RNA-Seq Bioinformatics Overview

From Raw Sequence to Gene Counts

Why RNA-Seq?

- Transcriptome: "Which genes are expressed in this sample?"

  - Differential Expression

  - Genome Annotation

- SNPs

- Gene Fusions

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- Transcriptome: "Which genes are expressed in this sample?"

  - Differential Expression

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In a Nutshell

Input
Sequences of RNA
Fragments

Output
Counts of Gene
Fragments
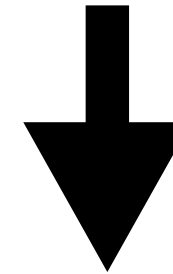
In a Nutshell

Input
Sequences of RNA
Fragments

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+
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@M00698:36:000000000-AFBEL:1:1101:15340:1413 1:N:0:0
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+
AABABFFFFFFCGGGGGGGGGGGFHHGFHHGFHGGGGGHHHHHGGGGFHGGFGGHHHCFGFFEFCGHHFHHHHGGAFGGFFFFHHHHHHHHHHHHGEEGGGHHB
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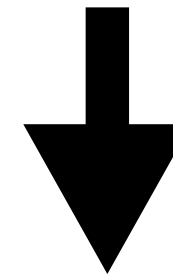
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+
AABABFFFFFFCGGGGGGGGGGGFHHGFHHGFHGGGGGHHHHHGGGGFHGGFGGHHHCFGFFEFCGHHFHHHHHGGAFGGFFFFHHHHHHHHHHHHGEEGGGHHB
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Output
Counts of Gene Fragments

Gene Name	Read Count
Gene A	5
Gene B	11
Gene C	0
Gene D	2

A Biblio-Archeologic Analogy

Fragments of a Library

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here were no proper cupboards for their clothes; what cupboards there were either would not close at -- 634360 Anna Karenina
ef secretary. She knew that he usually went out quickly to his office, and she wanted to see him bef -- 782396 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
t. All her arrangements had to be modified because they could not be carried out, and they were modi -- 1252025 Anna Karenina
ns in general, and I believe that's just why philanthropic institutions always give such poor resu   -- 1699588 Anna Karenina
oys running, playing at horses. Seryozha! And I'm losing everything and not getting him back. Yes,   -- 1837882 Anna Karenina
he nursery. And he was indeed crying. She heard him and hastened. But the faster she went, the loude -- 1897819 Anna Karenina

ides her body's flaws calling under her brown shawl from an archway where dogs have mired. Her fancy -- 90509   Ulysses
eupon Punch Costello dinged with his fist upon the board and would sing a bawdy catch _Staboo Stabel -- 803339 Ulysses
spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 870689 Ulysses

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
here were no proper cupboards for their   lose at -- 634360 Anna Karenina
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 _Staboo_ 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

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   e he -- 235828 A Tale of Two Cities
prison--seemed to strike across the eart   ry m -- 340478 A Tale of Two Cities
him to act in anything without her quiet   he e -- 490073 A Tale of Two Cities
 Indivisible. Liberty, Equality, Fratern   ner -- 562830 A Tale of Two Cities
recatory manner, "the anguish of his dau   e ob -- 682808 A Tale of Two Cities

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

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

 him. I could not look at him without fe   , -- 165528 Anna Karenina
er hand shook more violently, but she di   it. -- 455094 Anna Karenina
here were no proper cupboards for their   e at -- 634360 Anna Karenina
ef secretary. She knew that he usually w   bef -- 782396 Anna Karenina
h to intensify the influence of her gaze   eara -- 1186098 Anna Karenina
t. All her arrangements had to be modifi   modi -- 1252025 Anna Karenina
ns in general, and I believe that's just   su -- 1699588 Anna Karenina
oys running, playing at horses. Seryozha   s, -- 1837882 Anna Karenina
he nursery. And he was indeed crying. Sh   oude -- 1897819 Anna Karenina

ides her body's flaws calling under her   ancy -- 90509   Ulysses
eupon Punch Costello dinged with his fist upon the board and would sing a bawdy catch _Staboo Stabel -- 803339 Ulysses
spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 870689 Ulysses
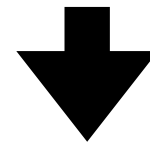
Book	Durham	Chapel Hill
A Tale of Two Cities	8	17
Huckleberry Finn	5	2
Alice's Adventures	3	1
Anna Karenina	9	2
Ulysses	3	2

RNA-Seq
for
Differential Expression Analysis

Bioinformatic Analysis

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Gene 1	0	9		2
Gene 2	5	0		12
…				
Gene M	0	0		3

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

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

FASTQ

- FASTA with Quality

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

FASTQ: Format

- **FASTA Format**

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- **FASTQ Format**

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FASTQ: Format

- **FASTA Format**

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

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- **FASTQ Format**

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FASTQ: Format

- **FASTA Format**

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- **FASTQ Format**

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FASTQ: Format

- **FASTA Format**

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- **FASTQ Format**

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Quality Score

FASTQ: Format

- **FASTA Format**

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- **FASTQ Format**

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FASTQ: Read Files

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

Quality Scores

Character	Phred Score	Probability of Error
I	40	10^{-4}
?	30	10^{-3}
5	20	10^{-2}
+	10	10^{-1}
&	5	0.32

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
+
AAABBFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

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
+
CCCCCFFFFBFFGGGGGGGGGCECGHHHHHHHHHHGGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

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
```

Demultiplexing

Undetermined_S0_L001_R1_001.fastq.gz
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHGHGHGHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG

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
+
CCCCCFFFBFFGGGGGGGGGCECGHHHHHHHHHGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHGHHHHHHHHHHHHEHGHHHGHHHGGGGGGG

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

Undetermined_S0_L001_R1_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

Undetermined_S0_L001_R2_001.fastq.gz

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

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

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

Demultiplexing

Undetermined_S0_L001_R1_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGHHHHHHHGHGHGHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

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
+
CCCCCFFFBFFGGGGGGGGGCECGHHHHHHHHHGHGGGGHGGCGCHHGFHGGGGHGGGGGHHHHHGHHHHHHHHHHHHEHGHHHGHHHHGGGGGGG
```

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

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

Demultiplexing

Undetermined_S0_L001_R1_001.fastq.gz
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG

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
+
CCCCCFFFFBFFGGGGGGGGGCECGHHHHHHHHHGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHHGHHHHHHHHHHHHEHGHHHGHHHGGGGGGG

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

Undetermined_S0_L001_R1_001.fastq.gz
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
TTACGCTAACAGGCGGTAGCCTGGCAGGGTCAGGAAATCAATTAACTCATCGGAAGTGGTGATCTGTTCCATCAAGCGTGCGGCATCGTCAAAACGCCC
+
ABBBABBBAFFFGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG

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
+
CCCCCFFFBFFGGGGGGGGGCECGHHHHHHHHHGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHGHHHHHHHHHHHEHGHHHGHHHGGGGGGG

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

8B_N

Demultiplexing

Undetermined_S0_L001_R1_001.fastq.gz

```
@M00698:36:000000000-AFBEL:1:1101:14738:1412 1:N:0:0
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+
ABBBABBBAFFFGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAAABBFFFFFFFGGGGGGGGGGHHHHHHHGHGHGHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
```

Undetermined_S0_L001_R2_001.fastq.gz

```
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+
ABBBABFFFFDBDGCG??FFGGGHGHFEG3EAEGGFHAE3GFBGGHGGGHHCFGHFGBGHFHHDFEGGHFHEFHHHH3BFGF0GFEGGGGGHHA/FGHFHHH
@M00698:36:000000000-AFBEL:1:1101:16483:1412 2:N:0:0
GCTTCTTCCGTACTCATGCGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCTGGTGTGAAACCTCCACGCCGGAT
+
CCCCCFFFBFFGGGGGGGGCECGHHHHHHHHGHGGGGHGGCGCHHGFHGGGGHHGGGGGHHHHHGHHHHHHHHHHHHEHGHHHGHHHGGGGGGG
```

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
```

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

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+

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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

+

AAABBFFFFFFFGGGGGGGGGGGGGHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGGHHHHHHHHHHHGGG

8B_N_R2_001.fastq.gz

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

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+

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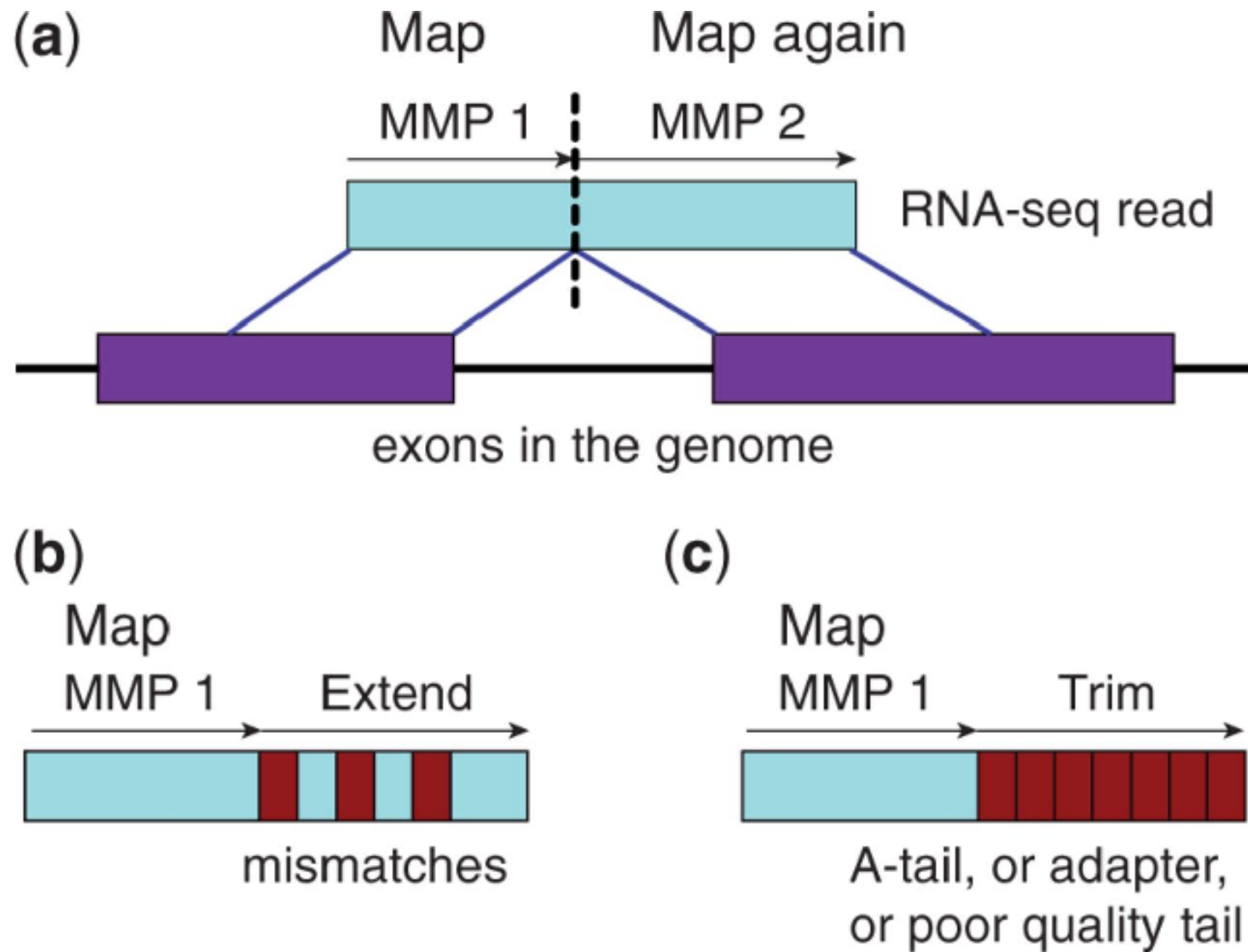| Step	Input	Output	Output Format
Pre-processing and QC	Raw Reads	Processed Reads	FASTQ
Mapping	Reads	Mapped Reads	SAM/BAM
Counting	Mapped Reads	Count Table	CSV

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
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```

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 (includes 10x specific TAGs)

SAM/BAM: Format

```
Coor        00000000011111   1111122222222223333333333444444
            12345678901234   567890123456789012345678901234 5
ref         AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1           TTAGATAAAGGATA*CTG
+r002          aaaAGATAA*GGATA
+r003       gcctaAGCTAA
+r004                         ATAGCT..............TCAGC
-r003                           ttagctTAGGC
-r001/2                                   CAGCGGCAT
```

SAM/BAM: Format

```
Coor        00000000011111   11111222222222233333333334444444
            12345678901234   567890123456789012345678901234
ref         AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1           TTAGATAAAGGATA*CTG                          Read Pair
+r002          aaaAGATAA*GGATA
+r003       gcctaAGCTAA
+r004                      ATAGCT..............TCAGC
-r003                          ttagctTAGGC
-r001/2                                         CAGCGGCAT
```

SAM/BAM: Format

```
Coor        00000000011111   11111222222222233333333333344444
            12345678901234   56789012345678901234567890012345
ref         AGCATGTTAGATAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT
+r001/1           TTAGATAAAGGATA*CTG
+r002         aaaAGATAA*GGATA
+r003      gcctaAGCTAA
+r004                         ATAGCT..............TCAGC
-r003                              ttagctTAGGC
-r001/2                                        CAGCGGCAT
```

```
@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

See a Real BAM

- `samtools view /hpc/group/chsi-mic-2022/data/pbmc8k/pbmc8k/outs/possorted_genome_bam.bam | less -S`

```
ST-K00126:314:HFYL2BBXX:7:1110:19837:23909    16    chr1    11919    1    98M    *    0    0    ATTTGCTGTCTCTTAGCCCAGACTTCCCGTGTCCTTTCCACCGGGCTTTTGAGAGGTCACAGGGTCTTGATGCTGTGGTCTTCATCTGCAGGTGTCTG    --A<<JFJA--A<7F<<FJFJ7FFJA77JJFJJAAJJJJJFJAJJFFFJFJJFAJJ<JFJJJFJJ7JFJJJJJAJ<JJ<FFJJ<-J<JP<FFJFF-AA    NH:i:4    HI:i:1
ST-K00126:314:HFYL2BBXX:8:2208:9161:21588     16    chr1    11973    1    98M    *    0    0    GGTCACAGGGTCTTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAAGCTGAGCACTGGAGTGGAG    JJJJJJJJJJJJJJJJJFJFJFJJJJJFJJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJFJJJFJJFJFJJJJAJF<<<A     NH:i:4    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1110:17056:1015     16    chr1    11973    1    98M    *    0    0    GGTCACAGGGTCTTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAAGCTGAGCACTGGAGTGGAG    JJFJJFJFFAJJJJJJAJJF-AAF-FFJFFJJJJFFJAFFFJFFJJJJJJJJFJJJFJJJFAJAJA<<FJFJJJ-A<AJJAAJFFFJA77JFFF<A     NH:i:4    HI:i:1
ST-K00126:314:HFYL2BBXX:8:2109:20831:46170    16    chr1    11979    1    98M    *    0    0    AGGGTCTTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAATCTGAGCACTGGAGTGGAGTTTTCC    )-F<--FJA<J<AJJFJFP<JJFFJAJAF<PJJFFFJA7-FFFFFJJFJFJJJFJ<JF7FF7JJA<JJFA77-JF<FJ<AJJFAA-A-JJ<JAA<<-    NH:i:3    HI:i:1
ST-K00126:314:HFYL2BBXX:8:1223:21602:40192    16    chr1    11979    1    98M    *    0    0    AGGGTCTTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAAGCTGAGCACTGGAGTGGAGTTTTCC    JF<F7JJJJJJJFJFJJF7JA<AJFFFAFA<A7FJFFF<JJ<<A<7FFJJJFJFFJJJJFFJA7JJFJJA7FJ<JJ<-FAFFJJAJFJJJJJJFFFA-    NH:i:3    HI:i:1
ST-K00126:314:HFYL2BBXX:8:1105:19958:32349    16    chr1    11979    1    98M    *    0    0    AGGGTCTTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAAGCTGAGCACTGGAGTGGAGTTTTCC    JJJJJJJJJJJJJJJJJJJJJFFJJJJJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJJFF<7A     NH:i:3    HI:i:1
ST-K00126:314:HFYL2BBXX:7:2219:19248:22995    16    chr1    11982    1    98M    *    0    0    GTCTTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAAGCTGAGCACTGGAGTGGAGTTTTCCTGT    FJJJJJJJJJJJJJJJJJJJJJJJJJFJJJJJFJJJJJJJJJJJFFJFJFJFJJJJJJAJFJFJJJJAFFJFJFAAAAFJA<AJJF<AJJJJA<FF<A    NH:i:3    HI:i:1
ST-K00126:314:HFYL2BBXX:7:2219:30411:8893     16    chr1    11985    1    98M    *    0    0    TTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAAGCTGAGCACTGGAGTGGAGTTTTCCTGTGGA    JJJAJJJJJFAJJJJJ7JFJJJJAFFJJJJJAJFFAJJJFFAJJJJ<JFFFJJJJFJ<JFJFFJ<FJJJFJFJFJJFA-JJ7FFJJJFJ<FJA<<<A    NH:i:3    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1225:5629:7345      16    chr1    11985    1    98M    *    0    0    TTGATGCTGTGGTCTTCATCTGCAGGTGTCTGACTTCCAGCAACTGCTGGCCTGTGCCAGGGTGCAAGCTGAGCACTGGAGTGGAGTTTTCCTGTGGA    JJJJJJJJJJJJJJJJJJJJJJJJJFFJJFFAJAFJJJJFFJJJFAFJJJJJFFJJJJJJJFAJJJJJJJJAJJFFFAA     NH:i:6    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1102:8186:29466     16    chr1    12117    0    98M    *    0    0    TCATCTTCTGGCCCCTGTTGTATGCATGTAACTGAATACCACAACCAGGCATAGGGGAAAGATTGGAGGAAAGATGAGTGAGAGCATCAACTTCTCTC    7<<FJJJJJJ<J<A--AFFFAAFFFJJJJJJJAA-JJFFFFJJJJJJJFJJJJJJJJJJJJJJJJFJF<<JJJAJFJJJJAJJJFJJJJJJJJFJPJFFFF<<<A    NH:i:6    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1210:3194:38433     16    chr1    12118    0    1S97M  *    0    0    ACATCTTCTGGCCCATGTTGTATTCATGTAACTTAATACCACAACCAGGCATAGGGGAAAGATTGGAGGAAAGATGAGTGAGAGCATCAACTTCTCTC    --7-FAJAJJFF<7-<JFJF-7F7-77A7-A<AAJ<FFJ-FAAA7-FAFFJJFJFF<FJ--<-<JJFFJFAA7FJF<--<JFJJJF<7JJJJJFA<AA    NH:i:6    HI:i:1
ST-K00126:314:HFYL2BBXX:8:2125:2098:7345      0     chr1    13982    0    98M    *    0    0    CCTGCAGCCTTGCTCCCTAACCTGCCCCACAGCCTTGCCTGGATTTCTATCTCCCTGGCTTCGTGCCCGTTCCTCCAAGTCGATGGCCACCTCCCTCCC    <<<<<FAJFJJJFFFJFJJJJJJJFJJJJJJJJJJJJFAFFJJF--FFJJJ<FFJFJFA77<F-F-7F7-7A<AF--AF-J-FJAAFFA----7F)<)<A    NH:i:6    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1215:9485:36921     0     chr1    13985    0    98M    *    0    0    GCAACCTTGCTCCCTAACCTGCCCCACAGCCTTGCCTGGATTTCTATCCCCCTGGCTTGGTGCCCGTTCCTCCAATTCGATGCCACCTCCCTCCCTCT    -A--<-<-7AJ<JJAJ<7AF-7A-AFA--777--77<--77-7FF<-7-A-7AA-7A<7--7AA--7--777<<7--7--FF-7<-A7-FFJ<-)))-    NH:i:6    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1214:4401:15487     0     chr1    13990    0    98M    *    0    0    CTTGCTCCCTAACCTGCCCCACAGCCTTGCCTGGATTTCTATCTCCCTGGCTTGGTGCCCAGTTCCTCCAAGTCGATGGCACCTCCCTCCCTCTCAACC    AAAFFJJJJJJJJJJFJJJJJ<JJJJJFJ<J7JAJJJJJJJJJJJJJJJJJJJJJJJFJJJJJJJFJJJJ<JJJFJJJJJJJJJFJJ     NH:i:6    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1125:25296:39101    0     chr1    13990    0    98M    *    0    0    CTTGCTCCCTAACCTGCCCCACAGCCTTGCCTGGATTTCTATCTCCCTGGCTTGGTGCCAGTTCCTCCAAGTCGATGGCACCTCCCTCCCTCTCAACC    -A7FFJJJJJJJJJJJJJJJJJJJAJJJJJJJJJF7JFFFJJJJJJJJJJJJFJ<FJ<-F-AJFFJJJJAFJJ<F<FFAFJJJFFJJJFJJJ<--FFJ    NH:i:6    HI:i:1
ST-K00126:314:HFYL2BBXX:8:1217:20862:36974    0     chr1    13996    0    98M    *    0    0    CCCTAACCTGCCCCACAGCCTTGCCTGGATTTCTATCTCCCTGGCTTGGTGCCAGTTCCTCCAAGTCGATGGCACCTCCCTCCCTCTCAACCAACTTGA    -A<FFJJJJJJJJJJJJJJJF7A7AAFAJAFFAFFF7FFFJ-<AA<7<-<FJJAAJFJ<JJAFF7FF<F-AAFJJJAJF<AJ<AFF<<FJ<JJJFAF    NH:i:5    HI:i:1
ST-K00126:314:HFYL2BBXX:8:2108:19613:23241    0     chr1    14005    0    98M    *    0    0    GCCCCACAGCCTTGCCTGGATTTCTATCTCCCTGGCTTGGTGCCAGTTCCTCCAAGTCGATGGCACCTCCCTCCCTCTCAACCACTTGATCAAACTCC    -<7-<FJFAJJJJFJJJJJ<AFF--7AFF--77<J7FFAJJ-F<FAAA-JJFFJFJJJJFFFF-A<FJ-A-<F7FAF<7-FJFJJJAA--7-FJJ---    NH:i:5    HI:i:1
ST-K00126:314:HFYL2BBXX:8:1219:25905:38328    0     chr1    14005    0    98M    *    0    0    GCCCCACAGCCTTGCCTGGATTTCTATCTCCCTGGCTTGGTGCCAGTTCCTCCAAGTCGATGGCACCTCCCTCCCTCTCAACCACTTGAGCAAACTCC    AAFFFJJJJJJJJJJJJJFJJJJJJJJJJJJJJJJJJJJJJJJFJFFJFJJJJJJJFJJFFFJJJJFJJFFFJJJJJJFJJJJFFJJA<AJ     NH:i:5    HI:i:1
ST-K00126:314:HFYL2BBXX:8:1223:10379:2633     0     chr1    14014    0    98M    *    0    0    CCTTGCCTGGATTTCTATCTCCCTGGCTTGGTGCCAGTTCCTCCAAGTCGATGGCACCTCCCTCCCTCTCAACCACTTGAGCAAACTCCAAGACATCT    A<<FFFJJJJJJJJJJFJJJJJJFJJJJJFFFJJJJFJJJFJJJJJ-AFJFA<FJJ7<JA<FFJJJJJJ7JJAAFJ<FAAJJJFJFJAFFJJJJJ<JJJ    NH:i:5    HI:i:1
ST-K00126:314:HFYL2BBXX:7:1228:5842:37994     0     chr1    14026    0    95M3S  *    0    0    TTCTATCTCCCTGGCTTGGTGCCAGTTCCTCCAAGTCGATGGCACCTCCCTCCCTCTCAACCACTTGAGCAAACTCCACGCCATCTTCTACCCCACCC    A-<F77AJJAFJF-<AFF<F-FJ<F-F7FJAA-FJJ-F-7F<FJJJ<<AFFFJA<JJJ-7FF-AAJJ--777--7F7<-F-7F7FJ<A7AJ<AJ))-A    NH:i:6    HI:i:1
```

See a Real BAM

- `samtools view /hpc/group/chsi-mic-2022/data/pbmc8k/`
  `pbmc8k/outs/possorted_genome_bam.bam | less -S`

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

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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

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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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Counting

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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
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spectacles offered by our streets, hideous publicity posters, religious ministers of all denominatio -- 870689 Ulysses
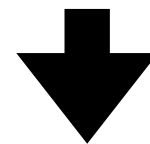
Counting

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 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

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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

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

scRNA-Seq

scRNA-Seq Bioinformatic Analysis

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+
ABBBABBBAFFFGGGGGGGGGGGHGGHGGGCG2GF3FFGHHHHHHGGFGHEHHGGGEHHHHAGGHHGHHHFFDHFHHHGEGGGG@F@H?GHH/GBEFGGG
@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGGHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGGHHHHHHHHHHHGGG
@M00698:36:000000000-AFBEL:1:1101:15928:1413 1:N:0:0
GTAAAGTCCTGAGTGATACCGGCAACTTTTACCCCCAGTCCCACTTTCGAACCGGCAAACATATCGGCAAAAGAGGCCGTGCCTGATTTAAAGCCGTAGGT
+
```

	Cell 1	Cell 2	…	Cell N
Gene 1	0	9		2
Gene 2	5	0		12
…				
Gene M	0	0		3

Bioinformatic Analysis

| Software	Step	Input	Output
cellranger mkfastq	• Convert • Demultiplex	BCL	FASTQ
cellranger count	• QC • Mapping • Counting • Cell Barcode • UMI	FASTQ	• BAM • Count Matrices • QC Metrics

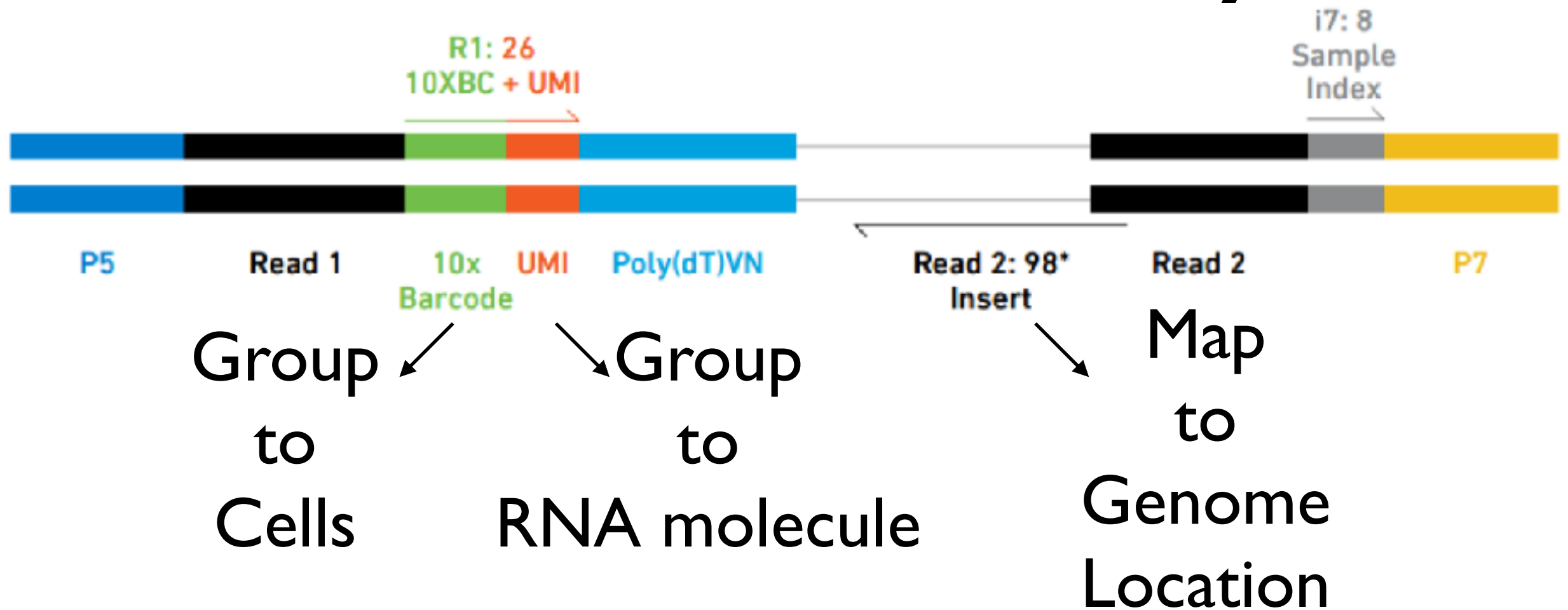# Multiplexing (Barcodes)
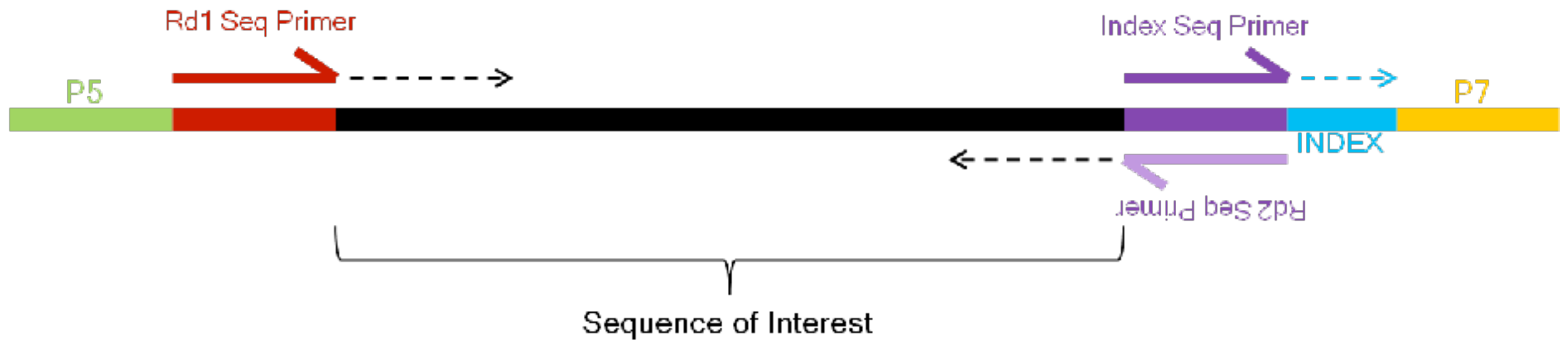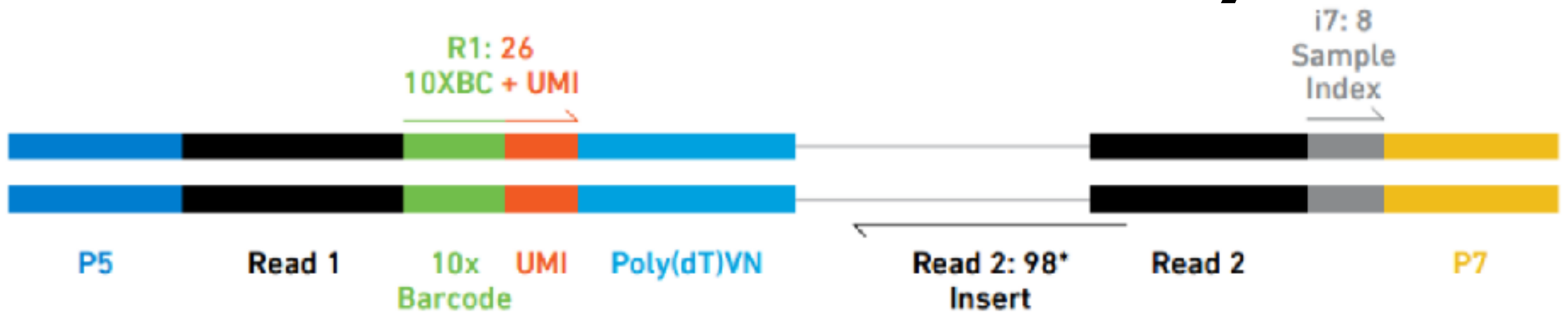
Bioinformatic Analysis

Bioinformatic Analysis

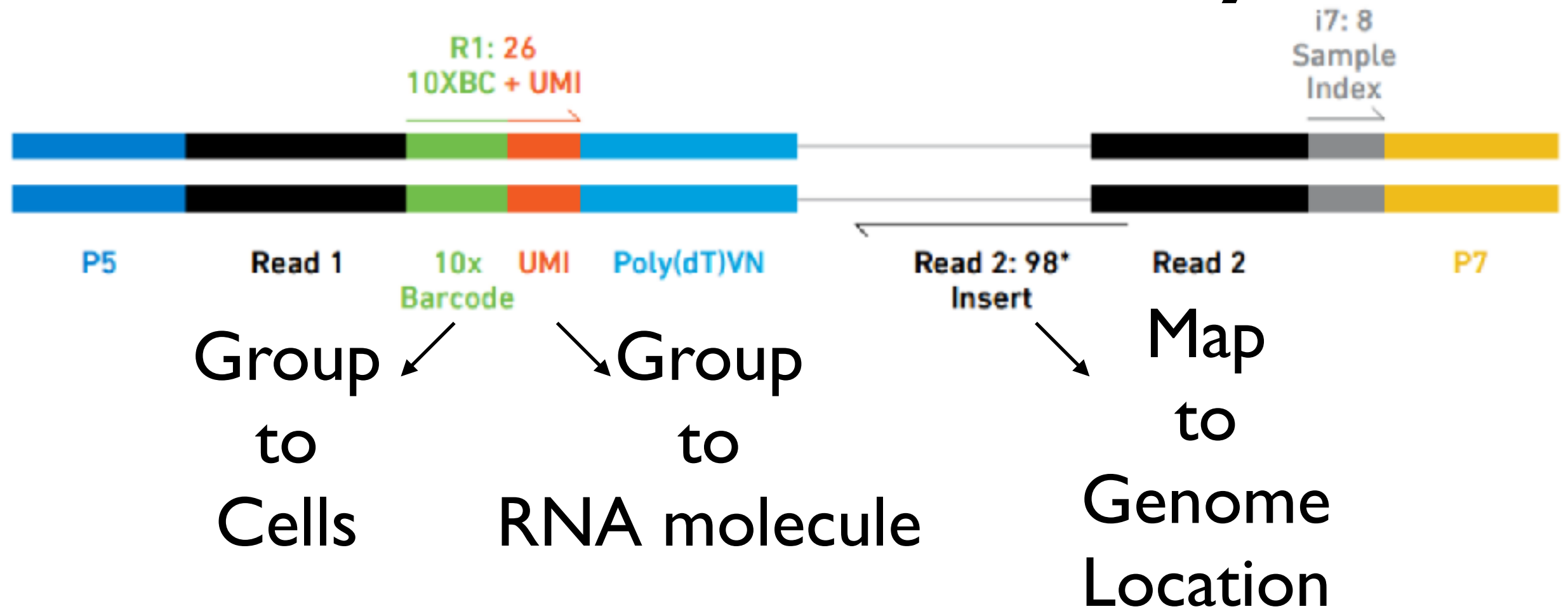# Bioinformatic Analysis

Bioinformatic Analysis

https://davetang.org/muse/wp-content/uploads/2018/06/10x_library_fragment.png
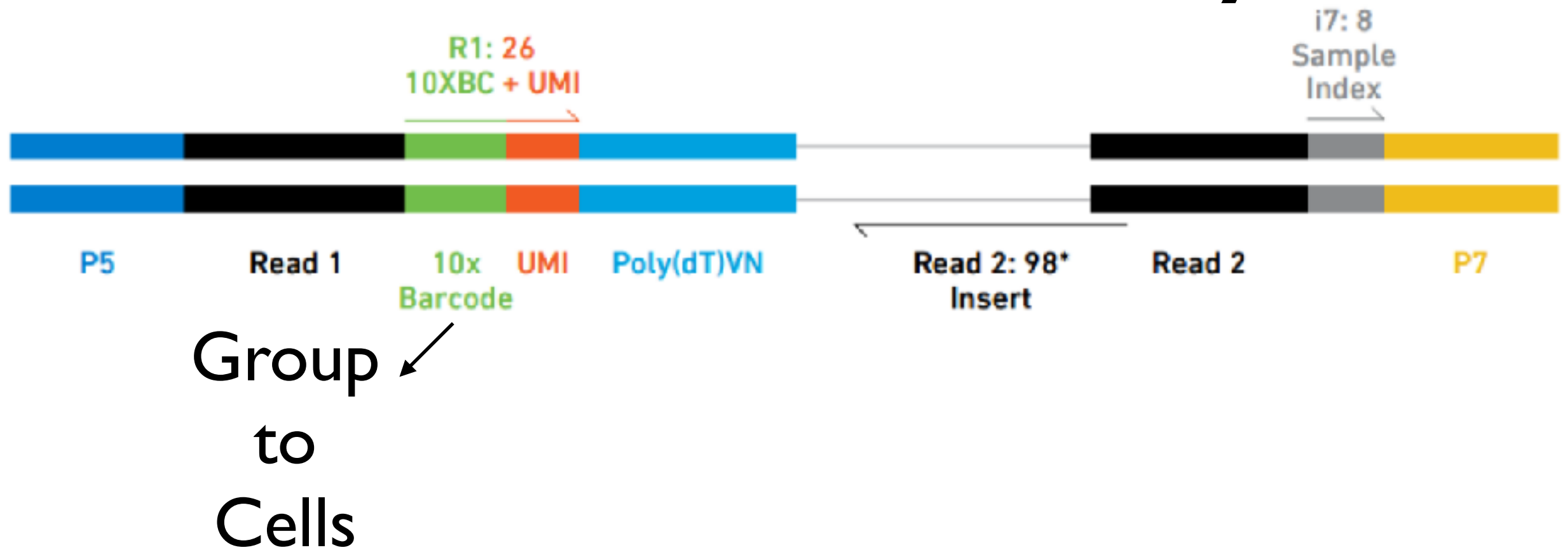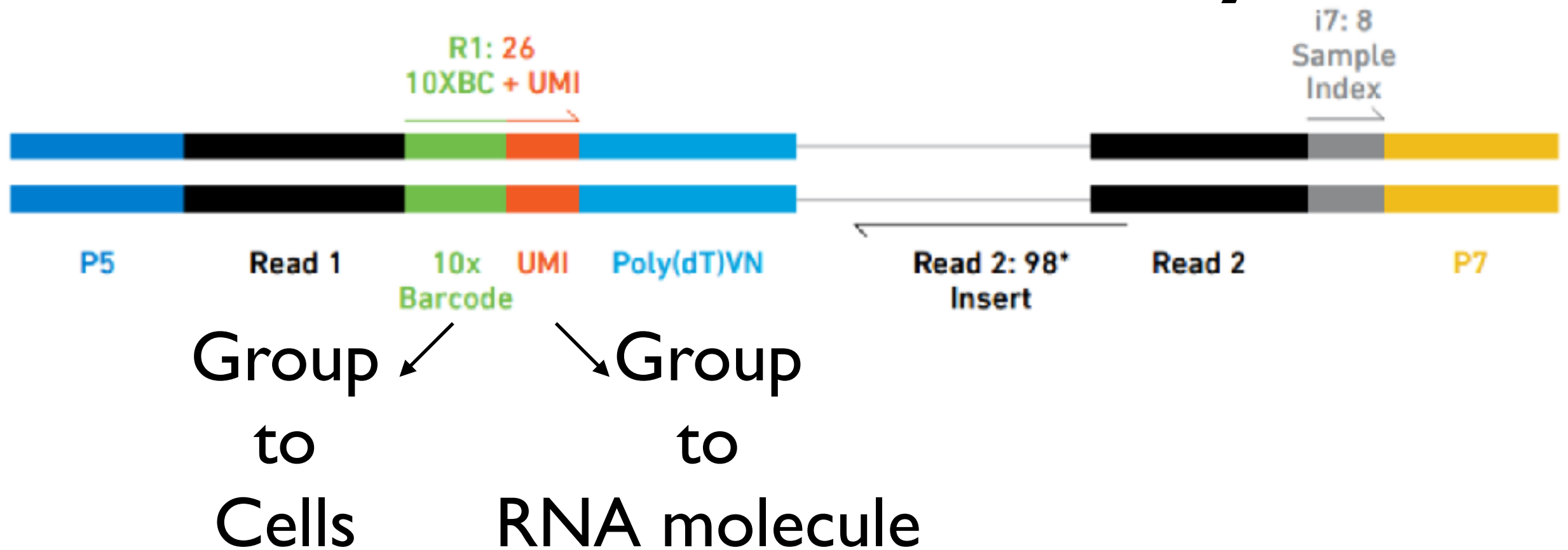
Bioinformatic Analysis

Filename	Read Type	Information
*_R1_001.fastq.gz	Forward	Cell Barcode and UMI
*_R2_001.fastq.gz	Reverse	Expressed RNA
*_I1_001.fastq.gz	Index	Sample (Index) Barcode

Key Details

Goldilocks & the Three Droplets
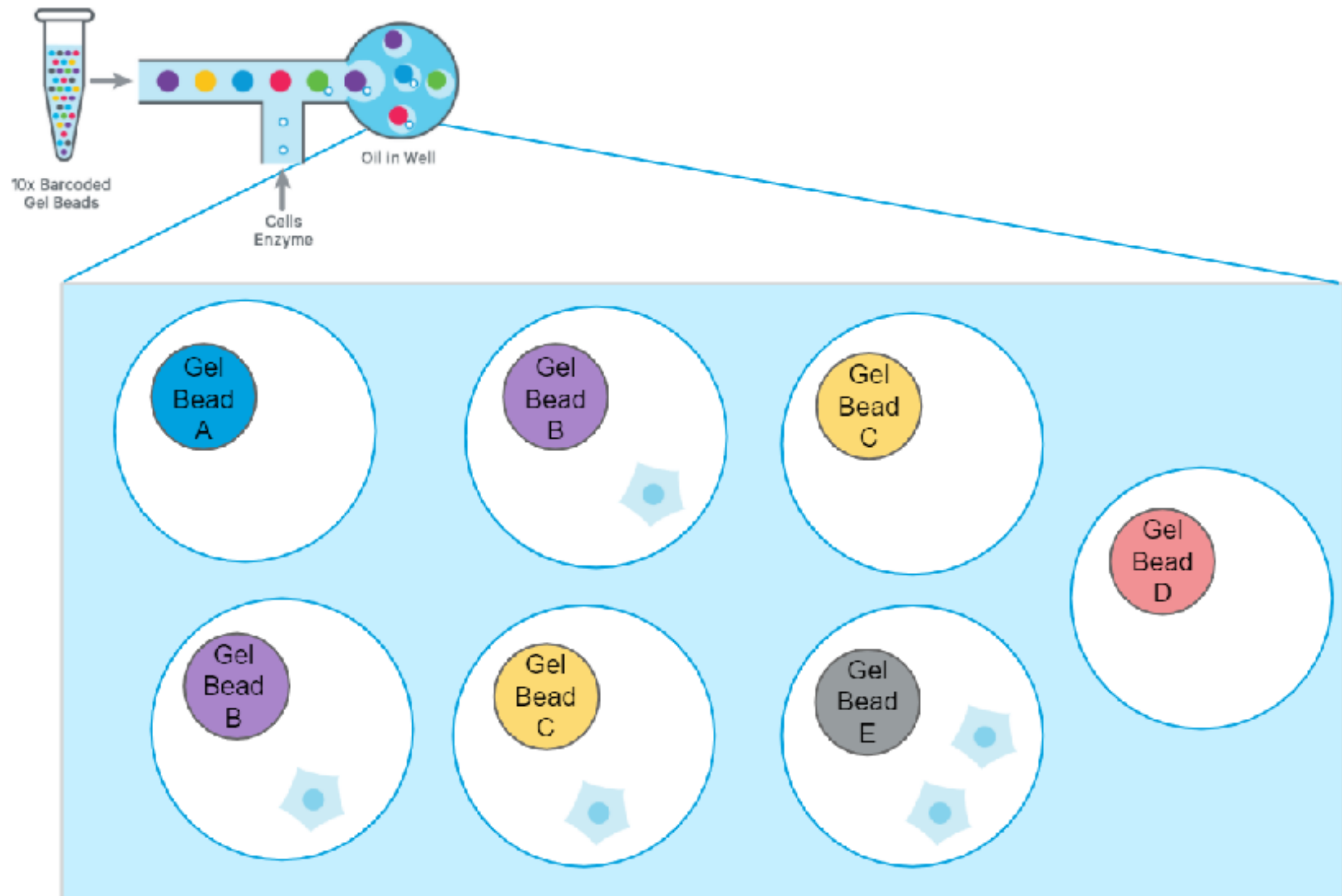
Goldilocks & the Three Droplets

Number of Cells	Expected Multiplet Rate (%)*
500	~0.4
1000	~0.8
5000	~3.9
10000	~7.6

Reference Genomes

- Genome Sequence (FASTA) and Genome Annotation (GTF) *Must be Built Specifically for cellranger*

- Download:

  - https://support.10xgenomics.com/single-cell-gene-expression/software/release-notes/build

- Build Your Own

  - `cellranger mkref`

  - https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/advanced/references