

Bioinformatics Overview

From Raw Sequence to Gene Counts

In a Nutshell

- Input: RNA Sequence Fragments
- Output: Gene Counts of Fragments

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: 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

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
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACCTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA
```

- **FASTQ Format**

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

FASTQ: Format

- **FASTA Format**

>NC_007779.1 Escherichia coli str. K-12 substr. W3110, complete genome

Header

AGCTTTTCATTCTGACTGCAACGGGCAATATGTCTCTGTGTGGATTAAAAAAGAGTGTCTGATAGCAGCTTCTGAACTG
GTTACCTGCCGTGAGTAAATTTAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCAGCCGGGGTCCCGCTGGCGCAATTGAAAACCTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA

- **FASTQ Format**

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

CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFBBBBFFGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGGGGGGHHHHGGGGGGHHHHGHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG

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GTTACCTGCCGTGAGTAAATTTAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACCTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA
```

Sequence

- **FASTQ Format**

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

```
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
```

+

```
AAABBFBBBBFFGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGGHHHHGGGGGGHHHHGHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG
```

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GTTACCTGCCGTGAGTAAATTTAAATTTTATTGACTTAGGTCACTAAATACTTTAACCAATATAGGCATAGCGCACAGAC
AGATAAAAATTACAGAGTACACAACATCCATGAAACGCATTAGCACCACCATTACCACCACCATCACCATTACCACAGGT
AACGGTGCGGGCTGACGCGTACAGGAAACACAGAAAAAAGCCCGCACCTGACAGTGCGGGCTTTTTTTTTTCGACCAAAGG
TAACGAGGTAACAACCATGCGAGTGTTGAAGTTCGGCGGTACATCAGTGGCAAATGCAGAACGTTTTCTGCGTGTTGCCG
ATATTCTGGAAAGCAATGCCAGGCAGGGGCAGGTGGCCACCGTCCTCTCTGCCCCGCCAAAATCACCAACCACCTGGTG
GCGATGATTGAAAAAACCATTAGCGGCCAGGATGCTTTACCCAATATCAGCGATGCCGAACGTATTTTTGCCGAACTTTT
GACGGGACTCGCCGCCGCCAGCCGGGGTTCCCGCTGGCGCAATTGAAAACCTTCGTCGATCAGGAATTTGCCCAAATAA
AACATGTCCTGCATGGCATTAGTTTGTGGGGCAGTGCCCGGATAGCATCAACGCTGCGCTGATTTGCCGTGGCGAGAAA
```

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CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTGCGGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBBFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGGHHHHGGGGGGGGHHHGHFFHHHHHHGHGGGGGGGGGGHHHHHHHHHHHHGGG
```

**Quality
Score**

FASTQ: Format

- **FASTA Format**

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

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

1. Demultiplex*
2. Quality Control
3. Trimming (adapter and quality)
4. Filtering**

* Often done at sequencing facility

** Must keep reads in sync

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Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
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A Shredded Library

he needs it to sleep somewhere. --Needs! Mr Bloom ejaculated, professing not the least surprise at 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,

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Mapped Fragments

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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
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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
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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
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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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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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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
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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      gcctaAGCTAA
+r004      ATAGCT.....TCAGC
-r003      ttagctTAGGC
-r001/2    CAGCGGCAT
```

SAM/BAM: Format

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

Read Pair

SAM/BAM: Format

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

**Chimeric
Read**

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
```

Split
Alignment

SAM/BAM: Format

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

@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

he needs it to sleep somewhere. --Needs! Mr Bloom ejaculated, professing not the least surprise at -- 1144052 Ulysses
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

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Book	Fragment Count
A Tale of Two Cities	8
Huckleberry Finn	5
Alice's Adventures	3
Anna Karenina	9
Ulysses	4

-- 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

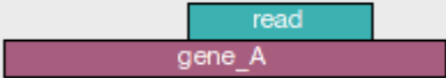
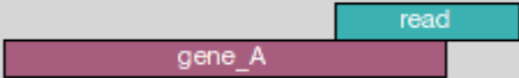


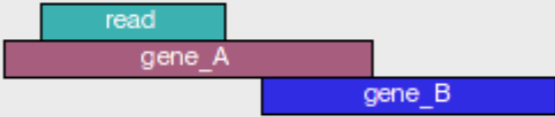
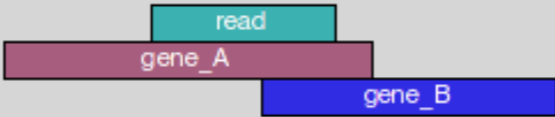

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-- 78968 Alice's Adventures
 -- 80787 Alice's Adventures
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 -- 803339 Ulysses
 -- 870689 Ulysses
 -- 1144052 Ulysses

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

Tophat

