Assignment 5

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Contents

[Description 1](#_Toc447010298)

[Application specifications 1](#_Toc447010299)

[Analysis of generated Graphs 2](#_Toc447010300)

[Mappability-output-205 4](#_Toc447010301)

[Source code 9](#_Toc447010302)

[com.bio.main.CheckMappabilityApp 9](#_Toc447010303)

[com.bio.main.GenerateReadsApp 10](#_Toc447010304)

[com.bio.main.MergeOutputApp 11](#_Toc447010305)

[com.bio.main.util.FileUtils 12](#_Toc447010306)

[com.bio.main.util.MappabilityUtils 18](#_Toc447010307)

[com.bio.pojo.Gene 22](#_Toc447010308)

[com.bio.pojo.Mappability 23](#_Toc447010309)

# Description

The objective of this assignment is to generate mappability of 250 gene sequences in scope of HG19.chr1. There are 3 different applications made for this purpose and here is a step by step guidance on how to achieve the goal (Take note that all the files must be placed under Assignment5/io folder as this is the main io folder for both input and output):

1. Generate Reads with different length by running the following application: /Assignment5/src/com/bio/main/GenerateReadsApp.java
2. Use the read files (reads50.fa for example) one by one and generate BowTie outputs and place them in io folder (length 50 BT output is called final-BTout50-v2-m1 in this assignment. Same naming convention was used for the rest).
3. Generate the mappability files by running the following application: /Assignment5/src/com/bio/main/CheckMappabilityApp.java
4. Merge the 3 mappability files (for example: final-output-50) generated from step 3 by running the following application: /Assignment5/src/com/bio/main/MergeOutputApp.java
5. The final file which can be used by R application will be named: Mappability-output-250

## 

## Application specifications

**Programming language:** JAVA

**Other required installations:** JRE 1.8 to run the application,

JDK 1.8 to compile the application.

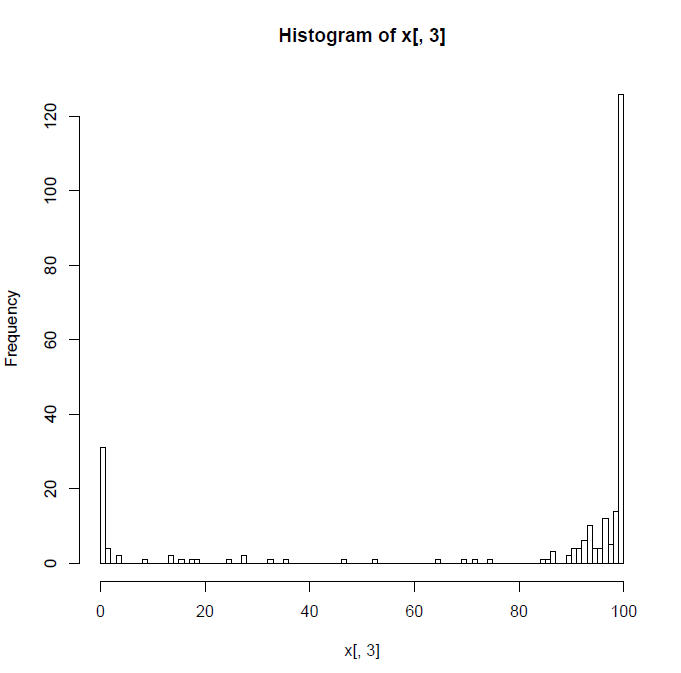
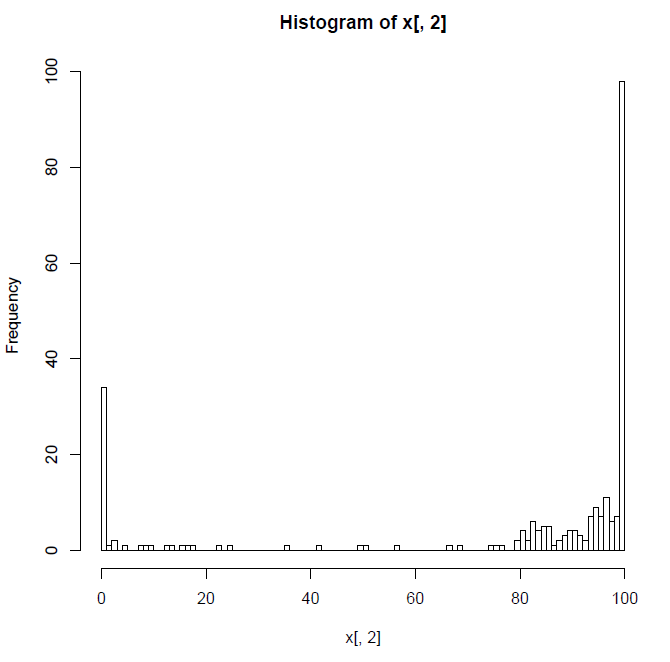
**Version Control:** Git (GitHub) - https://github.com/momazia/Bioinformatic/tree/master/Assignment5

**IDE:** Eclipse (Mars 4.5.1)

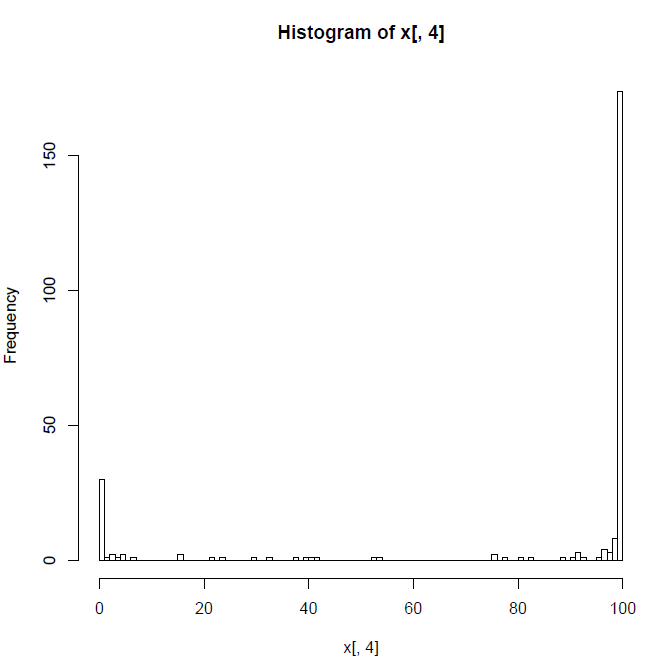
**Build automation tool:** Maven (Refer to /Assignment5/pom.xml for the list of the libraries used)

## Analysis of generated Graphs

As an individual study on each of mappabilities of the different lengths, by looking at their Histogram graphs, the most mappabilities are distributed on either 100 or zero percent. It also shows that 100% mappabilities in all 3 different lengths are having the most occurrences in the output.

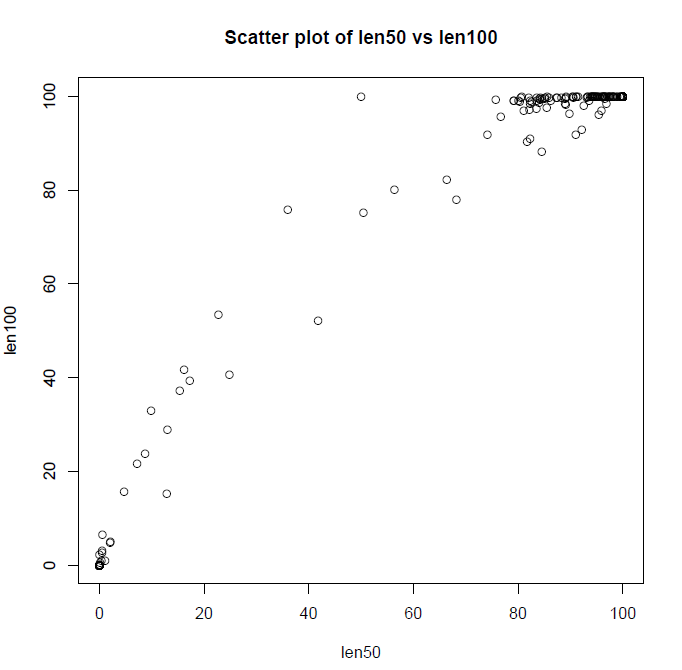
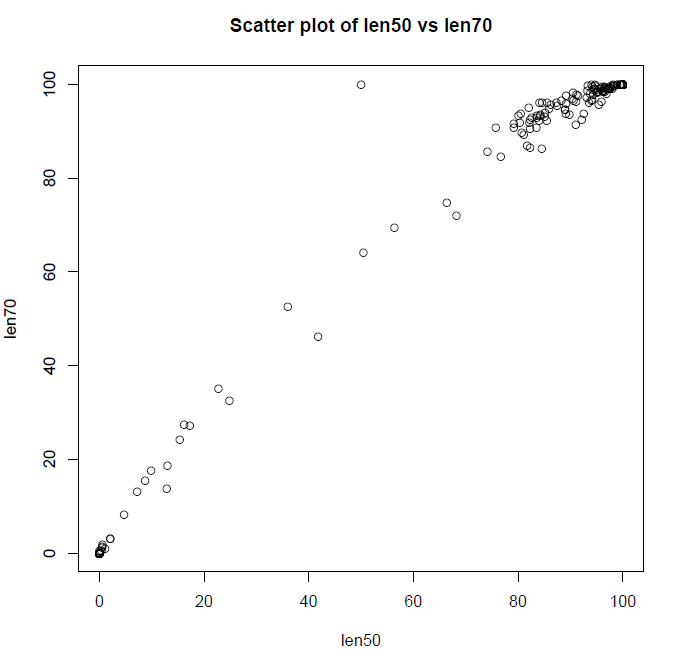


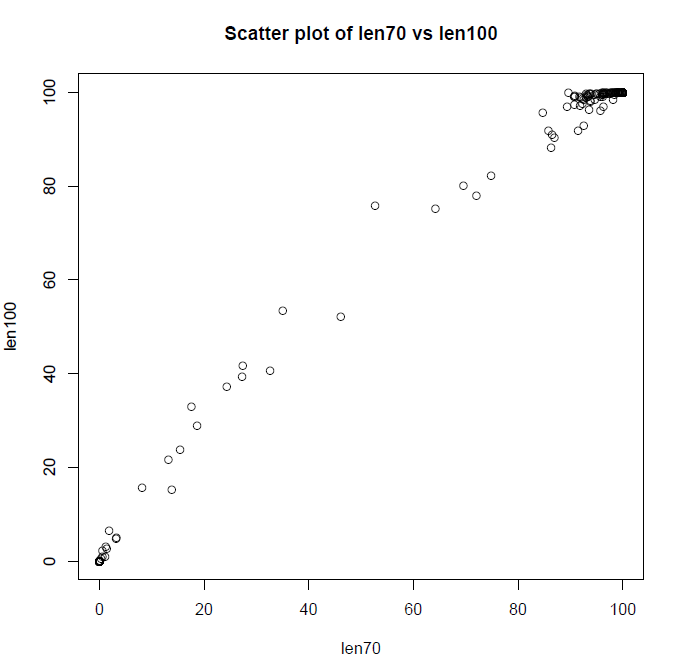
Length 50 Length 70



Length 100

By comparing scatter plot graphs of all the possible combinations between the 3 different read lengths, it is most obvious that the longer the length of a read, the higher the mappability of the read is. Because in none of the graphs below, we have a single dot under the line , where the smaller read length is presented on in all the graphs.





# Mappability-output-205

geneID readLen50 readLen70 readLen100

chr1.19629201.19638640.NM\_003689.- 85.4633 92.2625 97.5375

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chr1.26364513.26372604.NM\_032513.- 96.1701 98.367 100.00

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chr1.2487804.2495267.NM\_003820.+ 99.5414 100.00 100.00

chr1.26606212.26608013.NM\_031286.+ 100.00 100.00 100.00

chr1.134772.140566.NR\_039983.- 0.5744 1.869 6.4618

chr1.22970117.22974603.NM\_172369.+ 100.00 100.00 100.00

chr1.31408535.31408623.NR\_033295.- 0.00 0.00 0.00

chr1.26226606.26233368.NM\_203399.- 97.3633 99.1185 99.94

chr1.33327868.33336414.NM\_001171940.- 93.5624 96.1779 99.195

chr1.13035542.13038381.NM\_001100631.+ 0.00 0.00 0.00

chr1.26226606.26232644.NM\_203401.- 97.0446 99.0116 99.9326

chr1.367658.368597.NM\_001005221.+ 0.00 0.00 0.00

chr1.761585.762902.NR\_024321.- 100.00 100.00 100.00

chr1.2517898.2522908.NR\_036638.+ 100.00 100.00 100.00

chr1.31441009.31441084.NR\_003066.- 100.00 100.00 100.00

chr1.1138887.1142089.NM\_004195.- 100.00 100.00 100.00

chr1.13641972.13648987.NM\_001098376.+ 0.00 0.00 0.00

chr1.12851545.12856777.NM\_023013.+ 15.3579 24.3269 37.1907

chr1.32826870.32827844.NM\_001167676.- 100.00 100.00 100.00

chr1.32799439.32801834.NM\_023009.- 100.00 100.00 100.00

chr1.20978259.20988037.NM\_005216.- 94.2954 97.7444 100.00

chr1.31838099.31845923.NM\_004102.- 97.7235 99.7679 100.00

chr1.27237974.27240567.NM\_021969.- 95.283 98.5341 100.00

chr1.25568739.25573985.NM\_020317.- 96.0939 99.5171 100.00

# Source code

## com.bio.main.CheckMappabilityApp

package com.bio.main;

import java.io.IOException;

import java.util.List;

import com.bio.main.util.FileUtils;

import com.bio.main.util.MappabilityUtils;

/\*\*

\* The main application to create different mappability files for the given BowTie outputs. All the files must be placed under /Assignment5/io folder.

\*

\* @author Mohamad Mahdi Ziaee

\*

\*/

public class CheckMappabilityApp {

// Constants to the file names

private static final String CHR1\_250\_FA = "chr1-250.fa";

private static final int TILE\_LENGTH\_50 = 50;

public static final String FINAL\_OUTPUT\_50 = "final-output-50";

private static final String FINAL\_B\_TOUT50\_V2\_M1 = "final-BTout50-v2-m1";

private static final int TILE\_LENGTH\_70 = 70;

public static final String FINAL\_OUTPUT\_70 = "final-output-70";

private static final String FINAL\_B\_TOUT70\_V2\_M1 = "final-BTout70-v2-m1";

private static final int TILE\_LENGTH\_100 = 100;

public static final String FINAL\_OUTPUT\_100 = "final-output-100";

private static final String FINAL\_B\_TOUT100\_V2\_M1 = "final-BTout100-v2-m1";

/\*\*

\* The main method to be executed.

\*

\* @param args

\*/

public static void main(String[] args) {

try {

// Reading the 250 chromosomes file.

List<String> chr1FileLines = FileUtils.getInstance().readFile(CHR1\_250\_FA);

MappabilityUtils.getInstance().checkMappability(FINAL\_B\_TOUT50\_V2\_M1, TILE\_LENGTH\_50, FINAL\_OUTPUT\_50, GenerateReadsApp.READS\_50\_FA, chr1FileLines);

MappabilityUtils.getInstance().checkMappability(FINAL\_B\_TOUT70\_V2\_M1, TILE\_LENGTH\_70, FINAL\_OUTPUT\_70, GenerateReadsApp.READS\_70\_FA, chr1FileLines);

MappabilityUtils.getInstance().checkMappability(FINAL\_B\_TOUT100\_V2\_M1, TILE\_LENGTH\_100, FINAL\_OUTPUT\_100, GenerateReadsApp.READS\_100\_FA, chr1FileLines);

} catch (IOException e) {

e.printStackTrace();

}

}

}

## com.bio.main.GenerateReadsApp

package com.bio.main;

import java.io.IOException;

import com.bio.main.util.FileUtils;

/\*\*

\* This application reads a chromose file (in this case, chr1-250.fa) and produces different Reads files for the given size and save them into the

\* destination file. All the files must be placed under /Assignment5/io folder.

\*

\* @author Mohamad Mahdi Ziaee

\*

\*/

public class GenerateReadsApp {

// The list of constants

private static final String CHR1\_250\_FA = "chr1-250.fa";

public static final String READS\_50\_FA = "reads50.fa";

public static final String READS\_70\_FA = "reads70.fa";

public static final String READS\_100\_FA = "reads100.fa";

private static final int READ\_LENGTH\_50 = 50;

private static final int READ\_LENGTH\_70 = 70;

private static final int READ\_LENGTH\_100 = 100;

/\*\*

\* Main application method to be executed.

\*

\* @param args

\*/

public static void main(String[] args) {

try {

FileUtils.getInstance().createReads(READ\_LENGTH\_50, READS\_50\_FA, CHR1\_250\_FA);

FileUtils.getInstance().createReads(READ\_LENGTH\_70, READS\_70\_FA, CHR1\_250\_FA);

FileUtils.getInstance().createReads(READ\_LENGTH\_100, READS\_100\_FA, CHR1\_250\_FA);

} catch (IOException e) {

e.printStackTrace();

}

}

}

## com.bio.main.MergeOutputApp

package com.bio.main;

import java.io.IOException;

import com.bio.main.util.FileUtils;

/\*\*

\* Merges the given mappability files into one file which could be used by R application for analysis.All the files must be placed under

\* /Assignment5/io folder.

\*

\* @author Mohamad Mahdi Ziaee

\*

\*/

public class MergeOutputApp {

private static final String MAPPABILITY\_OUTPUT\_250 = "Mappability-output-250";

/\*\*

\* The main method to be executed.

\*

\* @param str

\*/

public static void main(String[] str) {

try {

FileUtils.getInstance().merge(MAPPABILITY\_OUTPUT\_250, CheckMappabilityApp.FINAL\_OUTPUT\_50, CheckMappabilityApp.FINAL\_OUTPUT\_70, CheckMappabilityApp.FINAL\_OUTPUT\_100);

} catch (IOException e) {

e.printStackTrace();

}

}

}

## com.bio.main.util.FileUtils

package com.bio.main.util;

import java.io.BufferedWriter;

import java.io.FileWriter;

import java.io.IOException;

import java.io.PrintWriter;

import java.nio.file.Files;

import java.nio.file.Paths;

import java.util.ArrayList;

import java.util.HashMap;

import java.util.List;

import java.util.Map;

import java.util.concurrent.TimeUnit;

import org.apache.commons.lang3.StringUtils;

import org.apache.commons.lang3.time.StopWatch;

import com.bio.pojo.Mappability;

/\*\*

\* The main utility class which is in charge of all the file related operations

\*

\* @author Mohamad Mahdi Ziaee

\*

\*/

public class FileUtils {

// Constants used for reading from and writing to the files.

private static final String ZERO = "0.00";

private static final String GENE\_ID\_HEADER = "geneID";

public static final String SEPARATOR\_DOT = ".";

public static final String SEPARATOR\_TAB = "\t";

public static final String CHR\_INDICATOR = ">";

public static final String IO\_PATH = "../Assignment5/io/";

private static FileUtils instance = null;

/\*\*

\* Private constructor for Singleton design pattern purpose. Declared private so it is not accessible from outside.

\*/

private FileUtils() {

}

/\*\*

\* Gets instance of this class. It will instantiate it if it is not done yet, once.

\*

\* @return

\*/

public static FileUtils getInstance() {

if (instance == null) {

instance = new FileUtils();

}

return instance;

}

/\*\*

\* Generates Reads for the given read length and save it into the output file name passed to this method. If the file output exists, it will

\* overwrite it.

\*

\* @param readLength

\* The read length

\* @param outputFileName

\* The output file name to save the reads to

\* @param chr1FileName

\* The chromosome source file to generate the reads from

\* @throws IOException

\*/

public void createReads(int readLength, String outputFileName, String chr1FileName) throws IOException {

System.out.println("Creating [" + outputFileName + "]...");

StopWatch stopWatch = new StopWatch();

stopWatch.start();

String outputPath = FileUtils.IO\_PATH + outputFileName;

Files.deleteIfExists(Paths.get(outputPath));

List<String> lines = readFile(chr1FileName);

String header = null;

PrintWriter out = new PrintWriter(new BufferedWriter(new FileWriter(outputPath, true)));

for (int i = 0; i < lines.size(); i++) {

String line = lines.get(i);

if (i % 2 == 0) {

// Reading the gene name

header = line;

} else {

// Reading the sequence

List<String> tiles = createTiles(readLength, line);

writeReadsToFile(header, tiles, out);

}

}

out.close();

stopWatch.stop();

System.out.println("[" + outputFileName + "] is done in [" + TimeUnit.MILLISECONDS.convert(stopWatch.getNanoTime(), TimeUnit.NANOSECONDS) + "] MILLISECONDS");

}

/\*\*

\* Writes the tiles given into the output file by adding a counter at the end of the gene name.

\*

\* @param header

\* Gene name

\* @param tiles

\* The list of tiles generated for a given gene sequence

\* @param out

\* the output file PrintWriter

\* @throws IOException

\*/

private void writeReadsToFile(String header, List<String> tiles, PrintWriter out) throws IOException {

int counter = 0;

for (String tile : tiles) {

// Writing the header

StringBuffer buffer = new StringBuffer();

buffer.append(header);

buffer.append(SEPARATOR\_DOT);

buffer.append(counter++);

out.println(buffer.toString());

out.println(tile);

}

}

/\*\*

\* Given a gene sequence and a read length, it generates all the possible tiles.

\*

\* @param readLength

\* Desired read length

\* @param line

\* The main string to create the tiles from

\* @return The list of tiles

\*/

public List<String> createTiles(int readLength, String line) {

List<String> result = new ArrayList<>();

if (readLength < line.length()) {

for (int index = 0; index < line.length() - readLength + 1; index++) {

result.add(line.substring(index, index + readLength));

}

} else {

result.add(line);

}

return result;

}

/\*\*

\* Given the file name, it reads all its content and put it into a list.

\*

\* @param fileName

\* File name to read

\* @return

\* @throws IOException

\*/

public List<String> readFile(String fileName) throws IOException {

return Files.readAllLines(Paths.get(FileUtils.IO\_PATH + fileName));

}

/\*\*

\* Writes the given map of mappabilities into the output file.

\*

\* @param result

\* A map which the key is the gene names and the value is the mappability related to that gene.

\* @param outputFileName

\* The name of the file to save the result into.

\* @param fileTileLength

\* The length of the tile, this is used to generate the header

\* @throws IOException

\*/

public void writeFile(Map<String, Mappability> result, String outputFileName, int fileTileLength) throws IOException {

String path = FileUtils.IO\_PATH + outputFileName;

Files.deleteIfExists(Paths.get(path));

PrintWriter out = new PrintWriter(new BufferedWriter(new FileWriter(path, true)));

// Printing the header

out.println(String.join(SEPARATOR\_TAB, GENE\_ID\_HEADER, "readLen" + fileTileLength));

for (String geneName : result.keySet()) {

Mappability output = result.get(geneName);

if (output == null) {

out.println(String.join(SEPARATOR\_TAB, geneName, ZERO));

} else {

out.println(String.join(SEPARATOR\_TAB, geneName, output.getMappability()));

}

}

out.close();

}

/\*\*

\* Merges any given number of files into one. It overwrites if the output already exists.

\*

\* @param outputFileName

\* The output file name.

\* @param inputFileNames

\* The list of input File names

\* @throws IOException

\*/

public void merge(String outputFileName, String... inputFileNames) throws IOException {

Map<String, List<String>> file = readFileIntoMap(inputFileNames);

String path = FileUtils.IO\_PATH + outputFileName;

Files.deleteIfExists(Paths.get(path));

PrintWriter out = new PrintWriter(new BufferedWriter(new FileWriter(path, true)));

// Printing the header

StringBuffer stringBuffer = createLine(file, GENE\_ID\_HEADER);

out.println(stringBuffer);

file.remove(GENE\_ID\_HEADER);

// Printing the body

for (String geneId : file.keySet()) {

stringBuffer = createLine(file, geneId);

out.println(stringBuffer);

}

out.close();

}

/\*\*

\* It constructs a line in merging process for each mappability type.

\*

\* @param file

\* The content of the whole mappabilities.

\* @param geneId

\* the Desired geneId which the line must be created for.

\* @return

\*/

private StringBuffer createLine(Map<String, List<String>> file, String geneId) {

StringBuffer stringBuffer = new StringBuffer();

stringBuffer.append(geneId);

stringBuffer.append(SEPARATOR\_TAB);

stringBuffer.append(String.join(SEPARATOR\_TAB, file.get(geneId)));

return stringBuffer;

}

/\*\*

\* For the given file names, it reads the files and put them into a map in which the key is the gene names and the value is the mappability

\* related to that gene.

\*

\* @param inputFileNames

\* @return

\* @throws IOException

\*/

private Map<String, List<String>> readFileIntoMap(String[] inputFileNames) throws IOException {

Map<String, List<String>> result = new HashMap<>();

for (String fileName : inputFileNames) {

List<String> lines = readFile(fileName);

for (String line : lines) {

String[] splittedLine = StringUtils.splitByWholeSeparator(line, SEPARATOR\_TAB);

String geneId = splittedLine[0];

if (!result.containsKey(geneId)) {

result.put(geneId, new ArrayList<>());

}

List<String> existingMappabilities = result.get(geneId);

existingMappabilities.add(splittedLine[1]);

result.put(geneId, existingMappabilities);

}

}

return result;

}

}

## com.bio.main.util.MappabilityUtils

package com.bio.main.util;

import java.io.IOException;

import java.util.HashMap;

import java.util.List;

import java.util.Map;

import java.util.concurrent.TimeUnit;

import org.apache.commons.lang3.StringUtils;

import org.apache.commons.lang3.time.StopWatch;

import com.bio.pojo.Gene;

import com.bio.pojo.Mappability;

/\*\*

\* The main utility class which is in charge of mappability related operations.

\*

\* @author Mohamad Mahdi Ziaee

\*

\*/

public class MappabilityUtils {

private static MappabilityUtils instance = null;

/\*\*

\* Private constructor for Singleton design pattern purpose. Declared private so it is not accessible from outside.

\*/

private MappabilityUtils() {

}

/\*\*

\* Gets instance of this class. It will instantiate it if it is not done yet, once.

\*

\* @return

\*/

public static MappabilityUtils getInstance() {

if (instance == null) {

instance = new MappabilityUtils();

}

return instance;

}

/\*\*

\* The main method to check the mappabilities of the given read file names.

\*

\* @param btOutputFileName

\* BowTie output file name

\* @param fileTileLength

\* Tile length

\* @param outputFileName

\* Output file name which the result will be saved into

\* @param readFileName

\* Reads file name

\* @param chr1FileLines

\* chromosome file name

\* @throws IOException

\*/

public void checkMappability(String btOutputFileName, int fileTileLength, String outputFileName, String readFileName, List<String> chr1FileLines) throws IOException {

System.out.println("Creating [" + outputFileName + "]...");

StopWatch stopWatch = new StopWatch();

stopWatch.start();

System.out.println("Reading [" + btOutputFileName + "]");

List<String> btOutputFileLines = FileUtils.getInstance().readFile(btOutputFileName);

System.out.println("Done reading [" + btOutputFileName + "]");

System.out.println("Reading [" + readFileName + "]");

List<String> readFileLines = FileUtils.getInstance().readFile(readFileName);

System.out.println("Done reading [" + readFileName + "]");

Map<String, Mappability> result = createMappability(btOutputFileLines, readFileLines, chr1FileLines);

FileUtils.getInstance().writeFile(result, outputFileName, fileTileLength);

stopWatch.stop();

System.out.println("[" + outputFileName + "] is done in [" + TimeUnit.MILLISECONDS.convert(stopWatch.getNanoTime(), TimeUnit.NANOSECONDS) + "] MILLISECONDS");

}

/\*\*

\* Creating a map which its key is a gene name and its value is the mappability. It also calculates the number of tiles which are within the gene.

\*

\* @param btOutputFileLines

\* BowTie output file name

\* @param readFileLines

\* Reads file content in a list

\* @param chr1FileLines

\* chromosome file name in a list

\* @return

\* @throws IOException

\*/

private Map<String, Mappability> createMappability(List<String> btOutputFileLines, List<String> readFileLines, List<String> chr1FileLines) throws IOException {

Map<String, Mappability> result = new HashMap<>();

for (String btOutputLine : btOutputFileLines) {

String[] splittedLine = StringUtils.split(btOutputLine, FileUtils.SEPARATOR\_TAB);

Integer tileStart = getTileStartIndex(splittedLine);

Integer tileEnd = getTileEndIndex(splittedLine);

Gene gene = getGeneId(splittedLine);

String geneName = gene.getName();

if (!result.containsKey(geneName)) {

result.put(geneName, new Mappability(0, getTotalNumberOfTiles(geneName, readFileLines)));

}

Mappability output = result.get(geneName);

if (isTileWithinGene(tileStart, tileEnd, gene)) {

output.addNumberOfMatches();

}

result.put(geneName, output);

}

// Add the missing tiles from output

for (int lineIndex = 0; lineIndex < chr1FileLines.size(); lineIndex++) {

if (lineIndex % 2 == 0) {

// Reading the first line

String geneName = StringUtils.substringAfter(chr1FileLines.get(lineIndex), FileUtils.CHR\_INDICATOR);

if (!result.containsKey(geneName)) {

result.put(geneName, null);

}

}

chr1FileLines.get(lineIndex);

}

return result;

}

/\*\*

\* Calculates the total number of tiles by counting the occurance of the gene name in the reads file content list passed.

\*

\* @param geneName

\* @param readFileLines

\* @return

\* @throws IOException

\*/

private Integer getTotalNumberOfTiles(String geneName, List<String> readFileLines) throws IOException {

int counter = 0;

for (String line : readFileLines) {

if (line.contains(geneName)) {

counter++;

}

}

return counter;

}

/\*\*

\* A tile is within a gene if its start and end indexes are within the gene's index range.

\*

\* @param tileStart

\* @param tileEnd

\* @param gene

\* @return

\*/

private boolean isTileWithinGene(Integer tileStart, Integer tileEnd, Gene gene) {

return gene.getStartIndex() <= tileStart && tileEnd <= gene.getEndIndex();

}

/\*\*

\* Extracts the geneID out of the splitted file line.

\*

\* @param splittedLine

\* @return

\*/

private Gene getGeneId(String[] splittedLine) {

String[] geneSplittedStr = StringUtils.split(splittedLine[3], FileUtils.SEPARATOR\_DOT);

return new Gene(Integer.valueOf(geneSplittedStr[1]), Integer.valueOf(geneSplittedStr[2]), StringUtils.substringBeforeLast(splittedLine[3], FileUtils.SEPARATOR\_DOT));

}

/\*\*

\* Gets the tile end index by looking at the 3rd element in the given splitted line

\*

\* @param splittedLine

\* @return

\*/

private Integer getTileEndIndex(String[] splittedLine) {

return Integer.valueOf(splittedLine[2]);

}

/\*\*

\* Gets the tile end index by looking at the 2nd element in the given splitted line

\*

\* @param splittedLine

\* @return

\*/

private Integer getTileStartIndex(String[] splittedLine) {

return Integer.valueOf(splittedLine[1]);

}

}

## com.bio.pojo.Gene

package com.bio.pojo;

/\*\*

\* A POJO to hold gene information like start and end indexes.

\*

\* @author Mohamad Mahdi Ziaee

\*

\*/

public class Gene {

private Integer startIndex;

private Integer endIndex;

private String name;

/\*\*

\* The main constructor.

\*

\* @param startIndex

\* @param endIndex

\* @param name

\*/

public Gene(Integer startIndex, Integer endIndex, String name) {

this.startIndex = startIndex;

this.endIndex = endIndex;

this.name = name;

}

public Integer getStartIndex() {

return startIndex;

}

public void setStartIndex(Integer startIndex) {

this.startIndex = startIndex;

}

public Integer getEndIndex() {

return endIndex;

}

public void setEndIndex(Integer endIndex) {

this.endIndex = endIndex;

}

public String getName() {

return name;

}

public void setName(String name) {

this.name = name;

}

}

## com.bio.pojo.Mappability

package com.bio.pojo;

import java.text.DecimalFormat;

/\*\*

\* A POJO to hold the data related to the mappability. It contains the number of matches and the tiles.

\*

\* @author Mohamad Mahdi Ziaee

\*

\*/

public class Mappability {

private Integer numberOfMatches;

private Integer numberOfTiles;

private static final DecimalFormat df = new DecimalFormat("#0.00##");

/\*\*

\* The main constructor.

\*

\* @param numberOfMatches

\* @param numberOfTiles

\*/

public Mappability(Integer numberOfMatches, Integer numberOfTiles) {

this.numberOfMatches = numberOfMatches;

this.numberOfTiles = numberOfTiles;

}

public void addNumberOfMatches() {

this.numberOfMatches++;

}

public Integer getNumberOfTiles() {

return numberOfTiles;

}

public void setNumberOfTiles(Integer numberOfTiles) {

this.numberOfTiles = numberOfTiles;

}

public Integer getNumberOfMatches() {

return numberOfMatches;

}

public void setNumberOfMatches(Integer numberOfMatches) {

this.numberOfMatches = numberOfMatches;

}

/\*\*

\* Calculates the mappability percentage by dividing numberOfMatches over numberOfTiles \* 100. It is formatted using #0.00## formatter.

\*

\* @return

\*/

public String getMappability() {

return df.format(numberOfMatches.doubleValue() / numberOfTiles.doubleValue() \* 100);

}

}