Habits

John Doe

March 22, 2005

Module 1: Technology

Overview

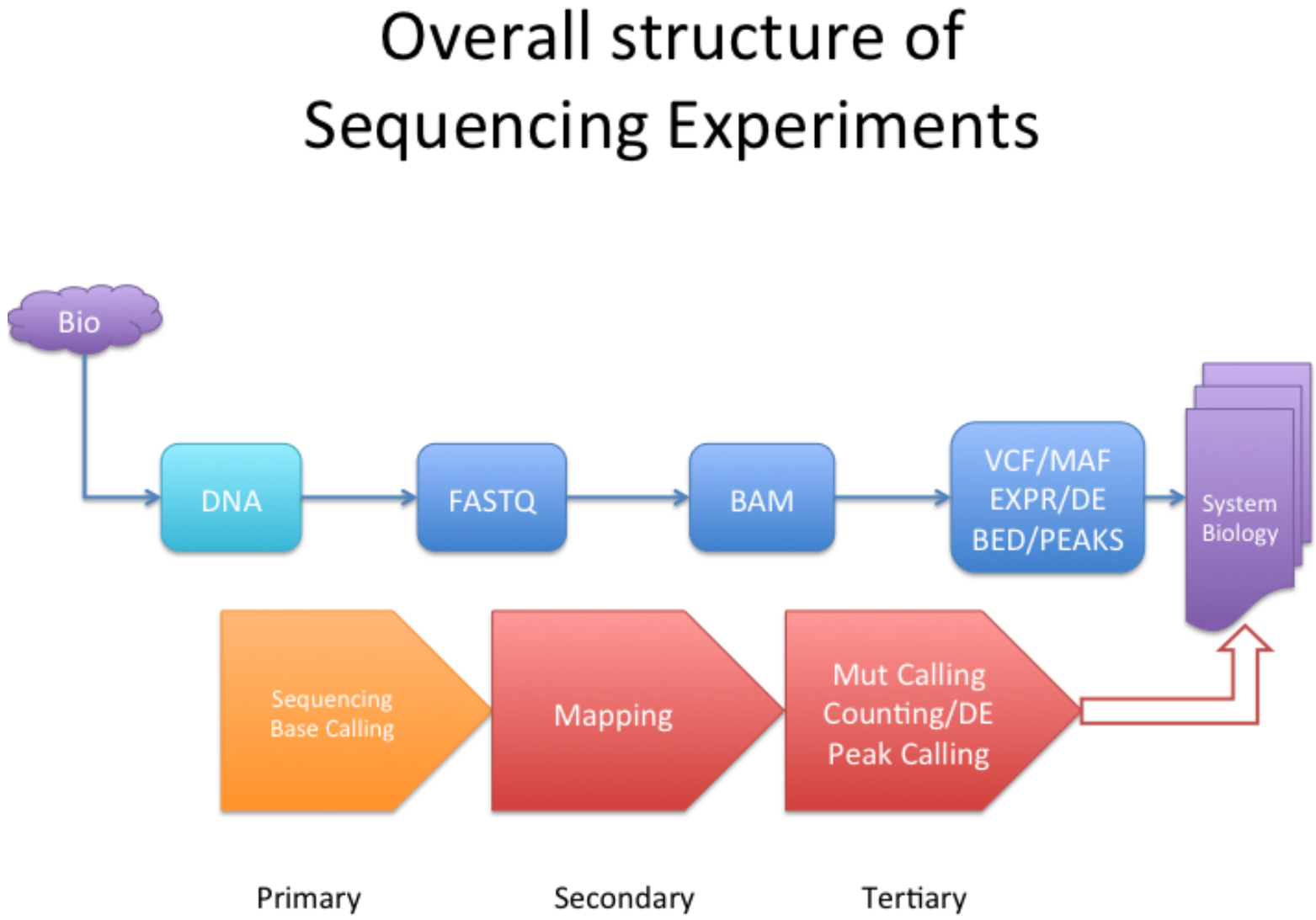


Figure 1:

Computer Stuff

- All the stuff in the middle involves heavy use of computers
  - No way to avoid it
- But to many that stuff in the middle is impenetrable
  - And often computer/math/physics types are not all that helpful

Obtuse and Obfuscated

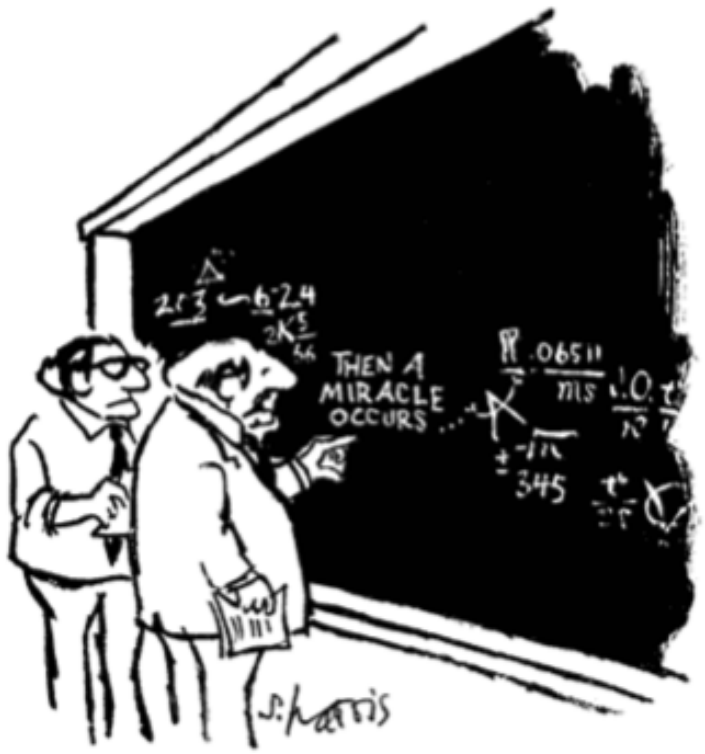


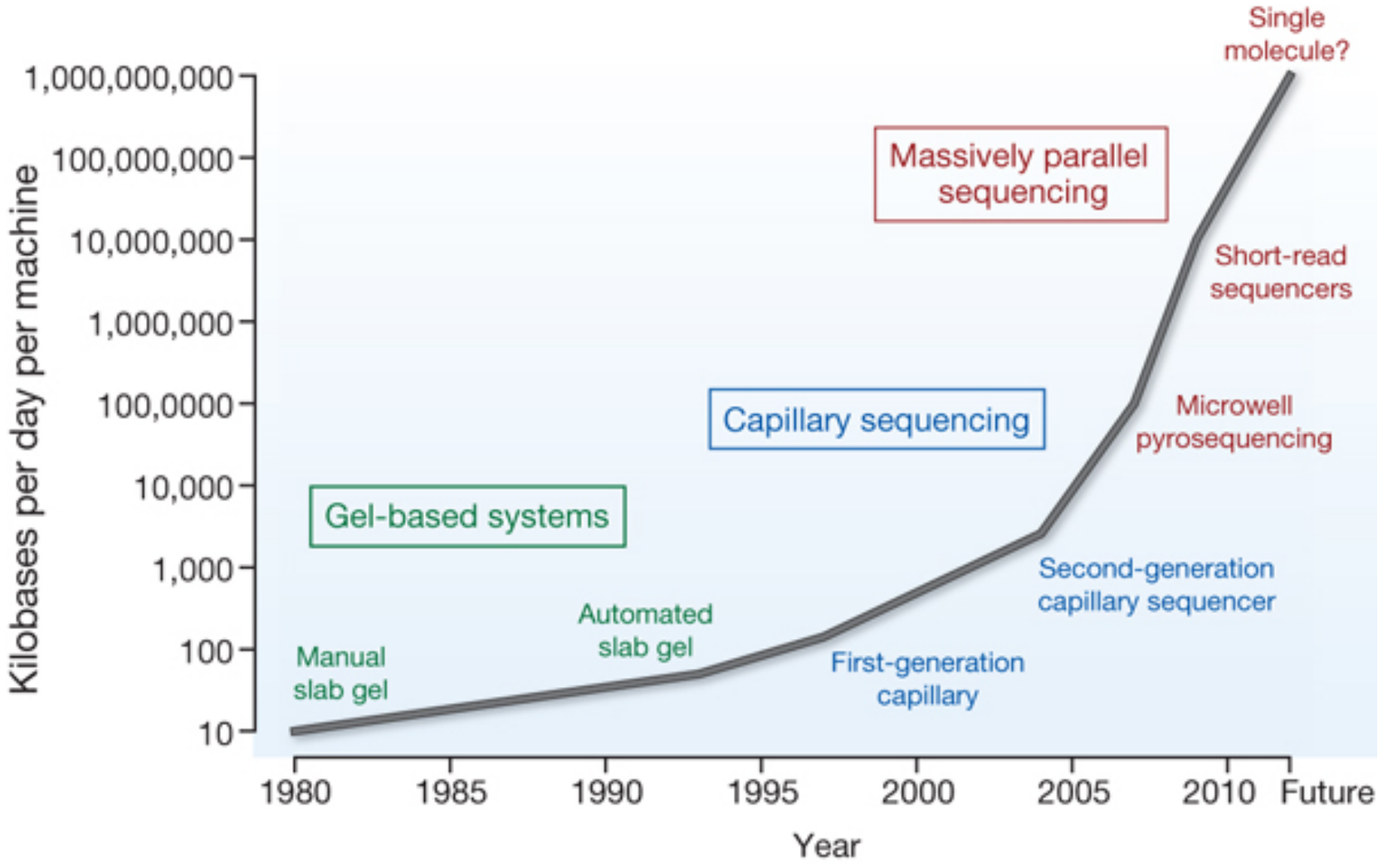
Figure 2:

Here to help

- Or at least try

Introduction to sequencing technologies

Manditory Growth Slide



MR Stratton *et al. Nature* **458**, 719-724 (2009)

Figure 3:

Multiple technologies

- Illumina
- SOLiD
- 454 (successor Ion Torrent)
- PacBio

Technology comparison

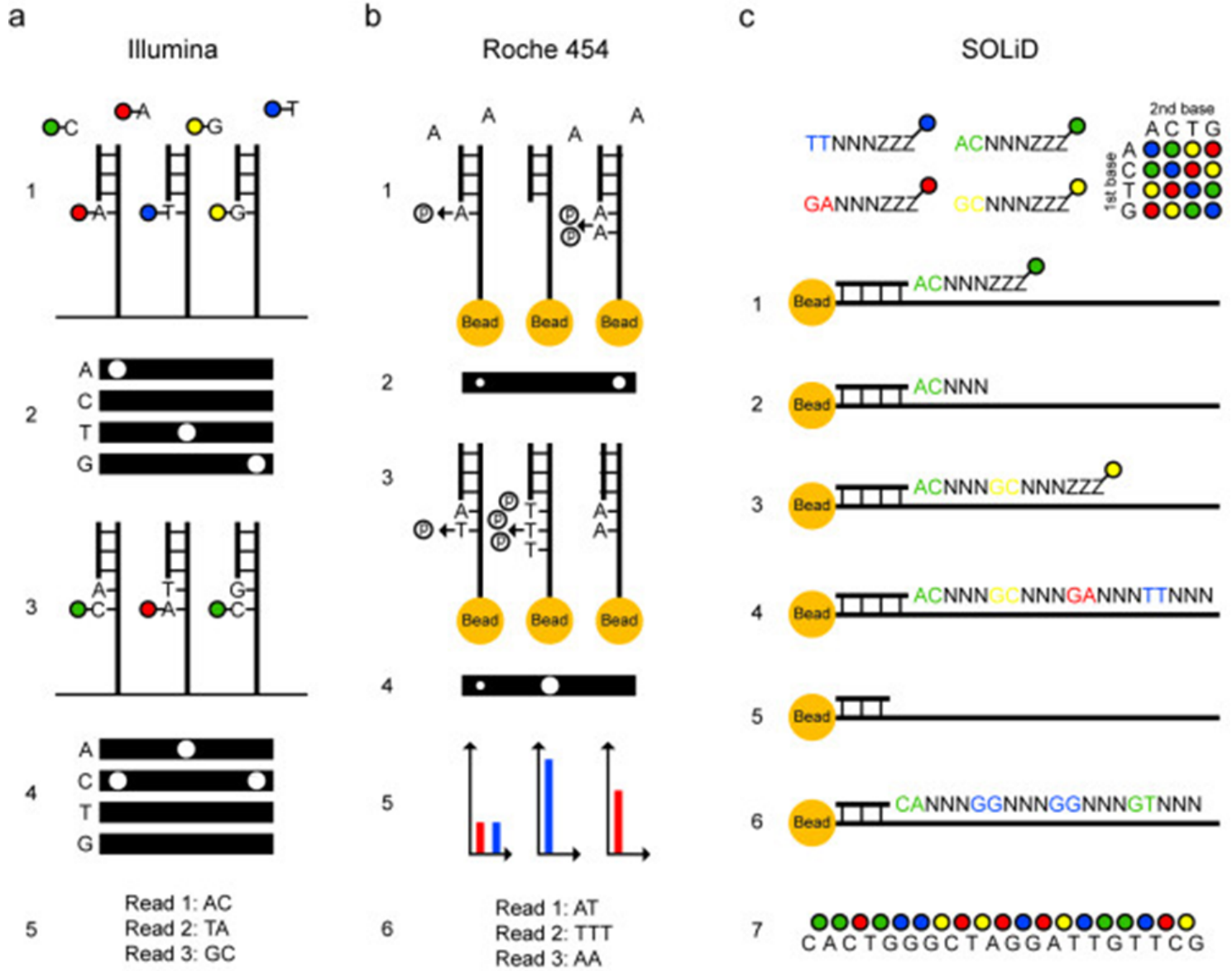


Figure 4: 3 major sequencing techs

Illumina

Sequencing by synthesis

Cyclic reversible termination

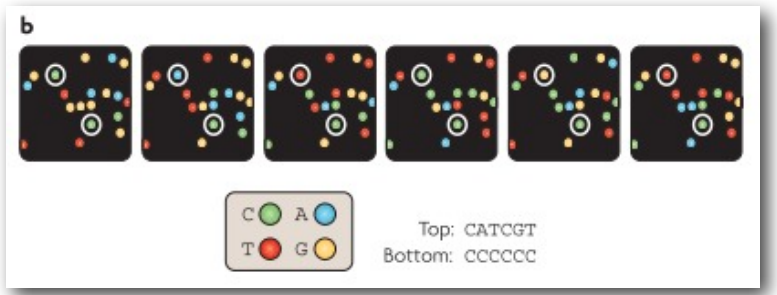
DNA synthesis is terminated after adding single nucleotide

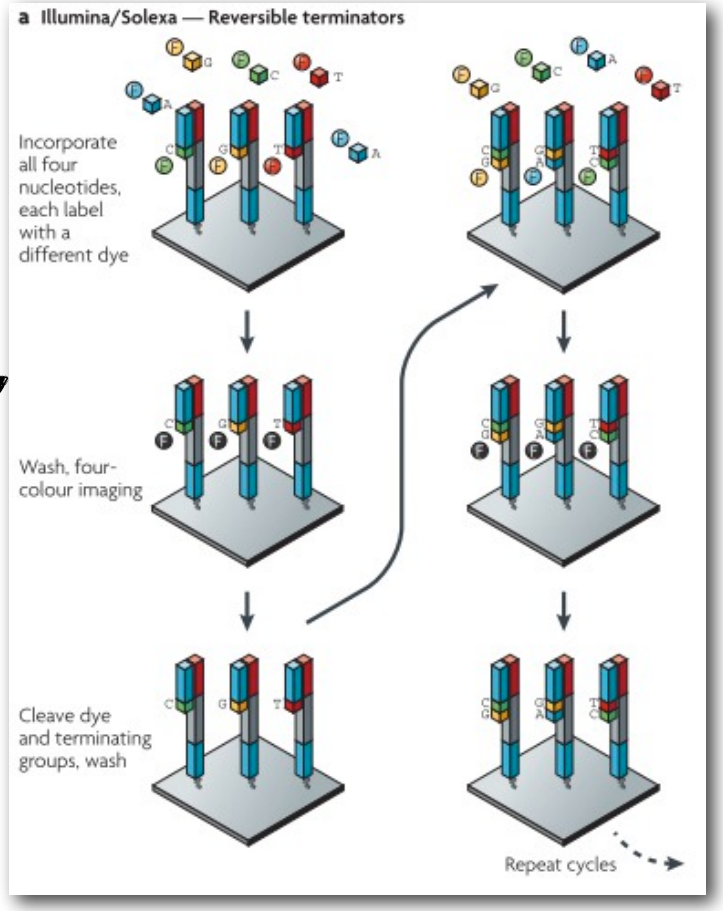
start/stop/start/stop/start/stop/...

Illumina: 4-colour

sequencing result

sequencing steps



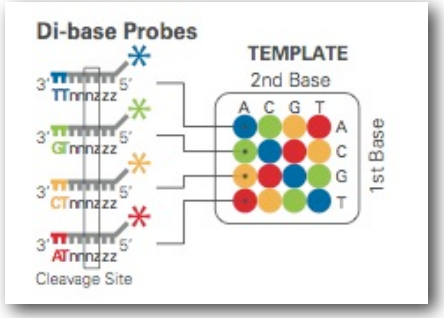


Metzker et al, 2010

SOLiD

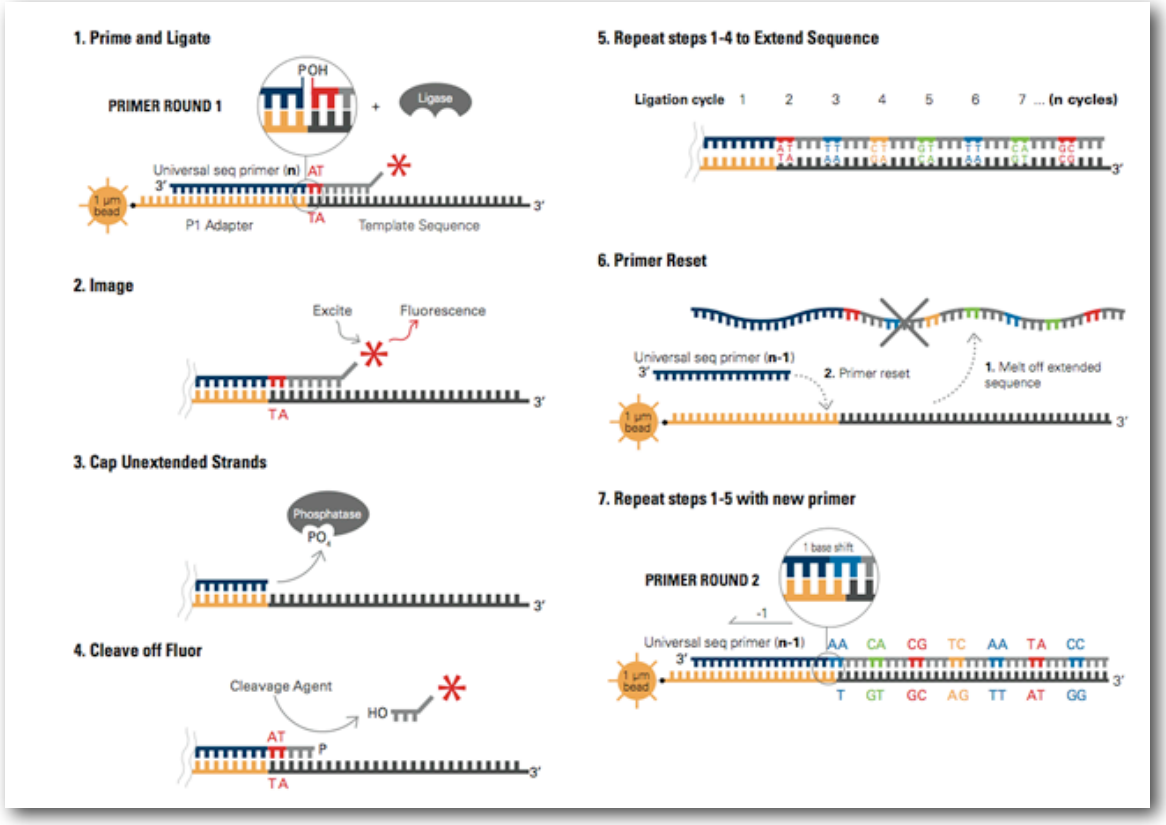
Sequencing by ligation

Sequencing by ligation



http://bit.ly/fPh22X

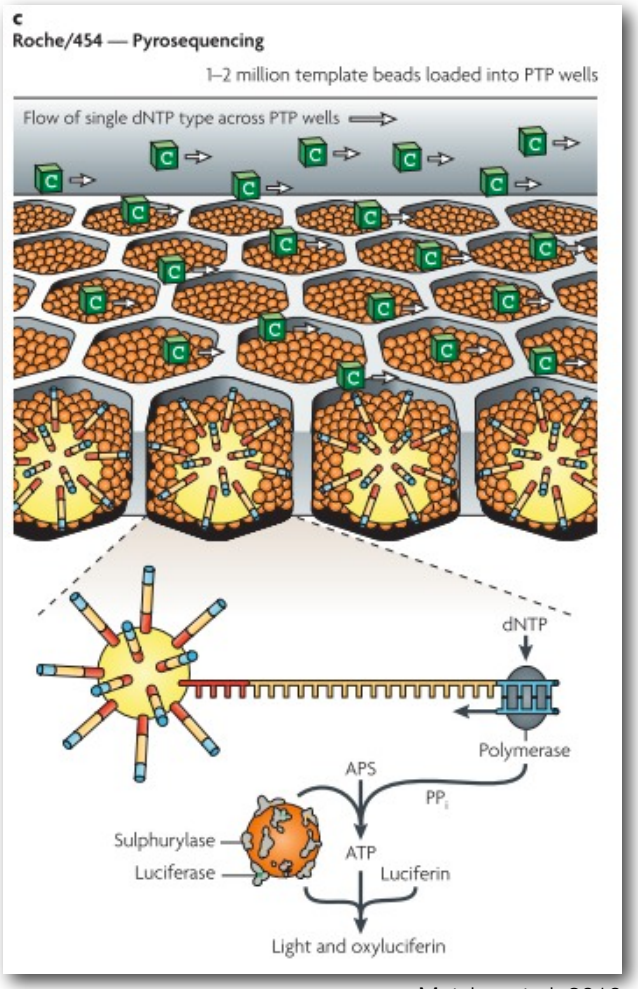
sequencing steps



454/IonTorrent

Pyrosequencing (H+ sequencing)

Pyrosequencing



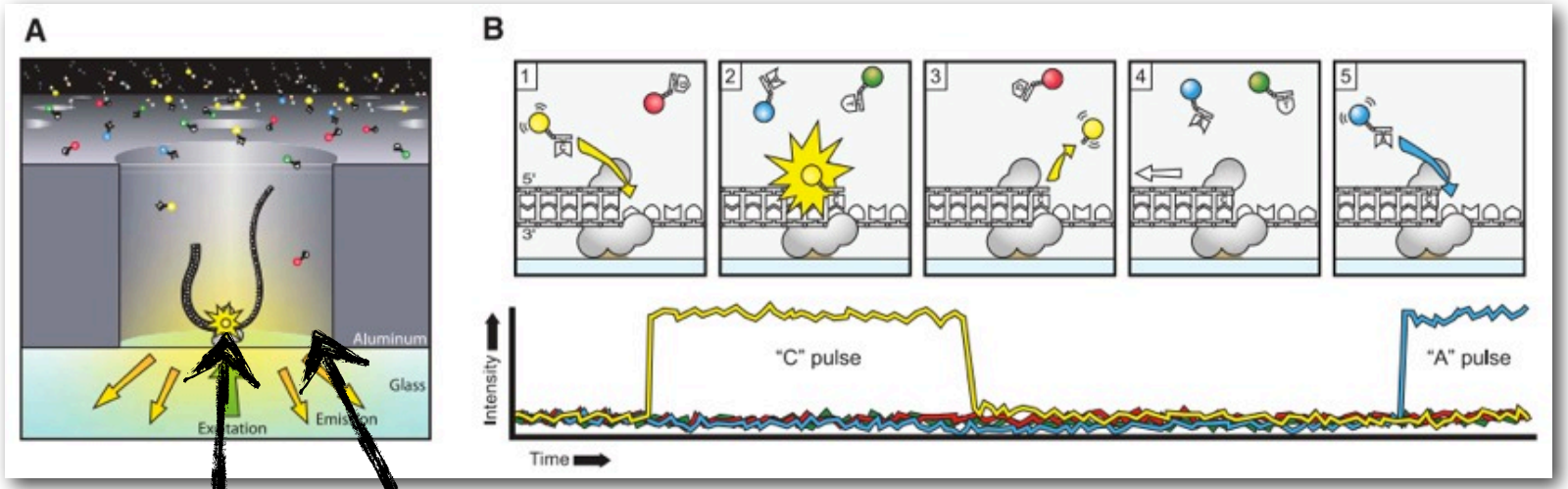
Metzker et al, 2010

Metzker et al, 2010

PacBio

Single molecule sequencing (sequencing by video)

Real-time sequencing



"ZMW" zero-mode waveguide

DNA polymerase

"strobe sequencing"

Accuracy

- Sanger $>$ SOLiD $>$ Illumina $>>$ 454/IonTorrent $>>$ PacBio
  - 454/IonTorrent problem with homopolymers
  - However with the exception of Sanger read length goes up as you move to the right. Less accuracy but longer reads

pyrosequencing homopolymer problem

- Affects 454 and IonTorrent
- Because it reads multiple runs of the same base in one cycle there is a signal to noise issue;
  - Need to discriminate $(N-1)/N$
    - threshold is like $(1/N)$
  - This gets very hard as N gets large
  - Practical limit 5-8 mers
  - But when 2mers are issues

Paired End Sequencing

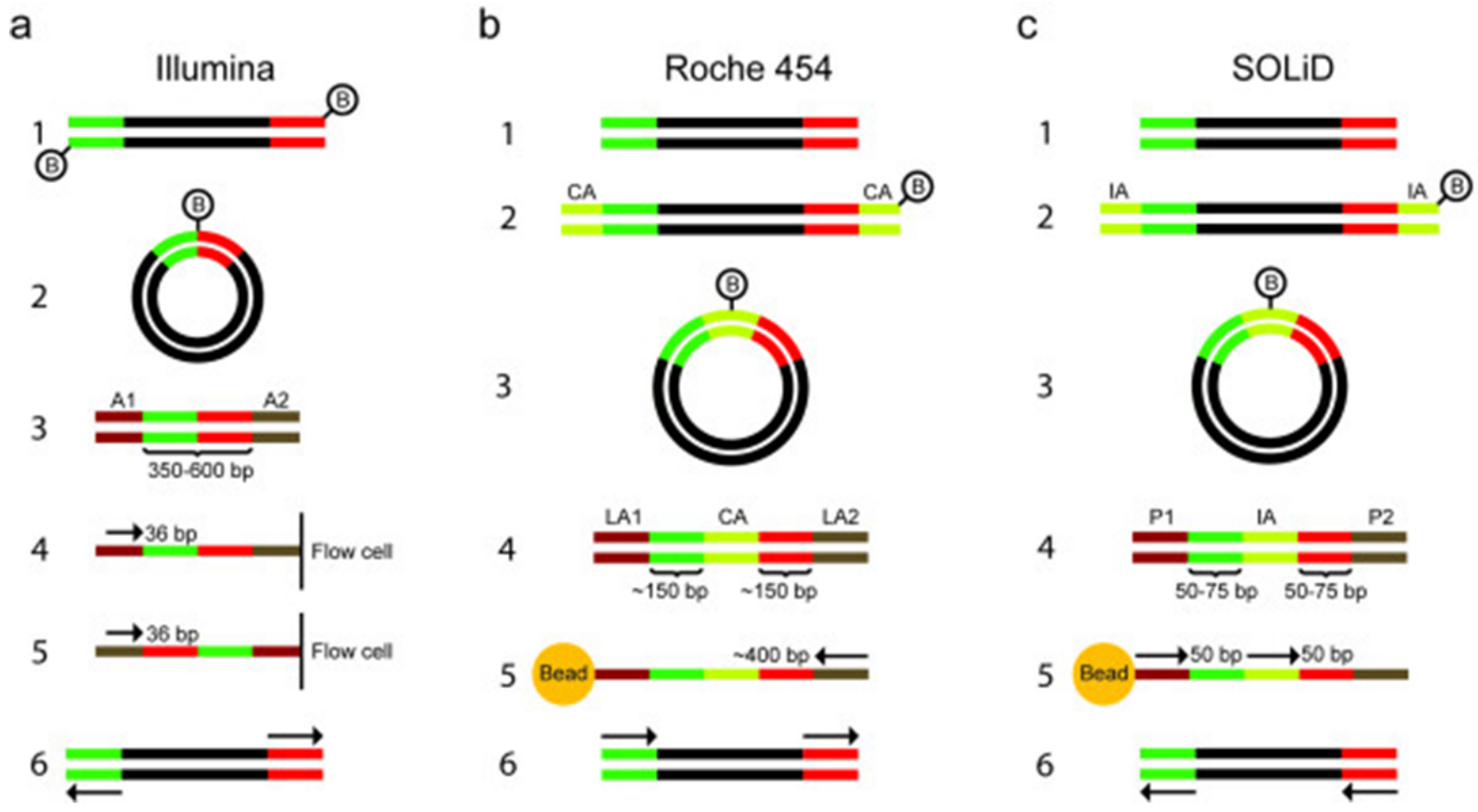


Figure 9: Various Paired End (Mate Pair) formats

Paired End Sequencing, II

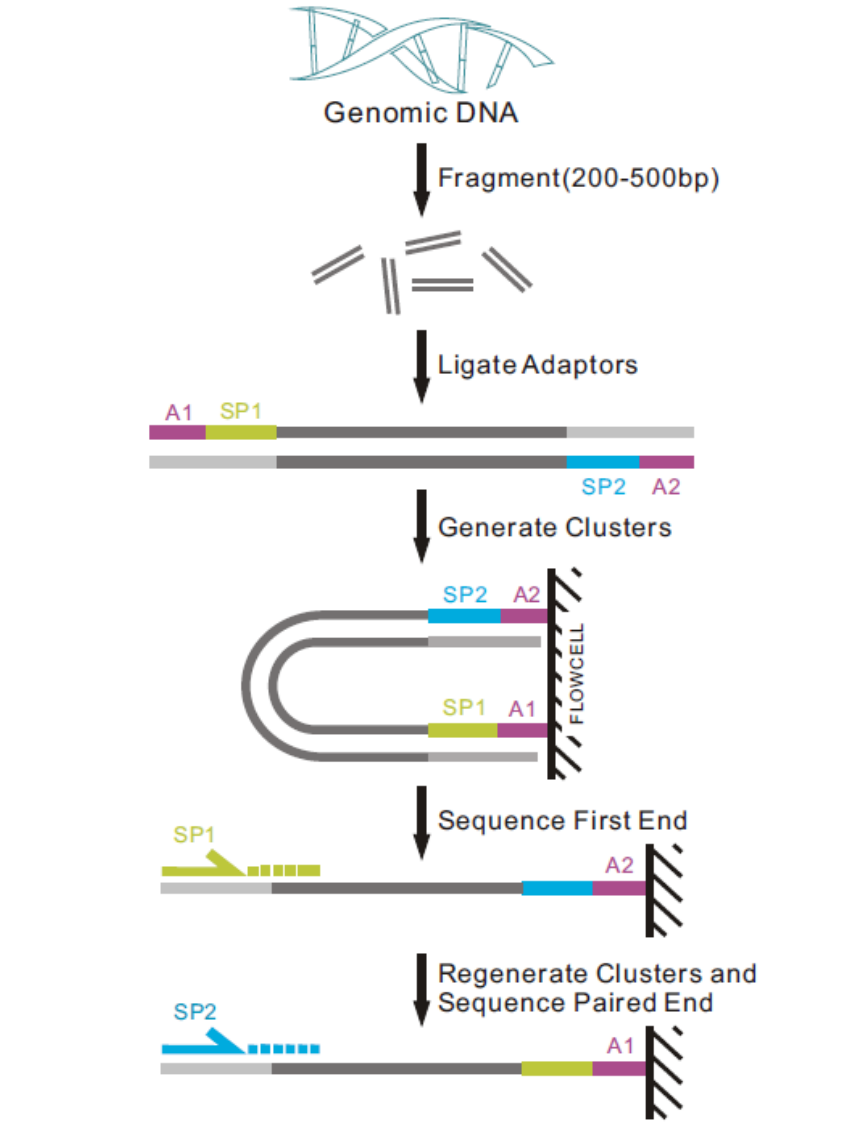


Figure 1-2-1 Pipeline of paired-end sequencing (www.illumina.com)

Figure 10:

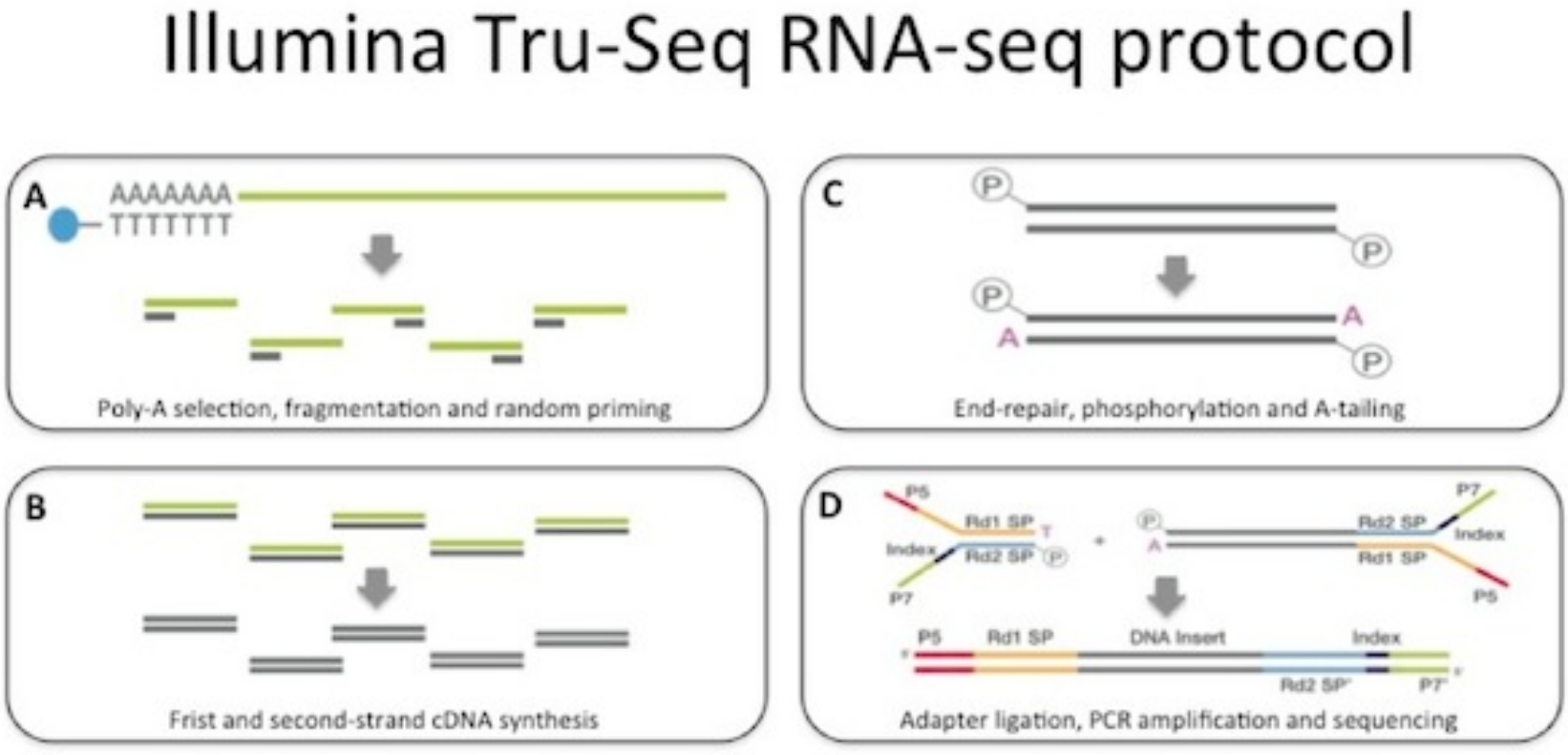
Applications of NGS

- RNAseq
- ChIPseq
- other not discussed
  - Whole Exome (targeted) sequencing (WES)
  - Whole Genome Sequencing (WGS)
  - BiSulfite
  - Target PCR based

RNAseq library types (for ChIPSeq guys)

- From a bioinformatics view you need to know (you really do)
  - Poly-A unstranded (Illumina TruSeq Poly-A Selection)
    - Unstranded
  - SMARTer Amplification
    - Strand Forward, FIRST_READ_TRANSCRIPTION_STRAND
  - KAPA mRNA Stranded
    - Strand Reverse, SECOND_READ_TRANSCRIPTION_STRAND
  - Ribo-minus (Illumina TruSeq RiboDeplete)
    - Strand Reverse, SECOND_READ_TRANSCRIPTION_STRAND

Illumina True Seq RNAseq



Library prep begins from 100ng-1ug of Total RNA which is poly-A selected (A) with magnetic beads. Double-stranded cDNA (B) is phosphorylated and A-tailed (C) ready for adapter ligation. The library is PCR amplified (D) ready for clustering and sequencing.

Figure 11:

Two different ChIP libraries

- From a bioformatics view you know
  - Focal Binding ChIP: le protein binding is strongly localized
    - Transcription Factors
  - Diffuse Binding ChIP: binding is weak-localized
    - Histone (chromotin) or Methyl binding factors
- MACS calls there model and non-model cases

ChIPseq library prep (for RNAseq guys)

- Cross-link DNA and proteins
- Isolate DNA & fragmentation
- Chromatin Immunoprecipitation
- Reverse cross-links and purify DNA
- Add adapters & sequence

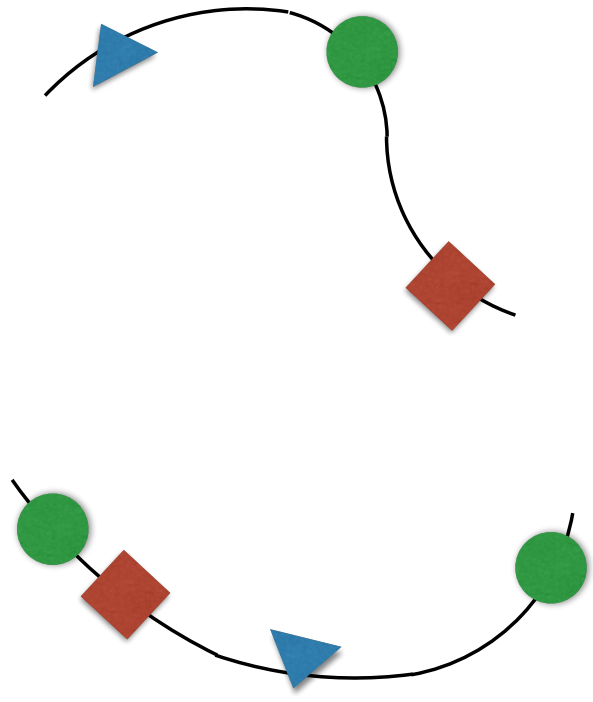


Figure 12:

ChIPseq library prep

- Cross-link DNA and proteins
- Isolate DNA & fragmentation
- Chromatin Immunoprecipitation
- Reverse cross-links and purify DNA
- Add adapters & sequence

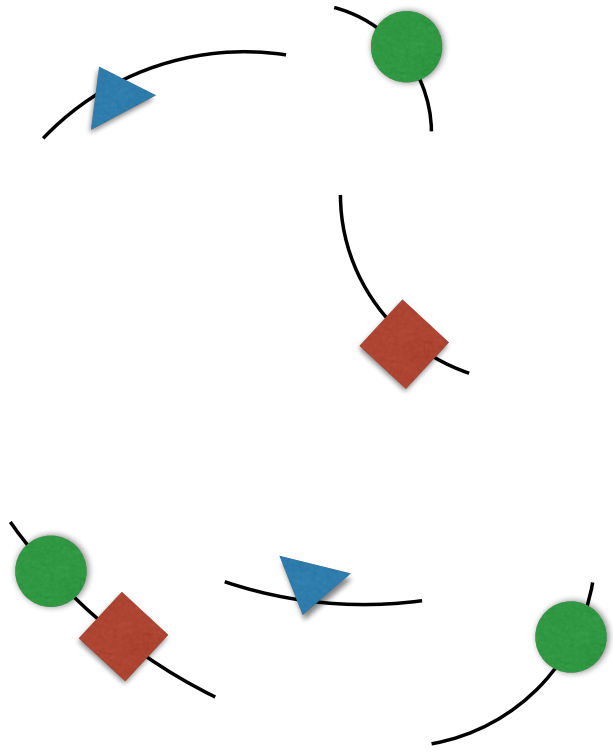


Figure 13:

ChIPseq library prep

- Cross-link DNA and proteins
- Isolate DNA & fragmentation
- Chromatin Immunoprecipitation
- Reverse cross-links and purify DNA
- Add adapters & sequence

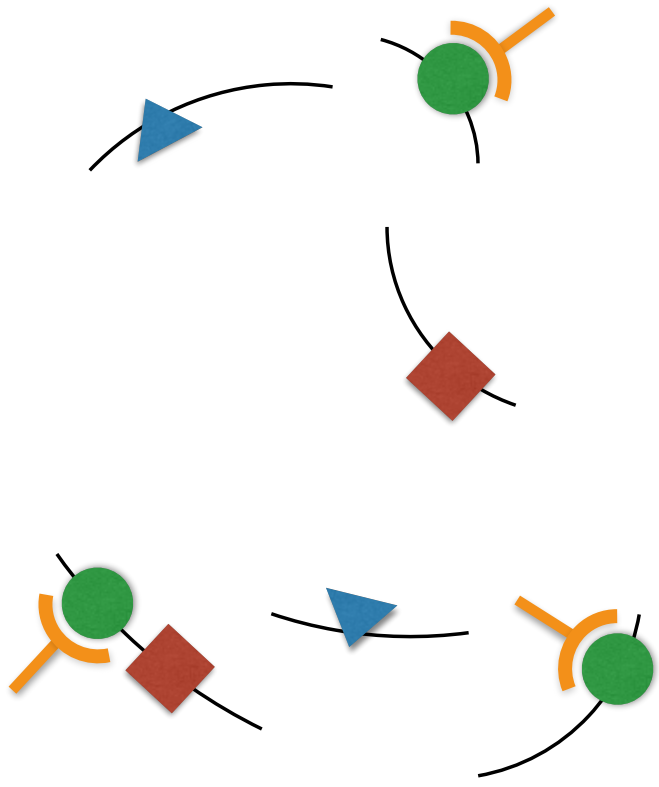
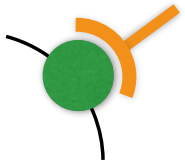
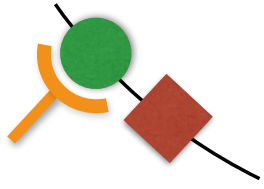


Figure 14:

ChIPseq library prep

- Cross-link DNA and proteins
- Isolate DNA & fragmentation
- Chromatin Immunoprecipitation
- Reverse cross-links and purify DNA
- Add adapters & sequence





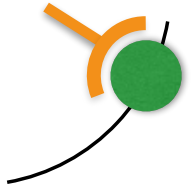


Figure 15:

ChIPseq library prep

- Cross-link DNA and proteins
- Isolate DNA & fragmentation
- Chromatin Immunoprecipitation
- Reverse cross-links and purify DNA
- Add adapters & sequence

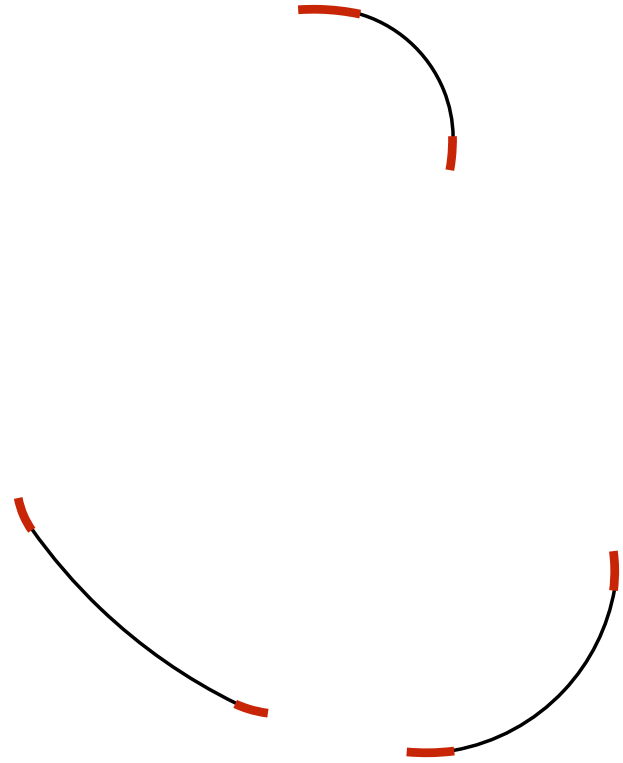


Figure 16:

Sequencing data file formats: FASTA/FASTQ

Original format: FASTA

- For both xNA (nucleotides) and AA (proteins)
- Basic structure:

```
>gi|31563518|ref|NP_852610.1| microtubule-associated
MKMRFFSSPCGKAAVDPADRCKEVQQIRDQHPSKIPVIIERYKGEKQ
LPVLDKTKFLVPDHVNMSELVKIIRRRLQLNPTQAFFLLVNQHSMVS
VSTPIADIYEQEKDEDGFLYMVYASQETFGFIRENE
```

FASTA, cont.

- Can encode multiple sequences

```
>SEQUENCE_1
MTEITAAMVKELRESTGAGMMDCKNALSETNGDFDKAVQLLREKGL
LVSVKVSDDFTIAAMRPSYLSYEDLDMTFVENEYKALVAELEKENE
IPQFASRKQLSDAILKEAEEKIKEELKAQGKPEKIWDNIIPGKMNS
MGQFYVMDDKKTVEQVIAEKEKEFGGKIKIVEFICFEVGEGLEKKT
>SEQUENCE_2
SATVSEINSETDFVAKNDQFIALTKDTTAHIQSNSLQSVEELHSST
ATIGENLVVRRFATLKAGANGVVNGYIHTNGRVGVVIAAACDSAEV
```

Extension to store quality of reads: FASTQ

- Change delimiter and add an additional line of quality information

```
@SEQ_ID
GATTTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTT
+
!''*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>
```

- the 4th line encodes the Quality value (Q) for each base

Q value / PHRED scale

- The q value is defined to be

$$Q = -10 \log_{10}(P_{err})$$

where P_{err} is the probability the base is *incorrect*

Q	Perr	Nerr
10	0.1	1 in 10
20	0.01	1 in 100
30	0.001	1 in 1,000
40	0.0001	1 in 10,000

Q encoding

- The Q value has over time been encoded in different ways

```
 SSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS.....................................................
 .........................XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....................
 ...............................IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII.....................
 ...................................JJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJ.....................
 LLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLLL.....................................................
 !"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
 |                          |    |        |                              |                     |
33                          59   64       73                             104                   126
 0.........................26...31.......40
                            -5....0........9.............................40
                                  0........9.............................40
                                     3.....9.............................40
 0.2.......................26...31........41

S - Sanger        Phred+33,  raw reads typically (0, 40)
X - Solexa        Solexa+64, raw reads typically (-5, 40)
I - Illumina 1.3+ Phred+64,  raw reads typically (0, 40)
J - Illumina 1.5+ Phred+64,  raw reads typically (3, 40)
    with 0=unused, 1=unused, 2=Read Segment Quality Control Indicator (bold)
    (Note: See discussion above).
L - Illumina 1.8+ Phred+33,  raw reads typically (0, 41)
```

Figure 17:

Q encoding

- ord(c)-33 ==> Q / ord is the ascii value for a character
- chr(Q+33) ==> Character

Quality Control (Manipulating FASTA files)

- FastQC toolkit: (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/)
- Show Samples

Unix Crash Course

History

- First shell, Ken Thompson, *1971* (44yr)
- First (?) UNIX shell, Bourne Shell [SH], Stephan Bourne, 1977
- C-Shell [TCSH], Bill Joy, 1978
- People (especially scientist) have been using some from of a shell to talk to computers for longer that most of people in this room were alive.
- Probably will still be using it after we are gone.
- Might be good idea to learn it (before SKYNET takes over)

Where to start

- Most Commonly Used Commands

fgrep	10.81%	**cd**	10.11%	**ls**	8.06%
more	7.77%	**cat**	6.39%	**rm**	3.53%
find	3.23%	xargs	2.69%	cut	2.67%
egrep	2.41%	mkdir	1.86%	sort	1.76%
git	1.66%	awk	1.66%	wc	1.58%
head	1.32%	**mv**	1.26%	bjobs	1.23%
sed	1.22%	*uniq*	1.19%	history	1.00%
vi	0.97%	**pwd**	0.90%	cp	0.90%
tr	0.86%	perl	0.77%	du	0.75%
samtools	0.68%	listCols	0.56%	zcat	0.56%
hg	0.54%	parseLSFLogs.py	0.49%	tee	0.48%
chmod	0.45%	rsync	0.43%	*ln*	0.43%
sudo	0.43%	diff	0.42%	bedtools	0.38%

Unix I/O conventions

- files / directories
- commands
- I/O redirection, pipes

Basic unix commands:

file / directory

- ls, cd, pwd, cat (more/less), rm, mv, mkdir, rmdir
- wild cards / glob patterns

Home directory:

```
# Go home
cd

# Show home direcotry
cd
pwd

# better (leaves you in where you are)

echo $HOME
```

Important intermediate commands

`history`

- list and rerun commands
- `!!` usually replaced by up-arrow (^p)
- history editting replaced with cut-and-paste

Important intermediate commands

`man`

- make sure to go over `man`
  - `man -k` == `apropos`

Important intermediate commands

`locate`

- make sure to explain caveat that database is **not** updated continuously (usually everyday)
- typically configure to not index user space
  - **NOT** like spotlight
- Mostly useful for system stuff
- works best with `grep`

Important intermediate commands

`fgrep`, `egrep`, `grep`

- `fgrep` == fast grep (`grep -F`)
- `egrep` == extended grep (`grep -E`)
  - regular expression crash course
  - like wild cards but different syntax

From man page:

> *Direct invocation as either `egrep` or `fgrep` is deprecated, but is provided to allow historical applications that rely on them to run unmodified.*

i.e., for old people

Unix cautions

- `mv` semantics can be deceptive

```
mv file1 file2
```

This renames `file1` to file `file2` but if `file2` exists it also deletes `file2`

- many people uses alias to redefine defaults to something more forgiving.

For beginners strongly suggest

```
alias mv="mv -n"
```

Probably should also do `alias rm="rm -i"`, but gets tedious pretty quickly

Parting thought

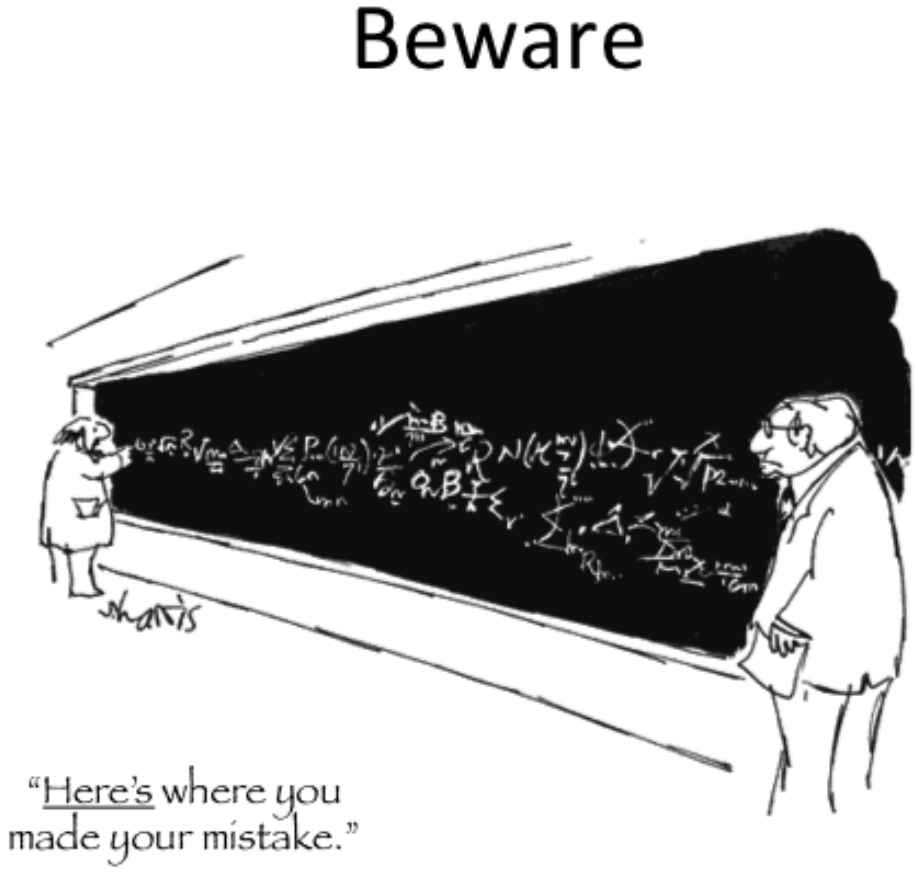


Figure 18: