Bioinformatics Overview

From Raw Sequence to Gene Counts

Rna-Seq Applications

- Differential Expression
- Transcriptome
- Genome Annotation
- “Bargain Exome"
  - SNPs
  - Gene Fusions

Rna-Seq Applications

- Transcriptome: “Which genes are expressed in this sample?”
  - Differential Expression
  - Genome Annotation
- SNPs
- Gene Fusions

RNA-Seq

- Bulk RNA-Seq
- Single-Cell RNA-Seq (scRNA-Seq)

In a Nutshell

- Input: RNA Sequence Fragments

- Output: Gene Counts of Fragments

Rna-Seq Analysis

A Biblio-Archeologic Analogy

Fragments of a Library

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Mapped Fragments

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Book	Fragment Count
A Tale of Two Cities	8
Huckleberry Finn	5
Alice's Adventures	3
Anna Karenina	9
Ulysses	3

Short Reads

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Paired-End Reads

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RNA-Seq
for
Differential Expression Analysis

Step	Input	Output	Output Format
Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
Mapping	Reads	Mapped Reads	SAM/BAM
Counting	Mapped Reads	Count Table	CSV

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Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
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FASTQ

- FASTA with Quality

- https://en.wikipedia.org/wiki/FASTQ_format

FASTQ: Filenames

"What's in a name? that which we call a **FASTQ**
By any other name would smell as sweet"

— *Romeo and Juliet*, William Shakespeare

FASTQ: Filenames

```
1C_TAAGGCGA_L006_R1_001.fastq.gz
1C_TAAGGCGA_L006_R1_002.fastq.gz
1C_TAAGGCGA_L006_R1_003.fastq.gz
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1C_TAGGCATG_L003_R1_001.fastq.gz
1C_TAGGCATG_L005_R1_001.fastq.gz
2C_CGTACTAG_L003_R1_001.fastq.gz
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FASTQ: Filenames

Sample Name	Barcode	Lane #	Read #	File Part
1C	TAAGGCGA	L006	R1	001.fastq.gz
1C	TAAGGCGA	L006	R1	002.fastq.gz
1C	TAAGGCGA	L006	R1	003.fastq.gz
1C	TAAGGCGA	L006	R1	004.fastq.gz
1C	TAGGCATG	L003	R1	001.fastq.gz
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2C	CGTACTAG	L003	R1	001.fastq.gz

FASTQ: Format

- **FASTA Format**

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>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
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AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
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Header Sequence

- **FASTQ Format**

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+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
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Quality Score

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+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
@M00698:36:000000000-AFBEL:1:1101:15928:1413 1:N:0:0
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+
1>AAAFFFFFFFGGGGGGGGCEGGGHHHHHFHHHHGGGHHHHHHHHHGHFA0FEGGGGGHGGHHHHEECEEHFEFH/E/E/>EGGHBHHHDGHHHHC<?E/1
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+
@BBCCFFFFFFFGGGGGGGGGGGGGHHDGHHHHHHHHHHGEGHGHHHGHHHHHHHHHHHEHHHHHHHGGHHHHHHHEGGHHGHHHHHHGGGGFHHHHHHHHHHH
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ABCCCCCCCFFFGGGGGGGGGGGHHGGGGGHHHHHHGGGGGHGGGGGFGFFHHHHHHHHGHHHHHHHHHHGHGGGGGFFAE>EGGGGGEGGHHHHGGHGGHHG
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AAACCCFFFFFFGGGGGGGGGGGHHHHHHHHHHHHHGGGGGGGGGGGHHHHHHHHHHHHHHHHHHHGGGGGGGHGHGGGGGFHHHHHHHHHGHHHHHHHHHHHHG
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AAABCCCCCFFFGGGGGGGGGGGGGHHHHHHHHHHHHHHHHHHGGGGHGGGGGHGFGHHHHHHHHHHGGGHGHHHHGGGGGGGGGGGGHHHGGGCGGGHHHHH?
@M00698:36:000000000-AFBEL:1:1101:17071:1419 1:N:0:0
CACGCCACCTTTATCCAGTTTGCGGCTTTGCGAAGTGGCGAGCCACAGCGCGTTTTCTTGCTGGCTATAAGCCATTTCGTAGGCACCTTTACCTACCGCTT
+
AABBBBBBFFFFGGGGGGGGGGGHGGGGGHHHGGGGFHHGGGGGGHHHHHHGGGFGGGHHHHHHHHHHGHHHHHHHHHHHHHHHHHGGHGHGHHHFHHHHHHGGGG
@M00698:36:000000000-AFBEL:1:1101:15606:1420 1:N:0:0
CGGGTTTTTAACTTTCAGCCACGGGCCACCGTCGATCAGTTCACCGCCAAACTCTTCACGCGCCAGCTGGTAGCCCCAGTCTTTAAACGCTCCTTCGGTGA
+
BBCDBBCCDCFFGGGGGGGGGGGGGGGGGHGGGGGGHGHHHHHHHHGGGGGHHHHHHHHHHHHGGGGGGHHHHHHHHHHGGGHHHHHHHHHHGGGGGHHHHGFGG
@M00698:36:000000000-AFBEL:1:1101:15945:1421 1:N:0:0
TCATTGTTGCGCGGTATTCGCGCCCGTTGGTCGAGTAGCAGAAGGGGATTTTAAACCGTTGTTTGCCGCTGGTGTCCTGCCAGCTGGTTTCATACTCTGG
+
?ABABFFFFBBBFGEEGGGGGGGGGGGHGHCHGCGGGHGHHHHHHHGGGGHGHHHHGHGEGDGHHHHHGGG?EB2GGHGHHHGHGHHHHGHHFHHHHHGGH
@M00698:36:000000000-AFBEL:1:1101:16329:1421 1:N:0:0
GCCCCGACAGCTGTATGCATAGCGATAAATTCCAGCAGGCCGGGACGCCGGTCTATTTTGCCCCAGAGTGTCAATGTTAGACTTGACGGACATTGTGCAG
+
3ABCCCCCCCCCFGGGGGGGGGGHGGHGGHHHHHHHHHHHHGGGGGGGGGGGGGGGHHHHHHHHHHGGGHHHHHHHGHHHHHHHHHHHHHGGGGGHHHHHHHHH

Quality Scores

- Go to Notebook:

HTS2018-notebooks/bioinformatics/quality_scores.ipynb

FASTQ: Read Files

Combined_R1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHGGG
```

Combined_R2.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

Combined_I1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
AGTTCC
+
CCCCDF
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CCTGTC
+
A11>>1
```

Step	Input	Output	Output Format
Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
Mapping	Reads	Mapped Reads	SAM/BAM
Counting	Mapped Reads	Count Table	CSV

Pre-processing

1. Demultiplex [often done at sequencing facility]
2. Quality Control
3. Trimming (adapter and quality)
4. Filtering [must keep reads in sync]

Demultiplexing

Undetermined_S0_L001_R1_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

Undetermined_S0_L001_R2_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGG
```

Undetermined_S0_L001_I1_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
AGTTCC
+
CCCCDF
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CCTGTC
+
A11>>1
```

7B_K

8B_N

Sample	Barcode
7A_K	AGTCAA
7B_K	AGTTCC
7C_N	CGTACG
8A_N	GAGTGG
8B_N	CCTGTC
8C_N	ATTCCT
7A_P	CGATGT

Demultiplexing

7B_K_R1_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
```

7B_K_R2_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
```

8B_N_R1_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

8B_N_R2_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGGCECGHHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

Quality Control

Trimming

Trim:

- Phred <20
- N's
- Adapter:
  - AAACCTCCACGCCGGATACCAGTCTAATCGGCTAGTC

SampleX_R1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

SampleX_R2.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

Filtering

Filter:

- Length <40
- Averge Phred <20
- N's > 3

SampleX_R1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

SampleX_R2.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGG
```

Maintaining Synchrony

SampleX_R1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

SampleX_R2.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGG
```

Maintaining Synchrony

SampleX_R1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

SampleX_R2.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFFBFFGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

Step	Input	Output	Output Format
Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
Mapping	Reads	Mapped Reads	SAM/BAM
Counting	Mapped Reads	Count Table	CSV

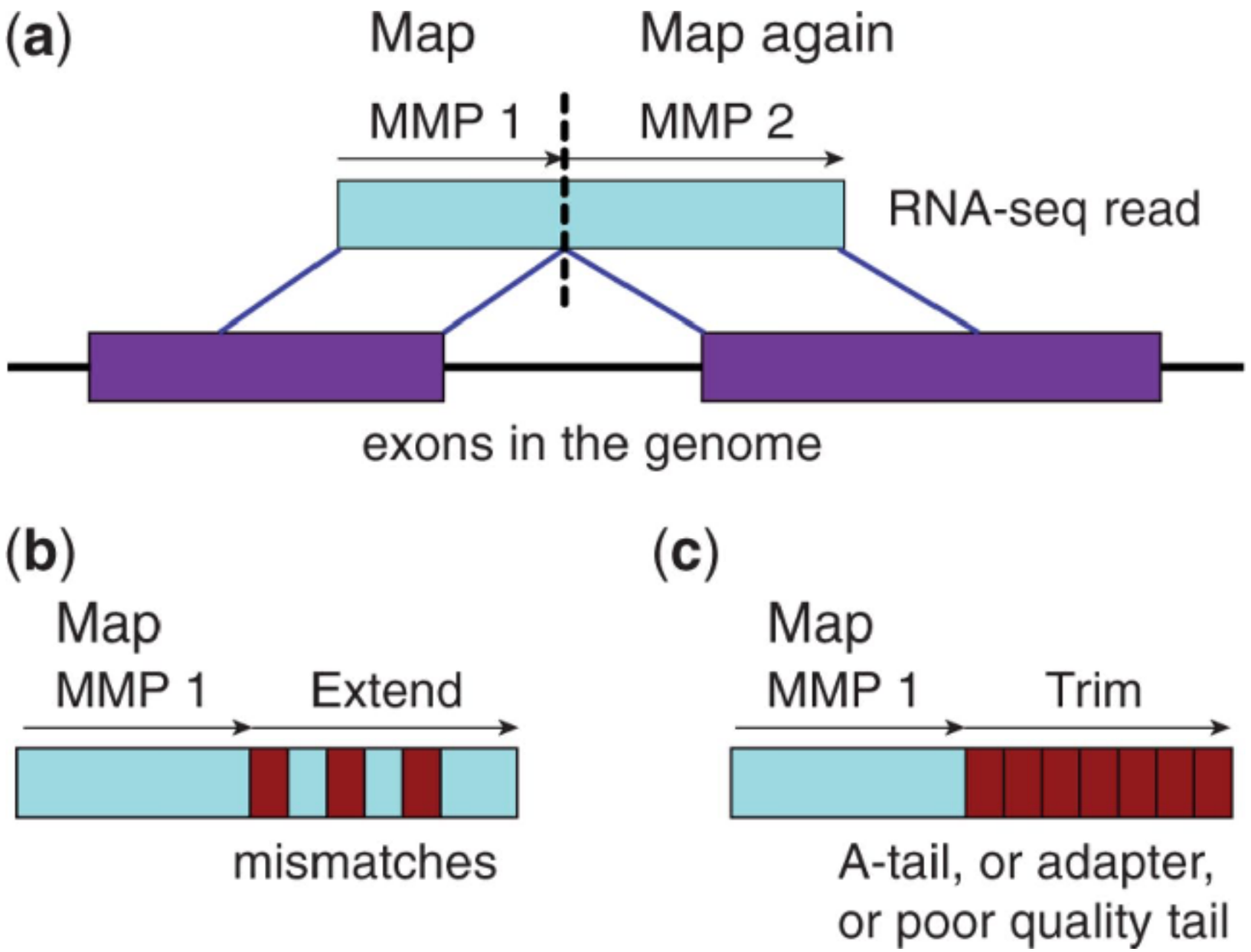
Fragments of a Library

ng on nights, it's us; we're going to set you free." Jim only had time to grab us by the hand and s
of this agreement. See paragraph 1.C below. There are a lot of things you can do with Project Guten
 with his head!"' 'How dreadfully savage!' exclaimed Alice. 'And ever since that,' the Hatter went
g, that chateau of Monsieur the Marquis, with a large stone courtyard before it, and two stone sweep
he nursery. And he was indeed crying. She heard him and hastened. But the faster she went, the loude
h to intensify the influence of her gaze. "Yes, they draw away all the sap and give a false appeara
 him. I could not look at him without feeling sorry for him. We both know him. He’s good-hearted,
ed, under various disguises of Art, through the portraits of every Drinking Age. "You are a little
eupon Punch Costello dinged with his fist upon the board and would sing a bawdy catch _Staboo Stabel
him to act in anything without her quiet aid), and the day passed quickly. Early in the evening he e
ed. 'Give your evidence,' said the King; 'and don't be nervous, or I'll have you executed on the sp
harge a fee for access to, viewing, displaying, performing, copying or distributing any Project Gute
ef secretary. She knew that he usually went out quickly to his office, and she wanted to see him bef
onsiderable. Then he said he must start in and "'terpret" it, because it was sent for a warning. ?
ides her body's flaws calling under her brown shawl from an archway where dogs have mired. Her fancy
recatory manner, "the anguish of his daughter, which must be a dreadful anguish to him!" "I have ob
uld be like, but it puzzled her too much, so she went on: 'But why did they live at the bottom of a
 Indivisible. Liberty, Equality, Fraternity, or Death! Who could that be with Mr. Lorry--the owner
ns in general, and I believe that’s just why philanthropic institutions always give such poor resu
here were no proper cupboards for their clothes; what cupboards there were either would not close at
he detected all around him, walked from one to another. The first was the best room, and in it were
t. All her arrangements had to be modified because they could not be carried out, and they were modi
er hand shook more violently, but she did not take her eyes off him, watching how he would take it.
s." It was done. "Well?" "Monseigneur, it is nothing. The trees and the night are all that are he
spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio
 sore, but I couldn't see no way out of the trouble. After all this long journey, and after all we
 she got to pumping me about England, and blest if I didn't think the ice was getting mighty thin so
 the morning all sound and right. So she had to be satisfied. But she said she'd set up for him
prison--seemed to strike across the earth, messieurs, to where the sky rests upon it!" The hungry m
oys running, playing at horses. Seryozha! And I’m losing everything and not getting him back. Yes,

Mapped Fragments

ng on nights, it's us; we're going to set you free." Jim only had time to grab us by the hand and s -- 471971 Huckleberry Finn
of this agreement. See paragraph 1.C below. There are a lot of things you can do with Project Guten -- ??????????
 with his head!"' 'How dreadfully savage!' exclaimed Alice. 'And ever since that,' the Hatter went -- 78968   Alice's Adventures
g, that chateau of Monsieur the Marquis, with a large stone courtyard before it, and two stone sweep -- 232648 A Tale of Two Cities
he nursery. And he was indeed crying. She heard him and hastened. But the faster she went, the loude -- 1897819 Anna Karenina
h to intensify the influence of her gaze. "Yes, they draw away all the sap and give a false appeara -- 1186098 Anna Karenina
him. I could not look at him without feeling sorry for him. We both know him. He’s good-hearted,   -- 165528 Anna Karenina
ed, under various disguises of Art, through the portraits of every Drinking Age. "You are a little -- 168896 A Tale of Two Cities
eupon Punch Costello dinged with his fist upon the board and would sing a bawdy catch _Staboo Stabel -- 803339 Ulysses
him to act in anything without her quiet aid), and the day passed quickly. Early in the evening he e -- 490073 A Tale of Two Cities
ed. 'Give your evidence,' said the King; 'and don't be nervous, or I'll have you executed on the sp -- 127701 Alice's Adventures
harge a fee for access to, viewing, displaying, performing, copying or distributing any Project Gute -- ??????????
ef secretary. She knew that he usually went out quickly to his office, and she wanted to see him bef -- 782396 Anna Karenina
onsiderable. Then he said he must start in and "'terpret" it, because it was sent for a warning. ? -- 176566 Huckleberry Finn
ides her body's flaws calling under her brown shawl from an archway where dogs have mired. Her fancy -- 90509   Ulysses
recatory manner, "the anguish of his daughter, which must be a dreadful anguish to him!" "I have ob -- 682808 A Tale of Two Cities
uld be like, but it puzzled her too much, so she went on: 'But why did they live at the bottom of a -- 80787   Alice's Adventures
 Indivisible. Liberty, Equality, Fraternity, or Death! Who could that be with Mr. Lorry--the owner -- 562830 A Tale of Two Cities
ns in general, and I believe that’s just why philanthropic institutions always give such poor resu   -- 1699588 Anna Karenina
here were no proper cupboards for their clothes; what cupboards there were either would not close at -- 634360 Anna Karenina
he detected all around him, walked from one to another. The first was the best room, and in it were -- 182459 A Tale of Two Cities
t. All her arrangements had to be modified because they could not be carried out, and they were modi -- 1252025 Anna Karenina
er hand shook more violently, but she did not take her eyes off him, watching how he would take it. -- 455094 Anna Karenina
s." It was done. "Well?" "Monseigneur, it is nothing. The trees and the night are all that are he -- 235828 A Tale of Two Cities
spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 870689 Ulysses
 sore, but I couldn't see no way out of the trouble. After all this long journey, and after all we -- 422461 Huckleberry Finn
 she got to pumping me about England, and blest if I didn't think the ice was getting mighty thin so -- 345525 Huckleberry Finn
 the morning all sound and right. So she had to be satisfied. But she said she'd set up for him   -- 558004 Huckleberry Finn
prison--seemed to strike across the earth, messieurs, to where the sky rests upon it!" The hungry m -- 340478 A Tale of Two Cities
oys running, playing at horses. Seryozha! And I’m losing everything and not getting him back. Yes,   -- 1837882 Anna Karenina

STAR



https://doi.org/10.1093/bioinformatics/bts635

Step	Input	Output	Output Format
Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
Mapping	Reads	Mapped Reads	SAM/BAM
Counting	Mapped Reads	Count Table	CSV

SAM/BAM

- Sequence Alignment/Map
- Specification: http://samtools.github.io/hts-specs/SAMv1.pdf
- BAM: Binary version of SAM

SAM/BAM

- Per read information:
  - Name
  - Position in genome
  - Quality of mapping
  - Insertions? Deletions?
  - Calculated Insert Length (pair-end only)
  - Sequence
  - Sequence Quality
  - Etc

SAM/BAM: Format

```
Coor      12345678901234  5678901234567890123456789012345
ref       AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1         TTAGATAAAGGATA*CTG
+r002         aaaAGATAA*GGATA
+r003       acctaAGCTAA
+r004                         ATAGCT..............TCAGC
-r003                               ttagctTAGGC
-r001/2                                        CAGCGGCAT
```

Read Pair

Chimeric Read

Alignment

```
@HD VN:1.5 SO:coordinate
@SQ SN:ref LN:45
r001   99 ref  7 30 8M2I4M1D3M = 37  39 TTAGATAAAGGATACTG *
r002    0 ref  9 30 3S6M1P1I4M *  0   0 AAAAGATAAGGATA    *
r003    0 ref  9 30 5S6M       *  0   0 GCCTAAGCTAA       * SA:Z:ref,29,-,6H5M,17,0;
r004    0 ref 16 30 6M14N5M    *  0   0 ATAGCTTCAGC       *
r003 2064 ref 29 17 6H5M       *  0   0 TAGGC             * SA:Z:ref,9,+,5S6M,30,1;
r001  147 ref 37 30 9M         =  7 -39 CAGCGGCAT         * NM:i:1
```

http://samtools.github.io/hts-specs/SAMv1.pdf

Step	Input	Output	Output Format
Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
Mapping	Reads	Mapped Reads	SAM/BAM
Counting	Mapped Reads	Count Table	CSV

Counting

ng on nights, it's us; we're going to set you free." Jim only had time to grab us by the hand and s -- 471971 Huckleberry Finn
 with his head!"' 'How dreadfully savage!' exclaimed Alice. 'And ever since that,' the Hatter went -- 78968   Alice's Adventures
g, that chateau of Monsieur the Marquis, with a large stone courtyard before it, and two stone sweep -- 232648 A Tale of Two Cities
he nursery. And he was indeed crying. She heard him and hastened. But the faster she went, the loude -- 1897819 Anna Karenina
h to intensify the influence of her gaze. "Yes, they draw away all the sap and give a false appeara -- 1186098 Anna Karenina
him. I could not look at him without feeling sorry for him. We both know him. He’s good-hearted,   -- 165528 Anna Karenina
ed, under various disguises of Art, through the portraits of every Drinking Age. "You are a little -- 168896 A Tale of Two Cities
eupon Punch Costello dinged with his fist upon the board and would sing a bawdy catch _Staboo Stabel -- 803339 Ulysses
him to act in anything without her quiet aid), and the day passed quickly. Early in the evening he e -- 490073 A Tale of Two Cities
ed. 'Give your evidence,' said the King; 'and don't be nervous, or I'll have you executed on the sp -- 127701 Alice's Adventures
ef secretary. She knew that he usually went out quickly to his office, and she wanted to see him bef -- 782396 Anna Karenina
onsiderable. Then he said he must start in and "'terpret" it, because it was sent for a warning. ? -- 176566 Huckleberry Finn
ides her body's flaws calling under her brown shawl from an archway where dogs have mired. Her fancy -- 90509   Ulysses
recatory manner, "the anguish of his daughter, which must be a dreadful anguish to him!" "I have ob -- 682808 A Tale of Two Cities
uld be like, but it puzzled her too much, so she went on: 'But why did they live at the bottom of a -- 80787   Alice's Adventures
 Indivisible. Liberty, Equality, Fraternity, or Death! Who could that be with Mr. Lorry--the owner -- 562830 A Tale of Two Cities
ns in general, and I believe that’s just why philanthropic institutions always give such poor resu   -- 1699588 Anna Karenina
here were no proper cupboards for their clothes; what cupboards there were either would not close at -- 634360 Anna Karenina
he detected all around him, walked from one to another. The first was the best room, and in it were -- 182459 A Tale of Two Cities
t. All her arrangements had to be modified because they could not be carried out, and they were modi -- 1252025 Anna Karenina
er hand shook more violently, but she did not take her eyes off him, watching how he would take it. -- 455094 Anna Karenina
s." It was done. "Well?" "Monseigneur, it is nothing. The trees and the night are all that are he -- 235828 A Tale of Two Cities
spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 870689 Ulysses
 sore, but I couldn't see no way out of the trouble. After all this long journey, and after all we -- 422461 Huckleberry Finn
 she got to pumping me about England, and blest if I didn't think the ice was getting mighty thin so -- 345525 Huckleberry Finn
 the morning all sound and right. So she had to be satisfied. But she said she'd set up for him   -- 558004 Huckleberry Finn
prison--seemed to strike across the earth, messieurs, to where the sky rests upon it!" The hungry m -- 340478 A Tale of Two Cities
oys running, playing at horses. Seryozha! And I’m losing everything and not getting him back. Yes,   -- 1837882 Anna Karenina

Counting

ed, under various disguises of Art, through the portraits of every Drinking Age. "You are a little -- 168896 A Tale of Two Cities
he detected all around him, walked from one to another. The first was the best room, and in it were -- 182459 A Tale of Two Cities
g, that chateau of Monsieur the Marquis, with a large stone courtyard before it, and two stone sweep -- 232648 A Tale of Two Cities
s." It was done. "Well?" "Monseigneur are he -- 235828 A Tale of Two Cities
prison--seemed to strike across the eart ungry m -- 340478 A Tale of Two Cities
him to act in anything without her quiet ng he e -- 490073 A Tale of Two Cities
 Indivisible. Liberty, Equality, Fratern owner -- 562830 A Tale of Two Cities
recatory manner, "the anguish of his dau have ob -- 682808 A Tale of Two Cities

onsiderable. Then he said he must start ing. ? -- 176566 Huckleberry Finn
 she got to pumping me about England, an thin so -- 345525 Huckleberry Finn
 sore, but I couldn't see no way out of all we -- 422461 Huckleberry Finn
ng on nights, it's us; we're going to se d and s -- 471971 Huckleberry Finn
 the morning all sound and right. So sh him   -- 558004 Huckleberry Finn

 with his head!"' 'How dreadfully savag er went -- 78968   Alice's Adventures
uld be like, but it puzzled her too much m of a -- 80787   Alice's Adventures
ed. 'Give your evidence,' said the King the sp -- 127701 Alice's Adventures

 him. I could not look at him without fe ted,   -- 165528 Anna Karenina
er hand shook more violently, but she di ke it. -- 455094 Anna Karenina
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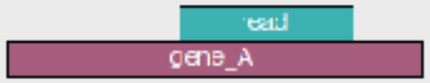
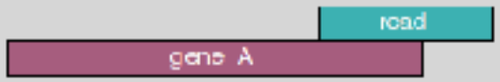
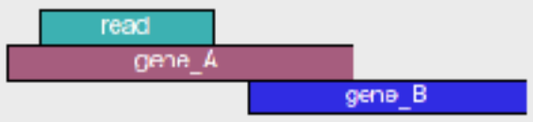
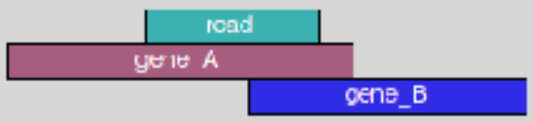
ef secretary. She knew that he usually w him bef -- 782396 Anna Karenina
h to intensify the influence of her gaze appeara -- 1186098 Anna Karenina
t. All her arrangements had to be modifi re modi -- 1252025 Anna Karenina
ns in general, and I believe that's just resu   -- 1699588 Anna Karenina
oys running, playing at horses. Seryozha Yes,   -- 1837882 Anna Karenina
he nursery. And he was indeed crying. Sh e loude -- 1897819 Anna Karenina

ides her body's flaws calling under her r fancy -- 90509   Ulysses
eupon Punch Costello dinged with his fist upon the board and would sing a bawdy catch _Stabbo Stabel -- 803339 Ulysses
spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 870689 Ulysses

Book	Fragment Count
A Tale of Two Cities	8
Huckleberry Finn	5
Alice's Adventures	3
Anna Karenina	9
Ulysses	3

Counting

	union	Intersection _strict	Intersection _nonempty
	gene_A	gene_A	gene_A
	gene_A	no_feature	gene_A
	gene_A	no_feature	gene_A
	gene_A	gene_A	gene_A
	gene_A	gene_A	gene_A
	ambiguous	gene_A	gene_A
	ambiguous	ambiguous	ambiguous

http://www-huber.embl.de/users/anders/HTSeq/doc/count.html

Step	Input	Output	Output Format
Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
Mapping	Reads	Mapped Reads	SAM/BAM
Counting	Mapped Reads	Count Table	CSV

CSV:
Comma-separated values

```
gene990,1
gene991,2
gene992,20
gene993,1
gene994,3
gene995,0
gene996,14
gene997,9
gene998,45
gene999,0
```

TSV:
Tab-separated values

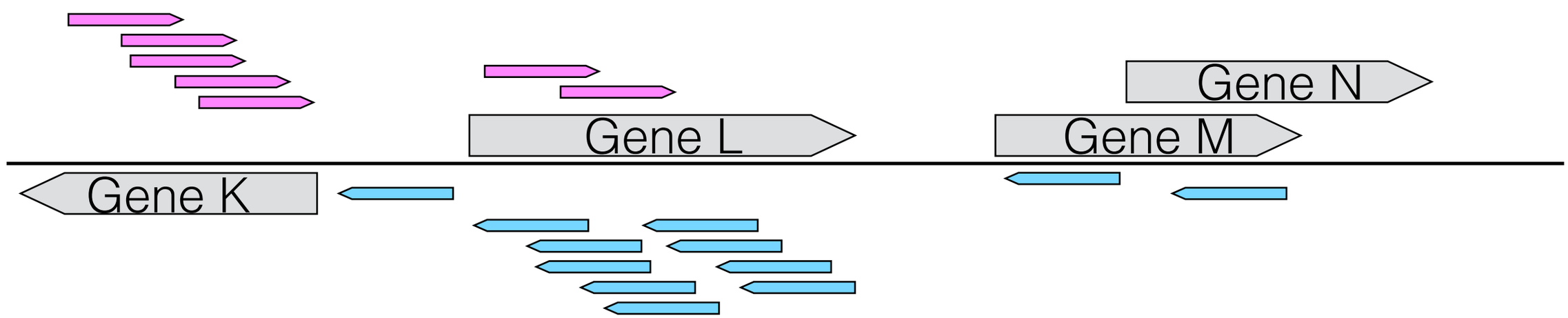
```
gene990 1
gene991 2
gene992 20
gene993 1
gene994 3
gene995 0
gene996 14
gene997 9
gene998 45
gene999 0
```

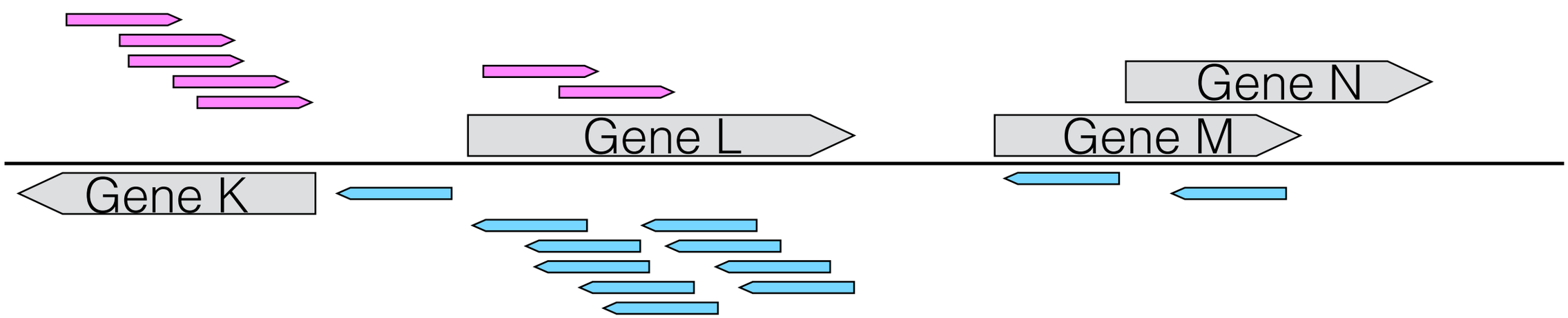
STAR Counts

	Unstranded Reads	Coding Strand Reads	Antisense Strand Reads
N_unmapped	3386	3386	3386
N_multimapping	149388	149388	149388
N_noFeature	472714	2327552	503625
N_ambiguous	138341	1266	1354
CNAG_04548	1	0	1
CNAG_07303	0	0	0
CNAG_07304	9	0	9
CNAG_00001	0	0	0
. . .			

STAR Counts

	Unstranded Reads	Coding Strand Reads	Antisense Strand Reads
N_unmapped	?	?	?
N_multimapping	?	?	?
N_noFeature	?	?	?
N_ambiguous	?	?	?
Gene K	?	?	?
Gene L	?	?	?
Gene M	?	?	?
Gene N	?	?	?



STAR Counts

	Unstranded Reads	Coding Strand Reads	Antisense Strand Reads
N_unmapped	?	?	?
N_multimapping	?	?	?
N_noFeature	1	17	3
N_ambiguous	1	0	1
Gene K	5	0	5
Gene L	11	2	9
Gene M	1	0	1
Gene N	0	0	0