

ASIC200 GENOMICS LECTURE (IN CLASS)

*(Note, for exam, **specific details outlined in the class lecture notes highlighted in red are fair game**). More important is that exam questions may involve queries that recognize your understanding of general trends (but not in a technical detail manner) in genetic technology development (i.e. it's really really fast). This material could also be very helpful in thinking about your projected future in the game design project.*

THE STORY SO FAR:

Basically, phenotypes from a living organism are heavily encoded from information found in the sequences derived from DNA, RNA and proteins, although of course, the environment also plays a significant role. This is why molecular biology is a large and important field of science since it's a term that covers research that looks at these types of molecules. An example, of course, includes the polymerase chain reaction (show slide of original paper, as well as data from the class).

MOLECULAR BIOLOGY (BACK IN THE DAY) ONE GENE AT A TIME.

Emphasize how molecular biology use to be done in a silo fashion. i.e. researchers tended to be experts in just "one" thing (one gene, protein, etc).

(Building off from the last video)

FYI: Sanger sequencing is good for getting about 1000 nucleotides of sequencing in a single reaction (<- which is kind of tiny if you want to sequence a genome!)

ANYWAY sequencing has allowed us to figure out some pretty remarkable stuff, such as the general structure of what a human gene might look, but more importantly, it has allowed us (from about the 1960s to the present day), to figure out some very fine tuned details about specific genes/proteins. i.e. we know a fair bit about how genes work, how the DNA code is organized, how genes are turned on, how they are turned off, how a single gene can actually have multiple roles.

BASICALLY, the research pipeline worked in this way for a while.

Whereby, you have:

- 1) an interesting organism,
- 2) with an intriguing phenotype which you try to functionally make sense of, 3) which is linked to a protein or a gene (or more)

4) which in turn is sequenced so that gain info how that gene might work exactly. And in this way, a single organism (such as a human) gets “figured out” one gene at a time, until you have a sense of how it all works together.

i.e. you get a sense of how all these genes might work in the context of the whole organism, in the context of the whole DNA code. NOTE that this is information culled from a variety of sources, not a single specimen. i.e. the data (from many samples) is “representative” of a species, but the data is not necessarily derived from a single sample of species. ALSO NOTE that the data has an added layer of complexity, because genes don’t tend to be an ALL or NONE thing. You have genes that may exist in different forms (different ALLELES) that work better, work slower, only work when such and such is just right. Everyone is different right? BOTTOM line, this mass of data, whilst useful, has many caveats. It represents a holistic “average” of what an organism is all about. ALSO, there is a strong bias on what gets studied. In many ways, the only things that get studied are the things which come with very noticeable phenotypes (i.e. lots of stuff just gets missed out – think about how your Facebook profile doesn’t quite present a full picture of you!

BECAUSE OF THIS, THE NOTION OF SEQUENCING AN ENTIRE GENOME SORT OF MAKES SENSE, AND THEREFORE PROVIDED THE NEXT STEP.

To discuss the implications of this, the drama of the human genome project is probably the best avenue of exploration.

THE (HUMAN) GENOME

First, a reminder of some definitions:

GENOME: In modern molecular biology and genetics, the **genome** is the entirety of an organism's hereditary information. It is encoded either in DNA or, for many types of virus, in RNA. The genome includes both the coding and the non-coding sequences of the DNA/RNA

GENOMICS: is a discipline in genetics concerning the study of the genomes of organisms.

HUMAN GENOME PROJECT (completed June 26th, 2000):

I’ll skim the dramatic bits (which I have a feeling may be discussed by Allen). Briefly...

PUBLIC PROJECT: In the U.S. most of the funds came from the National Institutes of Health – referred to as the “public project” about \$3 billion dollars set over 15years. Dr. Francis Collins, director of the National Human Genome Research

(started in 1990) “The Human Genome Initiative is a worldwide research effort with the goal of analyzing the structure of human DNA and determining the location of the estimated 100,000 human genes...” initial draft proposal for NIH funding of human genome project. ~30 different human cell libraries.

FOR-PROFIT: most famous enterprise is Celera (Latin for “speed”) Genomics Group.

SPEED MATTERS: J. Craig Venter, and NIH scientist frustrated at the slow pace of sequencing the genome leaves NIH in 1992, to form The Institute for Genomic Research (TIGR) with wife Claire Frasier. Venter and others developed a technique termed SHOTGUN SEQUENCING which relies heavily on automated DNA sequencing machines, and in 1995 are the first group in the world to sequence a complete genome (*Haemophilus influenzae*, a bacteria that causes the flu)

1998 Venter teamed up with PE Biosystems / Applied Biosystems (ABI) to form *Celera*. Goal sequence the human genome by 2001 (2 years before completion by the HGP, and for a mere \$300 million (about a tenth of the public project).

Sample derived from DNA samples taken from 5 individuals. Celera says it used one man's DNA as the foundation for its work. (This turned out to be Dr. Venter himself)

In the end, both project streams agreed to share data to help complete the human genome sequence by mid 2000. This was approximately sequence that worked out to about 10 fold coverage (i.e. actually tried to sequence the sucker 10 times!) for 90% of the total sequence.

SOME KEY FINDINGS

- The HGP has revealed that there are probably about 25,000 to 40,000 (since updated to a count of ~20,500 human genes) *This is really quite amazing! That something as complicated as a living organism such as a human can be derived from the actions and mechanisms of this few working parts! Point in comparison is to realize that total number of different lego pieces has been argued to be greater (see <https://www.quora.com/How-many-types-of-LEGO-bricks-parts-are-there>).*

- Human genome is remarkably similar to other genomes in terms of total gene numbers and gene functions, although most genes are more complex. (Comparative Genomics) *This, folks, was already sort of known but the HGP really segmented this view. That there is value in working with other simpler organisms to pose biological questions that may be applicable to human biology.*

Use e.coli as an example, in that e.coli is just way less maintenance to look after and study, than say a human.

- Between 1.1% to 1.4% of the genome's sequence codes for proteins. Nonfunctional regions appear to account for ~97%. 12% of human genomic DNA is due to copy number variations – CNVs *The main thing here is to recognize that an awful lot (~97% of the DNA sequence) appears to be completely useless*

- ~2 million single nucleotide polymorphisms - SNPs (~0.1 to 0.3% of total genome)

SNPs are pretty important. They deserve their own section!

LOOKING AT SNPs

A single nucleotide polymorphism is a DNA sequence variation occurring when a single nucleotide — A, T, C, or G — in the genome (or other shared sequence) differs between (human) members.

SNPs are extremely useful because they are a significant element that differentiates one human genome from another. In other words, if we want to sort out why genetic differences result in differences between people, there is (conceptually) no longer a need to sequence the entire genome. **JUST LOOK AT SNPs**, since they represent a key part of what's different between genomes.

Plus, there's a really cool way to look at SNPs, actually millions of them (at once).

First, a quick rehash of the structure of DNA.

“The double helix structure of DNA is not unlike square dancing in an overly homophobic community”

The double stranded nature of the DNA molecule is actually very useful, because it infers the notion that if you know the sequence of one strand, you can predict the sequence of the other strand (due to the complementation of different nucleotides – A pair with Ts, Gs with Cs)

Powerful because you can look for interactions between complementary sequences.

FOR EXAMPLE: (in class we'll reenact by using the square dancing situation as our metaphor for SNPs. Our sequence of interest will be composed of the instructors and TAs in the course)

“...Let’s say we are interested in finding out whether a person’s genome has 1 specific SNP (i.e. specific single nucleotide polymorphisms). In our demo let’s pretend this SNP maps to an appreciation for black coffee...”

Of course, in this example we’re only looking at 1 SNP (and in reality these probes are not just 4 (persons) nucleotides in length, they tend to be around the 20+ nucleotide length). But what about 3 SNPs (this is shown in a slide with some other coffee related options), 100 SNPs, or maybe a thousand? Or let’s say even a million IN ONE EXPERIMENT. If you can do a look for binding with a million different SNP sequences, then you’ve got yourself a pretty powerful system of looking at SNPs. And remember, SNPs represent a significant portion of what makes one person’s genome different from another!

Anyway, all to say that such experiments – looking at millions of SNPs in one go is indeed possible. We call these things DNA CHIPS or MICROARRAYS.

As well, this is a powerful form of GENOTYPING.

(definition) **GENOTYPING**: is the process of determining information about the genes (genotype) of an individual by examining the individual's DNA sequence by using biological experiments (such as looking for whether the presence of specific SNPs correlate to a specific phenotype – we also did a genotyping experiment using PCR in our lab exercise)

The ability to genotype in this way is actually very powerful. For instance, if a SNP is well defined – i.e. if you have this SNP, then that means you have this trait – then you can use this for predictions.

OR, it also allows you to more quickly and efficiently infer linkage between a trait and DNA sequence, sometimes simply by looking at differences or similarities in DNA sequences and attempting to correlate trait differences and or similarities. i.e. “Hey, this SNP always crops up when looking at this phenotype – maybe it has something to do with the phenotype.”

NOTE that this is looking for a correlation trend, and correlation DOES NOT equal CAUSATION but if the correlation is very striking, and spans over a massive sample number, then it’s probably going to be interesting enough for you to want to check it out further.

ALSO: Bring up the HAPMAP project (public project to characterize all possible human SNPs – up to about 10 million so far), as well as services like “23andme” (Allen will get into this – this is the \$99 genotyping service (which essentially looks at a variety of medically relevant SNPs).

Also highlight how 23andme is a DNA service that essentially tracks SNPs

BETTER WAYS TO JUST SEQUENCE THE HECK OUT OF A SINGLE SAMPLE

Although looking at SNPs is a powerful way of quickly characterizing a large number of elements in a person's genome, it stands to reason that if you could just sequence the **whole** genome of many individuals, perhaps even all individuals, then you would have an even stronger data set from which to correlate (and therefore identify) DNA sequences that result in certain traits/phenotypes.

I.e. instead of each sample being represented by 2 million SNPs, each sample is instead represented by 3 billion nucleotides! Obviously, you need some serious computer power to be able to look at this effectively – but guess what? Computers have had that processing power for a while now.

In fact, the first full personal human genomes were sequenced and published in 2007. There were of Craig Venter and James Watson. There's also the completed **1000 genome project**, which had 1000 human genomes (basically volunteers) sequenced by 2012, where all public data in the hopes of being able to correlate phenotypes/traits of these 1000 individuals to their genetic code.

And sequencing technology is getting better all the time. A wave of technology development led to new methods and devices which are often collectively referred to as **NEXT GENERATION SEQUENCING**. In fact, the speed in which sequencing is increasing is often compared to *Moore's Law* (law of transistors – whereby The quantity of transistors that can be placed inexpensively on an integrated circuit has doubled approximately every two years).

From the two graphs, you can see how sequencing technology is improving from both an output point of view (how many letters we can get and fast it takes to get it), as well as a cost per Mbp point of view. (Mbp = 1000,000bp, letters). It's actually improving **FASTER** than Moore's Law!

HOW IS THIS POSSIBLE? Various new technologies that allow for this. Go back to the Sanger example. With that experiment, you can get about 1000 letters in a single experiment, which will take about a full day. However, that is one tube, one experiment – you can imagine, it's pretty straightforward to (say) work with 10 tubes. This means, that in a single day, you can get 10x1000letters of data = 10,000bp. Conceptually, this means I can increase my sequencing output if I just have the opportunity to do more reactions in a single go.

However, this also raises costs – i.e. instead of chemicals for one tube, I need chemicals for 10 tubes (or however many reactions I do, since they are all in separate tubes) **MEANING** that the challenge one has (in increasing the amount of code determined, as well as keeping costs down) is: Can I mimic the data of

millions of reactions, but all in one place (one tube). This is what we'll go over with some more technical detail next week (i.e. it's very cool)

Anyway, THESE are what the new technologies are all about! At their heart, they are tricks to allow sequencing to be done in a massively parallel fashion – i.e. millions, if not billions of sequencing reactions at the same time. (I'll highlight Illumina's Solexa platform if there's time).

If you want to learn a little more about the various different Next Gen Sequencing techniques, here are two 5 min videos outlining the basic protocol of two of the most common platforms:

Illumina: <https://www.youtube.com/watch?v=fCd6B5HRaZ8&>

Ion Torrent: <https://www.youtube.com/watch?v=WYBzbxlfuKs&>

THE NET RESULT, is that research is now at a place where getting LOTS of sequencing data for relatively cheap costs is doable. Will show some graphs to illustrate these trends.

In any event, currently biological research is being propelled by these technologies, because ultimately, they allow you to get massive amounts of raw data (DNA, or RNA code). The trick is correlate this raw data with phenotypic observation, using computer tools, and use statistics to assess potential validity.

BUT, when your data sample consists of 3 billion variables (i.e. the individual letters of code, or even if you're looking at 10 million different SNPs), it's actually not that difficult to find correlations that are irrelevant, i.e. false correlations or the simple fact that if you're looking at 3 billion different things then of course, you're gonna find correlations, but of course, most of them would be statistically coincidental.

(See Unicorn versus Horse example)

How do you fix this problem with false correlations. Well you need to sequence **more** genomes, so that you have more samples to look for correlations. However, the issue with this, is that as fast and cheap as sequencing is, it was still a bit too expensive to do at a scale that statistically approaches numbers of samples needed.

But this speed and cost is changing: and fast. And with that, I'll leave you with the following stats so that you can compare how things have changed in even the last few years, we've taught this course...

1990: Several machines to sequence the human genome. Est. time and cost: 15 years and \$3 billion

1998: Celera (shotgun estimate). 3 years and \$300 million.

2003: Human Genome Project finished. Final price tag for a “draft” version was \$150 million.

2006: ~\$14 million.

2012: One machine can sequence an entire human genome in about 8 days at a cost of about \$10,000

2013: One machine can sequence an entire human genome in about 3 days at a cost of about \$5,000

2014: One large scale set up (HiSeq X Ten) can sequence an entire human genome’s worth of data in about 1 day at a cost of \$1,000 (set up is tens of millions of dollars).

2015: Oxford Nanopore MINion sequencer released for sale (sequencing device at ~\$1000, but 30% error rate)

2016: One machine can sequence an entire human genome in about 1 day at a cost of about \$1,200

2017: Illumina NovaSeq 6000: ~48 genomes per 45hrs (3000Gb). less than \$1000 each (this includes set up costs ~\$1000,000). Illumina keeps talking about an end goal of \$100 per human genome.

2018: Illumina just released their iSeq (~\$20,000 for the device). *1.2Gbp output in 9 hours.*

MINion error rate with appropriate software analysis currently can be dropped to about 4.25% to 0.6% (other platforms is < 0.05%)

Also, check out the MinION nanopore sequencing set-up (<https://www.youtube.com/watch?v=3UHw22hBpAk>)

BASICALLY...

All of this fast progress in sequencing is conceivably making it easier to “find” and/or “correlate” sequences to interesting phenotypes. And once you find them, you can focus your efforts on studying the functional aspects of that specific area of the genome. i.e. this stretch of DNA shows a really strong significantly significant correlation to this phenotype. OK, let’s look at that sequence more closely – does it encode for a protein, and if so, does that protein’s function make sense in terms of the phenotype... and so on.

Not only does this increase in sequencing potential lead to greater statistical confidence in correlating specific DNA sequences with phenotypes, it also gives us a very precise profiling tool.

(Dave actually forgot to mention this section in class, but will bring it up during the Q&A)

This in turn has led to the idea of PERSONAL GENOMICS, or PERSONAL MEDICINE (also known as PRECISION MEDICINE).

Definition (by way of wiki): is a medical model that proposes the customization of healthcare, with medical decisions, treatments, practices, or products being tailored to the individual patient (i.e. what we know from their genomic sequences, environment, and lifestyle).

Use coffee example to illustrate this, as well as bringing up the current problems with drug efficacy testing.

The other thing to note is that... If we're getting profoundly better at figuring out what sequences can do what, is there a way where we can directly change the code to suit our needs (needs being cure diseases, or produce GM variants). And this is where another piece of story has gotten very interesting lately.

NOW THAT YOU KNOW THE CODE, HOW ABOUT THE ABILITY TO EDIT THAT CODE IN YOUR GENOME?

CRISPR/Cas

It's here folks – easy, cheap, genome editing. Highlight, how genome editing means being able to make the change “directly in the cell” as oppose to making the DNA change in a test tube, and then trying to get it to insert into the host genome.

CRISPR is basically big news, because gene editing got a lot easier and cheaper with this discovery.

Highlight the original papers, and the story of Jennifer Doudna (discovered it first); and Feng Zhang (figured out how to get it to work in mammalian cells – i.e. possible use in human medicine).

Dave's notes on biology of CRISPR/cas systems:

Essentially, what you have here is a bacteria immune system that was discovered in the late 80s where incoming foreign (and hence bad) DNA from viruses got into bacteria to wreck havoc. However, it was noted that some of this foreign viral DNA was chopped up, and then integrated into special areas of the bacteria's own genome (these are CRISPR areas). In a way, it was a mechanism

for the bacteria to “remember” these viral sequences, since they are being kept in these CRISPR areas.

From there, it was discovered that another bacterial protein (Cas9) was capable of holding on to these “saved” viral sequences, and use them as homing devices to cause DNA cutting. i.e. Cas wants to cut DNA, but only cuts DNA sequences that matches the viral sequences – basically, a proto memory Immune system!

Anyway, what folks found is that this CRISPR/Cas system works really really really well in other cell types (most notably mammalian systems), which has made this genome editing tool all the rage in recent years. So much so, that there’s a brutal patent war going on (lots of money at stake), as well as various declared moratoriums on the use of the system in human cells (in essence, because it’s a little too easy to use). Allen will talk more on this.

Meaning that with this technology, you can do the following:

- Change the DNA code within a **cell’s** genome relatively easily.
- You can do this by way of CRISPR/cas making a very precise cut at the targeted sequence.
- When the cell tries to fix this, it tends to make small errors at these cut sites. These errors inadvertently mess up the sequence and can often shut down a gene and it’s ability to make the protein. Therefore, the editing results in a loss of a specific gene activity/protein.
- Or you can include additional DNA sequences, which the cell often tries to insert when trying to fix the initial cut. Therefore, the editing can result in a gain of DNA sequences, and therefore potential gain of specific gene activity/protein (this might include fixing something that was initially the wrong sequence).

AS USUAL, THINGS AREN’T SIMPLE

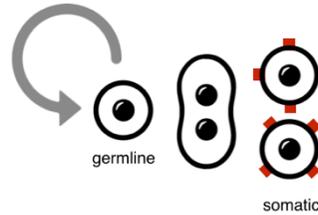
Some issues with CRISPR/cas:

Technical:

It’s not *that* easy. There’s a fair bit of a hype around the methodology, in that it is way easier than previous gene editing tools, but still requires trained hands, and considered expert thought.

It’s not perfect - both in terms of it working 100% of the time (i.e. sometimes, **no edit occurs**); but also not perfect in the sense that **mistakes** can be made (where the wrong part of the genome is edited).

This is an amazing system for **basic** research. i.e. I'm working on a cell/model organism and I want to see what happens when I change the DNA code. CRISPR/cas has made this research query much much easier (this is why you see it being used more and more, and goes hand in hand with the sequence data. i.e. **great for testing the seq to phenotype correlations**) BUT, as a tool for gene therapy, or genetic modification, then... **things get a little tricky.**



Ethical:

With the issue of errors/mistakes in what gets edited, what is the appropriate risk to use this for **gene therapy** (i.e. medical uses). Does the degree or seriousness of the **disease** factor in? And where do **enhancements** fit? For that matter, what is the line between a treatment and an enhancement?

One also needs to consider the cell type. Specifically, is it a **somatic** cell target, or a **germline** cell target? Editing a germline cell also comes with additional risks.

As these tools get cheaper and more efficient, and essentially easier to do, then can we assume that **policy** can provide appropriate regulations to make sure unethical uses are avoided? There's already a discussion around concepts of whether even **expert scientists have the necessary training to navigate the ethical considerations.**

Finally: your DNA sequence is also a bit more complicated than just the code in your "genome."

Briefly:

Epigenetics: The letters (A's, T's, C's, and G's) in your human genome don't dictate everything. Sometimes, how certain regions of your DNA are folded up will matter (i.e. the shape of the DNA, not the letters). Sometimes, the environment will cause chemical additions to your code, which in turn modifies how it's read and utilized by your cell. All to say, that there is yet another layer of complexity that determines how genes are turned on and off.

Mitochondrial DNA: Your cells also have additional DNA that is found in the mitochondria (an organelle involved in the energetics of your cell). It's kind of like a genome plug-in and codes for a few important things, and is inherited in a strictly maternal fashion. Still, it means that human cells could (with manipulation) have DNA derived from 3 different places.

Mosaicism: Not all your cells have the same exact genome code. Cells undergo mutations all the time, and these cells in turn, grow and divide, so that a person

may actually have clusters of cells with slight sequence differences. In fact, there is evidence to suggest that some of your cells may even be maternal in origin.

Microbiomes: There are at least as many bacteria, as there are human cells in/on your body. This means that being “human” can be thought of as being a community of living organism. Turns out, this community (in its various places) plays a huge role in how you function.