



Dolan DNA Learning Center
Cold Spring Harbor Laboratory

Detecting a Transposon in Corn



Funded by NSF

Detecting a Transposon in Corn

REAGENTS, SUPPLIES, AND EQUIPMENT CHECKLIST (for 24 students)

Reagents

- ☐ Maize seeds from cross of *Bz/bz* heterozygotes
- ☐ Edward's buffer
- ☐ 100% isopropanol
- ☐ Tris/EDTA (TE) buffer
- ☐ *BZ1/BZ2* primer/loading dye mix*
- ☐ *BZ1/Ds* primer/loading dye mix*
- ☐ Ready-to-Go™ PCR Beads
- ☐ Mineral oil (depending on thermal cycler)
- ☐ DNA Marker pBR322/*Bst*NI (0.075 µg/µl)*
- ☐ Agarose
- ☐ 1x TBE electrophoresis buffer
- ☐ Ethidium bromide solution (1 µg/ml) OR
- ☐ *CarolinaBLU*™ Gel & Buffer Stain
- ☐ *CarolinaBLU*™ Final Stain

Supplies and Equipment

- ☐ Planting pots or tray
- ☐ Plastic dome lid or plastic wrap
- ☐ Potting soil
- ☐ Micropipets and tips (1 µl to 1000 µl)
- ☐ 1.5-ml microcentrifuge tubes
- ☐ Microcentrifuge tube racks
- ☐ Microcentrifuge for 1.5-ml tubes
- ☐ Plastic pestles
- ☐ Thermal cycler
- ☐ Water bath for agarose (60°C)
- ☐ Electrophoresis chambers, trays, combs
- ☐ Electrophoresis power supplies
- ☐ Latex gloves
- ☐ Staining trays
- ☐ UV transilluminator (ethidium bromide staining)
- ☐ White light box (*CarolinaBLU*™ staining)
- ☐ Camera or photo-documentary system
- ☐ Permanent markers
- ☐ Containers with cracked or crushed ice
- ☐ Cool white fluorescent light (optional)
- ☐ Vortexer (optional)
- ☐ Masking tape
- ☐ Water

*Store at –20°C.

Ready-to-Go™ PCR Beads incorporate *Taq* polymerase, dNTPs, and MgCl₂. Each bead is supplied in an individual 0.5-ml tube or a 0.2-ml tube.

Detecting a Transposon in Corn

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STUDENT LAB INSTRUCTIONS

INTRODUCTION

Throughout the first half of the 20th century, geneticists assumed that a stable genome was a prerequisite for faithfully transmitting genes from one generation to the next. Working at Cold Spring Harbor Laboratory in the post-WWII era, Barbara McClintock found quite a different story in maize



Barbara McClintock at Cold Spring Harbor Laboratory, about 1950. Courtesy Cold Spring Harbor Laboratory Archives.

(corn). She observed numerous “dissociations” – broken and ring-shaped chromosomes – and traced the source of these mutations to two related loci, “dissociator” (*Ds*) and “activator” (*Ac*) on the short arm of chromosome 9.

Equipped only with her maize crosses and a light microscope, she showed that *Ac* and *Ds* are mobile genetic elements that transpose, or jump, from one chromosome location to another.

McClintock also offered genetic proof that *Ac* moves independently, but *Ds* depends on *Ac* for transposition. She showed that transposons may inactivate gene expression

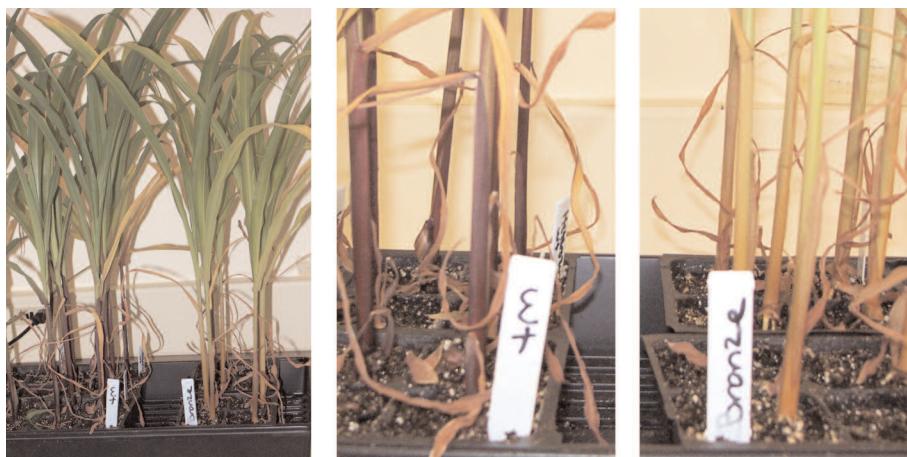
by inserting into a gene, or may reactivate expression by jumping out. Thus, McClintock explained color variegations, such as speckled kernels, that had intrigued botanists for centuries. (See Concept 32 of *DNA From the Beginning*, www.dnafb.org, for an animation explaining McClintock’s elucidation of the *Ac/Ds* system.)

Today the *Ac/Ds* system is an important tool in gene discovery, allowing scientists to characterize genes for which no biological role is known. In a process known as transposon mutagenesis, *Ac* and *Ds* elements are crossed into a corn strain to produce *Ds* insertions in genes. The *Ac/Ds* mutagenesis system also works well in a number of other plants including tobacco, tomato, and the model plant *Arabidopsis thaliana*.

This laboratory investigates a bronze (*bz*) mutant of maize to analyze the molecular relationship between genotype and phenotype. The *Bz* gene encodes an enzyme that catalyzes an important step in the biosynthesis of anthocyanins – red, purple, and blue pigments in plants. Wild-type *Bz* kernels are dark purple, while a transposon insertion in the *Bz* gene abolishes anthocyanin production, leaving kernels dark yellow or *Bronze*. The *bz* mutation also affects stem and leaf coloration.

In the lab, DNA is isolated from corn plants, and the *Bz* locus is amplified using polymerase chain reaction (PCR). Two primer sets – one specific to the wild-type allele (*BZ1/BZ2*) and one for the mutant allele bearing the *Ds* insertion (*BZ1/Ds*) – amplify DNA fragments that can be readily differentiated by agarose gel electrophoresis. Bioinformatics analysis maps the *Ds* insertion in the gene and identifies the protein inactivated in the bronze mutant.

Wild-type and *Bronze* (*bz*) mutant plants



Wild-type (purple) and bronze kernels.
Cob 637 (*Bz/Bz*) and cob 638 (*bz/bz*) are from homozygous self crosses. Cob 637/638 is a heterozygote (*Bz/bz*) from a cross between 637 and 638.

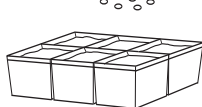


- Dooner, H. K., Weck, E., Adams, S., Ralston, E., Favreau M., et al., (1985). A molecular genetic analysis of insertion mutations in the bronze locus in maize. *Mol. Gen. Genet.* **200**: 240–246.
- Edwards, K., Johnstone, C., and Thompson, C. (1991). A Simple and Rapid Method for the Preparation of Plant Genomic DNA for PCR Analysis. *Nucleic Acids Res.* **19**: 1349.
- Fedoroff, N., Wessler, S., and McClure, M. (1983). Isolation of the Transposable Maize Controlling Elements *Ac* and *Ds*. *Cell* **35**: 235–242.
- McClintock, B. (1951). Chromosome Organization and Genic Expression. *Cold Spring Harbor Symp. Quant. Biol.* **16**: 13–47.

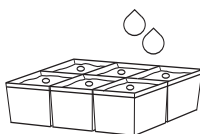
LAB FLOW

I. PLANT MAIZE SEED

PLANT
seeds



WATER
seeds



2-3 WEEKS

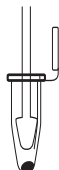


II. ISOLATE DNA FROM MAIZE

ADD
leaf
tissue



GRIND



ADD
Edward's
buffer



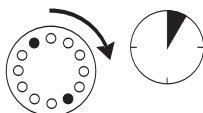
GRIND



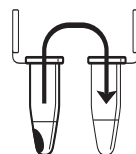
VORTEX



CENTRIFUGE



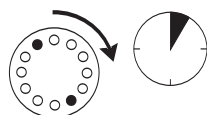
TRANSFER
supernatant



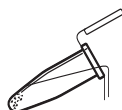
ADD and MIX
Isopropanol



CENTRIFUGE



POUR OFF
supernatant



REMOVE
supernatant



DRY



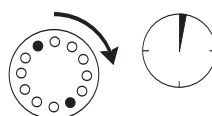
ADD
TE buffer



RESUSPEND
DNA



CENTRIFUGE



leaf
DNA
extract

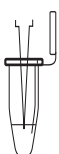


III. AMPLIFY DNA BY PCR

ADD
primer/
loading
dye mix



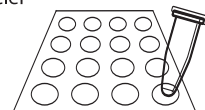
ADD
DNA



ADD
mineral oil
(if necessary)

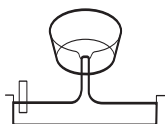


AMPLIFY
in thermal
cycler



IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

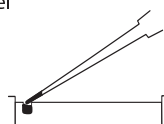
POUR
gel



SET



LOAD
gel



ELECTROPHORESE
130 volts



METHODS

I. PLANT MAIZE SEED

To extract DNA from plant tissue, you must plant the corn seeds 2-3 weeks prior to DNA isolation and PCR. Depending upon growing conditions, you may observe the phenotypic differences between mutant and wild-type plants in as little as 1 week.

Reagents (per student station)	Supplies and Equipment
Maize seeds from a self cross of a <i>Bz/bz</i> heterozygote plant	Planting pots and tray Plastic dome lid or plastic wrap Potting soil Water

1. Moisten the potting soil. Fill planting pots evenly with soil, but do not pack tightly.
2. Fit pots into the tray, but leave one corner space empty to facilitate watering.
3. Plant seeds 0.5 inches below the soil using your finger. Plant one seed per pot, so they will grow better and plant phenotypes can be readily observed.
4. Cover pots with plastic dome lids or plastic wrap to maintain humidity during germination. (Remove covers 3–4 days after planting.)
5. Add 1/2 inch of water to tray, using the empty corner space. Water regularly to keep soil damp, but do not allow soil to remain soggy.
6. Grow the plants close to a sunny window at room temperature (20–22°C) or slightly warmer. If the plants are grown under cold conditions, it may take longer to discern the different phenotypes.
7. Harvest plant tissue for PCR when the difference in phenotype between wild-type and *bz* plants becomes evident. Plants homozygous for the *bz* mutation have bronze pigmented stalks, while wild-type plants have dark purple stalks. You may wish to continue to grow the plants after you have harvested tissue for DNA isolation and amplification; the phenotypic differences between mutant and wild-type plants become more obvious over time.

To prevent the soil from drying out, keep a small amount of water in the tray at all times.

For optimum growth, provide constant (24-hour) fluorescent lighting.

II. ISOLATE DNA FROM MAIZE

Reagents (per student station)	Supplies and Equipment
Wild type or mutant maize plant	Plastic pestles
Edward's buffer, 1 ml	Permanent marker
100% isopropanol, 1 ml	Two 1.5-ml microcentrifuge tubes
Tris/EDTA (TE) buffer, 1 ml	Micropipet and tips (100-1000 µl)
	Microcentrifuge tube racks
	Microcentrifuge
	Vortexer (optional)
	Container with cracked or crushed ice

Your instructor will assign a maize plant to you.

When fully ground, the sample should be a green liquid.

Detergent in the Edward's buffer dissolves lipids of the cell membrane.

This step precipitates nucleic acids, including DNA.

Centrifugation pellets the nucleic acids. The pellet may appear as a tiny teardrop-shaped smear or particles on the bottom side of the tube underneath the hinge. Don't be concerned if you can't see a pellet. A large or greenish pellet is cellular debris carried over from the first centrifugation.

You will use 2.5 µl of the DNA extract for the PCR reactions in Part III. The crude DNA extract contains nucleases that will eventually fragment the DNA at room temperature. Keeping the sample cold limits this activity.

1. Obtain a wild-type or *bz* mutant maize plant, and record its phenotype.
2. Harvest two pieces of leaf tissue approximately 1/4 inch in diameter. (The large end of a 1000-µl pipet tip will punch disks of this size.) Place tissue in a clean 1.5-ml tube, and label with your group number.
3. Grind the plant tissue by twisting it with a clean plastic pestle for 1 minute *forcefully* against the inner surface of the 1.5-ml tube.
4. Add 400 µl of Edward's buffer to the tube. Grind briefly to remove tissue from the pestle and to liquefy any remaining pieces of tissue.
5. Vortex the tube for 5 seconds, by hand or machine.
6. Place the tube in a balanced configuration in a microcentrifuge, and spin for 2 minutes to pellet any remaining cell debris. Spin longer if there is still unpelleted debris.
7. Transfer 350 µl of supernatant to a fresh tube labeled with plant phenotype and group number. Be careful not to disturb the pelleted debris when transferring the supernatant. Discard the old tube containing the precipitate.
8. Add 400 µl of isopropanol to the tube of supernatant.
9. Close the tube and mix by inverting it several times, then leave at room temperature for 3 minutes.
10. Place your tube and those of other teams in a balanced configuration in a microcentrifuge, with the hinge of the cap pointing outward. (During centrifugation nucleic acids will gather on the side of the tube underneath the hinge.) Spin for 5 minutes at maximum speed to pellet the DNA.
11. Carefully pour off and discard the supernatant from the tube. Then, *completely* remove the remaining liquid with a medium pipet set at 100 µl.
12. Air dry the pellet by letting the tube sit open for 10 minutes to evaporate any remaining isopropanol.
13. Add 100 µl of TE buffer to the tube and dissolve pelleted DNA by pipetting in and out. Take care to wash down the side of the tube underneath the hinge, where the pellet formed during centrifugation.
14. DNA may be used immediately, or stored at -20°C until you are ready to continue with Part III. Keep the DNA on ice during use.

III. AMPLIFY DNA BY PCR

Reagents (per student station)	Supplies and Equipment
*Wild type or <i>bz</i> maize DNA (from Part II)	Permanent marker
* <i>BZ1/BZ2</i> primer/loading dye mix, 30 μ l	Micropipets and tips (1-100 μ l)
* <i>BZ1/Ds</i> primer/loading dye mix, 30 μ l	Microcentrifuge tube rack
Ready-To-Go™ PCR Beads	Container with cracked or crushed ice
Shared Reagents	Thermal cycler
Mineral oil, 5 ml (depending on thermal cycler)	
*Store on ice	

The primer loading dye mix will turn pink as the Ready-To-Go™ PCR Bead dissolves.

If the reagents become splattered on the wall of the tube, pool them by pulsing in a microcentrifuge (use appropriate adapters) or by sharply tapping the tube bottom on the lab bench.

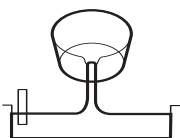
Mineral oil prevents the PCR mix from evaporating and condensing on the tube cap during cycling. Most modern thermal cyclers have heated lids that prevent condensing and DO NOT require the addition of mineral oil.

- Set up *BZ1/BZ2* reaction:
 - Obtain a PCR tube containing a Ready-To-Go™ PCR Bead. Label with the primer set (e.g. "*BZ*") and your group number.
 - Use a micropipet with a fresh tip to add 22.5 μ l of the *BZ1/BZ2* primer/loading dye mix to your tube. Allow bead to dissolve.
 - Use a micropipet with a fresh tip to add 2.5 μ l of maize DNA (from Part II). Insert the pipet tip directly into the PCR mix, eject the DNA, and insure that the tip comes out empty.
- Set up *BZ1/Ds* reaction:
 - Obtain a PCR tube containing a Ready-To-Go™ PCR Bead. Label with the primer set (e.g. "*Ds*") and your group number.
 - Use a micropipet with a fresh tip to add 22.5 μ l of the *BZ1/Ds* primer/loading dye mix to your tube. Allow the bead to dissolve.
 - Use a micropipet with a fresh tip to add 2.5 μ l of maize DNA (from Part II). Add DNA directly into the PCR mix.
- If necessary, top the reactants in the PCR tubes with one drop of mineral oil. Be careful not to touch the dropper tip to the tube or reactants, or subsequent reactions will be contaminated with DNA from your preparation.
- Store samples on ice until you are ready to begin thermal cycling.
- Program the thermal cycler for 30 cycles of the following profile. The program may be linked to a 4°C hold step after the 30 cycles are completed.

Denaturing step:	94°C	30 seconds
Annealing step:	55°C	30 seconds
Extending step:	72°C	30 seconds
- After cycling, store the amplified DNA at –20°C.

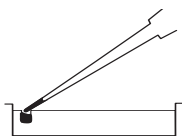
IV. ANALYZE PCR PRODUCTS BY GEL ELECTROPHORESIS

Reagents (per student station)	Supplies and Equipment
*Maize PCR products (from Part III)	Micropipets and tips (1-100 µl)
1× TBE, 300 ml	1.5-ml microcentrifuge tube
2% agarose in 1× TBE, 50 ml	1.5-ml microcentrifuge tube rack
	Gel electrophoresis equipment
Shared Reagents	Masking tape
*pBR322/BstNI marker, 130 µl	Power supply
Ethidium bromide (1 µg/ml), 250 ml	Staining trays
or	Latex gloves
CarolinaBLU™ Gel & Buffer Stain, 7 ml	UV transilluminator (for use with ethidium bromide)
CarolinaBLU™ Final Stain, 250 ml	White light transilluminator (for use with CarolinaBLU™)
	Digital or instant camera (optional)
	Water bath (60°C)
*Store on ice	Container with cracked or crushed ice



Avoid pouring an overly thick gel, which is more difficult to visualize. The gel will become cloudy as it solidifies.

Do not add more buffer than necessary. Too much buffer above the gel channels electrical current over the gel, increasing running time.



Expel any air from the tip before loading. Be careful not to push the pipet tip through the bottom of the sample well.

1. Seal the ends of the gel-casting tray with masking tape, and insert a well-forming comb.
2. Pour 2% agarose solution to a depth that covers about $\frac{1}{3}$ the height of the open teeth of the comb.
3. Allow the gel to solidify completely. This takes approximately 20 minutes.
4. Place the gel into the electrophoresis chamber, and add enough 1× TBE buffer to cover the surface of the gel.
5. Carefully remove the combs, and add additional 1× TBE buffer to just cover gel and fill its wells – creating a smooth buffer surface.
6. Use a micropipet with a fresh tip to add 25 µl of the BZ1/Ds sample/loading dye mixture into your first assigned well of a 2% agarose gel. (If you used mineral oil during PCR, pierce your pipet tip through the layer of mineral oil to withdraw the PCR products and leave the mineral oil behind in the original tube.)

MARKER	PLANT 1		PLANT 2		PLANT 3	
pBR322/ BstNI	BZ1/Ds	BZ1/BZ2	BZ1/Ds	BZ1/BZ2	BZ1/Ds	BZ1/BZ2
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

7. Use a micropipet with a fresh tip to add 25 µl of the BZ1/BZ2 sample/loading dye mixture into your second assigned well of a 2% agarose gel.
8. Load 20 µl of the molecular weight marker (pBR322/BstNI) into one well. (Alternatively, use appropriate amount of a 100-bp DNA ladder.)
9. Run the gels at 130 V for approximately 30 minutes. Adequate separation will have occurred when the cresol red dye front has moved at least 50 mm from the wells.



Destaining the gel for 5-10 minutes in tap water leeches unbound ethidium bromide from the gel, decreasing background and increasing contrast of the stained DNA.

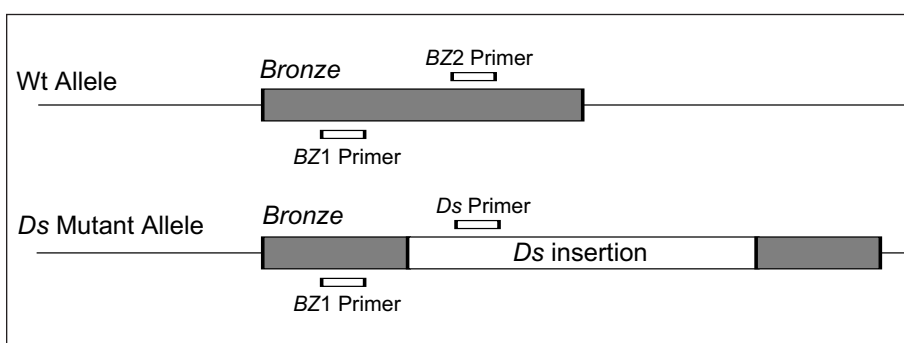
Transillumination, where the light source is below the gel, increases brightness and contrast.

10. Stain the gel in ethidium bromide or *CarolinaBLU*[™]:
 - a. For ethidium bromide, stain 10-15 minutes. Decant stain back into storage container for reuse, and rinse gel in tap water. Use gloves when handling ethidium bromide solution and stained gel, or anything that has ethidium bromide on or in it. Ethidium bromide is a known mutagen and care needs to be taken when using and disposing of it.
 - b. For *CarolinaBLU*[™] staining, follow directions in the Instructor Planning section.
11. View gel using transillumination, and photograph using a digital or instant camera. Draw an image of the bands on the gel if a camera is not available.

RESULTS & DISCUSSION

This laboratory investigates the *Bronze* (*Bz*) gene of *Zea mays* to show the molecular relationship between genotype and phenotype. The *bz* mutation seen in this lab was created by a *Ds* transposon insertion and produces a change in plant coloration that affects all plant organs, including stalk, leaves, and seed epidermis.

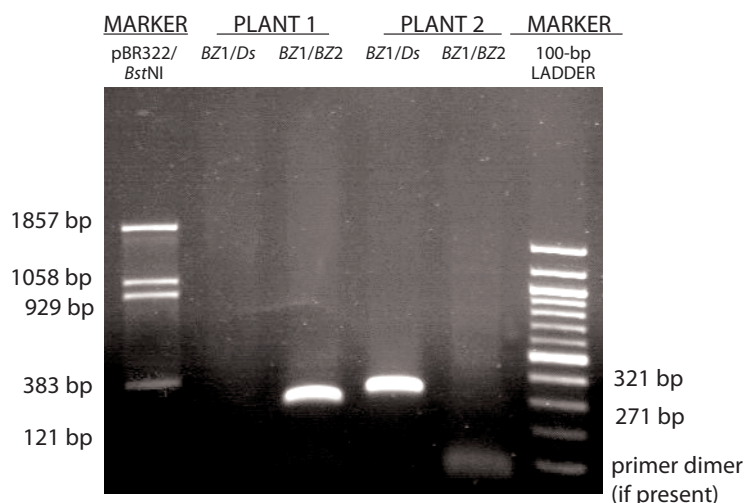
DNA was isolated from maize plants, and the *Bz* locus was amplified using polymerase chain reaction (PCR). Since the *Ds* insertion at the *Bz* locus is too large to amplify across, two sets of primers were used. One set (*BZ1/BZ2*) straddles the *Ds* insertion site and only amplifies the wild-type (*Wt*) allele. The second set (*BZ1/Ds*) only amplifies the *Ds*-insertion allele, with one primer located in the *Bz* gene and one located in the *Ds* transposon.



PCR amplification of the *BZ* locus

- Describe the purpose of each of the following steps or reagents used in DNA isolation (Part II):
 - grinding with pestle.
 - Edward's buffer.
 - Tris-EDTA (TE) buffer.
- What is the purpose of performing each of the following PCR reactions:
 - BZ1/BZ2*?
 - BZ1/Ds*?
- The photo on the following page shows an example of expected results. Indicate in the chart below which result is derived from Plant 1 and which from Plant 2, and complete the entries for genotype and phenotype. Which of the three rows is not represented in the gel? (Remember that your own samples can yield any of the combinations for *BZ1/BZ2* and *BZ1/Ds* in the table below.)

321bp (<i>BZ1/Ds</i>)	271 bp (<i>BZ1/BZ2</i>)	Plant Genotype	Plant Phenotype	Sample
present	present			
present	absent			
absent	present			



4. View the image of the stained gel containing your sample and those of other students. Orient the photograph with wells at the top. Interpret each lane of the gel.
 - a. Scan across the photograph of your gel to get an impression of what you see in each lane. You should notice that virtually all experiment lanes contain one prominent band.
 - b. Now locate the lane containing the pBR322/BstNI marker on the left hand side of the gel. Working from the well, locate the bands corresponding to each restriction fragment: 1,857 bp, 1,058 bp, 929 bp, 383 bp, and 121 bp (may be faint or not visible). (If you used the 100-bp marker, locate the lane containing the 100-bp ladder, with the fastest migrating band equal to 100 bp and each successive band 100 bp larger.)
 - c. The amplification product of the mutant *bz* allele (321 bp) should align just ahead of the 383-bp fragment of the pBR322/BstNI marker (or between the 300-bp and 400-bp fragments of the 100-bp ladder).
 - d. The amplification product of the wildtype *Bz* allele (271 bp) should align between the 121-bp and 383-bp fragments of the pBR322/BstNI marker (or between the 200-bp and 300-bp fragments of the 100-bp ladder).
 - e. It is common to see one or two diffuse (fuzzy) bands of RNA and/or primer dimer at the bottom of the gel. RNA may be found at approximately the position of the 121-bp fragment of the pBR322/BstNI marker (or the 100-bp marker of the 100-bp ladder). RNA is the largest component of nucleic acid isolated from plant tissue. Primer dimer is an artifact of the PCR reaction that results from two primers overlapping one another and amplifying themselves. Primer dimer is approximately 50 bp, and should be in a position ahead of the 121-bp marker fragment.
 - f. Additional faint bands, at other positions in the gel, occur when the primers bind to chromosomal loci other than the *Bz* locus,

giving rise to "nonspecific" amplification products.

5. Based on analysis of the agarose gel, what is the genotype of your plant?
6. How would you interpret a lane in which you observe primer dimer, but not the *bz* or wild-type alleles?
7. Would you classify the *bz* mutation as recessive or dominant? Explain your reasoning.
8. Based on the genotypic distribution of the plants analyzed by the class, what genotype was the parental plant? Why may the genotype distribution observed by your class deviate from what is expected under Mendel's laws of inheritance?

BIOINFORMATICS

Biological information is encoded in the nucleotide sequence of DNA. Bioinformatics is the field that identifies biological information in DNA using computer-based tools. Some bioinformatics algorithms aid the identification of genes, promoters, and other functional elements of DNA. Other algorithms help determine the evolutionary relationships between DNA sequences.

Because of the large number of tools and DNA sequences available on the Internet, experiments done *in silico* ("in silicon," or computer) now complement experiments done *in vitro* (in glass, or test tube). This movement between biochemistry and computation is a key feature of modern biological research.

In Part I you will use the Basic Local Alignment Search Tool (BLAST) to identify sequences in biological databases and to make predictions about the outcome of your experiments. In Part II you will identify the sequences of the BZ protein and the *BZ1/BZ2* amplicon. In Part III you will discover what chromosome the *Bz* gene is located on. In Part IV you will determine where in the *Bz* gene the *Ds* transposon is inserted into to produce the bronze phenotype. In Part V you will learn about the function and evolutionary history of the BZ protein.

The following primer sets were used in the experiment:

<i>BZ1</i>	5'-CGAATGGCTGTTGCATTCCAT-3' (Forward Primer)
<i>BZ2</i>	5'-ACGGGACGCAGTTGGCAGGA-3' (Reverse Primer)
<i>BZ1</i>	5'-CGAATGGCTGTTGCATTCCAT-3' (Forward Primer)
<i>Ds</i>	5'-TCTACCGTTCCGTTTCCGTTT-3' (Reverse Primer)

I. Use *BLAST* to Find DNA Sequences in Databases (Electronic PCR)

1. Initiate a BLAST search.
 - a. Open the Internet site of the National Center for Biotechnology Information (NCBI), www.ncbi.nlm.nih.gov/.

- b. Click on *BLAST* in the top speed bar.
 - c. Under *Basic BLAST* click on *nucleotide blast*.
 - d. Enter the *BZ1/BZ2* primer set sequences into the window. These are the query sequences.
 - e. Remove any non-nucleotide characters (anything that's not A, C, G, or T) because they will not be recognized by the BLAST algorithm.
 - f. Under *Choose Search Set* check *Others (nr etc.)*.
 - g. Click on *BLAST* and the query sequences are sent to a server at the National Center for Biotechnology Information in Bethesda, Maryland. There, the BLAST algorithm will attempt to match the primer sequences to the millions of DNA sequences stored in its database *GenBank*. This may take only a few seconds, or more than a minute if a lot of other searches are queued at the server.
2. The results of the BLAST search are displayed in three ways as you scroll down the Results page:
- a. First, a graphical overview illustrates the significance of matches and how they align with the query sequence. Matches of differing lengths are coded by color.
 - b. This is followed by a table listing *Sequences producing significant alignments*, or hits, with GenBank *Accession* numbers, sequence *Descriptions*, *Scores*, *E-values*, and the like. (The *Expectation* or *E-Value* is the number of alignments with the query sequence that would be expected to occur by chance in the database. The lower the *E-Value* the higher the probability that the hit is related to the query. What does an *E-value* of 0.025 indicate? Note the names of any *significant alignments* that have *E-values* less than 0.1. Do they make sense?)
 - c. Next, the section *Alignments* provides detailed views of each primer sequence (*Query*) aligned to nucleotide sequence it matches within the various GenBank search hits (*Sbjct*). Notice that the first match to the forward primer (nucleotides 1-22), and to the reverse primer (nucleotides 23-43) are located in the same GenBank datasheet (*gb | Accession number*). Which positions do the primers match in this *subject* sequence?
 - d. What can you say about the rest of the alignments in the list?
3. Calculate the predicted length of the product that the primer set will amplify in a PCR reaction (*in vitro*) as follows:
- a. In the *Alignments* section locate the alignment for *gb | DQ493652.1*.
 - b. Click the GenBank accession number *gb | DQ493652.1* and scroll to the bottom of the datasheet – this is the sequence the term *Sbjct* (for subject) refers to.
 - c. Return to the alignments for *gb | DQ493652.1* in the *Alignments* section using your browser's Back button.

- d. The lowest and highest nucleotide positions in the subject sequence indicate the borders of the amplified sequence in the PCR reaction. Subtract one from the other to arrive at a good approximation for the length of this sequence stretch.
- e. However, the actual length of the amplified fragment *includes* both ends, so add 1 nucleotide to the result to determine the exact length of the PCR product amplified by the two primers.

II. Identify the *Bz* Amino Acid Sequence and the *BZ1/BZ2* Amplicon

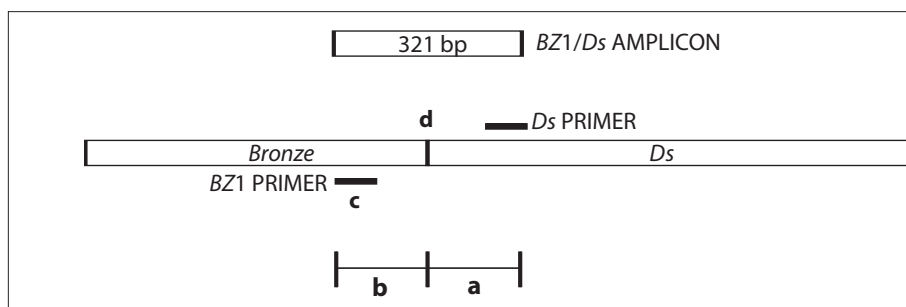
1. Click the Genbank accession *gb* | *DQ493652.1*-link to open the sequence datasheet.
2. At the top of the report, under *LOCUS*, is basic information about the sequence, including its basepair length, database accession number, source, and references. Describe the source and size of the sequence in which your BLAST hit is located.
3. The middle part, under *FEATURES*, contains annotations of sequence features such as genes, mRNA, and coding sequence (*CDS*), with their beginning and ending nucleotide positions. For example, the sequence in this GenBank record is encoding a protein from position 76 to 598 and from position 699 to 1591. What do you think the “,” in (76..598,699..1591) stands for? Using the nucleotide positions that match the primers (as determined in I.3.d above) identify the feature(s) the two primers span. What is the name of the locus?
4. Look at the *CDS* feature. “Join” shows the coordinates of coding exons that are spliced together for translation into a polypeptide chain. Each entry, such as 76..598, gives the first and last nucleotides of one exon. How many exons are in the *Bz* gene? How many introns? Where does the reverse primer end? Which part of the *Bz* gene does the amplicon span? (The use of brackets – “<” or “>” – indicates that the feature discussed extends beyond the nucleotide position indicated.)
5. The final part of the *CDS* entry is labeled */translation* which lists the 1-letter abbreviations for the amino acids specified by the coding sequence. Copy the entire amino acid sequence, and paste it into a text document for future use.
6. At the bottom of the report is the entire nucleotide sequence of this DNA sequence. Highlight all the nucleotides from the beginning of the forward primer to the end of reverse primer. Copy this sequence and paste it into a text document for future use. Delete all non-nucleotide characters (anything that’s not A, C, G, or T). What does this sequence resemble in the PCR reaction *in vitro*?
7. Examine another hit, *gb* | *AF391808.2*, and see whether the results you worked out above hold steady. Using this datasheet identify the chromosome *Bz* is located on and some of *Bz*’s neighbor genes.

III. Use Map Viewer to Determine the Chromosome Location of the *Bz* Gene

1. Return to the NCBI home page, then click on *Map Viewer* located in the *Hot Spots* column on the right.
2. Under *Plants* click on *Zea mays* (corn).
3. Enter "*bz1*" into the *Search for* window and click *Find*.
4. Small horizontal red bars on chromosomes indicate the positions of hits. What chromosome is the *bz1* gene located on?
5. Click the number underneath the chromosome to zoom in the locus.
6. Barbara McClintock worked on a number of genes involved in corn anthocyanin biosynthesis, some of which she mapped to chromosome 9. Can you identify any genes in the vicinity of the *Bz* (*bz1*) locus? Click their names or, if provided, *acc*-links to find more information for these genes. What can you find out about some of them? (For an idea what genes to look out for view the animation at <http://www.dnaftb.org/dnaftb/32/concept/index.html>.)

IV. Determine the Insertion Site of the *Ds* Transposon

1. The PCR results show that the *BZ1/Ds* amplicon, from *bz* mutants, is approximately 320 nucleotides long. This amplicon is composed partly of sequence from the *Bz* gene and partly of sequence from the *Ds* transposon. After determining how many nucleotides of the amplicon are from the *Ds* transposon, you can estimate the position "d" at which *Ds* inserted into the *Bz* gene.
2. Conduct a *nucleotide BLAST* search with the *BZ1/Ds* primers. What hits match the *BZ* primer? Can you identify a hit that is in a *Bz* gene, yet also matches the *Ds* primer?



3. Locate the alignment for *Zea mays bz-m2(Ac)* (*gb* | AF355378) and determine the length of the sequence fragment the two primers span.
4. Then, identify a hit in a *Ds* transposon (i.e. *gb* | AF332952.1) and determine how far from the edge the *Ds* primer maps. Use *gb* | DQ493652.1 to label the diagram with the answers to Questions a-c below to help you determine the *Ds* insertion site in the *Bz* gene (d.).

- a. How far into the *Ds* sequence does the *Ds* primer reach?
 - b. How many *Bz* nucleotides are in the *BZ1/Ds* amplicon?
 - c. What is the first nucleotide position of the *BZ1* primer in the *Bz* amplicon?
 - d. What is the insertion position of *Ds*?
5. Return to the mRNA or CDS entries in the datasheet for the *Bz* gene (II.4. above) and determine where in the *Bz* gene the *Ds* transposon is inserted.
 6. Examine the annotation in the datasheet *gb | AF391808.2* (the same as in II.7. above) and see whether you can find additional transposons inserted into this sequence. What are some of the names of your finds?

V. Use BLAST to Determine the Function of the *Bz* Protein

1. Return to the BLAST page at NCBI, www.ncbi.nlm.nih.gov/BLAST/. This time, click on *protein blast*.
2. Copy the *Bz* amino acid sequence from your text file (II.5. above), and paste it into the *Search* window.
3. Click on *BLAST* to send the amino acid sequence to the NCBI server.
4. An algorithm quickly scans the query sequence to identify functional domains (regions of the protein) that are conserved in different organisms. If a *conserved domain* is being displayed, click it to get some quick information about it. What do you find?
5. Close the *Conserved domains* pop-up window and return to the BLAST Results page.
6. What can you tell from the graphical overview?
7. Scroll down to the list of *Sequences producing significant alignments*. What can you conclude from the *E-values*, the titles of the hits, and the *gb-links*?
8. Scroll down to the *Alignments* to see just how well this amino acid sequence matches those from other organisms.

Secondary Plant Metabolites

Plants synthesize a number of substances that are not required to maintain basic metabolism. These “secondary plant metabolites” include phenolic acids, lignins, tannins, terpenoids (such as carotenoids), and flavonoids (such as anthocyanins). Secondary metabolites play important roles in the interaction between plants and the environment. Tannins, for example, deter predators. Anthocyanins and carotenoids produce a variety of colors in stalks, seeds, and fruits – which may attract animals for pollination and seed dispersal.

Most of these compounds require complex biosynthetic pathways, involving numerous genes. Some genes encode enzymes that work at various steps in the pathway, converting one product into the substrate for another enzyme. Other genes encode transcription factors that regulate the activity of enzyme-producing genes. Do an Internet search for *anthocyanin biosynthesis* to get an idea of the complexity of the secondary metabolism.

The *bz* mutation disables the production of an enzyme involved in the final step of anthocyanin biosynthesis. Thus, Bronze mutants accumulate the unfinished precursor of the purple pigment, which gives them their distinctively colored stalks and kernels.

As with many mutations in corn, *bz* is caused by a transposon insertion. In McClintock’s *Ac/Ds* system, the *Ac* element encodes a functional transposase gene, whose product is required for transposition. *Ds* elements are mutated copies of *Ac*, typically with internal truncations that destroy the transposase gene. Thus, the *Ds* element is incapable of transposing on its own and produces *stable* mutants.

However, *Ds* can still be trans-activated by a transposase encoded by an *Ac* element elsewhere in the genome. Thus, *Ds* mutations are *unstable* in the presence of an active *Ac* element, cycling between mutant and wild-type states. This cycling typically produces spotted or striped coloration.

INFORMATION FOR INSTRUCTOR

CONCEPTS AND METHODS

This laboratory can help students understand several important concepts of modern biology:

- The relationship between genotype and phenotype.
- The integration of *in vitro* experimentation and *in silico* computation.
- The effects of transposable elements to mutagenize and tag genes.
- The genetic control of plant metabolism.

The laboratory uses several methods from modern biological research:

- DNA extraction and purification.
- Polymerase chain reaction (PCR).
- Gel electrophoresis.
- Bioinformatics.

INSTRUCTOR PLANNING, PREPARATION, AND LAB FINE POINTS

The following table will help you to plan and integrate the four parts of the experiment.

Part	Day	Time	Activity
I. Plant Maize seeds	2-3 weeks prior to lab	15-30 min.	Lab: Plant Maize seeds
II. Isolate DNA	1	30 min. 30-60 min.	Pre-lab: Set up student stations Lab: Isolate corn DNA
III. PCR-amplify DNA	2	30-60 min. 15-30 min. 70+ min.	Pre-lab: Set up student stations Lab: Set up PCR reactions Post-lab: Amplify DNA in thermal cycler
IV. Analyze Amplified DNA	3 4	30 min. 30 min. 30+ min. 20+ min. 20 min. to overnight 20 min.	Lab: Prepare agarose solution; cast gels Load DNA samples into gels Electrophorese samples Post-lab: Stain gels De-stain gels Photograph gels

I. PLANT MAIZE SEED

Maize seeds must be planted 2-3 weeks before the date anticipated for DNA extraction and amplification by PCR. Two 1/4 inch diameter leaf disks are required for each experiment, but multiple small leaves and even whole plantlets can be used.

Light fixtures for growing maize should be fitted with at least two 40-watt "daylight" bulbs (not cool white). The following products suggested for maize cultivation are available from Carolina Biological Supply Company (www.carolina.com). Products from garden stores may be substituted.

Standard Poly-Tray Without Holes (54 x 27 x 6 cm tray)	item number 66-5666
Poly-Flats (6-cm deep cells that can be separated into individual pots)	
24-Cell Tray	item number 66-5669
36-Cell Tray	item number 66-5670
Poly-Flats Humidity Dome	item number 66-5674
Redi Earth Soil	item number 15-9701

II. ISOLATE DNA FROM MAIZE

The reagents for DNA isolation are pre-aliquoted into six portions, each of which is shared between two student teams.

Assign each team a number at the outset of the experiment. This will make it easier to mark and identify the several types of small tubes used in the experiment.

Make sure that each student team extracts DNA from either wild-type or *bz* mutant plants and that both types of plants are being examined by the class as a whole.

The cell walls of living plant tissue are typically broken up by grinding with a mortar and pestle. This can be accomplished directly in a 1.5-ml tube with the plastic pestle provided in the kit.

Pre-lab Setup (per student team)

- Wild-type or *bz* corn plant
- Edward's buffer, 500 μ l
- Isopropanol, 500 μ l
- Tris/EDTA (TE) buffer with RNase, 200 μ l (thaw and store on ice)

- 2 1.5-ml microcentrifuge tubes
- Permanent marker
- 1 plastic pestle
- Micropipet and tips (100-1000 μ l)
- Microcentrifuge tube rack
- Container with cracked or crushed ice

Shared Items

- Microcentrifuge
- Vortexer (optional)

III. AMPLIFY DNA BY PCR

The *Ds* insertion at the *Bz* locus is too large to amplify across, so a single primer set cannot amplify both the wild-type and insertion alleles. Thus, this experiment amplifies the wild-type and insertion alleles in separate PCR reactions using two different sets of primers (see RESULTS & DISCUSSION). One primer set (*BZ1/BZ2*) spans the *Ds* insertion site and thus amplifies only the wild-type *Bz* allele. The *BZ2* primer is moved out of amplifiable range in chromosomes with a *Ds* insertion. So, to amplify the insertion allele, the *BZ1* primer is paired with a second primer, *Ds*, which is located within the *Ds* transposon.

Each Ready-To-Go™ PCR Bead contains reagents so that when brought to a final volume of 25 µl the reaction contains 2.5 units of *Taq* DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, and 200 µM of each dNTP.

The lyophilized *Taq* DNA polymerase in the Ready-To-Go™ PCR beads become active immediately upon addition of the primer/loading dye mix. In the absence of thermal cycling, "nonspecific priming" at room temperature allows the polymerase to begin generating erroneous products, which can show up as extra bands in gel analysis. *Therefore, work quickly! Be sure the thermal cycler is set and have all experimenters set up their PCR reactions as a coordinated effort. Add primer/loading dye mix to all reaction tubes, then add each student template, and begin thermal cycling as quickly as possible. Hold reactions on ice until all are ready to load into the thermal cycler.*

Each primer/loading dye mix incorporates the appropriate primer pair (0.26 pico-moles/µl of each primer), 13.8% sucrose, and 0.0081% cresol red. The inclusion of loading dye components, sucrose and cresol red, allows the amplified product to be directly loaded into an agarose gel for electrophoresis. The primer/loading dye mix may collect in the tube caps during shipping; pool the reagent by spinning the tubes briefly in a microcentrifuge or by tapping the tube ends on the desktop.

PCR amplification from crude cell extracts is biochemically demanding, and requires the precision of automated thermal cycling. However, amplification of the *Bz* locus is not complicated by the presence of repeated units. Therefore, the recommended amplification times and temperatures will work adequately for all types of thermal cyclers.

Pre-lab Setup (per student team)

- Maize DNA, from Part II (store on ice)
- 30 µl *BZ1/BZ2* primer/loading dye mix (thaw and store on ice)
- 30 µl *BZ1/Ds* primer/loading dye mix (thaw and store on ice)
- 2 Ready-To-Go™ PCR Beads (in PCR tubes)

- Permanent marker
- Micropipet and tips (1-100 µl)
- Microcentrifuge tube rack
- Container with cracked or crushed ice

Shared Items

- Thermal cycler
- Mineral oil, 5 ml (depending on thermal cycler)

IV. ANALYZE AMPLIFIED DNA BY GEL ELECTROPHORESIS

Enough reagents should be provided to prepare and electrophorese 6 gels.

Prepare a 1× concentration of TBE by adding 75 ml of 20× concentrated stock into 1,425 ml of deionized or distilled water. Mix thoroughly.

Prepare a 2% agarose solution by adding 2 g of agarose to 100 ml of 1× TBE in a 500 ml flask or beaker. Heat the flask or beaker in a microwave oven (approximately 4 minutes) or in a boiling water bath (approximately 15 minutes) until the agarose is completely dissolved. You should no longer see agarose particles floating in solution when the beaker is swirled. Allow the agarose to cool to approximately 60°C, and hold at this temperature in a hot water bath. Cover beaker or flask with aluminum foil, and skim any polymerized "skin" off the top of the solution before pouring. Notice there is enough agarose to pour 6-7, 50-ml gels. There is enough marker to run 6 gels.

The combination of cresol red and sucrose in the primer mix functions as loading dye, so that amplified samples can be loaded directly into an agarose gel. This is a nice time saver. However, since it has relatively little sugar and cresol red, this loading dye is more difficult to use than typical loading dyes. So, encourage students to load carefully.

Plasmid pBR322 digested with the restriction endonuclease *Bst*NI is an inexpensive marker and produces fragments that are useful as size markers in this experiment. The size of the DNA fragments in the marker are 1,857 bp, 1,058 bp, 929 bp, 383 bp, and 121 bp. Use 20 µl of a 0.075 µg/µl stock solution of this DNA ladder per gel. Other markers or a 100-bp ladder may be substituted.

View and photograph gels as soon as possible after appropriate staining/destaining. Over time, the small-sized PCR products will diffuse through the gel and lose sharpness. (If absolutely necessary, gels can be wrapped in plastic wrap and stored at 4°C for up to 24 hrs.)

Pre-lab Setup (per student team)

- Maize PCR products from Part III (store on ice)
- 2% agarose in 1× TBE (hold at 60°C), 50 ml per gel
- 1× TBE buffer, 300 ml per gel

- 1.5 ml microcentrifuge tubes
- Micropipets and tips (1-20 µl; 20-100 µl)
- Microcentrifuge tube rack
- Electrophoresis chamber and power supply
- Latex gloves
- Staining tray
- Container with cracked or crushed ice

Shared Items

- Water bath for agarose solution (60°C)
- pBR322/*Bst*NI markers (thaw and store on ice)
- Ethidium bromide (1 µg/ml), 250 ml
- or
- CarolinaBLU™ Gel & Buffer Stain, 7 ml and CarolinaBLU™ Final Stain
- Transilluminator with camera

CarolinaBLU™ STAINING

POST-STAINING

1. Cover the electrophoresed gel with the *CarolinaBLU™* Final Stain and let sit for 20–30 minutes. Agitate gently (optional).
2. After staining, pour the stain back into the bottle for future use. (The stain can be used 6–8 times.)
3. Cover the gel with deionized or distilled water to destain. Chloride ions in tap water will cause the staining to fade.
4. Change the water 3 or 4 times over the course of 30–40 minutes. Agitate the gel occasionally.
5. Bands that are not immediately present will become more apparent with time, and will reach their maximum visibility if the gel is left to destain overnight in just enough water to cover the gel. Gels left overnight in a large volume of water may destain too much.

PRE-STAINING

CarolinaBLU™ can also be used to stain the DNA while it is being electrophoresed. Pre-staining will allow students to visualize their results prior to the end of the gel run. However, post-staining is still required for optimum viewing.

To pre-stain the gel during electrophoresis, add *CarolinaBLU™* Gel and Buffer Stain in the amounts indicated in the tables below. Note that the amount of stain added is dependent upon the voltage used for electrophoresis. *Do not use more stain than recommended. This may precipitate the DNA in the wells and create artifact bands.*

Gels containing *CarolinaBLU™* may be prepared one day ahead of the lab day, if necessary. However, gels stored longer tend to fade and lose their ability to stain DNA bands during electrophoresis.

Use the table below to add the appropriate volume of *CarolinaBLU™* stain to the agarose gel:

Voltage	Agarose Volume	Stain Volume
<50 Volts	30 ml	40 µl (1 drop)
	200 ml	240 µl (6 drops)
	400 ml	520 µl (13 drops)
>50 Volts	50 ml	80 µl (2 drops)
	300 ml	480 µl (12 drops)
	400 ml	640 µl (16 drops)

Use the table below to add the appropriate volume of *CarolinaBLU™* stain to 1× TBE buffer:

Voltage	Agarose Volume	Stain Volume
<50 Volts	500 ml	480 µl (12 drops)
	3000 ml	2.88 ml (72 drops)
>50 Volts	500 ml	960 µl (24 drops)
	2600 ml	5 ml (125 drops)

ANSWERS TO DISCUSSION QUESTIONS

- Describe the purpose of each of the following steps or reagents used in DNA isolation (Part II):
 - grinding with pestle. **Grinding with the pestle breaks the cell walls of the maize tissue.**
 - Edward's buffer. **The detergent component of Edward's buffer, sodium dodecyl sulfate, dissolves lipids that compose the cell membrane.**
 - Tris-EDTA (TE) buffer. **Tris-EDTA (TE) buffer provides conditions for stable storage of DNA. Tris provides a constant pH of 8.0, while EDTA binds cations (positive ions) that are required for DNase activity.**
- What is the purpose of performing each of the following PCR reactions:
 - BZ1/BZ2*? **Detects the presence of the wild-type *Bz* allele.**
 - BZ1/Ds*? **Detects the presence of the mutant *bz* allele bearing a *Ds* transposon insertion.**
- Place Plants 1 and 2 of the example gel in the proper cells of the chart below, and complete the entries for genotype and phenotype.

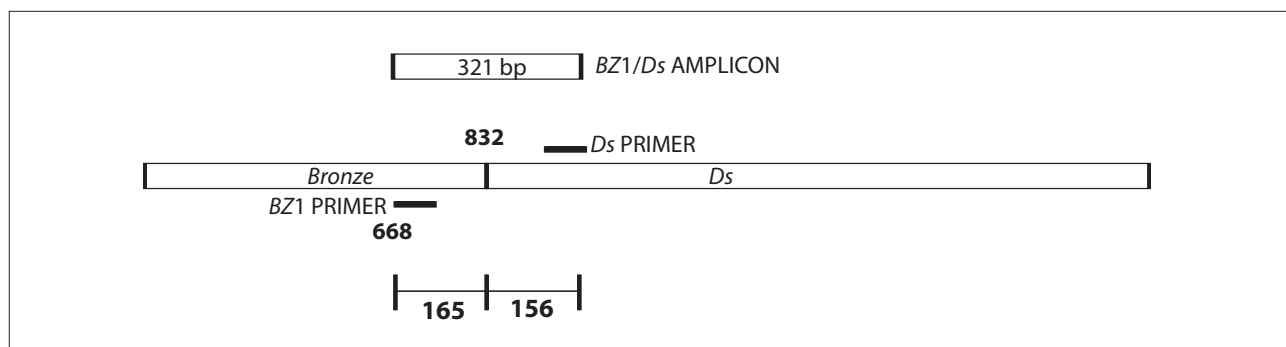
321 bp (<i>BZ1/Ds</i>)	271 bp (<i>BZ1/BZ2</i>)	Plant Genotype*	Plant Phenotype	Sample
present	present	<i>bz/Bz</i>	wild-type	not shown in gel
present	absent	<i>bz/bz</i>	mutant	Plant 2
absent	present	<i>Bz/Bz</i>	wild-type	Plant 1

- Based on analysis of the agarose gel, what is the genotype of your plant? **If you observe only the smaller band, the plant is homozygous for the wild-type allele (-/-). If you observe only the larger band, the plant is homozygous for the mutant *bz* allele (+/+). If you observe both bands, the plant is heterozygous (+/-).**
- How would you interpret a lane in which you observe primer dimer, but not the *bz* or wild-type alleles? **The presence of primer dimer confirms that the reaction contained all the components necessary for amplification, but there was insufficient template to amplify target sequences.**
- Would you classify the *bz* mutation as recessive or dominant? Explain your reasoning. **The *bz* mutation is recessive. Plants that are heterozygous do not demonstrate the mutant phenotype. Only plants that are homozygous for the *bz* mutation demonstrate the bronze phenotype.**
- Based on the genotypic distribution of the plants analyzed by the class, what genotype was the parental plant? Why may the genotype distribution observed by your class deviate from what is expected under Mendel's laws of inheritance? **First, count the number of +/+, +/-, and -/- plants, and express it as a ratio. Then, draw Punnett squares for the six kinds of parental crosses, which produce three different genotypic ratios. The observed ratio of +/+, +/-, and -/- plants should be closest to the 1:2:1 ratio predicted by a cross between two heterozygous parents. Small sample size or bias toward picking one phenotype will cause deviations from the expected ratio.**

(Maize has the ability to self-pollinate. The seeds for this experiment, in fact, came from a parental plant that self-pollinated. Think about the kind of self-crosses that are possible for plants of each of the three genotypes!)

ANSWERS TO BIOINFORMATICS QUESTIONS

- I.2. b. What does the *E-value* of 0.025 mean? Note the names of any significant alignments that have *E-values* less than 0.1. Do they make sense? **An *E-Value* of 0.025 denotes a probability of 25×10^{-3} or 25 in 1,000 to match the hit sequence just by chance. Many hits have *E-values* of less than 0.1, most are in corn. They all contain the *Bz* locus and, so, make sense.**
- I.2. c. Which positions do the primers match in the subject sequence? **The sequence between positions 668-689 matches the forward primer (*BZ1*), and the sequence between positions 918-938 matches the reverse primer (*BZ2*).**
- I.2. d. What can you say about the rest of the alignments in the list? **There are many BLAST hits that contain good matches to both primers.**
- I.3. d. The lowest and highest nucleotide positions in the subject sequence indicate the borders of the amplified sequence in the PCR reaction. Subtract one from the other to arrive at a good approximation for the length of this sequence stretch. **270 nucleotides (938-668).**
- I.3. e. However, the actual length of the fragment includes both ends, so add 1 nucleotide to the result to determine the exact length of the PCR product amplified by the two primers. **271 nucleotides.**
- II.2. Describe the source and size of the sequence in which your BLAST hit is located. **The BLAST search identified matches to the forward and the reverse primers within a 1,617 basepair DNA fragment from *Zea mays*.**
- II.3. What do you think the “” in (76..598,699..1591) stands for? Using the nucleotide positions that match the primers (as determined in I.3.d above) identify the feature(s) the two primers span. What is the name of the locus? **The “” in (76..598,699..1591) signifies an intron. The sequences matching the two primers overlap with three annotated features that begin at nucleotide positions 76: a gene and its corresponding mRNA and CDS (coding sequence). The gene is identified as *bz*, and its product is a UDPG-flavonoid 3-O-glucosyltransferase.**
- II.4. How many exons are in the *Bz* gene? Where does the reverse primer end? Which part of the *Bz* gene does the amplicon span? **The *Bz* gene contains 2 exons. The reverse primer ends at position 938, in the second exon. The forward primer begins at position 668, in the intron; the two primers therefore span a sequence from the intron into the second exon.**
- II.6. What does this sequence represent? **The amplicon, or amplified product, expected from the PCR reaction.**
- II.7. Using this datasheet identify the chromosome *Bz* is located on and some of *Bz*'s neighbor genes. **The *Bz* gene is located on chromosome 9 and is flanked by *Stk1-McC*, a serine threonine kinase gene, and *Stc1*, a sesquiterpene cyclase gene.**
- III.4. On what chromosome have you landed? **Chromosome 9, as expected.**
- III.6. Can you identify any genes in the vicinity of the *Bz* (*bz1*) locus? What can you find out about some of them? **For example *c1* was studied by McClintock too, who found this gene to be crucial for Anthocyanin biosynthesis in corn.**
- IV.2. What hits match the *Bz* primer? Do these hits also have matches to the *Ds* primer? What can you conclude? **Most hits are from corn (*Zea mays*), and many mention *Ds* or the related transposon *Ac*. Several are cloning vectors used in *Ds* mutagenesis. There also are hits that contain matches to both primers simultaneously, thus the *bz* mutant sequence is in the database.**
- IV.3. Examine the hit for *Zea mays bz-m2(Ac)* (*gb* | AF355378) and determine the length of the DNA sequence the two primers span. **The length of the *BZ1/Ds* amplicon is 321bp.**



IV.4. Label the following diagram to help you determine the *Ds* insertion site in the *Bz* gene.

- Scroll to the *Alignments* section and focus on one of the complete *Ds2* sequences. How far into the *Ds2* sequence is the *Ds* primer located? **156 bp.**
- How many *Bz* nucleotides are in the *Bz1/Ds* amplicon? **321 bp – 156 bp = 165 bp.**
- What is the first nucleotide position of the *BZ1* primer in the *Bz* amplicon? **668.**
- What is the insertion position of *Ds*? **668 + 164* = 832. (*Nucleotide #668 in the gene and nucleotide #1 of the amplicon align with each other; therefore the insertion point stretches only 164 nucleotides beyond #668.)**

IV.5. Return to the mRNA or CDS entries on the datasheet for the *Bz* gene, to determine which exon or intron the *Ds* transposon is inserted into. **The transposon is inserted in the second exon of the gene.**

IV.6. Find additional transposons inserted into this sequence. What are some of the names of respective finds? **Corn chromosomes seem to be riddled with transposons and retrotransposons. The sequence in *gb* | *AF391808.2* contains transposons such as "Misfit CACTA," "Zeon 1," "Tourist-Zm1" and "Stowaway-Zm3." Examples of retrotransposons in this sequence are "Prem 2a," "Huck 1," "Opie 2" and "RIRE 1."**

V.4. Follow links to get some quick information about a domain. What do you find? **The protein product of the *Bz* gene (BZ) contains a UPDG domain.**

V.6. What can you tell from the graphical overview? **The BLAST hits show strong homology across the entire protein.**

V.7. What can you conclude from the *E-values*, the titles of the hits, and the *gi*-links? **Extremely low *E-values* across a range of organisms (from *Arabidopsis*, *Petunia*, *Oryza* (rice), Grape, Strawberries) indicate that the flavonoid 3-O-glucosyltransferase domain, and other parts of the BZ protein, have been strongly conserved among plants through evolutionary time.**