Do-It-Yourself Immunoglobulin Gene Rearrangement

Resource Type: Curriculum: Classroom

Publication Date: 9/30/2002

Authors

Wendy Gorman
Biology
Northland College
Ashland, WI 54806
US
Email: wgorman@northland.edu

Abstract

This exercise provides hands-on experience in visualizing and understanding the difficult concept of immunoglobulin gene rearrangment and isotype switching. Students loop, ligate (tape), and cut strips of paper DNA to simulate the looping and joining of heavy chain gene segments. Students begin with a strip of germ line DNA, rearrange the DNA, form a primary RNA transcript, process it into mRNA, translate a nascent polypeptide, and produce the final heavy chain. At the completion of this activity students will understand immunoglobulin gene rearrangements and isotype switching enough to start with nascent DNA and create any isotype of immunoglobulin.

Activity

Invitation for User Feedback. If you have used the activity and would like to provide feedback, please send an e-mail to MicrobeLibrary@asmusa.org. Feedback can include ideas which complement the activity and new approaches for implementing the activity. Your comments will be added to the activity under a separate section labeled "Feedback." Comments may be edited.

INTRODUCTION

Background.
This exercise requires that students are familiar with the basics of gene expression: transcription, RNA processing, and translation. Students should have also been introduced to the process of gene rearrangements involved in the formation of immunoglobulin light and heavy chains. I don't dwell on this rearrangement in lecture because the information they have been given in lecture will make sense when they loop and join the DNA themselves. I usually introduce the concept of gene rearrangement by using the analogy of choosing a meal at a restaurant: there are limited options for appetizer (J), main course (C), vegetable (V), and dessert (D), but different combinations create a large variety of meals (completed heavy chain).

PROCEDURE

Materials.

- Scissors, inexpensive kiddie scissors work fine
- Transparent tape
- Markers (optional)
- Diagram sheet of human heavy chain immunoglobulin DNA, 3 per student
  Immunoglobulin diagram (GIF)

Student Version.
You have been given a sheet of paper with printed gene segments for the human immunoglobulin (Ig) heavy chain. This diagram represents gene segments in the **germ line** DNA that is found on chromosome 14 and is identical in all cells. For simplicity, not all gene segments are shown because in humans there are actually 65 V segments, 27 D segments, 6 J segments, and 10 C segments. During the process of immunoglobulin production in B lymphocytes, these segments become rearranged so that the DNA sequence in these cells actually changes and is no longer identical to the germ line DNA. The different combinations of gene segments that occur during DNA rearrangement create the tremendous diversity of immunoglobulins found in the immune system.

To start this exercise you must first assemble your germ line DNA by cutting the diagram into strips and taping the strips together in order, with the L-V segments on the left (strip "a"), the D and J segments in the middle (strip "b"), and the C segments on the right (strip "c" and "d"). To make visualization easier, color the J and D segments a different color; you may color the other segments if you wish. The principles demonstrated in this exercise for the immunoglobulin heavy chain in B lymphocytes also apply to the synthesis of light chain in B lymphocytes and the chains of the T receptor in T lymphocytes because the process of gene rearrangement is similar.

DNA rearrangement
Early stages of gene rearrangement: D-J, then V-D-J
The early stages of gene rearrangement create the variable portion or domain of the immunoglobulin heavy chain. The

combination of the V, D, and J gene segments creates up to 11,000 possible heavy chain variable domains, much like the selection of different items from a menu create a variety of complete meals. Although any V, D, and J segment can join, DNA sequences called recombination signal sequences (RSS) associated with the V, D, and J segments ensure that the segments join correctly and that they are juxtaposed in the process of rearrangement. The RSS's are complementary (depicted in the diagram as circle or concave structures) so that a V segment RSS will only join with an RSS of a D and not a J or another V.

1. Take your DNA strip and decide which D gene segment you want to join with which J gene segment. As far as we know, the choice of D and J segments is a random process in the lymphocyte. Bring the RSS (circle structure) of that D gene segment to overlap with the RSS (concave structure) of the J gene segment by looping your DNA. Your D gene segment should abut the J gene segment, but each one of you will join your segments a little differently. Each B cell joins gene segments a little differently as well, and this contributes to immunoglobulin diversity. Using your high-tech scissors, cut out the looped DNA. The section of excised DNA is now no longer part of the chromosomal DNA and can be recycled (literally!).
2. Next, decide which V gene segment you want to join with the combined D-J segment. Notice that each V segment has an associated L segment. The L is a leader sequence that permits the transport of the immunoglobulin heavy chain into the endoplasmic reticulum, therefore you must keep the L gene segment associated with the V gene segment you have chosen until the heavy chain polypeptide is made. Overlap the RSS (concave structure) of a V gene segment with the available RSS (circle structure) associated with the D-J. You may have now lost a considerable amount of DNA. This is your **rearranged DNA**, which retains all of the constant gene segments and may still have some V and J segments that were not used. There should be no unused D segments left.
3. Your choice of V-D-J segments to join will most likely be different from all other students in the class. Each one of you represents a different B lymphocyte in the body, each B lymphocyte undergoing a different variable domain gene rearrangement, but each student B lymphocyte now committed to their particular rearrangement.

Making the RNA transcript and protein

Joining the V-D-J gene segment with a constant m and d gene segment

While the V-D-J gene segments have been joined in a specific way, the C gene segments that represent the constant domain of the Ig have remained unaltered in the DNA. Now the V-D-J becomes associated with a C gene segment. The choice of C gene segments, however, is not random but is under the influence of cytokines from the T lymphocytes. The C segment with which the V-D-J is associated determines the isotype of the immunoglobulin and the particular job that immunoglobulin will perform in the body, such as attracting macrophages (IgG), activating complement (IgM), or being transported to a newborn baby via breast milk (IgA). The first isotypes produced by a B lymphocyte are IgM and IgD and their synthesis will therefore involve the m or d constant gene segments. The association of V-D-J with m or d gene segments does not involve further rearrangement of the DNA however, because it occurs after the DNA is transcribed to RNA.

1. You will serve as the transcriber of DNA to RNA. Copy (transcribe) a portion of your rearranged DNA onto a blank strip of paper, starting just to the left of the Leader sequence associated with your joined L-V-D-J gene segments and ending just after the Cd gene segment. This is your **primary transcript** of RNA and it will include both the m and d gene segments as well as unused J segments and the "s" sequence near m. This transcript is not yet mRNA and cannot leave the nucleus to be translated into a polypeptide until it is further processed.
2. To make an mRNA, decide first if you want your primary transcript to make a m heavy chain or d chain. A single primary transcript can only make one or the other, but within the nucleus of a B lymphocyte there will be many copies of this primary transcript and some will become mRNA for the m chain and some will become mRNA for the d chain depending upon how the RNA transcript is cut and spliced. Notice that your particular V-D-J gene combination **remains the same** (and antigen specificity remains the same) whether you make an mRNA for the m or d chain.

    a. To make a m mRNA, join your L-V-D-J gene segment with the Cm segment and cut out any looped DNA in between. Cut off the Cd. Remove the small intron between the leader and V-D-J.

    b. To make a d mRNA, join your V-D-J gene segments with the C d segment and cut out any looped DNA in between, including the small intron between the leader and the V-D-J.

3. In order for the mRNA to be translated into a polypeptide further processing must occur; a polyadenylation tail and a methylguanosine cap must be added to either end, then the mRNA must be transported from the nucleus to the cytoplasm where it associates with a ribosome near the endoplasmic reticulum. If you wish, you may add a string of A's to the C segment and a cap to the L segment of your transcript.
4. Make your m or d heavy chain polypeptide by first copying (translating) your mRNA onto another strip of paper. This represents the nascent heavy chain polypeptide. The translated Leader sequence of the nascent heavy chain polypeptide permits it to enter the endoplasmic reticulum where the heavy chain associates with another heavy chain and two light chains to make a complete immunoglobulin. The leader sequence is enzymatically removed from the heavy chain polypeptide shortly after it enters the endoplasmic reticulum and is not present in the complete immunoglobulin. Cut off the Leader sequence to make your completed heavy chain. Your final paper strip representing the heavy chain should be considerably shorter than the DNA at the start of this exercise.

Isotype switching

Isotype switching changes the isotype of immunoglobulin that a B lymphocyte produces but does not change the antigen specificity of the immunoglobulin. Once a B lymphocyte has undergone V-D-J gene rearrangement it is committed to recognizing a specific antigen, but association of that rearrangement with a particular C gene segment can change. That can change where the immunoglobulin encounters the antigen (e.g., in the lymph node or in the intestinal mucosa) or what it does with the bound antigen (e.g., attracting neutrophils or attracting complement). Note on your DNA strip, one of the C gene segments, yCe, is called a pseudogene because this gene is similar to Ce but is no longer functional.

1. Take your rearranged DNA strip. This time, instead of making a m or d heavy chain, you are going to switch isotypes and make a g2 heavy chain. Again, keep in mind that your V-D-J gene combination, and therefore antigen specificity, remains **exactly the same**.
2. Loop your DNA strip so that the Cg2 switch sequence (rectangle containing an "s" to the right of the Cg2 segment) is next to the m switch sequence. Tape these together, cut out the looped DNA and recycle the DNA that has been removed. This represents another stage of gene rearrangement in the DNA. Cm, Cd, Cg3, Cg1, yCe, and Ca1 were in the loop of DNA that was removed, and they are gone forever in that particular B cell.
3. Take the DNA strip and transcribe the DNA into a primary transcript as you did before by transcribing just to the left of the L sequence of the V-D-J and ending to the right of the Cg2 segment. Your transcript will contain L-V-D-J, unused J segments, the switch sequences, and Cg2.
4. To make mRNA, remove the small intron between the Leader sequence and V-D-J and the intron (including the switch sequences) between V-D-J and Cg2.
5. To make an IgG2 heavy chain polypeptide, translate your arrangement onto another strip of paper. Cut off the leader sequence from your nascent polypeptide. This represents your IgG2 heavy chain.
6. Suppose you now want to switch isotypes again, there are just four constant gene segments left on your DNA: Cg2, Cg4, Ce, and Ca2. To switch to IgE isotype, bring the Ce switch site sequence next to the Cg2 switch sequence, tape together, and remove the looped DNA. This is yet another gene rearrangement, and the Cg2 and Cg4 gene segments are now gone.
7. To make a primary transcript of IgE, transcribe your DNA as before, starting from the left of the V-D-J Leader and ending to the right of the Ce.
8. Remove the introns to make the mRNA.
9. Translate the mRNA by making a copy of the mRNA and trimming off the leader to make an IgE polypeptide.
10. If you wanted to, you still have the option of switching to IgA2 **only**.

Test your understanding
You should have completed three polypeptides for three different heavy chain isotypes, each with the same variable region but a different constant region. To receive credit for this exercise you will perform the rearrangement for another specific chain. Each person in the class will be assigned a different heavy chain configuration. Each person will therefore be analogous to a different B lymphocyte in the body.

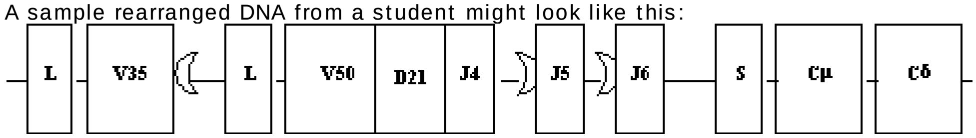
1. From the stack of note cards, take one. Construct your assigned heavy chain polypeptide as you did before using a new DNA strip. The end result should match the heavy chain on the card.
2. To be truly analogous to a B lymphocyte, you would have to construct millions of copies of your particular heavy chain polypeptide!
3. When you have completed your assignment, attach it to the note card and write your name on the card.

Instructor Version.
This exercise is intended to allow students to make mistakes because they can easily go back and start over, and to have fun while learning a difficult concept. Students may work alone or with a partner for the initial part of the exercise but should work alone when they construct their assigned polypeptide. Encourage students to help each other. A few students request extra copies of the DNA so they can practice further on their own. The DNA diagram can be enlarged when it is photocopied.

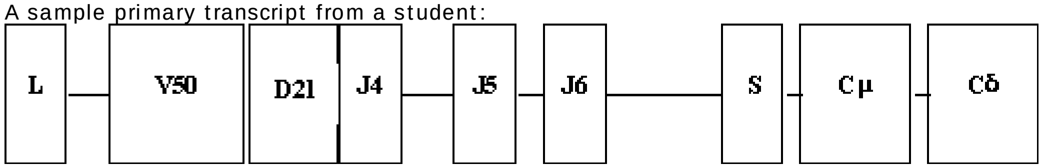
1. A sample note card might look like this:

    Provide the primary transcript and final polypeptide for the following:
    V35-D15-J5-Cg4

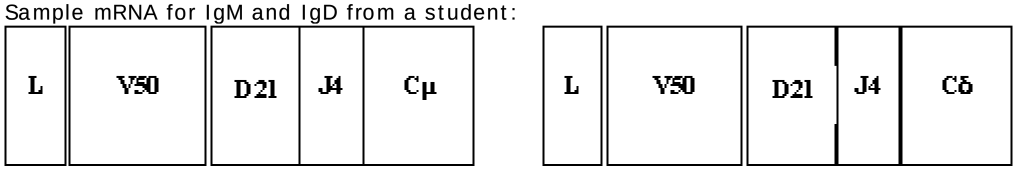
2. A sample rearranged DNA from a student might look like this:



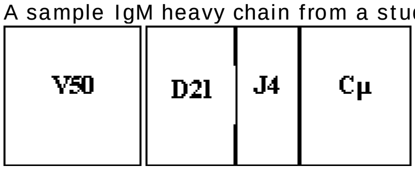
3. A sample primary transcript from a student:



4. Sample mRNA for IgM and IgD from a student:



5. A sample IgM heavy chain from a student:



Safety Issues.
None. Use blunt scissors if pointed ones are an issue.

ASSESSMENT and OUTCOMES

Suggestions for Assessment.
Assessment of student understanding and success in creating an immunoglobulin heavy chain starting with DNA is their ability to form the correct finished product. Student progress can easily be monitored during the exercise, allow students to make corrections on their DNA strip or start with a new strip, if necessary. The final product can be quickly checked. I give students points for completing the exercise, and they can repeat it until it is done correctly.

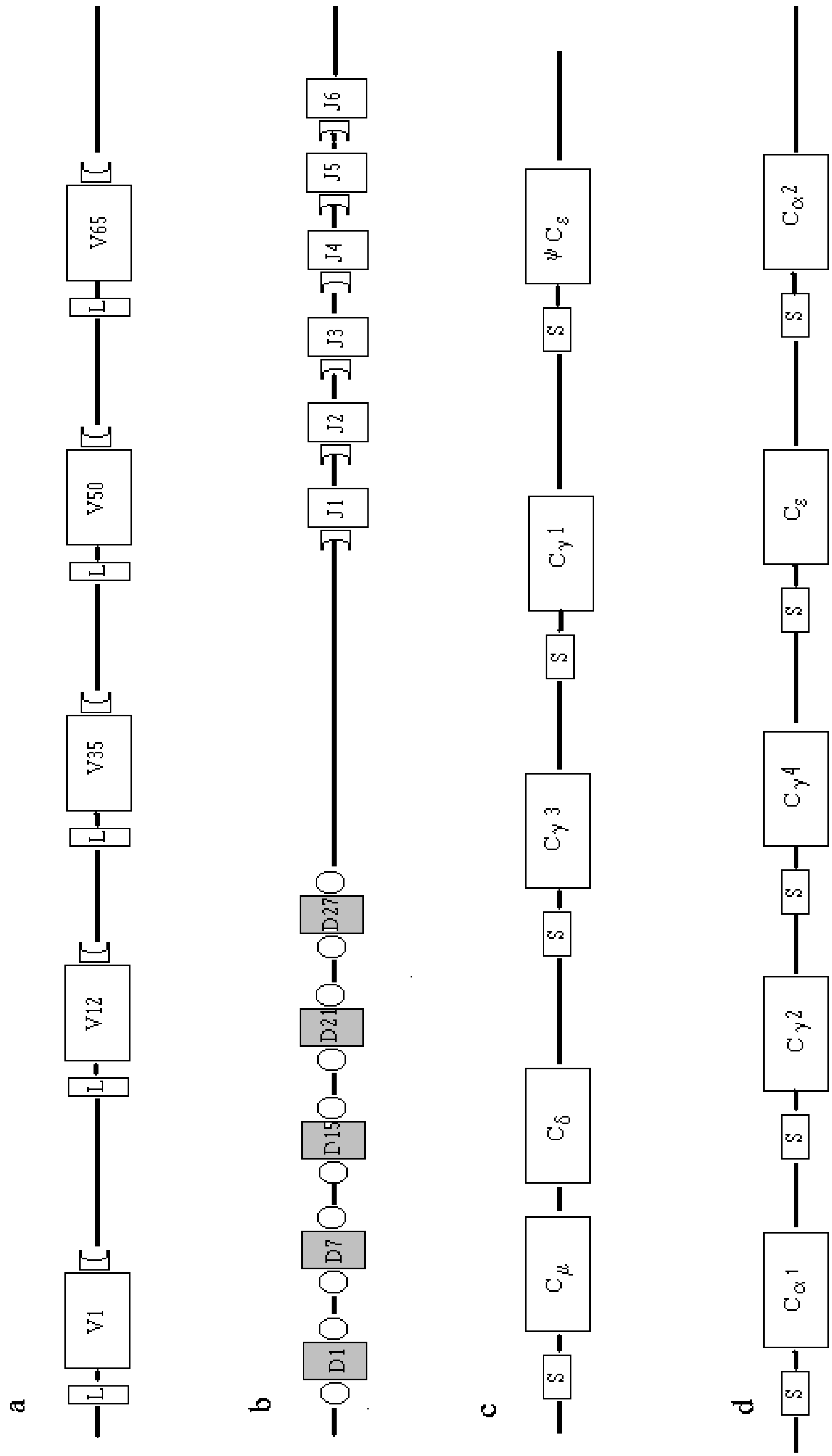
Field Testing.
I have done this activity about six times in an immunology class. Students have responded positively, particularly those who had difficulty in following the cutting and splicing as it was presented in lecture and in their textbook.

Student Data.
This exercise is so defined that all students initially finish with the same products and their final individual polypeptide is easily checked.

SUPPLEMENTARY MATERIALS

Immunoglobulin heavy chain gene segments of germ-line DNA (PDF FILE)

Human immunoglobulin heavy chain gene segments of germ-line DNA



Immunoglobulin heavy chain gene segments of germ-line DNA