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Oligonucleotide Design for Whole Genome Tiling Arrays

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Graduate Program in Computer Science
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

OLIGONUCLEOTIDE DESIGN FOR WHOLE GENOME TILING ARRAYS

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by

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Abstract

Oligonucleotides are short, single-stranded fragments of DNA or RNA, designed to readily bind with a unique part in the target sequence. They have many important applications including PCR (polymerase chain reaction) amplification, microarrays, or FISH (fluorescence in situ hybridization) probes.

While traditional microarrays are commonly used for measuring gene expression levels by probing for sequences of known and predicted genes, high-density, whole genome tiling arrays probe intensively for sequences that are known to exist in a contiguous region.

Current programs for designing oligonucleotides for tiling arrays are not able to produce results that are close to optimal since they allow oligonucleotides that are too similar with non-targets, thus enabling unwanted cross-hybridization. We present a new program, BOND-tile, that produces much better tiling arrays, as shown by extensive comparison with leading programs.

Keywords: DNA, oligonucleotide, tiling arrays, microarrays

Acknowlegements

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Chapter 1

Introduction

Oligonucleotides are short, single-stranded fragments of DNA or RNA, designed to readily bind with a unique part in the target sequence. They are labelled in a way that allows identification, e.g., using fluorescence, thus permitting the detection of their target. It is therefore essential that good oligos do not cross-hybridize with non-target sequences. Oligonucleotides have many important applications including PCR (polymerase chain reaction) amplification, microarrays, or FISH (fluorescence in situ hybridization) probes.

While traditional microarrays are commonly used for measuring gene expression levels by probing for sequences of known and predicted genes, high-density, whole genome tiling arrays probe intensively for sequences that are known to exist in a contiguous region. Whole genome tiling arrays have many advantages such that the high reproducibility among arrays, unbiased and complete genomic coverage, multiple and potential overlaps, and probes representing transcription factor binding regions.

There are several programs for designing whole genome tiling oligonucleotide probes, such as ArrayDesign [7] and OligoTiler [3]. The main procedure of the leading programs for detecting cross-hybridization is based on several derivative tools of the suffix array [17] and BLAST (Basic Local Alignment Search Tool) [2]. For this reason, they are not able to produce results that are close to optimal since they allow oligonucleotides that are too similar with non-targets, thus enabling unwanted cross-hybridization. In addition, they can not produce the maximum number of good

oligonucleotides, thus not being able to detect every unique fragment.

In order to design better oligonucleotides, we present a new program, BOND-tile, which uses the method of spaced seed-based similarity search and employs multiple spaced seeds [15] computed by SpEED [9]. The proposed BOND-tile algorithm is designed to search the non-overlapping unique oligonuleotide probes for whole genome tiling, considering factors such as:

- Oligo placement
- Similarity between an oligonucleotide and its non-target sequences
- Melting temperature
- GC-content
- Heuristic oligo selection

The new BOND-tile program designs 100% good oligonucleotides without any noisy output, and finds the largest number of unique oligonucleotides. It produces significantly better oligonucleotides as shown by extensive comparison with leading programs.

The thesis is organized as follows. After a brief introduction of the biological background and computer science methods in Chapter 2, we describe completely the new BOND-tile algorithm in Chapter 3, and perform the comparison against ArrayDesign and OligoTiler in Chapter 4, concluding with a brief discussion of the achievements in Chapter 5.

Chapter 2

Genome Tiling

In this chapter, we introduce the basic concepts and definitions. It includes three sections. The first section introduces molecular biology terminology and concepts. The second section explains the concept of oligonucleotides and their applications. Finally, the central problem of the thesis, whole genome tiling, is introduced, together with some of the leading programs for solving it.

2.1 Molecular biology primer

In order to understand the concepts of the thesis, we present a brief introduction to basic biological background.

2.1.1 Organisms and cells

Every organism, a living system, is composed of cells. A cell is the functional unit and basic structure of organisms. Organisms are classifed into unicellular and multicellular. Cells have two types, eukaryotic and prokaryotic. Prokaryotic cells can live independently, while eukaryotic cells are components of multicellular organisms. The most important difference between eukaryotic and prokaryotic cells is that eukaryotic cells include their organelles which are divided and wrapped by their membrane. The organelles are the main site of occurring specific metabolic activities. Cells exchange

information and make decisions through complex networks of chemical interaction which are called pathways [1].

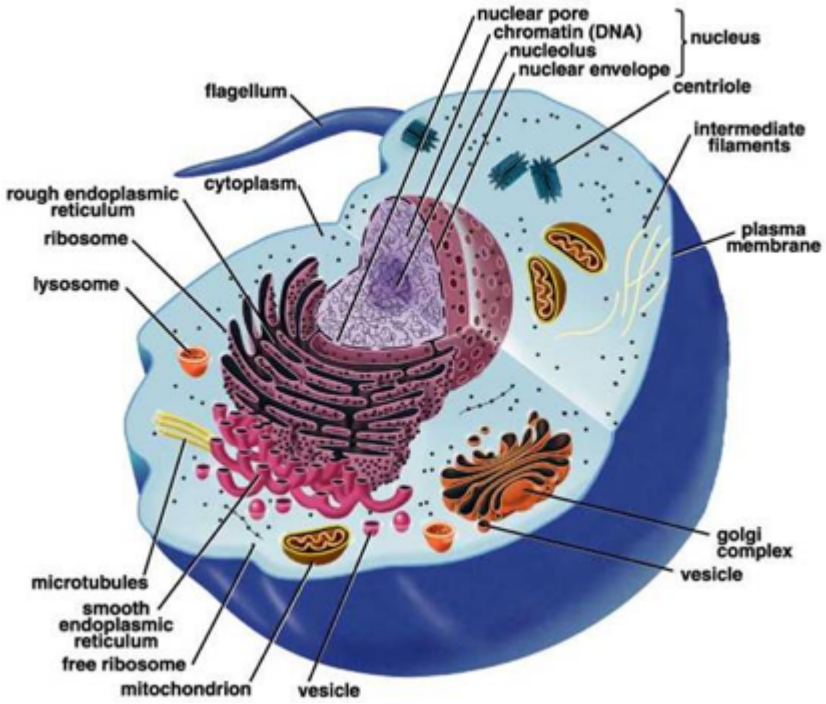


Figure 2.1: An eukaryotic cell. (from Wikipedia)

2.1.2 DNA, RNA, and proteins

DNA, *deoxyribonucleic acid* (Fig. 2.2), is a biological macromolecule that contains the genetic instructions to guide biological development and vital functions. The four bases found in DNA are adenine (A), cytosine (C), guanine (G) and thymine (T).

RNA, *ribonucleic acid* (Fig. 2.2), is also a biological macromolecule which is constructed from nucleotides and chemically similar to DNA. The four bases found in RNA are adenine (A), cytosine (C), guanine (G), and uracil (U). In the cell, according to the different structures and functions, RNA is classified into three categories, namely tRNA, rRNA, and mRNA. mRNA is the template of protein synthesis based on translation from a DNA sequence; tRNA identifies the genetic code on the mRNA and is the amino acid transporter; rRNA is contained in the ribosome which is the machinery of protein synthesis [1].

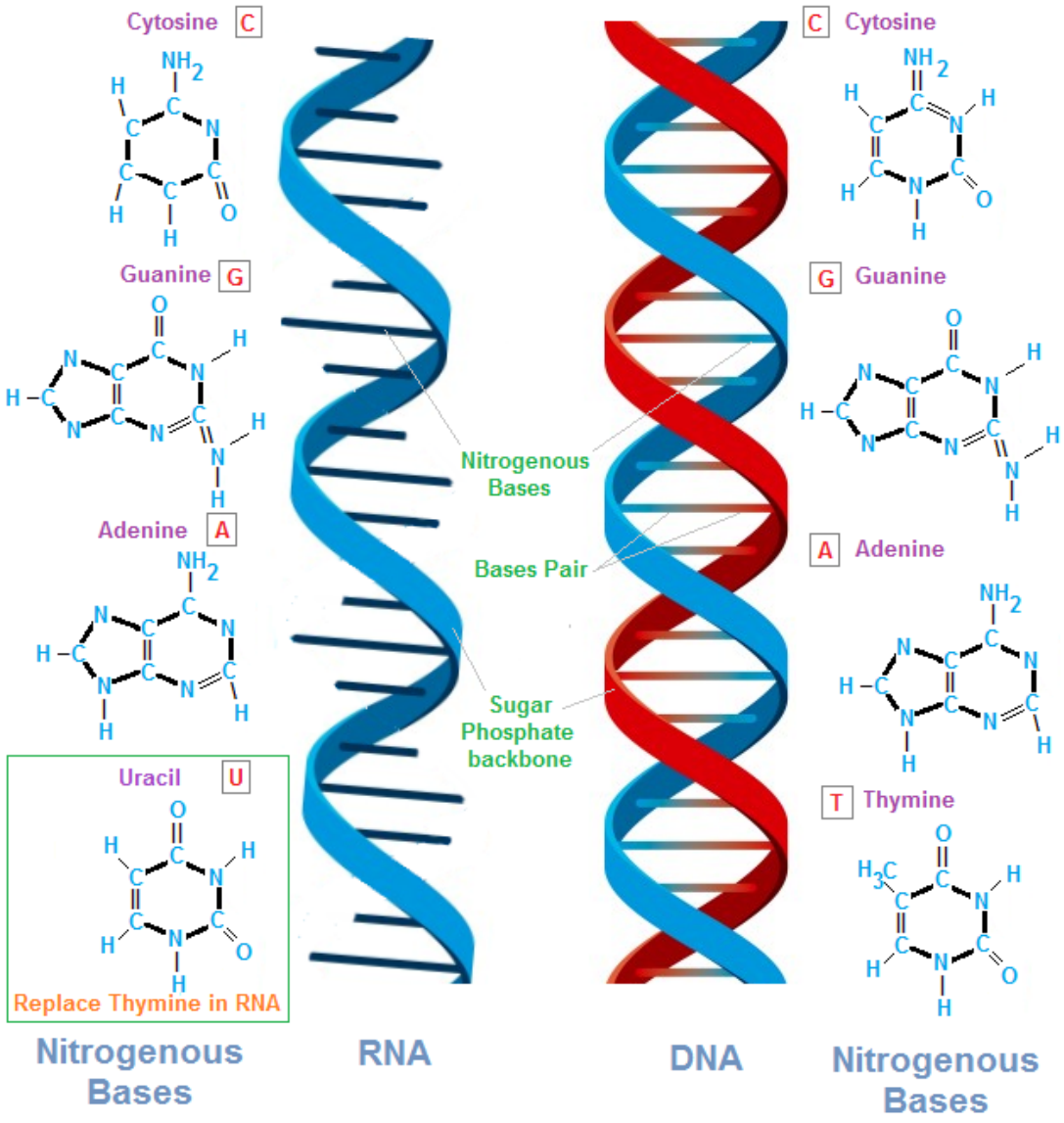


Figure 2.2: Structure of DNA and RNA.
(http://chemistry.tutorvista.com/biochemistry/nucleic-acids.html)

Proteins are biological molecules composed of amino acids. They are the functional molecules of the cell and can be classifed into groups such as structural proteins, enzymes and transmembrane proteins. Biochemists often identify four distinct aspects of a protein's structure (presented in Figure 2.3): primary structure, secondary structure, tertiary structure, and quaternary structure.

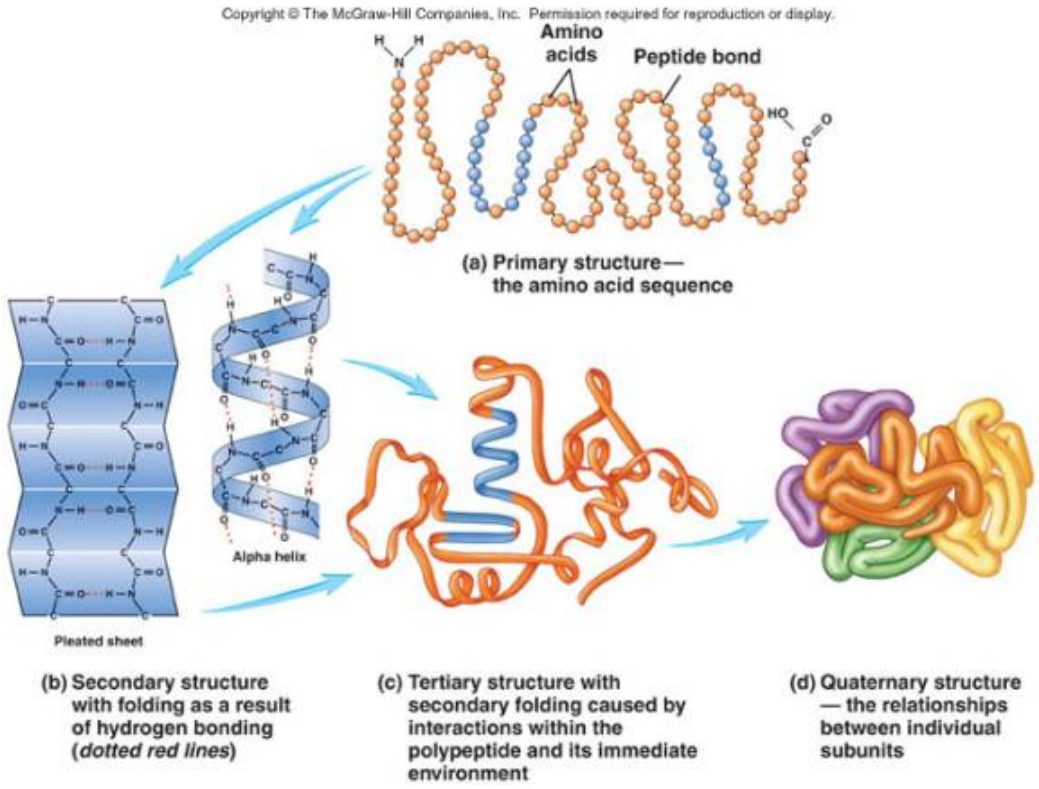


Figure 2.3: Structure of proteins. (http://www.entrenandotualimentacion.es/2013/07/03/suplementacion-con-whey-protein-en-ancianos/)

2.1.3 Genome, chromosome, and gene

The genome of an organism includes a complete set of DNA sequences. The DNA is packaged into thread-like structures called chromosomes.

A gene is a piece of DNA that carries genetic information. The process of forming proteins is guided by genetic information. Genes are the basic units of heredity to control the characters of an organism.

The relationship between genome, chromosomes and genes is shown in Figure 2.4:

2.1.4 Thermodynamics of DNA

DNA is normally a double stranded macromolecule. The strands are held together by hydrogen bonds (Fig. 2.5). Thus, the double-stranded structure of DNA is formed

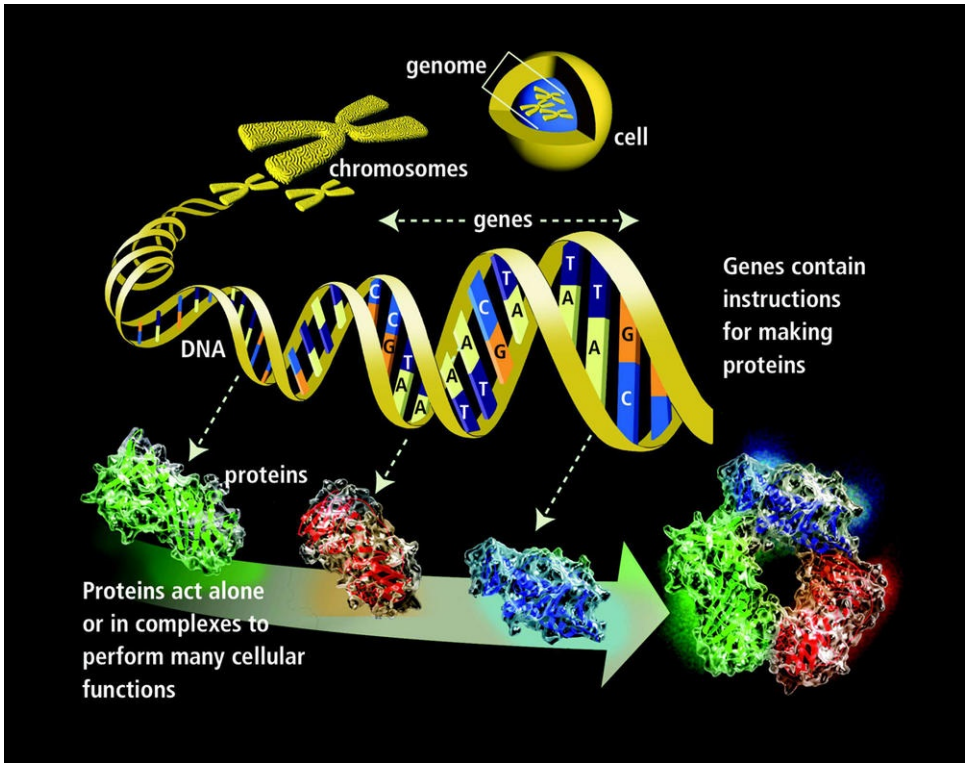


Figure 2.4: Relationship of genome, chromosome and genes
The genome (inside the cell) contains all of an organism's genetic instructions. (http://www.broadinstitute.org/blog/word-day-genome)

by binding two complementary DNA sequences together. This phenomenon is called hybridization. Double-stranded DNA can be separated into two single strands by heating. This process is called DNA melting. During the melting of DNA, hydrogen bonds between complementary bases will be broken. The melting temperature, T_m, indicates the temperature at which the number of single-stranded molecules and double-stranded molecules is the same.

In DNA, the pairs A=T have two hydrogen bonds, while C≡G have three hydrogen bonds (Fig. 2.6). Therefore, C≡G is stronger than A=T. The GC content is the percentage of C≡G pairs in DNA. Limits imposed on the GC content constrain the range of melting temperature. Generally, sequences with a higher percentage of GC base pairs have a higher T_m than AT-rich sequences do.

Several methods are available to calculate the melting temperature. The Nearest Neighbour model is the most widely used approach [20, 23] which will be described

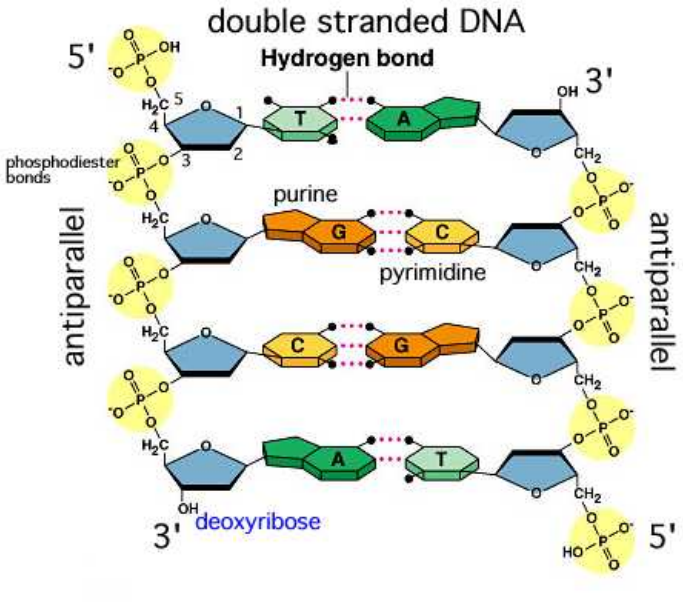


Figure 2.5: Double-stranded DNA. (from City University of New York) The 5' end and the 3' end refer to the two distinctive ends of DNA.

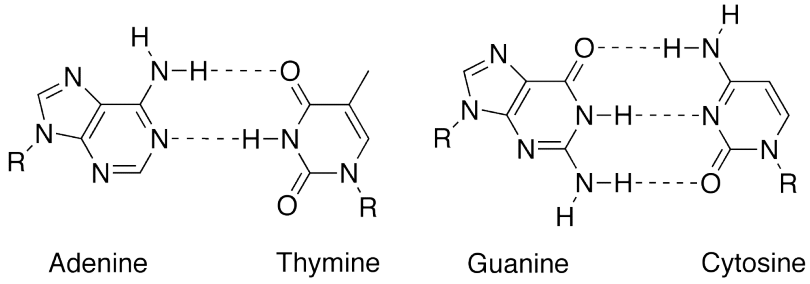


Figure 2.6: Hydrogen bonds between AT and GC base pairs. (from Wikipedia)

in detail in Chapter 3.

2.2 Oligonucleotides and applications

2.2.1 Oligonucleotides

A DNA oligonucleotide, abbreviated oligo, is a single-stranded chain of DNA that is designed to bind uniquely to a target region (Fig. 2.7). A good oligo should not cross-hybridize with non-target sequences.

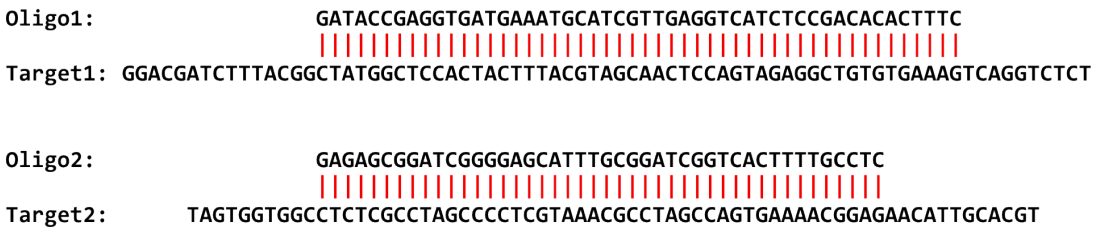


Figure 2.7: An example of oligos hybridized with their target DNA sequences.

2.2.2 Applications of oligonucleotides

Oligo have many important applications, some of the most important ones of which are listed below:

- PCR (polymerase chain reaction) amplification; oligos are used as primers whose sequence is complementary to the 5' end of the sequence targeted for amplification.

- Microarrays; these are collections of microscopic DNA spots attached to a solid surface. The DNA sequences attached are the oligos. By specific target hybridization, simultaneous measurement of the expression of large number of genes is performed (Fig. 2.8).

- FISH (Fluorescence In Situ Hybridization) probes; oligos labelled with fluorescent dyes are used so that hybridization can be detected by fluorescence microscopy. This method can be used for detecting and localizing DNA or RNA within cells and tissues.

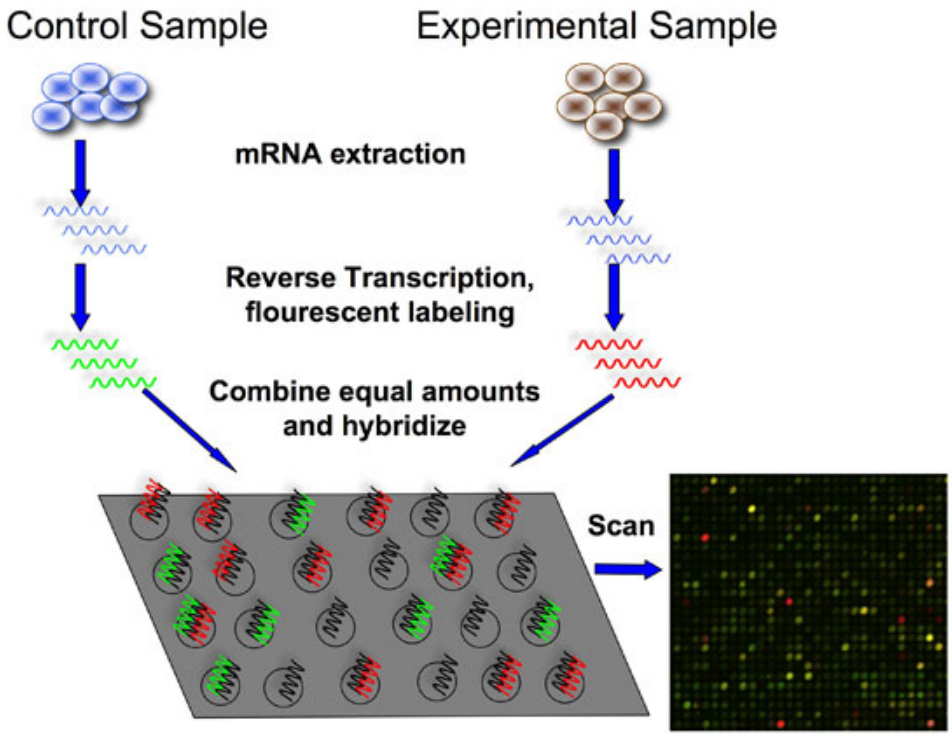


Figure 2.8: A typical DNA microarray co-hybridization (2 dye) experiment. (http://bitesizebio.com/articles/introduction-to-dna-microarrays/)

2.3 Whole Genome Tiling

DNA microarrays are an effective technology commonly used for measuring gene expression levels in biological and medical areas. Microarrays use one or several probes for every gene. They probe for sequences of known and predicted genes.

Microarray chips have a branch referred to as tiling arrays. Instead of probing for sequences of known or predicted genes that may be scattered throughout the genome, whole-genome tiling arrays probe intensively for sequences that are known to exist in a contiguous region (Fig. 2.9). In order to cover entire genomes, the density of probes needs to be much higher than the traditional microarrays.

The significant advantages of tiling arrays over the traditional ones includes the high reproducibility among arrays, unbiased and complete genomic coverage, multiple and potential overlaps, and probes representing transcription factor binding regions [18]. Therefore, whole-genome tiling arrays are a useful tool in genome-wide associa-

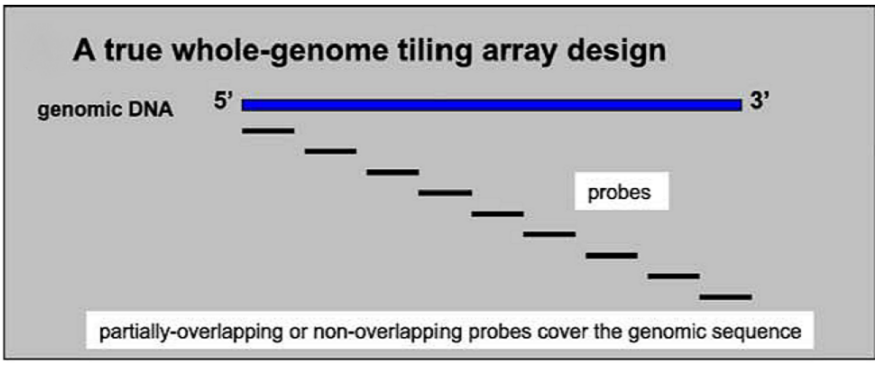


Figure 2.9: Unbiased whole-genome tiling array designs [18].
Probes may be partially overlapping or nonoverlapping and tiled end to end.

tion studies for detection of genetic variants.

There are several approaches, presented in Figure 2.10, using whole-genome high-density oligonucleotide tiling arrays for transcriptome characterization and genome analysis.

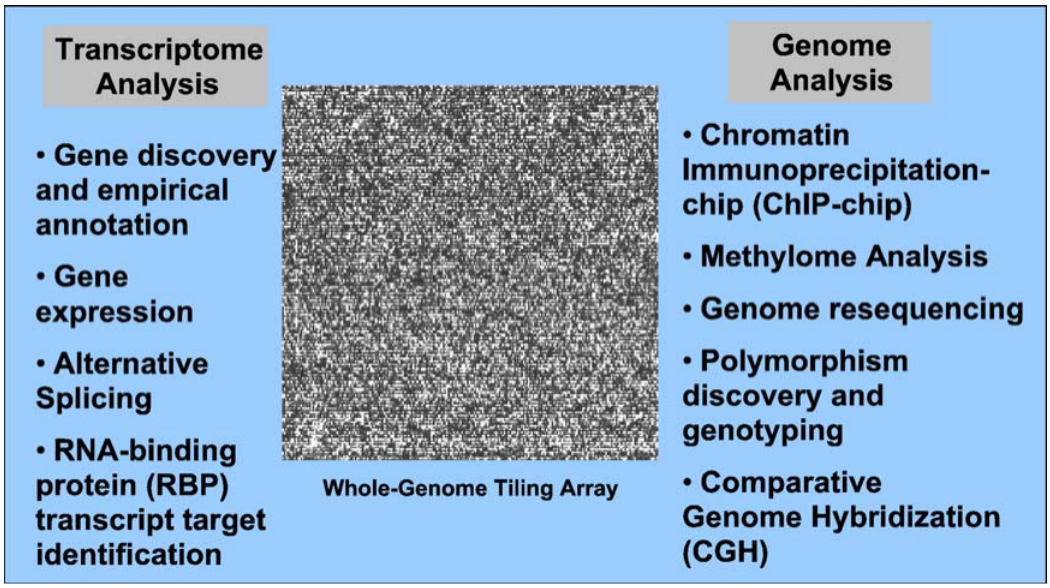


Figure 2.10: Whole-genome high-density tiling arrays provide a universal data capture platform for a variety of genomic information [18].

2.4 Sequence alignments

We present in the following sections two notions from computer science that are necessary for understanding some of the whole genome tiling approaches. Sequence alignment is a method widely used for comparing strings in order to find regions of high similarity between them. Evolutionary related sequences have regions of high similarity which show the structural and functional relationship between them.

Alignments are of two types: global and local. A global alignment is an alignment in which every element in the two sequences is involved. A local alignment finds similar regions (substrings) between the two sequences.

An earlier global alignment algorithm, based on dynamic programming, was developed by the Needleman and Wunsch [19]. An example is shown in Figure 2.11.

		A	**C**	**T**	**G**	**A**	**T**	**T**
	0	-2	-4	-6	-8	-10	-12	-14
A	-2	**2**	0	-2	-4	-6	-8	-10
C	-4	0	**4**	**2**	0	-2	-4	-6
G	-6	-2	2	1	**4**	2	0	-2
C	-8	-4	0	-1	**2**	1	-1	-3
A	-10	-6	-2	-3	0	**4**	2	0
T	-12	-8	-4	0	-2	2	**6**	**4**

The alignment:

A C T G – A T T

A C – G C A T –

Figure 2.11: Needleman-Wunsch alignment of two sequences; the dynamic programming matrix and the optimal alignment.

The Needleman-Wunsch algorithm may miss short but highly similar regions because the optimal global alignment may choose a path that outweighs them. There-

fore, local alignments are often more useful for detecting the homology of sequences. Smith and Waterman [25] developed an algorithm, also based on dynamic programming, to find the substring pair with the maximum similarity score. An example is given in Figure 2.12. The Smith-Waterman method finds the subsequence with the highest score.

		G	C	C	C	T	A	G	C	G
	0	0	0	0	0	0	**0**	0	0	0
G	0	1	0	0	0	0	0	**1**	0	1
C	0	0	2	1	1	0	0	0	**2**	0
G	0	1	0	1	0	0	0	1	0	**3**
C	0	0	2	1	2	0	0	0	2	1
A	0	0	0	1	0	1	1	0	0	1
A	0	0	0	0	0	0	2	0	0	0

The alignment:

G C G

G C G

Figure 2.12: Smith-Waterman alignment of two sequences;
the dynamic programming matrix and the best local alignment.

2.5 Text indexing

Text indexing is the general procedure of constructing a data structure from a given text such that certain operations can be performed efficiently. The best known text indexes are the suffix tree [26] and the suffix array [17].

The suffix tree of a string is a tree whose edges are labelled with substrings such that every suffix of the string corresponds to a unique path from the root to a leaf. Suffix trees can do many string operations in linear time that otherwise are very

difficult. An example of a suffix tree is shown in Figure 2.13.

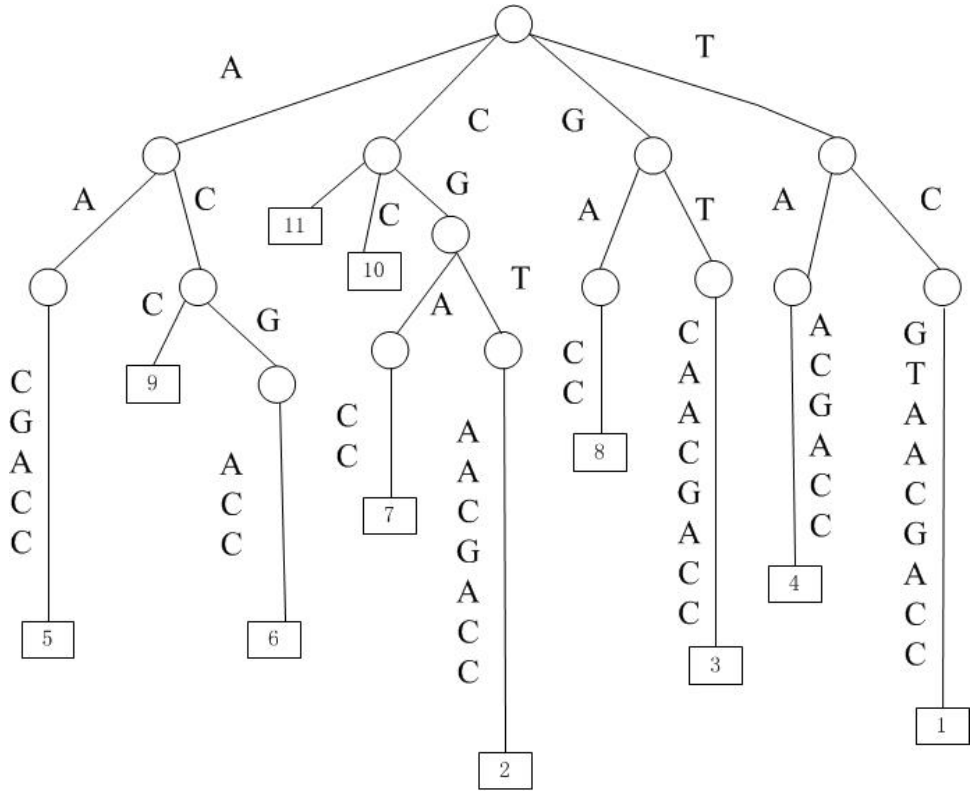


Figure 2.13: The suffix tree for the string TCGTAACGACC;
the leaves are labelled by the starting positions of the corresponding suffixes.

The suffix array (SA) of a string is defined as an array of positions sorted in the lexicographical order of the corresponding suffixes of the string. The longest common prefix (LCP) of two consecutive suffixes in the suffix array stores the length of the longest shared prefix. Figures 2.14 gives an example of a suffix array and a LCP array.

2.6 FM-index

The FM-index [5] is a compressed full-text substring index referring to an opportunistic data structure based on the Burrows-Wheeler transform.

i	SA$[i]$	Suffix$_{\mathrm{SA}[i]}$	LCP$[i]$
1	5	AACGACC	0
2	9	ACC	1
3	6	ACGACC	2
4	11	C	0
5	10	CC	1
6	7	CGACC	1
7	2	CGTAACGACC	2
8	8	GACC	0
9	3	GTAACGACC	1
10	4	TAACGACC	0
11	1	TCGTAACGACC	1

Figure 2.14: The suffix array (SA) for the string TCGTAACGACC; the third column gives the suffixes and the last the LCP array.

2.6.1 Burrows-Wheeler transform

The Burrows-Wheeler transform (BWT) is an algorithm applied in data compression. The algorithm was invented by Michael Burrows and David Wheeler [4]. It transforms a string of characters into a permutation of similar characters. When using this method to convert a string, the algorithm only changes the order of the characters in the string without changing any value of its characters. If the original string contains some substrings that appear several times, then there will be several continuously repeated characters on the transformed string, which would benefit compression.

BWT includes the following steps in order to implement the transformation:

- Create a table whose rows are all possible rotations of the string.
- Sort all rows in lexicographical order.
- Extract the last column of the table as the output.

The output contains some consecutive repeated characters so that it is easier to compress. In addition, this transformation can be reversed without any additional data being stored. Therefore, the BWT improves the efficiency of text compression algorithms. An example is given in Figure 2.15.

Transformation				
Input	All Rotations	Sorting All Rows in Alphabetical Order	Taking Last Column	Output Last Column
^BANANA|	^BANANA| |^BANANA A|^BANAN NA|^BANA ANA|^BAN NANA|^BA ANANA|^B BANANA|^	ANANA|^B ANA|^BAN A|^BANAN BANANA|^ NANA|^BA NA|^BANA ^BANANA| |^BANANA	ANANA|^B ANA|^BAN A|^BANAN BANANA|^ NANA|^BA NA|^BANA ^BANANA| |^BANANA	BNN^AA|A

Figure 2.15: Burrows-Wheeler transform for the string "^BANANA|". The string " ^BANANA |" is transformed into "BNN ^AA | A" (the red vertical bar indicates the 'end of file' pointer). (from Wikipedia)

2.6.2 The FM-index

The FM-index uses the Burrows-Wheeler transform along with the Suffix Array data structure such that it allows compression of the input string, simultaneously permitting fast substring queries. The most important advantage of the FM index is that it uses significantly less space compared with the suffix array.

An example of how the FM-index finds the position of a substring without uncompressing is shown in the following steps:

- Transforming the string "acaacg$" into the BWT string "gc$aaac" through Burrows-Wheeler matrix of the string (the $ indicates the 'end of file' pointer), synchronously recording the corresponding suffix array (Fig. 2.16).

- The substring "aac" is searched for using the BWT string and the alphabetical order row. The pairs of continuing arrows indicate the region of the BWT that includes all positions that correspond to the current suffix of the substring searched for. The process is represented in Figure 2.17.

- If the substring "aac" is found in at least one row, its positions are obtain by the corresponding value of suffix array shown above (Fig. 2.16).

Thus, "aac" is located in the third position of the given string "acaacg$".

F						L	SA
$	a	c	a	a	c	g	7
a	a	c	g	$	a	c	3
a	c	a	a	c	g	$	1
a	c	g	$	a	c	a	4
c	a	a	c	g	$	a	2
c	g	$	a	c	a	a	5
g	$	a	c	a	a	c	6

Figure 2.16: FM-index table for the string "acaacg$" via the Burrows-Wheeler matrix that contains all rotations of the string sorted lexicographically. The 7th column gives the BWT output string and the last records the corresponding SA.

Figure 2.17: Steps to match the substring "aac" in the string "acaacg$" [13].

2.7 Leading programs for genome tiling

A significant amount of research has been performed for designing tiling oligonucleotide probes. In this chapter, we present the top two current algorithms for whole genome tiling arrays.

2.7.1 ArrayDesign

ArrayDesign [7] constructs whole genome tiling arrays by defining a measure of quality for oligonucleotide probes named the uniqueness score (U). It is shown that U is equivalent to the number of shortest unique substrings in the probe. A greedy algorithm is given to design whole genome tiling arrays using probes that maximize U. The outline of the ArrayDesign algorithm is as follows:

Defining the uniqueness score

To determine the uniqueness score, the minimum unique prefixes are computed at all possible positions. This is reduced to a congruent relationship between the minimum unique substrings and the distinct end positions of minimum unique prefixes (Fig. 2.18). The relationship is that counting the distinct end positions of minimum unique prefixes helps computing the number of minimum unique substrings. In Figure 2.18, the sequence of each minimum unique prefix in the windows is shown. Computing the number of minimum unique substrings gives the uniqueness score for every window of a fixed length.

Computing minimum unique prefixes

ArrayDesign uses an algorithm based on a simple technique to compute minimum unique prefixes. The uniqueness problem involves the comparison of a huge number of uniqueness queries against the same data set. A solution based on the FM-index was developed. ArrayDesign first constructs the Burrows-Wheeler transform, then uses a multiway merging procedure which synchronously reads all suffix arrays [17] from left to right. After each merging step, the next suffix is acquired in the sorted order of all suffixes and obtain the corresponding character of the Burrows-Wheeler transform and the other related information. After the FM-index is stored, the uniqueness problem can be solved.

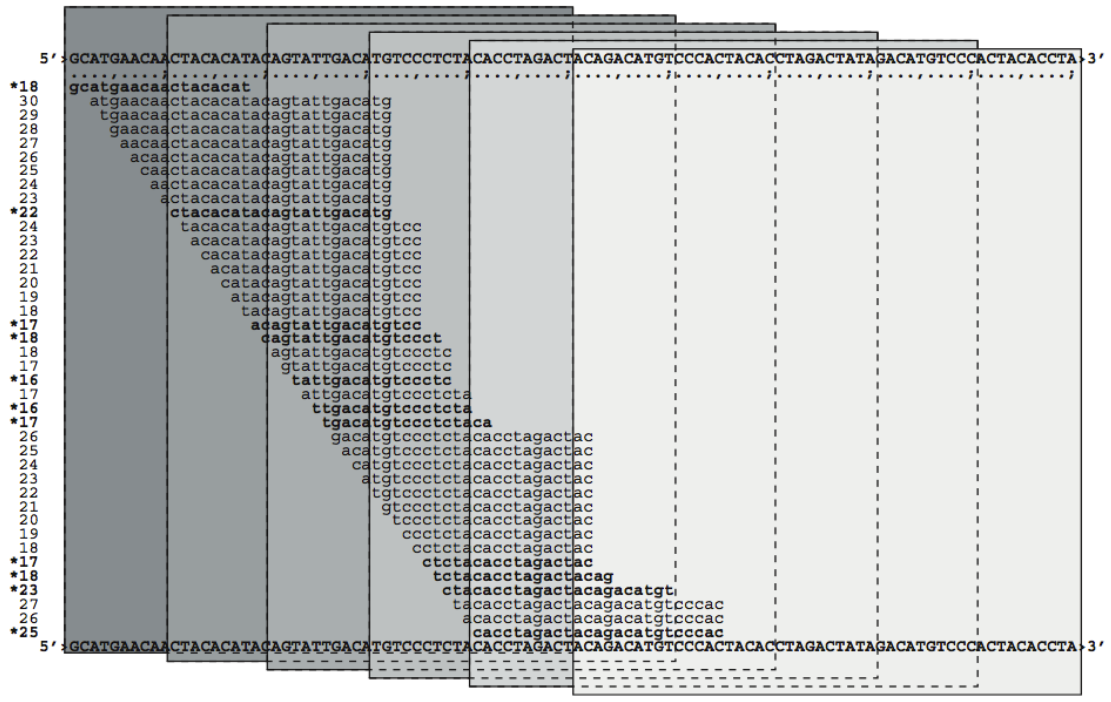


Figure 2.18: Uniqueness scoring function [7] (exemplified by Mus musculus, chr17:3028401-3028500). The minimum unique prefixes for each window are illustrated in the sequences shown. In each window of a fixed length, counting the number of minimum unique substrings helps calculating the uniqueness score. The minimum unique substrings, which are indicated by stars, are used to add to the uniqueness score. The uniqueness scores for the six windows shown are 7, 9, 7, 4, 1, 0, respectively.

Validating the uniqueness score

A collection of 50-mer probes from the NimbleGen whole genome tiling array was used to validate the uniqueness score. For each of these probes, the uniqueness score is determined as explained above and the number of hybridization-quality alignments for each probe to the genome is computed using BLAT [12] (BLAST-Like Alignment Tool).

Probe selection algorithm

The optimal probes are calculated (as shown in Figure 2.18) using a greedy selection strategy, which considers also a number of additional restrictions on the probes.

Figure 2.19 illustrates the process of the probe selection algorithm.

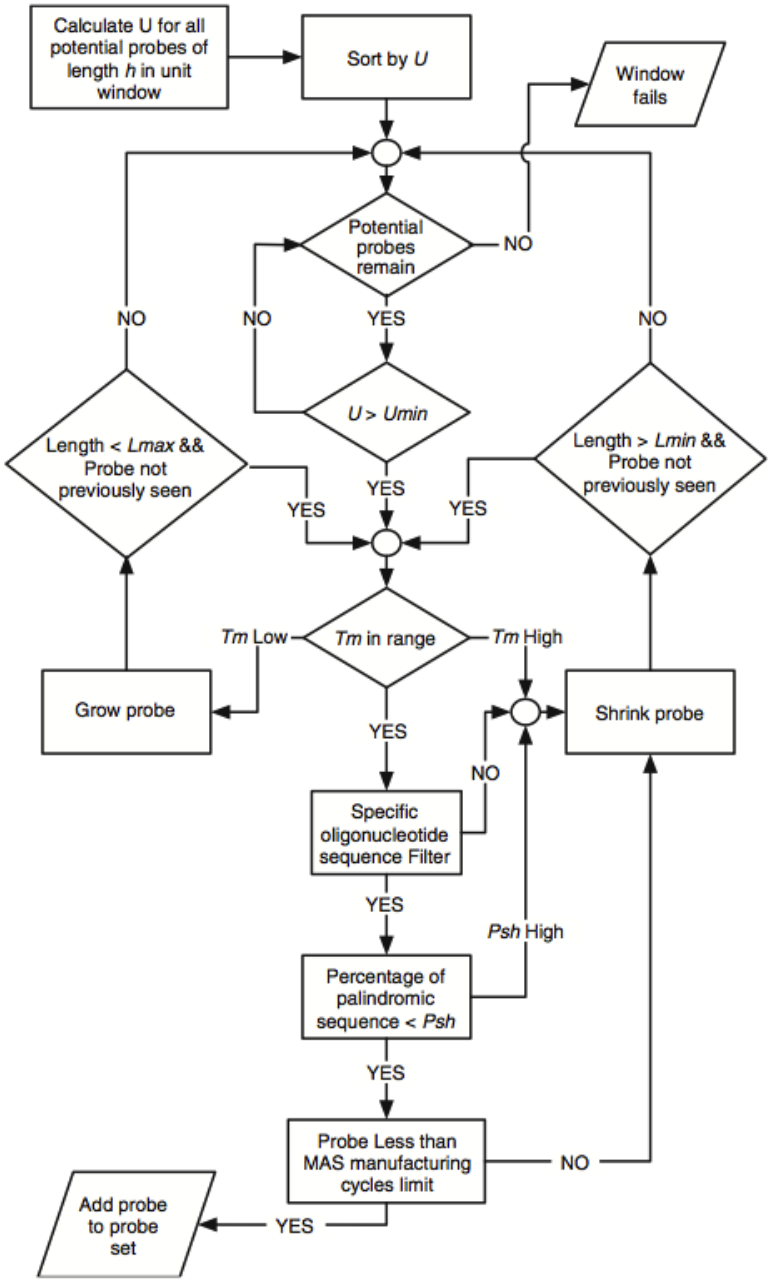


Figure 2.19: Probe selection algorithm [7].

The parameters that are considered in the probe selection step are:

- Ensuring that the uniqueness score is higher than a given U threshold.

- Calculating the melting temperature using the following salt-adjusted approximation [22]:

$$T_m = 81.5 + 16.6 \times log(c(Na^+)) + 0.41 \times f_{GC} - 600/N \quad (2.1)$$

where $c(Na^+)$ is the salt concentration, f_{GC} is the frequency of GC's, and N is the overall number of nucleotides in the sequence.

- Making sure not to include any specific runs which produce unsatisfactory oligonucleotides (e.g., more than 6 consecutive G's).

- Checking that the fragment can only contain less than a pre-set percentage of palindromic sequence to eliminate probes with significant self-hybridization potential.

- Choosing the probes with less than a given maskless array synthesis (MAS) manufacturing cycle limit.

2.7.2 OligoTiler

OligoTiler [3] determines the similarity degree of many oligonucleotide sequences from large genomes. It gives two algorithms for finding an optimal tile path. The first, implemented by a dynamic programming approach, finds the optimal tiling in linear time and space and the second uses a heuristic search to decrease the space complexity to satisfy a constant requirement. The main methods and considered factors of OligoTiler are shown below.

Sequence similarity and single-copy tiling

This design of tiling arrays involves computing the degree of similarity of any given oligonucleotide sequence in a large genome in order to generate a single-copy tile path which is represented on the array (Fig. 2.20). This intends to identify and eliminate non-unique sequences to reduce the potential cross-hybridization of sequences

elsewhere in the genome. Due to the problem of memory constraints and sequence mismatches, OligoTiler uses a BLAST-like scheme.

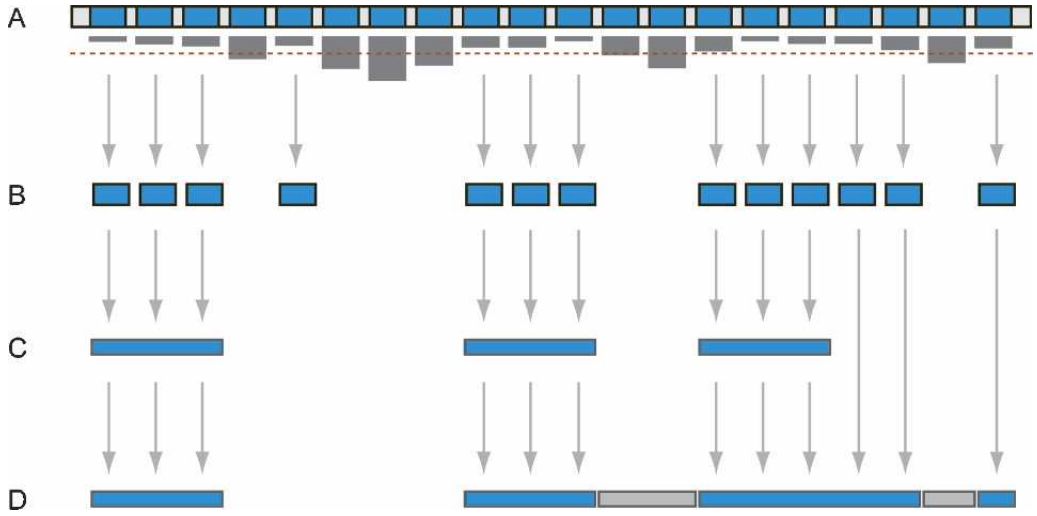


Figure 2.20: The problem of sequence similarity in tiling genomic DNA [3]. (A) Calculating the level of similarity (shown by the grey bars). The sequences are eliminated from the tiling path when the sequence redundancy surpasses the pre-set threshold (shown by the dashline). (B) Avoiding redundant or repetitive sequence, simultaneously retaining sufficient sequence tiling. (C) With the minimum tile connecting with their contiguous tile, the level of non-repetitive sequence coverage decreases. (D) The tiling algorithms is enabled to involve a few redundant sequences in an optimal fashion aiming at acquiring a higher percentage of non-repetitive DNA.

Repeat identification and low-complexity filtering

On a global scale, OligoTiler adopts libraries of known repeats identified in genomic DNA through comparison of sequences using BLAST [2] (Basic Local Alignment Search Tool). Using software such as RepeatMasker (`http://ftp.genome.washington.edu/RM/RepeatMasker.html`) is the most convenient way to accomplish it.

Tiling resolution

An important factor, tiling resolution, involves determining how to subdivide the remaining non-repetitive DNA segments and how densely oligonucleotides should rep-

resent it. It makes use of shifting the starting position of each probe by a few nucleotides so that the windows can overlap the previous oligonucleotides coordinates (Fig. 2.21). In Figure 2.21, the strategy of using single-base offset placement provides a finer-resolution sequence tiling. In addition, it can indicate more accurately where hybridizing sequences are located on the chromosome.

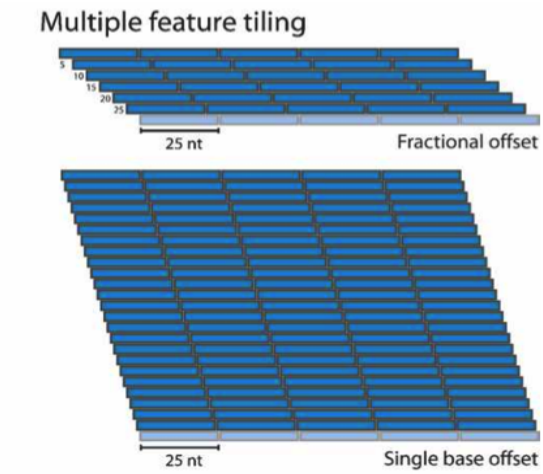


Figure 2.21: Multiple Feature Tiling [3]. Overlapping tiles using fractional offset (e.g., one 25-mer probe placed every 5 nucleotides) and single-base offset placement.

Thermodynamic properties of oligonucleotide probes

One of the factors of OligoTiler in selecting oligonucleotide sequences for tiling arrays is the predicted hybridization affinity [23]. For sequences over thirteen nucleotides, hybridization affinity is almost the equivalent of calculating the melting temperature using this formula:

$$T_m = 64.9 + 41 \times (n_G + n_C - 16.4)/(n_A + n_T + n_G + n_C) \quad (2.2)$$

where $n_{[A,C,G,T]}$ denotes the number of each nucleotide in the DNA sequence.
For more precise calculations, OligoTiler uses a base-stacking approach for the exact

sequence [21]:

$$T_m = [\Delta H(kcal/^\circ C \times Mol)/\Delta S + R \times ln([oligo]/2)] - 273.15^\circ C \quad (2.3)$$

In order to increase the consistency of T_m which corresponds to each probe, computing the melting temperature helps shifting the replacement of oligonucleotide within every non-repetitive region. OligoTiler selects the individual probe from each available region such that its computed T_m is closer to the optimal temperature than the unsatisfactory region. The universal set of oligos can be shifted all together so that their aggregate T_m can be optimized.

Additional Parameters

Other parameters considered to create oligonucleotides can be set by the user:

- "IR region" considers how OligoTiler deals with the potential inverted repeats at the two ends of an oligo. It gives a limit of how many characters at the two ends should be checked in order to avoid inverted repeats.
- "IR require" considers the same parts as above. It means the number of matching base pairs that must be checked within the "IR region" such that the start position of the oligo will be shifted.

Chapter 3

BOND-tile

In this chapter, we describe our new program for whole genome oligo tiling: BOND-tile. We explain first why the current approaches connot produce the best oligo sets. Then, we introduce the notion of spaced seeds, that is central to our new design.

3.1 Problems with previous designs

Two leading programs for whole genome tiling arrays were described in the last section of Chapter 2. We point out several problems with their design, common to all existing tools, not just the two we have described.

The first, and most important problem, is hybridization with non-targets. DNA hybridization happens between complementary sequences. However, the complementarity does not have to be perfect for strong hybridization to take place. Since oligos are supposed to bind only to their targets, their complementary sequences must be very different from any non-target sequence. The identity percentage denotes the percentage of matches between two sequences. Usually, identity of 75% or lower is required in order to prevent binding to non-targets [11]. Differently put, the identity between the complement of the oligo and any non-target must be less than 75%.

The existing programs succeed only partially at achieving this goal. Some use various heuristics, with limited success, others use BLAST [2] in order to find similarities and eliminate them. BLAST however has limitations in finding similarities.

For instance, BLAST finds only 40% of the similarities of length 50 and identity 75%. Its sensitivity is shown as the pink line in Figure 3.3.

The second problem is that the current programs do not find the highest possible number of oligos, as it will be seen in our evaluation.

Third, the methods used in evaluating these programs suffer from the same problem of not being able to thoroughly look for similarities. For example, the survey [14] uses BLAST which, as we indicated above, is inappropriate.

These problems appear also in programs designing traditional oligonucleotides and they were corrected by the recent BOND program [10] (BOND stands for Basic OligoNucleotide Design). Using the same method, of spaced seed-based similarity search, we extend here the BOND program to solve the more difficult problem of whole genome tiling. Our new program is called BOND-tile.

3.2 Spaced seeds

A full alignment procedure, the dynamic programming algorithm of Smith-Waterman, was used before fast algorithms were developed. The Smith-Waterman algorithm has perfect sensitivity but its quadratic time complexity makes it impossible to apply for large sequences. Hence, heuristics approaches that can identify similarity fast are needed. The Basic Local Alignment Search Tool [2], BLAST, became the most widely used algorithm in searching similarities. It is assumed that high similarity implies sharing a long substring which results in local alignment of the two sequences. The development of BLAST was based on this idea. BLAST uses a consecutive seed 11111111111 to select 11 consecutive positions which are identical. A '1' denotes a match. The 11 consecutive matches are called a hit. BLAST finds pairs of such matching strings (hits) and then attempts to extend the similarity both ways (Fig. 3.1). Consecutive matches are easy to find, but BLAST may miss some high similarity regions with a few mismatches. (As an example, one can imagine a similarity where all positions match, except for every eleventh one. The similarity is about 91%, yet it is undetected by BLAST.)

Figure 3.1: BLAST will extend the consecutive matches to find more similarities.

PatternHunter [16] presented a homology search algorithm by using an non-consecutive spaced seed to check matches between sequences, which has a higher sensitivity. PatternHunter's seed is 111*1**1*1**11*111; a '1' denotes a match and '*' represents a "don't care" position. In other words, it only checks the positions directly corresponding to 1's, while ignoring the positions corresponding to *'s. The weight of a seed refers to the number of 1's. The total number of 1's and *'s is the length of a seed. The probability of a hit depends only on the weight of the seed. So, between non-spaced and spaced seeds with the same weight, the expected number of hits is the same. Spaced matches have higher sensitivity because of less overlapping between hits. Put another way, with the shifting of a seed, spaced seeds need more matches to detect another hit. Therefore, consecutive seeds suffer from hit clustering and thus spaced seeds can detect more similarities. Figure 3.2 illustrates the comparison between non-spaced and spaced seeds.

Figure 3.2: Comparison between non-spaced and spaced seeds.
A hit of the contiguous seed (left) requires only one more match for another hit at the next position, whereas for the spaced seed (right) six additional matches are needed.

In order to dramatically increase the sensitivity, a set of several seeds can be ap-

plied together for homology searching, which is called multiple spaced seeds. Using multiple seeds can reach almost perfect sensitivity [15]. To design an efficient similarity check, we need optimal multiple spaced seeds but they are hard to compute. Several heuristic algorithms have been designed to calculate multiple spaced seeds, but all of them have exponential time complexity except SPEED [9, 8]. This algorithm calculates highly sensitive multiple spaced seeds and was used to compute the seeds employed by BOND-tile. Figure 3.3 illustrate the comparison of BLAST's seed and multiple spaced seeds computed by SPEED which was used by the BOND program for designing oligos.

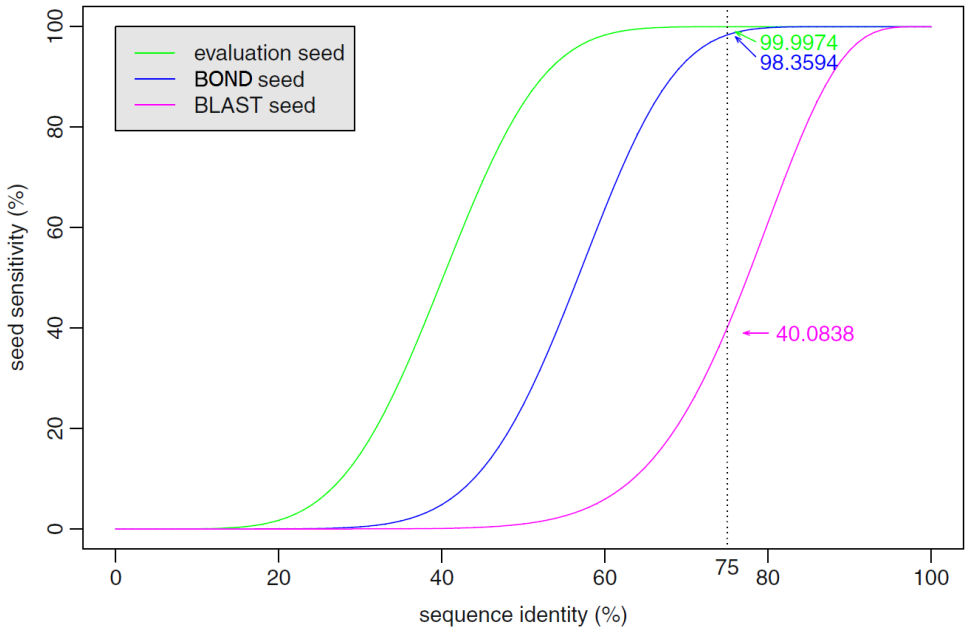


Figure 3.3: Comparing sensitivity of space seed and BLAST seed [10].
The green line shows weight 6 multiple spaced seeds; the blue line shows weight 9 multiple spaced seeds; the pink line shows the BLAST seed;

3.3 The BOND-tile algorithm

In this section, we explain in detail the BOND-tile algorithm for designing oligonucleotide probes for whole genome tiling arrays.

3.3.1 The outline of BOND-tile algorithm

Oligo placement

For whole genome tiling array, the genome is divided into a large number of contiguous windows with fixed length. Every oligo is searched from each window of the whole genome. There are two methods to search the oligos. One is searching overlap-allowed oligos and another is searching non-overlapping oligos. Figure 3.4 shows the two approaches. We choose the latter method to design BOND-tile as it produces better results. The two methods are described below:

- The overlap-allowed oligos can start from every position of their window in the whole genome such that the oligos in consecutive windows may overlap. Put another way, the start position of an oligo may happen within the previous oligo.

- The non-overlapping oligos are defined such that consecutive oligos have no overlap. Every oligo is fully contained in its target window. This method is named EasyCut.

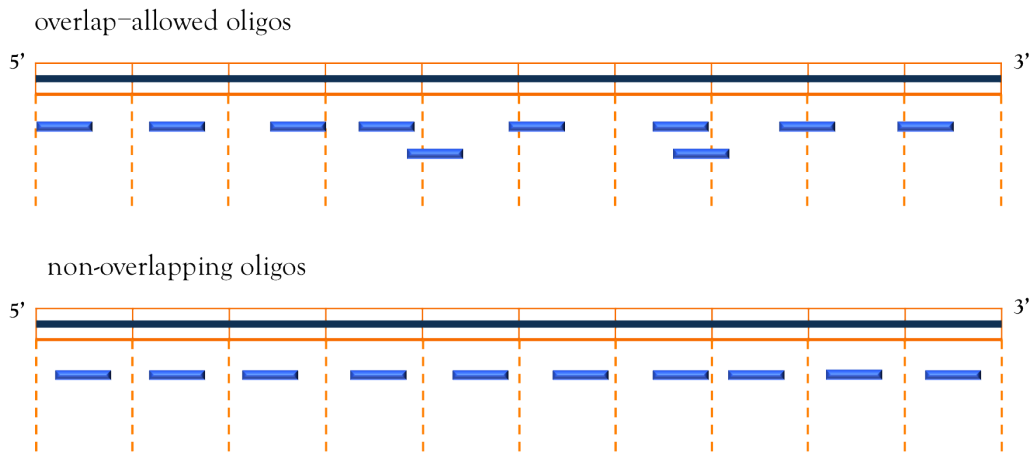


Figure 3.4: The overlap-allowed oligos and the non-overlapping oligos in a genome. Long dark lines denote the chromosomes, yellow boxes denote the windows in the genome, and blue bars denote the oligos.

Outline of BOND-tile

The proposed BOND-tile algorithm is designed to search non-overlapping unique oligonuleotide probes from each contiguous window (EasyCut).

BOND-tile marks every position in the genome as a possible candidate or not for an oligo ending at that position. Every step deals only with candidate regions in the genome. Once a position is marked as non-candidate, the remaining steps will not consider it.

Then main steps of BOND-tile are presented below.

- Encoding the input sequences
- Fast elimination of high-similarity (spaced seed-based)
- GC-content management
- Melting temperature evaluation
- Intensive elimination by homology search (spaced seed-based)
- Final oligo extraction

Every step of the algorithm is explained in detail in the following subsections.

3.3.2 DNA encoding

BOND-tile encodes the input sequences in binary code, using two bits per nucleotide (see Table 3.1).

Table 3.1: Encoding of genome sequence

Nucleotide base	Binary encoding
A	00
C	01
G	10
T	11

Then, each chromosome sequence is stored in an array of unsigned 8-bit integers; each value in the array will encode four consecutive nucleotides. Since an oligo is searched for in any window of a fixed length, each chromosome sequence is divided into these interval, which determine the available range for each potential oligo.

The data encoding in the algorithm has two advantages. The main one is to enable the use of bitwise operations. Instead of comparing the nucleotide bases one by one, we can consider the genome sequences as chunks of 64 bits corresponding to 32 nucleotide bases each time.

Also, it saves three quarters of the memory usage. Encoding each base requires only two bits, while, in regular storage, every nucleotide base needs one byte due to the character data type. This is helpful and efficient in managing large genomes.

3.3.3 GC content

GC-content refers to the proportion of G (guanine) and C (cytosine) in a DNA sequence. The genome of one organism, or specific segments of DNA and RNA, has specific GC-content. To evaluate the GC-content, we developed an algorithm which determines the thresholds of the minimum and maximum GC-content through a user-defined schema. In the proposed algorithm, the default values are set as 30% and 70% respectively. It can be reset by the user.

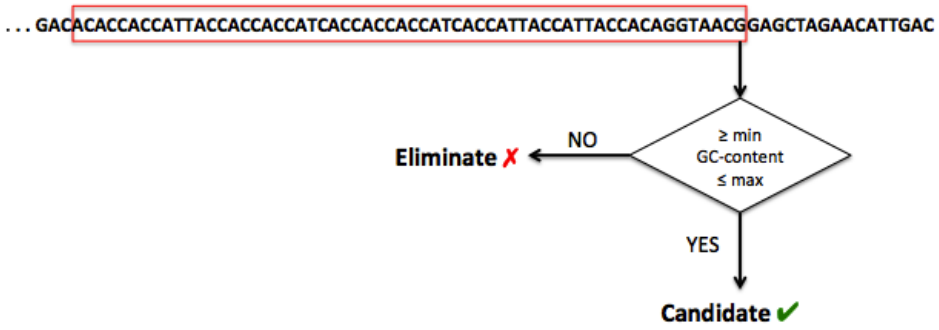


Figure 3.5: GC-content evaluation process.

It can be seen from Figure 3.5 that the management of GC-content is conducted from the right end of the encoded genome, screening to the left end. Among the whole procedure, the percentage of guanine and cytosine will be examined; meanwhile some oligo candidate positions whose GC-content percentage is outside the predefined range will be eliminated. Every end position of eliminated subsequences will be marked as non-candidate.

3.3.4 Melting temperature

The melting temperature, T_m, is one of the key parameters for designing the oligo probes. To maximise uniformity for the obtained oligonucleotide set, melting temperatures of oligonucleotides need to differ only slightly. The Nearest Neighbour model is the most widely used approach to calculate the melting temperature by applying it with the parameters from SantaLucia [23] or Rychlik [20]. BOND-tile uses this approach to compute the T_m[6, 24]:

$$T_m = \frac{\Delta H}{R\ln(\mathrm{C}/4) + \Delta \mathrm{S}} - 273.15 + 12\log[\mathrm{Na}^+] \tag{3.1}$$

where ΔH, enthalpy(k.cal/mol), denotes the total energy exchange between the system and its surrounding environment, the ideal gas constant is denoted by R = 1.987 (cal/mol.K), C denotes the molar concentration of oligo sequence, ΔS, entropy(cal/mol.K), is the energy spent to achieve self-organization by the system, and $[Na^+]$ represents the molar concentration of salt. The values of ΔH and ΔS are presented in Table 3.2.

The melting temperature of the candidate at every position will be computed and stored. The ones falling outside the user defined range are considered as non-candidates. The range of melting temperatures is set by the user.

Table 3.2: Nearest-Neighbor parameters for DNA/DNA duplexes (SantaLucia [23])

Stack (5'3'/3'5')	**ΔH** ($\frac{kcal}{mol}$)	**ΔS** ($\frac{cal}{mol.K}$)
AA/TT	-7.9	-22.2
AT/TA	-7.2	-20.4
TA/AT	-7.2	-21.3
CA/GT	-8.5	-22.7
GT/CA	-8.4	-22.4
CT/GA	-7.8	-21.0
GA/CT	-8.2	-22.2
CG/GC	-10.6	-27.2
GC/CG	-9.8	-24.4
GG/CC	-8.0	-19.9
Init. w/term. G/C	0.1	-2.8
Init. w/term.	2.3	4.1
A/T Symmetry correction	0	-1.4

3.3.5 Similarity search

The spaced seed-based similarity search involves finding and investigating all hits corresponding to all spaced seeds. This can be a time consuming task and, in order to speed up the process, we shall perform the search in two phases. In the first phase, for each seed, only one position of each hit is considered. This helps eliminating vary fast large regions of high similarity. In the second phase, all possible hits are considered, thus using the full power of the seeds.

BOND-tile employs the set of eight multiple spaced seeds of weight 10 shown in Figure 3.6. These seeds are selected by SPEED [9, 8].

111*111*1*111**
111*1**1***1***11*11**
1111*1***1****11*1*1**
1111****1*1***1*11*1**
1111***1**1**1****111**
11*11*******1*1**1***111**
11*1*1****1*****1***1**111**
11*1*1***1*****1*****11*11**

Figure 3.6: The set of multiple spaced seeds used in the homology search phase.

Hash table construction

Given a spaced seed s, an s-mer is a sequence of symbols that contains nucleotides for the 1-positions and zeros for the $*$-positions. For example, if $s = 11*1**1$, then an example of an s-mer is AC0G00T. The don't care positions '$*$' are ignored by having 0's in those places. A hit is given by a pair of identical s-mers. Therefore, we store all s-mers of each seed in a hash table. Since the nucleotides are encoded in binary, the s-mers are simply sequences of bits. To enable fast computation, we use a bitwise AND between the seed and the DNA sequences. The seed is suitably encoded by 1 $\rightarrow$ 11, $* \rightarrow$ 00.

A hash table is created for each of the eight spaced seeds. Linear probing is used for handling hash collisions. The algorithm checks the bases starting from the right end of the encoded genome and slides to the left end. The s-mer's integer value of each position in the genome will be searched by using the seed model. If found, a hit is discovered and investigated for potential similarity.

Phase I: Fast elimination

In the fast elimination phase, BOND-tile looks quickly for the potentially unsatisfactory oligo positions, and eliminates them after finding high-similarity regions.

BOND-tile calculates the s-mer integer value of each position in the genome, then searches the hash table for its hash value. If the corresponding hash value does not exist in the hash table, the BOND-tile algorithm uses this hash value to be the hash key and inserts the related position into the hash table. In the case that the hash value already exists in the hash table, that means a 'hit' is found.

After finding a hit, BOND-tile extends the matches corresponding to the hit both left and right as long as the similarity level of the two regions is above the user-specified threshold. BOND-tile eliminates positions of the two regions by marking them as non-candidates. Figure 3.7 shows how the extension works. The default value of similarity threshold is 75%. It can be reset by the user.

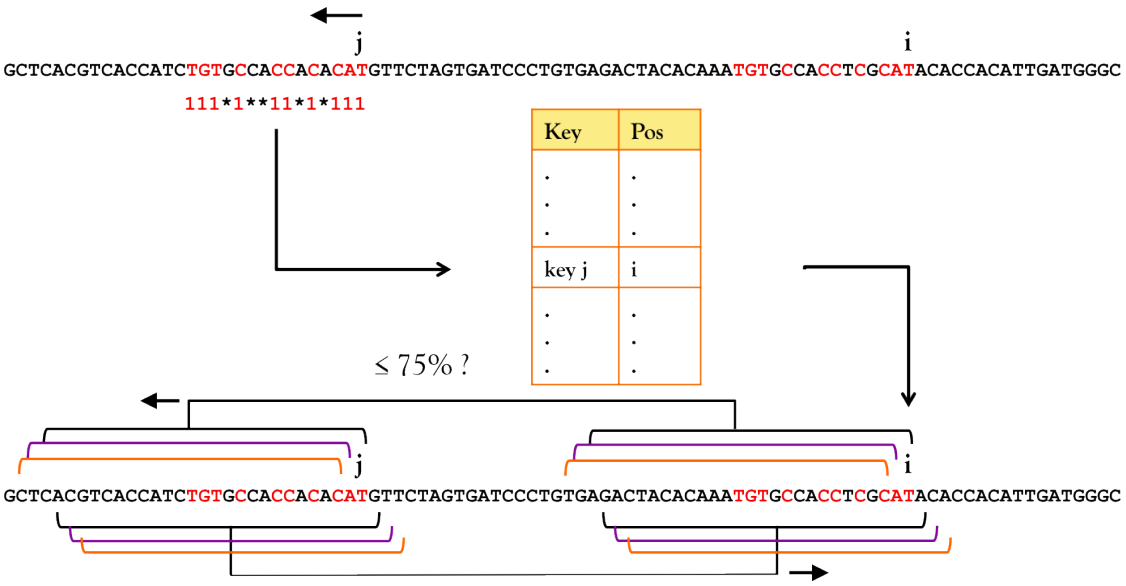


Figure 3.7: The process of similarity elimination.

Phase II: Intensive search and elimination

In the intensive elimination phase, BOND-tile first calculates the s-mer integer value of each position, and inserts this integer value as a hash key and synchronously input all the related positions into the hash table. In case the obtained integer value can be found as a hash key, only the related position will be put into the position array which corresponds to this hash key.

After all the hash tables are completely created, the algorithm will scan every candidate position in each window using a heuristic that tried the more likely ones first (details in Section 3.3.6). For each position considered, all hits of all s-mers corresponding to all eight seeds are investigated. That is why this phase is called intensive.

Differrence between fast and intensive elimination

To clarify, in the fast searching stage, only one position for each corresponding hash key is recorded in hashtable, which is the last checked position compared with the

last occurrence of the corresponding hit. In the intensive elimination, all candidate positions and their corresponding hash values are kept in the hash table to be prepared for similarity check. In addition, in every oligo candidate, the s-mer integer of every position will be used to search for similarity.

3.3.6 Oligonucleotide selection

In the selection phase, BOND-tile subdivides the genome into a large number of contiguous windows of a fixed length. The purpose is to select non-overlapping (EasyCut) unique oligonuleotide probes from each window. BOND-tile checks each tiling window to eliminate all unsatisfactory sequences and select the unique oligonuleotide probes.

Recall that BOND-tile stores every position in the genome, whether it is a candidate or not. To obtain the maximum number of unique oligo probes, the algorithm will execute all steps by checking every possible position in each tiling window. In addition, to maintain high quality of oligo probes as well as good speed, before running the search step of intensive elimination, BOND-tile chooses the middle position of a region of consecutive candidates, since it is expected to have the lowest similarity with any other region. For example, if the length of one region of consecutive candidates is $\boldsymbol{l}$ and the end position of this region is $\boldsymbol{j}$, the priority selected position is $\boldsymbol{j}$ - $\boldsymbol{l/2}$. The process is shown in Figure 3.8.

As shown in the upper part of Figure 3.8, there are several candidate sequences (green lines) and non-candidate sequences (red lines). The end positions of those sequences are marked as green ticks and red crosses. The longest region of consecutive green ticks, which means the longest region of consecutive candidates, is chosen to be considered. In the lower part, the end position of the considered region is $\boldsymbol{j}$ and the length of the region is $\boldsymbol{l}$. Then the first picked candidate we try is shown in the green box. Its end position is $\boldsymbol{j}$ - $\boldsymbol{l/2}$. After selecting the preferred candidate, we use intensive elimination phase to verify that it is a good oligo. When an oligo is found in one window, it is added to output and that window no longer investigated.

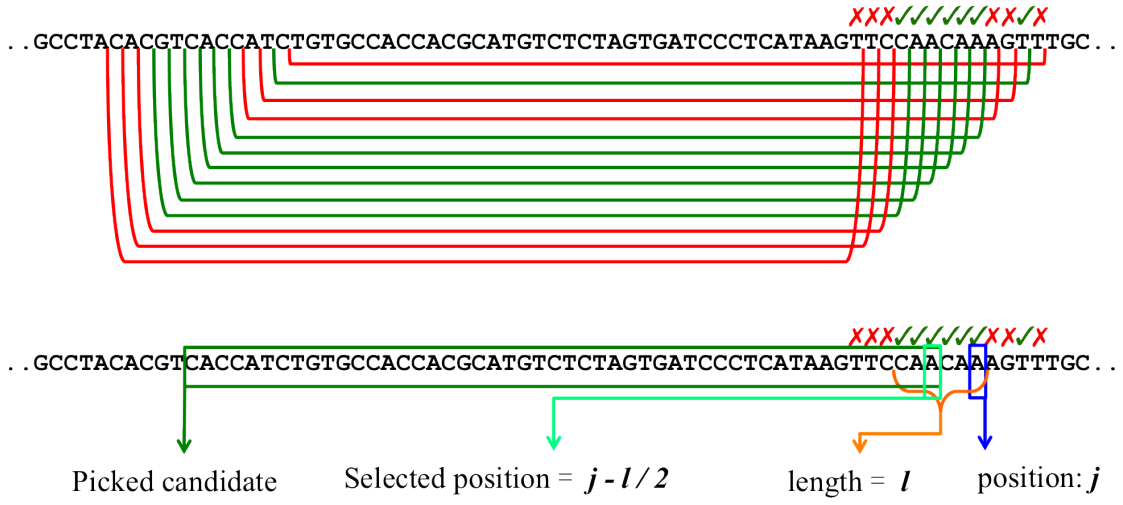


Figure 3.8: The process of selecting oligonucleotides.

Chapter 4

Evaluation

This chapter presents the evaluation of experimental results. We compare the performance of the BOND-tile algorithm with two leading programs for designing whole genome tiling arrays, ArrayDesign [7] and OligoTiler [3].

4.1 General setup

After implementing the whole genome tiling program, BOND-tile, several evaluation programs focusing on assessing the performance of BOND-tile were written as well. In this chapter we evaluate the computational results of ArrayDesign, OligoTiler and BOND-tile by comparing them according to the following factors:

- Identity with closest non-target (similarity check),
- Melting temperature,
- GC-content,
- Distance between consecutive oligos,
- Time,
- Space.

In all plots shown in this chapter, blue parts denote the results of ArrayDesign, purple (light or dark) parts denote OligoTiler's and green denote BOND-tile's.

For a meaningful comparison, all candidate programs have to be run with the same parameters. Since we could not control some parameters for OligoTiler (it is available through a website interface), those parameters had no limits imposed. The parameters are set as shown in Table 4.1:

Table 4.1: Commom factors for Evaluation.

	Defined Value		
Parameters	ArrayDesign	OligoTiler	BOND-tile
Oligo length	60bp	60bp	60bp
Window size	150bp	150bp	150bp
Overlapping allowed	No(EasyCut)	Very little (default, web)	No(EasyCut)
GC content	No Limit	N/A	No Limit
Melting temp	No Limit	N/A	No Limit
Secondary structure	No Limit	IRregion=0 IRrequire=0	Turned Off
Everything else	Default Values	Default Values	Default Values

4.2 Datasets

We use the following data sets as our input files. The FASTA file of *Drosophila melanogaster* DNA chromosome 2L is downloaded from the pdf site: `ftp://ftp.ensembl.org/pub/release-67/fasta/drosophila_melanogaster/dna`. The unmasked FASTA file of *Trichoderma reesei* genome v.2.0 is downloaded from the Department of Energy Joint Genome Institute website (JGI): `http://genome.jgi-psf.org/Trire2/Trire2.home.html`. We also use NCBI Build 36 of the *Mus musculus* DNA chromosome 17 downloaded from release 40 of Ensembl (`ftp://ftp.ensembl.org/pub/release-40/mus_musculus_40_36a/data/fasta/dna/`). The information concerning the input data sets used in the experiments is given in Table 4.2.

Table 4.2: Input data sets used for evaluation.

Species model	Genome size	Chromosome	Description
Drosophila melanogaster	23011544 bp	2L	*Drosophila melanogaster* BDGP5 67 dna chromosome 2L
Trichoderma reesei	33454791 bp	All	*Trichoderma reesei*V2 AssembledScaffolds
Mus musculus	95177420 bp	17	*Mus musculus* NCBIM36 40 dna chromosome 17

4.3 Operation Environment

The proposed algorithm is implemented in C++, and parallelized using openMP.

All computations were performed on the SHARCNET (Shared Hierarchical Academic Research Computing Network) which is a high performance computing consortium. We used some nodes of the "shadowfax" cluster whose characteristics are given below:

- Processor: Intel Xeon E6-2620 @ 2.0GHz, 2 sockets, 12 cores
- RAM: 256GB
- Operating System: Linux version CentOS 6.3
- Compiler: gcc version 4.8.1

4.4 Non-target similarity: good and bad oligos

The most important property, and the most difficult to achieve, of the oligos is low similarity with non-targets. The input file of the evaluation program is the set of the oligo probes produced by each algorithm. The output is the maximum similarity level with non-targets, for each oligo. The evaluation program is very similar with the similarity search phase of BOND-tile, except that we use much more sensitive seeds (see Fig. 4.1). The seeds were also computed using SpEED [9]. Their sensitivity is shown by the green curve in Figure 3.3.

111*1*11
111**111**
11*1***1*11**
1*1*1****111**
1*11**1***1*1**
11*1****1**1*1**
11*1****1***1*1**
11****1****1**11**

Figure 4.1: The set of multiple spaced seeds used in the closest non-target similarity evaluation.

If an oligo probe has more than 75% similarity with non-target regions, we mark it as a bad oligo, otherwise it is considered good. The evaluation program runs fairly slow because we use the multiple spaced seeds of weight six so that the evaluation program has high sensitivity, which results in a large number of hits.

The experimental results are shown in Tables 4.3-4.5. The percentage of good oligos computed by BOND-tile is 100%, while for the others, it varies between 70% and 97%. In addition, BOND-tile always obtains more good oligos than the other two programs. The difference is particularly big for the largest dataset where BOND-tile found almost 30% more good oligos than OligoTiler and 24% more than ArrayDesign.

Table 4.3: Good oligos comparison for *Drosophila melanogaster*.

Algorithm	Total Oligos	Good Oligos	% of Good Oligos
ArrayDesign	153409	**143260**	93.38
OligoTiler	153410	**142457**	92.86
BOND-Tile	144707	**144707**	100.00

Table 4.4: Good oligos comparison for *Trichoderma reesei*.

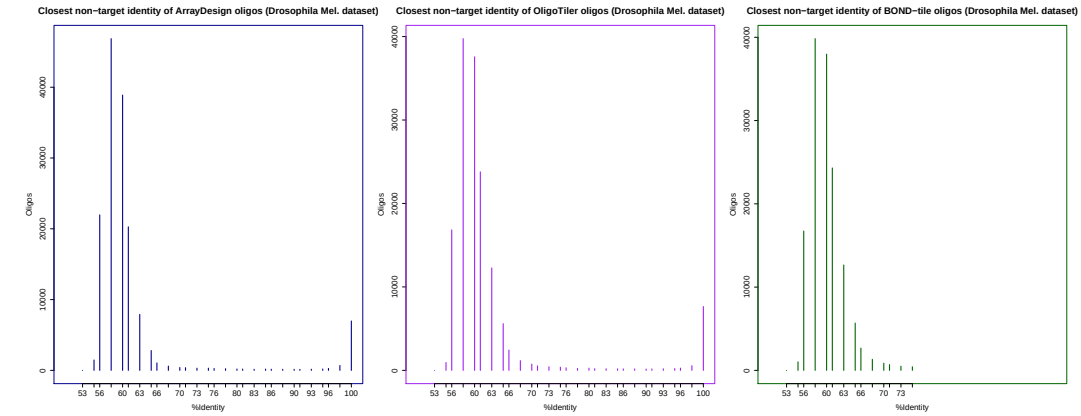
Algorithm	Total Oligos	Good Oligos	% of Good Oligos
ArrayDesign	222721	**216736**	97.31
OligoTiler	222793	**215724**	96.83
BOND-Tile	218319	**218319**	100.00

Table 4.5: Good oligos comparison for *Mus musculus*.

Algorithm	Total Oligos	Good Oligos	% of Good Oligos
ArrayDesign	608461	**462396**	75.99
OligoTiler	608397	**430023**	70.68
BOND-Tile	499396	**499396**	100.00

The comparison for non-target similarity evaluation of ArrayDesign, OligoTiler and BOND-tile is illustrated in Figures 4.2-4.10.

Figure 4.2-4.4 show the distribution of the oligos computed by the three programs with respect to the percentages identity with the closest non-target. We show both histograms and box-and-whiskers plots. BOND-tile has no oligos at identity higher than 75%. For the largest dataset, the distribution of the oligos of BOND-tile is significantly better than both the other programs (see Fig. 4.4, bottom). Also, ArrayDesign is slightly better than OligoTiler.



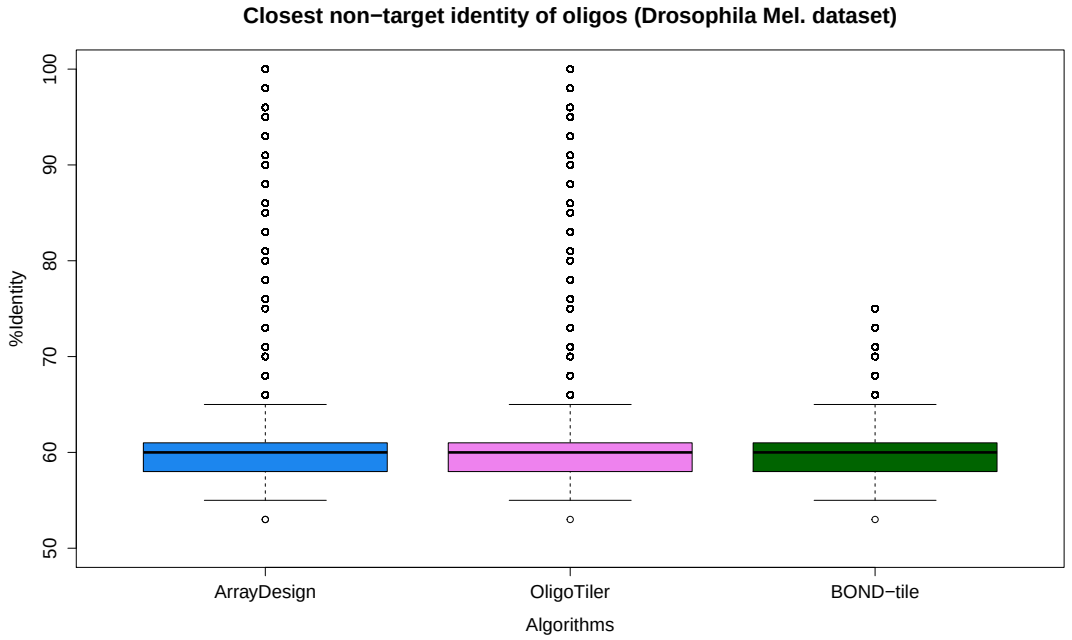


Figure 4.2: Closest non-target identity distribution for the *Drosophila melanogaster* dataset.

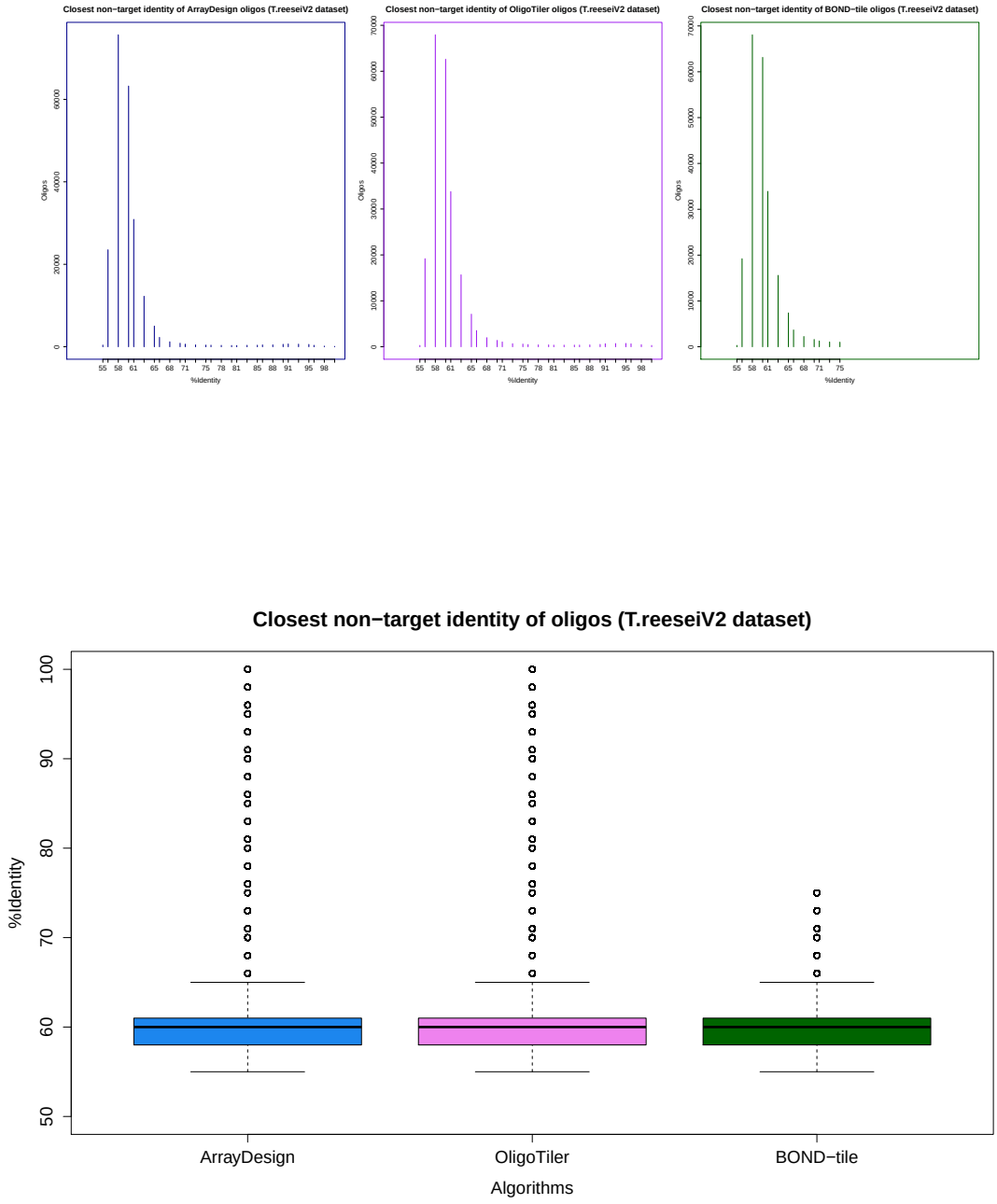


Figure 4.3: Closest non-target identity distribution for the *T.reesei* dataset.

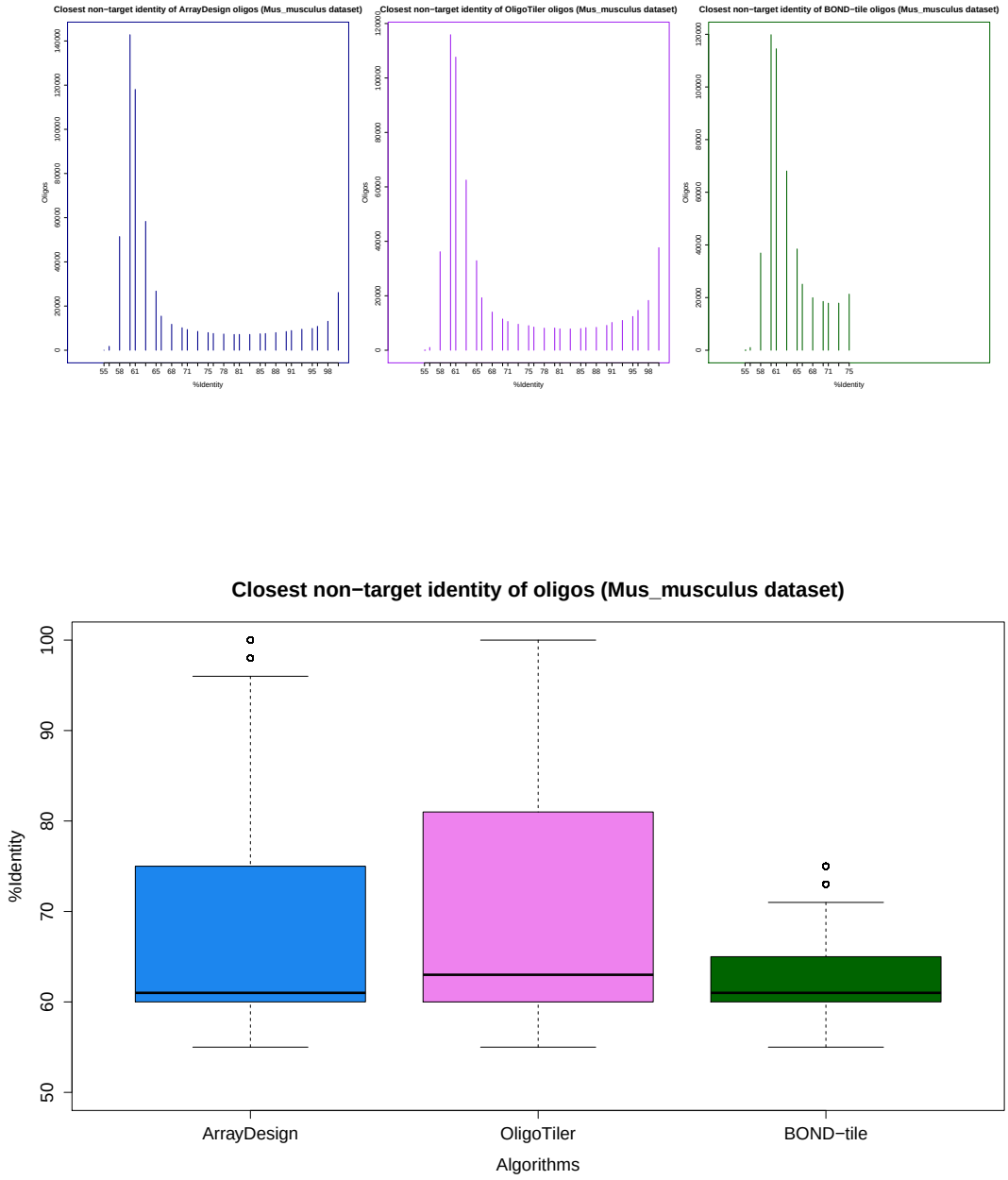


Figure 4.4: Closest non-target identity distribution for the *Mus musculus* dataset.

The histograms for the identity between 75-100% are shown in Figures 4.5-4.7. The same information is presented in the form of line graphs in Figures 4.8-4.10, this time with all three programs on the same graph for better comparison.

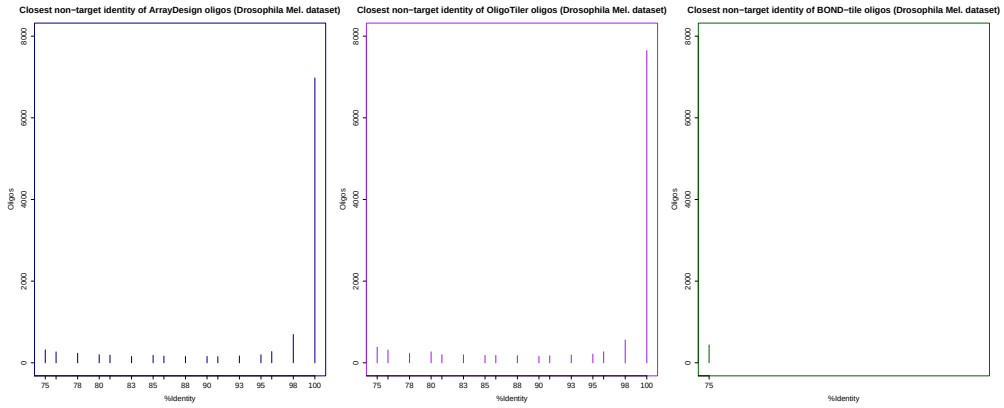


Figure 4.5: Closest non-target identity distribution for the *Drosophila melanogaster* dataset; the region of 75-100% identity.

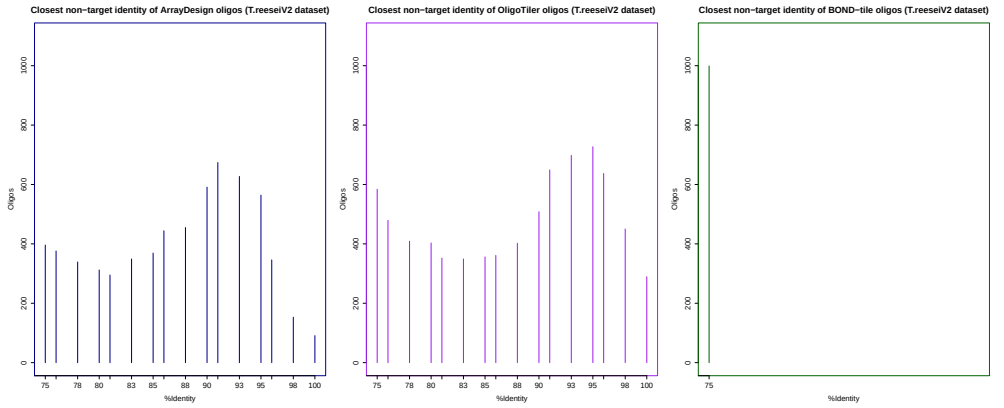


Figure 4.6: Closest non-target identity distribution for the *T.reesei* dataset; the region of 75-100% identity.

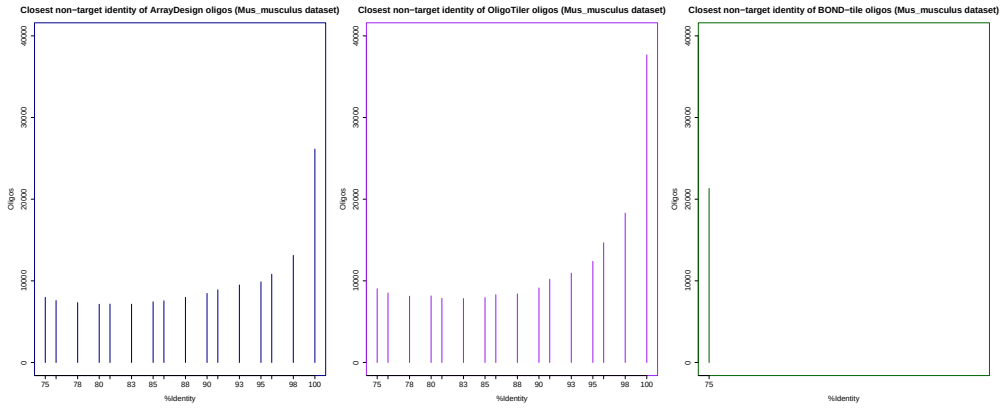


Figure 4.7: Closest non-target identity distribution for the *Mus musculus* dataset; the region of 75-100% identity.

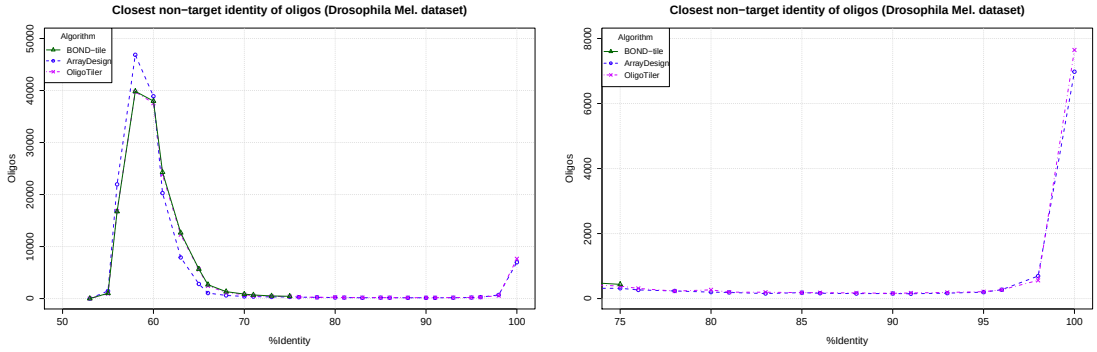


Figure 4.8: Closest non-target identity distribution for the *Drosophila melanogaster* dataset; all identity levels (left) and 75-100% identity (right).

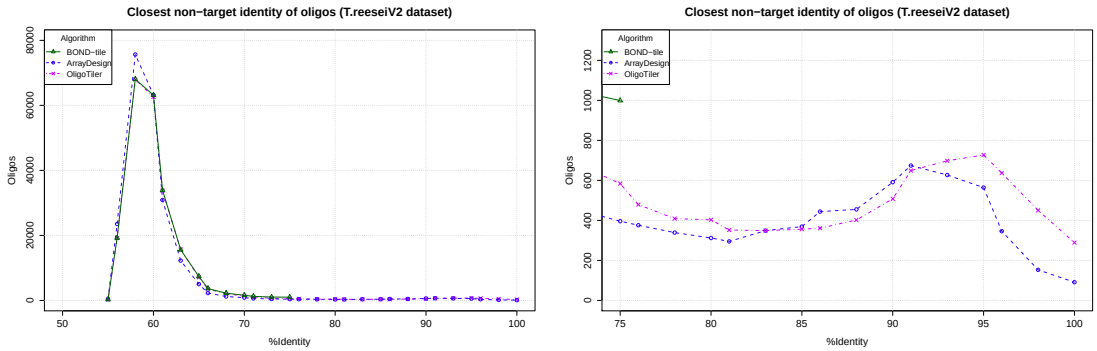


Figure 4.9: Closest non-target identity distribution for the *T.reesei* dataset; all identity levels (left) and 75-100% identity (right).

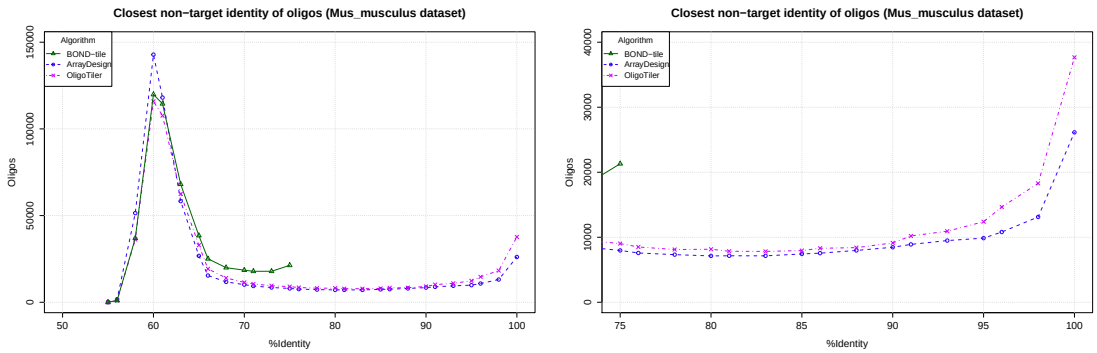
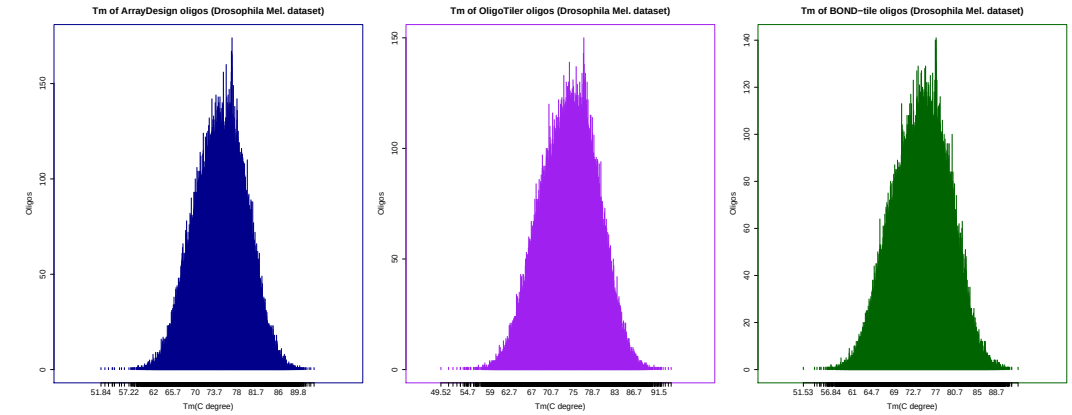


Figure 4.10: Closest non-target identity distribution for the *Mus musculus* dataset; all identity levels (left) and 75-100% identity (right).

4.5 Melting temperature

As shown in Figures 4.11-4.13, the distribution of the melting temperature is fairly similar among the three candidate programs.



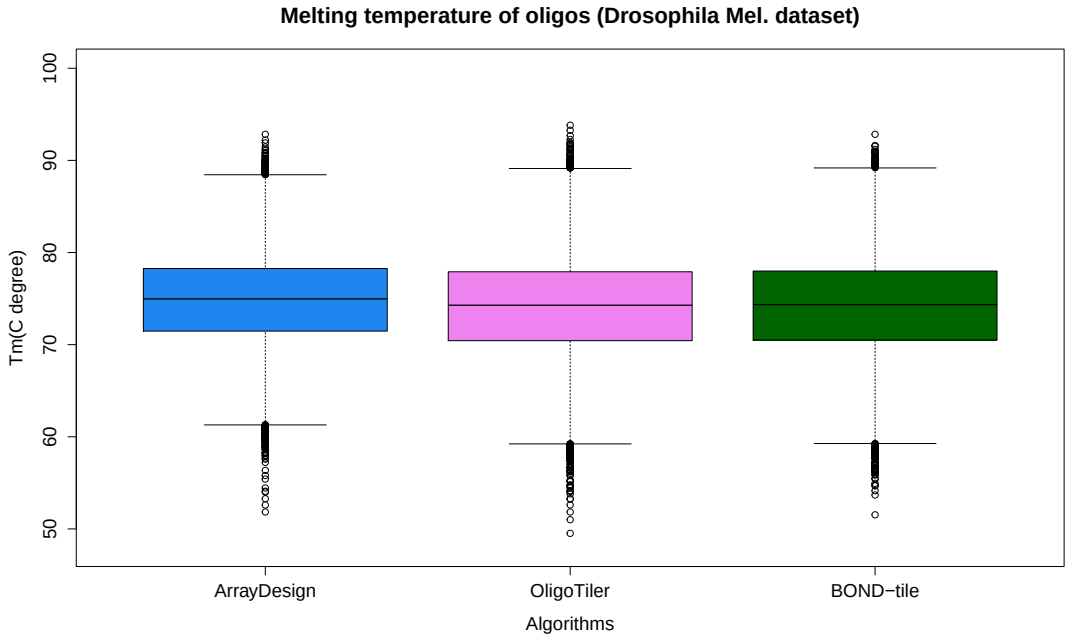
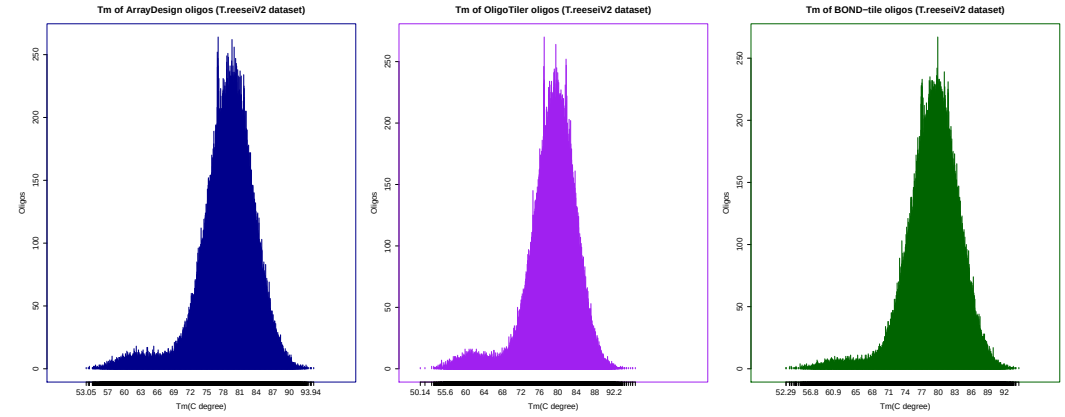


Figure 4.11: Melting temperature distribution for the *Drosophila melanogaster* dataset.



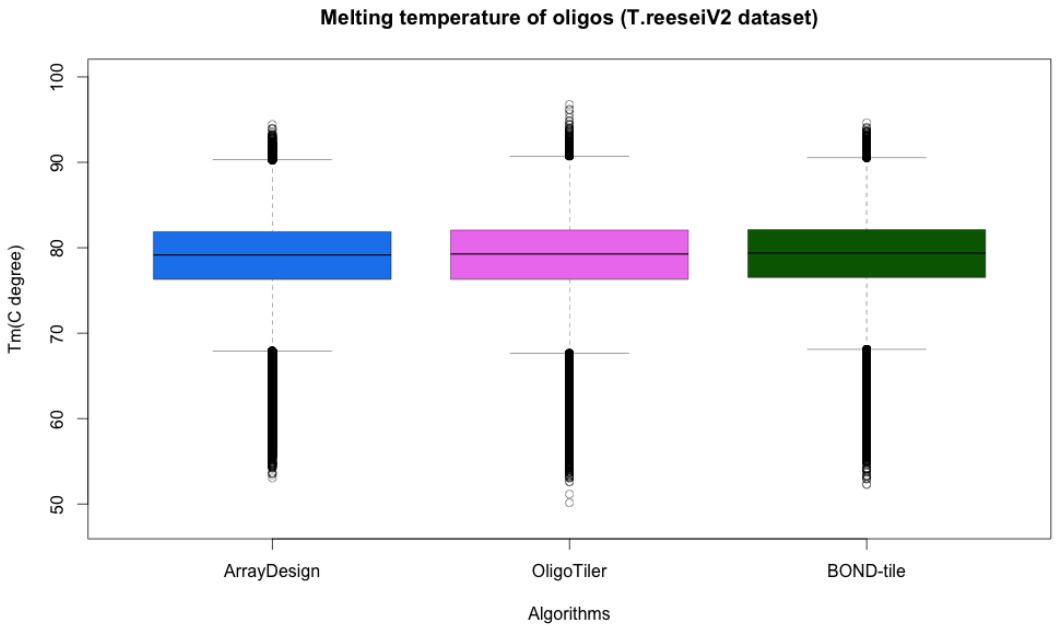
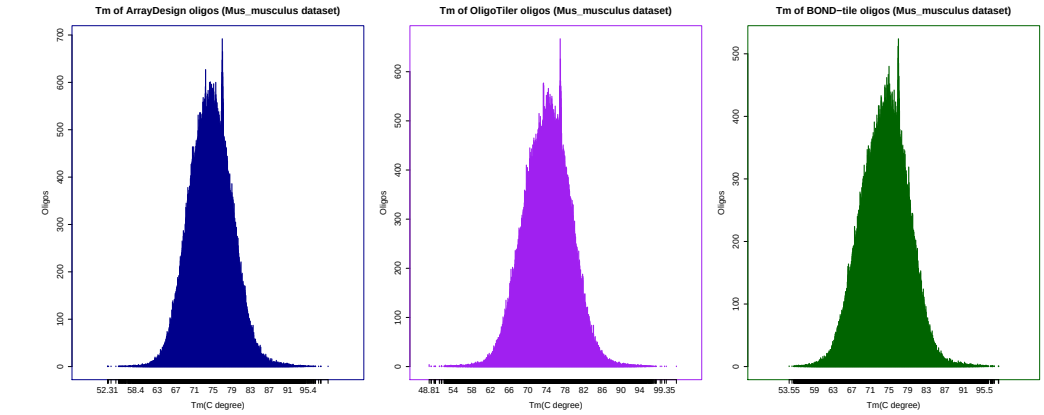


Figure 4.12: Melting temperature distribution for the *T.reesei* dataset.



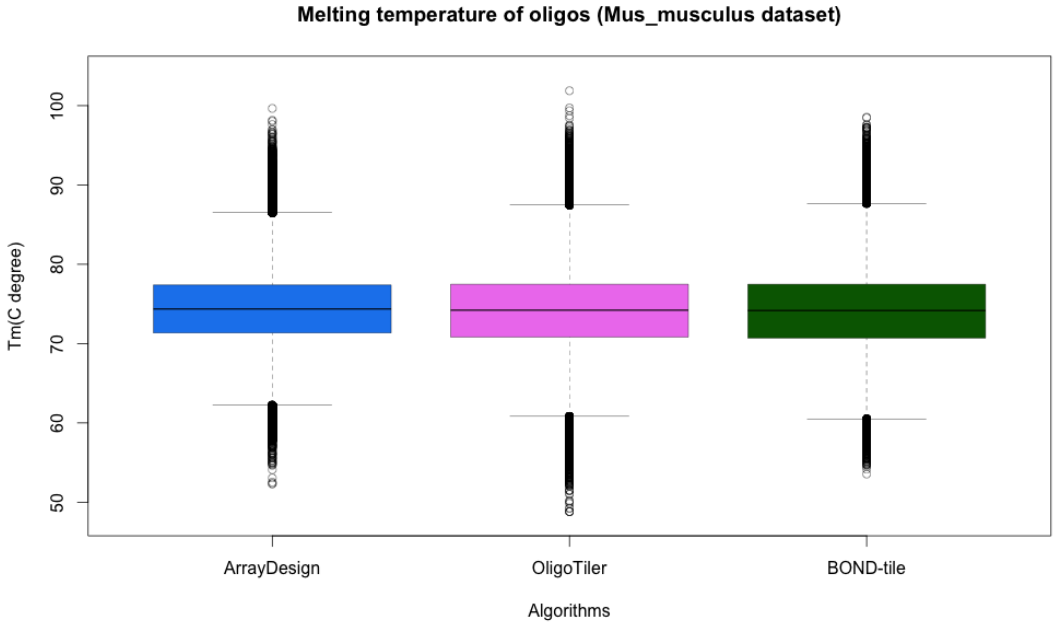


Figure 4.13: Melting temperature distribution for the *Mus musculus* dataset.

4.6 GC content

Figures 4.14-4.16 present the comparison of GC content parameter, and, again there is no significant difference among the three candidate programs. The range of OligoTiler is slightly wider than the others.

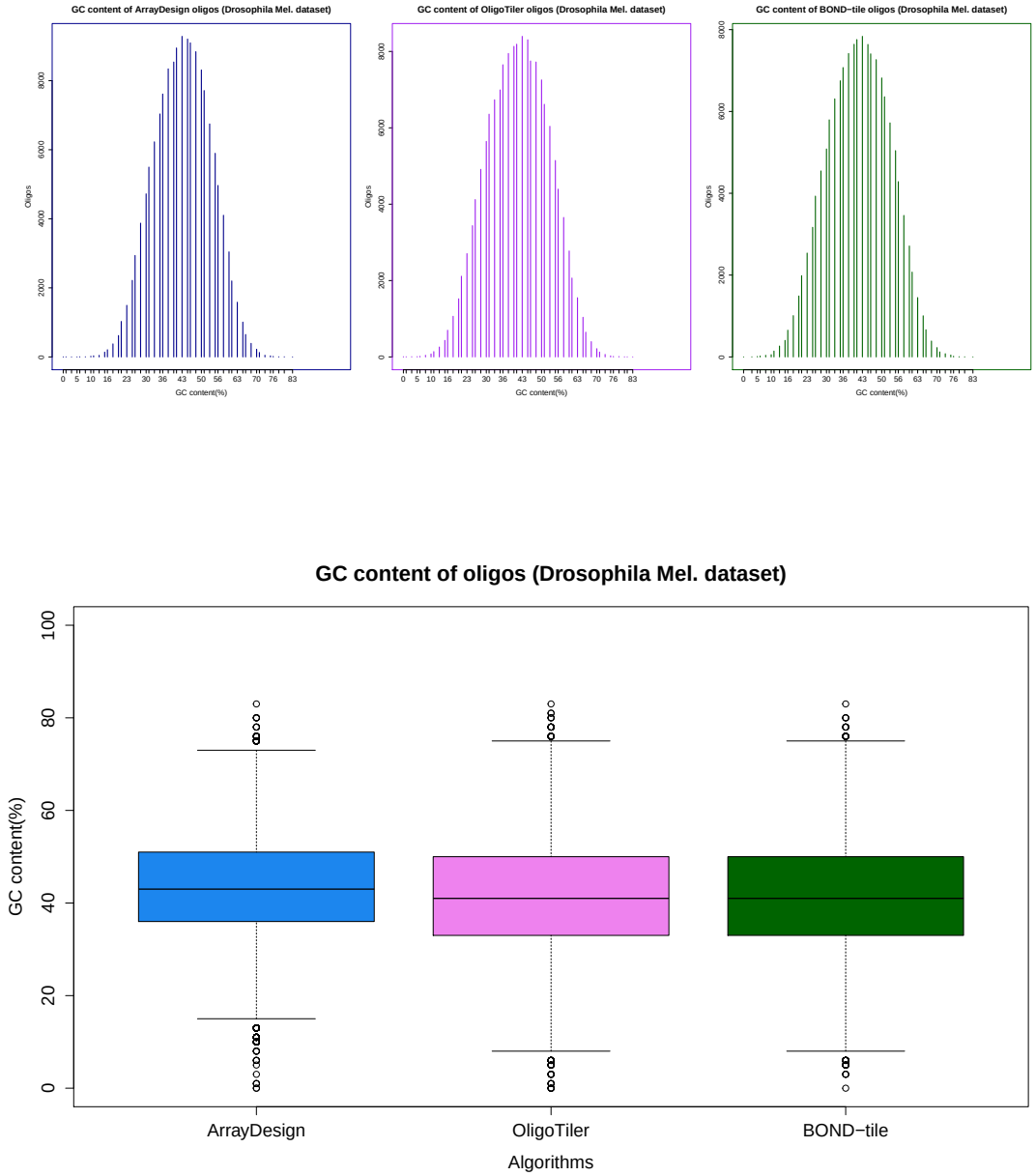


Figure 4.14: GC content distribution for the *Drosophila melanogaster* dataset.

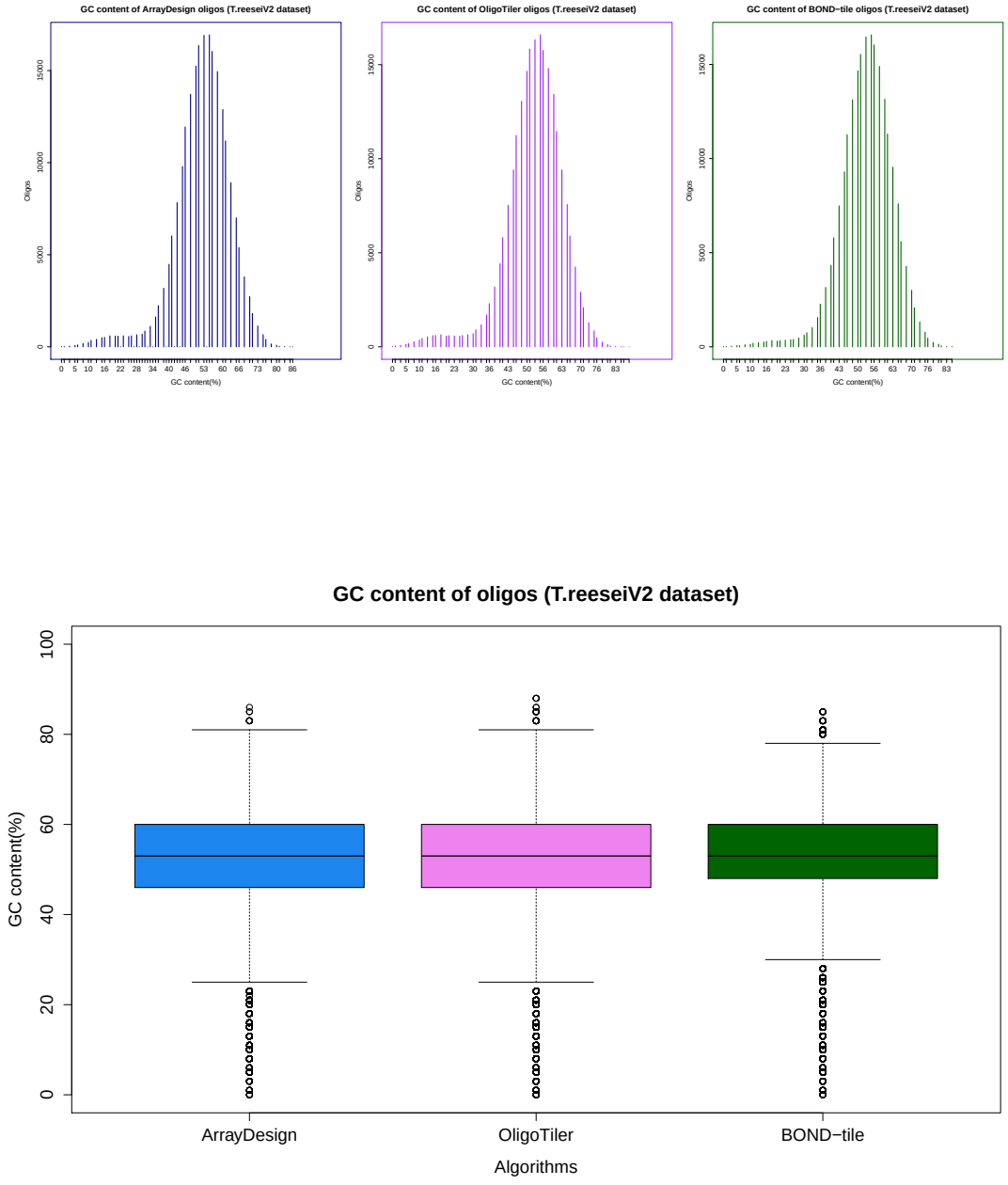


Figure 4.15: GC content distribution for the *T.reesei* dataset.

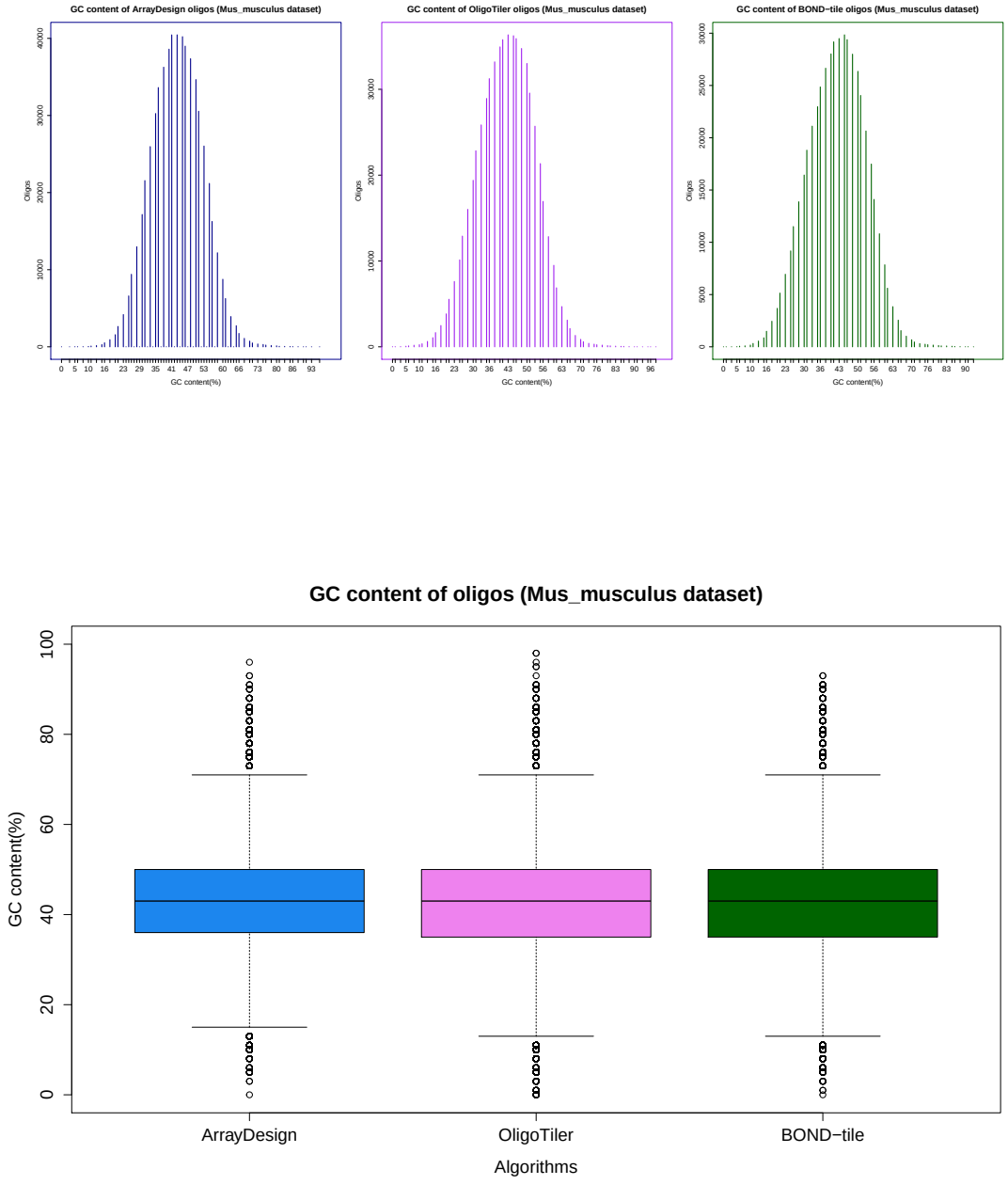
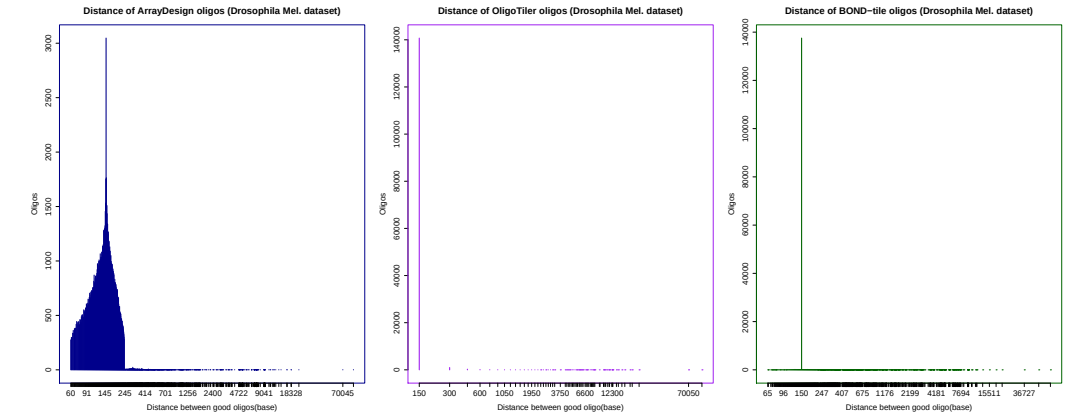


Figure 4.16: GC content distribution for the *Mus musculus* dataset.

4.7 Distance between oligos

Figures 4.17-4.19 illustrate the distance, in base pairs, between the contiguous good oligos. In other words, we eliminate all bad oligos produced by ArrayDesign and OligoTiler before calculating the oligo distances. It can be seen that the oligo distance parameter of BOND-tile is slightly better than OligoTiler and both are much better than ArrayDesign.



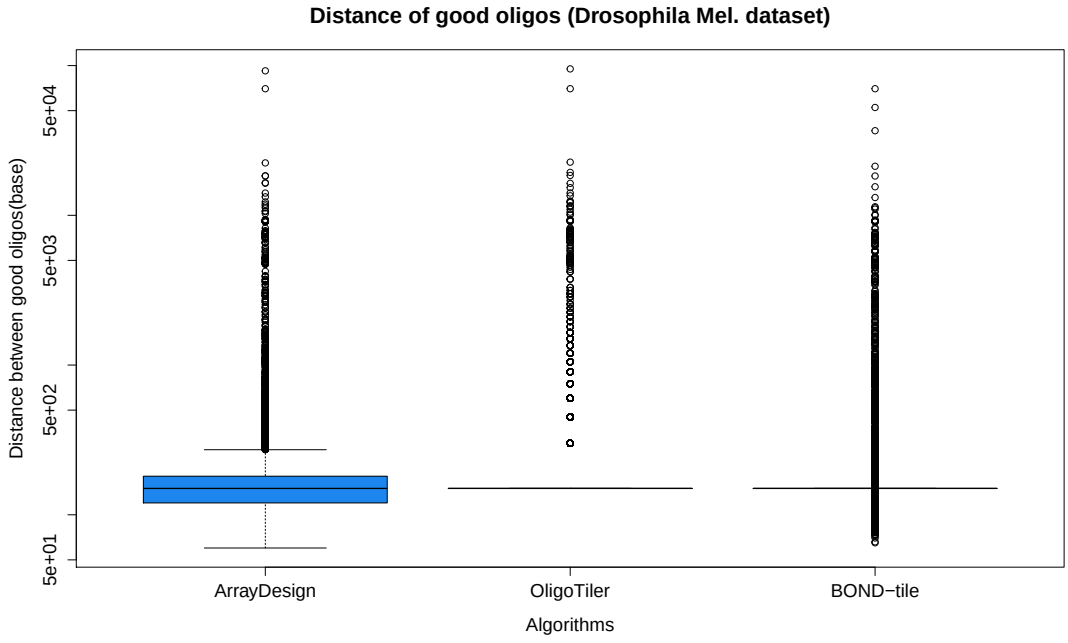
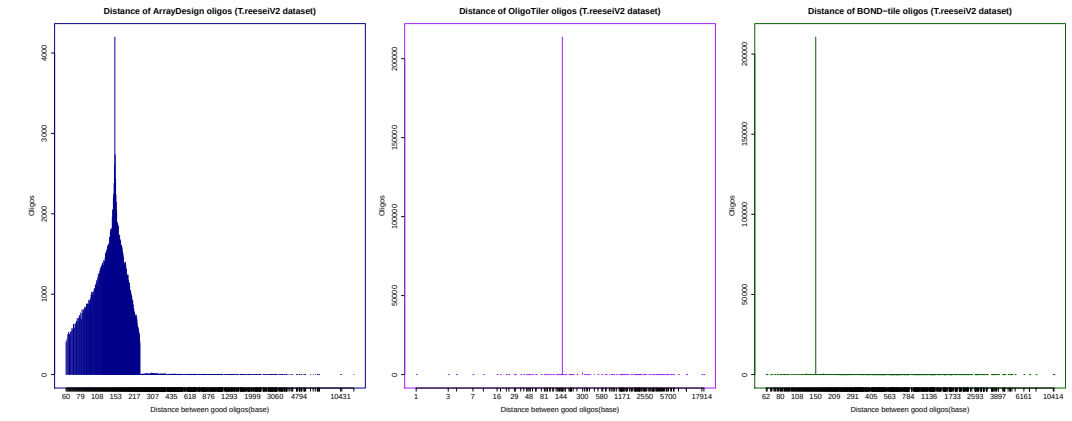


Figure 4.17: Good Oligo Distance distribution for the *Drosophila melanogaster* dataset.



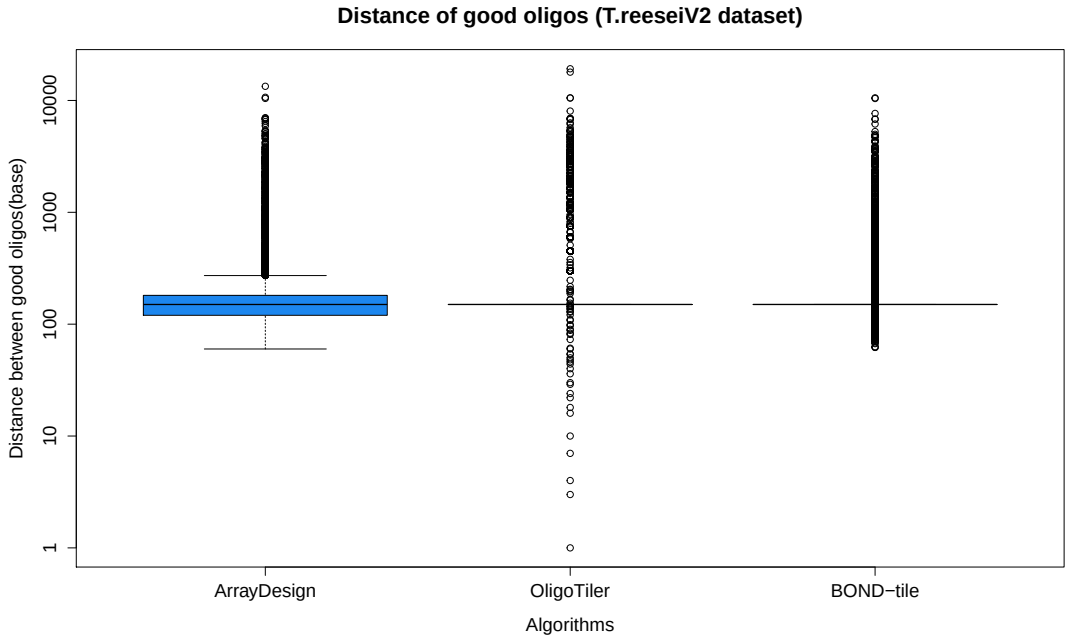
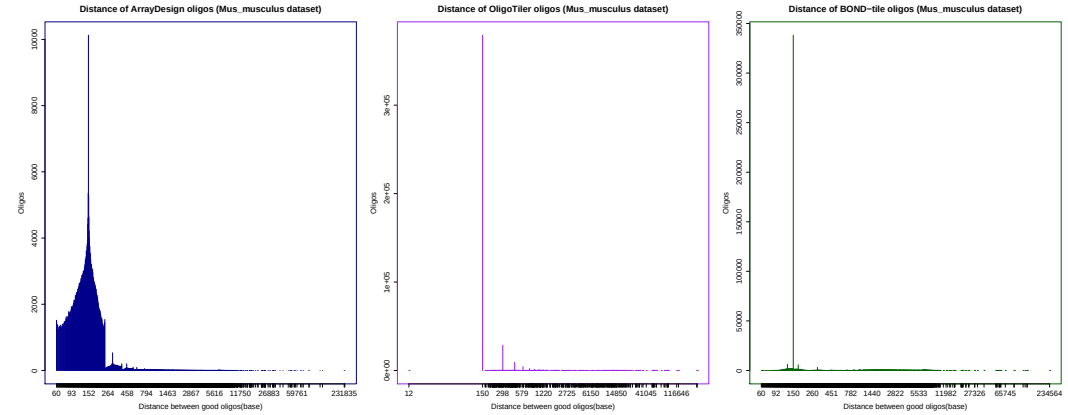


Figure 4.18: Good Oligo Distance distribution for the *T.reesei* dataset.



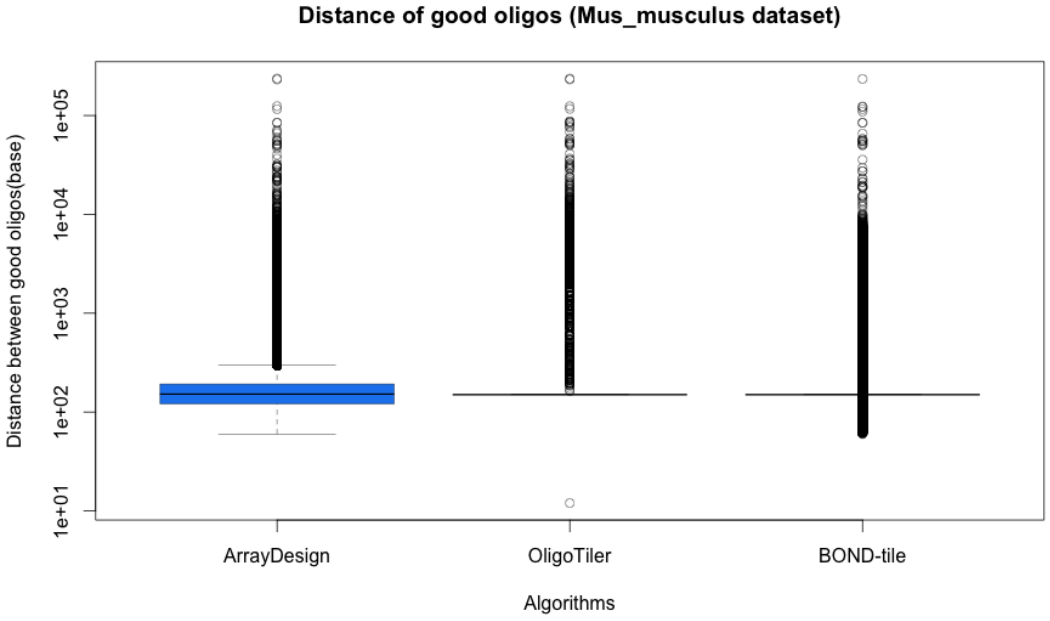


Figure 4.19: Good Oligo Distance distribution for the *Mus musculus* dataset.

4.8 Time and space

The running time and memory usage are presented in Tables 4.6-4.8. The space for OligoTiler could not be obtained and the time is the response time on the web interface. The space used by BOND-tile is between 2 and 4 times larger than ArrayDesign.

BOND-tile and OligoTiler have similar speed for the two smaller datasets, and much faster than ArrayDesign. For the largest dataset, BOND tile becomes very slow, probably due to the very simple collision resolving scheme. (We did not have time to try something else.)

Considering the fact that designing of tiling oligos is not done very often, all times and memory requirements are perfectly acceptable.

Table 4.6: Time and memory for the *Drosophila melanogaster* dataset.

Algorithm	Time(s)	Memory(Mb)	Total Oligos	Good Oligos
ArrayDesign	3212	763.4	153409	**143260**
OligoTiler	about 60	(web)	153410	**142457**
BOND-Tile	56	1731	144707	**144707**

Table 4.7: Time and Memory for the *Trichoderma reesei* dataset.

Algorithm	Time(s)	Memory(Mb)	Total Oligos	Good Oligos
ArrayDesign	4687	846.5	222721	**216736**
OligoTiler	about 120	(web)	222793	**215724**
BOND-Tile	94	2252	218319	**218319**

Table 4.8: Time and Memory for the *Mus musculus* dataset.

Algorithm	Time(s)	Memory(Mb)	Total Oligos	Good Oligos
ArrayDesign	13683	1343	608461	**462396**
OligoTiler	about 900	(web)	608397	**430023**
BOND-Tile	32241s	5298	499396	**499396**

Chapter 5

Conclusion

In this thesis, we have considered the problem of designing oligonucleotides for whole genome tiling. The results of previous methods are far from satisfactory since some of the produced oligos have cross-hybridization with non-targets. The highest possible number of oligos is not found. Also, the previous evaluations are not sensitive enough.

After pointing out the shortcomings of the existing programs, we have designed a new one, BOND-tile, that produces significantly better oligonucleotides. Through the evaluation, we have conducted a comprehensive analysis and comparison between the existing programs and BOND-tile. BOND-tile shows its advantage in finding the largest number of unique probes placed at equal distance between adjacent oligonucleotide. The running time of BOND-tile seems to grow fast with the size of the input but it is likely the increasing degree of repetition that causes the problem and which will be solved by using double hashing.

In addition, our design can be customized in various ways, such as, for instance, allowing oligos having identity with non-targets over 75%. Moreover, such oligos can be computed in such a way as to minimize the identity with non-targets. Also, the melting temperature interval can be automatically computed (assuming its size is give by the user) such that the total number of oligos found is maximized.

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