Info update – 15-20 minutes max

* outline
* learning/engaging activity
* using white board effectively
* take home message
* uses examples from the literature

Outline:

1. Advances in sequencing technology

2. Range of applications

3. General library prep workflow

4. Sequencing by synthesis

5. Other technologies

6. Learning activity

Human genome project – completed in 2001-03

* Used Sanger sequencing
  + Capillary gel sequencing bits of fluorescently tagged dntps
* 15 years of intense effort
  + one person’s genome
* Cost $3 billion

HiSeq XTen Platform – 2014

* in one day they can sequence 45 whole human genomes at $1000/genome
* Uses a flow cell (glass slide) each reaction happens on a tiny dot and there are 8 “lanes” on each cell
* Range of applications
  + whole genome sequencing, RNAseq, CHIPseq (chromatin immune precipitation sequencing), targeted/capture sequencing

Library Prep Workflow

* depends on which method you are using…
  + what are you interested in?
    - demographic history, adaptive genetic variation, gene expression variation
  + Diferent parameters
    - length of reads
    - # reads
    - Read distribution
  + Extraction for DNA/RNA(change to cDNA)
  + Fragment sample (break it into smaller chunks)
  + Ligate adapters
    - individual barcodes telling you which sample it is or if it is a pool, population/treatment
  + Add sequencing adaptors
  + PCR

Sequencing by synthesis

* Add samples to lanes
* each lane has oligos that your sequencing adapters attach to… so you get segments of attached DNA
* forward and reverse oligos
* Segments arc over…? (bridge amp (this is just for amplification, cluster gen (each cluster of amplified DNA belongs to a sample) \*\*\* look back on this
* every cluster gets a snap shot of which nucleotide is added

Whatever you are sequencing

* You will likely be using illumine sequencing
* 90% global data

Combine technologies to get the benefit of both

* get long reads from PacBio SMRT (not quite as accurate but easier to piece together)
  + Benefit of long reads:
* Get depth of coverage using illumine short reads (much more accurate but harder to assemble