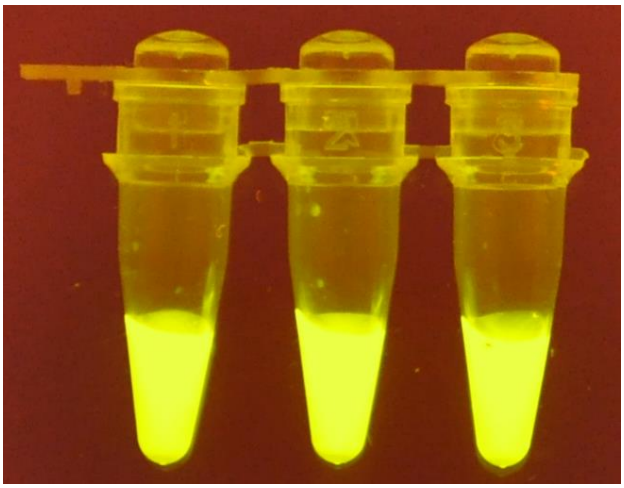
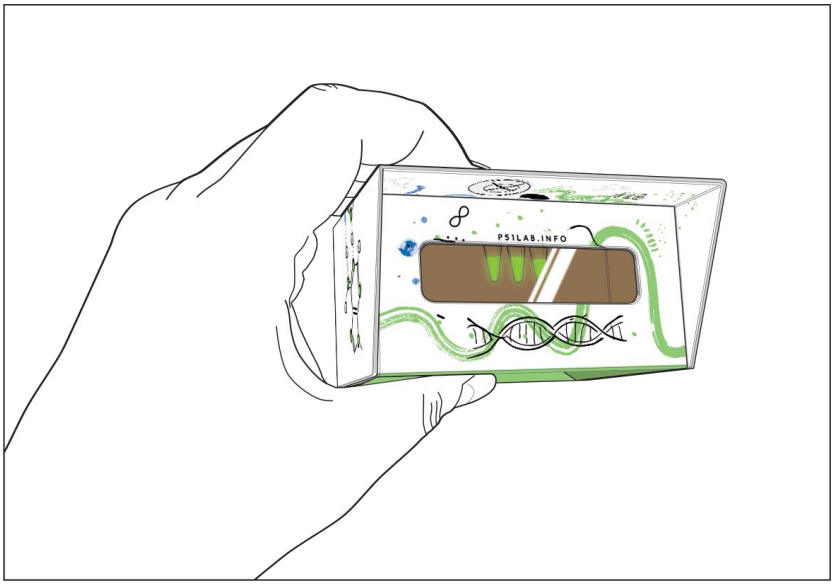
miniPCR™ DNA Glow Lab™:
Exploring DNA Structure



For use with P51™ Molecular Viewer



(or other blue light illuminator[1])

[1] Compatible with blue light transilluminators such as blueGel™, blueBox™ and other 460-480 nm illuminators.

Instructor's Guide Contents

Overview

The sentence "This structure has novel features which are of considerable biological interest" may be one of science's most famous understatements. It was published in April 1953 in the *Nature* article where James Watson and Francis Crick revealed the structure of DNA, the molecule that carries genetic information. Watson and Crick (and Maurice Wilkins) shared a Nobel Prize for determining that DNA is a double stranded helix, held together by specific base pairing. They also predicted, correctly, that at times the base pairs separate allowing DNA to perform functions that are essential for life. **In this lab, students will use a fluorescent dye to investigate the conditions that influence DNA structure and its transition from double helix to single strand, and vice versa.**

1. Quick guide: Preparatory activities

Suggested for 8 student groups (4 students per group)

Note: We recommend diluting DNA Dye in Buffer 1 as close to its expected use as possible. *Once diluted in buffer 1, DNA Dye is stable at room temperature for up to two hours*. If diluted DNA Dye is kept at room temperature for more than two hours, significant loss in fluorescence is possible. *Dye that has been diluted in Buffer 1 will retain its activity for up to 72 hours if kept on ice, refrigerated at 4°C, or frozen at -20°C and protected from light*.
Other reagents are stable at room temperature for up to 72 hours.

Gloves and protective eyewear should be worn for the entirety of this lab.

- **Add "Concentrated DNA Dye" to "Buffer 1"**
  - Add entire contents (70 µl) of "Concentrated DNA Dye" to the vial labeled "Buffer 1" (*Note*: contents may be slightly less than 70 µl due to evaporation of the solvent, but this will not affect results).
  - Cap and invert several times to mix.
  - If not using immediately, store on ice or in the refrigerator.
    *Note: Make sure Concentrated DNA Dye is fully melted before using. Concentrated DNA Dye is dissolved in DMSO and may be frozen at 4°C. Hold in a clenched fist if not fully melted.

- **Mix "Unknown" DNA**
  - Label a single 1.7 ml microcentrifuge tube "Unknown"
  - Aliquot 44 µl "Buffer 2" into the tube.
  - Add 4 µl 50:50 AT:GC DNA to the tube.
  - Pipette up and down gently to mix.
  - This tube will remain with the teacher until activity C.

- **Aliquot reagents into labeled 1.7 ml microtubes (6 tubes per lab group)**
  Each group will need the following reagents:
  - Buffer 1 (with Concentrated DNA Dye added) ---------- 275 µl
    (**Label "Dye"**, 1 tube per group. If using a 200 µl pipette, aliquot 135 µl twice.)
  - Buffer 2 ---------- 255 µl
    (**Label "Buffer"**, 1 tube per group. If using a 200 µl pipette, aliquot 125 µl twice.)
  - AT rich DNA (**label as Tube C**. 1 tube per group.) ---------- 40 µl
  - GC rich DNA (**label as Tube A**. 1 tube per group.) ---------- 40 µl
  - 50:50 AT:GC DNA (**label as Tube B**. 1 tube per group.) ---------- 50 µl
  - 100 mM **NaOH** (1 tube per group.) ---------- 65 µl

- **Distribute strip tubes by group (Three 8-tube strips per lab group)**
  - Each group will need three 8-tube strips with caps.
  - Cut two 8-strips in half with scissors for each group, or pass out three 8-tube strips to each group and have students cut two strips in half.
    - One 4-tube strip will be used in the temperature investigation.
    - Three 4-tube strips will be used in the pH investigation.
    - One 8-tube strip will be used in the DNA concentration investigation.

2. Synopsis

Can you discover the conditions that will make double stranded DNA denature and re-anneal?

This lab allows students to investigate DNA base pairing and the conditions that will cause the DNA double helix to separate into single strands (denaturation), and then come together again (annealing). Under normal conditions, DNA is a double stranded molecule held together by hydrogen bonds between complementary nitrogenous bases. This lab uses a fluorescent dye that binds selectively to double stranded DNA (dsDNA). Under blue light, this dye will fluoresce when bound to dsDNA, but will not bind and not fluoresce in the presence of single stranded DNA (ssDNA).

Students will test conditions that influence hydrogen bond formation and dissolution between Watson-Crick base pairs in complementary DNA strands. First, students will use temperature to differentiate between AT-rich DNA, GC-rich DNA, and a DNA molecule with balanced ATGC content, and will establish the melting temperature (T_m) of each sample. Second, they will alter the pH of samples in order to establish a relationship between pH, DNA composition and Watson-Crick base pairing. Finally, students will estimate the concentration of an unknown sample of DNA.

- **Techniques utilized:** Micropipetting, fluorescence detection of DNA.
- **Time required:** One 90 minute period or two 45 minute periods. Can be modified to run in one 45 minute period if necessary.
- **Reagents needed:** miniPCR DNA Glow Lab™ (KT-1900-01).
- **Suggested skill level:** Familiarity with DNA structure and DNA base pairing.

3. Learning goals and skills developed

Student Learning Goals – students will be able to:

- Empirically test the structural properties of DNA models.
- Identify unknown samples of DNA based on thermodynamic properties.
- Demonstrate thermodynamic differences between Watson-Crick base pairs.
- Relate the role of hydrogen bonding to DNA stability.
- Establish the melting/annealing temperature of a DNA strand.
- Demonstrate the effect of pH on Watson-Crick base pairs.
- Estimate the concentration of DNA in an unknown sample.

Scientific Inquiry Skills – students will:

- Create hypotheses and make prediction about results.
- Compare experimental results to their predictions.
- Make conclusions based on their experimental results about their hypothesis.

Molecular Biology Skills:

- Micropipetting.
- Fluorescence detection of DNA.

4. Standards alignment

Next Generation Science Standards - Students who demonstrate understanding can:

- HS-LS1-1 Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.
- HS-LS3-1 Ask questions to clarify relationships about the role of DNA and chromosomes in coding the instructions for characteristic traits passed from parents to offspring.
- HS-PS2-6 Communicate scientific and technical information about why the molecular-level structure is important in the functioning of designed materials.

Common Core English Language Arts Standards

- WHST.9-12.2 Write informative/explanatory texts, including the narration of historical events, scientific procedures/ experiments, or technical processes.
- WHST.9-12.9 Draw evidence from informational texts to support analysis, reflection, and research.
- RST.9-10.3 Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks, attending to special cases or exceptions defined in the text.
- RST.9-10.7 Translate quantitative or technical information expressed in words in a text into visual form (e.g., a table or chart) and translate information expressed visually or mathematically (e.g., in an equation) in words.
- RST.11-12.7 Integrate and evaluate multiple sources of information presented in diverse formats and media (e.g., quantitative data, video, multimedia) in order to address a question or solve a problem.

AP biology learning objectives

- LO3.1 The student is able to construct scientific explanations that use the structures and mechanisms of DNA and RNA to support the claim that DNA, and in some cases RNA, are the primary sources of hereditary information
- LO3.5 The student can explain how heritable information can be manipulated using common technologies.
- LO4.1 The student is able to explain the connection between the sequence and the subcomponents of a biological polymer and its properties
- LO4.2 The Student is able to refine representations and models to explain how the subcomponents of a biological polymer and their sequences determine their properties.
- LO4.3 The Student is able to use models to predict and justify that changes in the subcomponents of a biological polymer affect the functionality of the molecule.

5. Background and significance

"This structure has novel features which are of considerable biological interest."

- A Structure for Deoxyribose Nucleic Acid
Watson J. and Crick F
Nature **171**, 737 (1953)

The structure of DNA is both remarkable and unique. The discovery of that structure remains one of the great advancements in modern science. In 1951, 23-year-old James Watson and 35-year-old student Francis Crick started working together to try to deduce the structure of the DNA double helix. When they published their paper less than two years later, Watson and Crick ushered in a biological revolution and beat out some of the world's most respected scientists in the race to do so.

When Watson and Crick began working together, it was widely understood that DNA was the genetic material and that DNA was a polymer, a long biological molecule made of smaller subunits, or nucleotide monomers. Their job was to figure out how those nucleotides fit together like a molecular puzzle in a way that made sense both biologically and chemically. Watson and Crick solved this problem by literally building models out of cardboard, aluminum, and other materials.

Watson and Crick based their models of DNA on the results produced by other scientists. Perhaps most notably, the X-ray diffraction results of Rosalind Franklin and her student, Raymond Gosling, suggested that DNA was helical, or spiral, in shape. In 1952, based on these results, Watson and Crick built their first model of the DNA double helix. It was not correct. Rosalind Franklin, who had a much better understanding of basic chemistry, pointed out that parts of their model were obviously backwards. Their version of the double helix had sections that would be repelled by water on the outside, where they would be in direct contact with water, and parts that would be attracted to water buried on the inside.

Watson and Crick went back to working on their models. Their major breakthrough occurred after meeting with Erwin Chargaff, a scientist whose work gave them insight into the ratio of different nucleotides used in DNA. On February 28, 1953, they assembled a structure that both worked chemically and instantly had clear and profound biological implications. While celebrating in a pub later that day, according to their own recollection, Crick proclaimed loudly "We have discovered the secret of life." The paper detailing their discovery was published in April, 1953.

The biological breakthrough of Watson and Crick's model was what we now call "complementary base pairing", or sometimes "Watson-Crick base pairing." In its most basic form, base paring says that each type of nucleotide found in one DNA strand will bind, or pair, with only one other type of nucleotide. If you know the order of nucleotides on one side of the double helix, you can automatically deduce the order on the other side of the double helix. Their base pairing model not only explained DNA's structure,

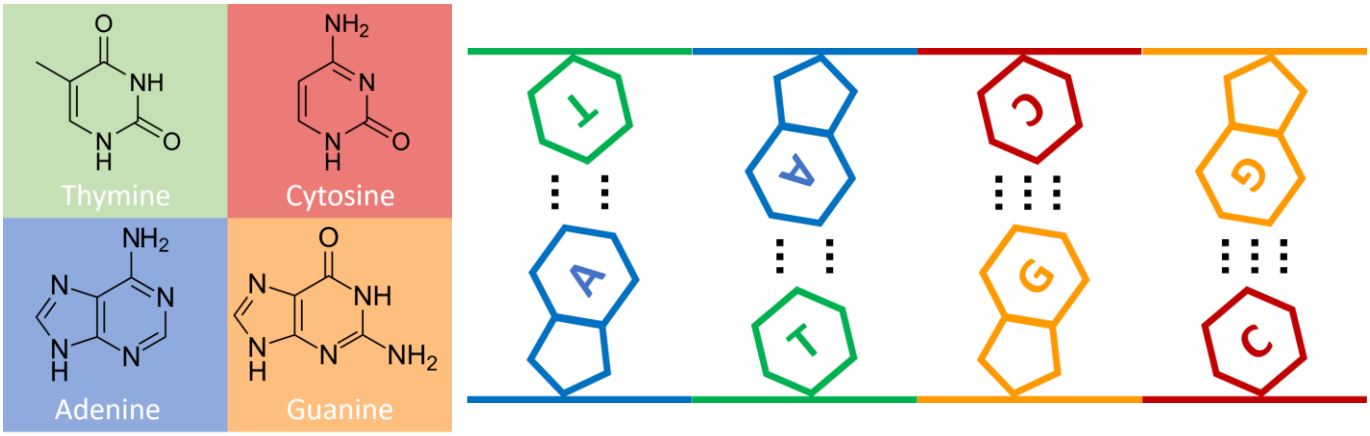
but also aspects of its function. Today, understanding this model of DNA base pairing is fundamental to understanding DNA structure, replication and transcription.

DNA structure – the basics

DNA is a double helix, a long spiral shaped molecule made of two strands twisted around each other. Each strand contains combinations of four nitrogenous bases, adenine (A), thymine (T), cytosine (C), and guanine (G). Along one strand of the double helix, these bases may be found in any order, but the order of the other strand of the double helix is strictly determined by the sequence of bases in the first strand and DNA base pairing rules.

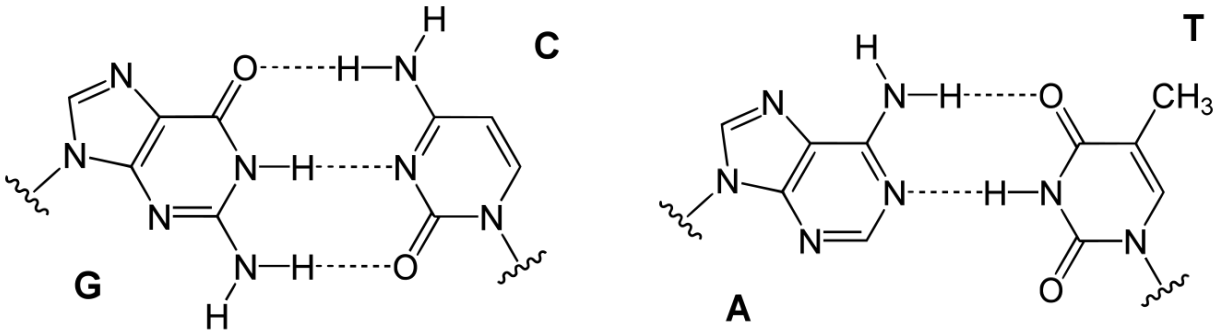
DNA base pairing is determined by two structural factors, nitrogenous base size and number and polarity of possible hydrogen bonds. DNA nitrogenous bases can be divided into two groups, purines and pyrimidines. Purines have a double ring structure; pyrimidines have a single ring structure. For DNA to maintain a constant width throughout the double helix, a double ring purine on one side of the helix can never match with another purine on the other side of the helix; together the two double ring bases would be too large and cause a bulge in the DNA. Likewise, a single ring pyrimidine nitrogenous base cannot be matched with another pyrimidine; together they would be too small to reach across the double helix. For this reason, when a double ring purine is found on one side of the helix, a single ring pyrimidine must be found on the other side of the helix. Adenine and guanine are purines, thymine and cytosine are pyrimidines. A simple way to remember this is that thymine, cytosine and pyrimidine are all spelled with the letter Y.

We can also separate purines and pyrimidines by the number and polarity of the hydrogen bonds they can make. Guanine can make three hydrogen bonds, adenine can make two bonds and the polarity of those bonds is opposite that of guanine. Cytosine can make three hydrogen bonds, thymine can make



Pyrimidines, thymine and cytosine, are single-ring structures. Purines, adenine and guanine, are double-ring structures. To maintain the proper width of the double helix, a purine must always bind with a pyrimidine. G:C pairs make three bonds. A:T pairs make two bonds.

two, again with opposite polarity. Knowing this, if we find adenine, a purine, on one side of the helix, we know it must bind with a pyrimidine to maintain the correct width of the helix, but it can only match with thymine because they share the correct number and polarity of bonds. Likewise, if we find cytosine, a pyrimidine on one side of the helix, we know it must bind with a purine to maintain the proper width, but it can only match with a guanine because they share the correct number and polarity of bonds. You can see the polarity of the bonds in the image below. In G:C pairings, the hydrogen atoms are located on opposite sides of the hydrogen bonds compared to A:T pairings.



Following these simple rules is the basis for understanding DNA structure and function. It explains how in DNA replication, to make a new double helix, you need only one side of the original strand. It explains how RNA, which follows similar base pairing rules, can be quickly and efficiently copied from a DNA template.

Hydrogen bonding and Watson-Crick base pairing

Hydrogen bonds occur when polar molecules are attracted to each other electrostatically. When a hydrogen atom bonds covalently to a highly electronegative atom such as oxygen or nitrogen, the electrons are not shared evenly between the two atoms. This creates a weakly positive hydrogen atom and a weakly negative oxygen or nitrogen atom. When the weakly positive hydrogen from one molecule is attracted to a weakly negative oxygen or nitrogen from another molecule, a hydrogen bond forms. You can think of hydrogen bonds like weak magnets. When the positive pole of one magnet is aligned with the negative pole of another magnet the attraction will pull the magnets together and hold them there. But because the magnets are relatively weak, it's not hard to pull them apart again.

Hydrogen bonds occur between many kinds of molecules. The hydrogens in water molecules are attracted to the oxygens in other water molecules, giving water the properties of surface tension and cohesion. Hydrogen bonds between amino acids in proteins are largely responsible for maintaining a protein's three-dimensional structure. In DNA, the bonds between base pairs pull the two strands of the double helix together.

Because hydrogen bonds are relatively weak, each individual bond can be broken fairly easily. But because the two strands of DNA are usually pulled together by so many individual base pairs, each with at least two of these bonds, the DNA double helix ends up being an incredibly stable molecule. That stability

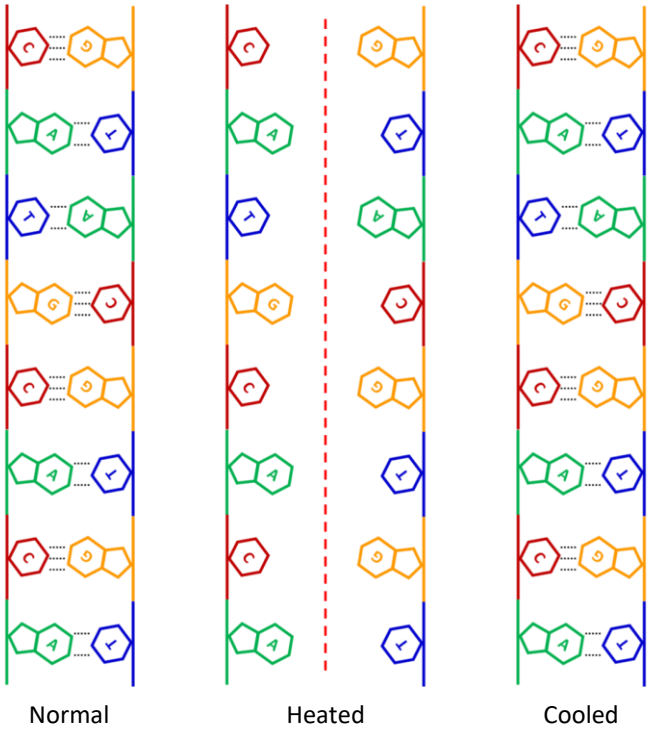
is a desirable attribute for hereditary material, but to be useful in transmitting that information, DNA can't be too stable. To copy DNA or to transcribe DNA into RNA, the hydrogen bonds must be temporarily split and DNA made single stranded. In the cell, this is done with enzymes. But changes in physical or chemical conditions can also affect the structure of DNA through changes in hydrogen bonding.

The stability of hydrogen bonds is most susceptible to changes in pH, temperature, and ion concentration. Because these bonds are relatively weak, relatively small changes in the thermal energy of a system can affect their stability. Heating molecules can weaken the attraction, while cooling hydrogen bonds can make them stronger. Changes in pH can also have a strong effect on hydrogen bonding. Acidic conditions have high levels of H^+ ions in solution. These H^+ ions can disrupt normal hydrogen bonding by binding to the negatively charged ends of polar molecules. Likewise, alkaline (basic) conditions have high levels of OH^- ions in solution. These OH^- ions will disrupt hydrogen bonds by being attracted to and bonding with the weak positive charge found on hydrogen atoms that are bound to oxygen or nitrogen.

Base pairing in biotechnology

Scientists can use two properties of the hydrogen bonds in DNA to their advantage. The first property is the specificity of hydrogen bonds, dictated by base pairing (A with T and C with G). The second is the ability to break and reform hydrogen bonds by altering the environment of a DNA molecule. For example, by simply altering temperature, scientists can temporarily break the hydrogen bonds of a DNA molecule and can later cause DNA to reform hydrogen bonds by cooling it down again.

In many procedures, scientists will add DNA of a known sequence to a sample of DNA. The sample can then be heated, *denaturing* the DNA double helix, that is, turning double stranded DNA into single strands. When the sample is cooled again, if the sequence the scientist added is complementary to one of the two strands in the sample, the strands can *anneal* (form a double strand) together. Denaturing and annealing, or breaking and rejoining the DNA double helix, are the basis of several techniques, including copying DNA in the polymerase chain reaction (PCR), using fluorescent probes to locate specific DNA sequence in *in situ* hybridizations and microarray experiments, and in DNA sequencing reactions.

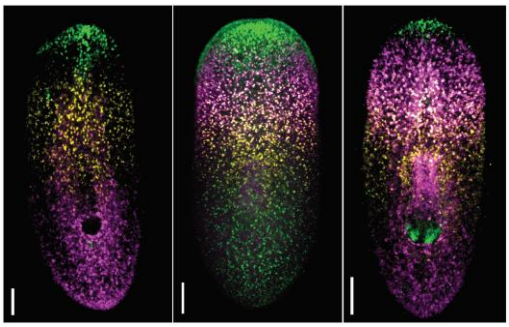


Double Stranded DNA will *denature* when heated. When cooled again, the strands will *anneal*.

As stated before, the number of hydrogen bonds varies depending on the nucleotide, and this difference can be important when designing experiments. G:C pairs share three bonds, while A:T pairs share only two. This difference plays into how easily the DNA strands can be denatured and how readily they will anneal. The more bonds between nucleotides, the stronger the hold between them - denaturing the strands becomes more difficult and already denatured strands will anneal more readily. The fewer bonds between the strands, the easier it is to denature the strands and the less readily they will reanneal. In experiments that require these steps, careful attention must be paid to the precise temperature, pH and salt concentration of the samples. Likewise, in experiments that require annealing of more than one sequence of DNA, like when using PCR primers, careful attention must be paid to make sure the GC and AT content of the two sequences is similar. Otherwise, the sequences may require very different conditions for annealing to occur.

Use of fluorescent dyes in biotechnology

Fluorescence can have many uses in the biology laboratory. Fluorescent proteins such as the green fluorescent protein (GFP) can be engineered to be produced by organisms, usually as a reporter molecule. Fluorescent in situ hybridization (FISH) uses fluorescent dyes to bind to nucleic acids inside intact cells and give beautifully colored pictures of showing gene expression patterns. Perhaps the most common use of fluorescent dyes, one you may have even done before in the classroom, is to visualize DNA in an agarose gel. The type of fluorescent molecule used and how that molecule finds its target varies in these examples, but the physical basis of the fluorescence is basically the same. When exposed to a particular wavelength of light, the molecule is excited and emits light at a different wavelength – one that is visible to the scientist.



Fluorescent *in situ* hybridization (FISH) image of *Planaria*

Another common use of fluorescent dyes is to measure the concentration of DNA in a sample. In this procedure, known concentrations of DNA are mixed with a dye that binds to double stranded DNA and the fluorescence is measured on a microplate reader or fluorospectrometer. Samples with unknown concentrations of DNA can then be read on the same reader and compared to the standard. Because the dye only binds to double stranded DNA, the advantage of this method of DNA quantification is that even samples with RNA, unincorporated nucleotides, or other materials in solution can be read accurately. Such other materials in solution would confound readings on a normal UV spectrophotometer as they would also be detected.

Today's lab

Watson and Crick deduced the structure of DNA by using X-ray crystallography results obtained by Rosalind Franklin. The conditions that cause DNA to denature and to anneal were later discovered using spectrophotometry. For practical reasons, both of these techniques are not viable in a classroom environment. This has left classrooms without and authentic laboratory investigation into the structure of DNA. For this reason, we have adapted the use of fluorescent dyes for student use to investigate conditions that affect DNA base pairing. In this lab, students will investigate Watson-Crick base pairing using a dye that only fluoresces when bound to double stranded DNA. Students will be given three unknown samples of DNA to identify, an AT-rich sequence, a GC-rich sequence and a sequence with equal proportions of A:T and G:C base pairs. Using a thermocycler or other heat source such as a water bath, students will establish the identity of each sample and then estimate the melting temperature (T_m) of each. Students will then create a series of samples with varying pH to test how pH, affects DNA base pairing. Finally, students will create a dilution series of a sample with known DNA concentration in order to estimate the concentration of an unknown sample through observing relative fluorescence.

Useful Vocabulary:

Hydrogen bond: Hydrogen atoms bound to electronegative elements such as oxygen or nitrogen will have a slight positive charge. This positive charge will cause them to be attracted to other electronegative elements, typically oxygen or nitrogen. The attraction between these atoms is referred to as a hydrogen bond.

Polarity: The separation of positive and negative charge. Hydrogen bonds form between polar molecules. In a hydrogen bond, the hydrogen will have a slight positive charge and the oxygen or nitrogen will have a slight negative charge.

Denaturation: The process by which a complex organic molecule loses its three-dimensional structure. For DNA, this means the two strands of the double helix will no longer bind together and will become single stranded.

Annealing: The formation of hydrogen bonds between complementary nucleotide sequences. Annealing between nucleotide strands will create double stranded molecules.

T_m / Melting temperature: The temperature at which 50% of the DNA in solution will be denatured. At this temperature, half the DNA will be double stranded; half will be single stranded. For DNA to be fully denatured, the temperature must be above T_m. For DNA to be fully annealed, temperature must be below T_m.

Annealing temperature: The temperature used in an experiment, usually PCR, to cause single stranded DNA to anneal and become double stranded. Annealing temperature is set by the scientist, but is usually around 5˚C below T_m.

Teacher's notes

This lab has been written and designed to be used in a classroom where students have access to miniPCR™ thermocyclers and a source of blue light illumination. The lab could be modified to use another heat source if a miniPCR™ thermocycler is not available. See the appendix for a suggested alternative protocol if using an alternative heat source. Any blue light source in the 460-480 nm range can be used. Blue light transilluminators such as blueGel™ or blueBox™ will give the brightest, clearest results. *P51™ molecular glow lab* has been designed to be an effective affordable option if you do not have other blue light illuminators in your classroom. If using blueGel™, simply use blueGel without the gel tray or buffer chamber. Using Fold-a-View™ will aid in seeing fluorescence in a lit room.

The more access different groups have to a programmable thermocycler, the more easily this lab will run and the more freedom students will have to experiment. The pH investigation and the determination of DNA concentration investigation do not require a thermocycler or other heat source. If access to a thermocycler or heat source is likely to be a bottleneck in the classroom, we recommend starting some groups on investigation B and C, while other groups start on investigation A. Identifying relative AT and GC content of tubes is possible in both the temperature investigation and the pH investigation.

When investigating pH, students will no doubt be tempted to try making the sample more acidic. In acidic conditions, however, the binding between the DNA and the DNA Dye is affected, resulting in a loss of fluorescence independent of the disruption of hydrogen bonds between DNA strands. Therefore, at low pH, a lack of fluorescence does not necessarily indicate that the DNA is in a single stranded state.

The ideal classroom set up for this lab will allow every group to have access to a heat source and blue light illuminator; however, because of the visual nature of this lab, it can also be run well as a demonstration.

6. Materials needed

Reagent	Volume included in kit	Amount needed per group	Storage	Checklist
Concentrated DNA Dye	70 µl	To be mixed with "Buffer 1"	4˚C	
Buffer 1	2.5 ml	275 µl (with Concentrated DNA Dye added)	4˚C	
Buffer 2	2.5 ml	255 µl	4˚C	
AT Rich DNA	400	40 µl	4˚C	
GC Rich DNA	400	40 µl	4˚C	
50:50 AT:GC DNA	500	50 µl	4˚C	
100 mM NaOH	650	65 µl	4˚C	
100 mM HCl (optional)	-	20 µl	Room temp.	

Supplied in Kit

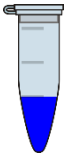
Equipment and Supplies	Checklist	
PCR tubes: 24 - 200 µl PCR microtubes – 3 strips of 8 tubes per group.		Supplied in Kit
Microcentrifuge tubes: 49 total tubes. 6 tubes for each group for distributing reagents, plus one tube for mixing unknown DNA sample.		Supplied in Kit
miniPCR™ thermal cycler: Other heat sources can be used but will require modifications to the protocol (see page 32).		Available at miniPCR.com
P51™ Molecular Fluorescence Viewer or other blue light Illuminator: eg. blueGel™ or blueBox™ transilluminator.		Available at miniPCR.com
Micropipettes 2-20 µl: one per lab group. 20-200 µl: one for the teacher.		Available at miniPCR.com
Disposable micropipette tips.		Available at miniPCR.com
Other supplies: ● Camera or phone with photo capabilities for recording results ● Disposable laboratory gloves ● Protective eyewear ● Permanent marker (fine tip is preferable) ● Small beaker for material disposal ● Ice bath (optional)		Available at miniPCR.com

7. Laboratory guide

Planning your time

This lab has 3 independent investigations for students to complete, and it is designed to run in **two 45-min** class period, or a single 1.5-hour period. Alternatively, different groups can perform different investigations simultaneously and share results, completing the activity in one class period.

Experimental timeline



Preparation: Dispense reagents and prepare equipment
- *20 min*

A **Temperature investigation**
- 40 min

STOP ***Stopping point:*** *If needed, lab can be broken into two periods. Diluted DNA dye must be kept frozen and will last up to 72 hours.*

B **pH investigation**
- 20 min

C **DNA concentration investigation**
- 20 min

Tubes that are heated to 95°C and above have the potential to pop open unexpectedly. Gloves and protective eyewear should be worn for the entirety of this lab.

This lab requires the use of 4-strip and 8-strip tubes. PCR tubes may be handed out as 8 strips. If a 4-strip is required, simply cut an 8-strip of tubes and caps in two using scissor or by twisting and pulling by hand.

A. Temperature investigation

1. **Label the tubes on a 4-tube strip.**
   - Label the tubes "**A**", "**B**", "**C**", and "N".
   - Use a fine tip permanent market to write on the side wall of the tube.

2. **Add reagents to your tubes.**
   - Add 10 µl **Buffer** to each tube.
   - Add 10 µl **Dye** to each tube.
   - Add 5 µl of **DNA** sample A, B, or C to the appropriate tubes.
   - Do not add any DNA to tube "N". This will serve as a No-DNA control.

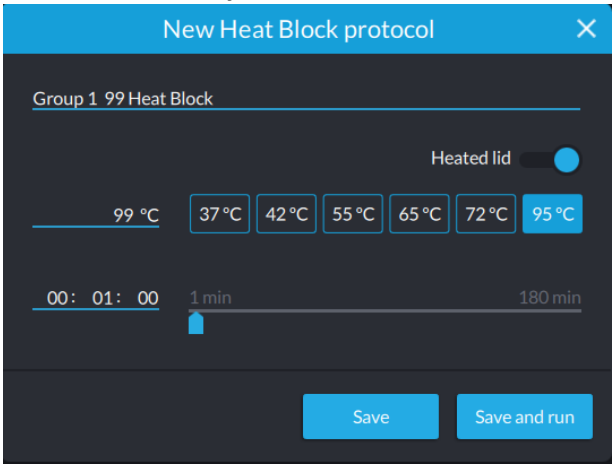
	Per tube
Buffer	10 µl
Dye	10 µl
DNA sample (A, B, or C)	5 µl
Final volume	***25 µl***

3. **Gently mix the reagents by pipetting up and down 3-4 times, cap the tubes.**
   - Make sure all the liquid volume collects at the bottom of the tube (lightly tap bottom of tubes on bench if needed.)
   - Tightly cap the tubes.

4. **View tubes in P51™ or other blue light illuminator (e.g. blueGel™, blueBox™, or other 480 nm light source).**
   - Darken the room or use a light blocking hood to better view the samples.
   - If possible record an image of the tubes.
   - Use this observation for future reference of tube brightness. Brightness observed will be considered a "5" or "maximum brightness" for comparison to future observations.
   - The no-DNA control will be a "0" or "minimal brightness" for comparison to future observations

Denature DNA and observe annealing.

1. **Open the miniPCR software app and remain on the "Protocol Library" tab.**
2. **Click the ⊕ (new protocol) button.**
3. **Select the "Heat Block" from the drop-down menu.**
4. **Enter a name for your protocol; for example, "Group 1 - 95 Heat Block".**
5. **Enter heat block temperature and time.**
   - Select 95˚C.
   - Set time to one minute or longer.



6. **Click "Save and run" (select the name of your miniPCR machine in the dialogue window if prompted) to finish programming the thermal cycler. Make sure that the power switch is in the ON position on the miniPCR.**

7. **Place tubes in the thermal cycler (or other heat source) and close the lid.**

8. **Allow tubes to remain at 95˚C for at least one minute (or up to three minutes.)**

9. **Carefully open the lid to remove tubes.**

   ⚠ *Be careful when opening and closing miniPCR, heat block and PCR lid will be hot.*

   - It is ok to open the miniPCR and remove tubes while the heat block program is still running.

10. **To view and record glowing DNA in tubes, quickly transfer tubes to P51™ or other blue light illuminator.**
    - Darken the room or use a light blocking hood to better view the samples.
    - Record an image of the DNA solution in the tubes if possible.
    - Observe and record the brightness of the DNA in each tube: does it appear to be at or close to full brightness, dimmer than full brightness, or has it stopped fluorescing?
    - Note that at 95˚C not all tubes may stop fluorescing completely, but clear differences between tubes can be observed.

11. **Continue viewing for up to two minutes at room temperature.**
    - Observe the tubes as they cool. Using a cell phone camera can aid in viewing. It can also be useful to record a video of the samples.
    - As the samples cool, DNA will begin to anneal. Annealing DNA can be recognized because the tubes will begin to fluoresce.
    - Record the order in which the different tubes begin to fluoresce.
    - AT rich DNA may not reach full fluorescence in the time allotted. This can be partly due to heat from the blue light illuminator keeping the tubes from fully cooling.

12. **Predict the contents of each tube based on relative time to fluoresce.**
    - AT rich DNA: tube ___
    - 50:50 DNA: tube ___
    - GC rich DNA: tube ___

Estimate approximate T_m using miniPCR™ in linear ramp mode.

1. **Open the miniPCR software app and remain on the "Protocol Library" tab.**

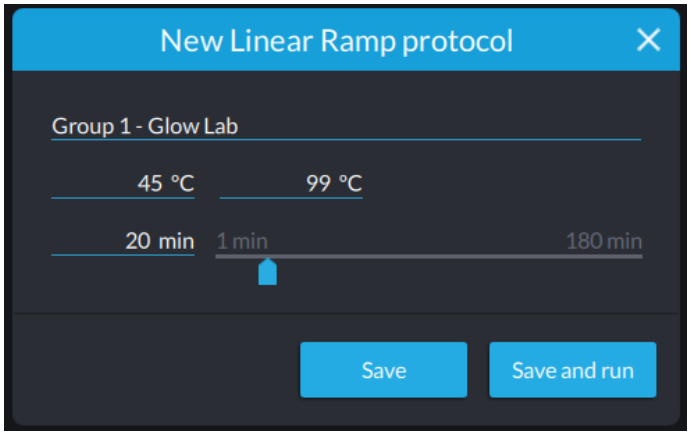
2. **Click the ⊕ (new protocol) button.**

3. **Select "Linear Ramp" from the drop-down menu.**

4. **Enter a name for the Protocol; for example: "Group 1 – Glow Lab"**

5. **Enter the Linear Ramp protocol parameters:**
   - **Start Temp:** 45°C
   - **End Temp:** 99°C
   - **Time** 20 min*

   * Time can vary between 10-20 minutes depending on class constraints. 20 minutes is recommended.



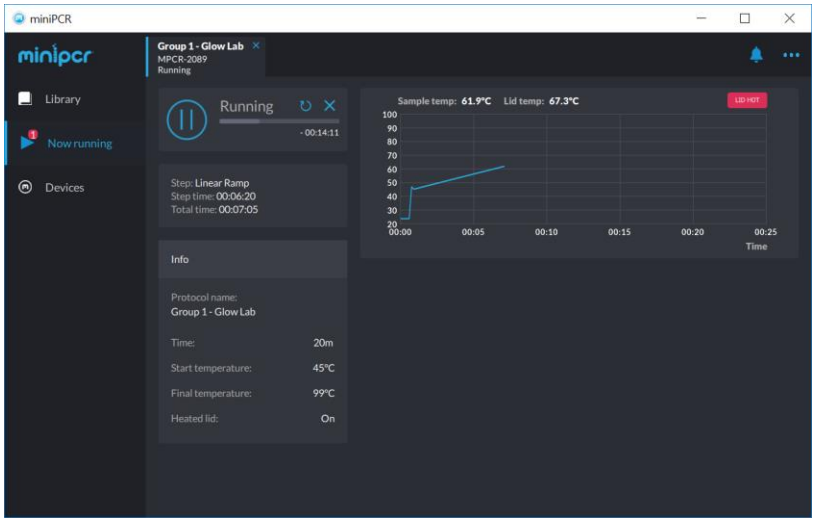
6. **Click "Save and run" (select the name of your miniPCR machine in the dialogue window if prompted) to finish programming the thermal cycler. Make sure that the power switch is in the ON position on the miniPCR.**

7. **Place the same tubes used in the previous investigation inside the miniPCR and close the lid.**

8. **As the linear ramp progresses, regularly remove tubes to view on P51™ or other blue light illuminator.**

   ⚠ *Be careful when opening and closing miniPCR, heat block and PCR lid will be hot.*

   - To remove tubes, simply open the miniPCR and lift tubes from wells.
   - Remove tubes at regular intervals (every minute for a ten-minute ramp, every 5 degrees for 20 minute ramps.)
   - Record the temperature and the time the tubes were removed.

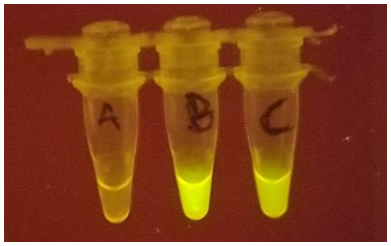


- It is OK to open the machine during the run. Do not cancel the program or turn off the machine. If desired, you may press the pause button to hold the temperature constant while the tubes are out of the thermal cycler.
- Replace tubes inside the miniPCR and close the lid when done viewing – wait for the next time or temperature interval until the reading.

9. To view and record glowing DNA in tubes, quickly transfer tubes to the blue light illuminator.

- Darken the room or use a light blocking hood to better view the samples.
- Record an image of the DNA solution in the tubes if possible.
- Observe and record the brightness of the DNA in each tube: does it appear to be at or close to full brightness, dimmer than full brightness, or has it stopped fluorescing?
- Record your observations by assigning a brightness value (1 = low brightness, 5 = maximum brightness) in the data logging table (see next section, "Data tables".)
- Tubes will begin cooling as soon as they are removed from the thermal cycler, so it is important to view the DNA as quickly as possible after removing from the miniPCR. Try to take the same amount of time between removing tubes from the heat source and viewing for every timepoint.

Example observation.
Tube A: minimal brightness - 1
Tube B: maximal brightness - 5
Tube C: intermediate brightness - 3



10. Quickly return tubes to the thermal cycler to continue heating.

⚠ *Be careful when opening and closing miniPCR, heat block and PCR lid will be hot.*

- Close and latch the lid.
- It is OK if the tubes are out of the machine for some of the linear ramp cycle. Make sure that tubes are in place for at least 30 seconds prior to each time you remove them. If the tubes have not been in the thermal cycler for a full thirty seconds, press the pause button to hold the temperature until 30 seconds is reached.

11. Continue regularly removing tubes and viewing in P51 or other blue light illuminator until the end of the linear ramp protocol is reached.

- Follow directions for viewing as above
- Record observations in the data logging table (see next section, "Data tables".)

12. Use observations to predict contents of tubes and melting temperatures (T_m) of each.

- Tubes contain AT rich, GC rich and 50:50 AT:GC DNA.
- T_m is the temperature at which 50% of the DNA strands in solution will be denatured.
- T_m should be approximated to a "3" (the intermediate value) on the brightness scale.

B. pH Investigation

Sodium hydroxide (NaOH) is a corrosive chemical that can cause skin and eye damage. Gloves and eye protection should be worn at all times.

**The "No-DNA Control" tube from the previous investigation can be separated from the other tubes on the strip and reused as a control for this investigation.*

1. **Label the three remaining 4-tube PCR strips** (200 µl thin-walled tubes) on the side wall.
   - Label the tubes in the first 4-strip A1, A2, A3, A4
   - Label the tubes in the second 4-strip B1, B2, B3, B4
   - Label the tubes in the third 4-strip C1, C2, C3, C4

2. **Add NaOH and/or Buffer to tubes.**
   - To tubes A1, B1, and C1, add 10 µl Buffer.
   - To tubes A2, B2, and C2, add 8 µl Buffer and 2 µl 100 mM NaOH.
   - To tubes A3, B3, and C3, add 5 µl Buffer and 5 µl 100 mM NaOH.
   - To tubes A4, B4, and C4, add 10 µl 100 mM NaOH.

3. **Add Dye to each labeled PCR tube.**
   - **Add 10** µl of Dye to each tube.

4. **Add 5 µl DNA to each tube.**
   - **Add 5** µl sample A to tubes A1, A2, A3 and A4
   - **Add 5** µl sample B to tubes B1, B2, B3 and B4
   - **Add 5** µl sample C to tubes C1, C2, C3 and C4

5. **Gently mix the reagents by pipetting up and down 3-4 times, and cap the tubes**
   - Make sure all the liquid volume collects at the bottom of the tube (tap lightly.)

All tubes will now contain 25 µl volume.

6. **Place tubes in P51™ or other blue light illuminator**
   - If possible record image with your camera.
   - Record brightness values in the "Data" section of your lab manual.

(continued on next page)

Optional

The following steps are optional to this investigation. These steps are independent of each other. You can do both, one, or neither of these.

7. Retest melting temperature of one of your samples.

- Set the thermocycler or heat block to a temperature 10-20˚C below what you previously established sample B melting temperature to be (in Activity A.)
- Place the sample B strip in the thermocycler and wait for at least one minute.
- Place the tube on a blue light illuminator and record your results.
- Compare to earlier observations during Activity A.

8. Add HCl to restore pH. (HCl is not supplied as part of the lab reagents.)

⚠ *Use caution - HCl should always be handled while wearing gloves and eye protection.*

- Add 2 µl of 100 mM HCl to tube A4, and place on the blue light illuminator.
- Continue adding HCl 2 µl at a time until fluorescence is achieved.

C. *Estimating DNA concentration - creating a 2-fold dilution series.*

Can you estimate how much DNA is an unknown sample?

1. **Label an 8-tube strip of PCR tubes**
   - Label the first seven tubes 1-7. Label the final tube "U" for unknown.

2. **To tube 1, add 10 µl of 50:50 AT:GC DNA (tube B).**

3. **Add 5 µl of Buffer to tubes 2-7.**

4. **Remove 5 µl from tube 1 and add it to tube 2.**
   - Pipette up and down gently three times to mix.

5. **Remove 5 µl from tube 2 and add it to tube 3.**
   - Pipette up and down gently three times to mix.

6. **Continue diluting samples in the 8-tube strip.**
   - Remove 5 µl of sample from tube 3 and add it to tube 4. Mix gently.
   - Remove 5 µl of sample from tube 4 and add it to tube 5. Mix gently.
   - Remove 5 µl of sample from tube 5 and add it to tube 6. Mix gently.

7. **Remove 5 µl of sample from tube 6 and discard.**
   - Tubes 1-6 should all have 5 µl of sample in each tube.
   - Tubes 1-6 now have a 2-fold serial dilution series.
   - Tube 7 should have no DNA and will serve as a blank control.

8. **Add 5 µl of the "Unknown DNA Concentration" to tube U.**
   - The teacher will have "Unknown DNA Concentration" at the front of the room.

9. **Add 10 µl of Dye to all 8 tubes.**

10. **Add 10 µl of Buffer to all 8 tubes.**

11. **Cap the 8-strip of PCR tubes and place in P51™ or other blue light illuminator.**
    - Darken the room, or use a light blocking hood to better view the samples.

12. **Estimate the concentration of DNA in the unknown from your dilution series.**
    - Concentration of 50:50 AT:GC DNA *before* adding to tube 1 was 1.5 µM (micromolar).
    - Brightness of the unknown sample is likely to not match any one tube in the dilution series exactly. Use a best approximation.

8. Data tables

A. *Temperature investigation – Linear ramp*

Record the temperature for each observation. For each temperature, assign a "Brightness Value" of 1-5 to each tube. 1=No Fluorescence. 5= Full Fluorescence. Use the brightest tube in your first reading at low temperature as your reference for a value of 5. Taking pictures of your samples can help in making comparisons and assigning brightness scores.

Time:		0													
Temperature:															
Brightness Value	DNA: A														
	DNA: B														
	DNA: C														

B. *pH investigation*

Record the Brightness Value (BV), for each tube.

NaOH added (µl)	0	2	5	10
DNA: A				
DNA: B				
DNA: C				

9. Study questions

A. Watson-Crick base pairing

1. In the following table, classify the four DNA bases as either double-ring or single-ring and as able to form either 2 hydrogen bonds or 3 hydrogen bonds.

	Double-Ring	Single-Ring
2 Hydrogen Bonds		
3 Hydrogen Bonds		

2. In the previous table, did you place the purines in the same row or the same column?

3. Only from the reading, what evidence do you have that hydrogen bonds are weaker than covalent bonds?

4. When trying to establish the three-dimensional structure of DNA, an early hypothesis was that like bound to like. That is, if adenine was on one side of the helix another adenine would be found on the other side of the helix. What aspect of the DNA base pairing rules makes this idea plausible? What aspect makes it unlikely?

5. When copying DNA, copying errors known as transitions, when a purine is switched for another purine or a pyrimidine is switched for another pyrimidine, are about ten times more common than transversions, when a purine is switched for a pyrimidine or *vice versa*. What does this say about the relative importance of the size of the nucleotide versus the number and polarity of the hydrogen bonds it can made in determining base pairing?

6. Why is it biologically important that the hydrogen bonds that hold DNA together can be broken relatively easily?

7. Scientists have found organisms capable of living on hydrothermal vents in temperatures even greater than 100˚C. In this type of extreme environment, from what you have learned about hydrogen bonding, what types of bases could help make the organisms' DNA more stable?

8. PCR primers are short (about 20 bases) sequences of single stranded DNA that are complementary to a known 20-base sequence located on either end of a DNA sequence a scientist is looking to copy. In the polymerase chain reaction (PCR), the reaction mix is heated, denaturing DNA, and then cooled to a specific temperature that will allow the primers to bind to their complementary sequences (annealing). A PCR experiment requires two different primers be added to the sample of DNA, a forward primer and a reverse primer. What would be the problem if one primer were an AT rich strand of DNA, and the other primer were a GC rich piece of DNA?

9. Consider again primer binding to a target DNA during PCR. If the annealing temperature is set too low, sometimes the primers will bind to sequences that are not perfect matches. Considering what you have learned about hydrogen bonds and temperature, why may this be so?

B. Post lab questions

1. Which sample do you think is the AT-rich sample; which is the GC-rich sample; which is the balanced ATGC DNA sample? Justify your answer using evidence from the lab.

2. What temperature did you estimate to be each sample's approximate melting temperature?

   *Note: Melting temperatures observed in this lab will lack precision. Give your best estimate.

3. Explain the above results in relation to Watson-Crick base pairing.

4. How did pH of your samples affect whether or not the DNA remained double stranded?

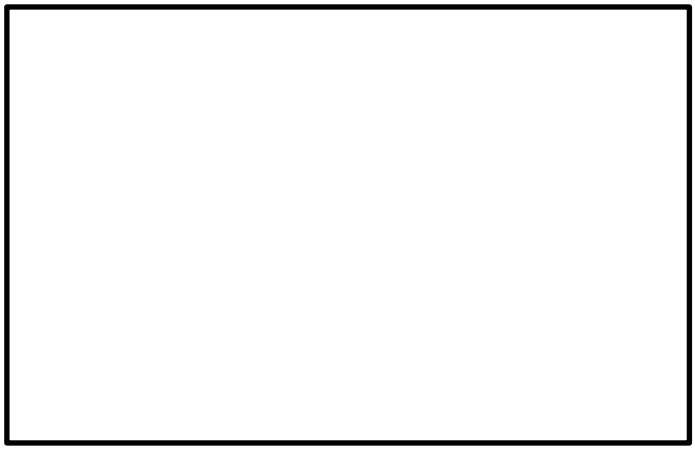
5. Explain your above results in relation to Watson-Crick base pairing.

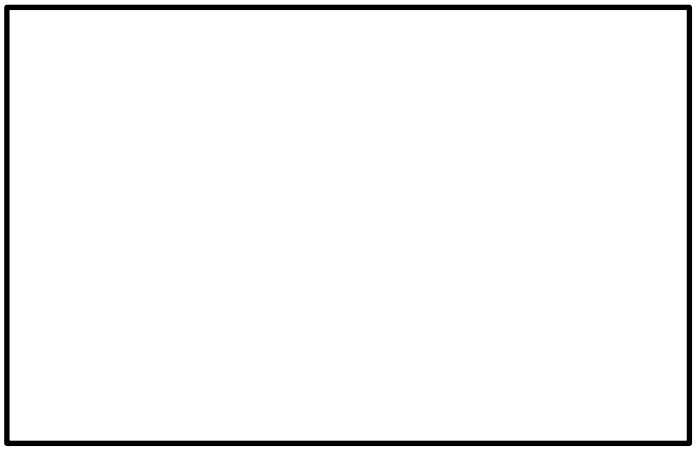
6. What do you think is the concentration of the unknown sample of DNA?

DNA Glow Lab story board

Directions:
Use at least three of the following six boxes to illustrate what occurred at the molecular level in this lab as if it were a comic strip. On the lines beside each box, describe what is happening in each drawing. Use and underline the following words or phrases: adenine, thymine, guanine, cytosine, denature, anneal, hydrogen bond, double helix, purine, pyrimidine.

DNA Glow Lab story board (cont.)





Story board rubric

	4 - Exceeds the Standard	**3** - Meets the Standard	**2** – Approaching the Standard	**1** – Does Not Meet the Standard
Illustrations	The illustrations clearly show the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides. The drawings can be understood without the aid of explanation.	The illustrations show the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides. All concepts are present, but one or two of the illustrations need the help of the written explanation to be clearly understood.	The illustrations show the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides, but some aspects may be unclear or missing. It may be difficult to understand several of the illustrations without the written description.	The illustrations show clear misunderstandings in the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides. It may be difficult to interpret the illustrations even with the help of the written descriptions
Vocabulary Usage	All underlined words are used in a context that makes it clear to the reader that the student has full mastery over their usage. There are no ambiguities or confusing statements. All words must be used.	The majority of underlined words are used in a context that demonstrates to the reader that the student understands their meaning. Some usage may reflect ambiguities or small confusion but there are no explicitly incorrect statements. All words must be used.	Some, but not all, of the words are used in a context that demonstrates their meaning. There may be some ambiguities and confusing statements, but few explicitly incorrect statements. One or two of the words may not be used.	The underlined words are generally misused or many of the words are not used at all.
Description of Process	From the written description alone, the student clearly demonstrates a solid understanding of the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides.	The student can describe the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides. There may be smaller details that are overlooked, but no major step is missed and no clear misconceptions are shown.	The student can outline the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides. There may be some larger details that are overlooked. The student may demonstrate some misconceptions.	The student has difficulty outlining the concept of base pairing and how physical and chemical conditions affect the hydrogen bonds between nucleotides. There may be some major misconceptions, or it is clear the student does not understand the process.

10. References and teaching resources

- "Base Pairing." *Wikipedia.*
- "Nucleic Acid Thermodynamics." Wikipedia
- "The DNA Double Helix Discovery" *HHMI Biointeractive* (video)
- "What is DNA?" Bozeman Science (video)
- "DNA Structure – Base Pair Hydrogen Bonding and Melting Temperature" Moof University (video)
- A Structure for Deoxyribose Nucleic Acid. Watson J.D. and Crick F.H.C. *Nature* 171, 737-738 (1953).
- Secret of Photo 51. Resources from PBS related to the *NOVA* documentary.
- Sexism in Science: Did Watson and Crick Really Steal Rosalind Franklin's Data? Cobb M. *The Guardian*. 23, June 2015.

11. Appendix- If a miniPCR™ or Linear Ramp mode is not available

The protocols in activity A of this lab were optimized for use on a miniPCR™ in Linear Ramp mode. These protocols could be modified to use a water bath or with other machines where a linear ramp protocol is not available. Below is a suggested protocol for use on a thermal cycler or other programable heat block or water bath with no ramp function. Activities B and C do not require a heat source or thermal cycler.

Tubes that are heated to 95° and above have the potential to pop open unexpectedly. Gloves and protective eyewear should be worn for the entirety of this lab.

1. **Incubate the tubes for at least 1 minute at 95˚C.**
   - Use a thermal cycler in Heat Block mode, a heat block, or water bath.
   - Close the heated lid on the PCR machine to avoid lids opening under pressure. Be aware that if using another heat source, PCR tube lids may pop open when heated.

2. **Remove tubes from heat block and *immediately* transfer to P51™ or other blue light illuminator.**
   ⚠ *Careful, heat block and PCR lid will be hot*
   - Tubes will begin cooling off as soon as they are removed from heat, so it is important to visualize the tubes within the first few seconds.
   - Darken the room or use a light blocking hood to better view the samples.

3. **If possible, record an image of the samples.**

4. **Continue viewing the samples for up to 2 minutes.**
   - Note changes in fluorescence over time.
   - Note the relative time taken for each sample to regain fluorescence
   - Move on once all three tubes have regained fluorescence. Note - maximum fluorescence is likely to be less than when originally viewed prior to heating.
   - Samples can be reheated to observe again if necessary.

5. **Use observations to predict contents of tubes.**
   - Tubes contain AT rich, GC rich and AT:GC DNA.

Identify the melting temperature of your samples

6. **Make sure all three samples have fully cooled.**
   - It can be helpful to briefly place the samples on ice or wave tubes in air for a short time to help cool.

7. **Using a programmable heat block: Try different temperatures to establish the melting temperature (T_m) of each different sample.**
    - Start by heating the sample to 45 ˚C. Repeat the above procedure, increasing the temperature of the heat block each time by 5-10 ˚C.
    - If a sample does not fluoresce immediately after removing it from the heat block and viewing on the illuminator, the sample is above T_m.
    - If a sample fluoresces immediately after removing it from the heat block and viewing on the illuminator, the sample is below T_m.
    - Remember to heat the samples for at least one minute to ensure thorough heating and denaturation.
    - Remember to transfer samples to the illuminator and to view as quickly as possible as the temperature of the sample will change as soon as it is removed from the heat block.
    - It may be easiest to start at a low temperature and work upwards to avoid having to cool samples in between temperatures.
    - Melting temperatures should be clearly distinguishable between samples and resolvable to approximately 5-10˚ accuracy.

8. **Using multiple water baths: Try different temperatures to establish the melting temperature (T_m) of each different sample.**
    - Use at least three water baths set at intervals between 55˚ and 95˚ C.
    - If only using three water baths, we recommend trying 70˚, 85˚, and 95˚ C.

12. Ordering Information

miniPCR DNA Glow Lab™ (KT-1900-01) **contains the following reagents:**

- Concentrated DNA Dye
- Buffer 1
- Buffer 2
- AT rich DNA sample
- GC rich DNA sample
- 50:50 AT:GC DNA sample
- 100 mM NaOH
- 1.7 ml microcentrifuge tubes
- PCR strip tubes

To order miniPCR DNA Glow Lab, the P51™ Molecular Fluorescence Viewer, or other lab kits:

- Call (781)-900-8PCR
- Email orders@minipcr.com
- Visit www.minipcr.com

Materials are sufficient for 8 lab groups, or 32 students

All components should be kept refrigerated at 4° C for long-term storage

Reagents must be used within 12 months of shipment

13. About miniPCR Learning Labs

This Learning Lab was developed by miniPCR™ in an effort to make molecular biology and genetics more approachable and accessible.

We believe that there is no replacement for hands-on experimentation in the science learning process. We also believe, based on our direct involvement working in educational settings, that it is possible for these experiences to have a positive impact in students' lives. Our goal is to increase everyone's love of DNA science, scientific inquiry, and STEM. We develop Learning Labs to help achieve these goals, working closely with educators, students, academic researchers, and others committed to science education.

Starting on a modest scale, miniPCR™ Learning Labs are designed to bring real scientific inquiry at an affordable price to the science classroom, and their use is growing rapidly through academic and outreach collaborations. See our complete line of miniPCR™ Learning Labs and innovative, affordable biotechnology equipment at minipcr.com.

Authors: Bruce Bryan M.S., Sebastian Kraves, Ph.D.