

Ecological Genomics Notes

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Info Update:

- Advantages in Seq tech.
- Range of applications:
 - WGS (whole genome sequencing)
 - RNAseq (sequencing RNA converted to cDNA)
 - Targeted capture seq. (string of probes mixed with sample, pulls immune related genes from organism and seq just those)
 - ChIPseq (chromatin immunoprecipitation seq, recognizes and antibody and pulls out all DNA bound to that protein)

Why one or the other?

Genetic variation

- phenotypes

number of samples

- population
- individual
- comparative studies
- model or not

Demographic history

Adaptive genetic variation

gene expression var.

length of reads

number of reads

distribution

Reads:

- short = 50bp
- long 100 bp, 150 bp, 300 bp (miseq)
- 10,000-60,000bp = SMRT

Single vs. paired end

- General library Prep. Workflow
 - extraction (DNA, RNA -> to cDNA)
 - fragment sample
 - ligate adaptors (individual barcodes)

- add seq. adaptors

Reduced Rep

- RNA -> coding
- GBS/RAD-seq
- near restriction sites
- Sequencing-by-synthesis (SBS)
 - bridge amp
 - cluster gen.
 - labeled dNTP (ATCG)
- Other Technologies
- Learning Activity

Human Genome Project (2001-2003)

- ABI = Sanger
- 15 years
- 1 genome (one person)
- \$3 billion

Uses PCR and sequences broken by faulty base pairs to work backwards

2014 X-Ten releases

-Hiseq by Illumina (look up video of how it works)
 -1 day
 -45 whole genomes
 -\$1000 bucks each

Sheet of glass with 8 lanes with flow cells... (look up)

Take home messages

- Likely using Illumina seq. (just library tech. changes) usually SBS
- adaptors are markers (barcodes) used to identify samples during sequencing
 - first thing that's sample and gives ID barcode = seq adaptor
 - alago is a sequence of DNA that is attached to plate binds to sample
- Model vs. non Model:
 - short reads (assembly to create long sequence based on short reads that shift)
 - denovo assembly -> computer program (added variability with mixed sample)
 - 15% error for SMRT can be reduced to less than 1 with repeated passes
 - illumina is much smaller but more accurate (0.05% error)
 - combine the two to have a higher degree of confidence

Paper Discussion:

Three advances in biology:

- Modern Synthesis (evolution (Darwin) and population genetics (Mendel))
- Watson and Crick (molecular biology DNA)
- Omics Era (genomics, proteomics etc.)

What do we think about this?

Phylogenies -> reducing error bars or reshaping question?

Do you throw out experimental design and scientific method for large scale shotgun blast sequencing? -> can still be used to do hypothesis driven science, but also something to be said for sending a telescope into deep space.

Most journals and funding sources require all seq data be made public - leads to a storage space issue -> genbank ran out of space and needed emergency funding from congress.

data are not reviewed very well some gen bank sequences are kinda rough

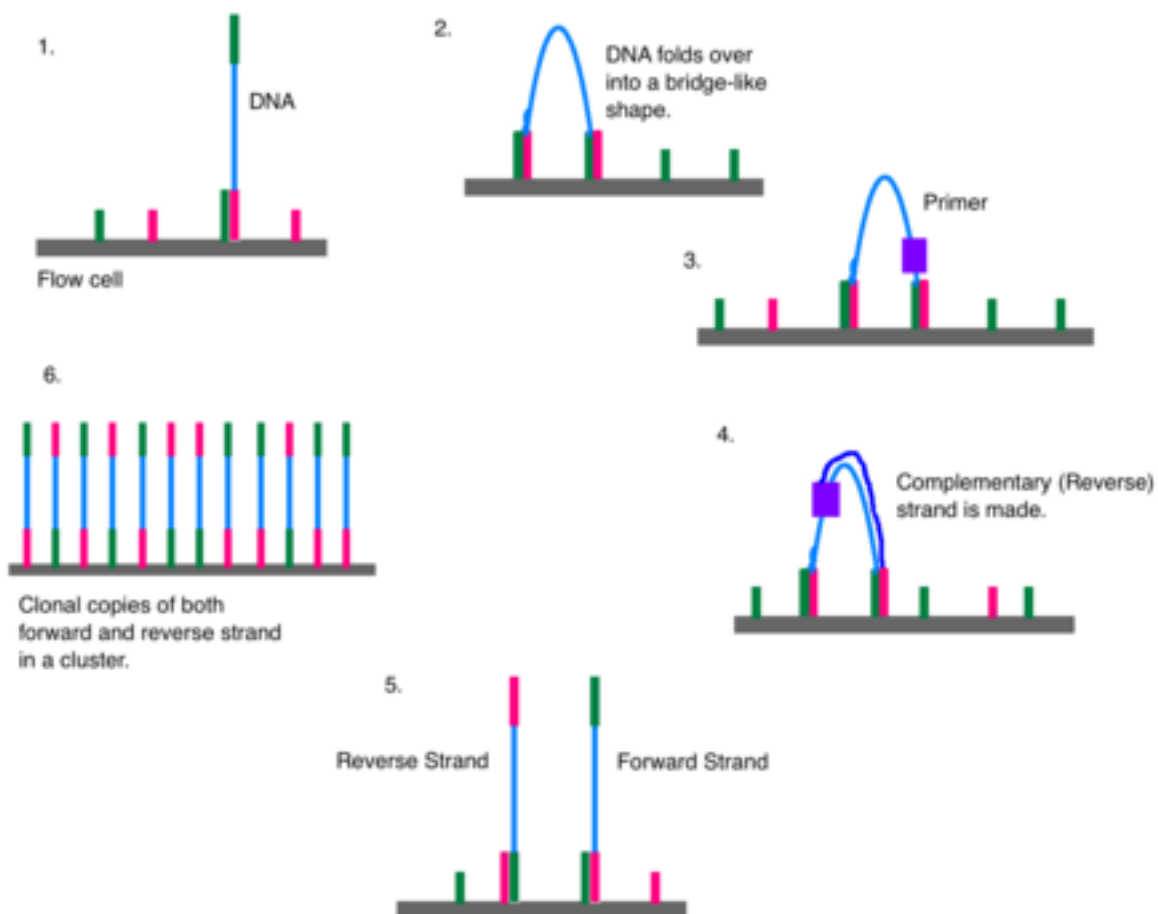


Figure 1: Illumina Sequencing