DNA Microarray Activity:
DNA Hybridization
Participant Guide

Description and Estimated Time to Complete

This activity provides the instructions for accessing an on-line tutorial on DNA Hybridization. DNA Hybridization is the process used to identify the degree of genetic similarity between pools of DNA. DNA Microarrays depend on the hybridization between single-stranded DNA (ssDNA) probes and ssDNA copies in control and test samples.

In order to get the most from this activity, you should have basic understanding of the DNA molecules, its four (4) nitrogenous bases (Adenine (A), Thymine (T), Guanine (G), and Cytosine (C)) and DNA base pairs (i.e., A-T, T-A, G-C, and C-G), which are the building blocks of DNA. (Refer to the References section for review sources.)

After completing the tutorial, this activity provides post-activity questions that allow you to demonstrate your understanding of the information presented in the tutorial.

Estimated Time to Complete
Allow at least 30 minutes to complete.

Introduction to DNA Hybridization

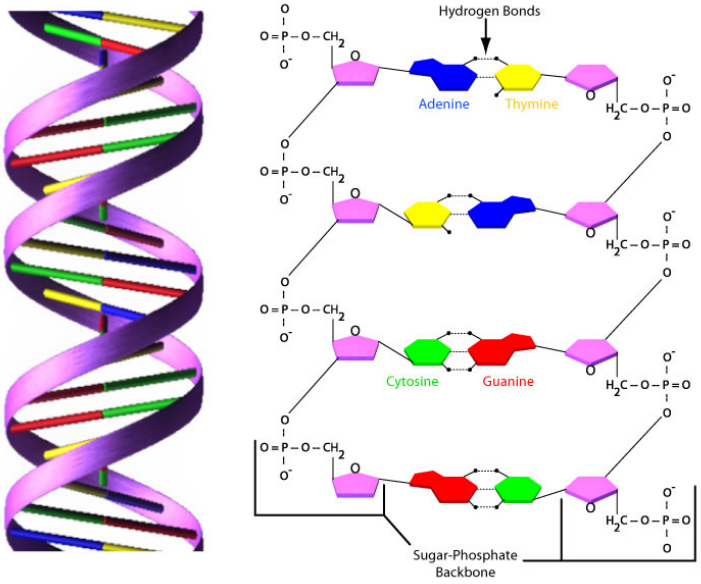
The ability to make DNA hybrids through DNA hybridization is used in standard techniques in molecular genetics such as the binding of oligonucleotide (a short nucleic acid polymer, typically with 20 – 50 bases or nucleotides) probes in a Southern blot and the annealing of primers in PCR (polymerase chain reaction).

- Southern blot is a technique used to detect a specific DNA fragment in a DNA electrophoresis gel; in other words, to locate a specific base or DNA sequence within an entire genome.
- PCR is used to amplify DNA sequences and to make numerous copies of specific DNA segments quickly and accurately.

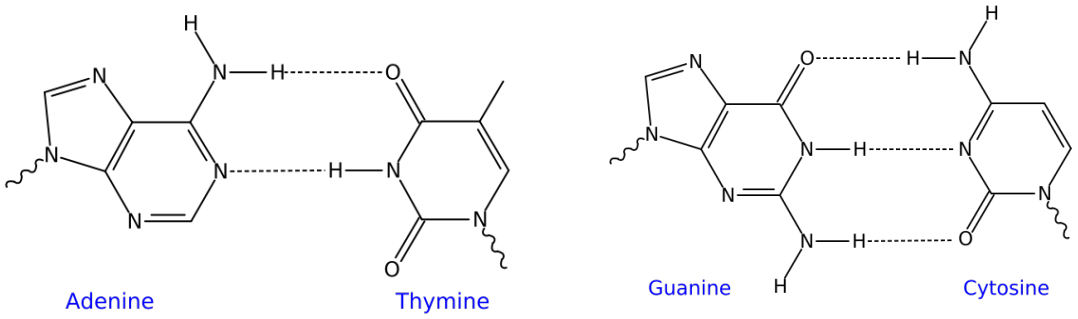
In each case, a synthetic single-stranded oligonucleotide is designed to search and find a complementary DNA sequence, then form a DNA hybrid. This process is similar to the "find" command in a word processing program. If the complementary DNA sequence to be found exists within the page of words, the oligonucleotide (*or oligo)* locates the word (or complementary DNA) and tags it. In DNA hybridizations, the oligo strand that carries a label (or sequence) for detecting the base-pairing of a hybrid molecule is called the probe.

The process of hybridization is similar to DNA replication. Hybridization refers to the process in which a double-stranded DNA (dsDNA) helix is denatured (or separated) into two, single stranded DNA (ssDNA) molecules by disrupting the hydrogen bonds that hold the two strands together.*(See the graphics below.)* Since hydrogen bonds are relatively weak, they can be disrupted by simply heating a DNA buffered solution and changing the solution chemistry with extremes of pH and high salt concentrations.

The image below illustrates a dsDNA molecule sequence and the hydrogen bonds that hold the two strands of the DNA together at each base-pairing (A-T, T-A, C-G, and G-C).

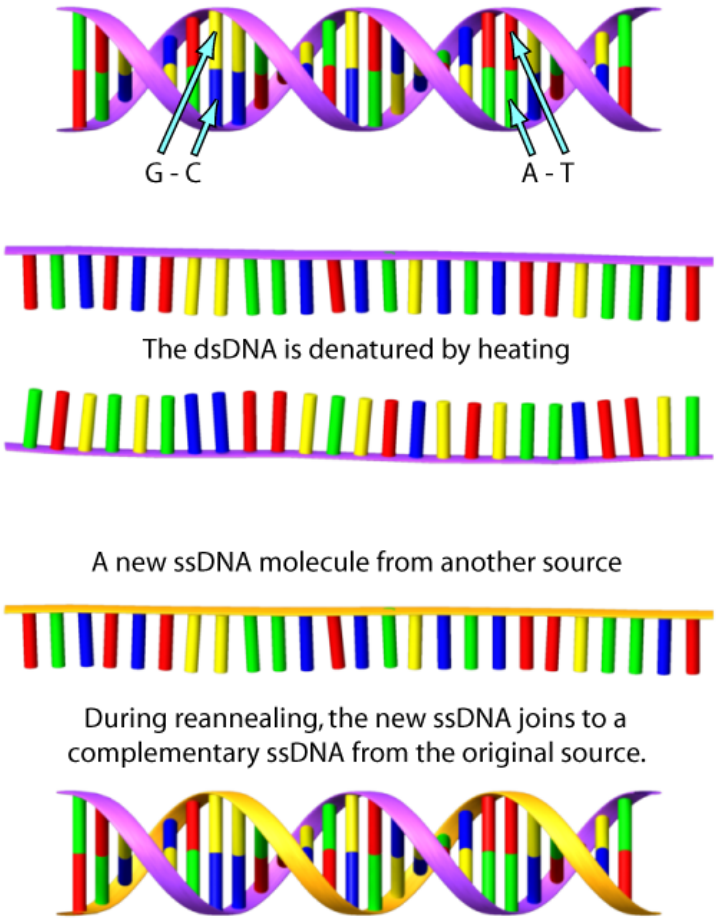


The graphic below shows the non-covalent hydrogen bonds between the pairs as dashed lines.
Left is an A-T base pair with two hydrogen bonds; right is a G-C base pair with three hydrogen bonds.



DNA denaturation is reversible. If the buffer conditions and temperature are slowly changed back to normal, the two strands of ssDNA will again bind to each other, reannealing the two single strands back to their original double-stranded structure.

"DNA hybridization" is when the denatured DNA molecules are cooled down in the presence of ssDNA molecules from another source. These DNA molecules from another source are introduced to the original DNA solution during the reannealing step. If the original ssDNA strands have sequences that are complementary to the introduced ssDNA strands, they can form dsDNA hybrid molecules with one strand from each (an original ssDNA and the source ssDNA). The following graphic illustrates the hybridization process.



Activity Objectives and Outcomes

Activity Objectives

- Explain how probes made of nucleotide sequences bind to complementary target DNA sequences.
- State at least two bioMEMS applications that study or utilize DNA hybridization.

Activity Outcomes

You will use the information gathered in this tutorial to explain how DNA hybridization occurs and its applications for bioMEMS.

Resources

Computer with high-speed Internet access.

Documentation

Your documentation should include all of the questions asked during each stage of this activity and your answer to each of these questions.

Documentation should also include the Post-Activity Questions and your answers.

Activity: DNA Hybridization

This activity will help you better understand the process of DNA hybridization and how it applies to bioMEMS applications. You will utilize the tutorial at The Molecular Workbench.

1. Go to The Molecular Workbench at http://workbench.concord.org/database

2. In the upper right corner **"Jump to Activity" # 265. Select "Student".** This should take you to an interactive called DNA Hybridization. (*NOTE: If you have a problem with the link, do a search within Molecular Workbench for DNA Hybridization.)*

3. Launch Activity (It may take a few minutes to download.)

4. Watch the modeling simulation.

5. When it stops and says "Probe Target Found", take a snapshot and describe the image you see. Record a sketch of the image and your description in this activity's documentation.

6. Resume simulation. Watch for a few more minutes and record what happens.

7. In the bottom left corner of the screen **select Southern Blot**. There are 5 parts to this tutorial. Complete all five starting with "Introduction". During this activity, record all questions and your answers for your documentation.

8. Use the "Back" button to return to the "Modeling DNA Hybridization" page or **Copy and paste** this URL (http://mw2.concord.org/public/student/lab/hybridization.cml) in the Molecular Workbench address box and RELOAD.

9. Select DNA double-helix (bottom left-hand corner). Use your cursor to rotate the molecule. Right click and change the "style" of the DNA double-helix in order to study it in different forms.

10. Answer the Post-Activity questions at the end of this activity.

Post-Activity Questions

1. What is the Southern Blot?

2. What is a nucleotide?

3. DNA hybridization makes use of base pairing between

   _____

4. Where is DNA hybridization currently found in bioMEMS technology? (State at least two applications)

5. What is another *possible* application of bioMEMS and DNA Hybridization?

Summary

Being able to see and study the DNA hybridization process has become a reality. BioMEMS devices are being designed to use this process for diagnostics and therapeutic applications.

References

[1] The Molecular Workbench at http://mw.concord.org/(Program funded by the National Science Foundation)
[2] SCME's BioMEMS Applications Overview Primary Knowledge unit
[3] SCME's DNA Overview Learning Module
[4] SCME's DNA Microarray Primary Knowledge unit

Support for this work was provided by the National Science Foundation's Advanced Technological Education (ATE) Program through Grants. For more learning modules related to microtechnology, visit the SCME website (http://scme-support.org/)

This Learning Module was developed in conjunction with Bio-Link, a National Science Foundation Advanced Technological Education (ATE) Center for Biotechnology @ www.bio-link.org.