Quiz 2

TRANSCRIPTOMICS

BIGNAUD AMAURY
30/03/2023
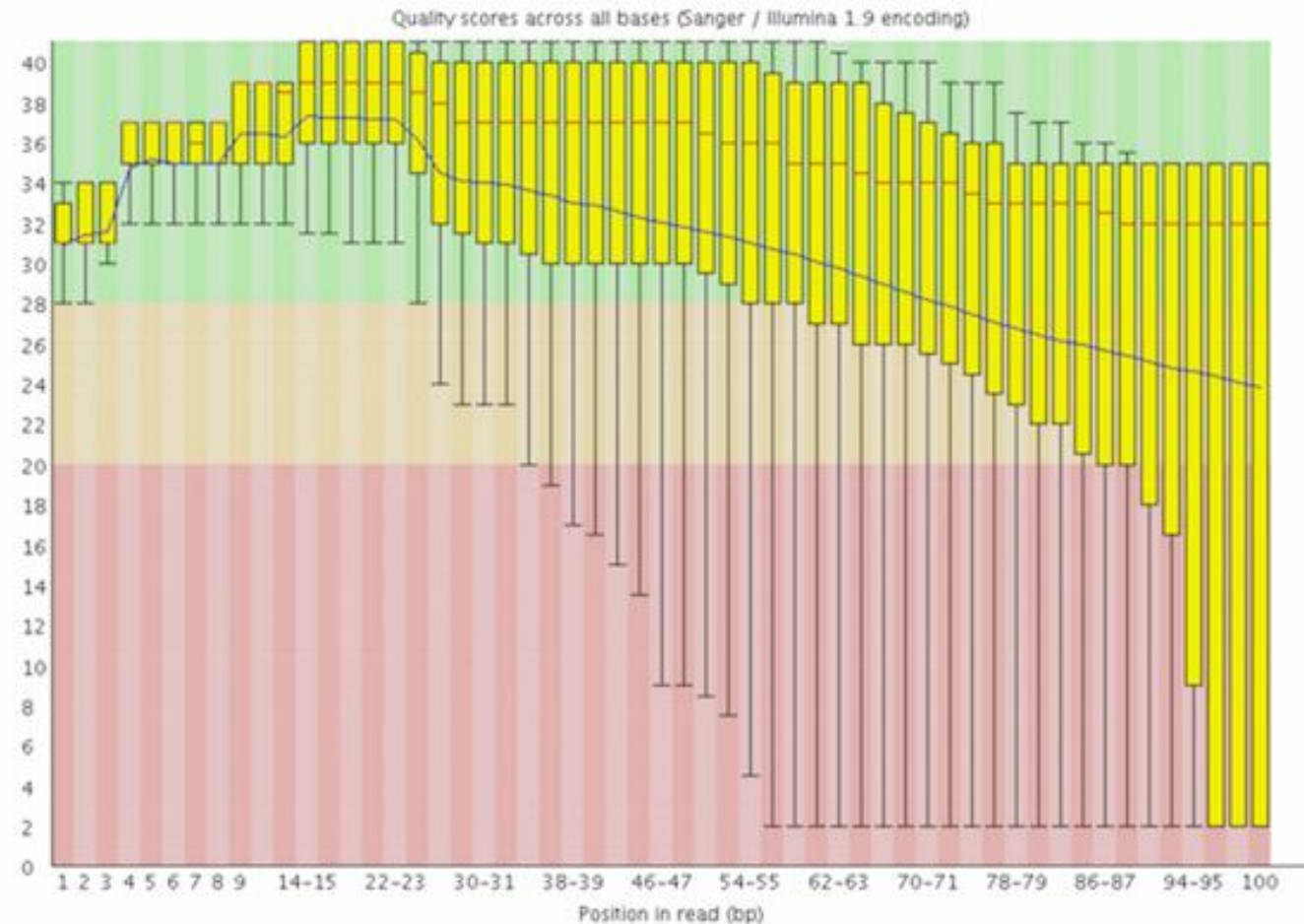
You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

A.   You sequencing is fine, we usually have a loss of quality at the end of the reads.

B.   The experiment has failed, I have to redo it.

C.   You have some issues, at the end of the reads, may be due a signal issue at the end of the run.

D.   You have some issues, at the end of the reads, may be due phasing issues at the end of the run.


Per base sequence quality

Question 1

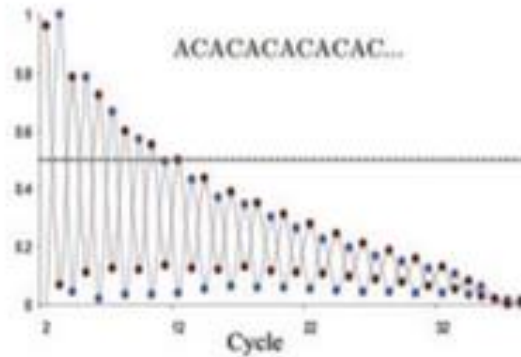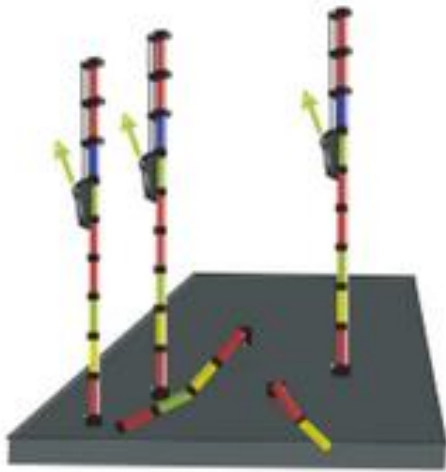You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

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Per base sequence quality
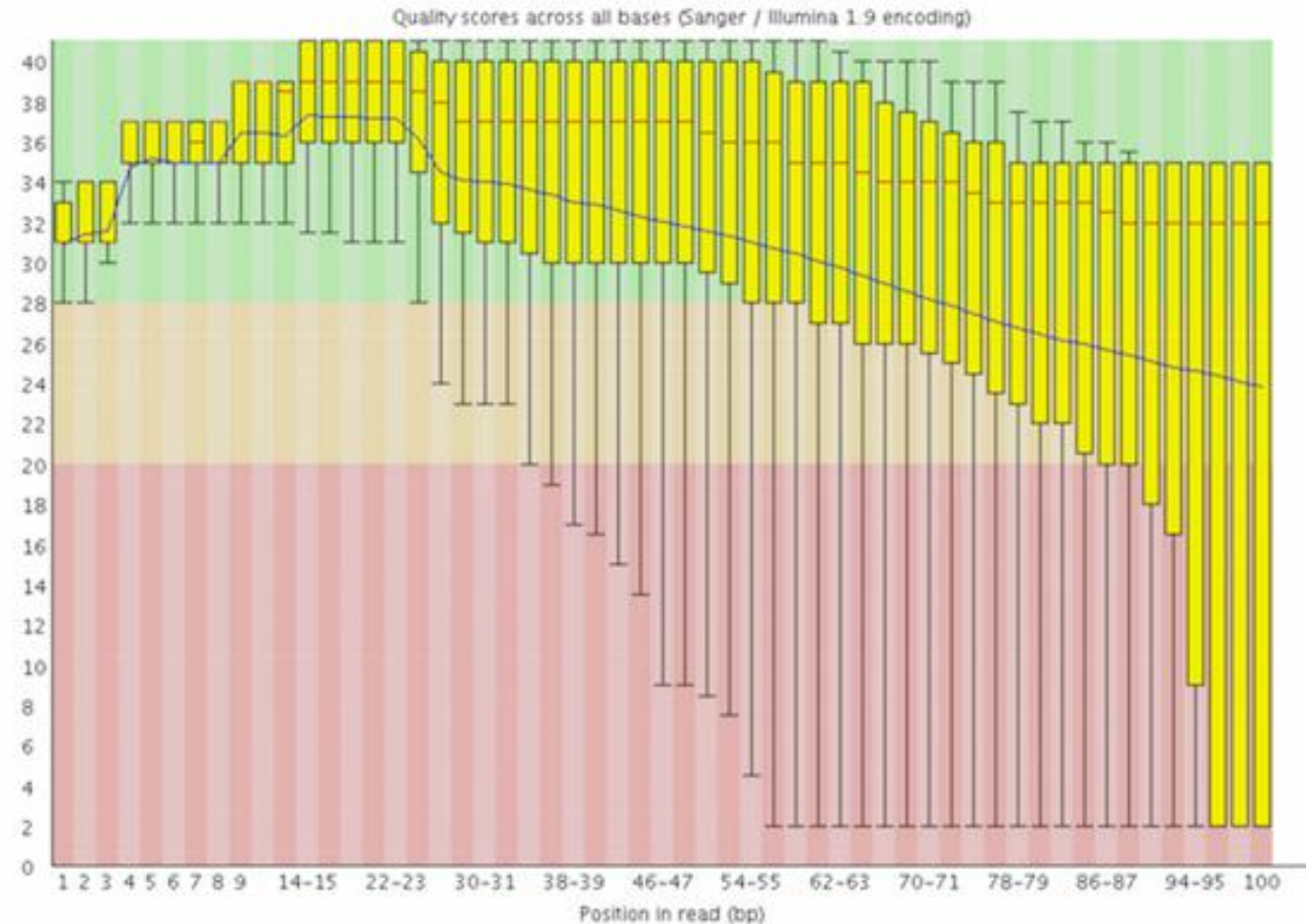
Question 1

- **Signal decay:** As sequencing proceeds, the fluorescent signal intensity decays with each cycle, yielding decreasing quality scores at the **3' end** of the read. This is due to:
    1. Degrading fluorophores
    2. A proportion of the strands in the cluster not being elongated

  Therefore, the proportion of signal being emitted continues to decrease with each cycle.

Question 1

- **Phasing:** As the number of cycles increases, the signal starts to blur as the cluster loses synchronicity, also yielding a decrease in quality scores at the **3' end** of the read. As the cycles progress, some strands get random failure of nucleotides to incorporate due to:
  1. Incomplete removal of the 3' terminators and fluorophores
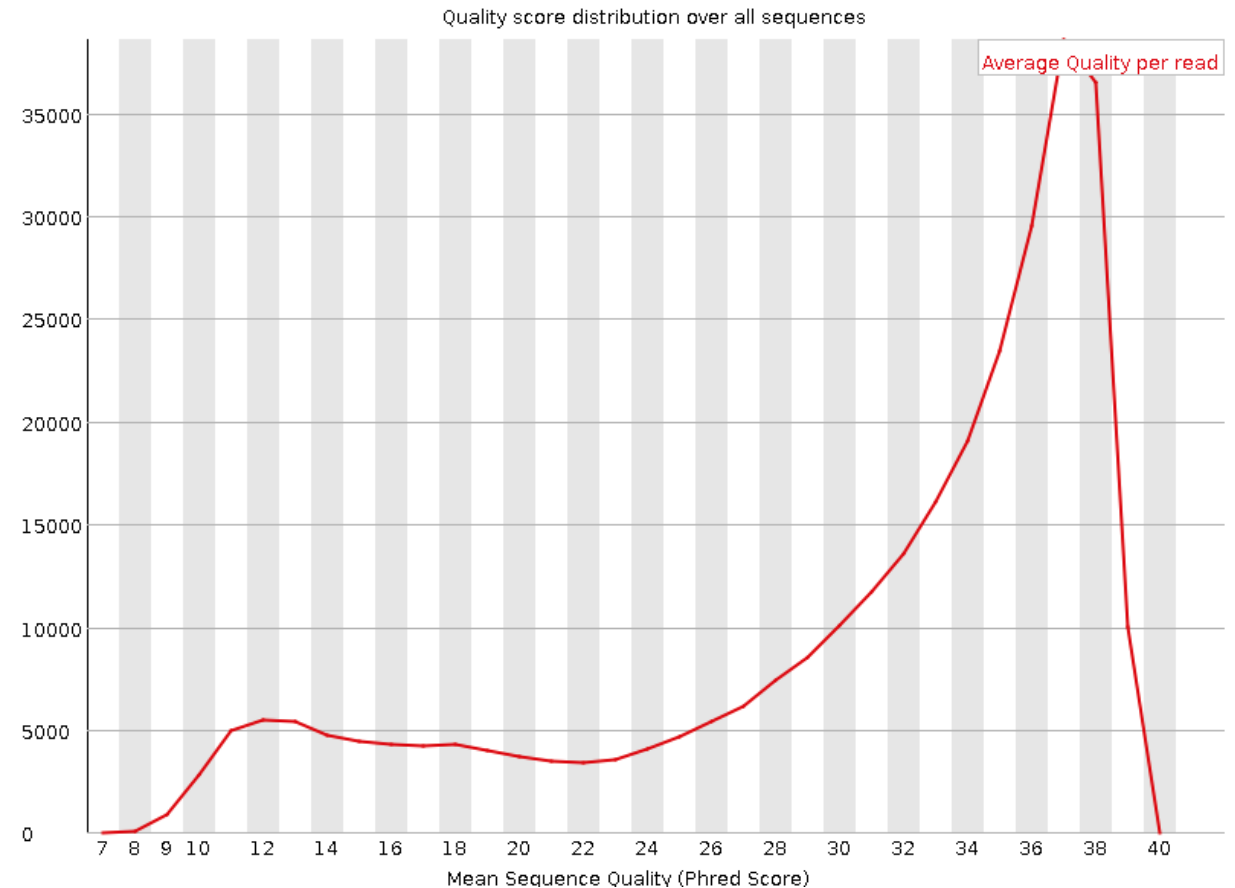 2. Incorporation of nucleotides without effective 3' terminators
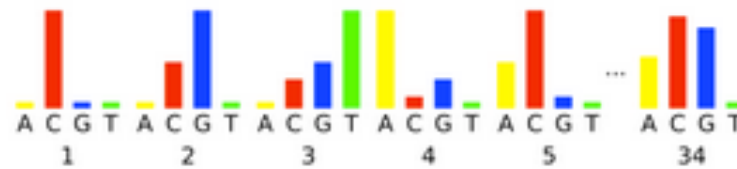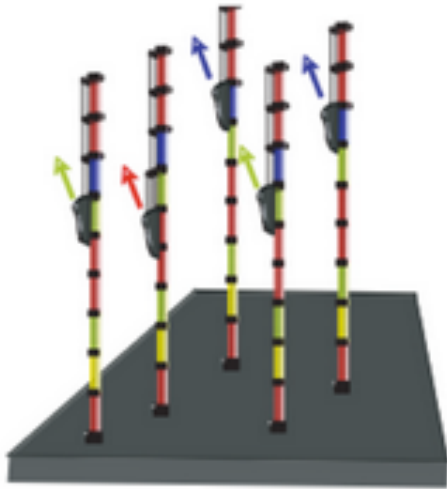
Question 2

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

A.   Nothing, this plot is useless.

B.   The experiment has failed, I have to redo it.

C.   We have a small portion of low-quality reads that we should removed before processing.

D.   We have a small portion of low-quality reads that we can keep before processing.

Per sequence quality scores

Quality score distribution over all sequences

Average Quality per read

Mean Sequence Quality (Phred Score)

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

❌ **A**.   Nothing, this plot is useless.

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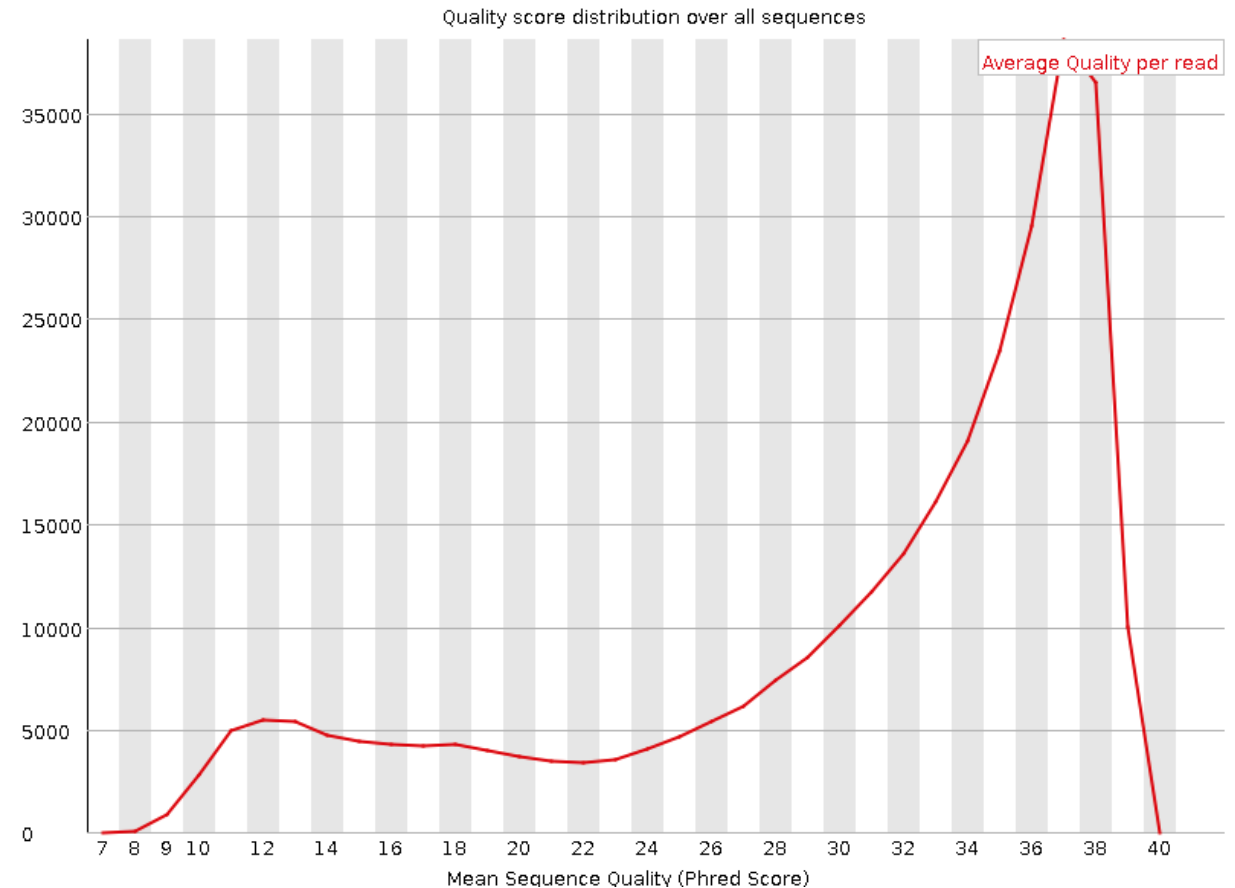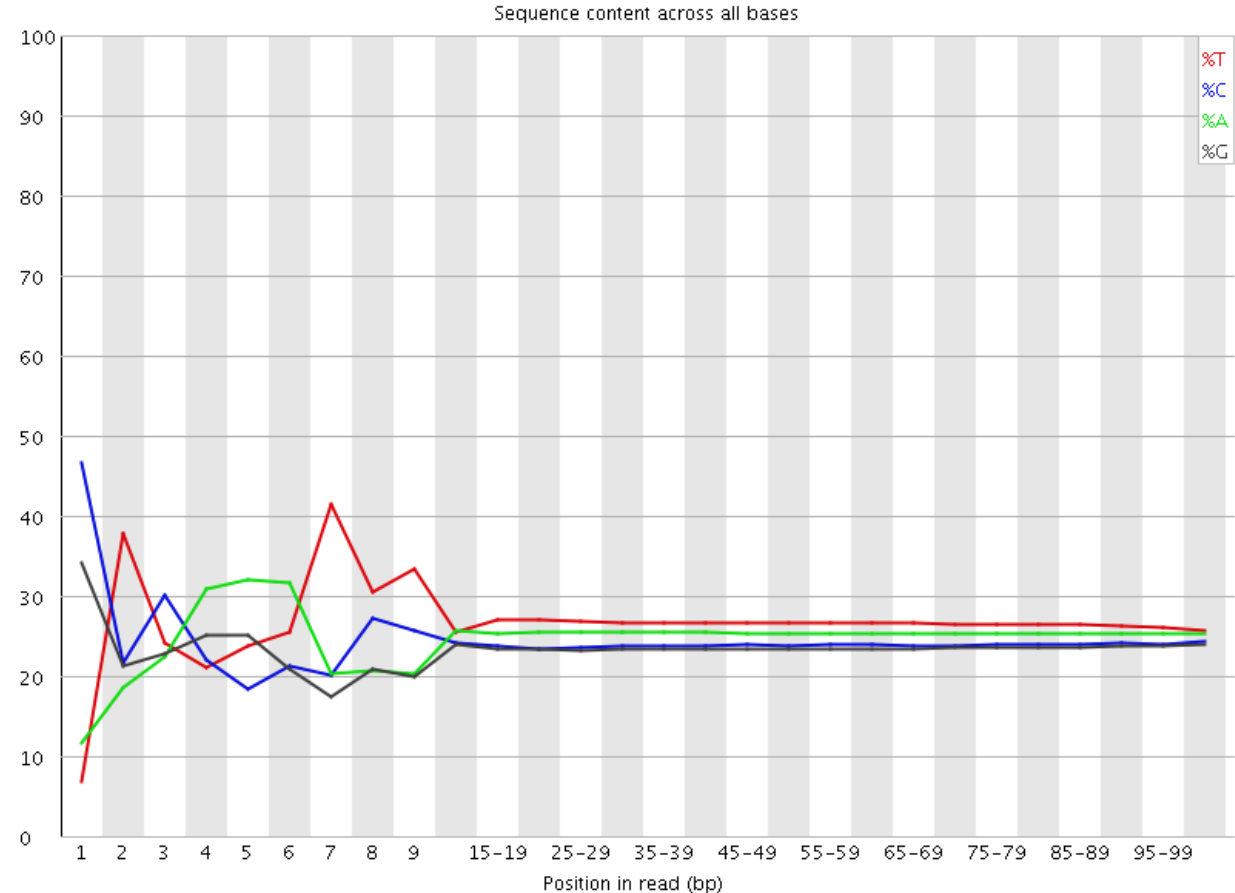

Per sequence quality scores

Quality score distribution over all sequences

Average Quality per read

Mean Sequence Quality (Phred Score)

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

A.   We have an adapters contamination.

B.   It may just be an artifact due to 'random' hexamer which are not so random.

C.   We have DNA contamination from another organism.

D.   The line at the end should be all equals. I have an issue about my library.
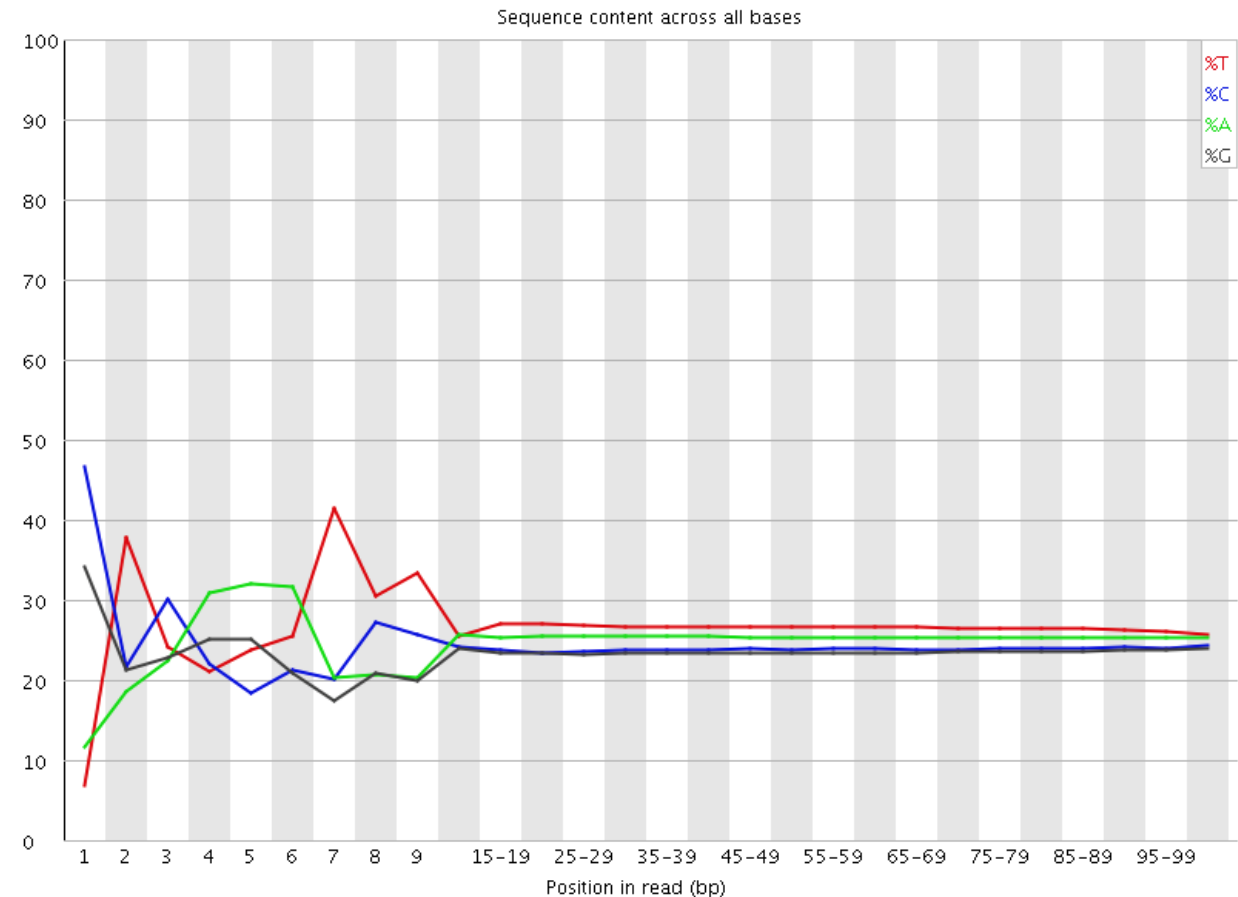

Per base sequence content

Question 3

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

A.   We have an adapters contamination.

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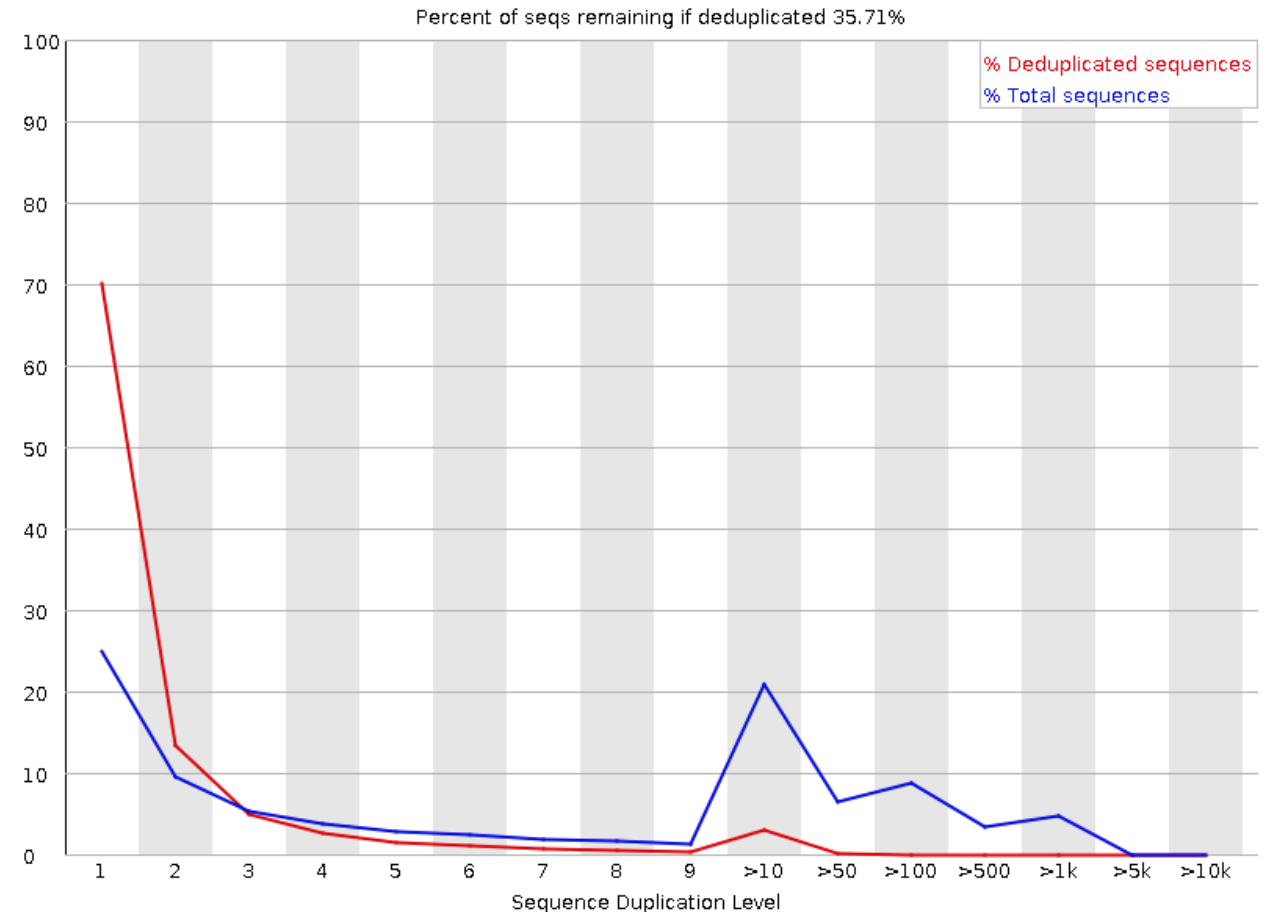

Per base sequence content

Question 4

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

A. There are always a lot of duplication event in RNAseq experiment.

B. We have an adapters contamination.

C. We have a rDNA contamination.

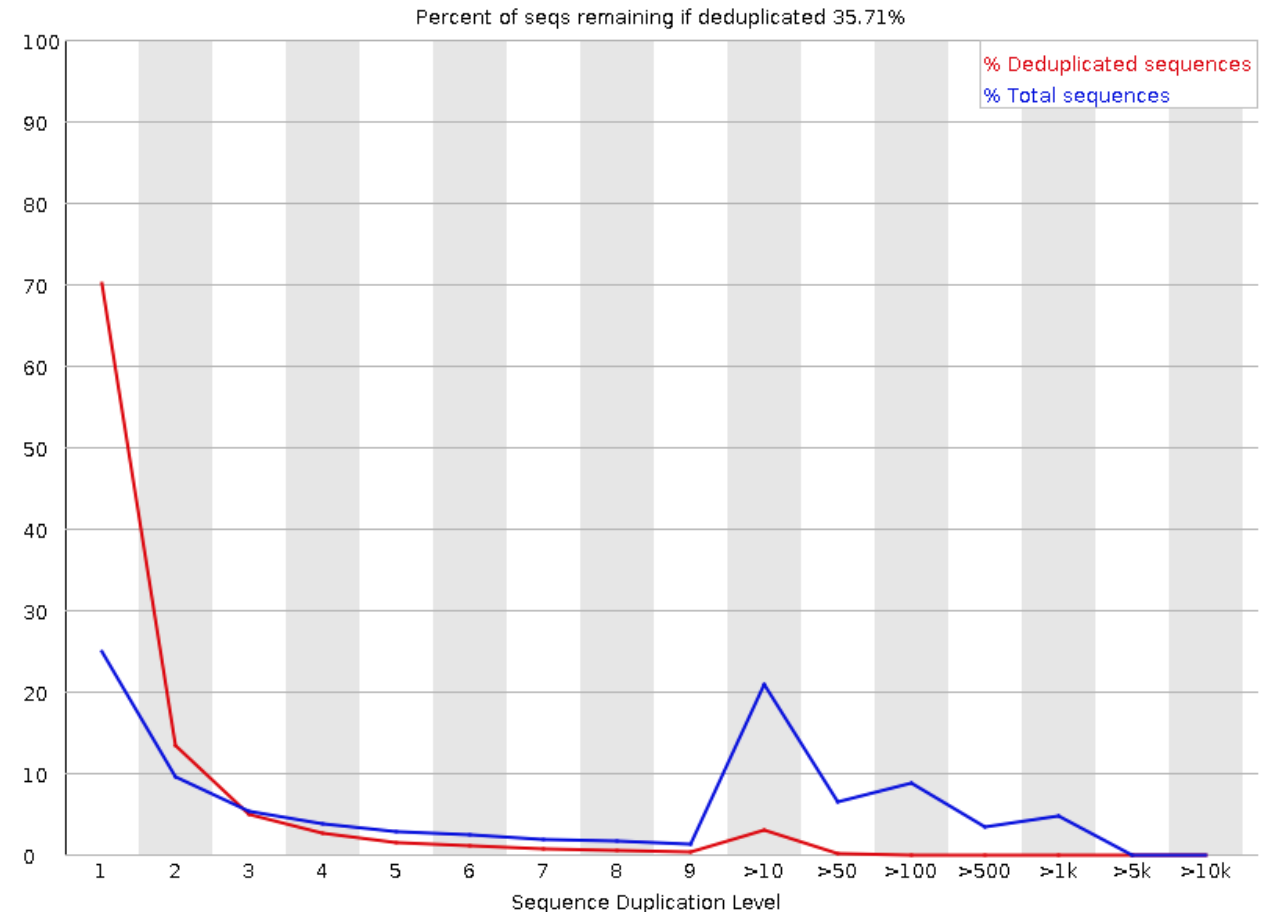**D.** I have a low complexity library, that I have sequence way too much.

Question 4

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

A.   There are always a lot of duplication event in RNAseq experiment.

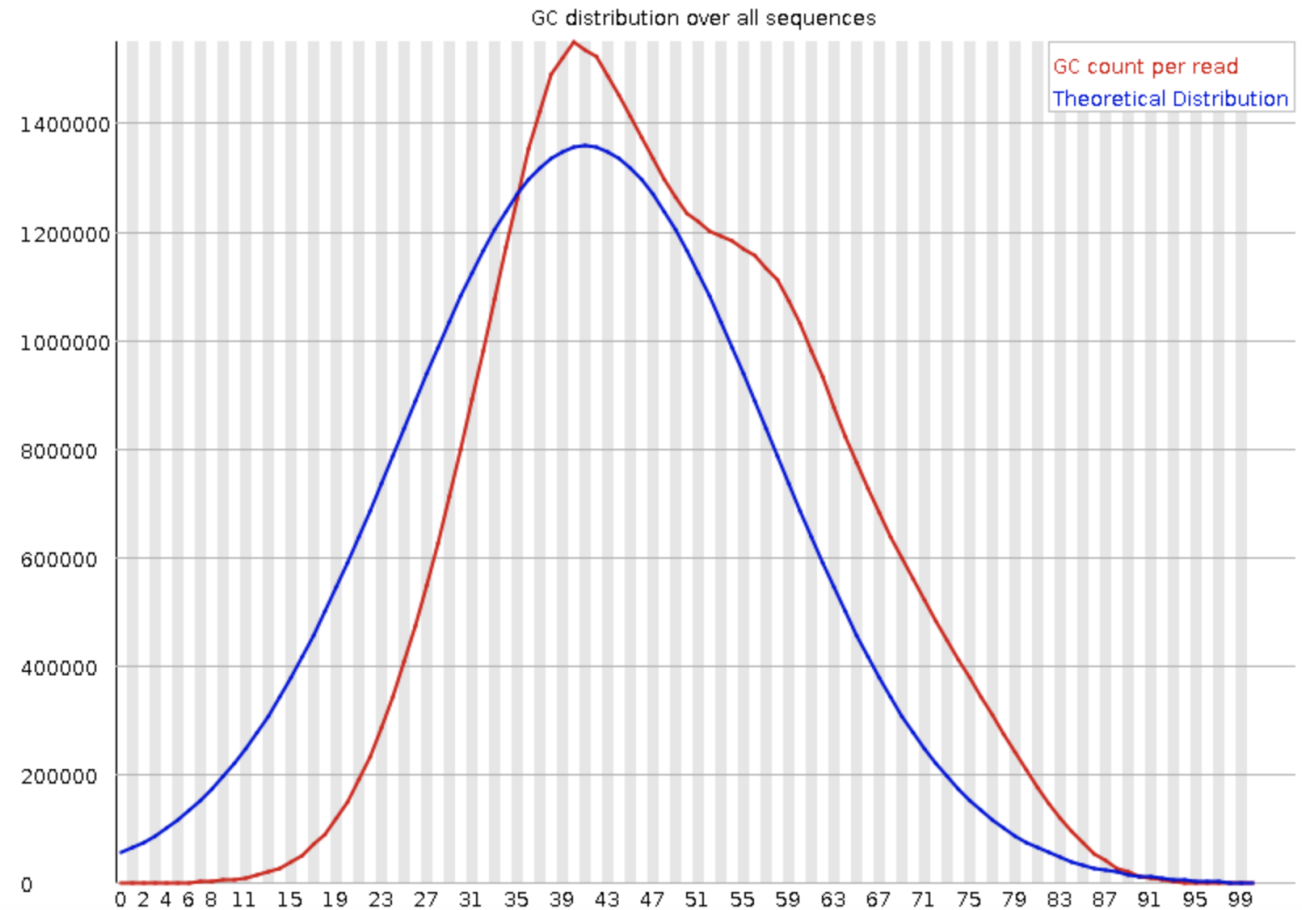**B**.   We have an adapters contamination.

C.   We have a rDNA contamination.

D.   I have a low complexity library, that I have sequence way too much.

Question 5

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?
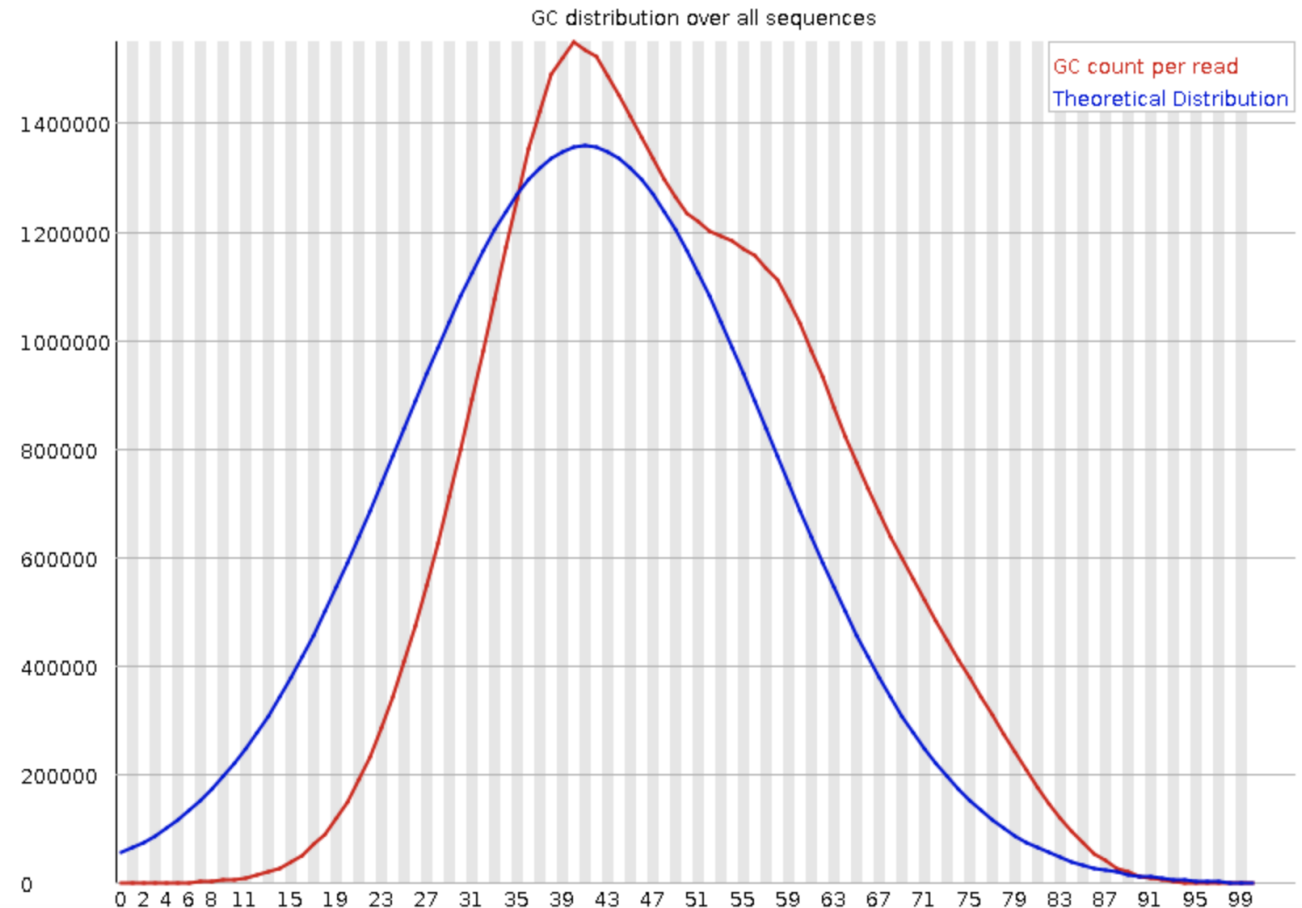
A. We have a contamination from another species.

B. We have an adapters contamination.

C. We have a rDNA contamination.

D. I can't know the origin of the contamination, I will blast my reads or/and do a fastq-screen.

Per sequence GC content

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

❌ **A**. We have a contamination from another species.

❌ **B**. We have an adapters contamination.

❌ **C**. We have a rDNA contamination.

✅ **D**. I can't know the origin of the contamination, I will blast my reads or/and do a fastq-screen.

❌**Per sequence GC content**

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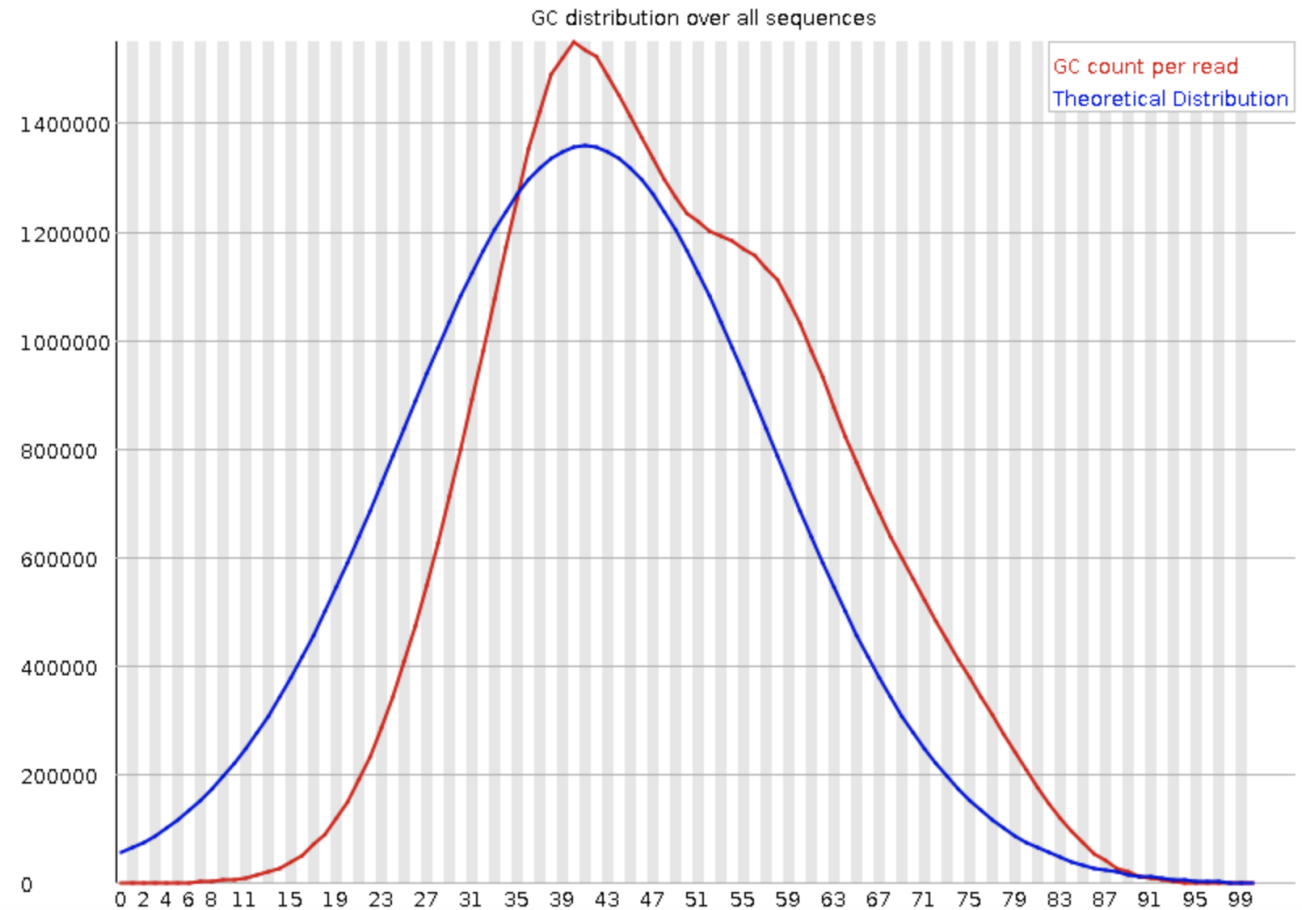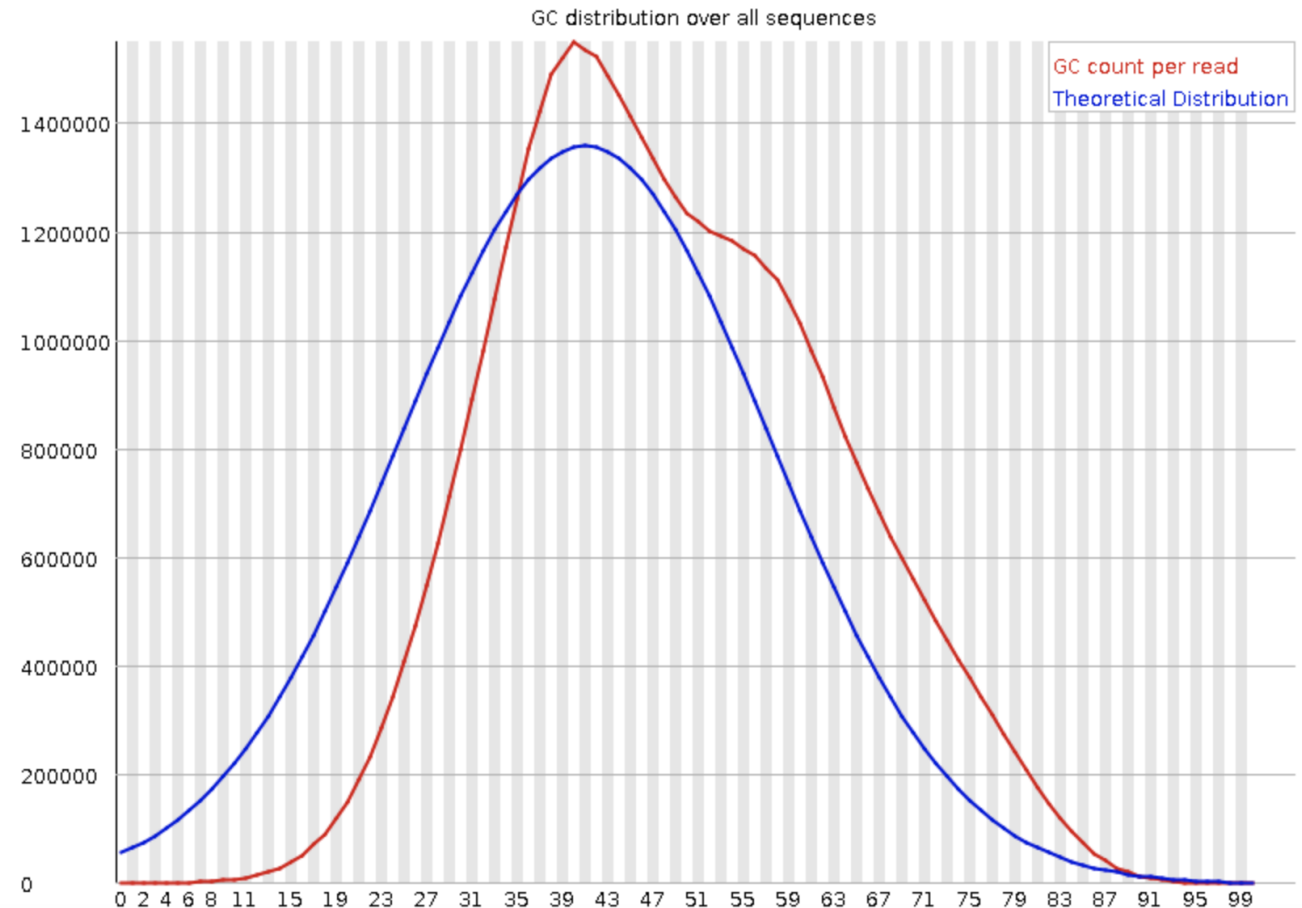**D**.   I can't know the origin of the contamination, I will blast my reads and do a fastq-screen.

Per sequence GC content

GC distribution over all sequences

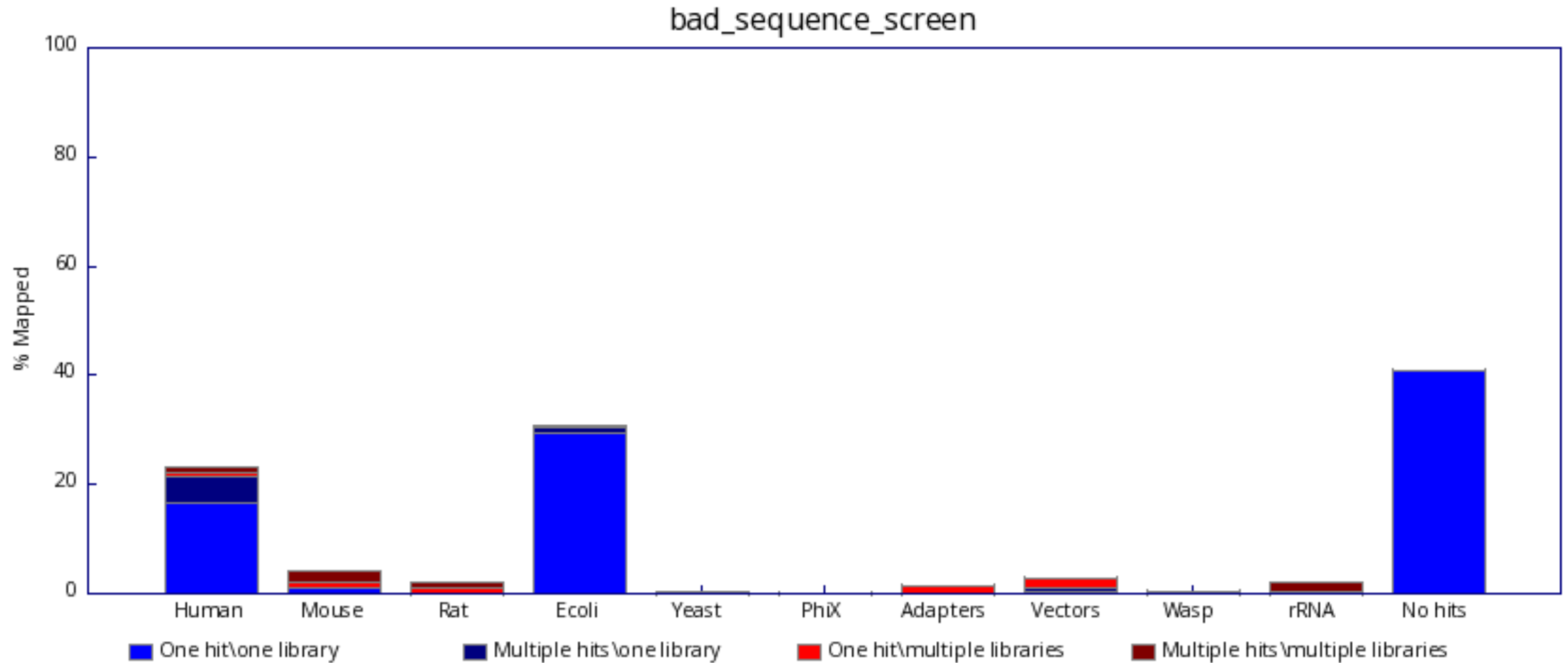GC count per read
Theoretical Distribution

Question 5

You did your fastqc on a RNAseq illumina library, you observed this plot. What do you deduce ?

A.   We have a contamination from another species.

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C.   We have a rDNA contamination.

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Per sequence GC content

GC distribution over all sequences

GC count per read
Theoretical Distribution

Question 5

bad_sequence_screen

Escherichia coli contamination of a human library.