Today, Lec 8

Mid-term: Tuesday Oct 20\textsuperscript{th}, in class

Go over reading, HW, Class notes.
Concepts—\( \sim \frac{1}{2}; \) write about them
Calculations— about \( \sim \frac{1}{2} \)

Today: DNA (& RNA) Sequencing
A revolution
[Tons of work for physicist-types]
Using DNA as a sequencer machine.

Use a Primer that you know starts somewhere
(Where is tricky. Sometimes know; often don’t.
But can sequence and then use overlapping to piece together)

Each time add in some dNTP (dATP, dCTP, dGTP, dTTT) with a fluorophore on it. Each type of dNTP has its own color.
Detect it (at single molecule level).
Cut off fluorophore so it diffuses away.
Wait for next dNTP to bind, fluorescent molecule detected, etc.
PacBio Method of DNA Sequencing
Technical Problems

Want a lot of signal, little noise (background)

Can you see an individual dye? (signal)

In particular, with a lot of background

1. Background— high concentration of unincorporated dye. (Need high conc. Because DNA polymerase needs this to go.)

2. Can you do it fast/slow enough?

   Analogy to seeing stars.
   Can you see a star in daylight?
To decrease your background, spatially confine your light: shine light where there is signal, and don’t shine light where there is background (due to unincorporated nucleotides.

They use surface chemistry where DNA polymerase works. Light focused to tiny (zeptoliters; $10^{-21}$L) chambers—100 nm wide. ~0.1-1 molecules Allows them to use relatively high conc. of nucleotides (0.1-10 μM)
Fig. 3. Long read length activity of DNA polymerase.

Fig. 2. Real-time detection of single-molecule DNA polymerase activity.

Fig. 4. Single-molecule, real-time, four-color DNA sequencing.
Alternative: looking for Genes
DNA Arrays/Bio-chips

“Inuk” died on an island off Greenland called Qeqertasussuk. He left bits of hair and bone that the permafrost preserved, including his complete genome.

Inuk’s genes reveal he was a fairly young man, robustly built to exist in a frigid climate, with A-positive blood, dark skin, brown eyes, and thick, black hair on a scalp genetically susceptible to baldness.

Can detect ancient treks: Comparing his genome to other living people, they deduced that he was member of the Arctic Saqqaq, the first known culture to settle in Greenland whose ancestors had trekked from Siberia around the Arctic circle in pursuit of game.

Contamination a big problem: The best place to find it is entombed in ice, where it is preserved by the cold and protected from contamination. Hair doesn't as readily absorb contaminants, and its surface can be bleached clean. They also tagged the millions of fragments of extracted DNA with a barcode-like sequence to distinguish them from stray modern human DNA.
Recall: RNA has 3 different structures, and uses. 
(pre-)mRNA, tRNA, rRNA

Re-read Unit 3!

1. Messenger RNA (mRNA)
   [~pre-mRNA: copy of DNA; take out introns-get mRNA]

2. transfer RNA (tRNA)
   [binds to amino acid and codon for mRNA] 3 bases correspond to unique amino acid.

3. ribosomal RNA (rRNA)
   [Makes up Ribosome, along with protein. Has catalytic activity— can form peptide bond.]

Can do PCR on mRNA
(instead of DNA to see what genes are expressed (and what exons...))
Exploring the new world of the genome with DNA microarrays

Can look at which proteins are expressed via mRNA

Normal cell → Cancer cell

mRNA

Fig. 1 Gene expression analysis using a DNA microarray.
Gene Arrays (“Chips”) can be made
Gene expression (i.e. RNA) can be detected
on genome-wide scale: revolution!!

(Non-fluorescent) genes put on chip at defined position.

2 procedures
1. RNA from cell of interest extracted and fluorescently labeled e.g. with red dye. Added to chip, hybridized. Unbound washed away. Which position lights up tells which gene is active in cell!

2. By using red from “normal” cell, green from test cell, can tell which genes are up-regulated (red) and which are down-regulated (green).

Extract and amplify RNA

Hybridize to microarray

Cancer cell

normal cell

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If spot green that gene is expressed in normal cell but not cancer cell.
If red: gene expressed in cancer cell, not normal cell. Yellow: both cells express.
Molecular portraits of human breast tumours

Gene chips can be used to follow genetic changes during cancer and cancer treatment.

**FIG. 1. Variation in expression of 1,753 genes in 84 experimental samples.**

Data are presented in a matrix format: each row represents a single gene, and each column an experimental sample. Green squares, transcript levels below the median; black squares, transcript levels equal to the median; red squares, transcript levels greater than the median; grey squares, technically inadequate or missing data.

Affymetrix, 1992. Marriage of Silicon and Genes. Semiconductor manufacturing techniques could be united with advances in combinatorial chemistry to build vast amounts of biological data on a small glass chip.

Gene Chips can be used to follow genetic changes during development.

Different spots light up, i.e. genes turned on at different times (developmental stages) in life cycle.
Molecular Evolution can be determined by DNA Sequences...or by protein sequences...or by protein structures
(Nice chapter in Berg, Tymoczko, Stryer, 5th ed.)

You and parents have same DNA by >>99.9%
You and me (unrelated humans) are 99.4% the same.
You and chimp: 99% the same.
We are related to a cauliflower! (about 50% DNA similarity)

Protein structures most closely related to function...best.
Can sometimes see similarities in structure even where a.a. or DNA sequences are very different ...hard to tell.

www.famous-scientists.net/jane-goodall.html

http://en.wikipedia.org/wiki/Common_descent
Example: Myoglobin
Oxygen-carrying protein in muscle.

Amino acid sequence

Humans & Chimps myoglobin differ
by 1 a.a. out of 153.

What’s the probability that two peptide sequences are identical based on random chance?

\[(1/20)^{153} \sim 0\]

Just as you and parent look alike because you came from parent, you and monkey...you and cauliflower have a common “parent”.
We are all related
Cauliflower, Whales, Chimps, Humans...
Genes: Whale and Humans have similar DNA sequence for Maleness.

Figure 6-27 The sex-determination genes from humans and whales are unmistakably similar. Although their body plans are strikingly different, humans and whales are built from the same proteins. Despite the length of time since humans and whales diverged, the nucleotide sequences of many of their genes are still closely similar. The sequences of a part of the gene encoding the protein that determines maleness in humans and in whales are shown one above the other, and the positions where the two are identical are shaded.

Essential Cell Biology, p. 215
Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

3. Related to today’s subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.