

Bioinformatics Overview

From Raw Sequence to Gene Counts

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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Counting

Book	Durham	Chapel Hill
A Tale of Two Cities	8	17
Huckleberry Finn	5	2
Alice's Adventures	3	1
Anna Karenina	9	2
Ulysses	3	2

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

FASTQ: Format

- **FASTA Format**

```
>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome
AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
GTTACCTGCCGTGAGTAAATTTAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
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AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
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Header
Sequence

- **FASTQ Format**

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Quality
Score

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+
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+
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+
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+
3ABCCCCCCCCFGGGGGGGGGGGGGHGGHGGHHHHHHHHHHHHGGGGGGGGGGGGGGGGGGHHHHHHHHHHHHHHHHHHHHHHHHHHHHGGGGGGHHHHHHHHH
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FASTQ: Format

FASTQ: Read Files

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+
ABBBABBBBAFFFGGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTTCGCGTGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFBBBBBBBBGGGGGGGGGGGGHHHHHHHHHGHGHHHHHHHHHHGGGGGGHHHHGGGGGGGGHHHHGHHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHHGGG
```

Combined_R2.fastq.gz

@M00698:36:000000000-AFBEL:1:1101:14738:1412 2:N:0:0
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+
ABBABFFFFDBDGC??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFH HHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFBFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG

Combined l1.fastq.gz

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

Bioinformatics Overview

From Raw Sequence to Gene Counts

Quality Scores

- Go to Jupyter:

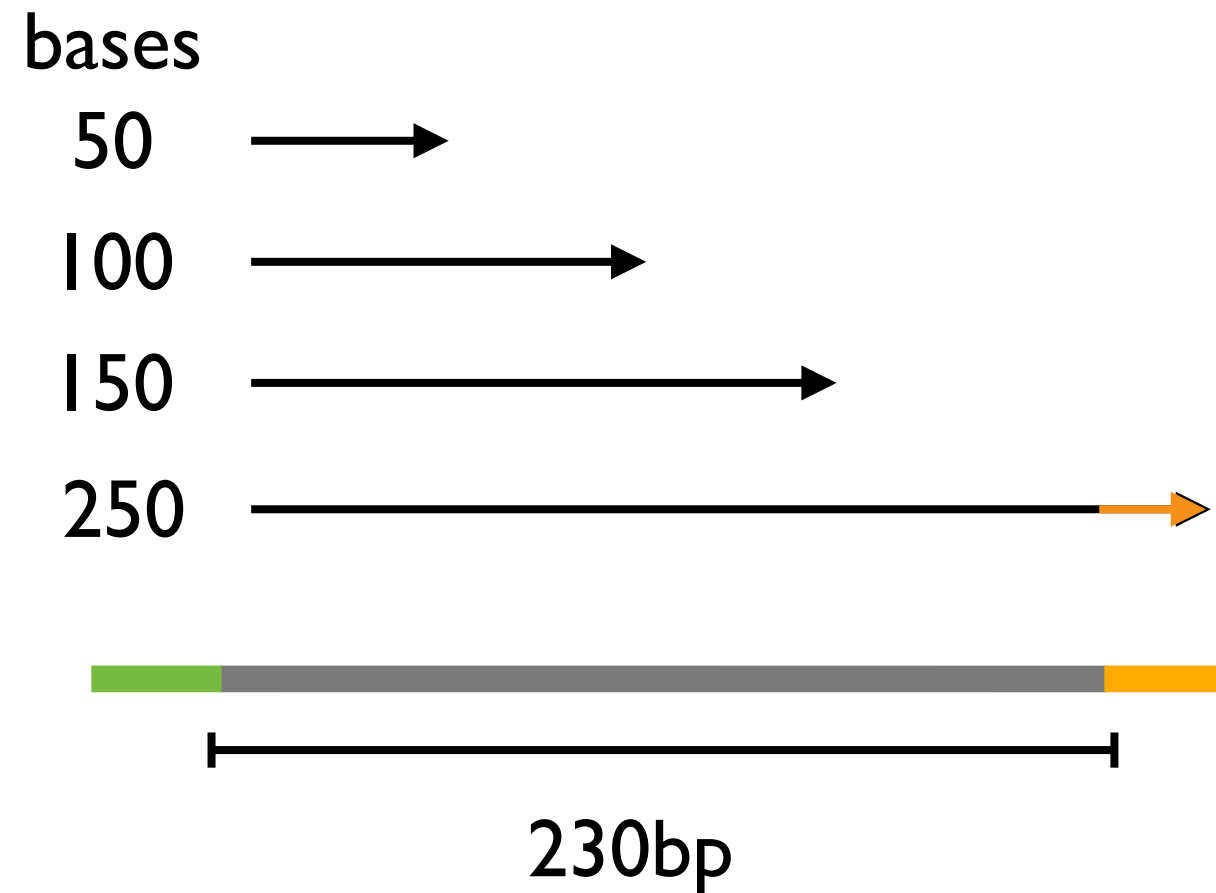
https://gitlab.oit.duke.edu/mic-course/2021-mic/-/blob/master/bioinformatics/quality_scores.Rmd

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]

Adapter Contamination



Trimming

Trim:

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

SampleX_R1.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
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+
ABBBABBBBAFFFGGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBBFFFFFGGGGGGGGGGGHGGHHHHHHHGGHGGHHHHHHGGGGGGHHHHGGGGGGGGHHHHGHHFFHHHHHHGGGGGGGGGGHHHHHHHHHHHHGGG
```

SampleX_R2.fastq.gz

```
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+
ABBABFFFFDBDGC??FFGGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFBFFFGGGGGGGGCECGHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGHHHHHHHHHHHHHHHHHEHGGHHHGGHHHGGGGGGG
```

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

TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+

ABBBABBBBAFFFGGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG

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

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+

AAABBFFFFFFFFFGGGGGGGGGGGHGGHHHHHHHHGGHGGHHHHHHGGGGGGGGGGGGHGGHHHFFHHHHHHGGGGGGGGGGGGHHHHHHHHHHHHGGG

SampleX_R2.fastq.gz

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

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+

ABBABFFFFDBDGC??FFGGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH

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

GCTTCTTCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+

CCCCCFFFBFFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGHHHHHHHHHHHHHHHEHGGHHHGGHHHGGGGGGG

Maintaining Synchrony

SampleX R1.fastq.gz

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

TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACATCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAACGCC

+

ABBBABBBBAFFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG

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

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG

+

AAABBBFFFFGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGHHHHGGGGGGHHHGHHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG

SampleX R2.fastq.gz

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

GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT

+

ABBABFFFDBDGC??FFGGGGHGFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFHFFFH3BFGF0GFEGGGGGHHA/FGHFHHH

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

GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT

+

CCCCFFFBFFGGGGGGGCECGHHHHHHHHHGGHGGGGHGCGCHGHFHGGGGHHGGGGHHHHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGG

Maintaining Synchrony

SampleX R1.fastq.gz

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

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG

+

AAABBBFFFFGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGHHHHGGGGGGHHGHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG

SampleX R2.fastq.gz

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

GGAAGATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGT

+

ABBABFFFDBDGC??FFGGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFH

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

GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT

+

CCCCFFFFBFFGGGGGGGCECGHHHHHHHHHGGHGGGGHGGCGCHHGFGGGGHGGGGGHHHHHHHHHHHHHHHHHEHGH HHGHHHHGGGGGG

Trimmed/Filtered Output

SampleX R1.fastq.gz

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

ABBBABBBBAFFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG

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

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG

+

AAABBBFFFFGGGGGGGGGGHHHHHHHHGHGHGHHHHHHHHGGGGHHHHGGGGGGHHGHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG

SampleX R2.fastq.gz

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

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+

ABBABFFFDBDGC??FFGGGGHGFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFH

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

+

CCCCFFFFBFFGGGGGGGCECGHHHHHHHHHGGHGGGGHGCGCHGFHGGGGHHGGGGHHHHHHGHHHHHHHHHHHHEHGH HH GHH HGGGGGG

Trimmed/Filtered Output

SampleX_R1.fastq.gz

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

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG

+

AAABBBFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHHHGHHHGGGGGHHHHGGGGGGGGHHHHGHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG

SampleX_R2.fastq.gz

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

GCTTCTTCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTG

+

CCCCCFFFBFFGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGGHHHHHHGHHHHHHHHHHHHHHE

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

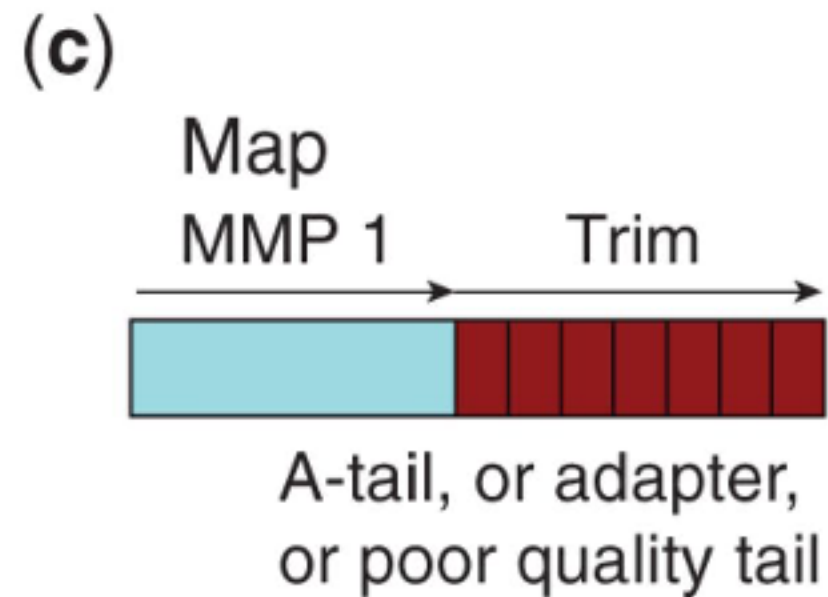
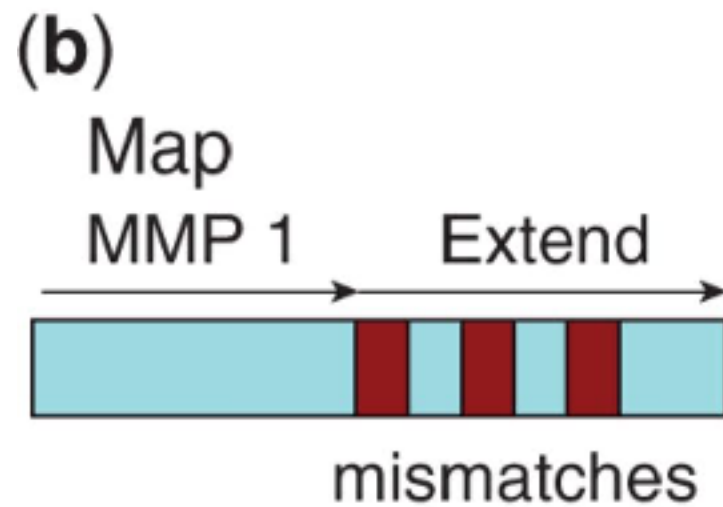
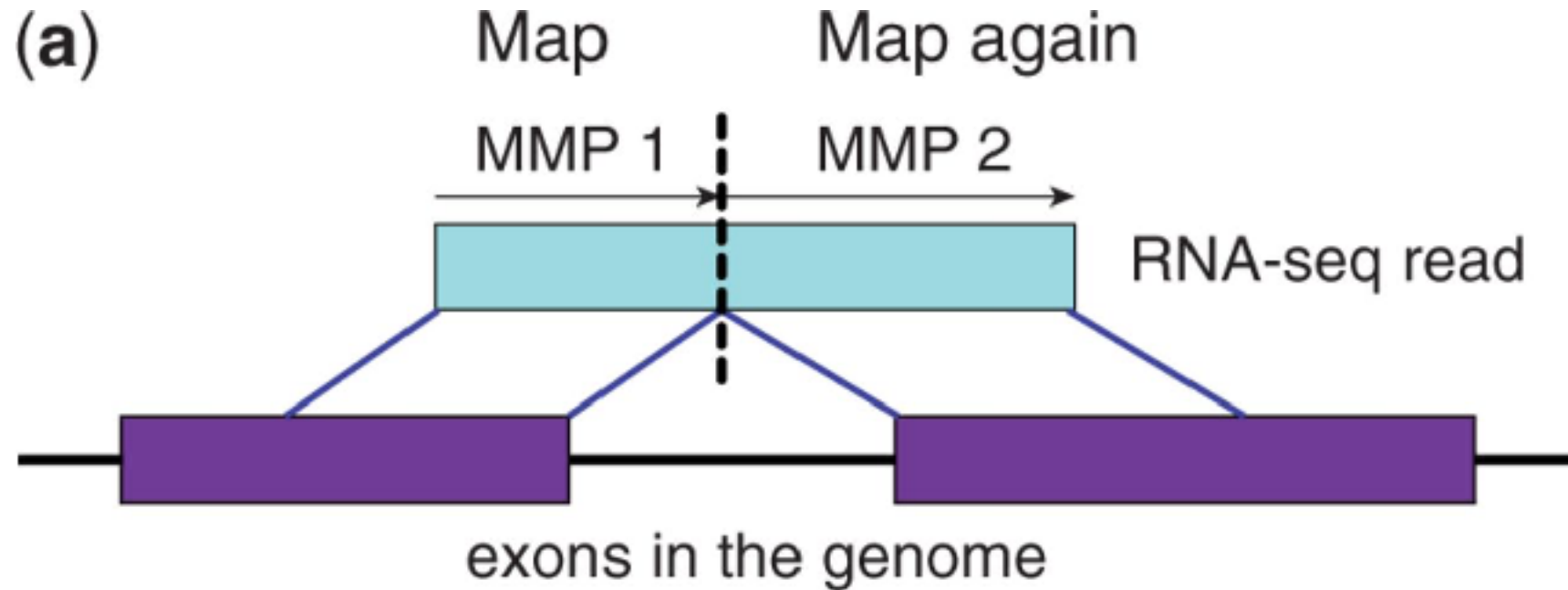
Fragments of a Library

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
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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
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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
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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
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



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

Coord	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

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

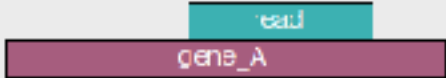
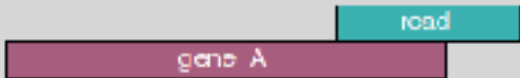


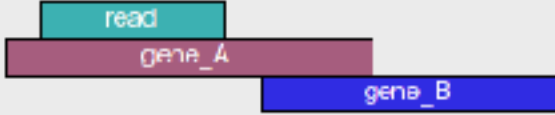
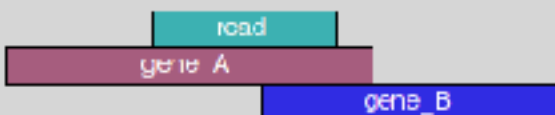
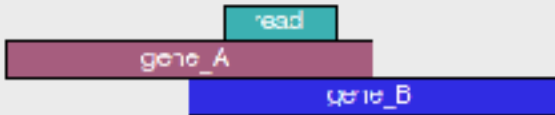
Counting

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
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h to intensify the influence of her gaze. "Yes, they draw away all the sap and give a false appeara -- 1186098 Anna Karenina
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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
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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
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 -- 232648 A Tale of Two Cities			
s." It was done. "Well?" "Monseigneur -- 235828 A Tale of Two Cities			
prison--seemed to strike across the eart -- 340478 A Tale of Two Cities			
him to act in anything without her quiet -- 490073 A Tale of Two Cities			
Indivisible. Liberty, Equality, Fratern -- 562830 A Tale of Two Cities			
recatory manner, "the anguish of his dau -- 682808 A Tale of Two Cities			
onsiderable. Then he said he must start -- 176566 Huckleberry Finn			
she got to pumping me about England, an -- 345525 Huckleberry Finn			
sore, but I couldn't see no way out of -- 422461 Huckleberry Finn			
ng on nights, it's us; we're going to se -- 471971 Huckleberry Finn			
the morning all sound and right. So sh -- 558004 Huckleberry Finn			
with his head!'" 'How dreadfully savag -- 78968 Alice's Adventures			
uld be like, but it puzzled her too much -- 80787 Alice's Adventures			
ed. 'Give your evidence,' said the King -- 127701 Alice's Adventures			
him. I could not look at him without fe -- 165528 Anna Karenina			
er hand shook more violently, but she di -- 455094 Anna Karenina			
here were no proper cupboards for their -- 634360 Anna Karenina			
ef secretary. She knew that he usually w -- 782396 Anna Karenina			
h to intensify the influence of her gaze -- 1186098 Anna Karenina			
t. All her arrangements had to be modifi -- 1252025 Anna Karenina			
ns in general, and I believe that's just -- 1699588 Anna Karenina			
oys running, playing at horses. Seryozha -- 1837882 Anna Karenina			
he nursery. And he was indeed crying. Sh -- 1897819 Anna Karenina			
ides her body's flaws calling under her -- 90509 Ulysses			
eupon Punch Costello dinged with his fist upon the board and would sing a daway catch -- 803339 Ulysses			
spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 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

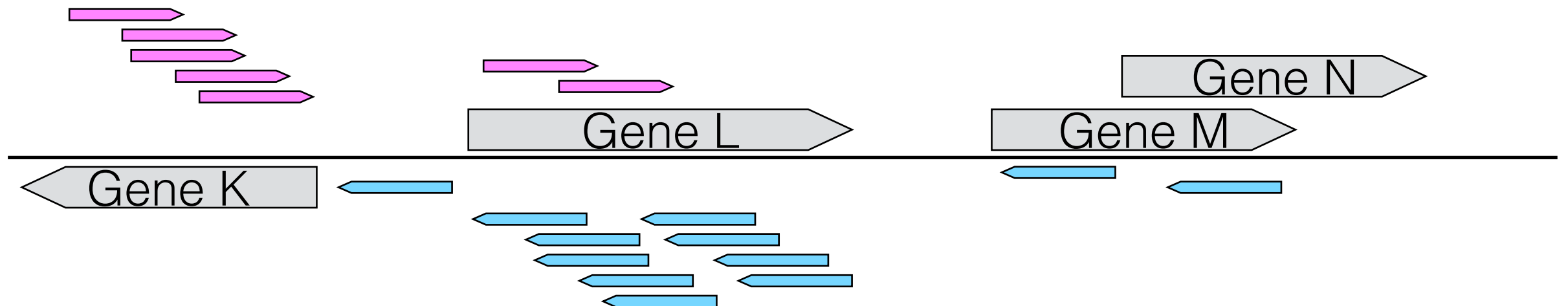
gene990	1
gene991	2
gene992	20
gene993	1
gene994	3
gene995	0
gene996	14
gene997	9
gene998	45
gene999	0

STAR Counts

	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

