Nanopore Studies of Single DNA Molecules

Joy Martin
Oliver Hazard Perry Middle School

Brown University
Physics Department
Molecular Biophysics Group
Purpose: To study the movement of DNA through a nanopore.
Inside the chip

Nanopore

Silicon chip
A nanopore is a tiny hole in the membrane of a silicon chip. It is about 1/10,000 the diameter of a human hair.
Size scale of a nanopore and DNA

- DNA is about 2 nanometers wide.
- The diameter of a nanopore is usually 2-10 nanometers.
DNA passing through a nanopore
Nanopore via Current Dip

Proof of One DNA Passing Through Nanopore

![Graph showing current dip](image-url)
The Future of Nanopore Research

- More knowledge about how DNA molecules move through nanopores

- More sensitive detection than just whole DNA molecules

- Detect small changes along each DNA molecule
  - Proteins bound along DNA molecules
Making DNA-Protein Binding Maps
Data vs. Dream

Data for DNA vs. Protein Molecules

Current (nA)

Time (ms)

The Dream

DNA binding proteins

predicted data

DNA enters

DNA exits

time
Something that should be easier to detect

- We made a 3-legged DNA molecule we call “Starfish”
- Starfish have an obvious midpoint
Something that should be easier to detect

When 2 of the “legs” pass through together, current readings should dip more.

This dip tells us it’s halfway through.
How to Make Starfish?
Background

- DNA is comprised of four nucleotide bases: Adenine (A), Thymine (T), Cytosine (C), and Guanine (G)
Complementary Base Pairs

- Adenine -- Thymine
- Guanine -- Cytosine
DNA Hybridization

When two perfectly complementary single strands come together to make one double strand.
Step 1

Heat circular Lambda DNA at 65°C to open the ring structure.
Sticky-Ends are formed when the ring breaks

- We can use these sticky ends to hybridize to other DNA.
- However, the DNA could also rehybridize to make unwanted circular or long DNA.
Closing Off One End

• We need to prevent lambda DNA from reforming its ring and from binding to other lambda DNAs

• We add a short piece of single stranded DNA (a primer) to close off one end.
Making Starfish
Step 2

- Hybridize two new primers together
- Hybridize three Lambda DNA with one pair of the new primers
- Starfish!
Ligation

• In order to make the newly formed bonds permanent:
• Add the enzyme ligase
An interesting mixture

- Soup of DNA, primer, DNA attached to primer, and ligase.
- Need to remove extra unattached primer and the ligase.
To remove ligase

- Add phenol-chloroform
- Put this tube of mixture in the microcentrifuge
- Pipette out the bottom layer
Remove essentially all remaining phenol

Add chloroform

Microcentrifuge

Pipette out bottom layer
Filter out the extra primers

- Use a micro-con filter
- The DNA with primers attached stay at the top
- Unattached primers and buffer filter through
Success!

• We have managed to create “Starfish” molecules, as shown on the right.

QuickTime™ and a YUV420 codec decompressor are needed to see this picture.
How will this research experience benefit my teaching abilities?

• Deeper scientific background knowledge of both content and procedures

• More aware and compassionate towards students that are having trouble grasping something new.
Acknowledgements

• NSF: GK-12 Program: “Physical Processes in the Environment”

• Karen Haberstroh

• Heather Johnson

• Brown University

• Providence Schools

• Jason Chan

• Jackson Del Bonis-O’Donnell

• Colin Horowitz

• Zhijun Jiang

• Walter Reisner

• Yongqiang Ren

• Charlie Wood

• Derek Stein