

7.36/7.91/20.390/20.490/6.802/6.874

**PROBLEM SET 2. BWT, Library complexity, RNA-seq, Genome assembly, Motifs, Multiple hypothesis testing (31 Points)**

**Due: Thursday, March 13<sup>th</sup> at noon.**

**Python Scripts**

All Python scripts must work on athena using /usr/athena/bin/python. You **may not assume availability of any third party modules** unless you are explicitly instructed so. You are advised to test your code on Athena before submitting. Please only modify the code between the indicated bounds, with the exception of adding your name at the top, and remove any print statements that you added before submission.

Electronic submissions are subject to the same late homework policy as outlined in the syllabus and submission times are assessed according to the server clock. **All python programs must be submitted electronically, as .py files** on the course website using appropriate filename for the scripts as indicated in the problem set or in the skeleton scripts provided on course website.

**Problem 1. Aligning reads to a genome using a Burrows Wheeler Transform and FM Index (9 points)**

For this exercise you will be implementing the core of a genome search function utilizing the Burrows Wheeler transform (BWT) and an FM-index. We have provided scaffolding code so that you can focus on the core of the algorithm. Please do not use Internet search tools to try to solve this problem – the point is for you to understand how the algorithm works.

You will need the coding and testing files from the course website (keep them in the same folder). This includes scaffold code, a 10kb segment of the yeast genome, reads which you will map to the genome, and an index for testing with correct output.

- (A) (7 pt.)** Complete the LF mapping code in the `_lf(self, idx, qc)` function and the search code in the `bounds(self, q)` function (both in `fmindex.py`).

To test your implementations we have provided the FM-index of an abbreviated version of the yeast genome in `test.index`. Running

```
% python fm-search.py test.index yeast_chr1_reads.txt out.txt
```

will place the mapped reads in `out.txt` and test your implementation. The correct output of this command is given in `test_mapped.txt` for you to check the correctness of your implementation. Your implementations of the `_lf(self, idx, qc)` and `bounds(self, q)` functions are the answer to 1.1. **Submit** `fmindex.py`.

- (B) (2 pt.)** Now let's make sure your implementation works on a larger genome. First you will build the FM-index. To build the index use the command:

```
% python fm-build.py yeast_chr1_10k.txt yeast_chr1_10k.index
```

Now let's search using the FM index with the code you wrote, and use it to map some reads

To map the reads:

```
% python fm-search.py yeast_chr1_10k.index yeast_chr1_reads.txt mapped_reads.txt
```

View the output:

```
% more mapped_reads.txt
example mapped_reads.txt (you will not have this same read):
ATGGGTATCGATCACACTTCCAAGCAACAC count:1 matches:[561]
...
```

**Submit** your `mapped_reads.txt` on course website as the answer to 1.2.

## Problem 2. Library Complexity (5 points)

Imagine you are responsible for sequencing DNA samples for your lab's latest important experiment. Using extensive simulations, you know that you need to observe at least 12 million unique molecules in order to test your current hypothesis. From previous experience, you know that each time a DNA library is constructed from a sample, it will contain exactly 40 million unique molecules (selected perfectly at random). You also know that *C. elegans*, your model organism, has a genome size of approximately 100 million base pairs.

You can have your sample sequenced in units called lanes. Each lane gives you 10 million reads, and a library can be sequenced on as many lanes as you want. However, ever-protective of your grant money, you want to achieve your experimental goals in the most efficient way possible. Suppose that each sample collection and library preparation step costs \$500 and that each sequencing lane costs \$1000.

- (A) (2 pt.)** Assume that each molecule in the library had equal probability of being sequenced. What is the most cost-effective experimental design (number of libraries and lanes sequenced for each library) for achieving your goal of observing 12 million unique molecules? Show your work.

**(B) (3 pt.)** Now suppose that there is variation in the selection probabilities across each molecule, which follows a negative binomial distribution with rate  $\lambda = 0.25$  (10 million reads divided by 40 million molecules) and variance factor  $k = 2$  (estimated from previous experiments). What is the most cost-effective experimental design for this situation? Show your work and comment on any differences between the two cases.

*Hint:* A more common formulation of the negative binomial distribution is in terms of failures  $n$  and a success probability  $p$ . This conversion is found in the lecture slides.

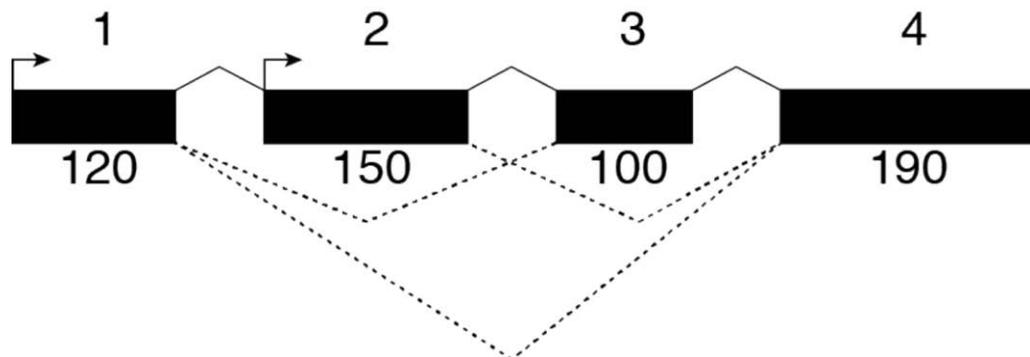
### **Problem 3. Differential gene expression (4 points)**

You are analyzing RNA-seq data to identify differentially expressed genes between two treatment conditions. You have three biological replicates in each of the two conditions for a total of 6 samples, and you process and sequence each of the samples separately.

**(A) (1 pts)** Imagine you first pool the sequencing results for each of the conditions, resulting in two pools. What kind of variation have you lost the ability to observe, and why might this variation be important?

**(B) (3 pts)** Devise an improved analysis strategy for these six samples and identify the sources of variation it can detect. Identify how you would estimate the mean-dispersion function for use in a negative binomial model of variation.

**Problem 4. RNA Isoform quantification (3 points)**



**Fig. 1**

Consider the gene structure in the above figure.

Exon numbers and sizes in nucleotides are indicated. The transcript can initiate at either of the arrows shown, and exons 2 and/or 3 can be spliced out.

**(A) (1 pt.)** How many possible isoforms of this gene could exist?

**(B) (1 pt.)** For each isoform, list the junction spanning RNA-seq reads that would support it.

**(C) (1 pt.)** Assuming single ended reads, what is the shortest read length that would guarantee the ability to unambiguously identify all isoforms of this gene if we require that a junction read must have minimum overlap of 5bp with each exon?

**Problem 5. de Bruijn graphs (5 points)**

Suppose you are interested in sequencing a particular RNA sequence. You opt to take a next generation sequencing approach and submit your sample to your local sequencing facility. You receive the following set of 6 bp reads in return, which are all in the same orientation.

AGCTGT, CAGCTG, TTCTGC, GCTGTA, TCAGCT, CTGTAT, TGTAGC, TTCAGC, CTGTAG, TTTCAG

**(A) (1 pt.)** Construct the corresponding de Bruijn graph with  $k = 5$

**(B) (1 pt.)** Simplify any chains in the graph. Remove any tips present in the graph.

**(C) (1 pt.)** Identify any bubbles in the graph. Resolve the bubbles by removing the path most likely to be caused by a sequencing error.

**(D) (1 pt.)** Which read(s) contain sequencing errors? Identify the error(s).

**(E) (1 pt.)** Write the sequence represented by the de Bruijn graph after the error correction steps.

**Problem 6. Modeling and information content of sequence motifs (5 points).**

To analyze gene evolution in three phylogenetic groups of protists, you collect samples of three different protist species, A, B, and C, that represent these lineages. You conduct both genome sequencing and cDNA sequencing from each and use spliced alignment of cDNAs to genomes to obtain sets of 10,000 confirmed 3' splice site (3'SS) sequences from each species. In all three species the invariant AG at the end of each intron is preceded by an 8 base polypyrimidine tract (PPT), with frequencies  $f_C = f_T = \frac{1}{2}$  at each position. Your goal is to develop probabilistic models of the PPT motif in each species for use in exon-intron prediction. Throughout this problem, unless instructed otherwise, you should describe the simplest possible model (fewest parameters) that accurately models the frequencies of all 8mers in the training data (and should therefore give good predictive accuracy). Information content of models should be calculated using the formula given in lecture:  $I = 2w - H(\text{model})$ , in bits, where  $w$  is the width of the motif and  $H(\text{model})$  is the Shannon entropy of the model. The abbreviation  $Y_8$  refers to 8mers that consist exclusively of pyrimidine (C or T) nucleotides.

**(A) (1 pt.)** In species A, all four dinucleotides CC, CT, TC, and TT occur equally often ( $f_{CC} = f_{CT} = f_{TC} = f_{TT} = \frac{1}{4}$ ) at each of the seven pairs of positions (1,2), (2,3), ..., (7,8), and each 8mer of the form  $Y_8$  occurs with frequency  $2^{-8}$ . In one sentence, describe a model for the PPT of species A. What is the information content of this model?

**(B) (1 pt.)** In species B, all four dinucleotides CC, CT, TC, and TT are equally likely ( $f_{CC} = f_{CT} = f_{TC} = f_{TT} = \frac{1}{4}$ ) at each of the seven pairs of positions (1,2), (2,3), ..., (7,8), but examining the frequencies of 8mers reveals that  $f_{T_8} = f_{C_8} = f_{(TC)_4} = f_{(CT)_4} = \frac{1}{4}$ . In one sentence, describe a model for the PPT of species B. What is the information content of this motif?

**(C) (3 pt.)** In species C,  $f_{CC} = f_{TT} = \frac{3}{8}$ ,  $f_{TC} = f_{CT} = \frac{1}{8}$  at each of the seven pairs of consecutive positions (1,2), (2,3), ..., (7,8), and the frequencies of all 8mers of the form  $Y_8$  are equal to  $3^{a+b}/Z$  where a is the number CC dinucleotides in the 8mer and b is the number of TT dinucleotides in the 8mer, and Z is the normalization constant that causes the frequencies to sum to 1. In one sentence, describe a model for the PPT of species B. What is the information content of this motif?

**(Extra 6.874 Problem) Multiple Hypothesis Testing (4 points)**

Differential expression analysis of RNA-seq data involves testing thousands of hypotheses in a single experiment. To limit false positives, it is necessary to adjust P-values. Two popular methods for doing so are Bonferroni correction and Benjamini-Hochberg.

Consider the following uncorrected p-values of 20 genes from a gene expression study in which we wish to identify differentially expressed genes, say using DEseq.

Gene	P-value
1	0.0002
2	0.0005
3	0.0040
4	0.0060
5	0.0070
6	0.0080
7	0.0090
8	0.0110
9	0.0120
10	0.0120

11	0.01500
12	0.02300
13	0.02400
14	0.03400
15	0.03900
16	0.04700
17	0.05000
18	0.05800
19	0.06000
20	0.09800

- (A) (1 pt.) Apply Bonferroni correction and list the genes that would be reported as differentially expressed at  $\alpha = 0.05$ . Show how you obtain the list.
- (B) (1 pt.) List the genes that would be reported as differentially expressed using Benjamini-Hochberg correction at  $\alpha = 0.05$ . Show how you obtain the list.
- (C) (2 pt.) How do the two lists differ in composition? What does this show about the stringency of these corrections?

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