Introduction to RNA-Seq Analysis

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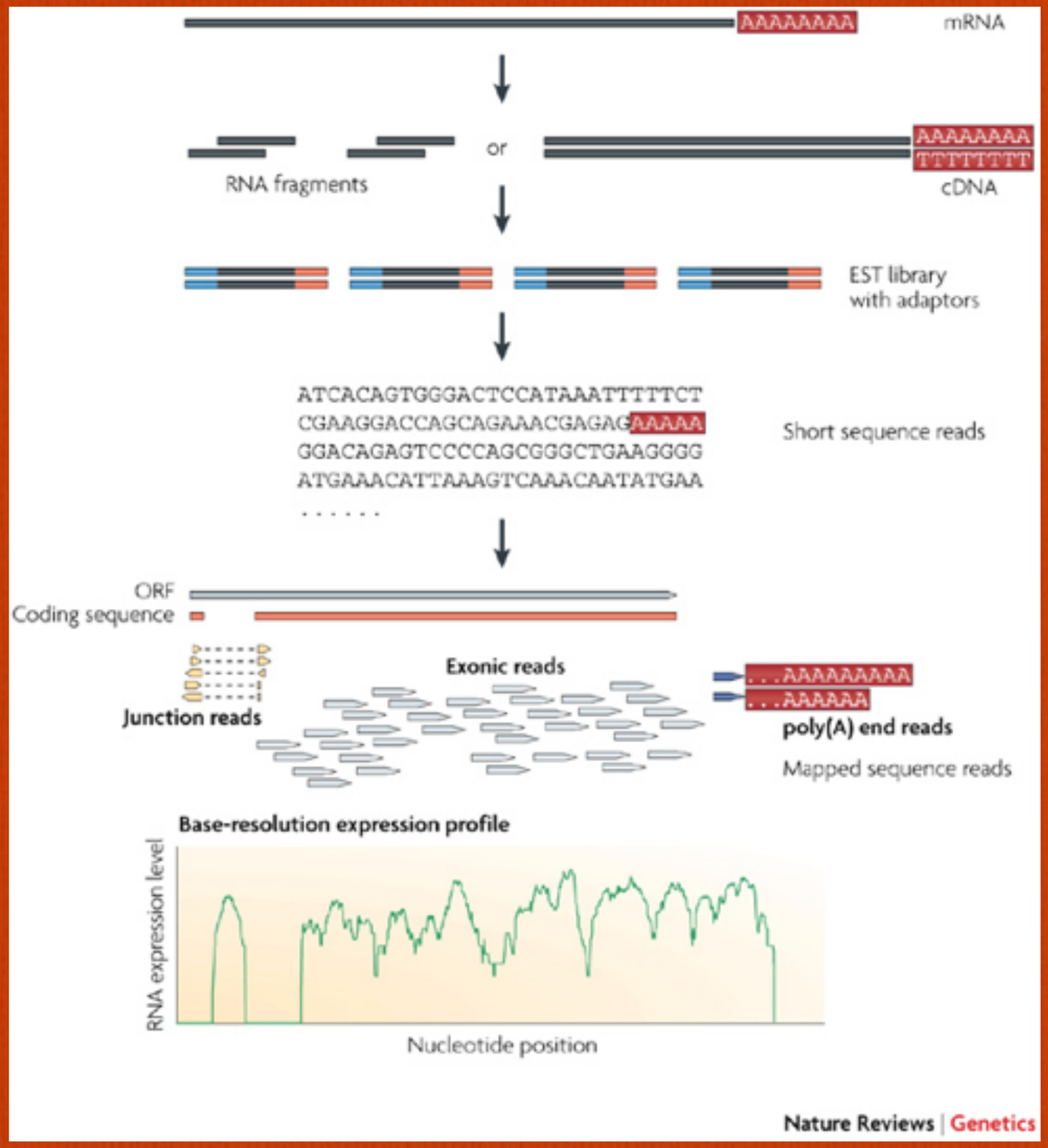
RNA-Seq


AAAAAAAA
mRNA
or
RNA fragments
AAAAAAAA
TTTTTTTT
cDNA
EST library
with adaptors
ATCACAGTGGGACTCCATAAATTTTTCT
CGAAGGACCAGCAGAAACGAGAGAAAAA
GGACAGAGTCCCCAGCGGGCTGAAGGGG
ATGAAACATTAAAGTCAAACAATATGAA
. . . . . .
Short sequence reads
ORF
Coding sequence
Exonic reads
. . .AAAAAAAAA
. . .AAAAAA
Junction reads
poly(A) end reads
Mapped sequence reads
Base-resolution expression profile
RNA expression level
Nucleotide position
Nature Reviews | Genetics

RNA-Seq

2cells_1.fastq

https://www.ebi.ac.uk/training/online/course/ebi-next-generation-sequencing-practical-course/rna-sequencing/rna-seq-analysis-transcriptome

```
@ERR022484.110 IL3_4946:5:1:15692:1051/1
CNGAAGCAAAGTGTGTGCGCGAAATGCTCGTCAGGAAGATCCAAGAGGAGTCGCTGCGCACTTACCTTTTCACCTA
+
A!AA=CCCDBHAG=HBHHEDGCFFADHADD>D>D@>@==>BA<=>:?=;>9<><8:::879988:877:9;<9;95
@ERR022484.142 IL3_4946:5:1:2246:1064/1
GTTTCTTTCTGCTTATTAAACATGTTGGCTTTGTCCTCCAGCTCCTTCTGTTTCTCCTTCCACATCTGGATTTCTT
+
BFFFFFFEFFFFFFFFF=E=@=EECBEFEAFEFDA?D<A=?B<;=A=<:;:==.<567;6%*.%/2511*344%0:
@ERR022484.297 IL3_4946:5:1:3383:1074/1
CAGACTTCATTTCATGTTTCATCCCAGCTCCATTGGGTTTGTCCTTTTGCCATTTCGATATGGCAGCAAAGATCCC
+
HHGGHHHHGHHHHEHGHHHHFGHHHDGDCFE=CEEEE;EEEB>=EDECA=;<AA?:=5;9?;;6:<:7782*<44:
@ERR022484.359 IL3_4946:5:1:10826:1077/1
TGAGAAGTTTTCCAGTGCCATTGGCTTGGTGTTGTTGGTCGAAGCCATTGCCGTGTTGGTGGGACCACCCGGAGCG
+
HGHHGCHDHHHHH@G?GGCCEGGED?@EE9@<B@==?=;;><99<:7;::::79;78994>71+7+22,/*6)20$
@ERR022484.374 IL3_4946:5:1:11790:1074/1
CTTTTCTCTTGGGGCTGTTTCCTCAGACATCAACTCTAGGTCAGAATCAGACTCTCCCTCATCAGAAGACCATGGG
+
HHHHHHHGGHGGGGHGGHHHHHEGCHGEFGCDBCCDGABE<AAB?@ECAB@D@=B@?A=@;==:=;:;99;99<9:
@ERR022484.391 IL3_4946:5:1:13841:1071/1
CCTTCCTCAAGGGTCTGAAAAAGGAATCTTGTCCAGGTTGTCCTCATCGCTGGCAGGCCAGAGAGTGAAAGACCTA
+
HHHHGHHHAHHHG=HCHHHHFGD@DGGGGGFCGEG@C<DB@CCCA=C=>@<=7<<<:=<9<893:7:)89:4788;
@ERR022484.399 IL3_4946:5:1:14919:1082/1
GCTGCAGCTGTGATTTTGGATCGTTCCAATCCTGGTTCAAGATGAACTCCTTGAGTCGTGGGAAGAAGCAAACGTT
+
EHHHHHHHHHHHHGHHHGGGEGGHGHFGHFGGGHHH@DDC@D=AD@>@BA?EDA<?;?B=?@=39?:9<:;6<:=:9
@ERR022484.417 IL3_4946:5:1:18221:1081/1
2cells_1.fastq
```

RNA-Seq

Main RNA-Seq Analysis Problems

- ☐ Reads Mapping
- ☐ Differential expressed gene calling
- ☐ Transcript expression profiling
- ☐ Isoform inference

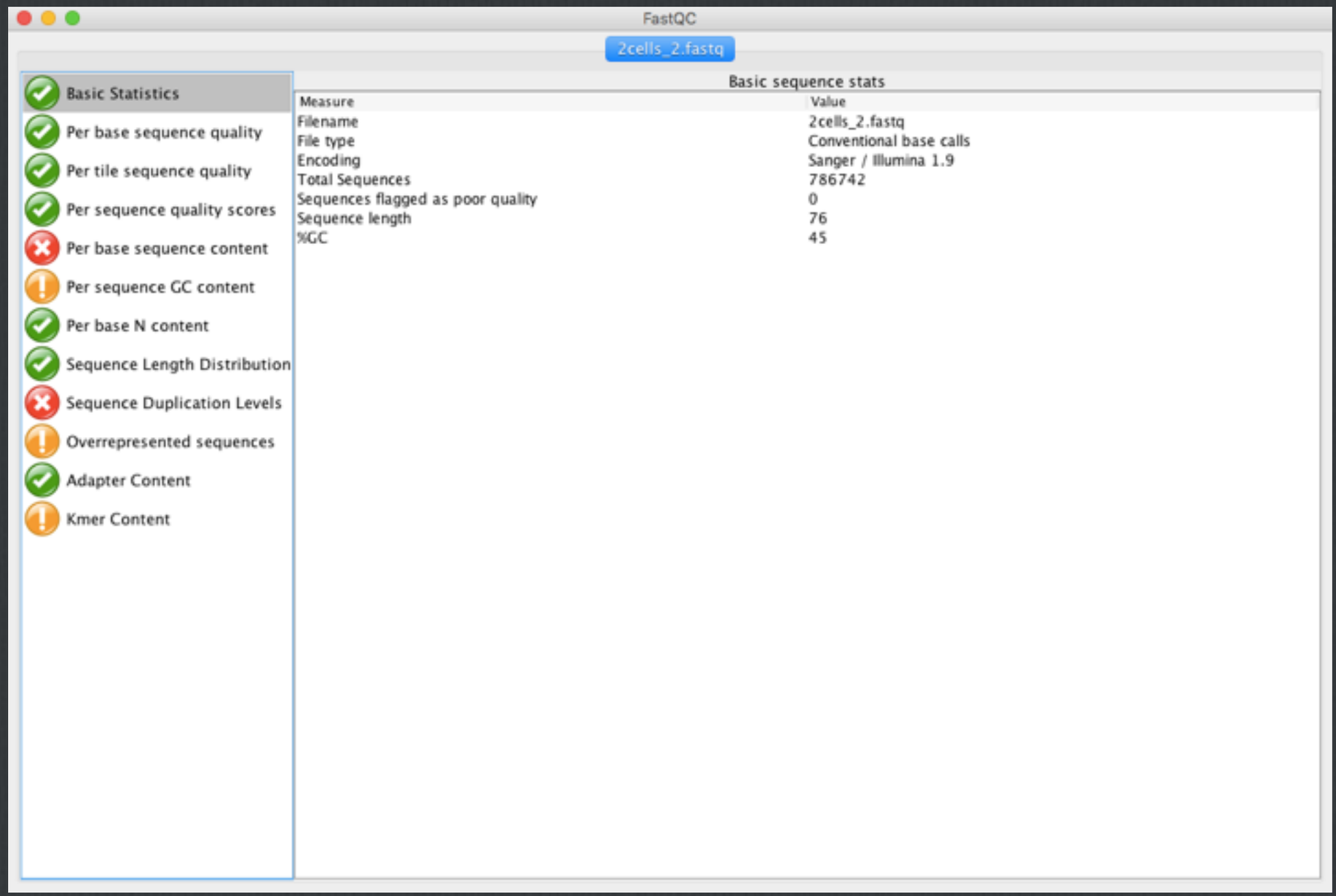
“Before starting analyze,

know your data first”

–Yiying Lang

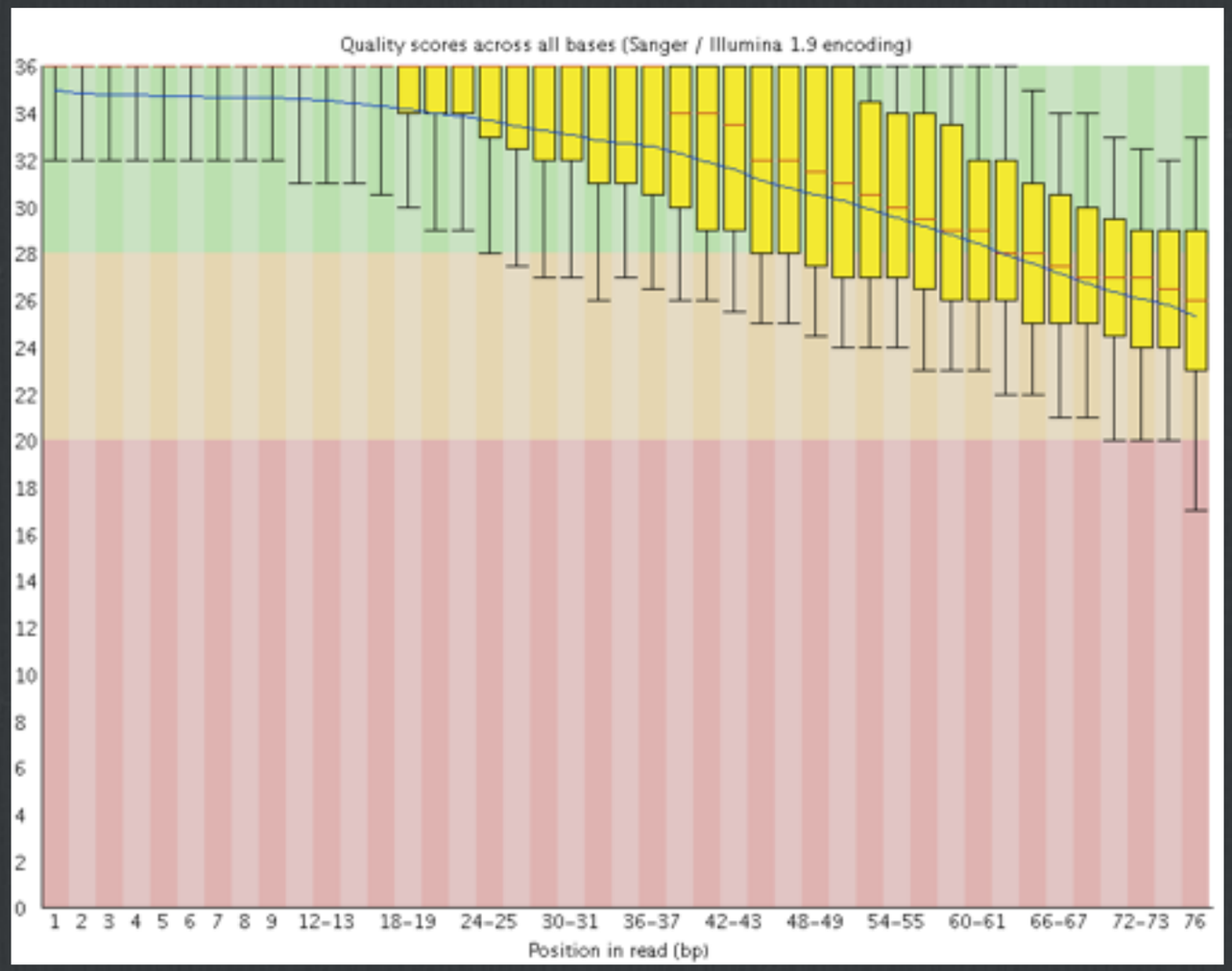
Quality Control —- FastQC

http://www.bioinformatics.babraham.ac.uk/projects/download.html#fastqc



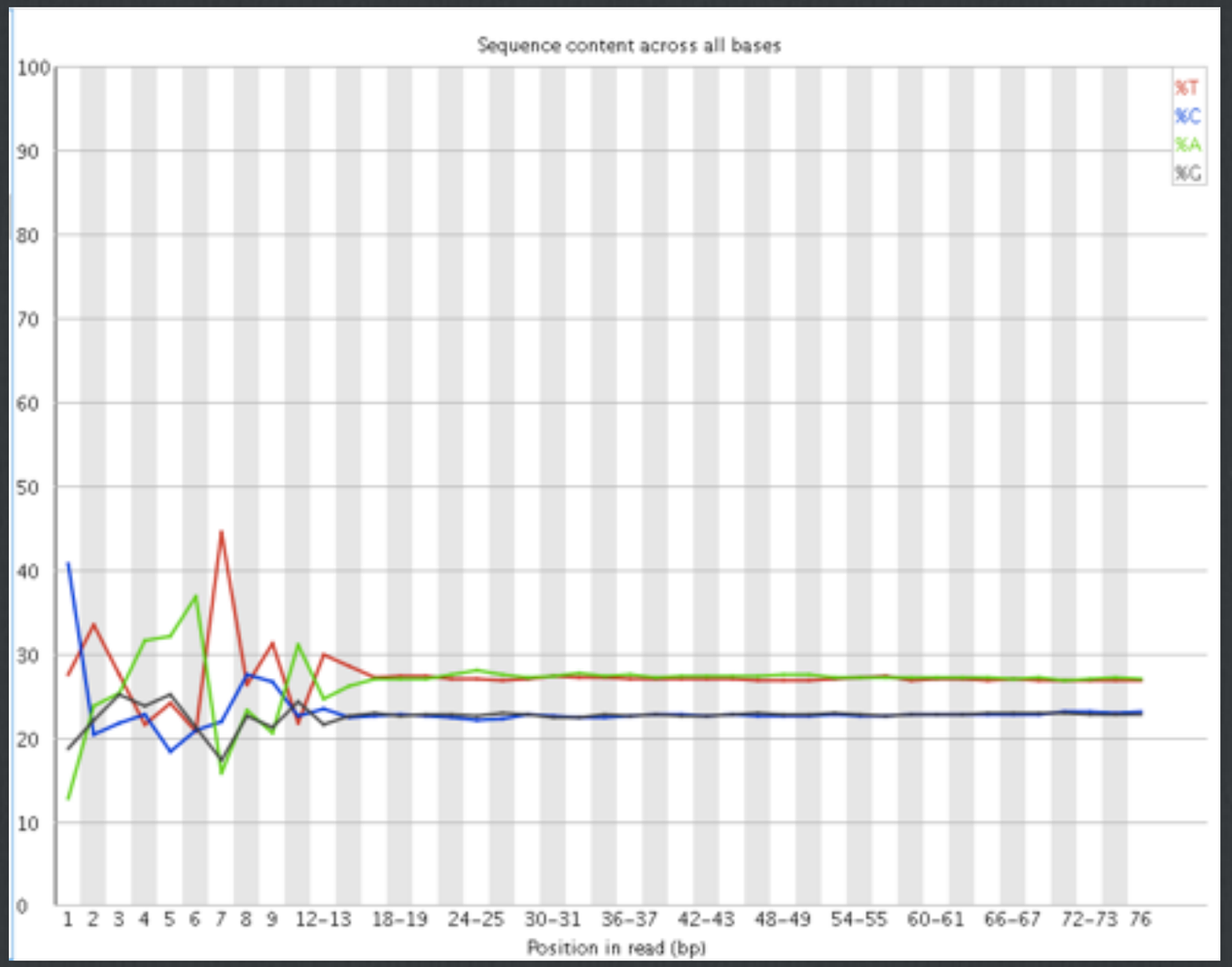
Quality Control —- FastQC

- ☐ Per Base Sequence Quality
- ☐ Per Base Sequence Count
- ☐ Per Base N Content
- ☐ Duplicate Sequence



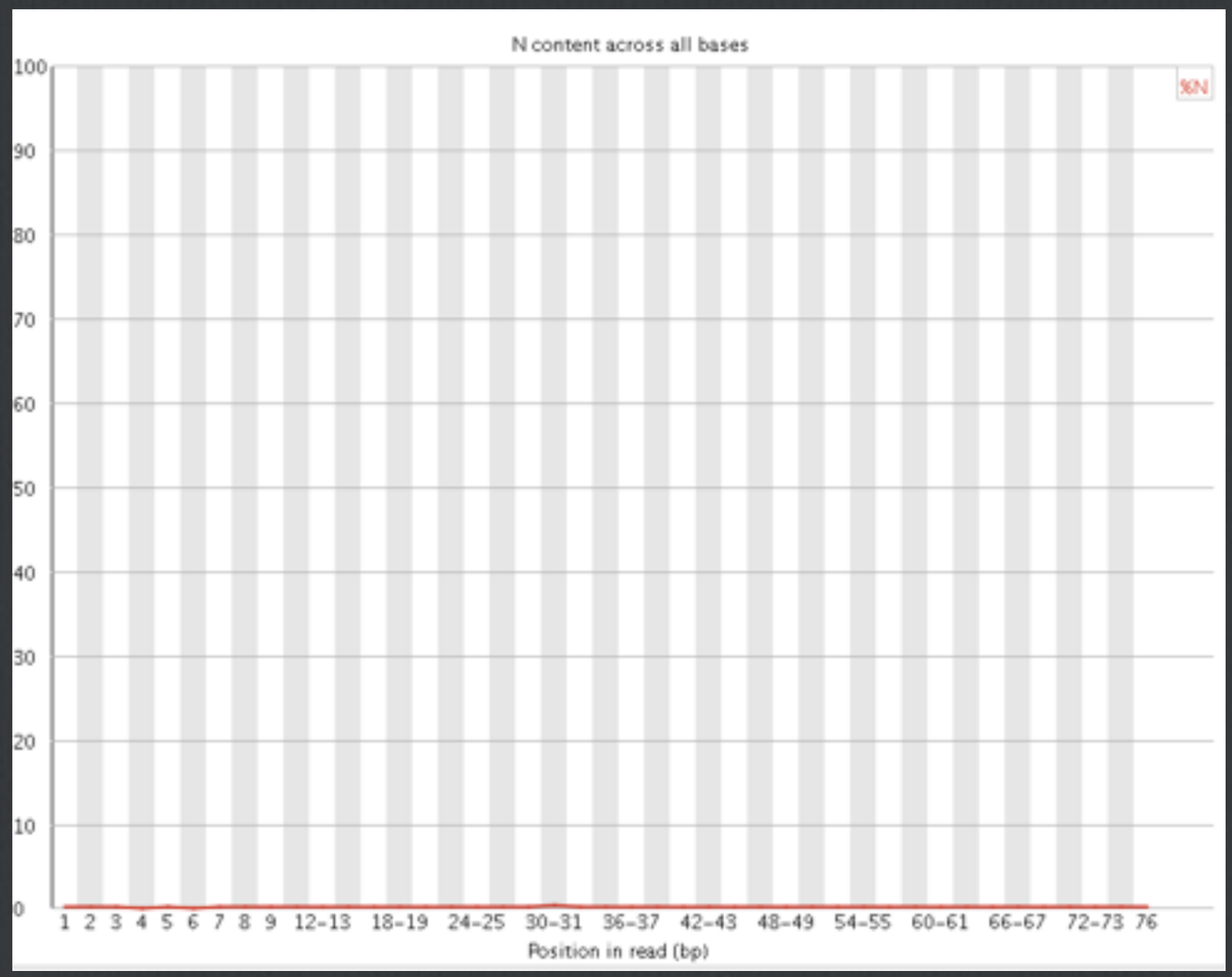
Quality Control —- FastQC

- ☐ Per Base Sequence Quality
- ☐ Per Base Sequence Count
- ☐ Per Base N Content
- ☐ Duplicate Sequence



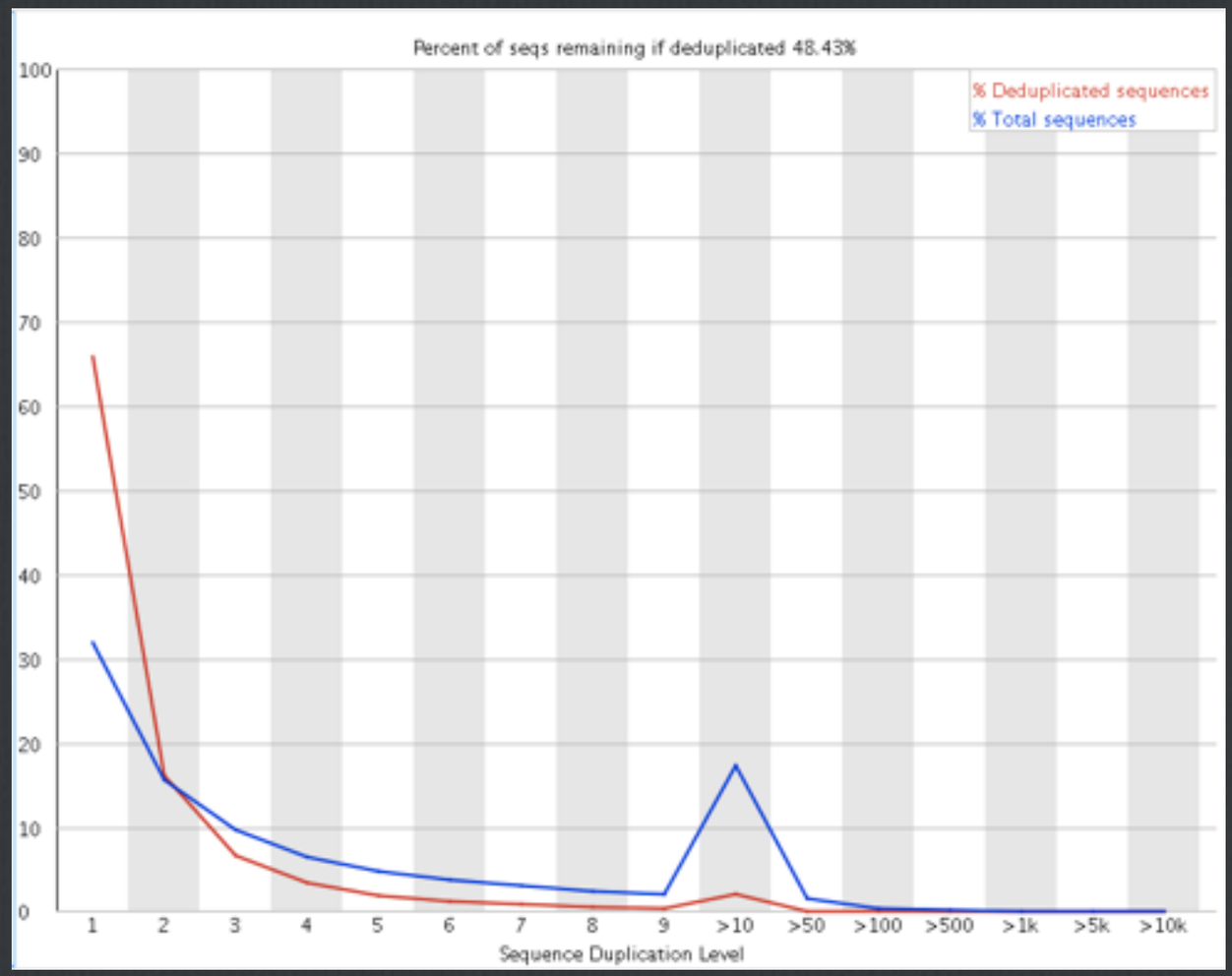
Quality Control —- FastQC

- ☐ Per Base Sequence Quality
- ☐ Per Base Sequence Count
- ☐ Per Base N Content
- ☐ Duplicate Sequence



Quality Control —- FastQC

- ☐ Per Base Sequence Quality
- ☐ Per Base Sequence Count
- ☐ Per Base N Content
- ☐ Duplicate Sequence



Sequence Depth and Coverage

$$\text{Sequence Depth} = \frac{\text{total number of bases generated}}{\text{size of genome}}$$

$$= \frac{(\text{number of reads}) \times (\text{average read length})}{\text{size of genome}}$$

Sequence Depth = 5X redundancy means on average each base has been read by 5 sequences .

GOOD: 10~15X

Bowtie, TopHat

- mapping reads to genome
- TopHat uses Bowtie
- Bowtie: DNA-Seq (ChIP-seq …)
- TopHat: RNA-Seq —— Alternative Splicing
- Bowtie1 reads length <= 50bp
- Bowtie2 reads length >= 50bp

The data we use

In the .zip file: 6 folders and 1 pdf

cuffdiff, cufflinks,tophat are the folders of result.

What do we use to generate these 3 results?

data

```
langyiying@LangYY ~/Desktop/RNA-seq/data ls
2cells_1.fastq*  2cells_2.fastq*  6h_1.fastq*  6h_2.fastq*
```

genome

```
langyiying@LangYY ~/Desktop/RNA-seq/genome ls -l
total 111496
-rwxrwxrwx 1 langyiying staff 51542283 Sep 22  2012 Danio_rerio.Zv9.66.dna.fa*
```

annotation

```
langyiying@LangYY ~/Desktop/RNA-seq/annotation ls
Danio_rerio.Zv9.66.gtf*  Danio_rerio.Zv9.66.spliceSites*
```

TopHat

build index

bowtie2-build Danio_rerio.Zv9.66.dna.fa ../test/index/ZV9

```
Wrote 12640132 bytes to secondary EBWT file: ../test/ZV9.rev.2.bt2
Re-opening _in1 and _in2 as input streams
Returning from Ebwt constructor
Headers:
    len: 50560508
    bwtLen: 50560509
    sz: 12640127
    bwtSz: 12640128
    lineRate: 6
    offRate: 4
    offMask: 0xfffffff0
    ftabChars: 10
    eftabLen: 20
    eftabSz: 80
    ftabLen: 1048577
    ftabSz: 4194308
    offsLen: 3160032
    offsSz: 12640128
    lineSz: 64
    sideSz: 64
    sideBwtSz: 48
    sideBwtLen: 192
    numSides: 263336
    numLines: 263336
    ebwtTotLen: 16853504
    ebwtTotSz: 16853504
    color: 0
    reverse: 1
Total time for backward call to driver() for mirror index: 00:00:49
```

```
langyiying@LangYY ~/Desktop/RNA-seq/test/index ls
ZV9.1.bt2  ZV9.2.bt2  ZV9.3.bt2  ZV9.4.bt2  ZV9.rev.1.bt2  ZV9.rev.2.bt2
```

TopHat

run TopHat

tophat2 -o test/tophat2 test/index/ZV9 data/2cells_1.fastq data/2cells_2.fastq

```
langyiying@LangYY ~/Desktop/RNA-seq tophat2 -o test/tophat2 test/index/ZV9 data/2cells_1.fastq data/2cells_2.fastq

[2015-11-20 04:14:27] Beginning TopHat run (v2.1.0)
-----------------------------------------------
[2015-11-20 04:14:27] Checking for Bowtie
                  Bowtie version:        2.2.5.0
[2015-11-20 04:14:27] Checking for Bowtie index files (genome)..
[2015-11-20 04:14:27] Checking for reference FASTA file
        Warning: Could not find FASTA file test/index/ZV9.fa
[2015-11-20 04:14:27] Reconstituting reference FASTA file from Bowtie index
  Executing: /usr/local/bin/bowtie2-inspect test/index/ZV9 > test/tophat2/tmp/ZV9.fa
[2015-11-20 04:14:31] Generating SAM header for test/index/ZV9
[2015-11-20 04:14:31] Preparing reads
         left reads: min. length=76, max. length=76, 786595 kept reads (147 discarded)
        right reads: min. length=76, max. length=76, 783800 kept reads (2942 discarded)
[2015-11-20 04:15:06] Mapping left_kept_reads to genome ZV9 with Bowtie2
```

TopHat

run TopHat

tophat2 -o test/tophat2 test/index/ZV9 data/2cells_1.fastq data/2cells_2.fastq

output path

TopHat

run TopHat

tophat2 -o test/tophat2 test/index/ZV9 data/2cells_1.fastq data/2cells_2.fastq

bowtie index

```
langyiying@LangYY ~/Desktop/RNA-seq/test/index ls
ZV9.1.bt2  ZV9.2.bt2  ZV9.3.bt2  ZV9.4.bt2  ZV9.rev.1.bt2  ZV9.rev.2.bt2
```

TopHat

run TopHat

tophat2 -o test/tophat2 test/index/ZV9 data/2cells_1.fastq data/2cells_2.fastq

data

```
langyiying@LangYY ~/Desktop/RNA-seq/data ls
2cells_1.fastq*  2cells_2.fastq*  6h_1.fastq*  6h_2.fastq*
```

TopHat

Arguments:

`<genome_index_base>`	The basename of the genome index to be searched. The basename is the name of any of the index files up to but not including the first period. Bowtie first looks in the current directory for the index files, then looks in the `indexes` subdirectory under the directory where the currently-running `bowtie` executable is located, then looks in the directory specified in the `BOWTIE_INDEXES` (or `BOWTIE2_INDEXES`) environment variable. Please note that it is highly recommended that a FASTA file with the sequence(s) the genome being indexed be present in the same directory with the Bowtie index files and having the name `<genome_index_base>.fa`. If not present, TopHat will automatically rebuild this FASTA file from the Bowtie index files.
`<reads1_1[,...,readsN_1]>`	A comma-separated list of files containing reads in FASTQ or FASTA format. When running TopHat with paired-end reads, this should be the *_1 ("left") set of files.
`<[reads1_2,...readsN_2]>`	A comma-separated list of files containing reads in FASTA or FASTA format. Only used when running TopHat with paired end reads, and contains the *_2 ("right") set of files. The *_2 files **MUST** appear in the same order as the *_1 files.

Options:

`-h/--help`	Prints the help message and exits
`-v/--version`	Prints the TopHat version number and exits
`-N/--read-mismatches`	Final read alignments having more than these many mismatches are discarded. The default is 2.
`--read-gap-length`	Final read alignments having more than these many total length of gaps are discarded. The default is 2.
`--read-edit-dist`	Final read alignments having more than these many edit distance are discarded. The default is 2.
`--read-realign-edit-dist`	Some of the reads spanning multiple exons may be mapped incorrectly as a contiguous alignment to the genome even though the correct alignment should be a spliced one - this can happen in the presence of processed pseudogenes that are rarely (if at all) transcribed or expressed. This option can direct TopHat to re-align reads for which the edit distance of an alignment obtained in a previous mapping step is above or equal to this option value. If you set this option to `0`, TopHat will map every read in all the mapping steps (transcriptome if you provided gene annotations, genome, and finally splice variants detected by TopHat), reporting

TopHat

```
langyiying@LangYY ~/Desktop/RNA-seq/test/tophat2 ls
accepted_hits.bam  align_summary.txt  deletions.bed  insertions.bed  junctions.bed  logs/  prep_reads.info  unmapped.bam
```

accepted_hits.bam
align_summary.txt
deletions.bed
insertions.bed
junction.bed
prep_reads.info
unmapped.bam
logs/

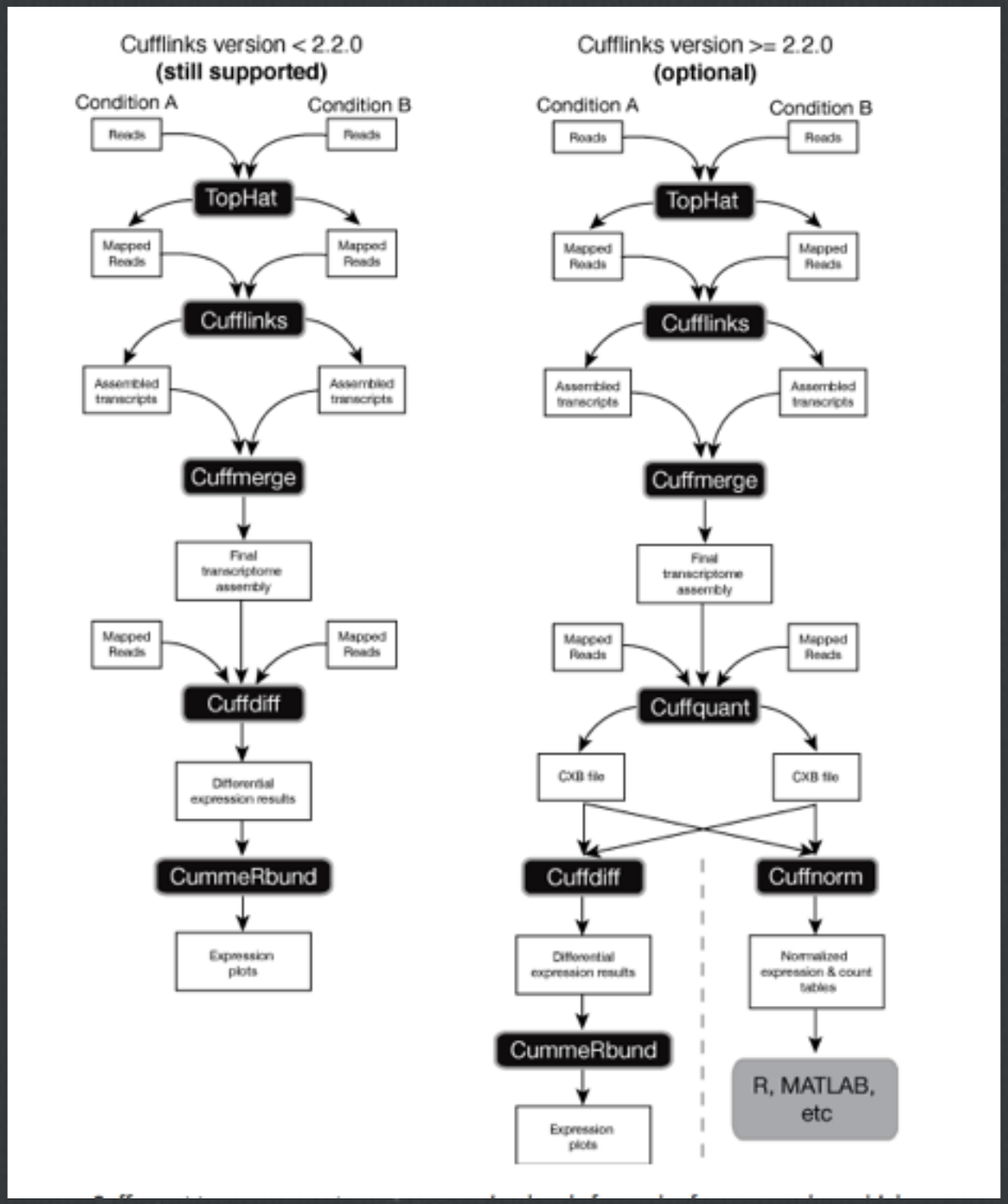
TopHat

```
langyiying@LangYY ~/Desktop/RNA-seq/test/tophat2 ls
accepted_hits.bam  align_summary.txt  deletions.bed  insertions.bed  junctions.bed  logs/  prep_reads.info  unmapped.bam
```

accepted_hits.bam → **Cufflinks**

align_summary.txt
deletions.bed
insertions.bed
junction.bed
prep_reads.info
unmapped.bam
logs/

Cufflinks — Assembly reads to transcripts



Cufflinks

cufflinks -o cufflinks tophat2/accepted_hits.bam

```
langyiying@LangYY ~/Desktop/RNA-seq/test cufflinks -o cufflinks tophat2/accepted_hits.bam
Warning: Your version of Cufflinks is not up-to-date. It is recommended that you upgrade to Cufflinks v2.2.1 to bene
links.cbcb.umd.edu).
[04:38:53] Inspecting reads and determining fragment length distribution.
> Processed 1961 loci.                         [*************************] 100%
> Map Properties:
>       Normalized Map Mass: 805667.00
>       Raw Map Mass: 805667.00
>       Fragment Length Distribution: Empirical (learned)
>                     Estimated Mean: 333.12
>                   Estimated Std Dev: 43.92
[04:39:03] Assembling transcripts and estimating abundances.
> Processed 1996 loci.                         [*************************] 100%
langyiying@LangYY ~/Desktop/RNA-seq/test
```

Cufflinks

Results:

genes.fpm_tracking
isoforms.fpkm_tracking
skipped.gtf
transcripts.gtf

Cufflinks

transcripts.txt

```
1  12  Cufflinks  transcript  12869  20204  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
2  12  Cufflinks  exon  12869  13196  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "1"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
3  12  Cufflinks  exon  13984  14019  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "2"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
4  12  Cufflinks  exon  14140  14172  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "3"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
5  12  Cufflinks  exon  15009  15111  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "4"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
6  12  Cufflinks  exon  15662  15836  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "5"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
7  12  Cufflinks  exon  16307  16426  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "6"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
8  12  Cufflinks  exon  16690  16809  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "7"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
9  12  Cufflinks  exon  17262  17313  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "8"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
10  12  Cufflinks  exon  17919  18046  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "9"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
11  12  Cufflinks  exon  18347  18424  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "10"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
12  12  Cufflinks  exon  18522  18670  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "11"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
13  12  Cufflinks  exon  18935  18980  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "12"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
14  12  Cufflinks  exon  19127  20204  1000  +  .  gene_id "CUFF.1"; transcript_id "CUFF.1.1"; exon_number "13"; FPKM "1332.3186006296"; frac "1.000000"; conf_lo "1276.359017"; conf_hi "1388.278184"; cov "128.176793";
15  12  Cufflinks  transcript  151995  152136  1000  .  .  gene_id "CUFF.2"; transcript_id "CUFF.2.1"; FPKM "69187.4683199782"; frac "1.000000"; conf_lo "29090.559643"; conf_hi "109284.376997"; cov "4234.808572";
16  12  Cufflinks  exon  151995  152136  1000  .  .  gene_id "CUFF.2"; transcript_id "CUFF.2.1"; exon_number "1"; FPKM "69187.4683199782"; frac "1.000000"; conf_lo "29090.559643"; conf_hi "109284.376997"; cov "4234.808572";
17  12  Cufflinks  transcript  276131  285220  1000  -  .  gene_id "CUFF.3"; transcript_id "CUFF.3.1"; FPKM "1471.7867031351"; frac "1.000000"; conf_lo "1391.195509"; conf_hi "1552.377897"; cov "157.075777";
18  12  Cufflinks  exon  276131  278083  1000  -  .  gene_id "CUFF.3"; transcript_id "CUFF.3.1"; exon_number "1"; FPKM "1471.7867031351"; frac "1.000000"; conf_lo "1391.195509"; conf_hi "1552.377897"; cov "157.075777";
19  12  Cufflinks  exon  278195  278362  1000  -  .  gene_id "CUFF.3"; transcript_id "CUFF.3.1"; exon_number "2"; FPKM "1471.7867031351"; frac "1.000000"; conf_lo "1391.195509"; conf_hi "1552.377897"; cov "157.075777";
20  12  Cufflinks  exon  278438  278629  1000  -  .  gene_id "CUFF.3"; transcript_id "CUFF.3.1"; exon_number "3"; FPKM "1471.7867031351"; frac "1.000000"; conf_lo "1391.195509"; conf_hi "1552.377897"; cov "157.075777";
21  12  Cufflinks  exon  279141  279322  1000  -  .  gene_id "CUFF.3"; transcript_id "CUFF.3.1"; exon_number "4"; FPKM "1471.7867031351"; frac "1.000000"; conf_lo "1391.195509"; conf_hi "1552.377897"; cov "157.075777";
22  12  Cufflinks  exon  281896  282089  1000  -  .  gene_id "CUFF.3"; transcript_id "CUFF.3.1"; exon_number "5"; FPKM "1471.
```

Cufflinks

http://cole-trapnell-lab.github.io/cufflinks/cufflinks/index.html

Running Cufflinks

Run cufflinks from the command line as follows:

`cufflinks [options] <aligned_reads.(sam/bam)>`

The following is a detailed description of the options used to control Cufflinks:

Arguments

`<aligned_reads.(sam/bam)>`

A file of RNA-Seq read alignments in the SAM format. SAM is a standard short read alignment, that allows aligners to attach custom tags to individual alignments, and Cufflinks requires that the alignments you supply have some of these tags. Please see Input formats for more details.

Cufflinks General Options

`-h/--help`

Prints the help message and exits

`-g/--GTF-guide <reference_annotation.(gtf/gff)>`

Tells Cufflinks to use the supplied reference annotation a GFF file to guide RABT assembly. Reference transcripts will be tiled with faux-reads to provide additional information in assembly. Output will include all reference transcripts as well as any novel genes and isoforms that are assembled.

`-M/--mask-file <mask.(gtf/gff)>`

Tells Cufflinks to ignore all reads that could have come from transcripts in this GTF file. We recommend including any annotated rRNA, mitochondrial transcripts other abundant transcripts you wish to ignore in your analysis in this file. Due to variable efficiency of mRNA enrichment methods and rRNA depletion kits, masking these transcripts often improves the overall robustness of transcript abundance estimates.

-g using a gtf file as guide

Cuffmerge

cuff merge [options] * <assembly_GTF_list.txt>

create assembly_list.txt first

assembly_list.txt

```
1  ../cufflinks/ZV9_2cells/transcripts.gtf
2  ../cufflinks/ZV9_6h/transcripts.gtf
3
```

cuffmerge -o cuffmerge/ assembly_list.txt

Cuffdiff

cuffdiff -o cuffdiff/ cuffmerge/merged.gtf
-b ../genome/Danio_rerio.Zv9.66.dna.fa
../tophat/ZV9_2cells/accepted_hits.bam ../tophat/ZV9_6h/
accepted_hits.bam

Cuffdiff

cuffdiff -o cuffdiff/ cuffmerge/merged.gtf
-b ../genome/Danio_rerio.Zv9.66.dna.fa
../tophat/ZV9_2cells/accepted_hits.bam ../tophat/ZV9_6h/
accepted_hits.bam

come from coffmerge

Cuffdiff

cuffdiff -o cuffdiff/ cuffmerge/merged.gtf
-b ../genome/Danio_rerio.Zv9.66.dna.fa
../tophat/ZV9_2cells/accepted_hits.bam ../tophat/ZV9_6h/
accepted_hits.bam

the genome

Cuffdiff

cuffdiff -o cuffdiff/ cuffmerge/merged.gtf
-b ../genome/Danio_rerio.Zv9.66.dna.fa
../tophat/ZV9_2cells/accepted_hits.bam ../tophat/ZV9_6h/
accepted_hits.bam

come from TopHat2

Cuffdiff

```
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 bias_params.info
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 cds.count_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 cds.diff
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 cds.fpkm_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 cds.read_group_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 cds_exp.diff
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 gene_exp.diff
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 genes.count_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 genes.fpkm_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 genes.read_group_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 isoform_exp.diff
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 isoforms.count_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 isoforms.fpkm_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 isoforms.read_group_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 promoters.diff
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 read_groups.info
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 run.info
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 splicing.diff
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 tss_group_exp.diff
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 tss_groups.count_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 tss_groups.fpkm_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 tss_groups.read_group_tracking
-rw-r--r-- 1 langyiying staff 0 Nov 20 05:28 var_model.info
```

It’s your time to play.