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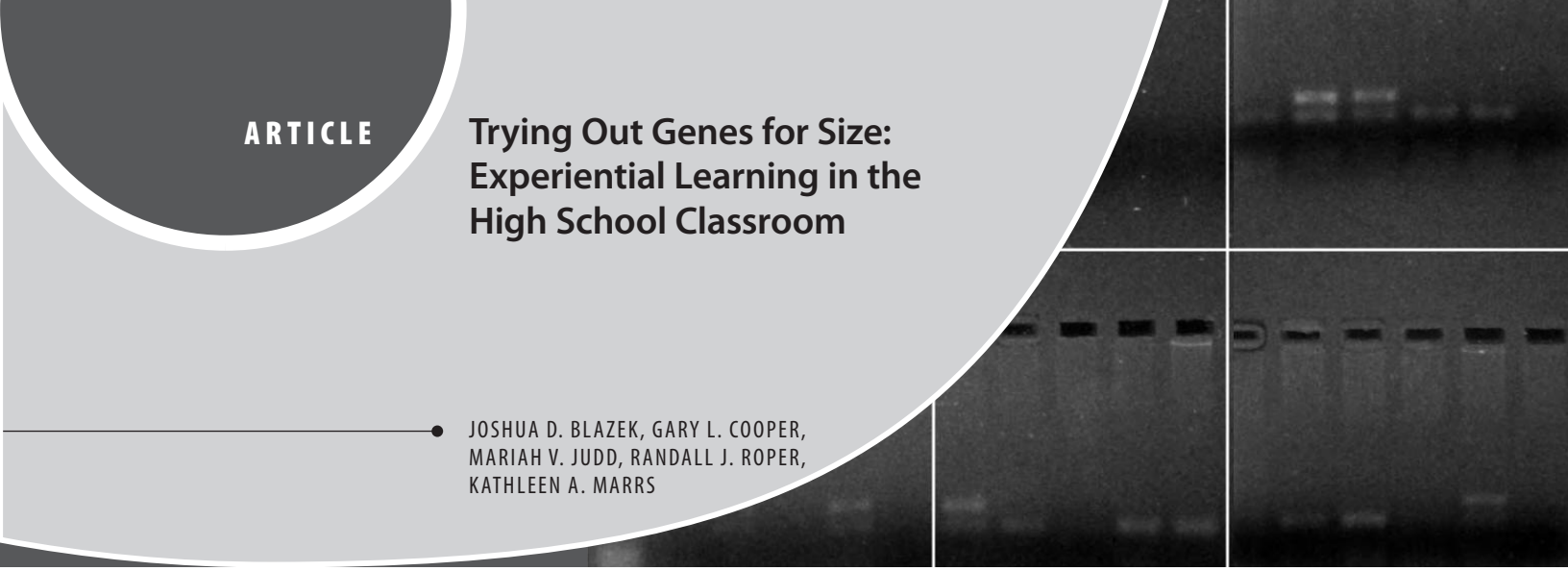
Trying Out Genes for Size: Experiential Learning in the High School Classroom



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Abstract

The National Science Foundation's GK–12 program provides a unique opportunity for STEM collaboration between the K–12 classroom and university research. This partnership benefits students through experiential learning, exposure to research, exceptional mentorship, and preparation for postsecondary education. Additionally, researchers gain valuable skills by explaining difficult scientific concepts to high school students and broadening their exposure to secondary education. We present graduate-research-based activities focused on understanding the genetic causes of Down syndrome. Modification of these activities could accommodate educational levels from middle school to entry-level college biology. This lesson involves several biological and data-collecting techniques. These experiential activities help students understand genetics and Down syndrome, and introduce basic scientific methodology and techniques useful for postsecondary education.

Key Words: *Down syndrome (DS); mouse model; trisomy; phenotype; genotyping; statistical analysis.*

Teaching molecular genetics to high school students is challenging. Students often have difficulty visualizing genetic and cellular mechanisms that translate to phenotypic expression. For many, this frustration creates a mind-set of "This is too hard" or "Why do I have to know this?" To make a seemingly difficult topic easier to understand, it is critical to provide students with interactive, hands-on activities that both spark their interest and provide valuable information in several different formats. Here, we highlight the benefits of collaboration between high school teachers and university researchers, emphasize the importance of using animal models in the study of human disorders, and provide a method for teaching molecular genetics in the high school or college classroom using current laboratory techniques.

Teaching molecular genetics to high school students is challenging.

○ Down Syndrome, a Familiar Disorder to Teach Genetics

The genetic disorder known as Down syndrome (DS) is one of the most commonly recognized chromosomal disorders and is a great model for teaching both basic and complex genetic topics, from karyotyping to signaling pathways and gene–phenotype relationships. DS occurs in approximately 1 in every 700 live births (Christianson et al., 2006) and results from the presence of an extra copy of human chromosome 21 (HSA21) or Trisomy 21. Individuals with DS exhibit various combinations and severity of more than 80 clinically defined phenotypes, including generalized growth deficits, cognitive impairment, craniofacial abnormalities, and cardiac anomalies (Richtsmeier et al., 2000; Van Cleve and Cohen, 2006; Van Cleve et al., 2006). It is hypothesized that these phenotypes are the consequence of an abnormal genetic cascade caused by the presence of extra chromosomal material in individuals with DS.

Animal models have long been used in the study of human disease and are the origin of many therapeutic treatments used for humans in the clinical setting. A number of mouse models have been created that contain triplications of several genes homologous (i.e., with the same or similar function) to those triplicated on HSA21 in humans with DS. These trisomic (having three copies of a chromosome or part of a chromosome) mice exhibit several DS phenotypes that parallel those observed in humans, including generalized growth deficits (Figure 1), cognitive impairment, and craniofacial abnormalities (Holtzman et al., 1996; Moore & Roper, 2007; Wiseman, 2009).

DS is a familiar disorder to most high school students, and many have had several encounters with individuals who have the disorder. Many students are aware that DS is caused by Trisomy 21 but do not understand how this triplication can lead to all the problems observed with DS. Students' familiarity with and basic knowledge of DS make it a relevant and engaging way to cover basic genetics as well as more complicated topics. Additionally, many students are unaware of the importance of using animal models to study human disease. When polled, a majority of the students were against the use of animals to study disease, which suggests a lack of understanding of how many therapeutic treatments are developed with the use of animal specimens. As a major part of the molecular genetics unit, students were

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Figure 1. A Ts65Dn mouse (left) next to a normal mouse (right).

introduced to a number of animal models of human disorders, and led through protocols very similar to those conducted in a university research laboratory. These activities included general discovery research, genotyping mice, and identifying the cellular localization of a regulatory molecule hypothesized to be important in the development of craniofacial phenotypes, including the formation of the mandible.

Activity 1: Discovering Genetic Disorders & How They Are Studied

Prior to starting the laboratory exercises, students were placed in groups and asked to research several human genetic disorders, including DS, cystic fibrosis, muscular dystrophy, celiac disease, sickle cell disease, and polycystic kidney disease. Students were asked to report to the class on the following: rates of occurrence, phenotypes, animal models used to study the disorder, and any treatments that have been developed for the disorder. After the reports were complete, a more in-depth lesson on DS was given to the students in preparation for the experimental portion of the lesson.

Activity 2: Genotyping Ts65Dn Mice to Determine Patterns of Inheritance

Prior to starting the activity, 11th- and 12th-grade AP Biology students were taught several topics in genetics, including meiosis, mitosis, DNA replication, and Mendel's laws of heredity. Additionally, the students were given an exploratory exercise to familiarize them with different biotechniques used in the processes of laboratory research and completed a basic lab dealing with the separation of molecules on the basis of size using gel electrophoresis. Activity 1 covered several Indiana State Biology Standards, including B.1.8 (mitosis/meiosis), B1.14 (cell signaling), B.1.21–24 and B.1.26–28 (Mendelian genetics, DNA replication, transcription, and translation).

Table 1. List of students' questions for Activity 1.

Students' questions
If a mother has a baby with DS, does she have a higher risk of having a second baby with DS?
If a female with DS has a child, does she have a higher risk of having a child with DS compared to the normal population?
Are there any risk factors for a mother having a baby with DS?

Female trisomic mice used in this example (Ts65Dn) have an extra, freely segregating segment of mouse chromosome 16, which contains several genes homologous to HSA21. According to Mendel's law of independent assortment, half of the gametes produced by the female trisomic mice will have an extra copy of the small segmental chromosome. Based on this, the hypothesis of this activity is that trisomic mothers should have normal and trisomic pups at an approximate 1:1 ratio. To begin the lab, students were given background information regarding the genetics of the mice and asked to uncover why this 1:1 ratio is expected. This exercise resulted in several interesting questions from the students, many related to the heritability of DS in humans (Table 1).

To test the hypothesis of Mendelian inheritance in the offspring of trisomic mothers, the students genotyped 30 pups from trisomic mothers and carried out statistical tests (on a larger group of laboratory data) to determine the ratio of trisomic to euploid (normal) offspring. The first step in the genotyping process was to train the students to properly use an adjustable-volume pipette to ensure accurate results. With polymerase chain reaction (PCR) and restriction digest protocols, students used reagents (1× PCR buffer [Invitrogen], 2.5 mM dNTP mix [Bioline], 2.5 mM $MgCl_2$ [Invitrogen], 0.2 µg/µL *Tag I* DNA polymerase [Bioline]) and DNA to set up the PCR reactions (primers: forward, 5′-AAATAGTAGCATCTCATGAGTG-3′; reverse, 5′-CATAGT-GCATCTTAGACAAGC-3′) and the DNA restriction (*Sac* 1 enzyme [Invitrogen]) digestion. See Lorenzi et al. (2010) for the complete protocol. The PCR and restriction digests were completed in the university laboratory, and the samples were brought back into the classroom the following day. The students were then given a hands-on tutorial on how to wet-load an agarose gel. The students loaded their samples into the gel for electrophoresis, and the gels were allowed to run for ~25 minutes. Images were produced using an imaging device in the university laboratory.

The restriction digest of the PCR product allows for the identification of normal versus trisomic mouse pups. Ts65Dn trisomic mice have an alteration in the genetic sequence of the extra chromosomal DNA that prevents the DNA from being cut by a restriction enzyme, resulting in two large bands in the gel as opposed to the one large band observed in euploid mice (Figure 2A; Lorenzi et al., 2010).

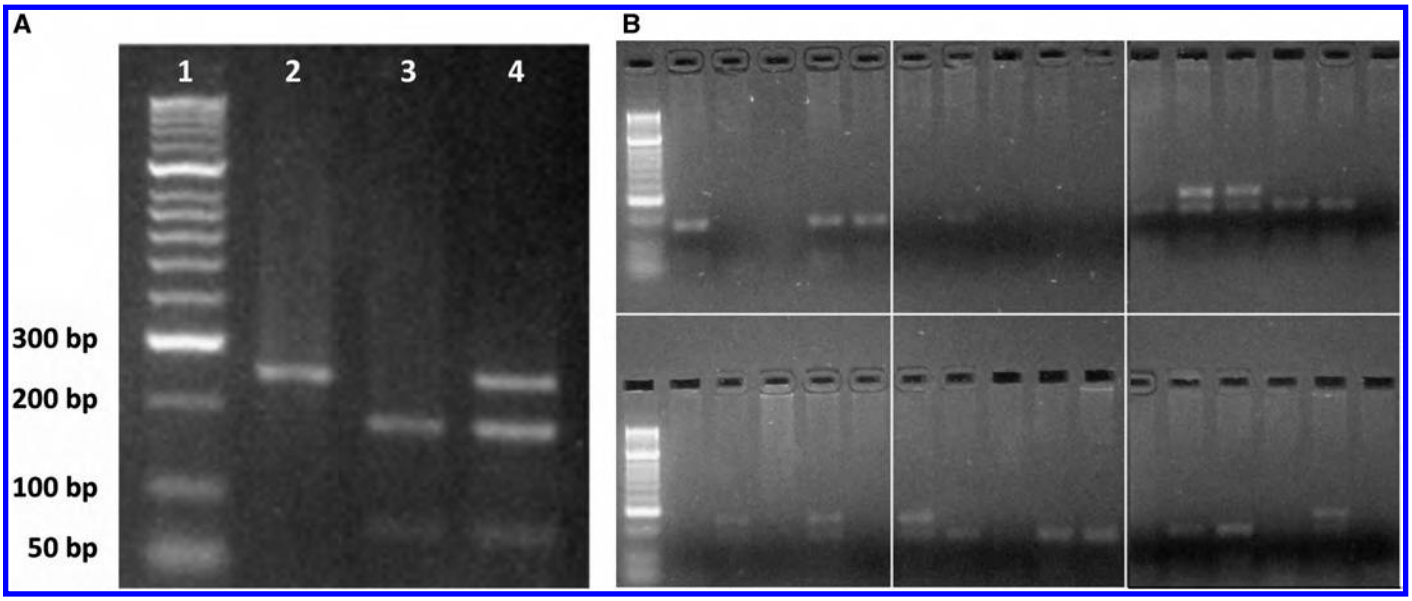

Figure 2. (A) An agarose gel with DNA ladder (lane 1), DNA control (lane 2), euploid DNA (lane 3), and trisomic DNA (lane 4) after restriction digest. Trisomic mice exhibit two major bands at approximately 250 and 150 bp (lane 4), whereas euploid mice have only one band at 150 bp (Lorenzi et al., 2010). (B) Gels loaded by the students in the classroom. Out of the six groups, 18 of the 30 genotypes were able to be determined from their gels (euploid, lanes 1, 4, 5, 7, 11, 14, 15, 22, 24, 25, 27, and 28; trisomic, lanes 12, 13, 17, 19, 21, and 30).

A

Genotype	O	E	[O-E]	$[O-E]^2$	$[O-E]^2/E$	χ^2
Euploid	72	52	20	400	7.69	15.38
Trisomic	32	52	20	400	7.69	

B

χ^2 Critical Values										
Tail probability p										
df	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.003	0.001
1	1.32	1.64	2.07	2.71	3.84	5.02	6.63	7.88	9.14	10.83
2	2.77	3.22	3.79	4.61	5.99	7.38	9.21	10.6	11.98	13.82

Figure 3. (A) Chi-square analysis conducted on the genotypes of 104 pups from trisomic mothers previously obtained in the university laboratory revealed a value of 15.38, which (B) translates to $P \leq 0.001$.

Each group of students (8 groups of 3) was given 5 different tubes of mouse DNA for PCR and digest. Of the eight groups of students that participated, six of the groups were able to successfully genotype at least one of the mice provided to them (Figure 2B).

Genotypes from the other two groups did not work because of a technical error, which served as a great teaching moment on the realities of scientific research. Because not all of the mice provided to the students were genotyped successfully (18 of 30 were successful: 12 normal and 6 trisomic), there were not enough data to conduct a meaningful statistical analysis. Because of this the students were asked to identify the bands on the gel and quantify the numbers of trisomic and euploid pups from a compilation of 104 genotypes of pups from trisomic mothers previously obtained in the university laboratory. The students analyzed their results using a chi-square goodness-of-fit test (χ^2 = [observed-expected]^2/expected). They used a table of chi-square values (Figure 3B) to determine the significance of their results, and their results are outlined in Figure 3A.

A consistent use of statistical analysis (i.e., Student's t-test and chi-square) throughout the school year led the students to have a strong understanding of P values and their subsequent meaning. Knowing that $\chi^2 = 15.38$ with 1 degree of freedom leads to $P \leq 0.001$, most of the students were able to draw the proper conclusion that the observed results were significantly different from those that were expected. Furthermore, the students were able to translate this into the context of the research by concluding that the ratio of trisomic to euploid pups from trisomic mothers did not obey Mendel's law of independent assortment. After interpreting these results, the students asked several questions and provided a number of well-thought-out hypotheses on why these mice do not exhibit Mendelian inheritance (Table 2), indicating the interest and critical thinking generated by the experience gained from the activity.

Table 2. Students' hypotheses for Activity 2.

Students' hypotheses
The extra genetic material leads to prenatal death in DS embryos resulting in an abnormal of ratio of trisomic:normal offspring.
Mice with DS are less fit to compete for the mother's milk and thus some die prior to genotyping.

Table 3. Students' questions for Activity 3.

Students' questions
Why do some individuals with DS have different phenotypes if they have the same disorder?
Do all of the genes that are triplicated in DS have an effect on an individual's overall phenotype?
How do genes cause certain phenotypes?
Why does having three copies of a chromosome lead to abnormal phenotypes?
Is Trisomy 21 the only example of a disorder caused by the presence of an extra chromosome?
Can a person have an extra copy of chromosome 21 and be normal?

Activity 3: Assessing the Nuclear Localization of Nfat in the Trisomic Mandibular Precursor

The foundation of this lesson was established by knowledge the students obtained during activities 1 and 2. During pre-laboratory exercises, the students asked several intuitive questions regarding why an extra copy of a chromosome results in phenotypic abnormalities (Table 3).

The goal of this activity was for the students to understand how genetic expression and, thus, phenotypes can be altered by having an abnormal number of chromosomes – a goal shared by many academic research labs that focus on DS. The mandible (the lower jawbone) is one of the structures altered in individuals with DS. It has been hypothesized that a number of DS phenotypes are caused, at least in part, by the disruption of a critical gene regulatory network related to the genes *Dyrk1a* and *Rcan1* (Arron et al., 2006). *Dyrk1a* and *Rcan1* are found in three copies in humans with DS and trisomic mice, and the excess expression of these genes is believed to alter the cellular localization and function of the Nfat nuclear transcription factor (Figure 4).

Assuming that the dysregulation of *Dyrk1a* and *Rcan1* negatively regulates Nfat nuclear localization, students were asked to generate a hypothesis about whether trisomic mice should have more or less nuclear Nfat in the mandibular precursor (unmineralized structure that ultimately becomes the mandible bone) compared with normal mice. To test this hypothesis, the students were taught the processes of immunohistochemistry (IHC) to determine the cellular localization of Nfat. Immunohistochemistry ("*Immuno*"-antibody, "*histo*"-cell, and "*chemistry*") is a process that uses the specific interactions between antibodies and cellular antigens to label a protein of interest, in our case Nfatc1.

The students conducted IHC on embryonic mouse sections that had been previously prepared in the university lab. The first step in the laboratory part of the lesson was to allow the students to observe and explore the slides containing sections of the mouse embryo using light microscopes (Figure 5A). This part of the activity generated numerous questions regarding different anatomical structures, and a few students even noticed some similarities between human and mouse development on the basis of observations from previous classes. After observations were finished, the students conducted the IHC protocol on several of the slides. They washed the slides, incubated them with the primary antibody against Nfat, and labeled them with a secondary antibody attached to a fluorescent probe (which binds to the primary antibody) in order to visualize the protein. Labeled sections were imaged in the university laboratory using a confocal microscope (Figure 5B). Additional samples from the university laboratory were provided to the students to complete their analysis.

To assess the cellular localization of Nfat, the students analyzed the mandibular precursor of embryo sections using processed images with areas of nuclear localization highlighted. The students then determined the percentage of cells that contained nuclear localization in both trisomic and normal embryos and were asked to process the data into a spreadsheet to derive a conclusion regarding the Nfat transcription factor based on their results and the statistical analyses.

It is important to note that the students' findings were novel, in that this quantification had not been done before in the laboratory. Letting the students know that they were gathering and analyzing actual laboratory data gave them a motivation and purpose that had been missing in previous years during the genetics unit. A compilation of the class data, assessing differences in numbers of cells with nuclear Nfat localization, preliminarily revealed that trisomic embryos had a lower percentage of cells with nuclear Nfat, compared with normal embryos, and a two-tailed t-test conducted by the students revealed that the results were significantly different (Figure 6; $P \leq 0.05$). On the basis of these results, the students were able to conclude that alterations in Nfat cellular localization may contribute to the mandibular phenotype associated with DS. Interestingly, further analysis in the university research laboratory confirmed these results.

○ Discussion

The general idea of bringing scientific research into the high school classroom initially seemed like a daunting task for both the students and the fellow/teacher. However, the success of these experiments in generating interest and critical thinking in the students suggests that collaborations forged between a high school and STEM (science, technology, engineering, and mathematics) graduate research (as demonstrated by the National Science Foundation's GK–12 program) are an effective method for teaching both general and complex biological topics, and they benefit all parties involved. Introducing university research into the classroom provides students with an interest in science and valuable laboratory skills that they will continue to use throughout their high school and college educations. Furthermore, introducing the use of animal models to these students helped them understand the importance of studying alternative models in the development of knowledge and therapeutic treatments

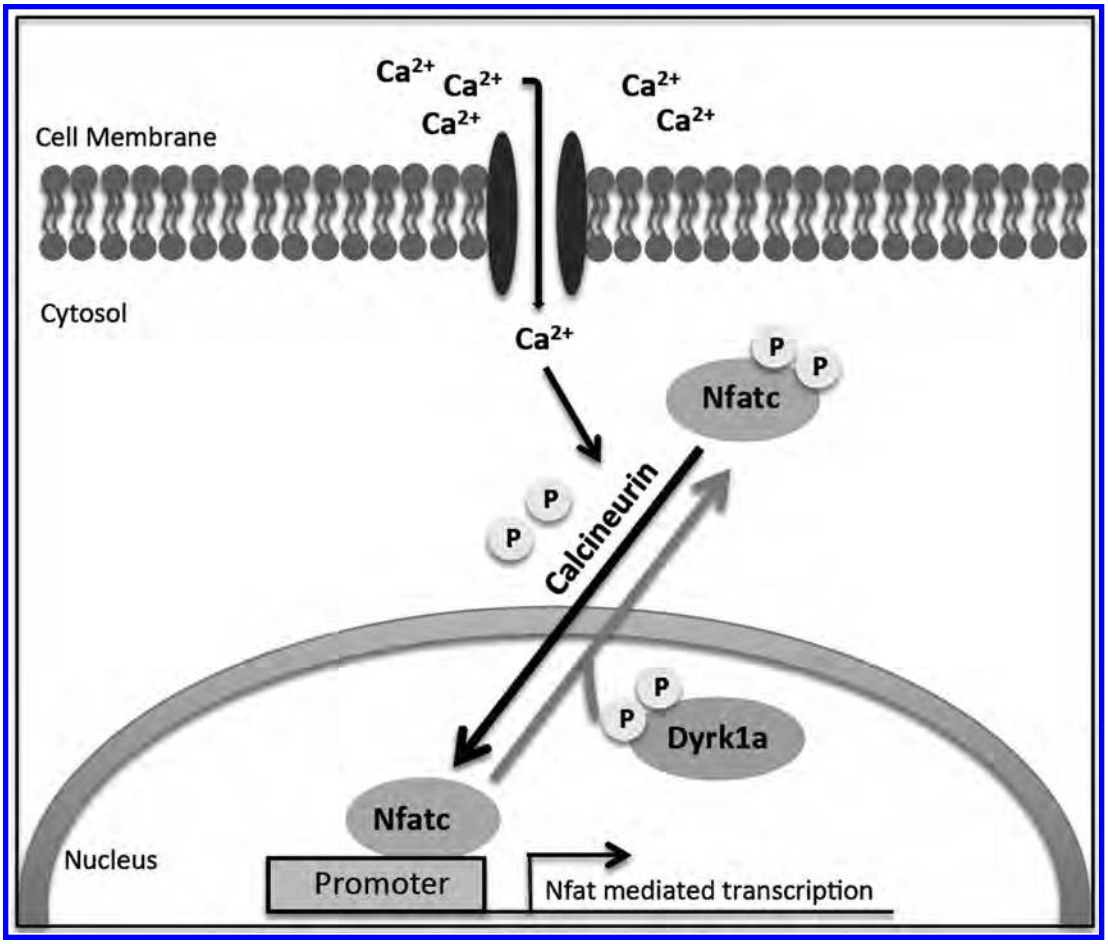

Figure 4. *Dyrk1a* negatively regulates the nuclear localization of Nfat, preventing Nfat from activating the transcription of target genes.

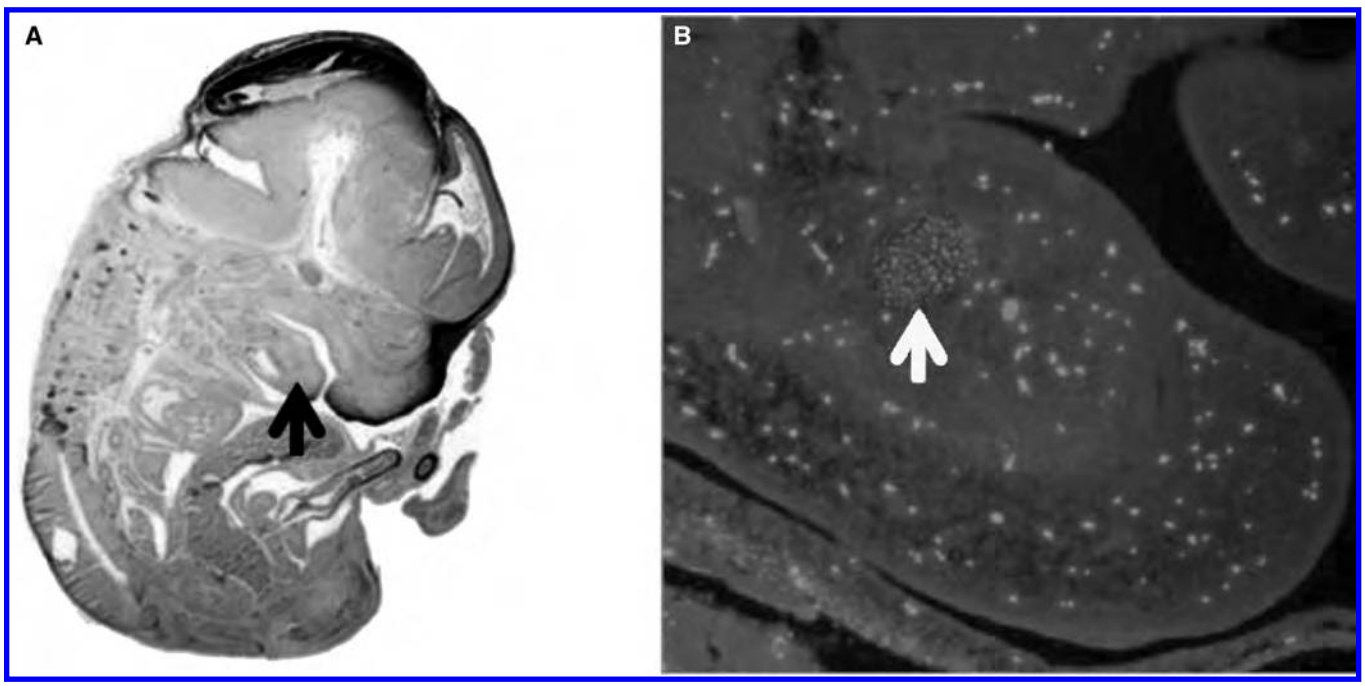

Figure 5. (A) Students observed the anatomical structure of sectioned mouse embryos prior to conducting immunohistochemistry and were asked to locate the mandible using a mouse atlas (black arrow). (B) Confocal image taken of the Nfat antibody-labeled mandibular precursor at 20×. The white arrow points to the area of interest known as Meckel's cartilage, which eventually forms into the mandible bone.

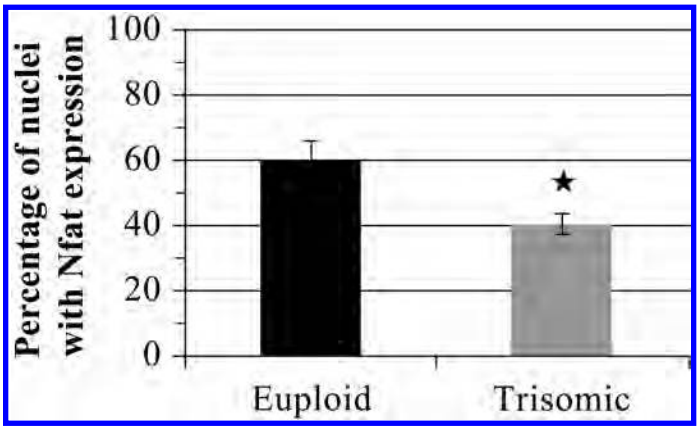

Figure 6. Students quantified the number of nuclei with Nfat localization in both groups (n = 6 euploid, n = 6 trisomic) of mice, revealing that trisomic mice have significantly less Nfat nuclear localization in the mandibular precursor than euploid mice (*P ≤ 0.05).

for human disorders. Contrary to what was observed at the beginning of the study, an exit poll revealed that a majority of students approved of and recognized the importance of using animal models to study human disorders. Additionally, many students who are interested in science think that attending professional school (medical or dental) after college is the only way to have a career in science. This encounter has provided them with a firsthand experience of an alternative way to pursue their passion of science (no matter what they are interested in) through research. Overall, several of the students who participated in the experiments displayed a newfound desire to participate in research laboratories early in their college careers, further validating the power and success of these activities.

The success of these activities was dependent on the resources of both the teacher and the university researcher, but many of these ideas can be easily translated into any high school science classroom. Many high school teachers may lack the resources necessary to conduct PCR, electrophoresis, and gel imaging, but a simple background lecture on each of these topics would provide students with valuable information regarding biotechnology. If trisomic DNA is not available, a simple restriction digest experiment conducted on purchased DNA would provide the students with experience in restriction enzymes and gel electrophoresis. Teachers could then explain the trisomic PCR and restriction digest, and subsequently have students analyze the compilation of imaged gels containing trisomic genotypes (supplementary information) and conduct statistical analyses as done in activity 2. Immunohistochemistry is a great way to teach antigen–antibody relationships in the immune system but is not feasible in most classroom scenarios. Other approaches could be used to demonstrate this same topic, including simple experiments using antibodies conjugated with an enzyme that, when bound to an antigen, changes colors in the presence of a substrate. With any of these projects, data can be collected and analyzed using statistical tests, an important concept that all students need to be familiar with before they enter college.

As a whole, this work highlights the importance of having STEM curricula like the GK–12 program to engage high school students in experiential learning. Collaborations between local high schools and graduate researchers provide an incredible learning opportunity for both the students and fellows. Although mutually beneficial to high school and university students, these collaborations may not have to be established by a fellowship or grant program. Teachers who practice near research institutions can and should establish partnerships with graduate students and use them as a resource to aid and improve their teaching methods through scientific research, because this benefits all parties involved. DS or any other human disorder can be used as an engagement activity before, during, or after a genetics unit. These types of activities draw interest from students and get them to think critically about real-life topics, all while allowing them to see the importance of basic biology in everyday life.

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