THE OLIGOPAINTS SCRIPTS SUITE MANUAL

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Last updated: 8/22/2012

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INTRODUCTION

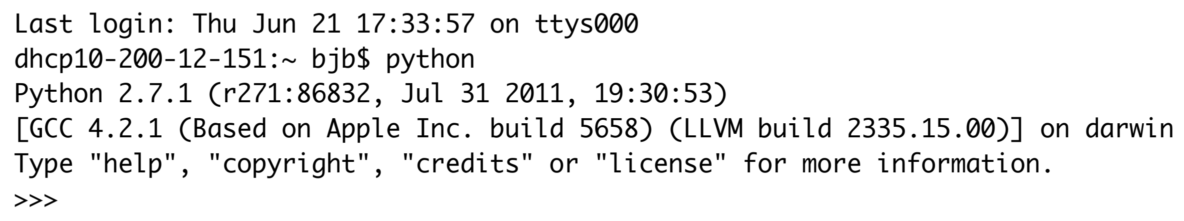
Welcome to the Oligopaints script suite. This manual will familiarize users with the database files that detail the genomic position and melting temperature (T_M) of Oligopaints probes and the python scripts created to manipulate them. The first section of the manual deals with the installation of Python and accessing the Command Line or Terminal to use the scripts. The next section contains detailed information on each individual script, followed by guided walkthroughs of common tasks one can complete using the scripts to process Oligopaints files.

INSTALLATION

Python 2.7 or later is required to use the Oligopaints scripts. Although some scripts may run using Python 3.0+, we encourage using Python 2.7 to avoid compatibility issues. To install Python, please navigate to http://www.python.org/download/releases/2.7.3/ and choose the appropriate installer for your operating system.

Our scripts are designed to run in a command-line environment on Mac OS X, Linux or UNIX operating systems, and can also be run on a Windows machine. Explanations of using OS X and UNIX/LINIX commands can be found Here and a list of commands Here; for Windows users an overview of the command prompt can be found Here and a list of commands Here.

You can test which version of Python you have by opening the terminal (Terminal in OS X, see below for Windows) and typing in “python”. If Python is installed, the program will open and the first line will show what version of Python is installed. The graphic below shows an OS X system running Python 2.7:



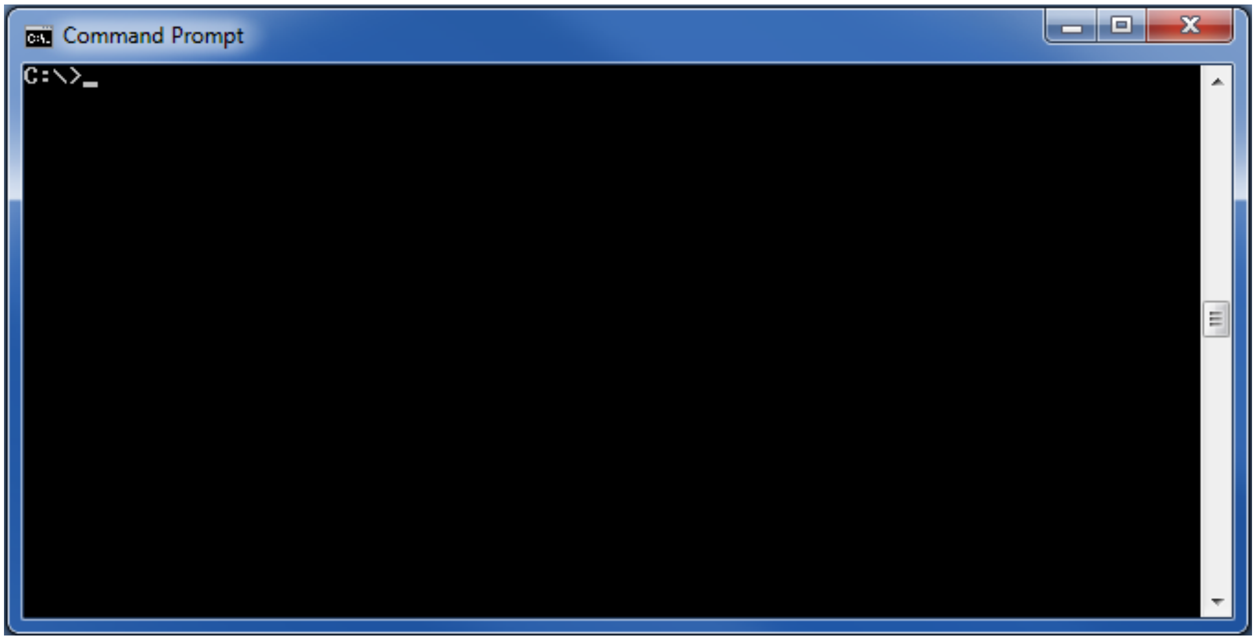
```
Last login: Thu Jun 21 17:33:57 on ttys000
dhcp10-200-12-151:~ bjb$ python
Python 2.7.1 (r271:86832, Jul 31 2011, 19:30:53)
[GCC 4.2.1 (Based on Apple Inc. build 5658) (LLVM build 2335.15.00)] on darwin
Type "help", "copyright", "credits" or "license" for more information.
>>>
```

Note: *The number after 2.7 refers to the minor version number and is not important for determining compatibility. As long as the first two numbers are 2.7, the correct version of Python has been installed.*

If Python 2.7+ has been installed successfully, you are now ready to use the Oligopaints scripts. The next sections will cover each script’s usage individually.

Windows Installation Notes

Proper installation of Python on a Windows OS can vary depending on the exact version of Windows being used (such as XP/Vista/7). We recommend reading the first two questions of the following FAQ during the installation process: http://docs.python.org/faq/windows.html
It is also important to be able to access a command-line interface for using the Oligopaints scripts. In most installations of Windows, the easiest way to do this is to open the Command Prompt. Usually, this program can be found in the start menu, selecting Start → Programs → Accessories → Command Prompt. The window should appear similar to the image below:



```
C:\>_
```

From this window, scripts can be executed using the examples shown later on in the manual.

Command Prompt/Terminal Commands

The Command Prompt (Windows) or Terminal (OS X/Linux/UNIX) window is a text-based, command-line interface with the computer. Commands are typed into the window, as opposed to using the mouse. When the Command Prompt or Terminal is first opened, a single line noting the current directory is displayed, followed by a symbol and then a space to type commands. This symbol may change depending on the computer, but is normally > for the Command Prompt and $ for the Terminal. In this document, we will use the $ symbol to represent a command line.

While the next section will explain specifically what commands are used to run python scripts, it is helpful to understand the basic commands that allow one to navigate the computer using the command prompt or terminal. The following commands should be typed in as written and followed by pressing the enter key, which will produce the desired action.

Command (Windows)	Command (OS X)	Command (Linux/UNIX)	Action
dir	**ls**	**ls**	Lists the files in the current directory.
cd	**cd**	**cd**	Changes the current directory to the directory listed after the command. For example, **> cd C:\** changes the directory to the C drive on the Windows command prompt.
help	**man**	**help**	Brings up the help manual for the Command Prompt/Terminal

A full listing of commands can be found at the following links for Windows (http://ss64.com/nt/), Mac OS X (http://ss64.com/osx/), and Linux/UNIX (http://ss64.com/bash/). It's important to note that although the commands for the Linux/UNIX terminal and the Mac OSX Terminal are similar, there are subtle differences between the programs.

Executable Scripts

The examples in the following guide show the scripts run in their executable form. Simply put, this means that there is a line in the script telling the computer that the script is a Python script, and must be deciphered and executed by the Python interpreter. When this line is present and working, the script is considered executable (Linux/UNIX users will note that the script must also have the appropriate permissions).

If a script is not executable, the user must explicitly tell the computer what program should be used to run the script. This is done by using the scripts in the following format:

```
python script.py
```

Instead of the format used in the examples:

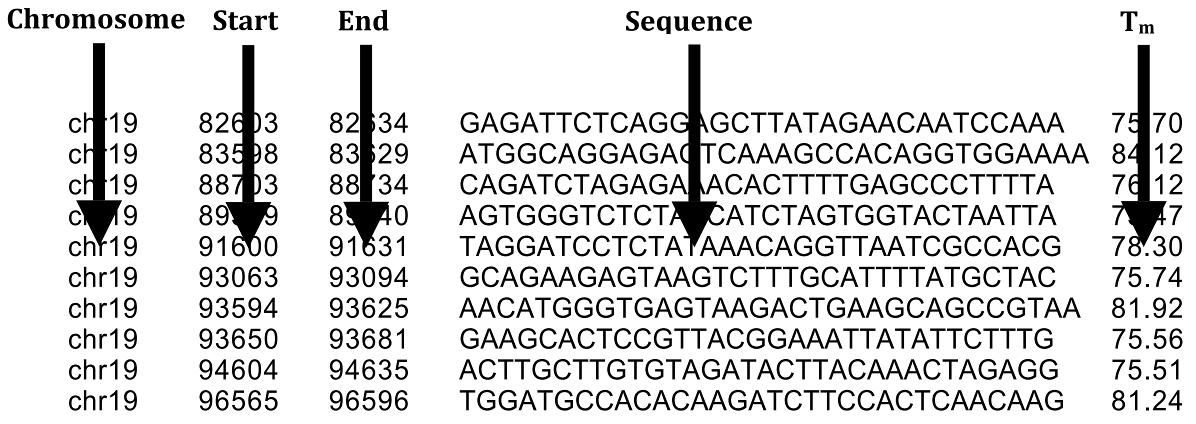
```
 ./script.py
```

Both forms are acceptable and the scripts will behave identically, regardless of which version is used. The scripts will attempt to automatically find the Python interpreter. However, it is often simpler for users to use the first, explicit format, which will avoid errors in case the script cannot properly find the interpreter.

Note: *If you would like to make the scripts executable, it is important to modify the shebang line (i.e. the line beginning with !# at the top of the file) in the beginning of the scripts to reflect the location of the interpreter in the current environment. Instructions for finding the location of the Python interpreter are in the Windows Python FAQ, for Windows users. OS X and Linux/Unix users should simply modify the line to wherever the correct installation of Python is located. If you chose to do this, make sure to overwrite the existing shebang line.*

Overview

This suite of scripts is primarily designed for working with Oligopaints .bed files, which are files containing the position of probes for each chromosome in the genome. For example, the hg19 folder contains chr1.bed, chr2.bed and so on, up to chrX.bed and chrY.bed. The dm3 folder contains chr2L.bed, chr2R.bed and so on. In this document, these files are referred to as a generic “chrN.bed” file. An example of the first lines of a chrN.bed file is shown below:



```
chr19   82603   82[illegible]34   GAGATTCTCAGG[illegible]AGCTTATAGAACAATCCAAA    75.70
chr19   83598   83[illegible]29   ATGGCAGGAGACTCAAAGCCACAGGTGGAAAA   84.12
chr19   88703   88734   CAGATCTAGAGA[illegible]ACACTTTTGAGCCCTTTTA     76.12
chr19   89[illegible]9   89[illegible]40   AGTGGGTCTCTA[illegible]CATCTAGTGGTACTAATTA     7[illegible]47
chr19   91600   91631   TAGGATCCTCTATAAACAGGTTAATCGCCACG   78.30
chr19   93063   93094   GCAGAAGAGTAAGTCTTTGCATTTTATGCTAC   75.74
chr19   93594   93625   AACATGGGTGAGTAAGACTGAAGCAGCCGTAA   81.92
chr19   93650   93681   GAAGCACTCCGTTACGGAAATTATATTCTTTG   75.56
chr19   94604   94635   ACTTGCTTGTGTAGATACTTACAAACTAGAGG   75.51
chr19   96565   96596   TGGATGCCACACAAGATCTTCCACTCAACAAG   81.24
```

Our files are *browser extendible data* files (.bed) files, a data format used by UCSC to store information about genomic reigons. These files are tab-delimited text files, which can be made using text editors such as Notepad (Windows) and TextEdit (OS X). When making .bed files, it is critical to make sure that the text editor is outputting plain text and not rich text or other formats, as the use of the latter can result in the inclusion undesirable hidden characters.

All .bed files contain at least 3 columns, which refer to the chromosome, start, and end of each region, with one region provided per line. Other columns can contain further information about the region. The following link to the UCSC genome browser provides a full description of the .bed format (http://genome.ucsc.edu/FAQ/FAQformat.html#format1). Although the later columns in the Oligopaints files do not correspond to the columns described in the definition, the files are fully compatible with the UCSC genome browser as the first 3 columns are preserved.

The scripts provided for working with chrN.bed files are described in the table below:

Script Name	Description
grabLines.py	Returns a specific range of lines from a chrN.bed file. E.g. the first 10,000 lines in the file.
grabRegion.py	Returns all the probes from a chrN.bed file falling within the specified chromosomal region. E.g. all of the probes within a chromosomal band or gene.
orderFile.py	Appends primer sequences to Oligopaints probes using user-specified

	primers and banding patterns.
probeRegion.py	Tiles a specified number of probes across a region of interest. E.g. 1,000 probes across a gene body.
probeNumber.py	Returns all possible contiguous sets of probes in a chrN.bed file that meet a user-specified number of probes. E.g. sets of 10,000.
sortFile.py	Sorts a given file by the specified column in either ascending or descending order.
windowSize.py	Returns all contiguous probe sets that cover a specified window size in kilobase pairs (e.g. 50 kb) from a chrN.bed file.

Options are a way to provide scripts the information they need on the command-line when the script is run. Options have a long form, which is the name of the option proceeded by two dashes, and a short form, which is usually the first letter of the option proceeded by one dash. All of the Oligopaints scripts contain three common options: --help, --interactive, and --output. Each script also has individual options so that necessary information can be provided on the command-line. It is possible to give the script only some of the necessary input on the command-line; if the script doesn't receive necessary user input or cannot understand it, it will run interactively and prompt the user for input. The options common to all Oligopaints scripts are detailed below:

-h, --help

When the "-h" or "--help" option is given with any script, information describing the usage and possible options for the script will be reported.

-i, --interactive

This option tells the script to run interactively, meaning that it will prompt the user for necessary information during the process of running the script.

-o, --output

Use this option to specify the name of the output file that the script should write to. For example:

```
./grabLines.py –o lines.txt
```

This will write the output of grabLines.py to the file "lines.txt". If the file does not exist in the working directory, it will be created. The script will warn the user if an existing file will be overwritten.

Each script has a default filename it will use if no output file is given by the –o option, which changes based on the options the user specifies. This output filename is described in the section for the script.

grabLines.py

A chrN.bed file contains hundreds of thousands of lines describing the location of probes along the chromosome. Often, it is convenient to work with a small subset of the lines in a given chrN.bed file. **grabLines.py** allows the user to reduce any chrN.bed file to the specified lines.

The ranges given must fall within the range of lines in the chrN.bed file; for example, a range of -1:40 would not be valid for a file containing only ten lines. The script will warn the user if the range appears to be invalid, and will not execute if more lines are specified than exist in the chrN.bed file.

Usage: grabLines.py [OPTIONS] chrN.bed

Input: chrN.bed

Output: chrN_lines_[start line]_to_[end line].bed

Option	Description
-h, --help	Brings up help menu
-i, --interactive	Runs script interactively
-o, --output	The name of the output file. If the file doesn't exist, it will be created.
-r, --range	The range of lines to grab, given as [start:end]. For example: **$ ./grabLines.py –r [5:50]**

Examples:

Retrieve the first 20 lines from chr13.bed and output to 20_lines.txt

```
$ ./grabLines.py –r [1:20] –o 20_lines.txt chr13.bed

Wrote to 20_lines.txt
```

Interactively use the script to get the 100th line to the end of chr2R.bed

```
$ ./grabLines.py –i chr2R.bed
Please enter the number of the first line needed [min = 1]: 100
Please enter the number of the last line needed [max = 313768]: 313768

Wrote to chr2R_lines_100_to_313768.bed
```

grabRegion.py

There are many tasks where it could be useful to find all probes within a chrN.bed file that fall into a certain region. The **grabRegion.py** script allows the user to intersect regions of interest with a chrN.bed file, returning all probes that fall into the region. The script also reports the probe density for the region as probes/kb. Advanced users may choose to use the intersectBed component of the bedtools package in place of this script (available here)

The script will accept the start and end coordinates for a region of interest given as an –r, --region argument and in the format [start:end]. This region must make sense for the file – if the coordinates given are outside the range of coordinates in the chrN.bed file, the script warn the user and correct the coordinates. When run interactively, the script will display the minimum start coordinate and maximum end coordinate possible for the chrN.bed file.

Usage: grabRegion.py [OPTIONS] chrN.bed

Input: chrN.bed

Output: chrN_region_[start coordinate]_to_[end coordinate].bed

Option	Description
-h, --help	Brings up help menu
-i, --interactive	Runs script interactively
-o, --output	The name of the output file. If the file doesn't exist, it will be created.
-r, --range	The range of lines to grab, given as [start:end]. For example: `$ ./grabRegion.py –r [20000:24000]`

Examples:
Get all probes from chr2R.bed between 20000th base and the 24000th base

```
$ ./grabRegion.py –i chr2R.bed
Please enter the region start coordinate [min = 3120]: 20000
Please enter the region end coordinate [max = 21145723]: 24000
Returned 45 probes with a density of 11.503 probes/kb

Wrote to chr2R_region_20000_to_24000.bed
```

Get all probes from chr16.bed from the 60000th base to 100 kb away, writing to chr16_probes.txt

```
$ ./grabRegion.py –r [60000:160000] –o chr16_probes.txt chr16.bed
Start coordinate is less than minimum start in file, correcting to 60081
Returned 838 probes with a density of 8.389 probes/kb

Wrote to chr16_probes.txt
```

orderFile.py

This script aids in preparing an order file by appending primer sequences to the probes in a chrN.bed file. The user can specify the number of primer pairs to be used and the probes that each pair is appended to. Primers can either be entered interactively, or given as a text file consisting of the forward primer sequence, the reverse primer sequence, and the range of probes for the primer pair separated by tabs. The sequences must be given 5’ to 3’, and the ranges should be given as start-stop, with multiple ranges separated by commas. The script will automatically convert the reverse primer to its reverse complement and warn if different primer pairs overlap in their range of probes. Note that a given primer sequence can be present in multiple primer pairs – for example, a common forward primer could be used with multiple reverse primers.

Usage: order.py [OPTIONS] chrN.bed

Input: chrN.bed

Output: chrN_order.bed

Option	Description
-h, --help	Brings up help menu
-i, --interactive	Runs script interactively
-o, --output	The name of the output file. If the file doesn’t exist, it will be created.
-p, --primer	A file containing one line for each primer being used. The forward and reverse sequences of each primer 5’ to 3’ and the range of probe lines being targeted must be separated by tabs and given in that order left to right. See the example below for details.

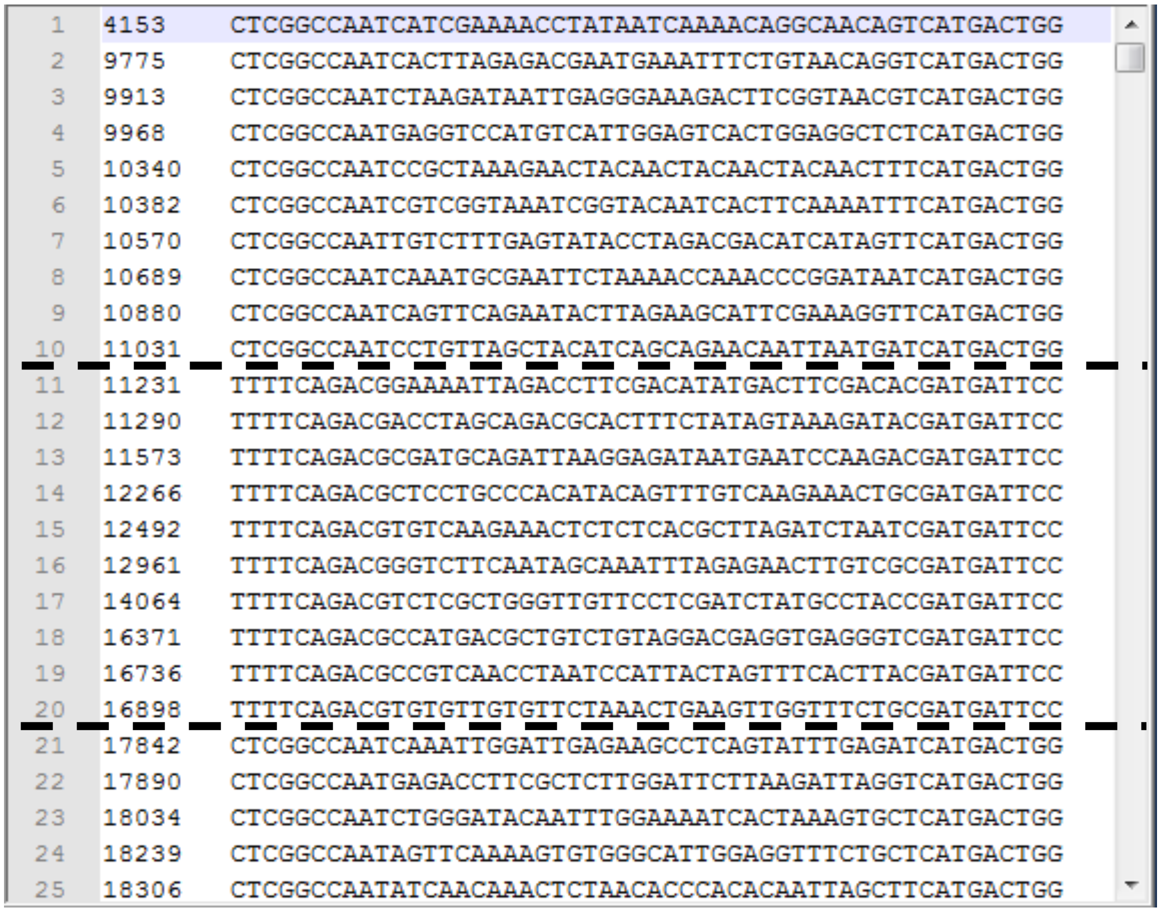
Examples:
Creating an order file for chr2R.bed, with a file containing the information required for two different primers, each targeted to a separate range of probes

```
CTCGGCCAAT	GGTCAGTACT	1-10,21-313768
TTTTCAGACG	CCTTAGTAGC	11-20
```

This file describes two primers: one that is targeted to the first 10 probes, and then the 21st probe to the last probe in the file, and one that is targeted to the 11th through 20th probes. The file is then passed to **order.py** using the –p option:

```
$ ./order.py –p primers.txt chr2R.bed
```

This will create a file called chr2R_order.bed in the current directory. An example of the output file is shown below:



```
 1  4153     CTCGGCCAATCATCGAAAACCTATAATCAAAACAGGCAACAGTCATGACTGG
 2  9775     CTCGGCCAATCACTTAGAGACGAATGAAATTTCTGTAACAGGTCATGACTGG
 3  9913     CTCGGCCAATCTAAGATAATTGAGGGAAAGACTTCGGTAACGTCATGACTGG
 4  9968     CTCGGCCAATGAGGTCCATGTCATTGGAGTCACTGGAGGCTCTCATGACTGG
 5  10340    CTCGGCCAATCCGCTAAAGAACTACAACTACAACTACAACTTTCATGACTGG
 6  10382    CTCGGCCAATCGTCGGTAAATCGGTACAATCACTTCAAAATTTCATGACTGG
 7  10570    CTCGGCCAATTGTCTTTGAGTATACCTAGACGACATCATAGTTCATGACTGG
 8  10689    CTCGGCCAATCAAATGCGAATTCTAAAACCAAACCCGGATAATCATGACTGG
 9  10880    CTCGGCCAATCAGTTCAGAATACTTAGAAGCATTCGAAAGGTTCATGACTGG
10  11031    CTCGGCCAATCCTGTTAGCTACATCAGCAGAACAATTAATGATCATGACTGG
11  11231    TTTTCAGACGGAAAATTAGACCTTCGACATATGACTTCGACACGATGATTCC
12  11290    TTTTCAGACGACCTAGCAGACGCACTTTCTATAGTAAAGATACGATGATTCC
13  11573    TTTTCAGACGCGATGCAGATTAAGGAGATAATGAATCCAAGACGATGATTCC
14  12266    TTTTCAGACGCTCCTGCCCACATACAGTTTGTCAAGAAACTGCGATGATTCC
15  12492    TTTTCAGACGTGTCAAGAAACTCTCTCACGCTTAGATCTAATCGATGATTCC
16  12961    TTTTCAGACGGGTCTTCAATAGCAAATTTAGAGAACTTGTCGCGATGATTCC
17  14064    TTTTCAGACGTCTCGCTGGGTTGTTCCTCGATCTATGCCTACCGATGATTCC
18  16371    TTTTCAGACGCCATGACGCTGTCTGTAGGACGAGGTGAGGGTCGATGATTCC
19  16736    TTTTCAGACGCCGTCAACCTAATCCATTACTAGTTTCACTTACGATGATTCC
20  16898    TTTTCAGACGTGTGTTGTGTTCTAAACTGAAGTTGGTTTCTGCGATGATTCC
21  17842    CTCGGCCAATCAAATTGGATTGAGAAGCCTCAGTATTTGAGATCATGACTGG
22  17890    CTCGGCCAATGAGACCTTCGCTCTTGGATTCTTAAGATTAGGTCATGACTGG
23  18034    CTCGGCCAATCTGGGATACAATTTGGAAAATCACTAAAGTGCTCATGACTGG
24  18239    CTCGGCCAATAGTTCAAAAGTGTGGCATTGGAGGTTTCTGCTCATGACTGG
25  18306    CTCGGCCAATATCAACAAACTCTAACACCCACACAATTAGCTTCATGACTGG
```

The first column displays the line number of each line in the file, while the dashed lines indicate a switch between one set of primers to the other. Just as the primers.txt file specified, the first primer set is used for probes 1-10 and 21-onwards, while the second primer set is used for probes 11-20.

Creating an order file for chr16.bed interactively, with the output file set to order_June1.txt

```
$ ./order.py –i –o order_June1.txt chr16.bed
Number of primers: 1
Enter primers 5' to 3'
Please enter the 1st forward primer: CCATACCA
Please enter the 1st reverse primer: GTGCCATC
The maximum probe range is 1-846611
Enter the range of probes for this primer to cover [ex. 1-30, 41-60]: 1-846611
Wrote to order_June1.txt
```

probeRegion.py

There are many reasons to want to target a region of interest with a specific number of probes. However, due to the density of the probe database, the region in question may contain more probes than the user actually needs. The **probeRegion.py** script allows the user to define the number of probes needed in a region, and returns a list of probes that tile across the region of interest. If there are fewer probes in the given region than requested, the **probeRegion.py** script will notify the user and allow them to either reduce the probe number or increase the region size.

Usage: probeRegion.py [OPTIONS] chrN.bed

Input: chrN.bed

Output: chrN_[number of probes]_probes_from_[start]_to_[end] .bed

Option	Description
-h, --help	Brings up help menu
-i, --interactive	Runs script interactively
-o, --output	The name of the output file. If the file doesn't exist, it will be created.
-p, --probes	The number of probes to pull out of the given region
-r, --region	The region to search in the chrN.bed file, given as [start:end]. For example: **$ ./probeRegion.py –r [1000:50000]**

Examples

Searching chr16.bed interactively to get 1000 probes in a 1 Mb window

```
 $ ./probeRegion.py –i chr16.bed
Please enter the number of probes per region [ex. 1000]: 1000
Please enter the start coordinate for the region [min = 60081]: 1000000
Please enter the end coordinate for the region [max = 90290462]: 2000000
Found 9696 probes in region
Tiling 1000 probes (out of 9696) evenly across region

Wrote to chr16_1000_probes_from_1000000_to_2000000.bed
```

An example of the output file is shown below:

```
chr16    1000118  1000149  CGAAAGACTCAGAGCCAATGACAGAGTCAGAG  80.43
chr16    1002045  1002076  CGCCTCGGCAAAGTCAGGCATGTGTGCGTGGA  89.15
chr16    1004124  1004155  CTTTAAGTAAGGAAGAGCTACTGCAAAGAGGT  77.34
chr16    1004642  1004673  TGGAAAGCCACCAGGAATGCCACGACTGGAAG  85.87
chr16    1005789  1005820  TCACCATTATGAAACACACCCTGAGGACCCTG  82.14
chr16    1007287  1007318  TGTCTGTGTAACTGTGCATGCTGGTTTGGAGT  82.57
chr16    1007835  1007866  AACTACACAGACAAAGAGCAGCCTTGGGCCGG  85.77
chr16    1008948  1008979  AAGGTTCGGGACAGCAGGAGTGCCCTCGCTGT  89.53
chr16    1009634  1009665  GCAACTCCGGAAAATGTTCTGAGTGGAGACCG  82.90
chr16    1010084  1010115  CCTCCCAGTGCCCAGACTGAAGTTTCTATGCT  83.54
```

PROBENUMBER.PY

This script allows the user to parse a chrN.bed file into a series of regions that meet a specified probe threshold. For example, one could find the regions in a chromosome that contain 1000 probes. The output file contains the coordinates for each region and the probe density (probes/kb) for the region.

The user can limit the search to a specific region of the chrN.bed file by providing start and stop coordinates using the-r, --region option.

Usage: probeNumber.py [OPTIONS] chrN.bed

Input: chrN.bed

Output: chrN_[number of probes]_probe_regions.bed

Note: *If the –r, --region option has been used, the file name will change to reflect the region searched, becoming chrN_[number of probes]_probe_regions_from_[start]_to_[end].bed*

Option	Description
-h, --help	Brings up help menu
-i, --interactive	Runs script interactively
-o, --output	The name of the output file. If the file doesn't exist, it will be created.
-p, --probes	The number of probes per region
-r, --region	The region to search in the chrN.bed file, given as [start:end]. For example: **$ ./probeNumber.py –r [1000:50000]**

Examples:

Searching chr16.bed interactively to find windows with 500 probes from the beginning of the file to 100,000 bases afterwards

```
 $ ./probeNumber.py –i chr16.bed
Please enter the number of probes per region [ex. 1000]: 500
Would you like to specify a region to search within the file? [Y/n]: y
Please enter the start coordinate for the region [min = 60081]: 60081
Please enter the end coordinate for the region [max = 90290462]: 160081

Wrote to chr16_500_probe_regions_from_60081_to_160081.bed
```

An example of the output file is shown below:

```
chr16    60081    130439   7.107
chr16    64122    130481   7.535
chr16    67574    130531   7.942
chr16    69680    130891   8.168
chr16    71255    130952   8.376
chr16    71407    130994   8.391
chr16    71480    131037   8.395
chr16    71522    131084   8.395
```

Finding all regions containing 1000 probes in chr2R.bed and writing to chr2R_windows.bed

```
$ ./probeNumber.py –p 1000 –o chr2R_windows.bed chr2R.bed

Wrote to chr2R_windows.bed
```

sortFile.py

There are many reasons a user may want to sort chrN.bed files. For example, one could sort a chrN.bed file to pull out probes with the highest T_m, or sort an output file to pull out the densest regions of a chromosome. While sorting small files is easily done using spreadsheet software, such as Excel, some files are too large to easily manipulate with such programs. The **sortFile.py** script allows the user to sort any tab-delimited text file by a column in ascending or descending order.

Usage: sortFile.py [OPTIONS] chrN.bed

Input: chrN.bed

Output: chrN_sorted.bed

Option	Description
-h, --help	Brings up help menu
-i, --interactive	Runs script interactively
-c, --columns	The column to sort the file by (e.g. -c 1 for the first column)
-s, --sort	The order in which the file should be sorted. The only two options are “a” or “d”, for ascending or descending order, respectively. Defaults to descending order.
-o, --output	The name of the output file. If the file doesn’t exist, it will be created.

Examples

Sorting chr2R.bed by T_m in descending order

The first ten lines of chr2R.bed before the sort:

```
chr2R    4153     4184     CATCGAAAACCTATAATCAAAACAGGCAACAG    76.12
chr2R    9775     9806     CACTTAGAGACGAATGAAATTTCTGTAACAGG    75.12
chr2R    9913     9944     CTAAGATAATTGAGGGAAAGACTTCGGTAACG    76.05
chr2R    9968     9999     GAGGTCCATGTCATTGGAGTCACTGGAGGCTC    83.59
chr2R    10340    10371    CCGCTAAAGAACTACAACTACAACTACAACTT    76.09
chr2R    10382    10413    CGTCGGTAAATCGGTACAATCACTTCAAAATT    77.03
chr2R    10570    10601    TGTCTTTGAGTATACCTAGACGACATCATAGT    75.72
chr2R    10689    10720    CAAATGCGAATTCTAAAACCAAACCCGGATAA    77.60
chr2R    10880    10911    CAGTTCAGAATACTTAGAAGCATTCGAAAGGT    76.24
chr2R    11031    11062    CCTGTTAGCTACATCAGCAGAACAATTAATGA    76.35
```

```
$ ./sortFile.py –c 5 chr2R.bed
Sorting chr2R.bed by the 5th column in descending order

Wrote to chr2R_sorted.bed
```

The first ten lines of chr2R_sorted.bed:

```
chr2R    20946954    20946985    TTGCCGTGTCATCCCGGAGGTGCGTGTGTCCG    89.99
chr2R    20936608    20936639    GAGTGTATGCTGGGAGTGCGGCCACCGTCCGG    89.99
chr2R    20826078    20826109    TGGAGGACGGCACCAAGCGCGAGGTGGTCAAG    89.99
chr2R    20775361    20775392    GAGCGCTGGAGGAACCACCGAGCCTTCCACCG    89.99
chr2R    20742317    20742348    TGTAGACCACTGGCGGTGCTGCACTCGGCCAC    89.99
chr2R    20718469    20718500    GTGTGTGCTCGAGGGAAGGGCGGCTTCTCCGG    89.99
chr2R    20476447    20476478    AACCTGGATGCGGGACTGGAGGCCACAGCGAC    89.99
chr2R    20462538    20462569    TCCGCAGTACCGCCCAGCTCATCCTGGCCAAC    89.99
chr2R    20461562    20461593    ATGGTGCAGACTGGCGATCCCACGGGAACGGG    89.99
chr2R    20458935    20458966    TTCTTGGCTCCGCTTCCTCCGCCGCCATCGTC    89.99
```

Interactively sorting chr16.bed by the start position (2nd column) in ascending order, and writing to sorted.txt

```
$ ./sortFile.py –i –o sorted.txt chr16.bed
Please enter the column to sort by [5 columns in file]: 2
Ascending or descending sort? [a/d]: a
Sorting chr16.bed by the 2nd column in ascending order

Wrote to sorted.txt
```

WINDOWSIZE.PY

This script allows the user to parse a chrN.bed file into windows of a certain size, reporting the coordinates, probe density (defined as probes/kb) and number of total probes for each window. The script attempts to find windows of the user-defined size, within a tolerance level that defaults to 10% of the window size. This tolerance level can be changed by the user, using the

–t, --tolerance option. The –s, --sort option sorts the output file by decreasing probe density, allowing the user to quickly find the densest regions of a given size for the chrN.bed file.

Usage: windowSize.py [OPTIONS] chrN.bed

Input: chrN.bed

Output: chrN_[window size]kb.txt

Option	Description
-h, --help	Brings up help menu
-i, --interactive	Runs script interactively
-o, --output	The name of the output file. If the file doesn't exist, it will be created.
-s, --sort	Sort output file by probe density in descending order
-t, --tolerance	The tolerance for variation in the window size. It defaults to 10% of the window size, but can be changed by providing the tolerance as a fraction of window size. Example: **$ ./windowSize.py –t 0.05**
-w, --window	The target window size, in kb. The script will attempt to find windows of that size, modified by the tolerance option.

Examples:

Finding the densest 10kb windows in chr16.bed with window size variation limited to 100 bp.

```
$ ./probeNumber –w 10 –t 0.01 -s chr16.bed

Sorting chr16_10kb.txt
Wrote to chr16_10kb.txt
```

An example of the output file, which gives the start and stop coordinates, probe density in probes/kb, and the number of probes in the window is shown below:

```
chr16    10033096    10043060    17.26    172
chr16    10033138    10043103    17.26    172
chr16    10035402    10045309    17.26    171
chr16    10035913    10045818    17.26    171
chr16    52216234    52226201    17.26    172
chr16    52216633    52226596    17.26    172
chr16    52216760    52226724    17.26    172
chr16    10035582    10045496    17.25    171
chr16    10035845    10045818    17.25    172
chr16    51020010    51029979    17.25    172
```

Walkthroughs

This section contains detailed walkthrough of three different tasks that can be completed using Oligopaints scripts. For each step involving a script, the terminal window will be shown with the script run interactively for greater clarity. Although the images shown will be from a UNIX Terminal, the scripts will behave in the same way using the same commands from a Mac OS X Terminal or a Windows Command Prompt.

Example 1: Designing 20,000 Probes to 19p12

In this first example, we are aiming to design a block of 20,000 probes to 19p12, using the chr19.bed file. First, we need to get the position of 19p12, which we can easily get from UCSC Genome Browser by typing “19p12” into the position bar when browsing the GRCh37/hg19 genome. We then define our region of interest (ROI) as chr19:20,000,001-24,400,000.

Next, we want to intersect our ROI with the chr19.bed file. We use **grabRegion.py** to do so:

```
$ ./grabRegion.py –l chr19.bed
Please enter the region start coordinate [min = 82603]: 20000001
Please enter the region end coordinate [min = 58128975]: 24400000
Returned 26927 probes with a density of 6.12 probes/kb

Wrote to chr19_region_20000001_to_24400000.bed
```

As shown above, we now have the file chr19_region_20000001_to_24400000.bed, which contains 26,927 probes. Since we only want 20,000 probes, we’ll narrow down our list by picking the top 20,000 with the highest T_m. We’ll use **sortFile.py** to sort our file by T_m, which is the fifth column in our file.

```
$ ./sortFile.py –i chr19_region_20000001_to_24400000.bed
Please enter the column to sort by [5 columns in file]: 5
Ascending or descending sort? [a/d]: d
Sorting chr19_region_20000001_to_24400000.bed by the 5th column in descending order

Wrote to chr19_region_20000001_to_24400000_sorted.bed
```

We now have our 26,927 probes sorted by T_m in descending order. Since we want probes with the highest T_m, we will take the first 20,000 lines from the sorted file and use those to order primers. In Linux/UNIX and OS X, the “head” command returns the first lines of a file, using the option -20000 to pick the first 20,000. This is used in the example below. On a Windows system, one could use **grabLines.py** to return the first 20,000 lines, or open the file in Excel and take only the first 20,000 rows. In addition, we’ll rename the file to chr19_20000_probes.bed to cut down on the length of the filename.

```
$ head –20000 chr19_region_20000001_to_24400000_sorted.bed > chr19_20000_probes.bed
```

Finally, we will create an order file with primer sequences appended to our oligos. The example below uses one primer pair, but multiple pairs can easily be used if so desired.

```
$ ./orderFile.py –i chr19_20000_probes.bed
Number of primers: 1
Enter primers 5' to 3'
Please enter the 1st forward primer: CTCGGCCAAT
Please enter the 1st reverse primer: TCATGACTGG
The maximum probe range is 1-20000
Enter the range of probes for this primer to cover [ex. 1-30, 41-60]: 1-20000

Wrote to chr19_20000_probes_order.txt
```

Our final result:

```
20000509     CTCGGCCAATTGGTACAGTCACTCACTGCAAGCTTCCCATCTCCAGTCATGA
20000737     CTCGGCCAATCCAAGCTTGGCCTGTTCAATTTATTCTGAATTCCAGTCATGA
20000831     CTCGGCCAATGCCATGCTGCCCAAATATTTTGTATACATTGTCCAGTCATGA
20001078     CTCGGCCAATTGCTACAGCCATTCCTGTTTGGATTACTGCAGCCAGTCATGA
20001120     CTCGGCCAATTCTGTAACATTTACCTTTAGACTCAGCAGACTCCAGTCATGA
20001178     CTCGGCCAATTTTCAGCATTTTATGTCATGGGAGACATTAGCCCAGTCATGA
20001963     CTCGGCCAATAAAAGCACAATGTTATACTGGGCAGCAGGAAGCCAGTCATGA
20002153     CTCGGCCAATAATCAGAACTGGTGGTATTTTGCTATGTCATCCCAGTCATGA
20002309     CTCGGCCAATCTTTGGCCAGAGAACATACAAAATTCTTTCCACCAGTCATGA
20003356     CTCGGCCAATACTGCATCCTCAACTCTAACTACACATAAGAGCCAGTCATGA
20003721     CTCGGCCAATTCATACCAAACAGAAGCCCTACAAGTGTGAAGCCAGTCATGA
```

EXAMPLE 2: DESIGNING 10,000 PROBES TO THE GENE BCKDK

In this second example, we want to target the gene BCKDK with 10,000 probes. We'll first get the genomic coordinates for BCKDK, ensuring that the genome build we get coordinates for is the same as the genome build used by Oligopaints (in this case, hg19/GRCh37). By typing BCKDK into the position bar, we search for all genes matching this name and pick the gene to get its coordinates. The coordinates for the gene are chr16: 31,119,662-31,124,112, and we'll use **grabRegion.py** to get the probes we need:

```
$ ./grabRegion.py –i chr16.bed
Please enter the region start coordinate [min = 60081]: 31119662
Please enter the region end coordinate [max = 90290462]: 31124112
Returned 57 probes with a density of 13.137 probes/kb
```

We immediately run into a problem. There are only 57 probes that cover our region of interest. In order to get around this, we can expand the flanks of our ROI to pick up more probes. We can use **probeRegion.py** to efficiently parse chr16.bed until we get a region that meets our threshold probe number of 10,000 probes:

```
$ ./probeRegion.py –i chr16.bed
Please enter the number of probes per region [ex. 1000]: 10000
Please enter the region start coordinate [min = 60081]: 30619662
Please enter the region end coordinate [max = 90290462]: 31624112
Found 9431 probes in region
Less probes found (9431) than requested (10000)
Would you like to change the region size? [Y/n]: y
Please enter the region start coordinate [min = 60081]: 30619662
Please enter the region end coordinate [max = 90290462]: 31874112
Found 13536 probes in region
Tiling 10000 probes (out of 13536) evenly across region

Wrote to chr16_10000_probes_from_30369662_to_31874112
```

As you can see above, we first try expanding our ROI by 500 kb on each side, giving us a spot of roughly 1 Mb. We enter the minimum number of probes/region, 10,000, and the new coordinates. However, this region only contains 9431 probes. We try again, using 750 kb flanks, and find 13536 probes in our new ROI. **probeRegion.py** then returns 10,000 probes tiled across our region of interest, out of the possible 13536.

Finally, we will use **orderFile.py** to get append one primer pair to all of our probes:

```
./orderFile.py –i chr16_10000_probes_from_30369662_to_31874112.bed
Number of primers: 1
Enter primers 5' to 3'
Please enter the 1st forward primer: ACCTGCATGCGG
Please enter the 1st reverse primer: CCGTGATCGTCA
The maximum probe range is 1-10000
Enter the range of probes for this primer to cover [ex. 1-30, 41-60]: 1-10000

Wrote to chr16_10000_probes_from_30369662_to_31874112_order.txt
```

And here's our order file, ready to go:

```
30027206    ACTAGTTGACGCGTTTGTTTTGTAGAGATGGGTCTTACTATGGTTACGGCATACGG
30027691    ACTAGTTGACGCACCGTTAGGTTTCCTTCGTGGCTCTCCAGAATTACGGCATACGG
30027733    ACTAGTTGACGCGCAGGACGAAGGGAGACACAGTGCAGTGTGAATACGGCATACGG
30027865    ACTAGTTGACGCTCCTGTGCTCTCTTCCCACGGTGCTTGCTCAATACGGCATACGG
30027926    ACTAGTTGACGCTGTTGAAGACAGGTAAGGATGAGGCTGGAGTTTACGGCATACGG
30028032    ACTAGTTGACGCGTCACCCAGTCATTCAGTAAATATTGACTGAATACGGCATACGG
30028127    ACTAGTTGACGCTCATAGGGAAGACAGACAAATACACAGACAATTACGGCATACGG
30028232    ACTAGTTGACGCGAGGGAACAAAGATTAACACCTCACTTCCCAGTACGGCATACGG
30028351    ACTAGTTGACGCTGTGCCAAAGCTGGGAGGCAGTCCAGAGTACGTACGGCATACGG
30028393    ACTAGTTGACGCAAAAGAAATCATTCTACCAGCCTGGTCAACATTACGGCATACGG
```

Example 3: Creating a Banding Pattern on chr2R With 15,000 Oligos

In this final example, we will use 15,000 probes to create a banding pattern on chr2R.bed. First, we'll use **probeNumber.py** to give us a list of regions containing at least 15,000 oligos across the entire chromosome:

```
./probeNumber.py –i chr2R.bed
Please enter the number of probes per region [ex. 1000]: 15000
Would you like to specify a region to search within the file [Y/n]: n

Wrote to chr2R_15000_probe_regions.bed
```

Next, we'll use **sortFile.py** to sort our file by probe density (the 4th column), allowing us to pull out the densest region:

```
$ ./sortFile.py –i chr2R_15000_probe_regions.bed
Please enter the column to sort by [4 columns in file]: 4
Ascending or descending sort? [a/d]: d
Sorting chr2R_15000_probe_regions.bed by the 4th column in descending order
Wrote to chr2R_15000_probe_regions_sorted.bed
```

The sorted file looks like this:

```
chr2R    19147407    20034670    16.906
chr2R    19147169    20034628    16.902
chr2R    19147127    20034575    16.902
chr2R    19147062    20034507    16.902
chr2R    19147019    20034465    16.902
chr2R    19146903    20034423    16.901
chr2R    19146380    20033924    16.901
chr2R    19146425    20033978    16.9
chr2R    19146289    20033862    16.9
chr2R    19146247    20033820    16.9
```

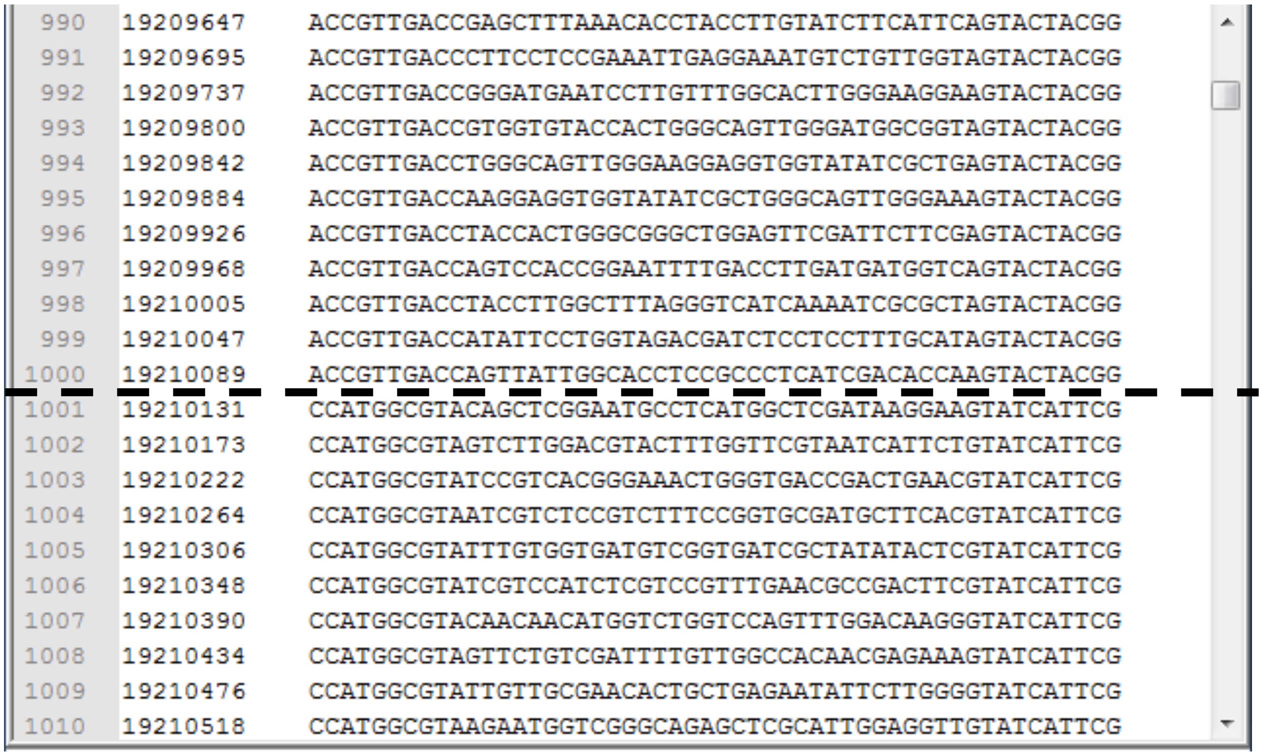
From this, we now know the region we want to target is between 19147407 and 20034670 on chr2R. We can use **grabRegion.py** to reduce chr2R.bed to the probes that lie in that region:

```
./grabRegion.py –i chr2R.bed
Please enter the region start coordinate [min = 3120]: 19147407
Please enter the region end coordinate [min = 58128975]: 20034670
Returned 15000 probes with a density of 16.906 probes/kb

Wrote to chr2R_region_19147407_to_20034670.bed
```

We want to use 3 different primers with our probes so that we can get a 3-color banding pattern. We'll divide up the region into 5 blocks. The first and fourth blocks will be targeted by the first primer; the second and fifth blocks by the second primer, and then the third block will be targeted by the third primer. Using **orderFile.py**, it is simple to generate an order file that meets our requirements:

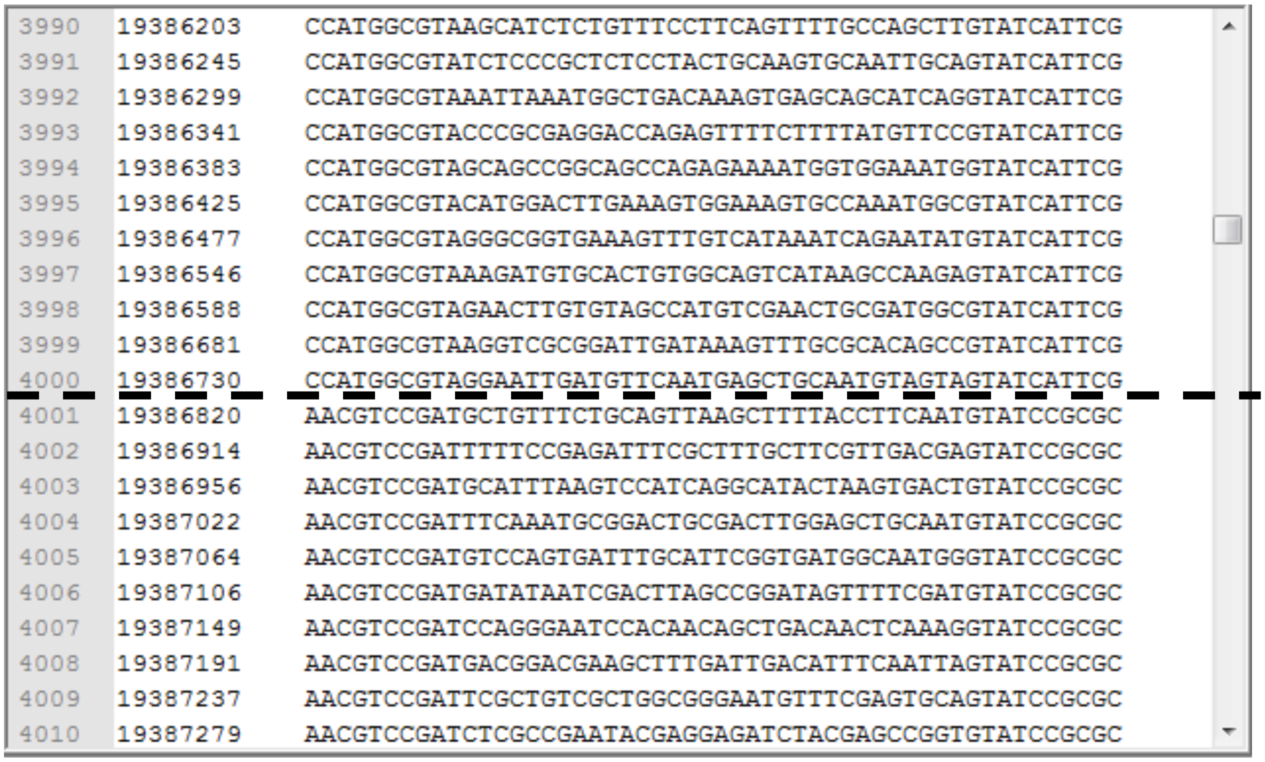
```
./orderFile.py –i chr2R_region_19147407_to_20034670.bed
Number of primers: 3
Enter primers 5' to 3'
Please enter the 1st forward primer: ACCGTTGACC
Please enter the 1st reverse primer: CCGTAGTACT
The maximum probe range is 1-15000
Enter the range of probes for this primer to cover [ex. 1-30, 41-60]: 1-1000,6001-9000
Enter primers 5' to 3'
Please enter the 2nd forward primer: CCATGGCGTA
Please enter the 2nd reverse primer: CGAATGATAC
The maximum probe range is 1-15000
Enter the range of probes for this primer to cover [ex. 1-30, 41-60]: 1001-4000,9001-15000
Enter primers 5' to 3'
Please enter the 3rd forward primer: AACGTCCGAT
Please enter the 3rd reverse primer: GCGCGGATAC
The maximum probe range is 1-15000
Enter the range of probes for this primer to cover [ex. 1-30, 41-60]: 4001-6000

Wrote to chr2R_region_19147407_to_20034670_order.txt
```

And a few shots of the resulting order file, showing the junctions between primers. The first column represents the line numbers, while the dotted line represents a switch between primer pairs:



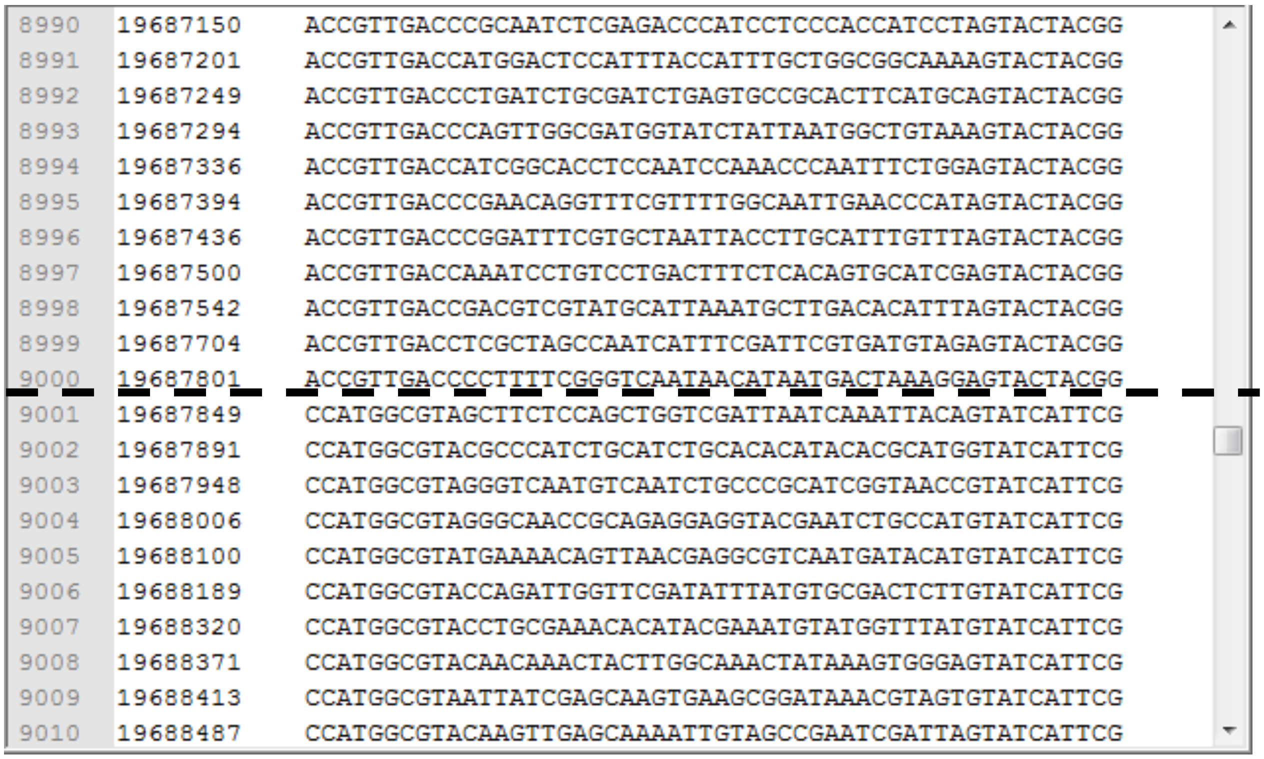
```
 990  19209647    ACCGTTGACCGAGCTTTAAACACCTACCTTGTATCTTCATTCAGTACTACGG
 991  19209695    ACCGTTGACCCTTCCTCCGAAATTGAGGAAATGTCTGTTGGTAGTACTACGG
 992  19209737    ACCGTTGACCGGGATGAATCCTTGTTTGGCACTTGGGAAGGAAGTACTACGG
 993  19209800    ACCGTTGACCGTGGTGTACCACTGGGCAGTTGGGATGGCGGTAGTACTACGG
 994  19209842    ACCGTTGACCTGGGCAGTTGGGAAGGAGGTGGTATATCGCTGAGTACTACGG
 995  19209884    ACCGTTGACCAAGGAGGTGGTATATCGCTGGGCAGTTGGGAAAGTACTACGG
 996  19209926    ACCGTTGACCTACCACTGGGCGGGCTGGAGTTCGATTCTTCGAGTACTACGG
 997  19209968    ACCGTTGACCAGTCCACCGGAATTTTGACCTTGATGATGGTCAGTACTACGG
 998  19210005    ACCGTTGACCTACCTTGGCTTTAGGGTCATCAAAATCGCGCTAGTACTACGG
 999  19210047    ACCGTTGACCATATTCCTGGTAGACGATCTCCTCCTTTGCATAGTACTACGG
1000  19210089    ACCGTTGACCAGTTATTGGCACCTCCGCCCTCATCGACACCAAGTACTACGG
1001  19210131    CCATGGCGTACAGCTCGGAATGCCTCATGGCTCGATAAGGAAGTATCATTCG
1002  19210173    CCATGGCGTAGTCTTGGACGTACTTTGGTTCGTAATCATTCTGTATCATTCG
1003  19210222    CCATGGCGTATCCGTCACGGGAAACTGGGTGACCGACTGAACGTATCATTCG
1004  19210264    CCATGGCGTAATCGTCTCCGTCTTTCCGGTGCGATGCTTCACGTATCATTCG
1005  19210306    CCATGGCGTATTTGTGGTGATGTCGGTGATCGCTATATACTCGTATCATTCG
1006  19210348    CCATGGCGTATCGTCCATCTCGTCCGTTTGAACGCCGACTTCGTATCATTCG
1007  19210390    CCATGGCGTACAACAACATGGTCTGGTCCAGTTTGGACAAGGGTATCATTCG
1008  19210434    CCATGGCGTAGTTCTGTCGATTTTGTTGGCCACAACGAGAAAGTATCATTCG
1009  19210476    CCATGGCGTATTGTTGCGAACACTGCTGAGAATATTCTTGGGGTATCATTCG
1010  19210518    CCATGGCGTAAGAATGGTCGGGCAGAGCTCGCATTGGAGGTTGTATCATTCG
```

The screenshot above shows the junction between the first primer pair and the second primer pair, at the 1000th line.



```
3990  19386203    CCATGGCGTAAGCATCTCTGTTTCCTTCAGTTTTGCCAGCTTGTATCATTCG
3991  19386245    CCATGGCGTATCTCCCGCTCTCCTACTGCAAGTGCAATTGCAGTATCATTCG
3992  19386299    CCATGGCGTAAATTAAATGGCTGACAAAGTGAGCAGCATCAGGTATCATTCG
3993  19386341    CCATGGCGTACCCGCGAGGACCAGAGTTTTCTTTTATGTTCCGTATCATTCG
3994  19386383    CCATGGCGTAGCAGCCGGCAGCCAGAGAAAATGGTGGAAATGGTATCATTCG
3995  19386425    CCATGGCGTACATGGACTTGAAAGTGGAAAGTGCCAAATGGCGTATCATTCG
3996  19386477    CCATGGCGTAGGGCGGTGAAAGTTTGTCATAAATCAGAATATGTATCATTCG
3997  19386546    CCATGGCGTAAAGATGTGCACTGTGGCAGTCATAAGCCAAGAGTATCATTCG
3998  19386588    CCATGGCGTAGAACTTGTGTAGCCATGTCGAACTGCGATGGCGTATCATTCG
3999  19386681    CCATGGCGTAAGGTCGCGGATTGATAAAGTTTGCGCACAGCCGTATCATTCG
4000  19386730    CCATGGCGTAGGAATTGATGTTCAATGAGCTGCAATGTAGTAGTATCATTCG
4001  19386820    AACGTCCGATGCTGTTTCTGCAGTTAAGCTTTTACCTTCAATGTATCCGCGC
4002  19386914    AACGTCCGATTTTTCCGAGATTTCGCTTTGCTTCGTTGACGAGTATCCGCGC
4003  19386956    AACGTCCGATGCATTTAAGTCCATCAGGCATACTAAGTGACTGTATCCGCGC
4004  19387022    AACGTCCGATTTCAAATGCGGACTGCGACTTGGAGCTGCAATGTATCCGCGC
4005  19387064    AACGTCCGATGTCCAGTGATTTGCATTCGGTGATGGCAATGGGTATCCGCGC
4006  19387106    AACGTCCGATGATATAATCGACTTAGCCGGATAGTTTTCGATGTATCCGCGC
4007  19387149    AACGTCCGATCCAGGGAATCCACAACAGCTGACAACTCAAAGGTATCCGCGC
4008  19387191    AACGTCCGATGACGGACGAAGCTTTGATTGACATTTCAATTAGTATCCGCGC
4009  19387237    AACGTCCGATTCGCTGTCGCTGGCGGGAATGTTTCGAGTGCAGTATCCGCGC
4010  19387279    AACGTCCGATCTCGCCGAATACGAGGAGATCTACGAGCCGGTGTATCCGCGC
```

The second screenshot above shows the junction between the first primer pair and the third primer pair at the 4000th line.



```
8990  19687150    ACCGTTGACCCGCAATCTCGAGACCCATCCTCCCACCATCCTAGTACTACGG
8991  19687201    ACCGTTGACCATGGACTCCATTTACCATTTGCTGGCGGCAAAAGTACTACGG
8992  19687249    ACCGTTGACCCTGATCTGCGATCTGAGTGCCGCACTTCATGCAGTACTACGG
8993  19687294    ACCGTTGACCCAGTTGGCGATGGTATCTATTAATGGCTGTAAAGTACTACGG
8994  19687336    ACCGTTGACCATCGGCACCTCCAATCCAAACCCAATTTCTGGAGTACTACGG
8995  19687394    ACCGTTGACCCGAACAGGTTTCGTTTTGGCAATTGAACCCATAGTACTACGG
8996  19687436    ACCGTTGACCCGGATTTCGTGCTAATTACCTTGCATTTGTTTAGTACTACGG
8997  19687500    ACCGTTGACCAAATCCTGTCCTGACTTTCTCACAGTGCATCGAGTACTACGG
8998  19687542    ACCGTTGACCGACGTCGTATGCATTAAATGCTTGACACATTTAGTACTACGG
8999  19687704    ACCGTTGACCTCGCTAGCCAATCATTTCGATTCGTGATGTAGAGTACTACGG
9000  19687801    ACCGTTGACCCCTTTTCGGGTCAATAACATAATGACTAAAGGAGTACTACGG
9001  19687849    CCATGGCGTAGCTTCTCCAGCTGGTCGATTAATCAAATTACAGTATCATTCG
9002  19687891    CCATGGCGTACGCCCATCTGCATCTGCACACATACACGCATGGTATCATTCG
9003  19687948    CCATGGCGTAGGGTCAATGTCAATCTGCCCGCATCGGTAACCGTATCATTCG
9004  19688006    CCATGGCGTAGGGCAACCGCAGAGGAGGTACGAATCTGCCATGTATCATTCG
9005  19688100    CCATGGCGTATGAAAACAGTTAACGAGGCGTCAATGATACATGTATCATTCG
9006  19688189    CCATGGCGTACCAGATTGGTTCGATATTTATGTGCGACTCTTGTATCATTCG
9007  19688320    CCATGGCGTACCTGCGAAACACATACGAAATGTATGGTTTATGTATCATTCG
9008  19688371    CCATGGCGTACAACAAACTACTTGGCAAACTATAAAGTGGGAGTATCATTCG
9009  19688413    CCATGGCGTAATTATCGAGCAAGTGAAGCGGATAAACGTAGTGTATCATTCG
9010  19688487    CCATGGCGTACAAGTTGAGCAAAATTGTAGCCGAATCGATTAGTATCATTCG
```

Finally, this screenshot shows the switch between the first primer pair and the second primer pair at the 9000th line.