

# Bioinformatics Overview

From Raw Sequence to Gene Counts

# Rna-Seq Applications

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

# 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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# Counting

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Book	Fragment Count	
A Tale of Two Cities	8	-- 168896 A Tale of Two Cities -- 182459 A Tale of Two Cities -- 232648 A Tale of Two Cities -- 235828 A Tale of Two Cities -- 340478 A Tale of Two Cities -- 490073 A Tale of Two Cities -- 562830 A Tale of Two Cities -- 682808 A Tale of Two Cities
Huckleberry Finn	5	-- 176566 Huckleberry Finn -- 345525 Huckleberry Finn -- 422461 Huckleberry Finn -- 471971 Huckleberry Finn -- 558004 Huckleberry Finn
Alice's Adventures	3	-- 78968 Alice's Adventures -- 80787 Alice's Adventures -- 127701 Alice's Adventures
Anna Karenina	9	-- 165528 Anna Karenina -- 455094 Anna Karenina -- 634360 Anna Karenina -- 782396 Anna Karenina -- 1186098 Anna Karenina -- 1252025 Anna Karenina -- 1699588 Anna Karenina -- 1837882 Anna Karenina -- 1897819 Anna Karenina
Ulysses	3	-- 90509 Ulysses -- 803339 Ulysses -- 870689 Ulysses



# 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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# FASTQ

- FASTA with Quality
- [https://en.wikipedia.org/wiki/FASTQ\\_format](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  
1C\_TAAGGCGA\_L006\_R1\_004.fastq.gz  
1C\_TAGGCATG\_L003\_R1\_001.fastq.gz  
1C\_TAGGCATG\_L005\_R1\_001.fastq.gz  
2C\_CGTACTAG\_L003\_R1\_001.fastq.gz



# 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
1C	TAGGCATG	L005	R1	001.fastq.gz
2C	CGTACTAG	L003	R1	001.fastq.gz

# FASTQ: Format

- **FASTA Format**

```
>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome  
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG  
GTTACCTGCCGTGAGTAAATTTAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC  
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT  
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTTCGACCAAAGG  
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG  
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCCGCCAAAATCACCAACCACCTGGTG  
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT  
GACGGGACTCGCCGCCGCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACCTTCGTCGATCAGGAATTTGCCCAAATAA  
AACATGTCCTGCATGGCATTAGTTTGTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA
```

Header  
Sequence

- **FASTQ Format**

```
@M00698:36:000000000-AFBE:1:1101:16483:1412 1:N:0:0  
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCAGATCCGGCG  
+  
AAABBBFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGGHHHHGGGGGGHHHHGHHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG
```

Quality  
Score



# Quality Scores

- Go to Notebook:

[HTS2018-notebooks/bioinformatics/quality\\_scores.ipynb](https://github.com/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
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTA ACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAACGCC
+
ABBBABBBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHHHHHHHHGGGGGGHHHHGGGGGGGGHHHHGHHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG
```

## Combined\_R2.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFFDBDGC??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFBFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGHHHHHHHHHHHHHEHGHHHHHHHHHHGGGGGGG
```

## 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
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCC
+
ABBBABBBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGTGGATAGCTTGTCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBBBBBBBBGGGGGGGGGGGGHHHHHHHHHGHGHHHHHHGGGGGGHHHHGGGGGGHHHHGHHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG
```

## Undetermined S0 L001 R2 001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
+
ABBABFFFDBDGC??FFGGGHGHEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHDFEGGHFHEFH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTCCGTACTCATGCGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCFFFBFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGHHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

## 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
```

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  
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAACGCCC  
+  
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHAGGHHGHHHFDFHFHHGEGGGG@F@H?GHH/GBEFGGG

## 7B K R2 001.fastq.gz

@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0  
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT  
+  
ABBABFFFFDBDGC??FFGGGHGHEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHDFEGGHFHEFH3BF0GFEGGGGGHHA/FGHFHHH

## 8B N R1 001.fastq.gz

@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0  
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTCCCCGATATTTCCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG  
+  
AAABBFFFFFFFGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGGGGGGHHHHGGGGGGHHHHGHHFFHHHHHGHGGGGGGGGHHHHHHHHHHHGGG

## 8B N R2 001.fastq.gz

@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0  
GCTTCTTCCGTAATCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT  
+  
CCCCFFFBFFGGGGGGGGCECGHHHHHHHHHGGHGGGGHGGCGCHGFGGGGHHGGGGHHHHHHHHHHHHHHHHHEHGHHGHHHHGGGGGGG

# Quality Control



# Filtering

Filter:

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

## SampleX\_R1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
```

```
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAACGCC
```

```
+
```

```
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGE GGGG@F@H?GHH/GBEFGGG
```

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

```
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
```

```
+
```

```
AAABBFBBBBFFFGGGGGGGGGGGHGGHHHHHHHGHGHGHHHHHGGGGHGGGGGGGGHGGHGHFFHHHHHHGHGGGGGGGGGGHGGHHHHHHHHHHGGG
```

## SampleX\_R2.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
```

```
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT
```

```
+
```

```
ABBABFFFFDBDGC??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHCFGHFGBGHFHDFEGGHFHEFH3BF0GFEGGGGGHHA/FGHFHHH
```

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

```
GCTTCTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
```

```
+
```

```
CCCCFFFFBFFFGGGGGGGGCECGHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHGGGGHGGHHHHHHHHHHHHHEHGHGGHHHHHGGGGGGG
```

# Maintaining Synchrony

## **SampleX\_R1.fastq.gz**

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0  
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAACGCC  
+  
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHHAGGHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG  
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0  
CTGCCAGTTGAACGACGGCAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG  
+  
AAABBBBBBBBBFGGGGGGGGGGGHHHHHHHHGHGHHHHHHGHHHGGGGGGHHHHGGGGGGHHHHGHHFFHHHHHHGHHGGGGGGGGHHHHHHHHHHHGGG
```

## **SampleX\_R2.fastq.gz**

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0  
GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT  
+  
ABBABFFFDBDGC??FFGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHDFEGGHFHEFH3BFGF0GFEGGGGGHHA/FGHFHHH  
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0  
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT  
+  
CCCCFFFBFFFGGGGGGGGCECGHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGHHHHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

# Maintaining Synchrony

## SampleX\_R1.fastq.gz

@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG

+

AAABBBBBBBBBGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGGGGGGHHHHGGGGGGHHHHGHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG

## SampleX\_R2.fastq.gz

@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0

GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT

+

ABBABFFFDBDGC??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFH3BFGFØGFEGGGGGHHA/FGHFHHH

@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0

GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT

+

CCCCFFFBFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGHHHHHHHHHHHHHHHEHGHGHGHGHGGGGGG

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

na 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 -- ??????????????  
er Secretary. She knew that he usually went out quickly to his office, and she wanted to see him bet -- 782596 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

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

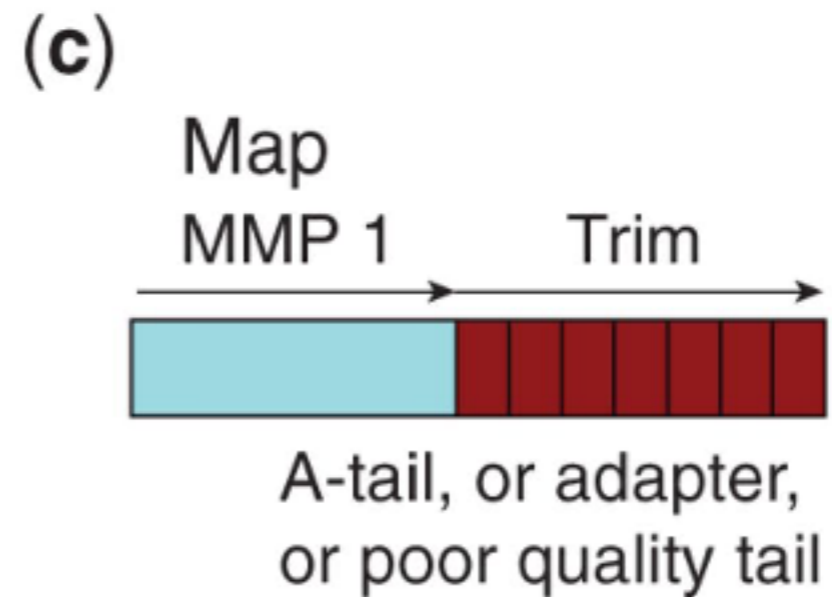
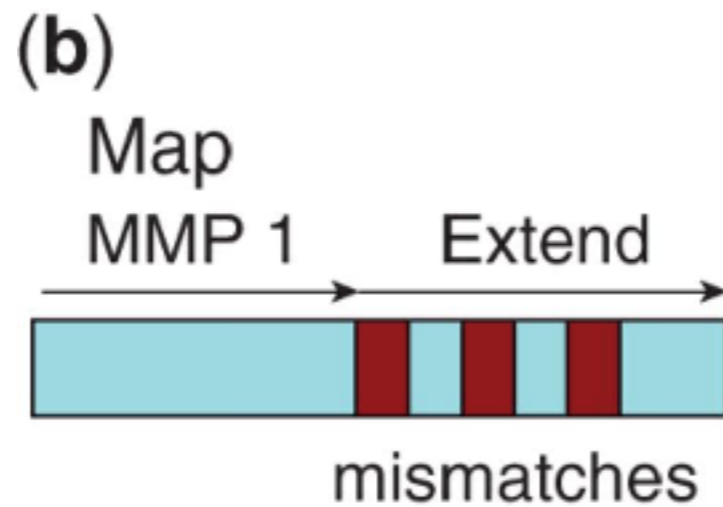
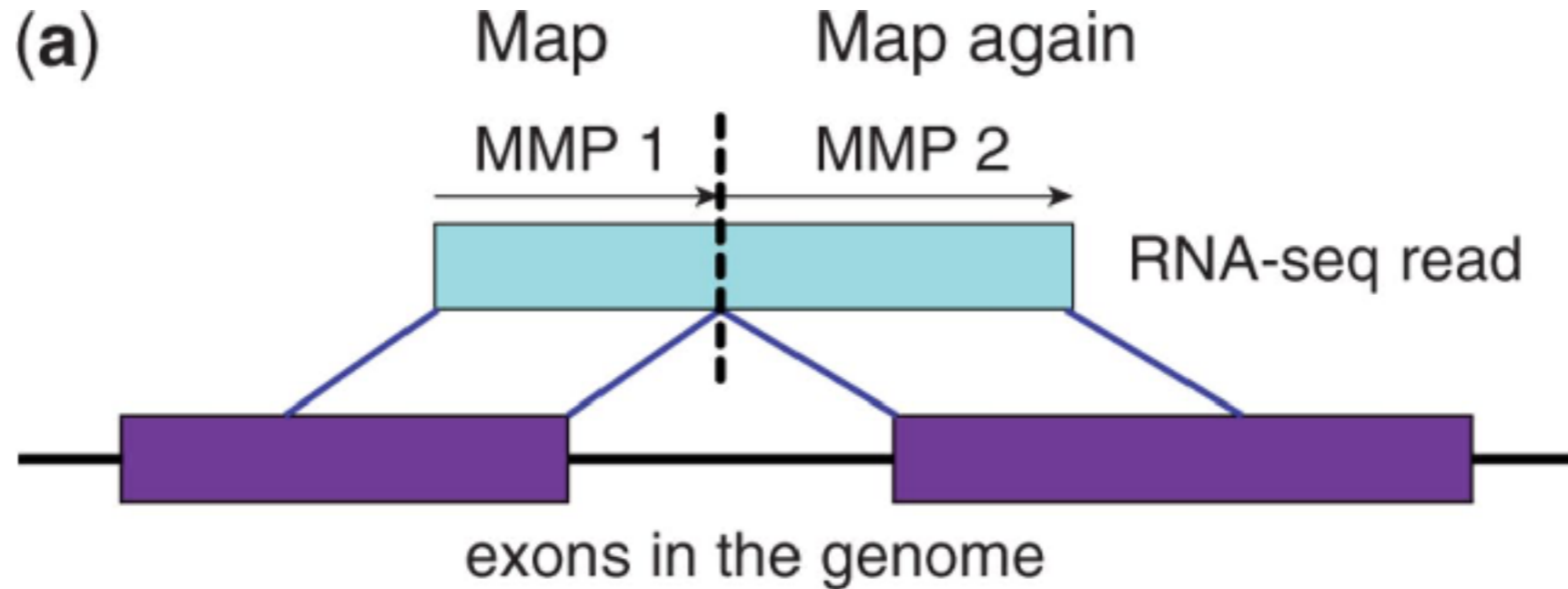
Chimeric  
Read

Alignment

```

@HD VN:1.5 S0: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
  
```

# STAR



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  
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spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 870689 Ulysses  
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she got to pumping me about England, and blest if I didn't think the ice was getting mighty thin so -- 345525 Huckleberry Finn  
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# Counting

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onsiderable. Then he said he must start  
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 sore, but I couldn't see no way out of  
 ng on nights, it's us; we're going to se  
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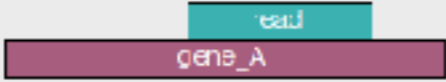
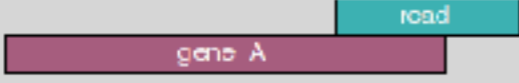
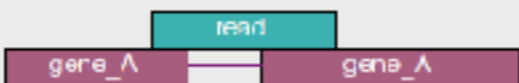

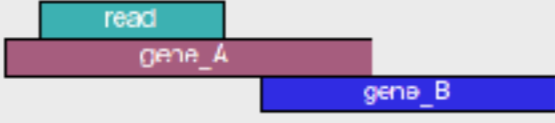
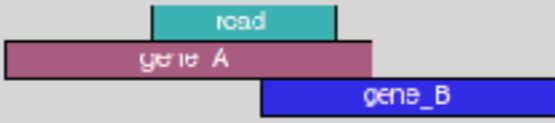
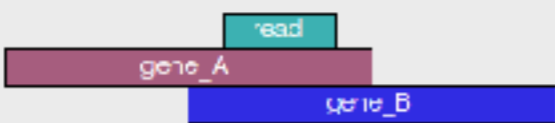
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 uld be like, but it puzzled her too much  
 ed. 'Give your evidence,' said the King

him. I could not look at him without fe  
 er hand shook more violently, but she di  
 here were no proper cupboards for their  
 ef secretary. She knew that he usually w  
 h to intensify the influence of her gaze  
 t. All her arrangements had to be modifi  
 ns in general, and I believe that's just  
 oys running, playing at horses. Seryozha  
 he nursery. And he was indeed crying. Sh

ides her body's flaws calling under her  
 eupon Punch Costello dinged with his fist  
 spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio

Book	Fragment Count		
A Tale of Two Cities	8	are he -- 235828	A Tale of Two Cities
Huckleberry Finn	5	ng he e -- 490073	A Tale of Two Cities
Alice's Adventures	3	owner -- 562830	A Tale of Two Cities
Anna Karenina	9	have ob -- 682808	A Tale of Two Cities
Ulysses	3	ing. ? -- 176566	Huckleberry Finn
		thin so -- 345525	Huckleberry Finn
		all we -- 422461	Huckleberry Finn
		d and s -- 471971	Huckleberry Finn
		him -- 558004	Huckleberry Finn
		er went -- 78968	Alice's Adventures
		m of a -- 80787	Alice's Adventures
		the sp -- 127701	Alice's Adventures
		ted, -- 165528	Anna Karenina
		ke it. -- 455094	Anna Karenina
		lose at -- 634360	Anna Karenina
		him bef -- 782396	Anna Karenina
		appeara -- 1186098	Anna Karenina
		re modi -- 1252025	Anna Karenina
		resu -- 1699588	Anna Karenina
		Yes, -- 1837882	Anna Karenina
		e loude -- 1897819	Anna Karenina
		r fancy -- 90509	Ulysses
		Stabel -- 803339	Ulysses
		-- 870689	Ulysses

# 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

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

	<b>Unstranded</b>	<b>Coding Strand Reads</b>	<b>Antisense Strand Reads</b>
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
. . .			