Bioconductor for Sequence Analysis

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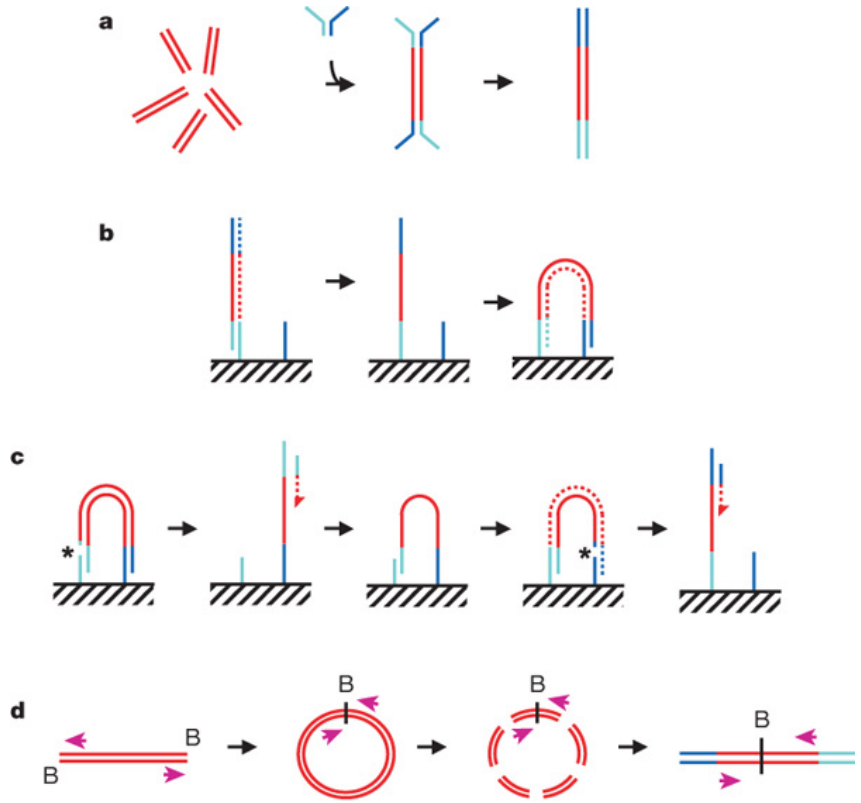
Sequencing: Work flows

1. Experimental design
2. 'Wet lab' sample prep
3. Sequencing
   - 100's of millions of reads
   - 30-150 nucleotides
   - Single and paired-end
   - Bar codes, lanes & flow cells
4. Alignment
5. Analysis: DNA, RNA, epigenetics, integrative, microbiome, ...



Bentley et al., 2008, Nature 456: 53-9

Experimental design and wet lab

- RNA-seq
  - Known gene / transcript diffential expression
  - Novel transcript discovery
  - Single- versus paired-end
- ChIP-seq
- Variants
  - Germline vs. somatic
  - Exome vs. whole genome
  - SNP vs. indel vs. structural
- Copy number
  - Low vs. high coverage

RNA-seq: single versus paired end

- Most analysis now paired-end
- Reads within exons
- A single end spanning exons: ‘junction reads’
- Reads spanning exons

```
@ERR127302.1703 HWI-EAS350_0441:1:1:1460:19184#0/1
CCTGAGTGAAGCTGATCTTGATCTACGAAGAGAGATAGATCTTGATCGTCGAGGAGATGCTGACCTTGACCT
+
HHGHHGHHHHHHHHDGG<GDGGE@GDGGD<?B8??ADAD<BE@EE8EGDGA3CB85*,77@>>CE?=896=:
@ERR127302.1704 HWI-EAS350_0441:1:1:1460:16861#0/1
GCGGTATGCTGGAAGGTGCTCGAATGGAGAGCGCCAGCGCCCCGGCGCTGAGCCGCAGCCTCAGGTCCGCCC
+
DE?DD>ED4>EEE>DE8EEEDE8B?EB<@3;BA79?,881B?@73;1?##########################
@ERR127302.1705 HWI-EAS350_0441:1:1:1460:13054#0/1
AAAACACCCTGCAATCTTTCAGACAGGATGTTGACAATGCGTCTCTGGCACGTCTTGACCTTGAACGCAAAG
+
EEDEE>AD>BBGGB8E8EEEGBGGGGBGGGGG3G>E3*?BE??BBC8GB8??:??GGDGDDD>D>B<GDDC8
@ERR127302.1706 HWI-EAS350_0441:1:1:1460:14924#0/1
CACCCAGTGGGGTGGAGTCGGAGCCACTGGTCCTGCTGCTGGCTGCCTCTCTGCTCCACCTTGTGACCCAGG
+
HHHHHGEEGEEADDGDBG>GGD8EG,<6<?AGGADFEHHC@>D@<@G@>AB@B?8AA>CE@D8@B=?CC>AG
@ERR127302.1707 HWI-EAS350_0441:1:1:1461:6983#0/1
CGACGCTGACACCGGAACGGCAGCAGCAGCAGGACGATTAAGACAAGGAGGATGGCTCCACAGACGCTCATG
+
GEEGEGE@GGGGGGEGGGGGBB>G3?33?8*;;79?<9@?DD8@DDEE888;-BB?.A##############
@ERR127302.1708 HWI-EAS350_0441:1:1:1461:10827#0/1
AAAGAAGGTCCTTGCAATAGACTGCCTCTGCTTGAGAACTTATGATGTAATTATTGCATGCTGCTAATATAC
+
GGGGGDDEBFGGGGGBE,DAGDDGGGEEEG<EEFDECFFEEEDE@<>ACEBEFDEEFE<EDC@E<EECCBEB
@ERR127302.1709 HWI-EAS350_0441:1:1:1461:7837#0/1
CAGCCACAGAACCACGGCACGGAAGACATGAGGCAGCATGCTCACGAGAGAGGTGAGGGTCTCCCCTCCAGG
+
HHGHHHH>DH:@.7@49;88G8>G>DDG@D>D@G@GE>@DDBDDG<A82?#######################
```

FASTQ files

`@ERR127302.1709 HWI-EAS350\_0441:1:1:1461:7837\#0/1`

- (Anntoation), machine, flow cell, tile, coordinates, end

`CAGCCACAGAACCACGGCACGGAAGACATGAGGCAGCATGCTCACGAGA...`

- DNA sequence, usually A, C, T, G, N (uncalled)

`HHGHHHH>DH:@.7@49;88G8>G>DDG@D>D@G@GE>@DDBDDG<A82...`

- Quality (approximately, $-\log_{10}(p)$) encoded as ASCII characters: 40 is approximately $p = 0.0001$, 30 is $p = 0.001$, etc.
- Different encodings – wikipedia
- Higher in the alphabet is better

```
##  !  "  #  $  %  &  '  (  )  *  +  ,  -  .  /  0  1
##  0  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16
##  2  3  4  5  6  7  8  9  :  ;  <  =  >  ?  @  A  B
## 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
##  C  D  E  F  G  H  I
## 34 35 36 37 38 39 40
```

Quality assessment

Assessment, e.g., fastqc

- Read length, duplication
- Nucleotide use: per cycle, GC content, N content, ...
- Quality: per cycle, per read
- Consistency across samples, without obvious treatment-specific associations

Remediation, e.g., trimmomatic

- Crop e.g., leading / tailing artifacts of sequencing protocol
- Trim based on quality

Alignment

Bowtie / Tophat / Cufflinks

- Bowtie2 – alignment
- Tophat – splice junction mapper
- Cufflinks / *cummeRbund* – isoform assembly & quantification

Other aligners

- SNAP – fast and accurate
- subread / *Rsubread* – memory efficient
- GSNAP / GMAP / *gmapR* – flexible and high quality alignments

BAM files

- Visualization with, e.g., IGV
- SAM / BAM (and other) specifications

```
ERR127302.25553011  403 chr14  19413639  1  72M
  =       19413589        -122
  CAAAGAATTGATTGAATTCATCAGGGCTAAAATCTCCAAAAATATACTGCGG...
  !#&&"%&$&%%&&%&"%***&'(')')")')%('#+++++++)'&%+*+*++...
  AS:i:-2 XN:i:0  XM:i:1  XO:i:0  XG:i:0  NM:i:1
  MD:Z:7C64 YT:Z:UU NH:i:3  CC:Z:=  CP:i:20145991
  HI:i:0
```

Field	Name	Value
1	QNAME	Query (read) NAME
2	FLAG	Bitwise FLAG, e.g., strand of alignment
3	RNAME	Reference sequence NAME
4	POS	1-based leftmost POSition of sequence
5	MAPQ	MAPping Quality (Phred-scaled)
6	CIGAR	Extended CIGAR string
7	MRNM	Mate Reference sequence NaMe
8	MPOS	1-based Mate POSition
9	ISIZE	Inferred insert SIZE
10	SEQ	Query SEQuence on the reference strand
11	QUAL	Query QUALity
12+	OPT	OPTional fields, format TAG:VTYPE:VALUE