volunteer can do a class of 10-16 alone. An experienced teacher helps. Beyond that, there should be 1 volunteer as lead and 1 other minimum / class of 16-20. There should be 1 lead and 3 others for a class of 30 if possible.

PRIOR KNOWLEDGE ASSUMED: DNA structure, some understanding of chemistry/electrolysis of water, may have been introduced to the PCR or restriction enzyme techniques previously. Senior High version: Suitable for a grade 11 and above biology or chemistry class or an "enriched" grade 8 or above science class. Junior High version: Suitable for any group that has done DNA 101, usually Grade 6 and up. When booking workshops, you can offer the DNA101 module to the teacher to cover prior to doing Module 5 on a separate day.

ACTIVITIES:
Pipetting and Gel electrophoresis

LEARNING OBJECTIVES:

1) WHAT THEY ARE TO DO?

The students will learn about the polymerase chain reaction technique for DNA fingerprinting and gel electrophoresis.

2) HOW THEY ARE TO DO IT?

a) Participate in a question and answer/discussion session about PCR (Power Point presentation).
b) How to use a micropipette.
c) How to load a submarine gel.
d) Load a real gel with a DNA sample or dye sample.
e) Observe results of real DNA samples and dye samples.
f) Interpret findings and discuss social implications.

3) HOW WE WILL KNOW THEY ARE SUCCESSFUL:

The participants will be successful if they participate in the discussion, load gels properly and anticipate the interpretation of gel results based on materials learned in all the modules prior.

MATERIALS

See list on pages 5 and 6 for everything in kit. Check contents upon arrival for materials against the shipping list. Besides reading this section, check out the DNA WhoDunnit? for Smarties list. If wet gels are received, store them at 4°C until use. All other materials, including DNA and SYBR Safe dye are stable at room temperature. For DNA WhoDunnit? alone, you will need all the equipment, materials and samples for Module 5. If adding the PCR activity, you will need that set of materials as well. Materials prep is presented in the methods section below and appendicies. Materials and methods are presented based on Day(s) Prior to Workshop, Day of Workshop (Set Up and Conducting), and Clean Up/Pack Up. ×Items not included in the kit.

METHODS

It is best for you to try this lab beforehand so you are familiar with the equipment.

🛠AT LEAST 1 DAY PRIOR TO WORKSHOP

1. Assemble gel trays for casting. Specific instructions for casting depend on gel box manufacturer. See specific instructions in Appendix 1 for the kit your received. For 1 of the TAE gels, only insert 1 comb, not two. For the second one, use two combs. For water gels, insert two combs in each tray.
2. Make 1 L of 1XTAE from the 50XTAE by diluting 20 mL to 1 L.
3. Determine if wet gels were provided with kit. If yes, determine the integrity of the wells in the gels. If unbroken, store gels at 4°C until your workshop. If broken, ♻you need to recast. To recast, remove gels from water or TAE (do not combine water and TAE gels, do not add additional water or buffer), break into smaller pieces, and put into the flask. Proceed to step 6.
4. If no wet gels were received, prepare gels according to the tables below.

🛠Amount and type of materials to prepare

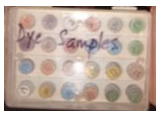
Gels will be recycled (used for more than one class, big event, or potentially sent to another site?)

How many kids at once?	Number of practice gels	Amount of agarose to water	Number of "real" TAE gels	Number of combs to use in TAE gels	Amount of agarose to TAE
8	1 Cut into 4	1.8-2 gm/120 mL water	1	1	1.8-2 gm/120 mL TAE
16	2 Cut into 8	3.6-4 gm/240 mL water	1	2	1.8-2 gm/120 mL TAE
24	3 Cut into 12	Both of above Cast 2 then 1	2	1 in one gel 2 in one gel	3.6-4 gm/240 mL TAE
32	4 Cut into 16	3.6-4 gm/240 mL water X2	2	2 in each	3.6-4 gm/240 mL TAE
36-40	Same as for 24 – have kids work in teams of 3-4		Same as for 32		
Mall version Whole day	8 or more Cut into 4s	3.6-4 gm/240 mL water each	4	2 in each	3.6-4 gm/240 mL TAE X2
☠If using the 250 mL flask in the kit, do not double up the volume. ×Use 500 mL flask. These guidelines are for 10x15 cm gels in the VWR gel boxes					

Gels will NOT be recycled

How many kids at once?	Number of practice gels	Amount of agarose to water	Number of "real" TAE gels	Number of combs to use in TAE gels	Amount of agarose to TAE
8	1 Cut into 4	1-1.2 gm/100 mL water	1	1	1-1.2 gm/100 mL TAE
16	2 Cut into 16	2-2.4 gm/200 mL water	1	2	1-1.2 gm/100 mL TAE
24	3 Cut into 24	Both of above Cast 2 then 1	2	1 in one gel 2 in one gel	2-2.4 gm/200 mL TAE
32	4 Cut into 32	2-2.4 gm/200 mL water X2	2	2 in each	2-2.4 gm/200 mL TAE
36-40	Same as for 24 – have kids work in teams of 3-4		Same as for 32		
☠If using the 250 mL flask in the kit, do not double up the volume. ×Use 500 mL flask. These guidelines are for 10x15 cm gels in the VWR gel boxes					

5. Mix powder agarose with buffer or water, swirl.
6. Microwave on full for ~1 min/100mL, swirl. Microwave until gel is fully dissolved. Watch the gel closely to prevent gel boiling over. Swirl. Let cool until you can hold onto the flask and the gel feels like hot bath water.
7. Cast the gel by pouring 100 - 120 mL into the casting tray. Let set at room temperature.
8. TAE gels: Remove from casting tray and store in container under 1XTAE. Keep extra TAE in 1L bottle to run the gels. Water gels: When set, remove from casting tray. Cut the gel into 4 smaller gels by cutting once vertically and once horizontally. ×Use scalpel blade, razor blade, knife, plastic ruler, etc. Store gels in containers under water. Keep all gels at 4°C until ready to use.
9. ♻Gels can be kept and used for up to 6 months by changing the water or TAE occasionally or recasting, if you plan on using the biotech more than once/year. You can even recycle in research gels from your institution ☠**IF they are NOT Ethidium Bromide gels**.
10. Ensure you have sufficient DNA samples and DNA ladder for demo purposes. See the table below. 🛠See Appendix X for more detailed preparation info and how to prepare your own DNA samples.
11. Ensure you have sufficient practice dye samples. If not, restock from tubes.

Samples (Module 5)	**Notes**	
Sample preparation is described in detail in Appendix 3.		
◆Practice Dye, single colour- ready to use for practice gels (1 box)	One tube is given to each pair/team of participants to learn pipetting, that DNA dye sinks, and how to pipette into a gel. Use 10 μL / well in practice water gels.	
Stock 6X Practice Dye More stock at National Office and at UofC	Dilute this stock 1 part + 5 parts water and refill the empty Practice Dye tubes (row above). Tube to make dilutions not provided. Disposable transfer pipettes (not provided) are great for this chore. Wear gloves.	
Determine what samples you will use based on number of participants. For small groups, you can use just DNA ladder and sample. For large groups and mall displays, use DNA sample and ladder in a few lanes and let participants load dye ladder and dye samples. For really young kids, dyes alone are sometimes much more fun for them to see. You can also shred the DNA you isolate from banana, onion or strawberry by pipetting up and down or using a syringe and needle. Add SYBR Safe 1 μL to 100 μL then 20 μL of loading dye.		
◆❍Dye Samples, mixed colours- ready to use for demo gels (1 box) Some kits have a stock of samples that can be diluted 1/6 as above. More stock at National Office and at UofC	Use 10 μL / well in TAE gel. Run with DNA samples. Allow participants to pick one to load onto the TAE gel and run. Each tube has a different combination of dyes in it to illustrate that DNA runs in different places based on size. Some of the dyes are fluorescent, so even if real DNA does not work on the blue light box, the dyes will.	 
◆❍Dye Ladder (few microfuge tubes, ready to use) More stock at National Office and at UofC	Use 10 μL / well in TAE gel. Use to demo loading in a gel box with dye samples. Tubes contain a combination of dyes that provides a ladder of colours.	

◆❍DNA Ladder dry (🛠stock dried for shipping, in black box or in water bottle. Stable in dark at RT.)	Usually dried down from 20 μL of prepared undyed ladder (0.5 μg load in 10 μL). Reconstitute with 20 μL water, 0.5 μL SYBR Safe and mix. Then add 4 μL of 6XPonceau S or 6XOrange G loading dye. Enough for two wells in a TAE gel.	
◆❍DNA samples dry (🛠dried for shipping) Reconstituted sample is stable at RT for several weeks, but may be quenched if left too long.	Usually dried down from pooled PCR products or bands purified from gel extracts. Concentration may vary…use 50 to 100 μL water to reconstitute. Add 1-2 μL SYBR Safe and mix. Then add 10 to 20 μL of 6XPonceau S or 6XOrange G loading dye. Load into TAE Gels at 10 μL / well. Load several wells.	In short labs, load and pre-run real DNA ladder and samples. Longer lab; participants can load and run with dyes.
◆❍DNA Ladder wet	You may receive DNA ladder purchased and prepared as above in the container from the manufacturer. Lucky you. Keep it in the dark and away from heat. Use 10 μL / well.	We have found that prolonged exposure of SYBR Safe to some dyes quenches the SYBR and, with the blue light box, the DNA gets harder to see.
◆❍DNA samples wet	You may receive PCR samples pooled and prepared as above. Luckier you. Keep it in the dark and away from heat. Use 10 μL / well. You can make your own by pooling samples. See Appendix 1.	
◆❍6XPonceau S or 6XOrange G loading dye (Ponceau S runs very fast, ~ 50bp size, in a gel. Orange G runs about 100 bp)	See above for the use.	
◆❍100X concentrated SYBR Safe DNA dye in 1xTAE in brown tube or black box	The purchased SYBR Safe is 10,000X meaning 1 μL in 100 mL gel. The SYBR Safe in the kit is supplied 1/100 in 1XTAE so you can add it to the samples directly. This stretches the most expensive reagent a lot further.	
NEW: Sample of DNA collection kit NEW in the Biotech kit. **For demonstration only.** EasiCollect: Buccal cell DNA collection kit provided by Whatman. This kit is in use by many police services. The cells are applied to FTA cards. FTA Cards contain chemicals that lyse cells, denature proteins and protect nucleic acids from nucleases, oxidative, and UV damage.		
The cost of SYBR Safe or other alternate dyes is coming down and it may be later in 2011, we will be able to afford to provide you with something you can add to the gel itself that will have no disposal issues and work as well with the blue light box as with a UV one. VWR is now selling a dye called Gel Green that should work in the gel and with the blue light box. You can get samples from your local VWR representative.		

DAY OF WORKSHOP – SET UP

1. Distribute /pair or trio of participants: 1 plastic gel tray with a practice gel just covered with water, 1 pipetter, 1 practice dye sample, ❍×1 3x3" piece of wax paper. For each two groups of students: 1 pack of tips, 1 tip waste bucket.
2. ☠Run the gels in an area of the lab/classroom with low traffic. Put TAE Gels in a quieter corner of lab or classroom in gel boxes, cover with 1xTAE, plug in power pack near. Keep DNA samples and dye samples near these gels. Start your real DNA samples ahead of starting the class for students for a shorter lab (55-60 min), run this gel at 80 to 100V. Watch it during the class period. Stop the gel when loading dye has progressed far enough. Place this gel on the blue light box and cover with the orange filter. Do not turn light on at this time.

CONDUCTING THE WORKSHOP

☠SAFETY:

1. No chewing gum, pencils, pens, fingers, or applying lip gloss or eating in the lab.
2. If performed in university labs, review fire routes from the area. If a fire alarm sounds, have one volunteer lead and the other be at the end of the group to ensure all participants are evacuated. Take the group to the nearest fire staging area and wait for an all clear before returning to the lab area. [Yes, it really messes up your timing when an alarm goes off – discuss what to cut with the teacher.]
3. If performed in university labs, only closed-toed shoes should be permitted. While there are no safety issues that require lab coats, it makes students feel very scientific. Lab coats may be required by some universities. [At UofC, we have our own box of lab coats for workshops, which is nice if you can arrange it. When visiting schools, we do not bring lab coats nor impose university safety rules on the local school. Consult with the teacher.]
4. Gloves for students are desirable, not mandatory, when handling the dye samples. There may not be enough in the kit for one pair for each student due to space and stocking issues. Gloves should be provided by a local chapter if possible. Wash hands before leaving classroom.
5. Caution with dye samples: Do not ingest. Wipe up any spills immediately. Avoid skin contact: Wash hands to remove. Again gloves would be desirable. See Appendix X for MSDS of sucrose-based loading dyes.
6. High voltage DC power source, ensure leads are correct and lid is on the gel properly. Participants should only be allowed to plug in gel box cables when the power pack is OFF.
7. Do not permit the lid to be removed during gel running.

1. Present the PowerPoint of Module 5. Review DNA in the cell, and introduce PCR technique as a way to amplify a particular region. See script with the PPT. Stop the presentation when you reach the lab break slide.

2. ☠**DISCUSS SAFETY ISSUES AGAIN.** Move participants to lab area if required. Be open to all questions during the lab time. If a question is a common one or one you should relay to all students, call for attention and discuss the question and answer.

3. Using pipetters:

a) Teach concept of very small volumes used . Picture a litre of milk – have students show you how big a liter is with their hands. State that a litre is 10 cm x 10 cm x 10 cm. Now divide by 1000 and get a milliliter; 1 cm x 1 cm x 1 cm. Now divided that by 1000 or 1 mm x 1 mm x 1 mm. That is a microlitre, which is the unit of volume being used in the lab. Pipetters in the kit could and should be set at 10 uL prior to use in the lab. You should not need to have students change the volume.

b) Teach the following by demo:

1. Using the tip – you only have one size – how to put it on.
2. How to take up and expel liquid – see notes below. Use practice dye or dye samples for demo and practice.
3. Ejecting tip

IMPORTANT - a way to teach pipetting:

- Participants in the workshop will not have seen pipetters before. Mention that they are expensive (>$100 each). Do not drop them. Hold onto them properly. Check that every student is holding it correctly.
- These are for pipetting wet solutions – DO NOT PUT PIPETTE HORIZONTAL OR UPSIDE DOWN WITH LIQUID IN IT.
- Demo the soft and hard stops. Use the words "Press gentle." and "Press hard". Modulate your voice – gentle is quiet and hard is louder.
- Be very sure to practice that hand up and down is different from thumb up and down. Practice a lot.
- Pipetting "song" – [no, there is no tune]

Hand up, thumb down gentle, gentle, gentle
(it is important to watch thumbs...if white and pressed, they are not going to be pipetting correctly.)
Hand down (tip down in dye), thumb up gentle, gentle
Hand up, move to new tube or back into same tube, don't thumb at all
Hand down, thumb down gentle, then thumb down hard
Thumb still down, hand up,
Now that hand is up, thumb up gentle.

4. **OPTIONAL – Observe mastery of pipetting:**
   Have students pipette 3-4 spots of 10 uL of water onto wax paper, plastic sheets, even the bench or table. Each bubble of water should be exactly the same size.

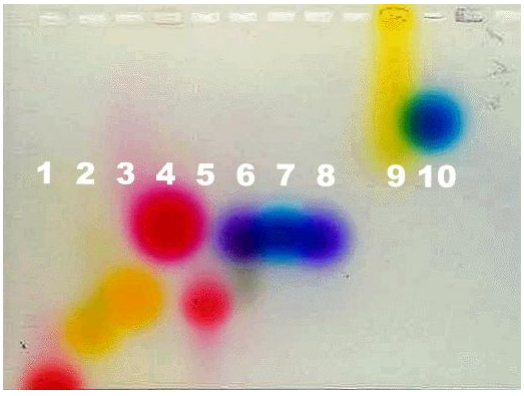
5. **Observe properties of dye loading dye:**
   Have students pipette 10 uL of dye into the water around the gel, not on or in the gel, by just injecting the dye under the surface of the water. The dye should sink. Ask why it sinks. Discuss that your DNA samples are in water, if you add water to water, the DNA would float away, so we add something to the dye…can they guess? The answer is syrup…we use glycerol or sugar syrup. Yes, they could drink the dye – we use sucrose, but their tongues would turn colour! [Don't let them do it!. The dyes used are the only chemicals in the whole workshop that have any chemical safety issues, but the amount is so minimal there are no known issues. If they get any on their fingers, wash it off.]

6. **Practice loading dye into gel wells:**
   You can demo the pouring of the gel by showing the comb and explaining that agarose is a powered just like Jello – heat and cast…yes it is thicker…yes it is edible, but YUK! They can touch the gels in front of them. Have students note the wells in the gels in front of them. Have them watch a demo before they proceed. Now they are to put the dye into the wells. IMPORTANT to convey that they must not wiggle side to side or they will break the gel, nor can they stab the gel as they will poke a hole and their DNA will leak out the bottom. Therefore, they should a) use two hands…the finger of one hand on the tip of the pipette that is in the other hand; b) put their elbows on the table; c) inject the sample into the top of the well rather than way down into the slot. CRITICAL – remind them to lift up their hand before they let their thumb go after pipetting the sample into the well, or they will suck it right back out again. Let each student load 2-3 wells in the practice gel. When the first team is complete, send them to the real gels with a new tip on their pipetter.

7. **Load dye samples into real gels:**
   Have students choose a dye sample and load it into the TAE gel. You may have to load one lane for them to see the wells when the gel is in the gel box. Load the dye ladders. You can also have 1-2 real DNA sample on the gel. When the whole class has pipetted, have the last group help put on the lid, plug in the gel and observe when you turn on the power. They should note the bubbles at the electrode. Run this gel at 100V. Involve the students who observed the start of the gel with electricity. Discuss bubbles.

Students can/should wash their hands and return to the class or lecture area.

8. **Resume the powerpoint presentation:**
   Important points about the presentation – variations in DNA between individuals leading to different sized pieces of DNA in the PCR products. The pattern for one set of primers yields two bands because chromosomes come in pairs. Pieces of the same size may indicate a family relationship. While the simple answer - appropriate for junior high – who dun the dirty deed is #5, it is important for senior high to cover family genetics vs population genetics and the implications of DNA technologies. See the script. The difference in timing between junior high and senior high levels is in the details in the powerpoint presentation, particularly in the interpretation of the gel and the level of materials discussed and demonstrated. Junior high level can also take 90 minutes to do by including DNA 101.



9. Once the presentation is complete, return to the lab area to observe the completed gels. Show and discuss DNA samples under blue light and dye gel fresh from the gel box. Some of the dyes used also fluoresce. You can discuss that all the dye samples looked pretty black and the electrophoresis has shown the presence of other colours…this is much like chromatography that can tell ink samples apart. NOTE: if the lab area is well lit, you may need to have a cardboard box to cover the blue light box. Cut a whole for viewing. The orange viewing screen on the blue light box will fog up with the heat generated by the box in the same way that the lid of the electrophoresis chamber does while running it. In order for students to see what is going on, you can coat the lids with soapy water. This will allow the fog of condensed water to drip off the lid easier.

To close, ask if there are any more questions. Have all volunteers relate how they may be using this technology in their research.

CLEAN UP AND PACK UP

1. Questions: Are gels to be recycled? No – discard the gels. ♻Yes – Is the next workshop immediately after the one you just completed? ♻Yes – Run the dyes and DNA out of the TAE gels by returning the gel to the gel box and turning the electricity on. You can run the gels at 150V for a short time without cooking them, but not too long. No – You can do run the dyes off the gel at your home location or place the gel(s) back into TAE and change the buffer once after 24 hours post workshop. Store the TAE gels in one water-tight container, cover with 1XTAE from the gel boxes and dispose of the rest. Pack up the practice gels in the other of the water-tight containers; add enough water to cover and keep the gels wet. Ensure that the boxes are full of gels or add some foam pieces to keep the gels from shifting during shipping.
2. Discard extra buffer and water. Return dye samples and practice dyes to their containers.
3. Rinse the erlenymer flask and cylinder. Briefly dry them, the gel boxes, and practice gel trays.
4. Dispose of tips into any waste disposal (there are no safety issues), or bring them back to your home institution for disposal.
5. Once you are at your home institution, unpack the gel boxes and gel trays to ensure they are dry before shipping. If you have time, be kind…restack the tip boxes with the tips from the stock bag. If you are going to be reusing the gels, store them at 4°C until next use or shipping. Please return the lock boxes that came with the kit and use containers of your own.
6. Prior to shipping, check in with LTS National if a) any supplies are needed to restock the kit and/or b) if it makes more sense for you to forward the kit to its next location or back to National office. Check the materials list for all contents. To arrange shipping, check with National office. Unless otherwise instructed, ship via ground.
7. Repack materials into the containers as you found them when you opened the kit. For the gel boxes, please replace the gel trays, rubber casting, electrical connecting wires and combs to the gel box and attach the lid securely. This is important to ensure that the gel boxes are not broken during shipping. Re-pack ALL the materials into the kit – another chapter is presuming that they will get the complete kit to their location. When everything is in the kit, it is packed tight and materials should not move if you shake/wiggle the box back and forth. If you don't have to work a bit to pack the binder, you haven't got it all in there! Pack the water tight containers so they will not shift during transport if you are sending them back to the national office or other chapter.

Module 5 Extra: PCR Set up

This section is the PCR set-up activity. Adding PCR adds 30 min to a workshop.

KEYWORDS: PCR, pipetting

TIMING: 20 minutes

PRIOR KNOWLEDGE ASSUMED: Completion of first ½ of Module 5. Do this activity before resuming the last part of Module 5. Suitable for a grade 11 and above biology or chemistry class or an "enriched" grade 8 or above science class.

ACTIVITY

This activity can be used to extend Module 5 to a full 2 hours by having students practice their pipetting to set up a PCR reaction.

LEARNING OBJECTIVES:

Just have a bit more fun pipetting. In this lab, they will learn how to change the volume on the pipetters. Volumes needed…5 uL and 45 uL. Learn how a PCR reaction would be set up.

1) WHAT THEY ARE TO DO?

Pipetting following the recipe sheets in the kit. Discuss with them that if they remember, the recipe for the PCR included buffer, dNTPs (DNA building blocks), primers, Taq and $MgCl_2$ and water. All those reagents are combined into one master mix. Discuss as well the need for positive and negative controls. Their job is to add the individual samples to the reagent in order to have samples for the next workshop.

2) HOW THEY ARE TO DO IT?

1. Once students have loaded their gel and the gel(s) are running AND you have sufficient time, have students proceed to set up a PCR reaction. If their PCR works, you will be using it with the next class, just like they are using DNA from the previous class (a Santa Claus-type lie).
2. Prior to **Module 5 lab**, distribute PCR strip tubes and caps, 7 DNA samples, MasterMix and water.
3. Students should pipette 45 uL into each of 8 tubes of the strip tubes. Then they can add 5 uLof each DNA sample to 1-6 of the samples of the strip tubes. To tube 7, they add the positive control. To tube 8, they should add water as a blank. They can then add the caps to the strip tubes.
4. Tell students you are taking their samples to your lab to amplify in a thermocycler. Put them on ice if have it and wish too. Don't worry about the samples…everything is water.

3) HOW WE WILL KNOW THEY ARE SUCCESSFUL?

If they get the 8 samples pipetted. They will tell you when they make mistakes.

Once the students have left, or you leave the classroom, dry out the strip tubes by flicking the water out. Restock the 7 DNA samples, MasterMix and water (blank) with water if needed and repack into the baggies. Welcome to the Martha Stewart method of Molecular Biology. [And that's a good thing.]

Module 6: Special Report

KEYWORDS: DNA techniques, *Listeria*.

TIMING: 20 minutes

PRIOR KNOWLEDGE ASSUMED: Completion of Module 5. Suitable for a grade 11 and above biology or chemistry class or an "enriched" grade 8 or above science class.

ACTIVITY
Powerpoint presentation showing a couple of other DNA fingerprinting techniques used to identify bacteria as part of an outbreak situation.

LEARNING OBJECTIVES:
Students will learn another PCR technique and one whole genome/restriction digest technique; both of which have been used and are currently in use during the Canadian *Listeria* outbreak of 2008

1) WHAT THEY ARE TO DO?
The students will view a PowerPoint presentation on the use of other DNA techniques.

2) HOW THEY ARE TO DO IT?
View a powerpoint presentation about some additional methods that are able to provide DNA fingerprinting information to answer an epidemiological question.

3) HOW WE WILL KNOW THEY ARE SUCCESSFUL?
The participants will be successful if they participate in discussion or ask relevant questions about the news story.

MATERIALS
PowerPoint Presentation

Module 7: Gene Expression

KEYWORDS: DNA techniques, gene expression, DNA Arrays, DNA chips.

TIMING: 20 to 30 minutes

PRIOR KNOWLEDGE ASSUMED: Completion of Module 5. Suitable for a grade 11 and above biology or chemistry class or an "enriched" grade 8 or above science class.

ACTIVITY

Powerpoint presentation with or without Flash presentation showing the use of DNA arrays to look at the genes expressed by yeast during beer or bread metabolism (anaerobic versus aerobic).

LEARNING OBJECTIVES:

Students will learn how genes are expressed under different conditions or in different conditions.

1) WHAT THEY ARE TO DO?

The students will view a PowerPoint presentation on the use of DNA arrays.

2) HOW THEY ARE TO DO IT?

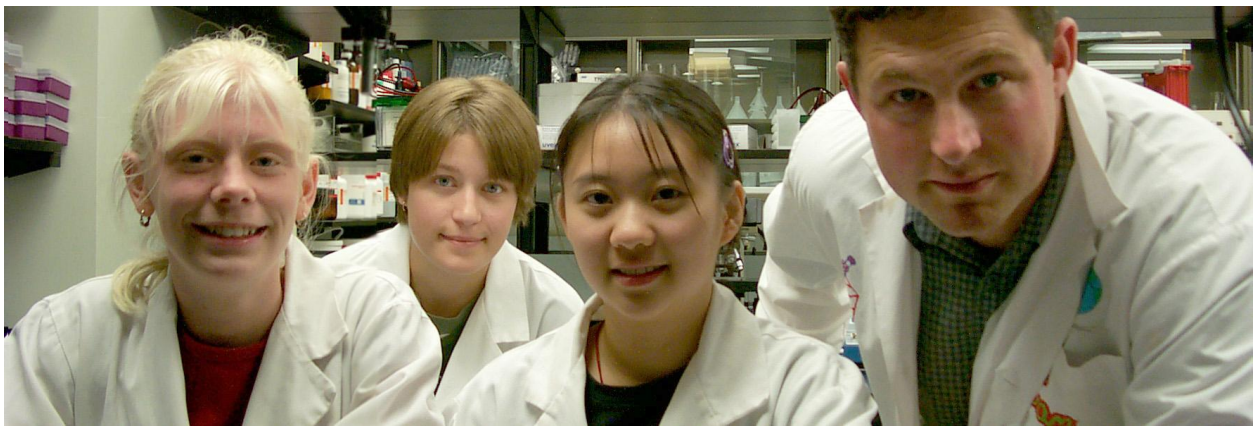
View a powerpoint presentation about gene expression arrays.

3) HOW WE WILL KNOW THEY ARE SUCCESSFUL?

The participants will be successful if they participate in discussion or ask relevant questions about the array technique.

MATERIALS

1. PowerPoint Presentation
2. Flash presentation (Flash produced by Dr. Malcolm A. Campbell, Davidson College and with permission).



Mall Module: DNA WhoDunnit? Mall Display

If you wish to do a interactive mall display activity with this kit, the information below will outline the set up and operation. The DNA isolation is based on Module 4 and loading DNA gels much like the junior high/elementary components of Module 5. Review both modules and practice components you are not comfortable doing.

BOOKING:

Book 1 to 2 eight foot tables. The number of tables is determined by the number of activities you plan on doing.

Access to an electrical outlet.
Access to water (washrooms work).
1 chair for each volunteer.
Plastic covers for tables if wooden.
Under as skylight to enable the visualization of DNA.
Freezer or Ice would be nice.

PACKING UP TO GO:

Bring alcohol and some kind of fruit, vegetable or meat (recommend calf thymus/veal sweetbreads because it is extremely soft meat or banana that can be fragmented with your fingers or by mixing in saline in a test tube. No need to use meat tenderizer with this!!! Pack into a cooler with lots of ice. The DNA isolation demo works best with iced alcohol.

SET UP:

1. If tables are wooden and plastic cover is not offered, first cover the tables. Autoclave bags make great table covers. Tape them to the table so that they don't slip during the show. In case there is a cover and it is white, bring some dark paper, construction paper or paper towels. In a pinch, we used black plastic disposable plates from a buffet to make black patches to go under the gels so that people could see the wells in the gels.
2. Determine best flow for walk up of people. If most people will be coming from the right (when you are behind the table, then start at that end with DNA isolation. If that location is dark, bring a desk light with you.
3. Central area – pipetting and gel loading practice. Set up 5 practice gel trays with 2 gels each and just cover with water. Space these about 2 feet apart. At each station, also put 1 10uL fixed pipette, box of tips, waste bucket, tube of practice dye. For the volunteers, have pipettes, tips, dye, waste bucket and demo gel.
4. At end opposite the DNA isolation end, set up gel boxes, powerpack and blue light box. This includes 1 gel box with 1xTAE buffer. The other can be used to demo how a gel is cast. Prior to demo starting, load one gel with dye samples and/or DNA samples. Run this gel so that the DNA and dyes have migrated far enough. HINT: If casting gels just for this event, only use one comb at the top to give a long gel. Place this gel on the blue light box. Place the whole blue light box and gel into a box with a hole cut for viewing as a dark "room". Put your second gel into the same gel box and load dye samples. Coat the inside of the gel box cover with dish soap to decrease condensation. Run this gel on the lowest power settings on your powerpack. When dyes run far down the gel, reload into the same wells and keep it running for the duration of the demo.

OPERATION:

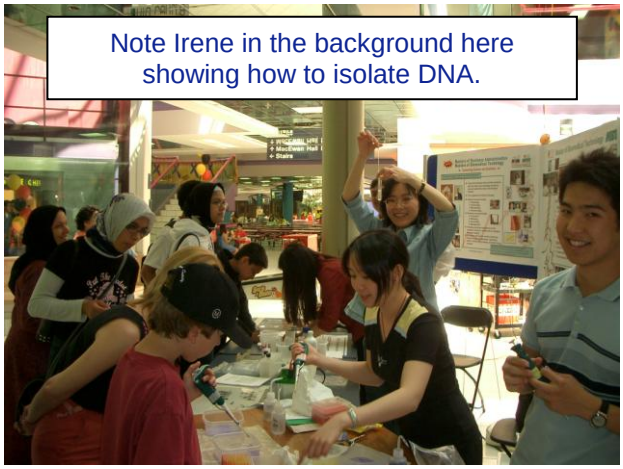
1. See above for the use of the gels. You will not always get everyone to start at the DNA isolation end, but do indicate the start. DNA isolation: Demo with clear glass or plastic tubes. Hold the DNA up to the light and have the observer lean close to see it. Show it stuck on the glass rod.
2. Move the participants to pipetting gels station. Have them put a tip on the pipetter, practice pushing the button to blow out the air and letting go to draw up. Have them do it in the water

where the gels are. Have them then pick up a sample dye. Show how to load it into the gel (you have to tell over and over to lift hand out of gel before letting thumb go…good luck on that one.).

3. Once they have loaded the gel, they can move on to look at the gel running and the DNA in the gel. If you have a DNA WhoDunnit? poster printed, you can go through the results of the gel as far as 1) lane 1 is crime scene sample, 2-8 are suspects, so who dunnit? 2) This type of assay works because everyone has a pattern for the two bands. Each band is inherited from mom or dad…if #3 is a kid, who are his parents. Go through the family relationship as described in Module 5 and leave it there.

APPLICATIONS:

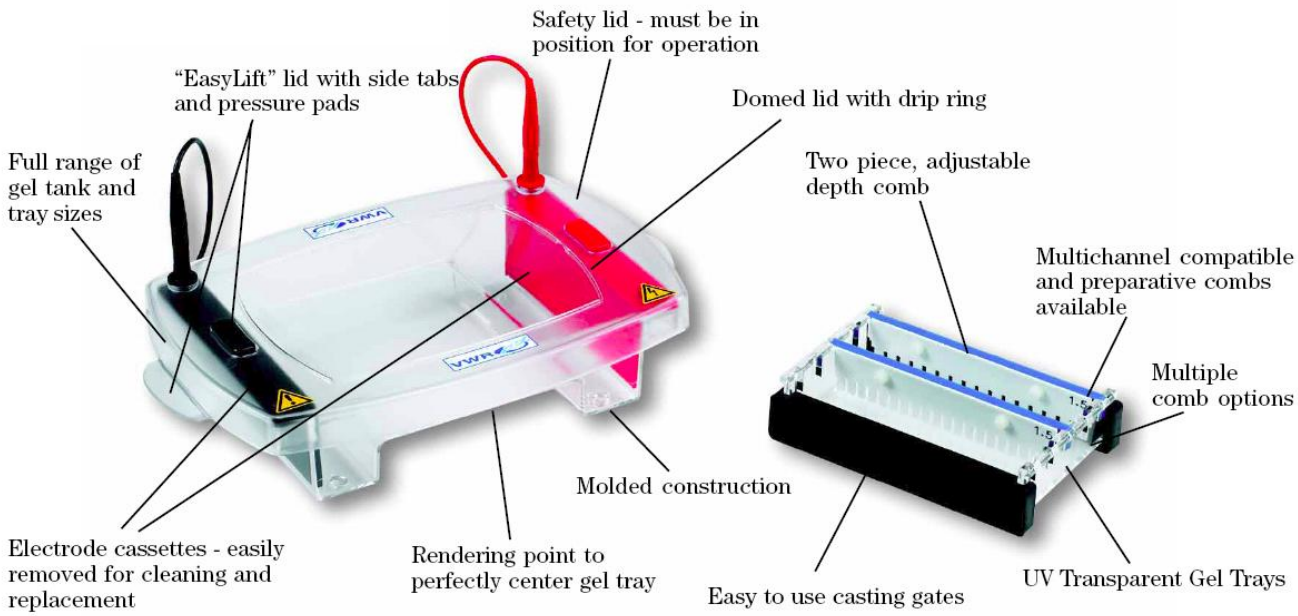
This demo display can be used to reach kids barely able to see over the table through to their great grandparents. When you are done the day, by counting pipette tips missing in the box, you will have a count of the number of people who did the activity. Remember to keep your volunteer tips out of that calculation. Besides the people who pipette, there are likely the same number again who will not, but will listen to everything you say, effectively doubling the number of people impacted by your presentation. [At UofC, we have done around 100 people / hour and more than 1000 in one day at our campus open-house.]



Note Irene in the background here showing how to isolate DNA.

Appendix 1. VWR gel boxes and pouring gels

LTS National Kits have the VWR gel boxes in use. This figure shows how the gel casting gates are assembled as well as the gel rig itself.



For those unfamiliar with the use of gel equipment and casting gels, please see the powerpoint that has pictures and explanations of all the steps in preparing the gels for the LTS workshops. A copy of that powerpoint has been included with this manual as a PPT Script file.

🛠Amount and type of materials to prepare

Gels will be recycled (used for more than one class, big event, or potentially sent to another site?)

How many kids at once?	Number of practice gels	Amount of agarose to water	Number of "real" TAE gels	Number of combs to use in TAE gels	Amount of agarose to TAE
8	1 Cut into 4	1.8-2 gm/120 mL water	1	1	1.8-2 gm/120 mL TAE
16	2 Cut into 8	3.6-4 gm/240 mL water	1	2	1.8-2 gm/120 mL TAE
24	3 Cut into 12	Both of above Cast 2 then 1	2	1 in one gel 2 in one gel	3.6-4 gm/240 mL TAE
32	4 Cut into 16	3.6-4 gm/240 mL water X2	2	2 in each	3.6-4 gm/240 mL TAE
36-40	Same as for 24 – have kids work in teams of 3-4		Same as for 32		
Mall version Whole day	8 or more Cut into 4s	3.6-4 gm/240 mL water each	4	2 in each	3.6-4 gm/240 mL TAE X2
☠If using the 250 mL flask in the kit, do not double up the volume. ×Use 500 mL flask. These guidelines are for 10x15 cm gels in the VWR gel boxes					

Gels will NOT be recycled

How many kids at once?	Number of practice gels	Amount of agarose to water	Number of "real" TAE gels	Number of combs to use in TAE gels	Amount of agarose to TAE
8	1 Cut into 4	1-1.2 gm/100 mL water	1	1	1-1.2 gm/100 mL TAE
16	2 Cut into 16	2-2.4 gm/200 mL water	1	2	1-1.2 gm/100 mL TAE
24	3 Cut into 24	Both of above Cast 2 then 1	2	1 in one gel 2 in one gel	2-2.4 gm/200 mL TAE
32	4 Cut into 32	2-2.4 gm/200 mL water X2	2	2 in each	2-2.4 gm/200 mL TAE
36-40	Same as for 24 – have kids work in teams of 3-4		Same as for 32		
☠If using the 250 mL flask in the kit, do not double up the volume. ×Use 500 mL flask. These guidelines are for 10x15 cm gels in the VWR gel boxes					

Appendix 2. Reagent preparation for kits supplies

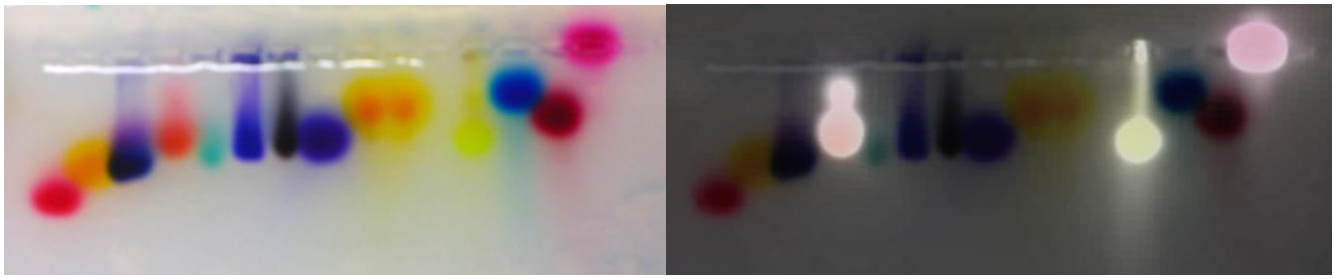
SAFETY PRECAUTIONS when working with powdered dyes. See Appendix 4 for MSDS. Wear laboratory gown, gloves, respiratory mask and eye protection. Avoid creating dust.

1. Stock and Diluted Practice Dye. Practice dye is used to show students how to load a gel. See page 17 and 18. Practice dye is used to teach a) pipetting, b) discovering that the loading dye's purpose is to make the DNA sink and c) practice loading practice gels.
   a. Stock supply in kit is 6X loading dye. This is usually 40% sucrose + 0.25% w/v of any negative or positive dye. In the current kits, this dye may be a mixture of stocks of various dyes that used to make the dye samples (see below) and is a good way to get rid of dyes that are left over from manufacturers.
   b. Dilute the stock dye as 1 part dye plus 5 parts water.
   c. Refill the 1.5 ml microfuge tubes with diluted dye.

2. Dye Samples. Before spending a lot of money on powdered dyes as listed below and dealing with the safety handling of dye prep, check if stocks are available at UCalgary. Most of the dyes listed were donated to LTS and dye stocks can be made available to other chapters. Dye samples are used instead of real DNA to demo the running of the gel electrophoresis. Each sample has a black dye plus 2-3 other colours. The black dye does not always hide the other colours, but often the mixture of colours belies the combination that was actually in the gel.
   a. Make a large stock of 10% sucrose loading dye by dissolving 100 grams of sucrose per 1L. Dissolve the sucrose in 500 mL. Heat 488 mL water (microwave or hotplate) and add to the first 500 mL + sucrose. Shake or stir with a magnetic stir bar. Add 10 mL of 2% Sodium Azide to prevent bacterial growth if dye samples may be stored for a very long time before being used. Add 20 mL of 50XTAE. Mix.
   b. Aliquot sucrose solution to 50 mL tubes or 100 mL bottles.
   c. Add 1 gm of most dyes per 100 mL of sucrose solution. Lighter coloured dyes should have a larger amount added (~2 gm) and darker dyes should have less (~0.8 gm). See table below for amounts, and position on a 1-1.2% agarose gel.
   d. Make a larger stock (500 mL) of black dye (Chlorazol Black or Naphthol BlueBlack).
   e. Run 10 μL of each dye in a lane of a 1-1.2% agarose gel until the fastest running dye, Ponceau S reaches the bottom or positive end of the gel.
   f. Examine each dye for intensity and add extra sucrose solution so that all dyes produce "bands" of approximately the same size and intensity. One gel is sufficient to keep testing the dyes by continuously running it so that previous loads of dyes run into the buffer chamber.
   g. If the position of the black dye is considered middle, then the dyes land slower or above the black, near the black dye or, faster and below the black dye.
   h. Mix equal amounts of black and one colour from above and one from below. The middle colours can also be added as desired. As each stock dye is around 1-2% to start, diluting each dye with another one leaves you with dye samples of ~0.25% to ~0.33% in 1% sucrose. The sucrose concentration is dilute enough that no further dilutions are required.
   i. Check the relative intensities and banding patters with an additional load of 10 μL of each dye sample mix on the 1-1.2% agarose gel. Adjust any dyes where one colour is fainter than the others by adding additional dye stock of that colour. Alternately, dilute the stock with additional sucrose solution if all dyes are too intense. See the gel below.
   j. Aliquoit dye sample mixes to 1.5 or 2 mL microfuge tubes and label with a number to indicate which sample is which and record the mix to check.

3. Dye Ladder. The dye ladder is a mixture of the various dyes that do produce a ladder like a DNA ladder will and does not contain 2 or more dyes that run in the same position. For each set of 20 or so of dye samples, prepare 4 tubes of dye ladder as that will be run with each gel.

Dye or Indicator	Position	Colour	Notes: Position given as top (nearest to wells) to bottom (farthest from wells).	Sigma order number
Acridine Red	0		Fluoresces red under UV and blue light. Very slightly positive.	A23609-5G
Xylene Cyanol	1		Use less dye powder	X4126-10G
Metanil Yellow	2			202029-100G
Cresol Red	3			114480-5G
Fluorescein	4		Fluoresces green/yellow under UV and blue light	46960-25G-F
Eosin Y	5		Fluoresces red under UV and blue light	E6003-25G
BromThymol Blue	5		Bluey purple in TAE	B0126-25G
Naphthol BlueBlack	5		Dark blue black	N3393-25G
Chlorazol Black E	5		True black	C1144-25G
Coomassie Blue	5		Use less dye powder	B0149-25G
Chlorphenol Red	5		True violet	199524
Light Green SF	6		Much greener in higher concentration	L5382-10G
Trypan Blue	7		Use less dye powder	T6146-25G
Orange G	8		Add extra dye powder	O3756-25G
Chromeazurol S	9		Add double dye powder	199532-25G
Ponceau S	10		Runs about 50 basepairs	P3504-10G



White light illuminated dye gel UV illuminated dye gel overlaid on previous picture.
In order: 10, 8, 7, Eosin, 6, Coomassie, Black, Bromphenol blue, 2, 4, 1, 3, 0.

White light UV light

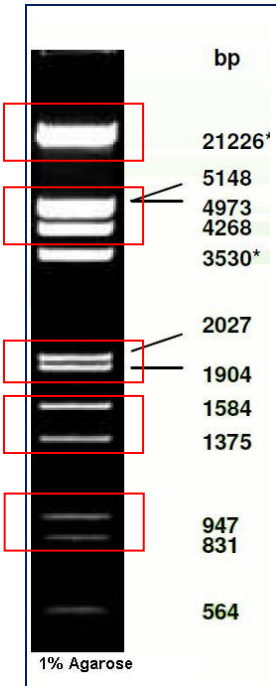
Dye mixtures tested. Note the bottom row 5 rightmost lanes. These dye mixtures are less concentrated than the other 25. Those were diluted with a stock sucrose solution prior to aliquoiting.

4. SYBRSafe (Invitrogen). SYBRSafe (Invitrogen) is a friendly DNA dye. It has none of the disposal issues of Ethidium Bromide or other Sybr products. SYBRSafe is purchased as a 10,000X concentrate, meaning you can add 10 μL to a 100 mL gel. For LTS use, dilute the SYBR Safe 1/100 with 1xTAE to add directly to DNA samples at 1/50 to 1/100. SYBR Safe is stable at room temperature as well as stable diluted TAE. See MSDS in Appendix 4.

5. PCR samples. One of the easiest ways to have PCR samples that students can load or that you load prior to a lab or mall demo can be done by collecting PCR samples from researchers who would otherwise throw them out. This includes collecting real-time or kPCR plates, or other kPCR tubes. Samples of similar band size can be pooled. For example, one lab we use is a clinical lab that runs a PCR daily that yields a 450 bp amplicon. This pool can be mixed with another pool that has a 250 bp amplicon thus replicating the DNA WhoDunnit? results that have two bands/ sample. For most workshops and because we have covered interpretation before they see their gel, the students do not care what they see, so long as they see DNA in the gel.
    a. Pooled PCR samples with loading dye already added: When you have pooled products of similar size, determine the volume you have roughly. Add 2 uL of SYBR Safe / 100 uL of PCR products. Samples are now ready to use. You may wish to do a test gel with 5 μL of sample / lane to determine if you need to load a larger volume. Note, some loading dyes quench the SYBRSafe – you may need to use more SYBRSafe or add SBYRSafe more than once if keeping the PCR pool for several days.
    b. kPCR samples from a plate or tube. If the reaction used SYBR Green, you may not need to add much more SYBR Safe to the pooled samples. Add 20 μL loading dye /100μL of pooled samples. You may wish to do a test gel with 5 μL of sample / lane to determine if you need to load a larger volume or need to add 1 μL SYBR Safe / 100 μL of PCR samples.

    For both situations above, the PCR products should be stored at 4°C.

6. 50X TAE buffer was purchased premade.



7. Prepared samples. It is possible to prepare fake PCR products by gel purifying bands from DNA ladders. While any DNA ladder can be used, lambda DNA cut with HindIII produces several intense bands that can be easily purified and represent the double band pattern in the DNA WhoDunnit? lab. In the picture here, suggested gel blocks for purification are shown. Advantage to having both large and smaller fragments is the advantage of being able to time your gel based on the lab time you have. For example, if you need to rush the gel run, you can use small size "PCR" products to ensure maximum separation in shortest time. If you have to walk away from the gel and run the risk of losing smaller bands to the buffer, then choose larger fragments for your PCR. Once you have the gel bands purified, pool them and check on a gel. You can aliquot sufficient to a tube to 100μL, vacuum or air dry them and keep/store/ship them at room temperature. Reconstitute with TAE, SybrSafe and loading dye as described above.

Lambda HindIII DNA marker with bands sizes in base pairs. The red boxes indicate gel blocks that can be used for gel purification preparation of fake PCR products. The largest piece, 21226 bp is a good sample to keep to ensure that DNA is seen in the gel even if it is not paired with another band. It can be paired with the 3530 bp band if you choose. It is difficult to separate the 4268, 4973 and 5148 bp bands, so the three are prepared together.

8. Gel purification kits: Protocol as provided by manufacturer. For the lambda fragments purified and supplied with the biotech kit this year, the kits used were obtained from VWR and manufactured by GE Life Sciences. The kits had expired and were unable to be sold and were donated to LTS for this project.

Appendix 3: MSDS and other Safety Information about kit contents

SAFETY PRECAUTIONS when working with powdered dyes.
Wear laboratory gown, gloves, respiratory mask and eye protection. Avoid creating dust.
The list of MSDS for the individual dyes in Appendix 1 is too long for this manual. See the separate file of MSDS sheets for one for each dye.

SYBR Safe Safety FAQs from http://www.invitrogen.com

How should I dispose of SYBR® Safe DNA gel stain?
Some institutions and municipalities have approved the disposal of SYBR® Safe DNA gel stain directly into their wastewater systems. However, disposal regulations vary—please contact your safety office or local municipality for disposal guidelines.

Is SYBR® Safe DNA gel stain really safe? Do I have to use gloves when I use it?
In numerous tests carried out by independent, licensed testing laboratories, SYBR® Safe DNA gel stain showed little or no genotoxicity and no acute toxicity. This stain is not classified as hazardous waste under US federal regulations; nevertheless, please exercise common safe laboratory practices when using this reagent.

Is SYBR® Safe DNA gel stain the same as SYBR® Green I dye?
All SYBR® dyes have similar spectral properties, but have different chemical compositions. SYBR® Safe DNA gel stain was specifically developed as a safer alternative to ethidium bromide. SYBR® Green I is an ultrasensitive stain for dsDNA, and SYBR® Green II is a highly sensitive stain for RNA and ssDNA. All SYBR® dyes are optimally excited by the Safe Imager™ blue-light transilluminator.

Can agarose gels be cast with SYBR® Safe DNA gel stain in them?
Yes. Simply substitute a SYBR® Safe DNA gel stain solution for the buffer when preparing the molten agarose. If using the 10,000X SYBR® Safe DNA gel stain concentrate, dilute the concentrated stain 1:10,000 in agarose gel buffer (e.g., 1X TBE or 1X TAE) and add the buffer/stain solution to the powdered agarose. The agarose/SYBR® Safe DNA gel stain mixture may be heated briefly in the microwave.

How should SYBR® Safe DNA gel stain spills be cleaned up?
Water and 70% ethanol should get rid of most stains from spills. For persistent stains, bleach may be used. Use a handheld UV light in the darkroom to check for any remaining stain.

How do I view DNA stained with SYBR® Safe DNA gel stain?
DNA stained with SYBR® Safe DNA gel stain can be viewed using a blue light transilluminator such as Invitrogen's Safe Imager™ instrument, or a standard UV transilluminator. If you plan to use the DNA for cloning, avoid exposing DNA stained with SYBR® Safe DNA gel stain to UV light.

What are the advantages of using blue light to view DNA stained with SYBR® Safe DNA gel stain?
Unlike UV light, blue light causes minimal damage to DNA and is therefore safer for you and better for your DNA sample. Use of SYBR® Safe DNA gel stain and the Safe Imager™ blue-light transilluminator gives improved cloning efficiency over DNA stained with ethidium bromide and exposed to UV light.

Where can I find more safety information about SYBR® Safe DNA gel stain?
Our website (www.invitrogen.com/SYBR Safe) contains all of our latest testing results.

Molecular Probes
invitrogen detection technologies

Material Safety Data Sheet

Revision Date: 22-Mar-2005

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code S33100
Product name SYBR Safe™ DNA gel stain in 0.5X TBE

Company/Undertaking Identification

MOLECULAR PROBES, INC.
29851 Willow Creek Road
Eugene, OR 97402
U.S.A.
++1 541 465 8300

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	EINECS-No.	Weight %	Classification
water	7732-18-5	231-791-2	> 96.9	-
SYBR Safe dye			< 0.1	-
TRIS Base	77-86-1	201-064-4	< 1	-
boric acid	10043-35-3	233-139-2	< 1	-
ethylenediaminetetraacetic acid, tetrasodium salt	64-02-8	200-573-9	< 1	-

3. HAZARDS IDENTIFICATION

The product is classified and labelled in accordance with Directive 1999/45/EC

Classification

Indication of danger
Not hazardous

R -phrase(s)
None

4. FIRST AID MEASURES

Skin contact Wash off immediately with plenty of water
Eye contact Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion Never give anything by mouth to an unconscious person
Inhalation Move to fresh air
Notes to physician Treat symptomatically

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media Dry chemical
Special protective equipment for firefighters Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions Use personal protective equipment
Methods for cleaning up Soak up with inert absorbent material

7. HANDLING AND STORAGE

Handling No special handling advice required
Storage Store at room temperature in the original container.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Exposure limits

Chemical Name	EU OEL (TWA)	EU OEL (STEL)	EU Skin Notation
water	-	-	-
SYBR Safe dye	-	-	-
TRIS Base	-	-	-
boric acid	-	-	-
ethylenediaminetetraacetic acid, tetrasodium salt	-	-	-

Chemical Name	Austria OEL (MAK)	Belgium (TWA)	Denmark (TWA)	Finland OEL (TWA)
water	-	-	-	-
SYBR Safe dye	-	-	-	-
TRIS Base	-	-	-	-
boric acid	-	-	-	-
ethylenediaminetetraacetic acid, tetrasodium salt	-	-	-	-

Chemical Name	France OEL (VME)	Germany OEL (TWA)	Ireland (TWA)	Italy OEL (TWA)
water	-	-	-	-
SYBR Safe dye	-	-	-	-
TRIS Base	-	-	-	-
boric acid	-	-	-	-
ethylenediaminetetraacetic acid, tetrasodium salt	-	-	-	-

Chemical Name	Netherlands OEL (MAC)	Spain OEL (TWA)	United Kingdom
water	-	-	-
SYBR Safe dye	-	-	-
TRIS Base	-	-	-
boric acid	-	-	-
ethylenediaminetetraacetic acid, tetrasodium salt	-	-	-

Engineering measures No special precautions required.

Personal protective equipment

Respiratory protection No special protective equipment required.
Hand protection Protective gloves.
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing
Hygiene measures Handle in accordance with good industrial hygiene and safety practice

Environmental exposure controls No special environmental precautions required

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** > 100 °C	**°F** > 212 °F
Autoignition temperature	**°C** No data available	**°F** No data available

Oxidizing properties Corrosivity, Method USEPA 150.1, Result: Not corrosive
Corrosivity, Method DOT-E 10904, Result: Not corrosive

Water solubility soluble

10. STABILITY AND REACTIVITY

Stability Stable.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
water	No data available	No data available	No data available
SYBR Safe dye	> 5,000 mg/kg Oral LD50 Rat	No data available	No data available
TRIS Base	5900 mg/kg Oral LD50 rat	No data available	No data available
boric acid	3450 mg/kg Oral mouse 2260 mg/kg Oral rat	No data available	No data available
ethylenediaminetetraacetic acid, tetrasodium salt	No data available	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes No information available.
Skin No information available.
Inhalation No information available.
Ingestion No information available.

Specific effects

Carcinogenic effects No information available.
Mutagenic effects Mutation Data: Cell transformation, Syrian hamster embryo (SHE) cells, negative; Forward gene mutation at the thymidine kinase (TK) locus, mouse lymphoma (L5178Y) cells, negative with and without metabolic activation; Chromosomal aberrations, human peripheral blood lymphocytes, negative with and without metabolic activation; Histidine reverse gene mutation, Ames assay, Salmonella typhimurium (TA97a, TA98, TA102, TA1538), positive with metabolic activation; Histidine reverse gene mutation, Ames assay, Salmonella typhimurium (TA97a, TA98, TA102, TA1538), negative without metabolic activation; Histidine reverse gene mutation, Ames assay, Salmonella typhimurium (TA100, TA1535, TA1537), negative with and without metabolic activation. .
Reproductive toxicity No information available.
Sensitization No information available.

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects Contains no substances known to be hazardous to the environment or not degradable in waste water treatment plants

Effects on waste water treatment plants
Chemical oxygen demand, 7020 ug/L (USEPA 410.1)
Ammonia as nitrogen, 253 ug/L (USEPA 350.1)
Total organic carbon, 2480 ug/L (USEPA 415.1)
Total phenolics, none detected (USEPA 420.1)
Organochlorine pesticides and PCBs, none detected (USEPA 608M)
Semi-volatile organic compounds, none detected (USEPA 625)
Volatile organic compounds, none detected (USEPA 624)
Priority Pollutant Metals, none detected (Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, Zn) (USEPA 200.7/200 series)

Aquatic toxicity Test Method: Fathead minnow CA Title 22 acute screening,
Result: Not hazardous or toxic to aquatic life

Mobility Completely soluble.
Biodegradation No information available.
Bioaccumulation Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

EWC waste disposal No No information available.

Dispose of in accordance with local regulations.

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

Indication of danger
Not hazardous

R -phrase(s)
None

S -phrase(s)
None

International Inventories

Chemical Name	EINECS	ELINCS	PICCS	ENCS
water	Listed	-	Listed	-
SYBR Safe dye	-	-	-	-
TRIS Base	Listed	-	Listed	Listed
boric acid	Listed	-	Listed	Listed
ethylenediaminetetraacetic acid, tetrasodium salt	Listed	-	Listed	Listed

Chemical Name	TSCA	CHINA	AICS	KECL
water	Listed	Listed	Listed	Listed
SYBR Safe dye	-	-	-	-
TRIS Base	Listed	Listed	Listed	Listed
boric acid	Listed	Listed	Listed	Listed
ethylenediaminetetraacetic acid, tetrasodium salt	Listed	Listed	Listed	Listed

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

SIGMA-ALDRICH

MATERIAL SAFETY DATA SHEET

Date Printed: 09/07/2010
Date Updated: 08/12/2010
Version 1.5

Section 1 - Product and Company Information

Product Name	GEL LOADING BUFFER
Product Number	G2526
Brand	SIGMA
Company	Sigma-Aldrich Canada, Ltd
Address	2149 Winston Park Drive Oakville ON L6H 6J8 CA
Technical Phone:	9058299500
Fax:	9058299292
Emergency Phone:	800-424-9300

Section 2 - Composition/Information on Ingredient

Substance Name	CAS #	SARA 313
GEL LOADING BUFFER	None	No

Ingredient Name	CAS #	Percent	SARA 313
BROMOPHENOL BLUE INDICATOR	115-39-9	0.05	No
SUCROSE	57-50-1	40	No
ETHYLENEDIAMINETETRAACETIC ACID	60-00-4	2.92	No
SODIUM DODECYL SULFATE	151-21-3	0.5	No
WATER	7732-18-5	56.53	No

Section 3 - Hazards Identification

HMIS RATING
HEALTH: 0
FLAMMABILITY: 0
REACTIVITY: 0

NFPA RATING
HEALTH: 0
FLAMMABILITY: 0
REACTIVITY: 0

For additional information on toxicity, please refer to Section 11.

Section 4 - First Aid Measures

ORAL EXPOSURE
If swallowed, wash out mouth with water provided person is conscious. Call a physician.

INHALATION EXPOSURE
If inhaled, remove to fresh air. If breathing becomes difficult, call a physician.

DERMAL EXPOSURE
In case of contact, immediately wash skin with soap and copious amounts of water.

EYE EXPOSURE
In case of contact with eyes, flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers. Call a physician.

Section 5 - Fire Fighting Measures

FLASH POINT
N/A

AUTOIGNITION TEMP
N/A

FLAMMABILITY
N/A

EXTINGUISHING MEDIA
Suitable: Water spray. Carbon dioxide, dry chemical powder, or appropriate foam.

FIREFIGHTING
Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Specific Hazard(s): Emits toxic fumes under fire conditions.

Section 6 - Accidental Release Measures

METHODS FOR CLEANING UP
Absorb on sand or vermiculite and place in closed containers for disposal. Ventilate area and wash spill site after material pickup is complete.

Section 7 - Handling and Storage

HANDLING
User Exposure: Avoid inhalation. Avoid contact with eyes, skin, and clothing. Avoid prolonged or repeated exposure.

STORAGE
Suitable: Keep tightly closed.

Section 8 - Exposure Controls / PPE

ENGINEERING CONTROLS
Safety shower and eye bath. Mechanical exhaust required.

PERSONAL PROTECTIVE EQUIPMENT
Respiratory: Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU). Respiratory protection is not required. Where protection is desired, use multi-purpose combination (US) or type ABEK (EN 14387) respirator cartridges.
Hand: Protective gloves.
Eye: Chemical safety goggles.

GENERAL HYGIENE MEASURES
Wash thoroughly after handling.

Section 9 - Physical/Chemical Properties

Appearance Physical State: Liquid

Property	Value	At Temperature or Pressure
pH	N/A	
BP/BP Range	N/A	
MP/MP Range	N/A	
Freezing Point	N/A	
Vapor Pressure	N/A	
Vapor Density	N/A	
Saturated Vapor Conc.	N/A	
Bulk Density	N/A	
Odor Threshold	N/A	
Volatile%	N/A	
VOC Content	N/A	
Water Content	N/A	
Solvent Content	N/A	
Evaporation Rate	N/A	
Viscosity	N/A	
Surface Tension	N/A	
Partition Coefficient	N/A	
Decomposition Temp.	N/A	
Flash Point	N/A	
Explosion Limits	N/A	
Flammability	N/A	
Autoignition Temp	N/A	
Refractive Index	N/A	
Optical Rotation	N/A	
Miscellaneous Data	N/A	
Solubility	N/A	

N/A = not available

Section 10 - Stability and Reactivity

STABILITY
Stable: Stable.
Materials to Avoid: Strong oxidizing agents.

HAZARDOUS DECOMPOSITION PRODUCTS
Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide.

HAZARDOUS POLYMERIZATION
Hazardous Polymerization: Will not occur

Section 11 - Toxicological Information

ROUTE OF EXPOSURE
Skin Contact: May cause skin irritation.
Skin Absorption: May be harmful if absorbed through the skin.
Eye Contact: May cause eye irritation.
Inhalation: Material may be irritating to mucous membranes and upper respiratory tract. May be harmful if inhaled.
Ingestion: May be harmful if swallowed.

SIGNS AND SYMPTOMS OF EXPOSURE
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Section 12 - Ecological Information

No data available.

Section 13 - Disposal Considerations

APPROPRIATE METHOD OF DISPOSAL OF SUBSTANCE OR PREPARATION
Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. Observe all federal, state, and local environmental regulations.

Section 14 - Transport Information

DOT
Proper Shipping Name: None
Non-Hazardous for Transport: This substance is considered to be non-hazardous for transport.

IATA
Non-Hazardous for Air Transport: Non-hazardous for air transport.

Section 15 - Regulatory Information

UNITED STATES REGULATORY INFORMATION
SARA LISTED: No

CANADA REGULATORY INFORMATION
WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR, and the MSDS contains all the information required by the CPR.
DSL: No
NDSL: No

Section 16 - Other Information

DISCLAIMER
For R&D use only. Not for drug, household or other uses.

WARRANTY
The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Inc., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

SIGMA-ALDRICH

sigma-aldrich.com

Material Safety Data Sheet

Version 5.0
Revision Date 06/22/2010
Print Date 09/07/2010

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Sodium azide
Product Number	:	S2002
Brand	:	Sigma-Aldrich
Company	:	Sigma-Aldrich Canada, Ltd 2149 Winston Park Drive OAKVILLE ON L6H 6J8 CANADA
Telephone	:	+19058299500
Fax	:	+19058299292
Emergency Phone #	:	1-800-424-9300

2. HAZARDS IDENTIFICATION

Emergency Overview

Target Organs

Heart, Central nervous system, Brain.

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by ingestion Highly toxic by skin absorption

GHS Label elements, including precautionary statements

Pictogram

Signal word: Danger

Hazard statement(s)

H300 + H310	Fatal if swallowed or in contact with skin.
H400	Very toxic to aquatic life.

Precautionary statement(s)

P264	Wash hands thoroughly after handling.
P273	Avoid release to the environment.
P280	Wear protective gloves/protective clothing.
P302 + P350	IF ON SKIN: Gently wash with plenty of soap and water.
P310	Immediately call a POISON CENTER or doctor/physician.

HMIS Classification

Health hazard:	4
Chronic Health Hazard:	*
Flammability:	0
Physical hazards:	0

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be fatal if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be fatal if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Formula : N_3Na
Molecular Weight : 65.01 g/mol

CAS-No.	EC-No.	Index-No.	Concentration
Sodium azide			
26628-22-8	247-852-1	011-004-00-7	-

4. FIRST AID MEASURES

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media
Carbon dioxide (CO2) Dry powder

Extinguishing media which shall not be used for safety reasons
Water

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Wear respiratory protection. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.

Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.

Methods and materials for containment and cleaning up
Pick up and arrange disposal without creating dust. Sweep up and shovel. Do not flush with water. Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Avoid contact with skin and eyes. Avoid formation of dust and aerosols.
Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.
Never allow product to get in contact with water during storage. Do not store near acids.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Components with workplace control parameters

Components	CAS-No.	Value	Control	Update	Basis

			parameters		
Sodium azide	26628-22-8	C	0.29 mg/m3	2007-07-06	Canada. British Columbia OEL
		C	0.11 ppm	2007-07-06	Canada. British Columbia OEL
		CEV	0.1 ppm 0.26 mg/m3	2005-12-17	Canada. Ontario OELs
		(c)	0.11 ppm 0.3 mg/m3	2009-04-30	Canada. Alberta, Occupational Health and Safety Code (table 2: OEL)
		C	0.11 ppm 0.3 mg/m3	2006-12-29	Canada. Quebec OELs
Remarks	A substance which may not be recirculated in accordance with section 108				
		(c)	0.29 mg/m3	2009-04-30	Canada. Alberta, Occupational Health and Safety Code (table 2: OEL)

Personal protective equipment

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face particle respirator type N100 (US) or type P3 (EN 143) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
Complete suit protecting against chemicals, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	crystalline
Colour	white

Safety data

pH	10 at 65 g/l at 25 °C (77 °F)
Melting point	275 °C (527 °F)
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	0.01 hPa (0.01 mmHg) at 20 °C (68 °F)
Density	1.850 g/cm3
Water solubility	65 g/l at 20 °C (68 °F) - completely soluble

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Conditions to avoid
An explosion occurred when a mixture of sodium azide, methylene chloride, dimethyl sulfoxide, and sulfuric acid were being concentrated on a rotary evaporator.

Materials to avoid
Halogenated hydrocarbon, Metals, Acids, Acid chlorides

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Sodium oxides

Thermal decomposition
300 °C

11. TOXICOLOGICAL INFORMATION

Acute toxicity
LD50 Oral - rat - 27 mg/kg

LC50 Inhalation - rat - 37 mg/m3
Remarks: Sense Organs and Special Senses (Nose, Eye, Ear, and Taste):Eye:Other. Behavioral:Convulsions or effect on seizure threshold. Lungs, Thorax, or Respiration:Structural or functional change in trachea or bronchi.

LD50 Dermal - rabbit - 20 mg/kg

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

Reproductive toxicity
no data available

Specific target organ toxicity - single exposure (GHS)
no data available

Specific target organ toxicity - repeated exposure (GHS)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be fatal if swallowed.
Skin	May be fatal if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Additional Information
RTECS: VY8050000

12. ECOLOGICAL INFORMATION

Toxicity

Toxicity to fish	LC50 - Lepomis macrochirus - 0.68 mg/l - 96.0 h
Toxicity to daphnia and other aquatic invertebrates.	EC50 - Daphnia pulex (Water flea) - 4.2 mg/l - 48 h

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Very toxic to aquatic life.

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material. Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN-Number: 1687 Class: 6.1 Packing group: II
Proper shipping name: Sodium azide
Reportable Quantity (RQ): 1000 lbs
Marine pollutant: No
Poison Inhalation Hazard: No

IMDG
UN-Number: 1687 Class: 6.1 Packing group: II EMS-No: F-A, S-A
Proper shipping name: SODIUM AZIDE
Marine pollutant: No

IATA
UN-Number: 1687 Class: 6.1 Packing group: II
Proper shipping name: Sodium azide

15. REGULATORY INFORMATION

DSL Status
All components of this product are on the Canadian DSL list.

WHMIS Classification

D1A	Very Toxic Material Causing Immediate and Serious Toxic Effects	Highly toxic by ingestion Highly toxic by skin absorption

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

Material Safety Data Sheet

SECTION 1 - IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND COMPANY:

Identification Of The Substance/Preparation:

Trade Name: AccuGENE® TAE Buffer, 50X
Chemical Name: Aqueous solution
Synonym: TAE Buffer
Canada PIN: None

Use Of The Substance: For R&D/experimental use only

Note: 50X TAE stock in use by LTS biotech kits varies varies in its source. Some was made at UofC, some purchased from BioRad, some from BioBasics. This MSDS was chosen to represent TAE from the various sources.

Company Identification:

Lonza Rockland, Inc.
191 Thomaston Street
Rockland, ME 04841
USA
Business Telephone: (207) 594-3400
Emergency Telephone: 703-527-3887 (US: 800-424-9300)

SECTION 2 - HAZARDS IDENTIFICATION:

Emergency Overview: Clear, colorless liquid. Can cause irritation to eyes, skin, and respiratory system.

Health Effects:

Inhalation: Mists may cause irritation to the upper respiratory system. Symptoms may include coughing and scratchy throat.
Skin: Can cause skin irritation. Symptoms may include redness, rash, and itching.
Eyes: Can cause eye irritation. Symptoms may include redness, tearing, and pain.
Ingestion: May cause nausea and discomfort.
Chronic Effects/Target Organ Effects: None known
Medical Conditions Aggravated By Exposure: None known

Environmental Effects: None known

SECTION 3 - COMPOSITION/INFORMATION ON INGREDIENTS:

Components	EINECS #	CAS #	Weight%	EU Classification
2-amino-2-(hydroxymethyl)-1,3-propanediol (TRIS)	201-064-4	77-86-1	10-15	Xi, R36/37/38
acetic acid	200-580-7	64-19-7	6	R10; C:R35
disodium EDTA dihydrate	205-358-4 (anhydrous form)	6381-92-6	5	Xn: R22; Xi: R36/37/38

SECTION 4 - FIRST AID MEASURES:

Inhalation: Follow standard care for a respiratory emergency as necessary. Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention immediately.
Skin: Follow standard care for skin contact as needed. Immediately wash thoroughly with soap and water. Remove contaminated clothing and shoes. Get medical attention if symptoms develop. Wash clothing and shoes before reuse, or discard in a manner which limits further exposure.
Eye: In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention immediately if irritation persists.
Ingestion: If swallowed, get medical attention immediately. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person.

Material Safety Data Sheet

SECTION 5 - FIRE FIGHTING MEASURES:

Extinguishing Media: Dry chemical, fire-fighting foam, water spray, or carbon dioxide based on surrounding materials.
Special Exposure Hazards: This product is water-based. Under fire conditions organic residues remaining after the water evaporates is expected to burn generating toxic and irritating gases and smoke. Evacuate area and fight fire from safe distance.
Special Protective Equipment: Wear approved pressure-demand self-contained breathing apparatus and full protective gear.

SECTION 6 - ACCIDENTAL RELEASE MEASURES:

Personal: Prevent skin/eye contact. Use personal protective equipment as needed. Isolate spill area, preventing entry by unauthorized persons.
Environmental Precautions: Minimize entry of material into sewers and drainage systems. Refer to permit discharge limitations if applicable.
Methods For Cleaning Up: Clean up spills immediately, observing precautions in the material safety data sheet and label. Use absorbent materials if needed and dispose into a chemical waste container.

SECTION 7 - HANDLING AND STORAGE:

Handling: Use with adequate ventilation as necessary. Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Follow all MSDS/label precautions. Follow same precautions when handling packaging that may contain product residues. Avoid contact with skin and eyes.
Storage: Keep container tightly closed when not in use. Store at 18-24°C.
Specific Uses: For R&D/experimental use only

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION:

Exposure Limit Values: Acetic acid: OSHA PEL/TWA: 10 ppm; ACGIH TLV/TWA: 10 ppm, TLV/STEL: 15 ppm
Exposure Controls: Use process enclosures, local exhaust ventilation, or other engineering controls as needed.
Respiratory Protection: Use an approved air-purifying respirator as needed. Consult with respirator manufacturer to determine respirator selection, use, and limitations.
Eye/Face Protection: Use safety glasses. Where contact with the eyes is likely, use chemical goggles.
Skin/Hand Protection: Use chemical gloves as needed. Use clean protective body covering clothing as needed to minimize contact with clothing and skin.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES:

Appearance:	Clear to colorless aqueous solution.
Odor:	None
Flash point:	Not available
Viscosity:	Not available.
Solubility:	Miscible in water
Density (water = 1): ..	Not determined
Partition coefficient: .	Not available
Boiling point:	~100°C
Melting point:	Not available
pH:	7~9
Other:	Not available.

SECTION 10 - STABILITY AND REACTIVITY:

Stability: Stable.
Conditions To Avoid: Avoid contact with strong oxidizers.
Materials To Avoid: Strong oxidizers.
Hazardous Decomposition Products: Oxides of carbon and nitrogen.

Material Safety Data Sheet

SECTION 11 - TOXICOLOGICAL INFORMATION:

2-Amino-2-(hydroxymethyl)-1,3-propanediol (TRIS): Oral LD50: 5900 mg/kg (rat)
Acetic Acid: Oral LD50: 3310 mg/kg (rat); Inhalation LC50: 11.4 mg/L/1H (rat); Dermal LD50: 1060 ug/kg (rabbit); Skin irritation: 50 mg/24H mild (rabbit)
Disodium EDTA Dihydrate (anhydrous form): Oral LD50: 2000 mg/kg (rat)

SECTION 12 - ECOLOGICAL INFORMATION:

Ecotoxicity: Acetic Acid: Freshwater Fish LC50: 88 mg/L/96H [static] (*P. promelas*), LC50: 75 mg/L/96 H (*L. macrochirus*); Microtox EC50: 8.8 mg/L; 5, 15, and 25 min (*P. phosphoreum*); Water Flea EC50: 95 mg/L/24H (*D. magna*).
Mobility: Acetic acid: Evaporation from dry surfaces is likely to occur. When spilled on soil, the liquid will spread on the surface and penetrate into the soil at a rate dependent on the soil type and its water content. Natural waters will neutralize dilute solutions to acetate salts.
Persistence And Degradability: Acetic acid: If released to water or soil, acetic acid will biodegrade readily. If released to the atmosphere, it is degraded in the vapor-phase by reaction with photochemically produced hydroyxl radicals (estimated typical half-life of 26.7 days). It occurs in atmospheric particulate matter in acetate form and physical removal from air can occur via wet and dry deposition.
Bioaccumulative Potential: Acetic acid: Acetic acid shows no potential for biological accumulation or food chain contamination.

SECTION 13 - DISPOSAL CONSIDERATIONS:

Dispose of unused product, residues, and containers according to local, regional, state, and national regulations.

SECTION 14 - TRANSPORT INFORMATION:

Not expected to be hazardous for transport

SECTION 15 - REGULATORY INFORMATION:

US Regulations:
This product meets the following SARA 311/312 hazard categories: acute.
This product contains the following SARA 302 Extremely Hazardous Substances: none.
This product contains the following SARA 313 Toxic Chemicals: none.
This product contains the following CERCLA Hazardous Substances: Acetic acid: 5,000 lbs.
Components of this product are listed on the TSCA inventory.
EU Regulations:
This preparation meets the dangerous preparations classification criteria under 1999/45/EC.
Xi: R36/37/38: Irritating to eyes, respiratory system and skin.
The following safety phrases apply:
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S37/39: Wear suitable gloves and eye/face protection.
Components of this product are listed on EINECS.
Canadian Regulations:
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and it contains the information required by the CPR.
This product meets WHMIS classification criteria D2B.
Components of this product are listed on the DSL.

Material Safety Data Sheet

SECTION 16 - OTHER INFORMATION:
NFPA/HMIS Ratings: Health = 2; Fire = 1; Reactivity = 0; NFPA/Special = None
Other Information: None
R-phrases used in section 2, but not listed section 15
R10: Flammable
R22: Harmful if swallowed.
R35: Causes severe burns.
MSDS Revisions: August 2008: Rev. 2.0

NOTE: This information is furnished without warranty, expressed, or implied, except that it is accurate to the best knowledge of Lonza Rockland, Inc. The data on this sheet relates only to the specific material designated herein. Lonza Rockland, Inc. assumes no legal responsibility for the use or reliance upon these data.

Agarose
Material Safety Data Sheet

Agarose used in LTS biotech kits comes from several different suppliers. The product is the "general use" agarose described here.

Ambion has evaluated all of its products to ensure compliance with OSHA regulations. Per section 1910.1200 of Title 29CFR, the following products are not considered hazardous and do not require separate material safety data sheets.

Agarose-LE (general use) 9040/9042 **Agarose HR (high resolution) 9044/9046**
Agarose-LM (low melt) 9048/9050

PHYSICAL DATA (Information in order Agaorse-LE, (Agarose-HR), ((Agarose-LM))

Appearance and Odor	White Crystalline Powder
Melting Point at 1.5% gel mix	88°C (66.8°C) ((65.2))
Specific Gravity	1.7
Gel Strength (Nikan)	3220 g/cm^2 (880 g/cm^2) ((870 g/cm^2))
Gel Strength (Cherry-Burrel)	1830 g/cm^2 (390 g/cm^2) ((410 g/cm^2))
pH in solution at 1.5% gel mix	6.6 (6.3) ((5.8))
Solubility in H_2O	Soluble in hot water

FIRE AND EXPLOSION HAZARD DATA (Information for all Agaroses)

Flash Point (test method)	205°C (auto ignition temp.)
Flammable Limits in Air(% by Volume)	LEL 0.4 oz/FT^3
Extinguishing Media	Media suitable to extinguish the surrounding fire such as H_2O or CO_2
Special Fire Fighting	Use Respirator, self contained breathing apparatus
Unusual Fire/Explosion Hazards	N/A

HEALTH HAZARD DATA

Effects of Overexposure	Excessive dust may be irritating to shin and eyes
Emergency First Aid Procedures	Wash affected area with water. Irrigate eyes for at least 15 minutes. If ingested, induce vomiting. Notify physician immediately.

REACTIVITY DATA

Stability	Stable
Incompatibility	None Known
Hazardous Decomposition Products	N/A
Hazardous Polymerization	Will not occur.

SPILL OR LEAK PROCEDURES

If released or spilled	Ventilate area. Prevent dust buildup. Place in container with lid. Clean area thoroughly
Waste Disposal Method	Dispose according to federal and local regulations

SPECIAL PROTECTION INFORMATION

Respiratory Protection	Not expected to require personal respirator usage. (NIOSH approved respirator if necessary)
Ventilation	Not expected to require any special ventilation. General lab hood if necessary.
Protective Gloves	Laboratory aprons and gloves. Store dessicated in freezer.
Eye Protection	Use general eye protection-goggles
Precautionary Labeling	None

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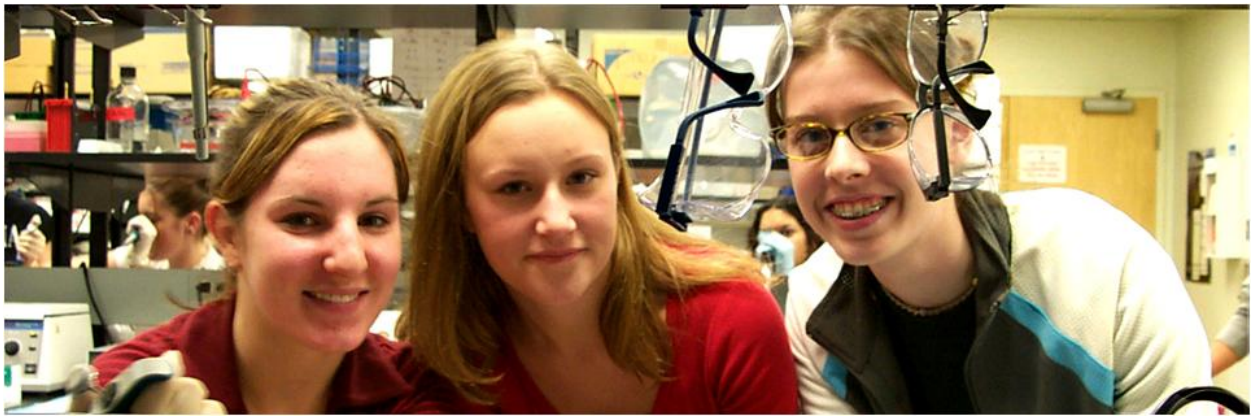
Appendix 4: DNA WhoDunnit? homework

In development. See CurioCity! In early 2011, some DNA WhoDunnit? homework should be showing up in CurioCity. Be sure to link your teachers to CurioCity for your high school classes.

Appendix 5: Additional Materials

The following pages provide materials for photocopying for your centre including:

DNA Isolation at home – assumes you have tubes to give away. Try to find if your university has a mechanism for "free to a good home" type of service. Alternately, a box of plastic test tubes is often possible to get by asking your local sales reps from VWR or Fisher to donate one.



Take home DNA Isolation (with tubes◆ not supplied – you may get them donated by suppliers or lab). Print 4 recipes/landscape page and cut to separate.

How to Isolate DNA at Home

1 cup (250 mL) of cold water with 1/2 tsp (2 mL) of salt
1/4 cup (50 mL) of dishwashing liquid diluted 1/10 with water (5 mL dish soap + 45 mL water)
Rubbing alcohol (on ice or in freezer)

1. Mush a bit of banana or 1 strawberry in some of the salt water. Let the pieces settle. Strain with cheesecloth. Get the solution cloudy, not so you can't see through it. You should be able to see this card through the solution, but not read the words.
2. Put 1 part (1 mL) in your test tube.
3. Add 1 part (1 mL) of dish soap solution. Mix very gently by finger flicking the tube. Avoid bubbles. The solution should clear.
4. Add 2 parts (2 mL) of ice cold rubbing alcohol as a layer over top of the soap/salt/fruit solution. The DNA is the white bits that float up into the soap layer. It can be covered in bubbles and look snotty. DNA floats. Proteins denatured by the soap sink.
5. Catch the DNA with a glass rod or wooden skewer. You can let it dry.
6. To prove it is DNA, as compared to fried egg white as a denatured protein, add your DNA to clean water in a clean test tube. If it dissolves, it is DNA. If it is denatured protein, like egg white, it will stay white and solid.

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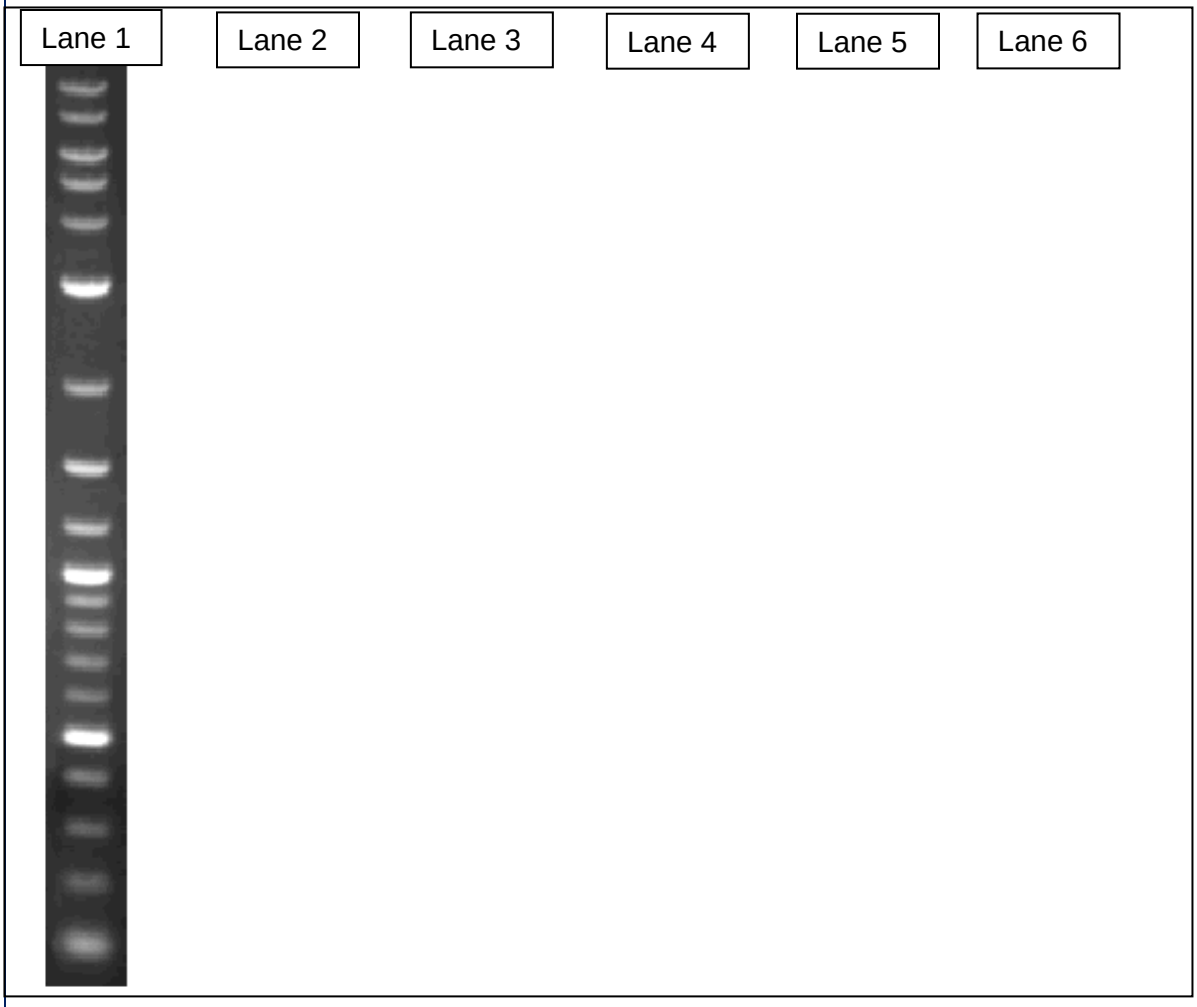
ACTIVITY SHEET: Gel Electrophoresis

In the space provided allow, draw the results of the gel electrophoresis using the “ladder” on the left to tell you where to draw the bands of DNA.
Gel Electrophoresis Analysis:



Fill in the information below with the sample contained in each of the lanes of your gel (victim, suspect 1...)

Lane 1: Ladder

Lane 2: _____

Lane 3: _____

Lane 4:_____

Lane 5:_____

Lane 6: _____

ELECTROPHORESIS BINGO CARD

B	I	N	G	O
What overall charge does a DNA molecule have?	Name three types of molecules that can be separated in a gel electrophoresis chamber	What size strands of DNA move more quickly in a gel?	What component of DNA is responsible for the charge on DNA?	True or false: Agarose is an extract of seaweed
What is the loading buffer used for?	What is the DNA ladder?	What does the acronym DNA stand for?	Other than gel electrophoresis what are other uses of agarose	The rate of DNA migration in a gel is dependent on what three factors?
Agarose is a polymer of what type of molecule? Sugar or Fat or Amino Acid	How is the powder of agarose converted into a gel?	**X**	What property of agarose makes it separate the DNA strands in an electric field?	What career do you want to do?
What are the three functional groups on the DNA molecule called?	How can one tell how large their DNA molecule is in a gel?	What direction does DNA migrate in an electrophoresis gel?	What is your name?	What are the names of the 4 DNA bases?
What chemical binds to DNA and makes DNA glow under fluorescent light?	What are the names of the Lu-LTSPP volunteers in your class today?	What is the name of the instrument used to dispense the DNA samples in the electrophoresis wells?	What types of DNA samples will be run in the gel today?	What is your teacher's name?

PCR BINGO CARD

B	I	N	G	O
What overall charge does a DNA molecule have?	What is the average number of cycles for a typical PCR reaction?10-2020-4050-100	Name one advantage of PCR?	Did the inventor of the PCR technique receive a Nobel Prize?	Is PCR a Canadian Innovation?
What is the technical name of the small DNA strands that initiate the reaction?	Who invented the Polymerase Chain Reaction technique?	What does the acronym DNA stand for?	What is the name of the machine that PCR reaction is conducted in?	What direction is DNA synthesized?
What is the optimal temperature for the enzyme that synthesizes DNA in the PCR?	What does the acronym PCR stand for?	**X**	At what temperature do DNA strands come apart?	True or False?The more DNA that is synthesized, the slower it migrates through the electrophoresis gel
What are the three functional groups on the DNA molecule called?	At what temperature do the primers that start the DNA polymerization reaction anneal?	What direction does DNA migrate in an electrophoresis gel?	Name one disadvantage of PCR?	What are the names of the 4 DNA bases?
Name an application of PCR.	What are the names of the LTSPP volunteers in your class today?	What is the name of the instrument used to dispense the DNA samples in the electrophoresis wells?	At what temperature do the hydrogen bonds on the DNA molecule disrupt and the double stranded DNA come apart?	What is the full name of the enzyme that synthesized DNA in a PCR reaction?

PCR BINGO CARD- answers

B	I	N	G	O
What overall charge does a DNA molecule have? **Negative** – the phosphate groups on the DNA molecule give it the charge	What is the average number of cycles for a typical PCR reaction? 10-20 **20-40** 50-100	Name one advantage of PCR? **Allows for amplification of DNA molecules over one million fold** – thus making it possible to detect DNA in small samples	Did the inventor of the PCR technique receive a Nobel Prize? **Yes in 1994 – Dr. Kary Mullis** won the Nobel Prize for Chemistry	Is PCR a Canadian Innovation? **No** – Dr Kary Mullis was working for the Cetus Biotechnology company in Berkley California when he made the PCR innovation.
What is the technical name of the small DNA strands that initiate the reaction? **Primers –** which are synthetic oligonucleotides, 15-30 base pairs long that have the corresponding DNA	Who invented the Polymerase Chain Reaction technique? **Dr. Kary Mullis** in 1984	What does the acronym DNA stand for? **D**eoxyribo**n**ucleic **A**cid	What is the name of the machine that PCR reaction is conducted in? **Thermocycler –** an incubation system that cycles DNA samples through different temperatures	What direction is DNA synthesized? **5' → 3'**
What is the optimal temperature for the enzyme that synthesizes DNA in the PCR? **72 °C** is the temperature at which TAQ polymerase is maximally active. This stage in the PCR reaction is known as the Extension Stage	What does the acronym PCR stand for? **Polymerase Chain Reaction**	**X**	At what temperature do DNA strands come apart? **92°C-96°C** is the temperature at which hydrogen bonds between base pairs come pair. This is also known as "denaturing" or "melting" of DNA.	True or False? The greater the amount of DNA, the slower it migrates through the electrophoresis gel **False –** The rate at which a molecule migrates through a gel is dependent on charge and size of the molecule not the amount
What are the three functional groups on the DNA molecule called? **Ribose** – sugar; **Phosphate group –** joins the sugars together to form the backbone; **Base –** Adenine, guanine, cytosine or thymine	At what temperature do the primers that start the DNA polymerization reaction anneal? **37°C-65°C** – is the annealing temperature of the primers	What direction does DNA migrate in an electrophoresis gel? **DNA** migrates from the negative end of the chamber to the positive end	Name one disadvantage of PCR? **Unwanted amplification of DNA products** from contamination or non-specific annealing of the primers	What are the names of the 4 DNA bases? Adenine, Guanine, Cytosine Thymine
Name an application of PCR. Forensics, Human Genome Project (diagnostic tests for diseases	What are the names of the LTSPP volunteers in your class today?	What is the name of the instrument used to dispense the DNA samples in the electrophoresis wells? **Micropipette**	At what temperature do the hydrogen bonds on the DNA molecule disrupt and the double stranded DNA come apart? **92°C-96°C**	What is the full name of the enzyme that synthesized DNA in a PCR reaction? ***Thermus aquaticus*** (**Taq**) polymerase