# **DNA Sequencing**

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# Nanopore Sequencing

## **Input Nanopore Sequencing Data (FASTQ File)**

```
fastqinfo('lambda_nanopore.fastq')
```

```
ans = struct with fields:
```

Filename: 'lambda\_nanopore.fastq'

FilePath: 'C:\Users\kabil\OneDrive - Drexel University\Academic\3 - Pre-Junior\1 - Fall Quarter\BMES 375

FileModDate: '14-Oct-2021 22:56:12'

FileSize: 10926131 NumberOfEntries: 947

#### reads = fastqread('lambda\_nanopore.fastq')

#### reads = $1 \times 947$ struct

Fields	Header	Sequence	Quality
1	'fdf910a5-7	'GTTGTGT	'/+++\$\$#
2	'a41fe7de-f	'AGTATGC	'#\$&#'&*</th></tr><tr><th>3</th><th>'baabbce4-9</th><th>'CACTAGG</th><th><b>'</b>\$.%'(.8</th></tr><tr><th>4</th><th>'42be897d-8</th><th>'CGTGTAC</th><th>'&\$#%'&</th></tr><tr><th>5</th><th>'9a42ec01-c</th><th>'ACTCTAT</th><th>'+&(.752</th></tr><tr><th>6</th><th>'3cd8595d-4</th><th>'GACTCGT</th><th>'&/%*.13</th></tr><tr><th>7</th><th>'56468e96-9</th><th>'ATTGCTA</th><th>'/**+-11</th></tr><tr><th>8</th><th>'5c1f6b93-0</th><th>'GTTACTA</th><th>'+)),*-G</th></tr></tbody></table>

Fields	Header	Sequence	Quality
9	'863bdf10-0	'ATGCTGT	'/##&'+0
10	'6fa01687-d	'CAGTATA	"#%)%#&
11	'ccc2bb13-2	'AGCTGAG	'+%,3698
12	'4282edf2-b	'ACTATTG	'/06556%
13	'50cd3e3d-3	'CTGTTTA	'\$"%%&%#
14	'd10220d5-d	'CATTCAC	'\$#"\$%#%
15	'1a10a492-6	'CGGTACT	'*%)*'6=
16	'7dc543fb-0	'GTTGTAC	')%(()02
17	'475608a9-b	'GTATGCT	'\$'\$\$\$,/
18	'e06a5c44-9	'ATTGCTG	'.\$%\$.76
19	'57d2b26a-2	'CGGTGTA	"\$%%%\$,
20	'8bc2f25a-1	'CACTTTA	'+/\$\$\$/8
21	'5bfdc711-8	'CACTTTA	'#*356BF
22	'99bf0895-c	'GTTGTAC	",/(-*1
23	'f30fcb5b-0	'CAGTATG	'%")&%#
24	'2b76be60-3	'GTTGTAC	'\$'+65;=
25	'4826d568-6	'GTTGTAT	'&%40/3)
26	'bd6ec2d0-5	'GTTGTAC	'%&\$\$\$
27	'65decd3f-c	'CGGTATG	'+%+-,))
28	'c0d54cc6-8	'CGGTATG	'+\$)-*)&
29	'dc5564fe-8	'GGTGTAC	'\$&\$#+-2
30	'6b3efec0-3	'ACTGAGC	'%')(**6
31	'5322227c-1	'CACTCGT	"+9;;2*
32	'23705b9e-5	'CAGTGTA	<b>'*</b> #( <b>'\$</b> %,
33	'7a635d85-3	'ACTGTAA	',\$\$#),+
34	'6b90d19d-5	'GGTATAC	'\$###\$#%
35	'9770040d-0	'CGATGTG	'&\$#%%&%
36	'75ba1b7c-5	'GTTGTAC	'\$'(\$\$%&
37	'918011e9-5	'GGTAGCT	'&'(&\$#(
38	'8ae02f94-c	'GATGTAC	'%\$+'"4
39	'85fb32ed-c	'ACTATTG	'-'0:8F5
40	'eb98718e-9	'CGATGTA	'+(###&
41	'ac9ae36b-1	'CGCTTCG	'##%'++/

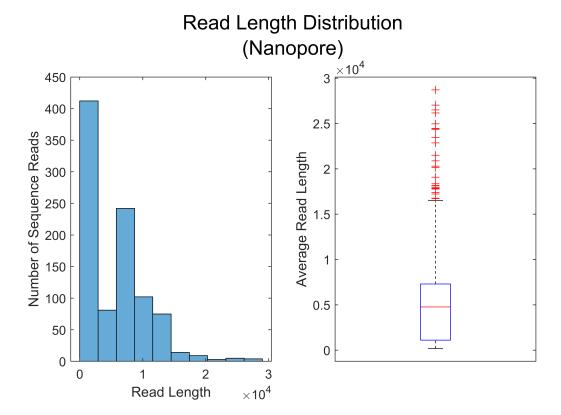
Fields	Header	Sequence	Quality
42	'4a42bec8-6	'CATTGTA	'\$#%')(&
43	'71d2f951-b	'CACTCTA	',+),06E
44	'b299d04f-7	'ATCATTG	'.%%\$%%%
45	'4e3c1746-a	'CGCATGG	"\$%-9;@
46	'53ab9c74-4	'GTTGTAC	'+45,,-,
47	'54c537c1-4	'CAGTGAC	'.#(\$%"%
48	'51169d15-c	'ACTGTGG	'-)+-7?A
49	'0bc40f6d-9	'CATTGTG	'%##+'%#
50	'035160b1-b	'CGTATTG	"%*5+++
51	'b37d0fc3-8	'CAATGCT	'.+&%%00
52	'705b9c80-4	'ACTGCTT	'0344))/
53	'e494ff3e-a	'CGGTATC	'("&,##
54	'ad586169-2	'CAATGTA	',%\$\$\$%*
55	'4005d8ae-3	'CAACACT	'&%%&')+
56	'2f2db06d-f	'CGGTAGC	'%&&%\$\$#
57	'9a5f92e1-d	'CGGTACT	'*&&('
58	'3ed9b28a-b	'CGGTATT	'0\$(%(\$%
59	'c27603ba-2	'GTTACTG	'&"2.3'
60	'ffbedb22-5	'CGGTATT	'.&))(\$%
61	'e2e40267-f	'GATGTAC	'%\$&\$%%)
62	'09bf2a04-f	'GGTATGC	'&&(&\$#%
63	'05d89c21-c	'GTTGTAC	'.67)+)4
64	'121e0af0-9	'GTTGTGC	'\$\$&%%(5
65	'9fff5139-9	'ATTGCTG	'3\$&&/7<
66	'e12c5c25-c	'CAAACTT	'\$&\$#"\$%
67	'6b7d8b39-4	'GTTGTAC	',#%#\$\$\$
68	'4489ecab-c	'GATGTGC	'\$#\$\$(&%
69	'1b42397a-e	'CATTGTA	""()'.,
70	'07dc7045-2	'CATTGTA	'-##\$%*1
71	'b2dabdf9-f	'CTTTAGT	'\$\$'(('%
72	'1549c5c2-b	'CAAACGT	'/-*%"#\$
73	'6ac83da8-3	'ACCATTC	'/#\$%\$/#
74	'124bfc77-a	'AGTATGC	'#\$&#\$\$'</th></tr></tbody></table>

Fields	Header	Sequence	Quality
75	'bb462264-8	'CGGTAGC	"\$%\$\$/0
76	'a3ac48d3-7	'GCGCTTT	'##\$%&''
77	'5470915f-e	'GTTCTTC	'*5/+(-2
78	'aca5810f-b	'GTTGTAA	'00.\$
79	'0025f8a3-9	'CAGTATA	'#&'\$%'%
80	'824f8dae-e	'CATTGTA	'\$#\$&%&&
81	'c0f5ef32-e	'GTTGTAC	'%02)(),
82	'2db70e97-7	'GTTGTAC	'-23/366
83	'fcfa06b7-6	'CTATGCT	'#\$%%\$12
84	'06d0d008-f	'CGGTGTA	')%\$%#%%
85	'fe768141-e	'ATCTATT	'.')0:9:
86	'30604dfc-a	'GTTGTAC	'),-\$));
87	'd7801ea9-c	'GGCATGC	'%#\$#\$"(
88	'f00524da-b	'ACTCTAT	'&.28C/+
89	'08a4e8dc-7	'CGGTGTA	"#%#\$&0
90	'ede50cae-6	'ACTGGGC	'201.'
91	'7d595274-1	'GTTGTGC	'-89))&\$
92	'42cb287b-6	'CACTATT	'\$.'3812
93	'07f28d79-a	'GTTGCTT	'%#\$#'/0
94	'ac18972e-0	'CGGTATT	'&\$\$%\$"\$
95	'a7dd4ba8-f	'GTTACTT	'-2(2667
96	'a497e1cc-b	'AAATTTG	'\$%#%))&
97	'ba4d675f-9	'GCTACTA	'%#%+-:>
98	'b44f057a-4	'CGTTGTA	'&%%'%\$%
99	'62cde812-b	'GTTGTAC	'&#%\$')+</td></tr><tr><td>100</td><td>'38179797-1</td><td>'CGTATGC</td><td>'%%&&*\$%</td></tr></tbody></table>

# **Read Length Distribution of All Sequencing Reads**

```
seqs_nano = {reads.Sequence}; % convert to cell array
readLen_nano = cellfun(@length, seqs_nano); % calculate read length

% Visualize results
figure('Position', [0 0 600 400]), sgtitle({'Read Length Distribution', '(Nanopore)'});
subplot(1,2,1), histogram(readLen_nano, 10);
xlabel('Read Length'), ylabel('Number of Sequence Reads');
```



**Caption:** The figure shows the distribution of sequence read lengths from the nanopore sequencing run. On the left is a histogram showing the frequency distribution of read lengths. On the right is a boxplot showing the median, upper and lower quartiles, and outliers of average sequence read length.

**Conclusion:** The average read length for the nanopore tends to be around  $0.5 \times 10^4$ . Although, there are some outliers with as high as  $2.5 \times 10^4$  reads.

## **Quality Score Distribution per Sequencing Read**

```
seqQ_nano = {reads.Quality}; % ASCII format
% Convert ascii to digits
seqQS_nano = cellfun(@(x) double(x) - 33, seqQ_nano, 'UniformOutput', false);

% Average, Median & Standard Deviation
avgQS_nano = cellfun(@mean, seqQS_nano);
medQS_nano = cellfun(@median, seqQS_nano);
stdQS_nano = cellfun(@std, seqQS_nano);

% Plot Distribution of Median and Average Quality
figure('Position', [0 0 600 400]), sgtitle({'Quality Score Distribution', 'Nanopore'});
subplot(1,2,1), histogram(medQS_nano, 10);
xlabel('Median Sequence Quality Score'), ylabel('Number of Sequence Reads');
subplot(1,2,2), boxplot(avgQS_nano), ylabel('Average Sequence Quality Score'), xticks([]);
```

#### **Quality Score Distribution** Nanopore Average Sequence Quality Score Number of Sequence Reads Median Sequence Quality Score

**Caption:** The figure shows the distribution of sequence quality scores from the nanopore sequencing run. On the left is a histogram showing the frequency distribution of quality scores. On the right is a boxplot showing the median, upper and lower quartiles, and outliers of average sequence quality score.

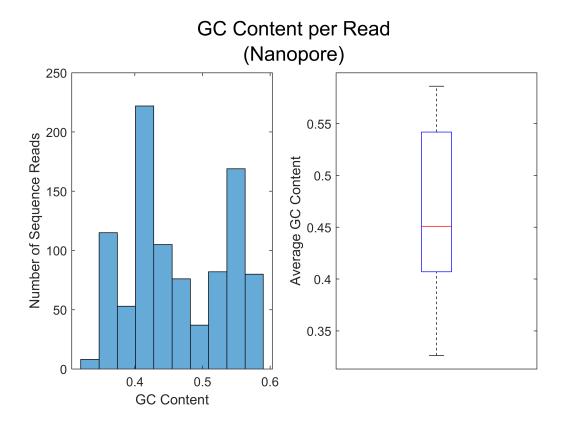
**Conclusion:** The average quality score for the nanopore tends to be around 18 and seems to follow a slightly skewed normal distribution.

## **GC Content Histogram per Sequence Read**

```
% Determine G and C content of reads
G_nano = cellfun(@(x) numel(strfind(x, 'G')), seqs_nano);
C_nano = cellfun(@(x) numel(strfind(x, 'C')), seqs_nano);

% Determine the GC content
GC_nano = (G_nano + C_nano) ./ readLen_nano;

% Visualize results
figure('Position', [0 0 600 400]), sgtitle({'GC Content per Read', '(Nanopore)'});
subplot(1,2,1), histogram(GC_nano, 10);
xlabel('GC Content'), ylabel('Number of Sequence Reads');
subplot(1,2,2), boxplot(GC_nano), ylabel('Average GC Content'), xticks([]);
```



**Caption:** The figure shows the distribution of GC content from the nanopore sequencing run. On the left is a histogram showing the frequency distribution of GC content. On the right is a boxplot showing the median, upper and lower quartiles, and outliers of average GC content.

**Conclusion:** The average GC content for the nanopore tends to be about 45%.

# Illumina Sequencing

# Input Illumina Sequencing Data (FASTQ File)

#### reads = 1×792838 struct

Fields	Header	Sequence	Quality
1	'M02486:	'AGGCAGA	'CCCCGG
2	'M02486:	'AATCAGC	'CCCCGG
3	'M02486:	'CCTCAAA	'CCCCGG

Fields	Header	Sequence	Quality
4	'M02486:	'GTAAGAT	'CCCCGG
5	'M02486:	'GGGCTGT	'CCCCGG
6	'M02486:	'TTCCAGC	'CCCCGG
7	'M02486:	'CTATTTA	'CCCCGG
8	'M02486:	'GACGTGT	'CCCCGG
9	'M02486:	'GCGCCGC	'CCCCGG
10	'M02486:	'GCCCAGC	'CCCCGG
11	'M02486:	'ATGATGA	'CCCCGG
12	'M02486:	'ATGCTGC	'CCCCGG
13	'M02486:	'AAACAAA	'CCCCGG
14	'M02486:	'GATGTGG	'CCCCGG
15	'M02486:	'GCTTTAA	'CCCCGG
16	'M02486:	'CTCACAT	'CCCCGG
17	'M02486:	'ACACAGA	'CCCCGG
18	'M02486:	'GCAACCG	'CCCCFG
19	'M02486:	'GTTCAAA	'CCCCGG
20	'M02486:	'TTTTAAA	'CCCCGG
21	'M02486:	'GGTAAAG	'CCCCGG
22	'M02486:	'GGTGAAT	'CCCCGG
23	'M02486:	'GACAAGA	'CCCCGG
24	'M02486:	'CCCCACA	'CCCCGG
25	'M02486:	'ATGTTGG	'CCCCGG
26	'M02486:	'CTTGCAG	'CCCCGG
27	'M02486:	'GACTCAG	'CCCCGG
28	'M02486:	'TATGATG	'CCCCGG
29	'M02486:	'TATGATG	'CCCCGG
30	'M02486:	'TCCCAAA	'CCCCGG
31	'M02486:	'GTATACC	'CCCCGG
32	'M02486:	'CTATCAC	'CCCCGG
33	'M02486:	'ATTCAAA	'CCCCGG
34	'M02486:	'ATTATGT	'CCCCGC
35	'M02486:	'ATAACAC	'CCCCGG
36	'M02486:	'GACCATA	'CCCCGG

Fields	Header	Sequence	Quality
37	'M02486:	'TGGTACA	'CCCCGG
38	'M02486:	'GCATAAC	'CCCCGF
39	'M02486:	'GAATAAT	'CCCCGG
40	'M02486:	'GATCACC	'CCCCGG
41	'M02486:	'GACTATA	'CCCCGG
42	'M02486:	'ACCAGAT	'CCCCGG
43	'M02486:	'GCTCTTT	'CCCCGG
44	'M02486:	'GAAAAGG	'CCCCGG
45	'M02486:	'TTTTTCA	'CCCCGC
46	'M02486:	'GCCATCA	'CCCCGG
47	'M02486:	'GTGTGTC	'CCCC-CF
48	'M02486:	'ATTCAAA	'CCCCGG
49	'M02486:	'CTGCCAC	'CCCCGG
50	'M02486:	'TGTGTGG	'CCCCGG
51	'M02486:	'GATCTGG	'CCCCGG
52	'M02486:	'GGTTTCT	'CCCCCGG
53	'M02486:	'AGCGAGA	'CCCCGG
54	'M02486:	'GTATTAA	'CCCCGG
55	'M02486:	'ACATTAG	'CCCCGG
56	'M02486:	'TCTCTAT	'CCCCGG
57	'M02486:	'AATGAGT	'CCCCGG
58	'M02486:	'GGTGCGC	'CCCCGG
59	'M02486:	'CTTAATT	'CCCC?FF
60	'M02486:	'GCTCCAT	'CCCCGG
61	'M02486:	'ATTTAAA	'CCCCGG
62	'M02486:	'GGGCTGA	'CCCCGG
63	'M02486:	'CTCGATT	'CCCCGG
64	'M02486:	'ATTTGGT	'CCCCGG
65	'M02486:	'GCCTCAT	'CCCCGG
66	'M02486:	'CGGTGTT	'CCCCGG
67	'M02486:	'GATATAG	'CCCCGG
68	'M02486:	'GAAGATA	'CCCCCAF
69	'M02486:	'ATTTTCA	'CCCCGG

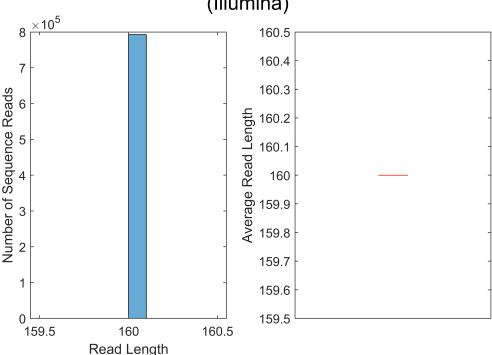
Fields	Header	Sequence	Quality
70	'M02486:	'TTATTAT	'CCCCGG
71	'M02486:	'TGCTGAT	' <ccccgg< th=""></ccccgg<>
72	'M02486:	'AAGTAAT	'9 <acb9f< th=""></acb9f<>
73	'M02486:	'GGTTTAG	'B@ACCGF
74	'M02486:	'ATATTGC	'CCCCGG
75	'M02486:	'GCCTGGG	'CCCCGG
76	'M02486:	'AGAAAAA	'@C@CCFG
77	'M02486:	'CGGGAGA	'CCCCGG
78	'M02486:	'AAGCTAT	'CCCCGG
79	'M02486:	'ATGCAAT	'CCCCGG
80	'M02486:	'GTGAGAT	'CCCCGG
81	'M02486:	'CGGTGGT	'CCCCGD
82	'M02486:	'TATTACG	'CCCCGG
83	'M02486:	'CATGTGC	'ACCCCGG
84	'M02486:	'GCCTTTA	'CCCCGG
85	'M02486:	'TCGTTTT	'CCC@CFF
86	'M02486:	'GGTATAA	'CCCCCGG
87	'M02486:	'GCACACA	'CCCCGG
88	'M02486:	'GTACGCC	'CCCCGG
89	'M02486:	'CTGTTGG	'CCCCGG
90	'M02486:	'GTATCGT	'CCCCGG
91	'M02486:	'CATTCCA	'CCCCCGG
92	'M02486:	'GGGATAA	' <bcccgg< th=""></bcccgg<>
93	'M02486:	'GTGAGTC	'CCCCGG
94	'M02486:	'AGCTTAT	'CC8CCGG
95	'M02486:	'TGATCGG	'CCCCGG
96	'M02486:	'ATTCAAC	'CCCCGG
97	'M02486:	'ACGGGAT	'CCCCGG
98	'M02486:	'GTATCGG	'CCCCGG
99	'M02486:	'GCATCAG	'CCCCGG
100	'M02486:	'GCCTAAT	'CCCCGG
	•	1	

Read Length Distribution of All Sequencing Reads

```
seqs_ilum = {reads.Sequence};
readLen_ilum = cellfun(@length, seqs_ilum);

% Visualize results
figure('Position', [0 0 600 400]), sgtitle({'Read Length Distribution', '(Illumina)'});
subplot(1,2,1), histogram(readLen_ilum, 10);
xlabel('Read Length'), ylabel('Number of Sequence Reads');
subplot(1,2,2), boxplot(readLen_ilum), ylabel('Average Read Length'), xticks([]);
```

# Read Length Distribution (Illumina)



**Caption:** The figure shows the distribution of sequence read lengths from the Illumina sequencing run. On the left is a histogram showing the frequency distribution of read lengths. On the right is a boxplot showing the median, upper and lower quartiles, and outliers of average sequence read length.

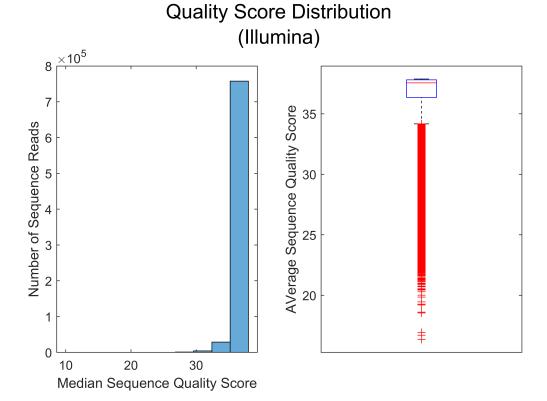
**Conclusion:** The read length is consistent at 160 bps for all samples.

#### **Quality Score Distribution per Sequencing Read**

```
seqQ_ilum = {reads.Quality}; % ASCII format
seqQS_ilum = cellfun(@(x) double(x) - 33, seqQ_ilum, 'UniformOutput', false);

% Average, Median & Standard Deviation
avgQS_ilum = cellfun(@mean, seqQS_ilum);
medQS_ilum = cellfun(@median, seqQS_ilum);
stdQS_ilum = cellfun(@std, seqQS_ilum);

% Plot Distribution of Median and Average Quality
figure('Position', [0 0 600 400]), sgtitle({'Quality Score Distribution', '(Illumina)'});
subplot(1,2,1), histogram(medQS_ilum, 10);
xlabel('Median Sequence Quality Score'), ylabel('Number of Sequence Reads');
```



**Caption:** The figure shows the distribution of sequence quality scores from the Illumina sequencing run. On the left is a histogram showing the frequency distribution of quality scores. On the right is a boxplot showing the median, upper and lower quartiles, and outliers of average sequence quality score.

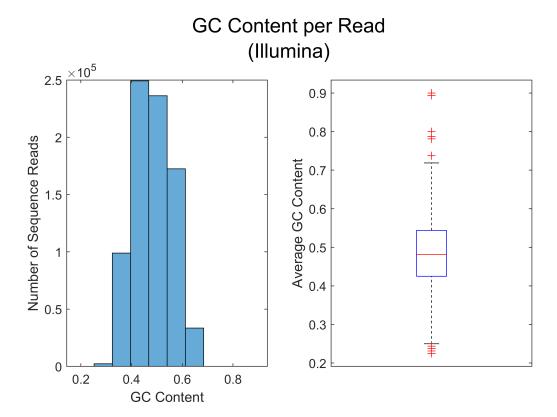
**Conclusion:** The quality scores remain relatively consistent at 36. However, there are several outliers with quality scores as low as 20.

#### **GC Content Histogram per Sequence Read**

```
% Determine G and C content of reads
G_ilum = cellfun(@(x) numel(strfind(x, 'G')), seqs_ilum);
C_ilum = cellfun(@(x) numel(strfind(x, 'C')), seqs_ilum);

% Determine the GC content
GC_ilum = (G_ilum + C_ilum) ./ readLen_ilum;

% Visualize results
figure('Position', [0 0 600 400]), sgtitle({'GC Content per Read', '(Illumina)'});
subplot(1,2,1), histogram(GC_ilum, 10);
xlabel('GC Content'), ylabel('Number of Sequence Reads');
subplot(1,2,2), boxplot(GC_ilum), ylabel('Average GC Content'), xticks([]);
```



**Caption:** The figure shows the distribution of GC content from the Illumina sequencing run. On the left is a histogram showing the frequency distribution of GC content. On the right is a boxplot showing the median, upper and lower quartiles, and outliers of average GC content.

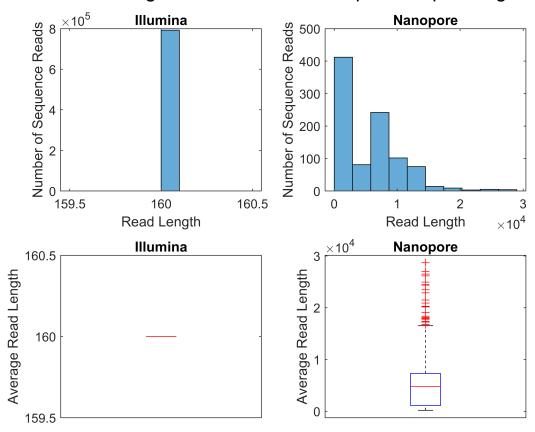
**Conclusion:** The average GC content for the Illumina sequencing run is roughle normally distributed with a mean of about 50%.

# Differences Between Illumina and Nanopore Sequencing

First difference: Plot the nanopore and Illumina data together using histogram and box plot (caption and conclusion)

```
figure('Position', [0 0 600 500]), sgtitle('Read Length for Illumina vs Nanopore Sequencing')
subplot(2,2,1), histogram(readLen_ilum, 10), title('Illumina');
xlabel('Read Length'), ylabel('Number of Sequence Reads');
subplot(2,2,3), boxplot(readLen_ilum), title('Illumina'), ylabel('Average Read Length'), xticks
subplot(2,2,2), histogram(readLen_nano, 10), title('Nanopore');
xlabel('Read Length'), ylabel('Number of Sequence Reads');
subplot(2,2,4), boxplot(readLen_nano), title('Nanopore'), ylabel('Average Read Length'), xticks
```

#### Read Length for Illumina vs Nanopore Sequencing



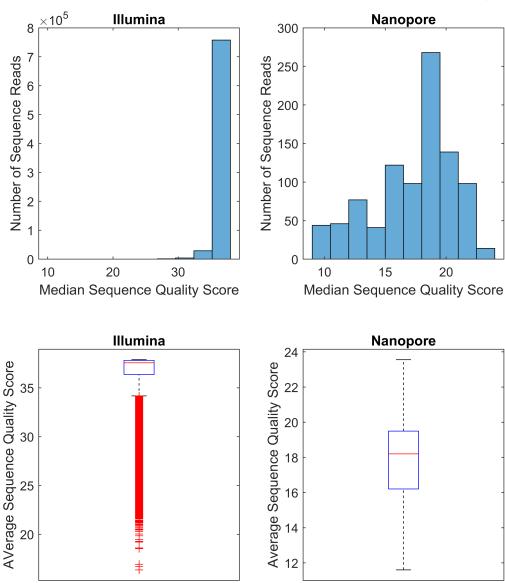
**Caption:** The figure shows histograms (top left & right) and boxplots (bottom left & right) showing the distibution of read lengths for illumina (left panes) and nanopore (right panes) sequencing.

**Conclusion:** Illumina sequencing produces reads with a consistent read length (in this case, 160bp), while nanopore sequencing produces reads of varying lengths. The read length for nanopore is also orders of magnitude larger than the read length for Illumina sequencing. This is due to underlying differences in the sequencing technologies. Illumina sequencing kits allow for a specific number of reads per cycle (usually in the range of 150 - 300bps), while nanopore sequencing allows much larger fragments of DNA to be sequenced at a time.

Second difference: Plot the nanopore and Illumina data together using histogram and box plot (caption and conclusion)

```
figure('Position', [0 0 600 700]), sgtitle('Quality Scores for Illumina vs Nanopore Sequencing
subplot(2,2,1), histogram(medQS_ilum, 10), title('Illumina');
xlabel('Median Sequence Quality Score'), ylabel('Number of Sequence Reads');
subplot(2,2,3), boxplot(avgQS_ilum), title('Illumina');
ylabel('AVerage Sequence Quality Score'), xticks([]);
subplot(2,2,2), histogram(medQS_nano, 10), title('Nanopore');
xlabel('Median Sequence Quality Score'), ylabel('Number of Sequence Reads');
subplot(2,2,4), boxplot(avgQS_nano), title('Nanopore');
```

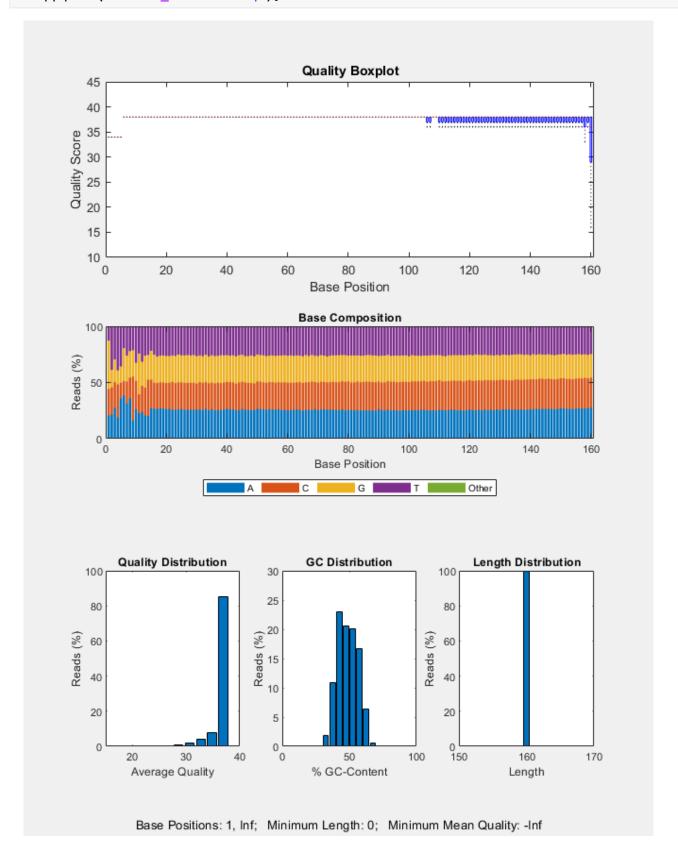
## Quality Scores for Illumina vs Nanopore Sequencing



**Caption:** The figure shows histograms (top left & right) and boxplots (bottom left & right) showing the distibution of quality scores for illumina (left panes) and nanopore (right panes) sequencing.

**Conclusion:** Illumina sequencing producces higher quality reads than nanopore sequencing. The quality scores are also significantly more consistent for Illumina sequencing. With nanopore quality cores being roughly normally distributed and illumina quality scores being mostly constant at 36, with a few outliers as low as 20.

# seqqcplot() on Illumina Data



%seqqcplot('lambda\_nanopore.fastq') %what happens and why?

The seqqcplot function does not work for nanopore sequencing data because the function currently does not support the encoding for nanopore.

# Filter Sequencing Reads

```
[outFile, in, out] = seqfilter('lambda_nanopore.fastq', 'Method', 'MeanQuality', 'Threshold', '
outFile = 1×1 cell array

{'C:\Users\kabil\OneDrive - Drexel University\Academic\3 - Pre-Junior\1 - Fall Quarter\BMES 375\bmes375.TonyOkel
in = 797
out = 150
```