What do we hope to learn from sequencing?

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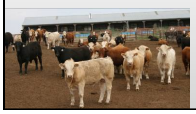
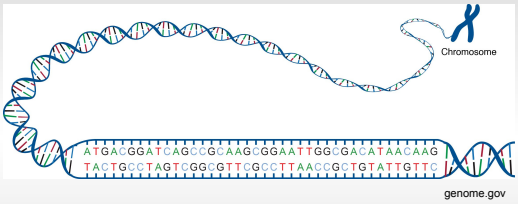


Genome sequence

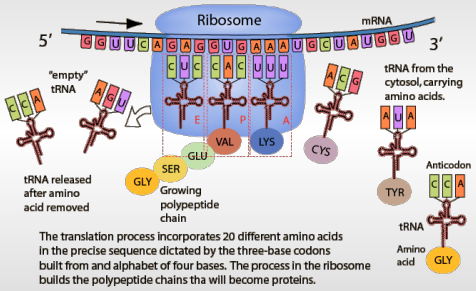


- Trying to read the base pairs (the code) along the whole genome

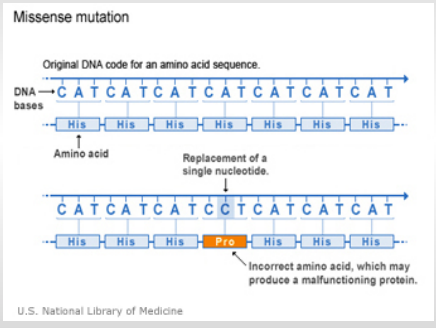
Whole genome sequencing

- Loosely defined as reading and recording all 3 billion bases in the cattle genome
  - Introns and intergenic regions
    - Do not code for any protein
    - Over 98% of genome
    - Likely some regulatory function
  - Exons
    - Remainder
    - Coding regions -- PROTEINS

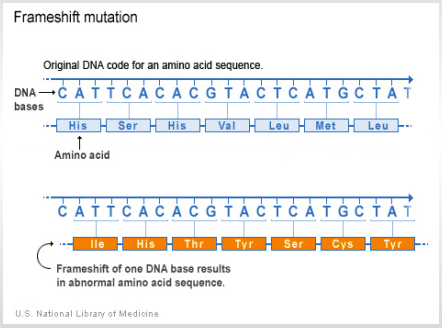
Exon - protein coding



Mutations – genomic variation



Mutations – genomic variation



First cattle genome sequence

- Dominette
  - USDA-ARS – Miles City Montana
  - Inbred Hereford female
- Impact
  - Reference Bovine genome
  - Cost over $53 million
  - Led to DNA chips (50K chip, etc.)
  - Base for further sequencing projects

USMARC sequence technology progression:

1992 Pharmacia manual slab gel – about 20,000 bases wk^{-1} $machine^{-1}$, about $7 per 1,000 base read; used for microsatellite sequencing ($7,000 per Mb)

1997 ABI 377 gel-based system – about 380,000 bases wk^{-1} $machine^{-1}$, about $3 per 800 base read; used for small scale gene sequencing and marker development ($3,750 per Mb)

1999 ABI 3700 capillary system – about 3.5 Mb wk^{-1}, about $2 per 600 base read; used for "large scale" EST sequencing, marker development and start of SNP discovery program ($3,300 per Mb)

2003 ABI 3730 capillary system – about 7.5 Mb wk^{-1}, about $0.80 per 600 base read; used for EST sequencing, BAC-targeted SNP discovery, lots of amplicon resequencing in breed panels ($1,300 per Mb)

Next generation sequencing

- Multiple platforms available

Next generation sequencing

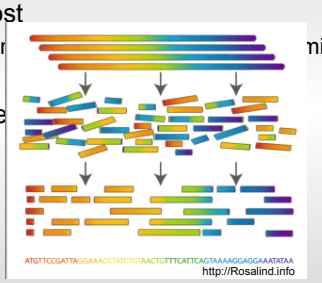
- Accuracy ranges from 87% to 99%
  - General trend is decreased accuracy with longer read-length
  - Generally read 50 to 1000+ bases per read
  - Easier to assemble longer reads
- Net effect is cheaper genome sequencing
  - Costs from $0.05 to $10 per million bases
  - Enough sequence for $150 whole genome

Some complications

- Initial cost
  - Sequencer costs from $80,000 to $1 million
- Genome assembly

Some complications

- Initial cost
  - Sequer illion
- Genome



Some complications

- Initial cost
  - Sequencer costs from $80,000 to $1 million
- Genome assembly
  - Overlap helps
    - Long reads better
    - Helps to compare to base sequence (other cattle)

Complications

- Tremendous amount of data
  - Hard to store, let alone manipulate
  - Makes assembly even tougher
  - Have to develop tools based on results
    - For example, genotyping based on new markers discovered in sequence (50K chip)
    - Hard to decide what 'differences' among animals are important
      - As with chips, phenotypes are still very important

Complications

- Multiple reads required
  - Reading 3 billion bases does not mean whole genome read
    - Each animal has two full genomes (diploid)
    - Genome is fragmented randomly into small pieces
    - Same section of genome likely read numerous times
    - Some advocate sequencing 20-50x when targeting whole genome
      - Focusing on one animal reduces impact of examining multiple animals (diversity, discovery, etc.)

Why bother?

- From a geneticist perspective:
  - Interested in sequencing to improve our chance at finding causal variation
    - Examine differences in sequence
    - Leads to finding markers and mutations
    - Mutations may change protein structure or protein regulation
  - Ultimately differences we see due to genetics lie with differences in the genome or its regulation

Two different strategies

- Compare genomes of diverse animals to see where they are different
  - Marker development, mutational candidates
  - Follow up with genotyping platform
- Associate differences in sequence among several animals with trait variation
  - Requires large numbers of sequenced animals
- First strategy cheaper, more restrictive

Functional variants

Impact	Effect	
High	SPLICE_SITE_ACCEPTOR	FRAME_SHIFT
	SPLICE_SITE_DONOR	STOP_GAINED
	START_LOST	STOP_LOST
	EXON_DELETED	RARE_AMINO_ACID
Moderate	NON_SYNONYMOUS_CODING	CODON_CHANGE
	CODON_INSERTION	CODON_DELETION
	UTR_5_DELETED	UTR_3_DELETED
	CODON_CHANGE_PLUS_CODON_INSERTION	
	CODON_CHANGE_PLUS_CODON_DELETION	
Low	SYNONYMOUS_START	NON_SYNONYMOUS_START
	SYNONYMOUS_CODING	NON_SYNONYMOUS_STOP
	SYNONYMOUS_STOP	START_GAINED
Modifier	All other effects	

Congolani et al., 2012

Summary

- Sequencing offers many possibilities
  - Move toward causal variation
  - Increase selection opportunities
  - Detect microbial interactions with economically relevant traits
  - Lower genotyping costs

Current efforts

- So we have potential for a lot of data
- Now what?

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