Case-It: Southern Blotting

# Sickle cell anemia

**Background**: Sickle cell anemia is a disease of red blood cells. It is caused by a mutation in the hemoglobin gene. A single base change results in a single amino acid substitution. This mutation causes the hemoglobin to change its conformation to a more elongated form under certain conditions, distorting the red blood cells and impairing their ability to carry oxygen. Sickle cell anemia is considered a recessive trait, since both chromosomes have to carry the mutation in order for the full blown disease symptoms to appear.

The sickle cell mutation also eliminates a restriction enzyme site - the recognition site for the enzyme MstII. To detect the sickle cell mutation, a patient’s DNA is digested with MstII and a Southern blot is performed using a probe corresponding to this region of the hemoglobin gene. The presence or absence of the sickle cell mutation can be determined based on the size of the fragment identified by the probe.

**Case A**: Steve and Martha are expecting their second child. They know that sickle cell anemia runs in both of their families. They want to know whether this child could be affected. Neither they nor their 10-year-old daughter, Sarah, have shown any symptoms of the disease. They decide to have DNA tests to determine the status of the fetus, as well as to find out whether they in fact are carriers of the disease gene.

DNA samples: Steve (father) Martha (mother)

Sarah (daughter) Fetus

Control DNA, homozygous for sickle cell mutation   
  
 Control DNA, homozygous normal, without sickle cell mutation

# Steps:

1. Open Case-It. Click “Lab Bench” in the lower right. Select “Quick Setup” from the set-up options on the *Tools* menu.
2. Run PCR Electrophoresis as normal. Begin by opening the DNA files by clicking *DNA* (from the menu bar) and *Select DNA Samples;* under the “Cases” folder, find the “Genetic Diseases” folder. Then open the “Sickle Cell Anemia Folder” and open “Case A”. Select **all** the DNA samples
3. Click on *Site* and *Open up to 4 enzyme tubes.* Select the **MstII** enzyme.
4. Add the enzyme to each tube of DNA and place the tubes (w/ tops closed) into the heating block. Hit “Power”
5. After the tubes have been removed and opened, add their DNA to the lanes of the gel and run the power on the gel.
6. Compare the genetic finger prints. Whose DNA matches the normal DNA? Whose DNA matches the mutated, sickle cell DNA?
7. Click on *Site*, and then *Open probe file for Southern blot*. Select the “Probe Sickle Cell”.
8. Click on *Run* (from the top menu bar) and then *Run Southern blot after running gel*. The Sickle Cell Probe should highlight the location of the normal and mutated genes for red blood cell shape.
9. Who is homozygous recessive for sickle cell anemia? Who is heterozygous? Who is homozygous dominant for normal?
10. Complete the questions below and turn this in.
11. If you finish early, continue to Cases B and C, or try another genetic disease.

Case-It: Southern Blotting - Questions

Name: Hour Date:

Date Assignment is due: *end of the hour*  Why late?   
 Day of Week Date If your project was late, describe why

1. From your testing, please indicate whether each is homozygous recessive (*has the disease*), heterozygous (*carries the disease but does not have it*), or homozygous dominant (*neither carries nor has the disease*).
   * Steve (father)
   * Martha (mother)
   * Sarah (daughter)
   * Fetus (not yet born)
2. What causes this disease?
3. How does Southern blotting enable us to test for this disease, and would it work for an infectious disease?

1. Why sickle cell anemia is considered a *recessive* disease?
2. What issues are raised by this type of testing? In other words, how could this information be abused?
3. Was it a good idea for Steve and Martha to test their unborn child? Why or why not?