Supplemental Data 1: my5C details, 5C methods and supplemental file descriptions.

This file contains a more detailed overview of the my5C web tools, a description of the 5C analysis presented in Figure 1 (including methods) and short descriptions of the contents of Supplemental Data 2-11.

Overview of my5C tools

My5C is composed of two main modules that allow researchers to manage 5C projects: My5C.primers facilitates the design of large sets of 5C primers; My5C.iHeatmap provides visualization and analysis tools for intuitive exploration of interaction maps obtained with these primers sets. My5C is accessed at
http://my5c.umassmed.edu/
where detailed tutorials for how to use my5C can also be found. Here we describe the overall features of my5C.

A 5C project starts by defining the genomic region(s) and the restriction enzyme that will be used. Users upload a FASTA file containing the DNA sequence of interest and choosing the restriction enzyme for the 5C experiment. There is no size limit to the genomic region and 5C designs for thousands of primers covering hundreds of megabases are possible. It is important to point out that there may be a limit to the number of interactions that can be reliably detected at a given depth of sequencing. We have obtained reliable 5C data, as evidenced by the accurate detection of positive controls, using 3,196 5C primers that combined detect 1,397,979 long- and short range interactions. This 5C analysis involved ~25 million paired end sequence reads. The field of deep-sequencing is still very much in development, and the number of reads that can be obtained per experiment will continue to increase in the future. Therefore, the number of interactions that can reliably be detected may well increase as well.

My5C.primers will design both forward and reverse 5C primers for every restriction fragment within the region of interest using user specified Tm and primer length. My5C.primers also calculates the repetitiveness of each 5C primer that is used as a filtering criteria later. The user also chooses the universal tails that will be attached to the forward and reverse primers (Figure 1).

Next, the researcher has the opportunity to determine for which fragments a forward primer is required and for which fragments a reverse primer. We have found that many laboratories are interested in studying the overall three-dimensional conformation of large genomic regions of biological interest. In that case an alternating primer design scheme[1] can be selected in my5C.primers.

Researchers may also wish to map all long-range interactions between two sets of genomic elements, *e.g.* between all promoters and all enhancers in the region of interest. Users can upload files that contain the genomic coordinates of these elements and can then instruct my5C.primers to select reverse primers for one set of overlapping restriction fragments and forward primers for the other. For the remaining fragments in the genomic region of interest the user can choose to use forward primers, reverse primers, an alternating set of forward and reverse primers or no additional primers.

It is important to point out that one can override any design decisions made by my5C.primers at any step along the design process. For any individual restriction fragment the user can change whether a forward or reverse primer is used or whether it is to be excluded. My5C.primers will not include a 5C primer for every possible restriction fragment due to the presence of repeats (typically 15-20% of fragments). My5C has pre-set thresholds that we have experimentally validated[1]. Using these thresholds less than 2% of primers typically end up giving data that has to be discarded later in the analysis. In our previously published work we had used no thresholds for primer design at all so that we could test performance of all primers. We found that certain primers that had low uniqueness scores yielded unreliable 5C data[1]. For my5C we have set default thresholds that would exclude primers with similar uniqueness scores to the ones that we had found to not yield reliable 5C data. It is important to point out that the user can alter the settings for acceptable repetitiveness allowing additional primers to be included in the design. Alternatively, the user can keep the default settings and select any

individual primer irrespective of its uniqueness to be included, *e.g.* when it corresponds to a critical restriction fragment of interest.

The quality of the 5C primers will affect the 5C data and therefore it is important to establish whether data obtained with these primers accurately reflect looping interactions, as compared to classical 3C. We have used 5C to analyze the human alpha- and beta-globin loci and the human IGF2 locus. These loci have previously been studied by 3C[1-5]. Our 5C data confirmed 100% of the loops detected by 3C (8 out of 8; data not shown) indicating a low false-negative rate. Estimates of false-positive rates cannot be given at this point as no true-negatives are known.

Researchers then assemble primer pools by combining sets of primers for different genomic regions that will be studied in a single 5C experiment. My5C.primers will check for any pairs of primers that can inappropriately form duplexes. These primers will be flagged and the user can decide to either include or exclude them.

Primer designs can be downloaded along with all other important information pertaining to the 5C project in a zip file. Importantly, my5C.primers also automatically designs a custom microarray probe set that can be used to detect all potential interactions that are detectable with the primer pool.

Upon obtaining experimental 5C interaction data (either by sequencing or by microarrays) researchers can return to my5C, upload their interaction data (using my5C.uploads) and explore it using my5C.heatmap which displays datasets as two-dimensional heatmaps with each datapoint corresponding to an unique interaction frequency between a fragment recognized by a 5C forward and reverse primer. An example is shown in Figure 1, which displays 5C data obtained by high-throughput sequencing for two 500 Kb regions: one on human chromosome 2 (ENCODE region Enr112 [6]) and one on human chromosome 13 (ENCODE region Enr112 [6]; see below for experimental for details). We note that my5C.heatmap can be used to visualize and analyze any chromatin interaction map, including those obtained with other methods.

5C typically generates very large interaction maps and the corresponding heatmaps can contain hundreds of thousands of datapoints, which complicates the ease with which users can visualize the data. Several features of my5C.heatmap facilitate the exploration of large 5C interaction maps, including the ability to zoom in at smaller genomic regions. Most importantly the heatmaps are fully interactive: when moving the cursor over the heatmap information is provided regarding the exact interaction at the cursor position. This information includes the detailed primer names and genomic positions of the interacting restriction fragments. When a specific interaction is clicked my5C.heatmap will display the signal strength and the complete interaction profiles across the entire dataset for each of the two interacting elements as line graphs.

Researchers may want to identify changes in chromosome conformation between different cells or conditions. my5C.heatmap contains various options that allow direct comparison of multiple datasets. First, for easy visual comparison two datasets can be displayed simultaneously. Second, users can display the difference, ratio or log ratio of any two datasets as heatmaps.

In order to identify specific long-range interactions my5C.heatmap enables users to identify elements that interact more frequently than expected for the level of background interactions. Background interactions are inversely proportional to the genomic distance between the interacting loci and reflect general chromatin fiber properties [7-9]. For each 5C dataset the average relationship between interaction frequency and genomic distance is automatically calculated using LOESS smoothing [10]. Based on this relationship an expected value can be calculated for each interaction. Users can display the ratio of observed and expected datasets and identify interactions that are significantly more frequent than expected, which may point to the presence of specific looping associations.

My5C.heatmap contains two options that are particularly useful for identifying higher order levels of chromosome organization. Using either raw or observed/expected values, users can smooth data along either axis of the heatmap (*e.g.* along forward or reverse primers) or both simultaneously. Users can also use sliding window analysis to convert 5C data into interaction maps that reflect interactions between genomic regions instead of interactions between individual restriction fragments. An example is shown in Figure 1c (right heatmap), which reveals some long-range interactions among distant regions, as is apparent by the clusters of increased interaction frequencies at some distance from the clear diagonal in the heatmap.

Finally, my5C.heatmap contains features that enable integrating chromosome conformation data with other genomic features. When a user clicks a position on the heatmap, links to the UCSC genome browser will appear that will lead the researcher to the corresponding positions in the relevant genome for rapid exploration of other publicly available annotations. Researchers can also upload a list of genomic annotations, e.g. transcription factor binding sites. My5C.heatmap will then identify which restriction fragments overlap any of these elements and then highlight 5C data obtained with these fragments in the heatmap using an alternative user-defined color scheme. Users also have the option to collapse the heatmap to display only interactions between these elements of interest.

Users can download any data that is displayed as a heatmap, whether this represents raw 5C datasets, ratios of two datasets, or windowed or smoothed data. Data can be exported as matrices in plain text files or as lists of pairwise interactions. The latter is useful as it can be uploaded into Cytoscape, a widely used and freely available software package for visualization and analysis of networks [11]. Perhaps most critically, data can also be downloaded in UCSC BED format. This file format can be uploaded to the UCSC genome browser for display of interaction data as multiple custom tracks in the genome browser (Figure 1e and Supplemental Data 11). This allows users to integrate their chromosome conformation data with the full set of publicly available genome annotations using all the tools available in the UCSC genome browser.

All 5C designs and all uploaded data are password protected to ensure that users can only access their own data. To further ensure confidentiality users can also opt not to store any primer designs or interaction data on the my5C server.

Description of the 5C analysis presented in Figure 1.

5C analysis of ENr112 and ENr132

5C primers were designed at *Hin*dIII sites using my5C.primers using an alternating primer design scheme (indicated in Figure 1b, top panel). Primers settings were: U-BLAST: 3; S-BLAST: 130: 15-MER: 1320; MIN_FSIZE: 40; MAX_FSIZE: 50000; OPT_TM: 65; OPT_PSIZE: 40. DNA sequence of the universal tails of Forward primers: CCTCTCTATGGGCAGTCGGTGAT; DNA sequence for the universal tails of reverse primers: AGAGAATGAGGAACCCGGGGCAG. In this particular design a 6 base barcode was included in between the specific part of the primers and the universal tail. This is currently not a standard feature of my5C. For ENr112 54 forward primers and 50 reverse primers were designed using an alternating design scheme (see Supplemental Data 12). For ENr132 we designed 10 reverse primers for restriction fragments overlapping transcription start sites, and 21 forward primers for all other restriction fragments in the region Supplemental Data 13).

3C was performed with *Hin*dIII as described by us before [1,12] using exponentially growing K562 cells. The conformation of ENr112 and ENr132 was analyzed as part of a larger 5C study that analyzed a total of 15 Mb of the human genome and that will be published elsewhere. 5C was performed in 20 reactions such that each contained an amount of 3C library that represents 200,000 genome equivalents and 0.5 fmol of each of 3,196 5C primers, including the set of 5C primers for ENr112 and ENr132. These primers interrogate a total of 1,397,979 pair-wise interactions. 5C ligation products were amplified using a pair of universal primers that recognize the common tails of the 5C forward and reverse primers (Primer 1: CCTCTCTATGGGCAGTCGGTGAT; primer 2: CTGCCCCGGGTTCCTCATTCTCT). To facilitate paired end DNA sequence analysis on the Illumina GA2 platform, Paired End adapters were ligated to the 5C library and further amplified for 18 cycles with the Illumina PCR Primer 1.0 and 2.0 using the Illumina PE protocol (Illumina manual 'Preparing Samples for Paired-End Sequencing, June 2008). The 5C library was then sequenced on the Illumina GA2 platform at the deep sequencing core at the University of Massachusetts Medical School. A single lane yielded 7,445,970 paired end reads of 36 bases each. A total of 4,919,991 paired end reads could be mapped back to pairs of forward and reverse 5C primers using Novoalign (www.novocraft.com).

This experiment yielded a total of 182,038 paired end reads for interactions in ENr112, and 58,326 paired end reads for interactions in ENr132. Interaction counts were uploaded to My5C and linked to specific primer sets for visualization in my5C.heatmap. These interactions are shown in Figure 1c. These datasets are also presented in Supplemental Data 9 and 10. The format of Supplemental Data 9 and 10 allows upload as a custom interaction dataset that is NOT linked to a specific primers set designed using my5C.primers.

References

1. Dostie, J. et al. Chromosome Conformation Capture Carbon Copy (5C): A Massively Parallel Solution for Mapping Interactions between Genomic Elements. *Genome Res.* 16, 1299-1309 (2006).
2. Vernimmen, D., De Gobbi, M., Sloane-Stanley, J. A., Wood, W. G. & Higgs, D. R. Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. *EMBO J.* 26, 2041-2051 (2007).
3. Tolhuis, B., Palstra, R. J., Splinter, E., Grosveld, F. & de Laat, W. Looping and Interaction between Hypersensitive Sites in the Active beta-globin Locus. *Mol Cell* 10, 1453-1465 (2002).

4. Murrell, A., Heeson, S. & Reik, W. Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. *Nat Genet.* 36, 889-893 (2004).
5. Kurukuti, S. et al. CTCF binding at the H19 imprinting control region mediates maternally inherited higher-order chromatin conformation to restrict enhancer access to Igf2. *Proc Natl Acad Sci U S A.* 103, 10684-10689 (2006).
6. ENCODE-consortium. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 447, 799-816 (2007).
7. Rippe, K. Making contacts on a nucleic acid polymer. *Trends Biochem Sci* 26, 733-740 (2001).
8. Dekker, J., Rippe, K., Dekker, M. & Kleckner, N. Capturing Chromosome Conformation. *Science* 295, 1306-1311 (2002).
9. Dekker, J. The 3 C's of Chromosome Conformation Capture: Controls, Controls, Controls. *Nat Methods* 3, 17-21 (2006).
10. Cleveland, W. S., Grosse, E. & Shyu, W. M. in *Statistical Models in S.* (eds. Chambers, J. M. & Hastie, T. J.) 309-376 (Chapman and Hall, London, 1993).
11. Shannon, P. et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13, 2498-2504 (2003).
12. Dostie, J. & Dekker, J. Mapping networks of physical interactions between genomic elements using 5C technology. *Nat. Protoc.* 2, 988-1002 (2007).

Supplemental Data 2
A tutorial describing in detail how to use my5C.primers. This tutorial can also be directly accessed through:
http://my5c.umassmed.edu/welcome/welcome.php?tab=primers

Supplemental Data 3
A tutorial describing in detail how to use my5C.uploads. This tutorial can also be directly accessed through:
http://my5c.umassmed.edu/welcome/welcome.php?tab=uploads

Supplemental Data 4
A tutorial describing in detail how to use my5C.heatmap. This tutorial can also be directly accessed through:
http://my5c.umassmed.edu/welcome/welcome.php?tab=heatmap

Supplemental Data 5
An example file in the format of a FASTA file that contains the DNA sequence and genomic information of a genomic region that users can upload to my5C.primers. The genomic region of this file corresponds to Enr112. The format of the file is as follows:
The file should contain a header line such as:

>hg18_dna range=chr2:51512209-52012208 5'pad=0 3'pad=0 strand=+ repeatMasking=none

This is the exact format UCSC outputs as DNA FASTA files.
Followed by the sequence:

AAAGAATACCCAGAAATGTGGAAGCAACCTTGGAACTGGGTAACAGACAG
AGGTTGGAGGAGTTTGGAGGCTTAGAAGAAGACAGAAAAATGTGGGAGAG

An example can be.
>hg18_dna range=chr2:51512209-52012208 5'pad=0 3'pad=0 strand=+ repeatMasking=none
AAAGAATACCCAGAAATGTGGAAGCAACCTTGGAACTGGGTAACAGACAG
AGGTTGGAGGAGTTTGGAGGCTTAGAAGAAGACAGAAAAATGTGGGAGAG
…
If you are using a DNA sequence not from the UCSC genome browser, you will only need to modify the header line to suit your exact region and include it as the first line of the FASTA file.

Supplemental Data 6
An example file for defining variable step sizes of alternating design schemes (arbitrarily chosen for Enr112). The format is as follows (tabbed delimited):

CHROMOSOME	START_POSITION	END_POSITION	NAME	SPACING_AMOUNT

SPACING_AMOUNT is a number field in BP amount.
20kb = 20000

An example is:

chr2	51512209	52012208	GLOBAL_40kb_spacing	20000
chr2	51612209	51912208	SEMI_20kb_spacing	10000
chr2	51712209	51852208	SPECIFIC_0kb_spacing	0

Supplemental Data 7
An example file with a list of genomic elements (arbitrarily chosen in Enr112) in the format required for upload in my5C.primers. Users can upload similar files describing elements of interest in order to design 5C primers for the overlapping restriction fragments.
The format is as follows (tabbed delimited)

CHROMOSOME	START_POSITION	END_POSITION	ELEMENT_NAME

An example is:

chr2	51512209	51522208	fake_gene1
chr2	51845055	51866223	fake_gene2

Supplemental Data 8
An example file for uploading interaction data to my5C.uploads linked to a primer pool.
You should use this option if you have a dataset generated by using a primer pool designed with the my5C.primers tool. This is the DEFAULT method of uploading data for most users.
The format is (tabbed delimited):

FORWARD_PRIMER_NAME	REVERSE_PRIMER_NAME	INTERACTION_COUNT

The primer names must match exactly to the names of the primers listed in the primer pool supplied in the my5C.primers zip file.

An example is:

5C_123_ENr112_FOR_73	5C_123_ENr112_REV_72	6171
5C_123_ENr112_FOR_62	5C_123_ENr112_REV_63	5233
5C_123_ENr112_FOR_26	5C_123_ENr112_REV_27	4629

This file would only correctly upload to primer pool exactly using the probe-set with ID# 123. The _123_ would change to whatever ID# your design is using (found in the output primer names).

The primer names should match exactly to the names of the primers listed in the primer pool supplied in the my5C.primers zip file. Sequencing reads can be mapped back directly to this primerpool FASTA file, yielding the correct primer names associated with each interaction pair. By using the name output from my5C.primers, all information regarding the specific fragment/primer can be referenced during upload.

NOTE: this file only serves as an example of this particular format and cannot be uploaded as there is no corresponding primer design in my5C.primers.

Supplemental Data 9
An example file for uploading interaction data to my5C.uploads linked to a *CUSTOM* primer pool. This is NOT the default method for uploading data. This method can be used for interaction data not created from a 5C design using my5C.primers. Any sort of interaction data can be used in this specified format, not just 5C data but also interaction data obtained with other methods.

This file describes 5C data we obtained for ENr112 in K562 cells, as described in the supplemental material. This file is a tab-delimited text file. This dataset corresponds to the data shown in figure 1. The numbers in this table are the DNA sequence counts that could be mapped to pairs of 5C primers for ENr112. The columns represent Reverse primers; the rows represent forward primers. The numbers correspond to the numbers of times a 5C ligation product of a specific pair of forward and reverse primers was sequenced. The names of the columns and rows (e.g. ENr112_FOR_2|hg18|chr2:51517721-51527793) indicate the primer name (ENr112_FOR_2); the genome that the primer recognized (hg18 represents the human genome assembly 18); and the chromosome number and genomic coordinates (chr2:51517721-51527793).

The format is (tab delimited):

Format will be a matrix of interactions, with headers attached to each row and column.
The format of the headers is:

NAME|ASSEMBLY|CHROMOSOME:STARTPOS-ENDPOS

An example is:
gene1|hg18|chr2:51517722-51527793

myData	gene1|hg18|chr2:51527935-51528740	gene2|hg18|chr2:51533038-51535102	gene3|hg18|chr2:51540236-51546080
re1|hg18|chr2:51517722-51527793	2739	292	261
re2|hg18|chr2:51528741-51533037	1305	3615	274
re3|hg18|chr2:51535103-51540235	43	679	1071

Supplemental Data 10
An example file for uploading interaction data to my5C.uploads linked to a *CUSTOM* primer pool. This is NOT the default method for uploading data. This method can be used for interaction data not created from a 5C design using my5C.primers. Any sort of interaction data can be used in this specified format, not just 5C data but also interaction data obtained with other methods.

This file describes 5C data we obtained for ENr132 in K562 cells, as described in the supplemental material. See legend of Supplemental Data 9 for details.

Supplemental Data 11
An example of data display in the UCSC genome browser. This is a TIFF file. The 5C data is for Enr112.

5C data for the Enr112 data (shown in Figure 2) was downloaded from My5C.iHeatmap in the BED format. This file format can be directly uploaded to the UCSC browser for display as a series of custom tracks in the browser. Each track displays an interaction profile (in the color scheme of My5C.iHeatmap) for a given restriction fragments (indicated as an orange bar).

Supplemental Data 12
DNA sequences of 5C primers used for analysis of the conformation of ENr112. This is the standard output of my5C.primers. This is a Tab-delimited text file. The columns display the following.

Column 1: Primer name. The name indicates whether the primer is Forward (FOR) primer or a Reverse primer (REV). The nomenclature is as follows: the name of the first forward primers is: 5C_123_ENr112_FOR_2. "5C_123" is a number that refers to the particular primer design in the MyPrimers database. "Enr112" is the name of the genomic region. "FOR_2" indicates that the primer is a forward primer and the number is the number of the *Hin*dIII fragment (numbered from the beginning of ENr112).

Column 2: Name of the genome region.

Column 3: Primer type (FOR = forward, REV = reverse).

Column 4: Genome assembly.

Column 5: The chromosome number the corresponding restriction fragment is on.

Column 6: Fragment_ID corresponds to the number of the restriction fragment, numbering starts at the beginning (5' end) of the genomic region.

Column 7: Primer_ID (1 or 2) corresponds to FOR and REV primers.

Column 8: Start position of the 5C primer (genomic coordinates).

Column 9: End position of the 5C primer (genomic coordinates).

Column 10: DNA sequence of the specific part of the 5C primer that anneals to the 3C library (see Figure 1).

Column 11: Length (bp) of the specific part of the primer.

Column 12: DNA sequence added to the 5' end of the specific part of Forward primers or 3' end of the specific part of reverse primers (filler sequence). This DNA sequence is added to equalize the length of all 5C primers.

Column 13: Length (bp) of the filler sequence shown in Column L.

Column 14: The melting temperature (Tm) of the specific part of the 5C primer.

Column 15: The GC percentage of the specific part of the 5C primers (sequence in column J).

Column 16: Start position of the corresponding restriction fragment (genomic coordinates).

Column 17: End position of the corresponding restriction fragment (genomic coordinates).

Column 18: Size of the corresponding restriction fragment (base pairs).

Column 19: ELEMENTID is a number that identifies any list of elements of interest the user had uploaded to MyPrimers and for which the specific 5C primer was designed.

Column 20: INTERSECTIONID is a number that identifies a specific element in the list of elements referenced in column S.

Column 21: E_NAME is the he name of the specific element (referred to in Column T) that has intersected with this fragment.

Column 22: The 15-mer frequency of the specific part of the primer plus the filler sequence. High 15-mer frequencies indicate a reduced uniqueness of the primer.

Column 23: BLAST count for the sequence of the primer containing the specific part + filler sequence (only 'exact' hits; exact means at least 20/23 bases align).

Column 24: BLAST count for the sequence of the primer containing the specific part + filler (exact+ similar hits; similar means any blast alignment).

Column 25: DNA sequence of the universal tail of the primer.

Column 26: Barcode sequence inserted at the 3' end of the universal tail (for Forward primers) or at the 5' end of the universal tail (for Reverse primers). Note that MyPrimers currently does not have the option to include barcodes. In this experiment 6-base barcodes were added to the 5C primers to facilitate mapping of DNA sequences. We have found that barcodes are not necessary and in the current version of myPrimers there is no option to include any barcodes in 5C primers.

Column 27: Barcode numerical code.

Column 28: Complete DNA sequence of the primer.

Supplemental Data 13
DNA sequences of 5C primers used for analysis of the conformation of ENr132. This is the standard output of my5C.primers. This is a Tab-delimited text file. See legend of Supplemental Data 12.

Supplemental Data 2
A tutorial describing in detail how to use my5C.primers. This tutorial can also be directly accessed through:
http://my5c.umassmed.edu/welcome/welcome.php?tab=primers

Dekker Lab Bioinformatics

my5C.primers manual

my5C.primers

my5C.primers is an online 5C tool for the rapid design of 5C primers. my5C allows complete control over extremely complex 5C design schemes.

Table of Contents

Getting Started

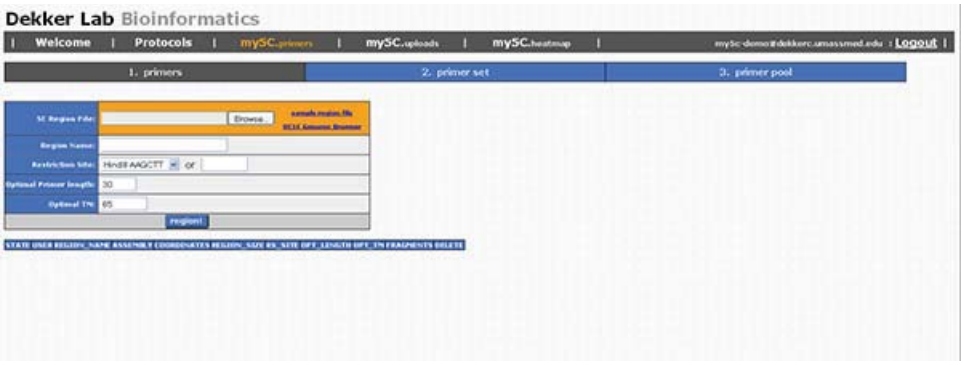
There are 3 main steps to 5C primer design.

- 1. Uploading a region of interest (ROI).
- 2. Creating a Primer Set(s).
- 3. Creating a Primer Pool.

There are options specific to your individual design at each step.
Here we will walk you through a simplified design process.

To start, click my5C.primers on the main menu of the website.

You should now see this screen.



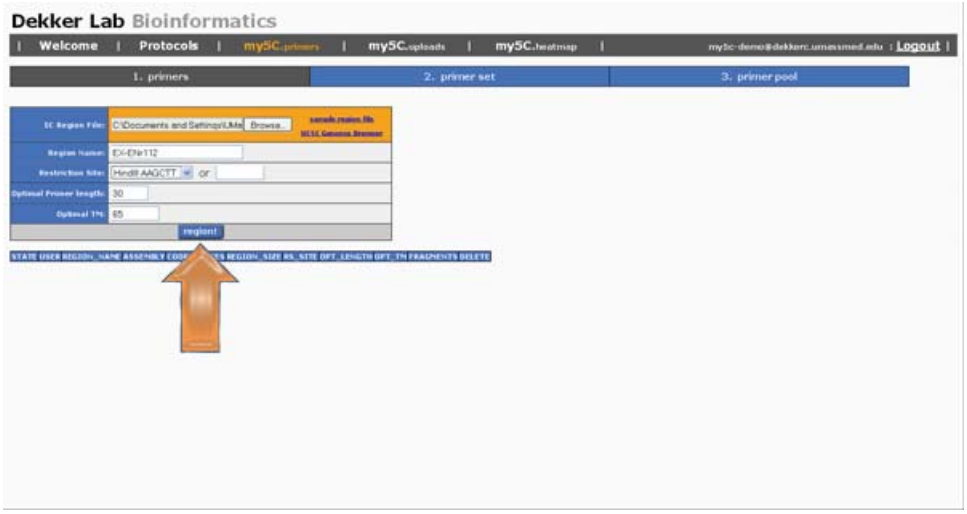
1. Uploading a region of interest (ROI).

You can now upload a UCSC FASTA format DNA file of your region of interest.
For this tutorial, we will use an ENCODE region as our sample region:
ENr112.
You can click the sample region or download the DNA from UCSC directly.

The UCSC FASTA header must be included in all uploaded files.

- 1. Browse to your region FASTA file.
- 2. Name your region.
- 3. Choose the restriction enzyme either from the dropdown, or insert a custom cutting sequence.
- 4. Choose your primer length.
- 5. Choose your optimal TM. (primers will be made as close to this TM as possible.)
    - TM is calculated using the 'Bre86' method.
    - Tm calculation based on Nearest Neighbor Thermodynamics.
    - 'Bre86': Breslauer et al. (1986) Proc. Natl. Acad. Sci. USA 83, 3746-3750.

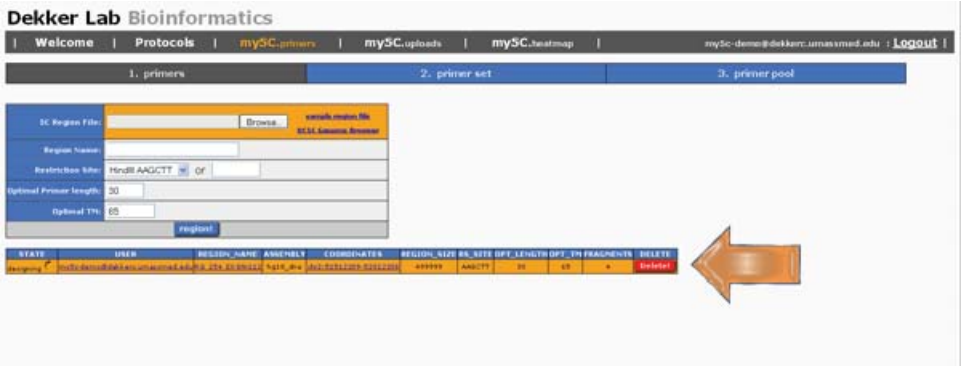
Once you have entered all the necessary information, click the *region!* button.



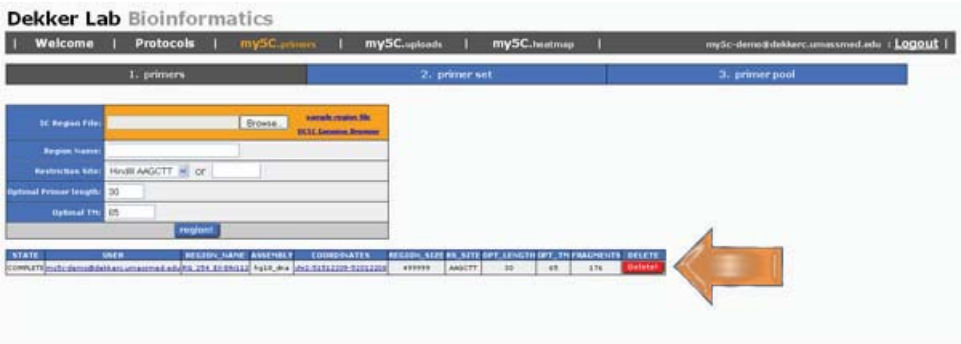
Primers are now being designed for your region in the background.

Both a forward and a reverse primer are designed for each individual restriction fragment and all statistics (TM,GC,BLAST etc) are calculated auton
In the next step you will narrow down your primers by selecting primers for specific fragments that interest you.

While your region is still in the design state, the entire row will turn orange.
If you refresh the page, you should see the number of FRAGMENTS slowly increase.
On average, a 1MB region takes ~3 minutes to design.



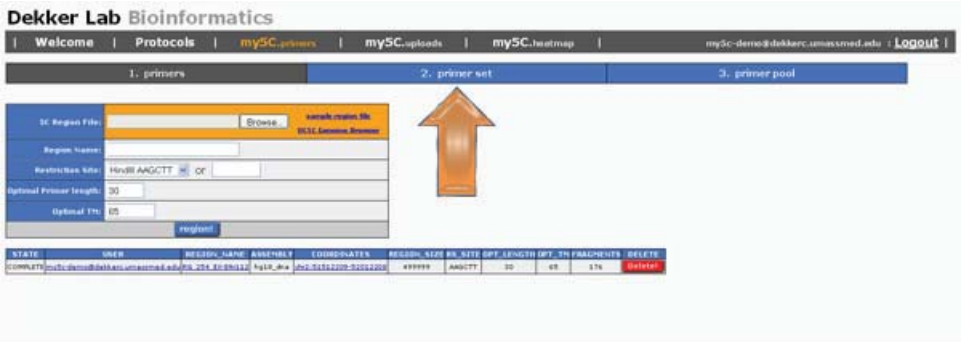
Once your design is complete, the row should turn grey, and the state should turn to **COMPLETE**.



You can now move onto step 2. Creating a Primer Set(s).

2. Creating a Primer Set(s).

Switch over to the primer set tab by clicking the **2. primer set** button from the my5C.primer menu



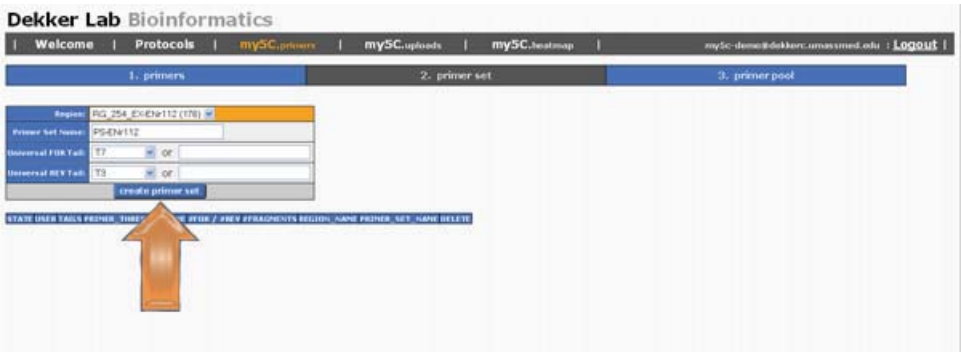
You should now see this screen.

Now you must create a **Primer Set** out of the primers you just designed.
A primer set is the filtered collection of primers that you plan to use during the 5C experiment.

- 1. Select the region you just designed primers for from the drop down.
- 2. Name your Primer Set.
- 3. Choose a forward PCR tail from the dropdown, or enter in a custom sequence (advanced users only).

- 4. Choose a reverse PCR tail from the dropdown, or enter in a custom sequence (advanced users only).

Once you have entered all the necessary information, click the *create primer set!* button.

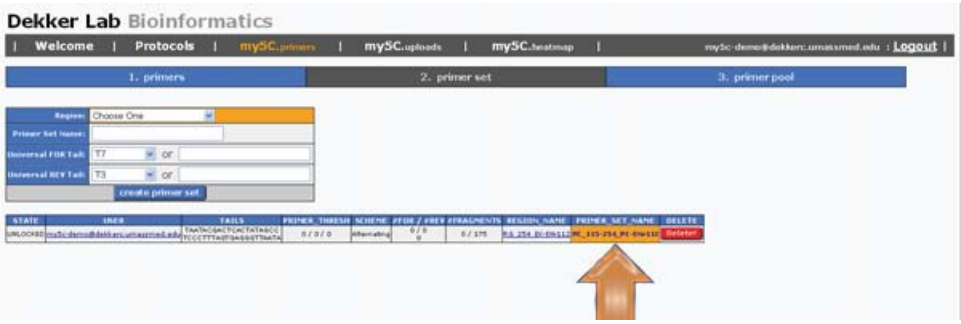


Your new Primer Set has now been created. Now we must go in and apply a primer layout.

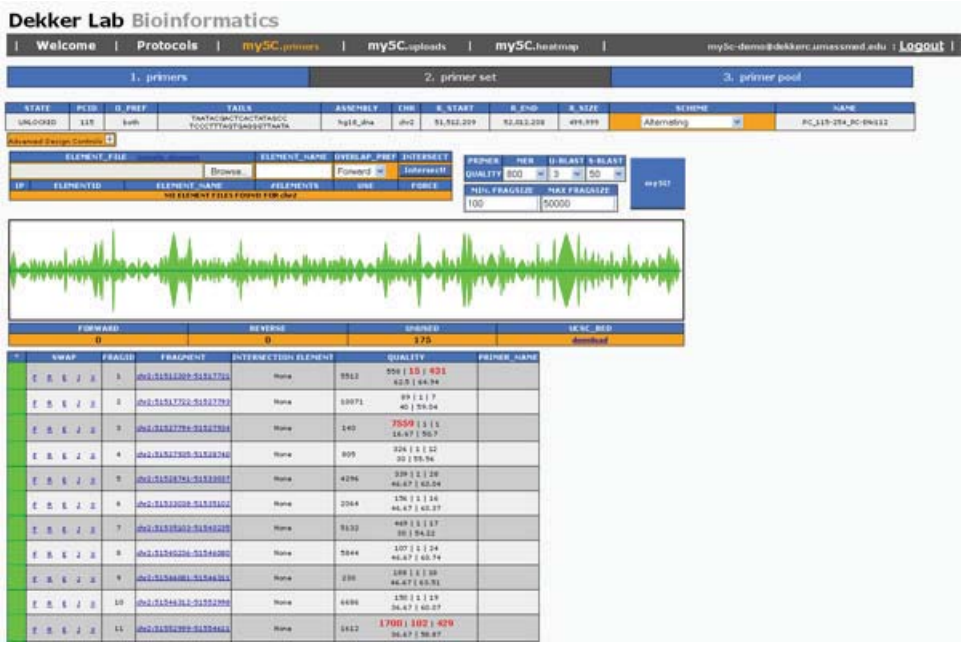
You will have the option to make any fragment with the region, either a forward or reverse primer for use in the 5C experiment. Initially, no fragments are made forward|reverse.

Click the orange box with your Primer Set name, to edit the primer set.

You will have the option to select either a forward or a reverse primer for any restriction fragment in the ROI. Initially, there is no prior selection of forward of reverse primers.



You should now be taken to the primer layout page for your primer set.



Main UI

This UI has 3 main parts.

- First the upper control section, containing all primer design options.
- Second the main primer layout image. Triangle Plot describing current layout.
- Third the individual fragment listing.

Control Section

Primer Layout Plot

Fragment Listing

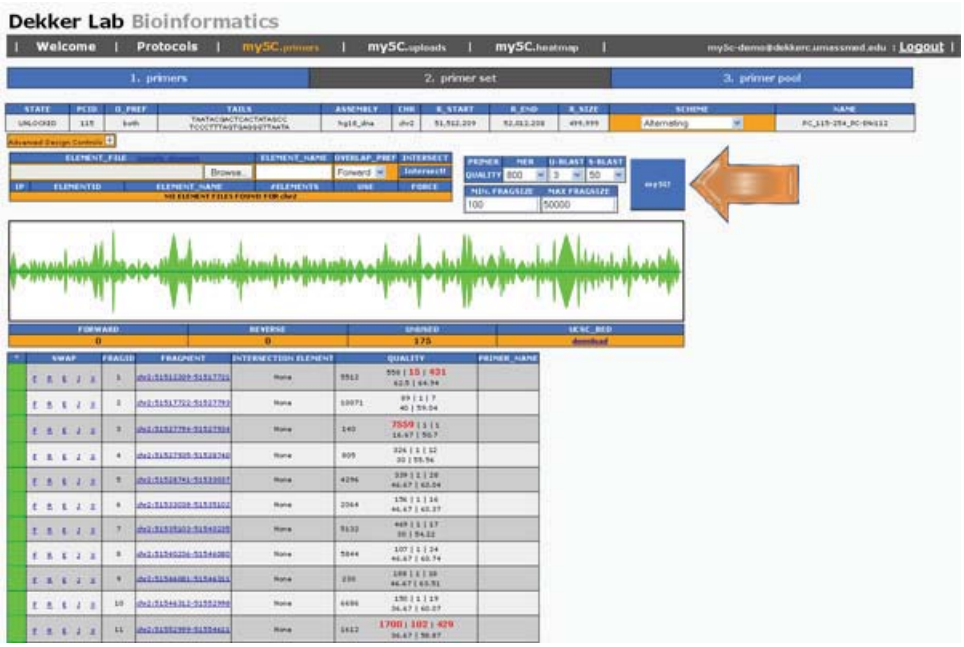
Control Section

There are a variety of options on this page.

- SCHEME: The scheme dropdown is your main control for your primer layout.
  - Alternating - Alternating forward and reverse design. Primer type switches on each sequential usable fragment.
  - Element vs Unknown - All usable fragments intersecting with a set of known 'elements' are made one primer type. Every other usable is made the other primer type.
  - Element vs Element - All usable fragments intersecting with a set of known 'A-elements' are made one primer type. All usable fragme intersecting with a set of known 'B-elements' are made the other primer type.
- ELEMENTS: You can upload 'element' files here.
- QUALITY: Repetitiveness thresholds for usable primer selection.
  - MER - Each 15MER in the primer is summed for # of genome-wide occurence. Then all 15MER counts are summed.
  - U-BLAST - A stringent blast only yielding near perfect alignments.
  - S-BLAST - A less stringent blast counting all similar alignments.
- MIN. FRAGSIZE - The minimum fragment size that is allowed to be considered usable.
- MAX. FRAGSIZE - The maximum fragment size that is allowed to be considered usable.

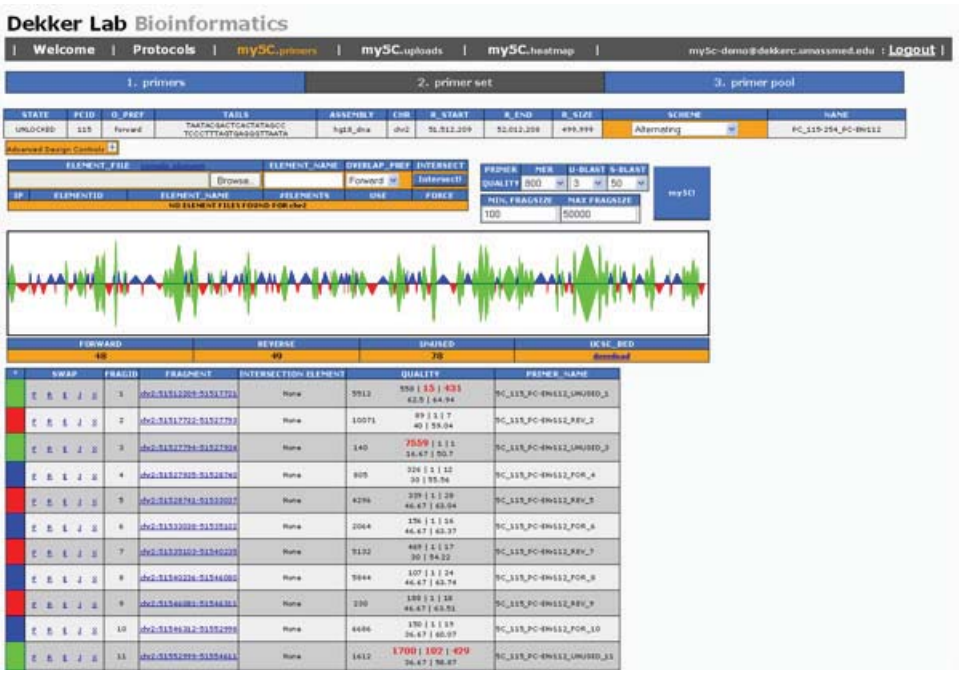
Initially, there is no design imposed on your region.

To create an 'alternating' design using all default values/threshoholds, make sure Alternating is selected in the scheme dropdown click my5C!

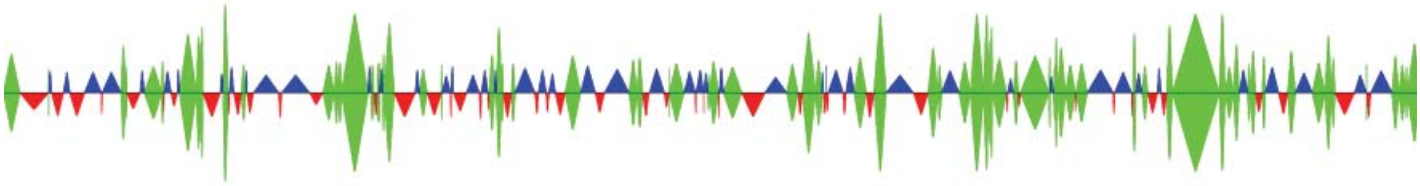


An Alternating design is now being calculated for your region.
All primers are first filtered by the quality scores and fragment sizes, and primers above the selected thresholds are excluded.
An alternating design is then imposed upon the region for all remaining, usable fragments.

Your output should now look like this:



Primer Layout Plot



Each triangle represents a restriction fragment in your region.
Triangles have 3 characteristics

- Width - corresponds to the size of the restriction fragment in bp.
- Height - corresponds to the repetitiveness of the primer. (small=not repetitive, big=very repetitive)
- Color - corresponds to type of primer used on restriction fragment.
    - Blue - means a forward primer was used on this restriction fragment.
    - Red - means a reverse primer was used on this restriction fragment.
    - Green - means no primer was used on this restriction fragment. (failed a quality test)

Primer Quality

- QUALITY: Repetitiveness thresholds for usable primer selection.
    - MER - Each 15MER in the primer is summed for # of genome-wide occurence. Then all 15MER counts are summed.
    - U-BLAST - A stringent blast only yielding near perfect alignments.
    - S-BLAST - A less stringent blast counting all similar alignments.
- MIN. FRAGSIZE - The minimum fragment size that is allowed to be considered usable.
- MAX. FRAGSIZE - The maximum fragment size that is allowed to be considered usable.

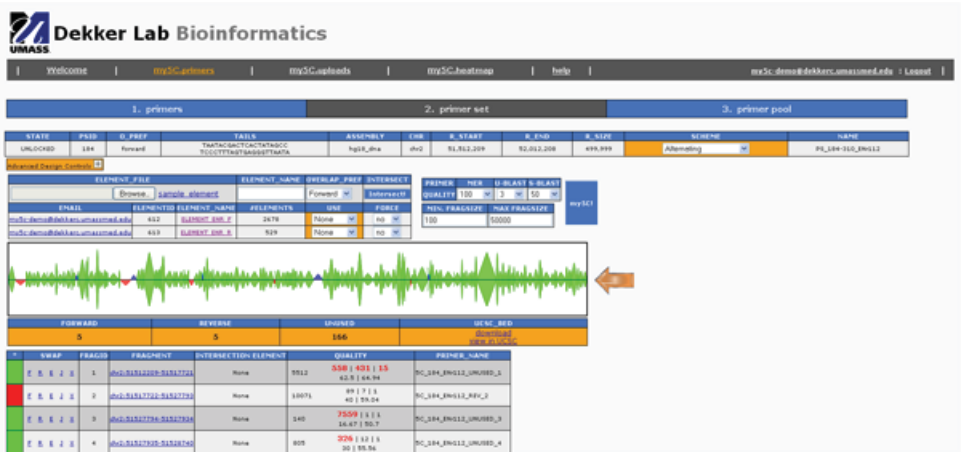
You have complete control over all primer quality thresholds.

We recommend using the default values as they have been experimentally tested to work best.

- 15MER
  - The 15MER value is a threshold for the total genome-wide occurrences of all consecutive 15MERS in each primer.
  - i.e. meaning that if 15 complementary bases are enough for a primer to find a target, how many possible 15MER targets does each p have.
- S-BLAST
  - The S-BLAST value is a threshold for how many similar blast hits each primer is allowed.
  - Similar blast hits are scored as > 2 mismatches and < (primer_length/2)
- U-BLAST
  - The U-BLAST value is a threshold for how many unique blast hits each primer is allowed.
  - Unique Blast hits are scores as any hit with up to 1 mismatch

By having 3 measures for primer repetitiveness, we try to account for all advantages/disadvantages of each technique.

Change the MER value from its default to a small value, such as 100.
Then re-calculate the design, press *my5C*



Note how few primers actually pass the set thresholds.

Experiment with different thresholds to use the most appropriate filtering parameters for your experiment.

Fragment Listing

FORWARD	REVERSE	UNUSED	UCSC_BED
48	49	78	download

*	SWAP	FRAGID	FRAGMENT	INTERSECTION ELEMENT	QUALITY		PRIMER_NAME
	F R E J X	1	chr2:51512209-51517721	None	5512	558 | 15 | 431 62.5 | 64.94	5C_115_PC-EN112_UNUSED_1
	F R E J X	2	chr2:51517722-51527793	None	10071	89 | 1 | 7 40 | 59.04	5C_115_PC-EN112_REV_2
	F R E J X	3	chr2:51527794-51527934	None	140	7559 | 1 | 1 16.67 | 50.7	5C_115_PC-EN112_UNUSED_3
	F R E J X	4	chr2:51527935-51528740	None	805	326 | 1 | 12 30 | 55.56	5C_115_PC-EN112_FOR_4
	F R E J X	5	chr2:51528741-51533037	None	4296	339 | 1 | 28 46.67 | 63.04	5C_115_PC-EN112_REV_5
	F R E J X	6	chr2:51533038-51535102	None	2064	156 | 1 | 16 46.67 | 63.37	5C_115_PC-EN112_FOR_6
	F R E J X	7	chr2:51535103-51540235	None	5132	469 | 1 | 17 30 | 54.22	5C_115_PC-EN112_REV_7
	F R E J X	8	chr2:51540236-51546080	None	5844	107 | 1 | 24 46.67 | 63.74	5C_115_PC-EN112_FOR_8
	F R E J X	9	chr2:51546081-51546311	None	230	188 | 1 | 18 46.67 | 63.51	5C_115_PC-EN112_REV_9
	F R E J X	10	chr2:51546312-51552998	None	6686	150 | 1 | 19 36.67 | 60.07	5C_115_PC-EN112_FOR_10
	F R E J X	11	chr2:51552999-51554611	None	1612	1700 | 102 | 429 36.67 | 58.87	5C_115_PC-EN112_UNUSED_11
	F R E J X	12	chr2:51554612-51554626	None	14	692 | 1 | 34 33.33 | 58.46	5C_115_PC-EN112_UNUSED_12
	F R E J X	13	chr2:51554627-51554891	None	264	1096 | 1 | 16 40 | 61.19	5C_115_PC-EN112_UNUSED_13
	F R E J X	14	chr2:51554892-51559483	None	4591	129 | 1 | 4 36.67 | 59.1	5C_115_PC-EN112_REV_14
	F R E J X	15	chr2:51559484-51560996	None	1512	192 | 1 | 27 30 | 57.38	5C_115_PC-EN112_FOR_15
	F R E J X	16	chr2:51560997-51567157	None	6160	1086 | 1 | 26 30 | 55.29	5C_115_PC-EN112_UNUSED_16

Each fragment is listed in a table format below the primer layout image.
For each fragment the following fields are listed:

- * - a color coded box showing which class the fragment is in (forward,reverse or unused)
- SWAP - 5 buttons that allow override control over any specific fragment.
  - F - force fragment to forward (overrides all other controls)
  - R - force fragment to reverse (overrides all other controls)
  - E - force fragment to either (overrides all other controls). (makes the fragment either a forward or a reverse, depending on SCHEME
  - J - force fragment to junk (unused) (overrides all other controls)
  - X - remove any forcing constraints imposed on fragment if one exists.
- FRAGID - Lists the fragment number as it is found in the region. (starts at 1)
- FRAGMENT - Lists the chromosome, start / end position of the fragment. This is a clickable link to UCSC genome browser.
- INTERSECTION_ELEMENT - Lists any ELEMENTS intersecting with the fragment, if an ELEMENT list was used.
- QUALITY - Lists quality scores of primer.
  - **Any number highlighted in red means that characteristic is above the current quality thresholds.**
  - First number = fragment size
  - Second number, top row = 15MER count
  - Third number, top row = S-BLAST count
  - Fourth number, top row = U-BLAST count
  - Second number, bottom row = GC %
  - Third number, bottom row = TM
- PRIMER_NAME - a unique primer name referencing the fragment / primer set.

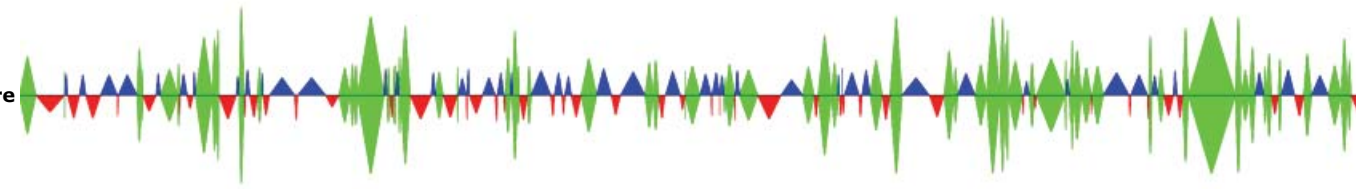
For example. If you press the **R** button on fragment # 13...

FORWARD	REVERSE	UNUSED	UCSC_BED
49	49	77	download

*	SWAP	FRAGID	FRAGMENT	INTERSECTION ELEMENT	QUALITY		PRIMER_NAME
	F R E J X	1	chr2:51512209-51517721	None	5512	558 | 15 | 431 62.5 | 64.94	5C_115_PC-EN112_UNUSED_1
	F R E J X	2	chr2:51517722-51527793	None	10071	89 | 1 | 7 40 | 59.04	5C_115_PC-EN112_REV_2
	F R E J X	3	chr2:51527794-51527934	None	140	7559 | 1 | 1 16.67 | 50.7	5C_115_PC-EN112_UNUSED_3
	F R E J X	4	chr2:51527935-51528740	None	805	326 | 1 | 12 30 | 55.56	5C_115_PC-EN112_FOR_4
	F R E J X	5	chr2:51528741-51533037	None	4296	339 | 1 | 28 46.67 | 63.04	5C_115_PC-EN112_REV_5
	F R E J X	6	chr2:51533038-51535102	None	2064	156 | 1 | 16 46.67 | 63.37	5C_115_PC-EN112_FOR_6
	F R E J X	7	chr2:51535103-51540235	None	5132	469 | 1 | 17 30 | 54.22	5C_115_PC-EN112_REV_7
	F R E J X	8	chr2:51540236-51546080	None	5844	107 | 1 | 24 46.67 | 63.74	5C_115_PC-EN112_FOR_8
	F R E J X	9	chr2:51546081-51546311	None	230	188 | 1 | 18 46.67 | 63.51	5C_115_PC-EN112_REV_9
	F R E J X	10	chr2:51546312-51552998	None	6686	150 | 1 | 19 36.67 | 60.07	5C_115_PC-EN112_FOR_10
	F R E J X	11	chr2:51552999-51554611	None	1612	1700 | 102 | 429 36.67 | 58.87	5C_115_PC-EN112_UNUSED_11
	F R E J X	12	chr2:51554612-51554626	None	14	692 | 1 | 34 33.33 | 58.46	5C_115_PC-EN112_UNUSED_12
	F R E J X	13	chr2:51554627-51554891	None	264	1096 | 1 | 16 40 | 61.19	5C_115_PC-EN112_REV_13
	F R E J X	14	chr2:51554892-51559483	None	4591	129 | 1 | 4 36.67 | 59.1	5C_115_PC-EN112_FOR_14
	F R E J X	15	chr2:51559484-51560996	None	1512	192 | 1 | 27 30 | 57.38	5C_115_PC-EN112_REV_15
	F R E J X	16	chr2:51560997-51567157	None	6160	1086 | 1 | 26 30 | 55.29	5C_115_PC-EN112_UNUSED_16

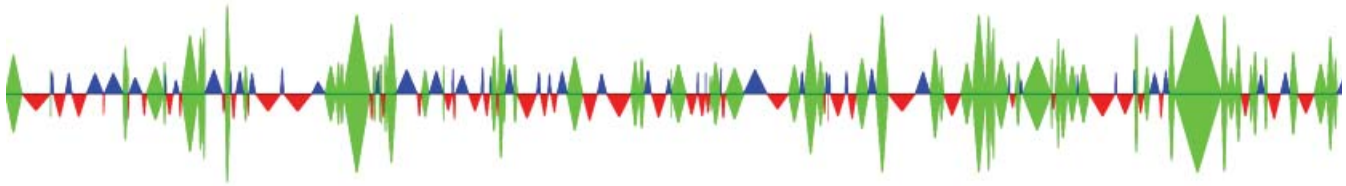
Fragment #13 is now forced to a reverse primer, regardless of its quality/size.
You will notice the entire row turns orange and the **R** button is highlighted to show it has been clicked.

Before



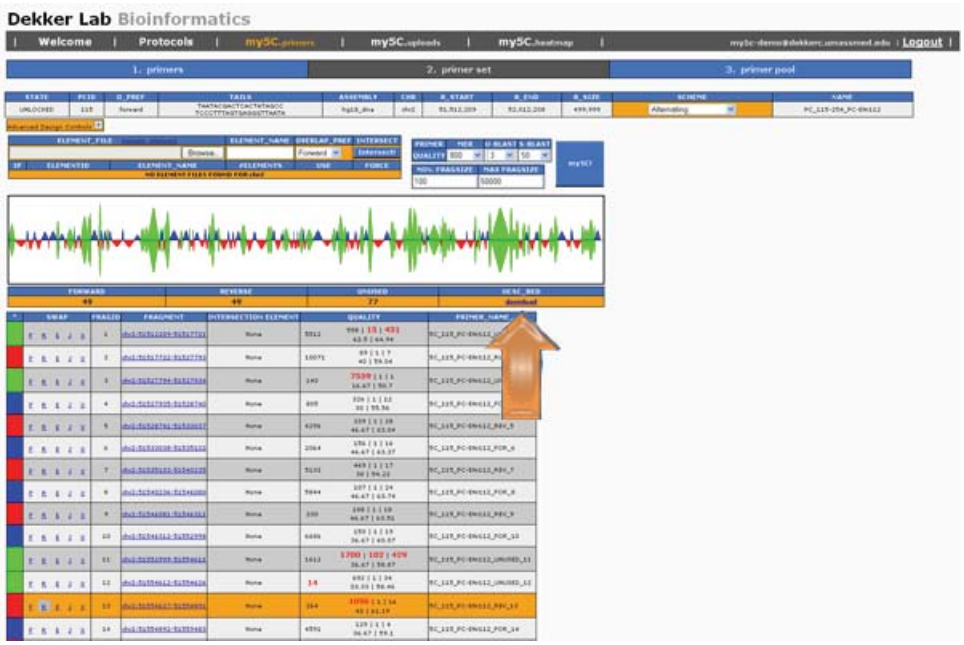
Also note the change in the alternating design; primers are rearranged to keep a true alternating layout.

After



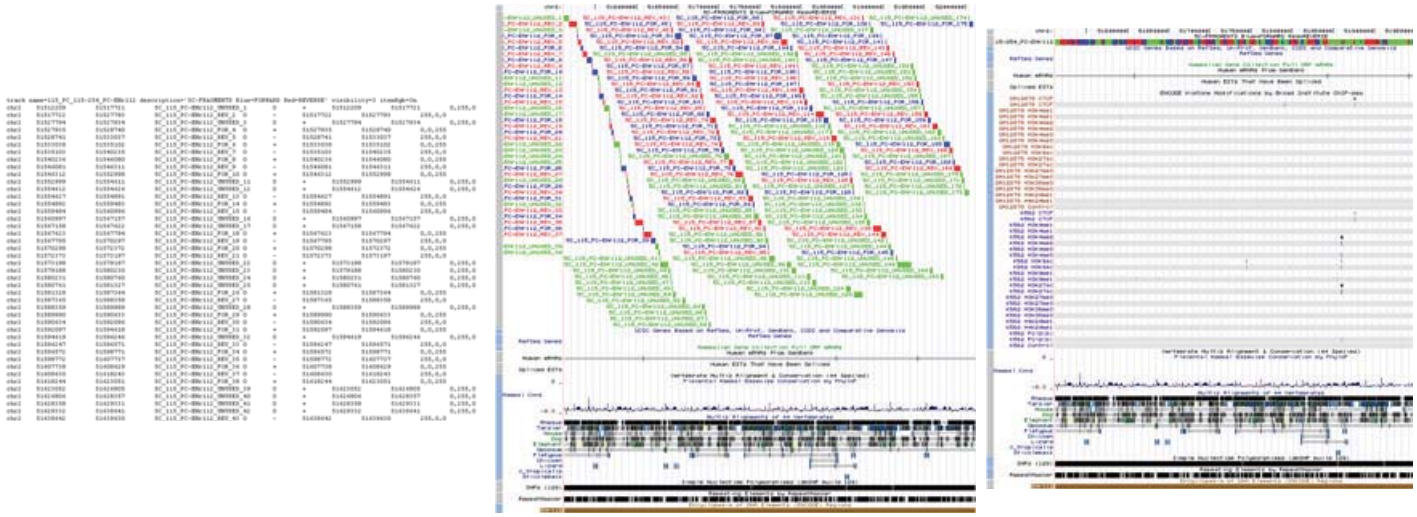
UCSC bed

If you click the bed file button, a bed file containing all fragments/primers within your region will be made available.



As you make changes to your design, this file is automatically updated to reflect the most recent layout.
Load this BED file into the genome browser to obtain the below result.
This can be used as an aid in the initial design and layout.

BED file **UCSC - pack** **UCSC - dense**



This sample design is now complete.

Advanced Design

Zoom

- Zoom - This field can be used to 'zoom' in on a region.
  - i.e. You can load a 1MB region for 5C design.
  - Then only design primers on a subset of that region by specifying a zoom parameter.
  - Zoom parameter is of the form: chrN:start-end (i.e. chr2:51712209-51912208)

Using zoom - chr2:51712209-51912208

Variable Step

- Variable Step - Sometimes for alternating designs, you would like to space your primers by some BP spacing.
  - i.e. You can perform an alternating design @ a 20kb resolution.
  - Meaning a primer will be placed in an alternating fashion every 20kb using a best-fit algorithm.

You can also specify multiply spacing constraints throughout the entire region of various bp sizes.
For instance you can give the entire region a 20kb spacing.
Then give a smaller subset of the region a 10kb spacing.
Then specify yet a smaller subset of the region to have a 0kb spacing (meaning a normal alternating).

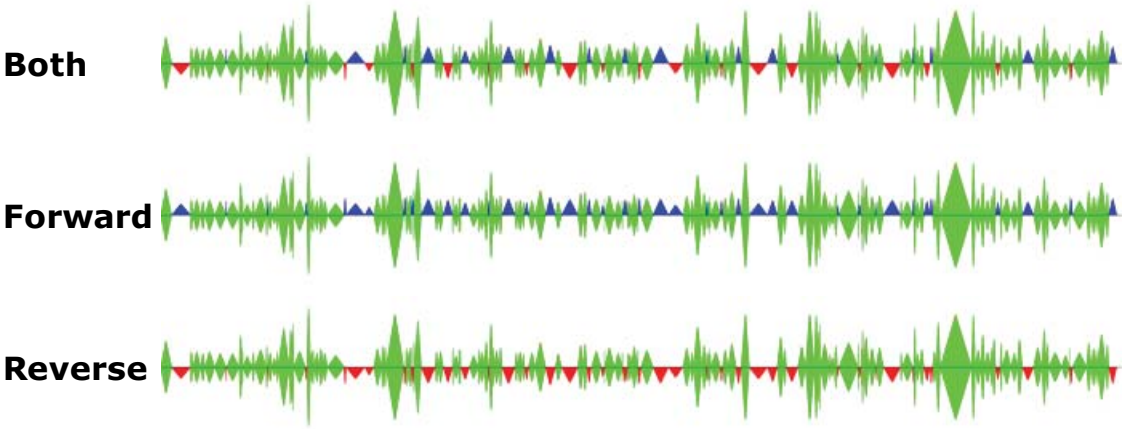
If there is any overlap between the spacing regions, the smallest available spacing size will be used.

Using the sample spacing file, you can produce the following results.

```
chr2    51512209    52012208    GLOBAL_40kb_spacing    20000
chr2    51612209    51912208    SEMI_20kb_spacing      10000
chr2    51712209    5185208     SPECIFIC_0kb_spacing   0
```

Format is tabbed delimited (chromosome - start - end - name - spacing(in bp))

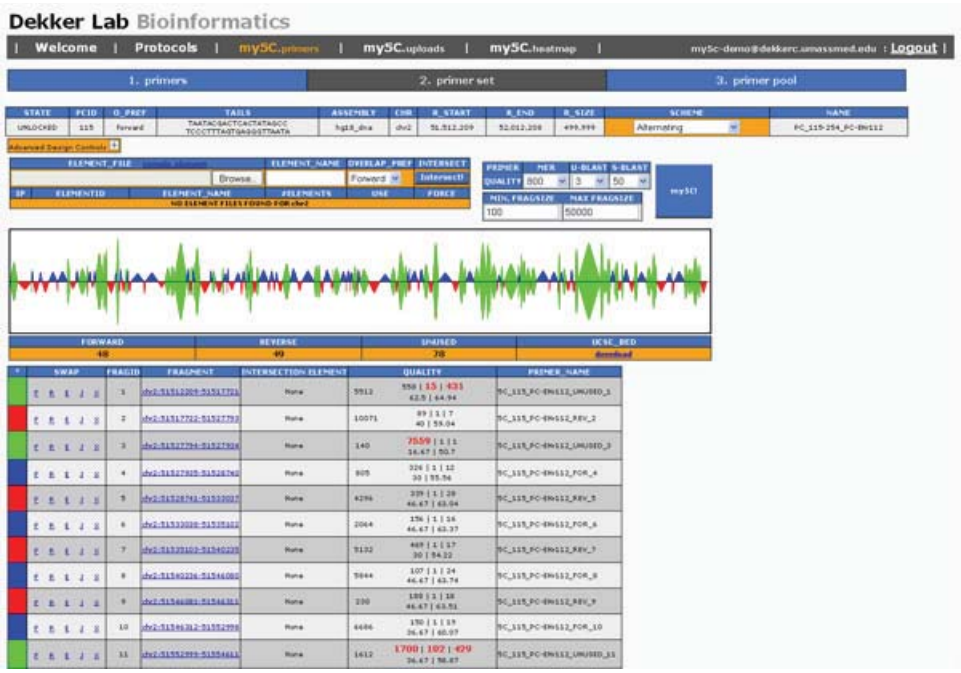
You can also specify the spacing to forwards only, reverse only, or in an alternating fashion (both) by using the dropdown.



Elements

- Element - A way to specify a set of elements to act as a specific primer type.
  - i.e. You can specify a set of elements (GENES), and make all intersecting fragments reverse primers.
  - You can then do one of two options.
    - Use an Element vs Unknown scheme to detect all interactions between genes and every other fragment in the region.
    - Use an Element vs Element scheme to detect the interactions between two sets of elements
    - i.e. genes vs some binding site.

Starting again with a normal alternating scheme:

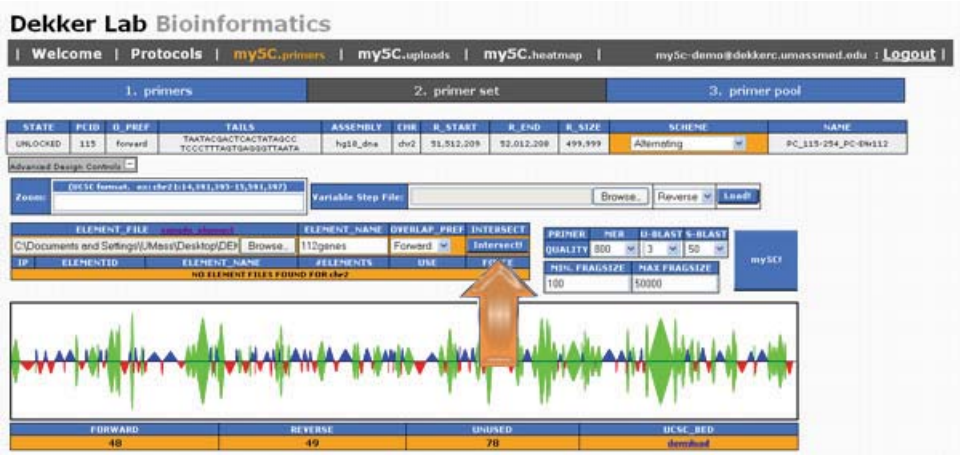


We will now upload an ELEMENT list using the sample element file sample element

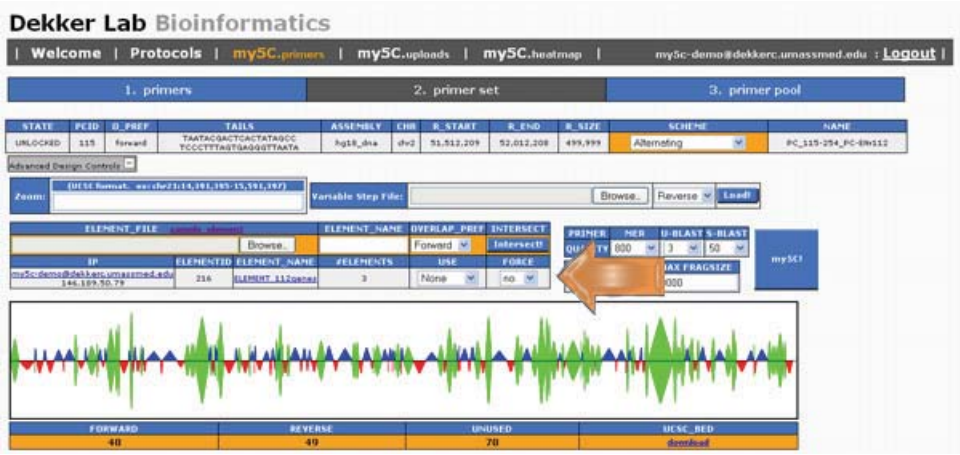
```
chr2    51512209        51522208        fake_gene1
chr2    51845055        51866223        fake_gene2
chr2    51597610        51612209        fake_gene3
```

Format for this file is 4 columns, tab delimited.
chromosome - start_position - end_position - name

Browse to your element file, enter an ELEMENT_NAME and press the intersect button.



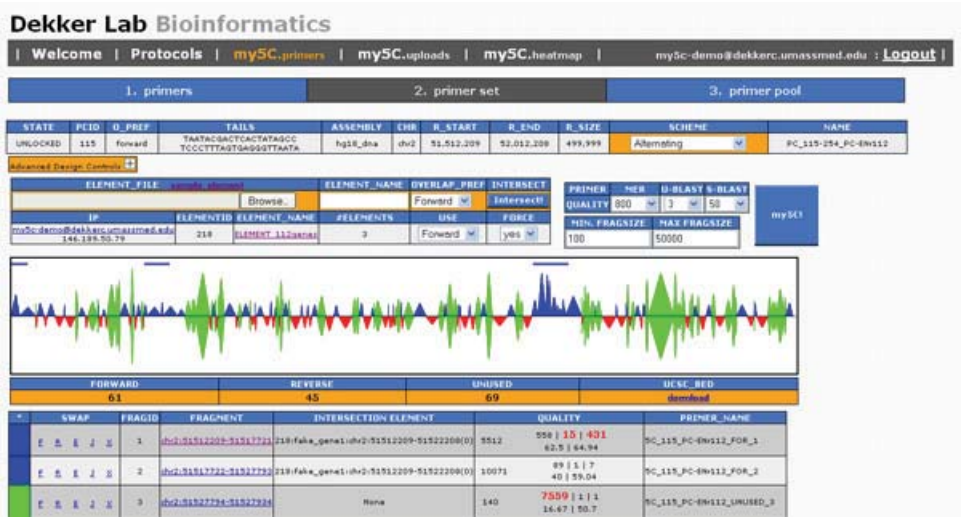
Any loaded element files that intersect with your current region will now be available in this new table.



To intersect your region with this set of elements, turn the USE column dropdown to Forward and click my5C!
Your design should now look like this:

Any usable fragments that intersect with your set of elements are made forward.
Also a blue bar representing the 'element' is now drawn on the primer layout plot.

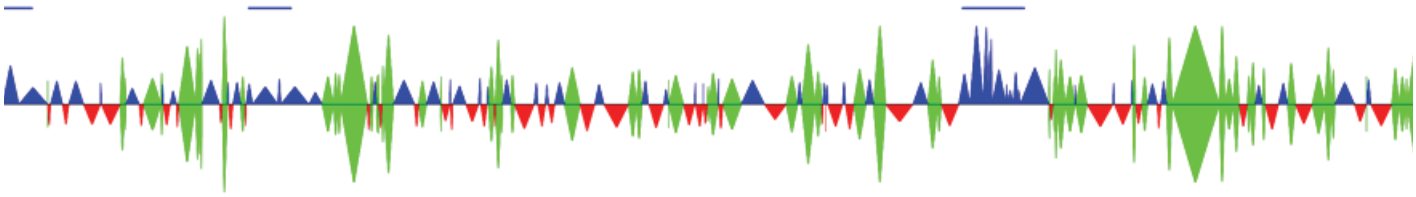
You may notice the * column in fragment #1 has turned yellow.
A yellow box means the fragment is being excluded due to filtering, however the fragment intersects with one of your element lists.
If you change the FORCE column to 'yes', any fragment overlapping with one of the 'elements' will be used regardless of any filtering.
Output should now look like:



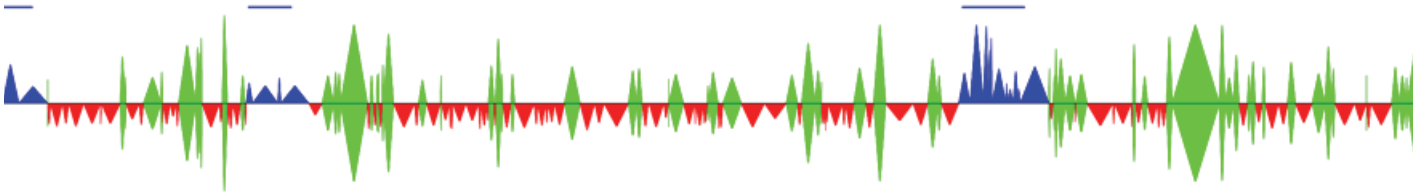
Design Schemes

You can also experiment by changing the SCHEME dropdown in the main control section

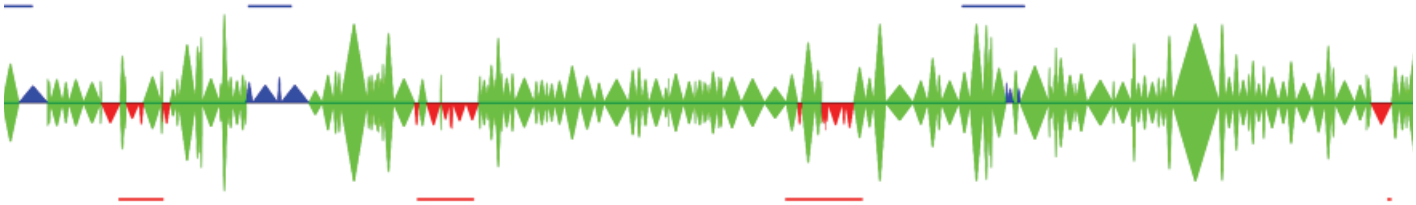
Forward Element (force) + Alternating



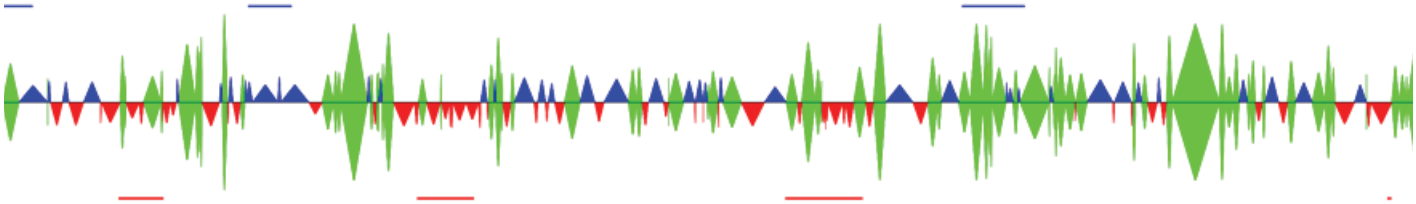
Forward Element (force) + Element vs. Unknown



Forward Element & Reverse Element + Element vs. Element



Forward Element & Reverse Element + Alternating



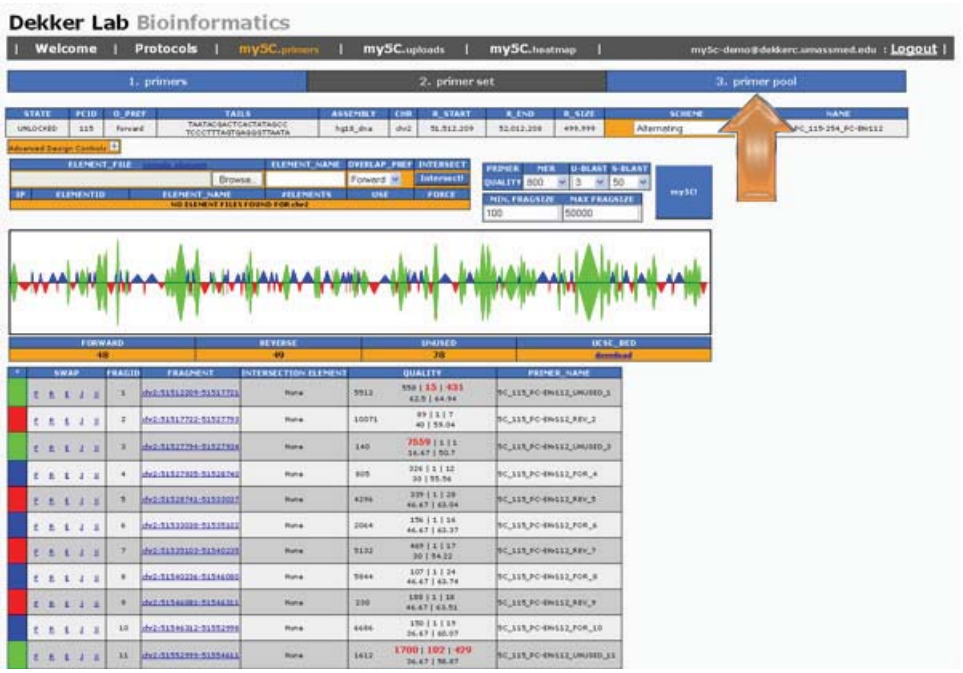
Please Note: You will need to load a new element list and use REVERSE, for an element vs. element design to be used properly.

Any combination of the above advanced controls can be used together yielding endless possibilities to the 5C design process

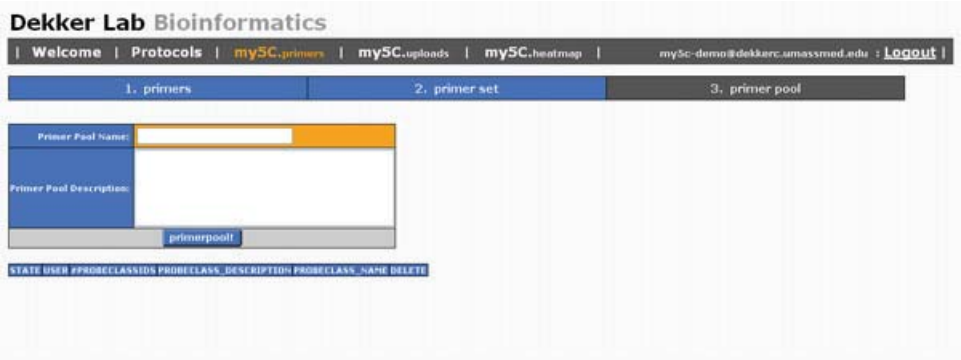
3. Creating a Primer Pool

When you are satisfied with the primer layout, the primer set design phase is complete and a primer pool can be created next.
A primer pool is a collection of primer sets that will be used in the 5C experiment.
This usually contains 1 or more main ROI and 1 control region, usually a gene desert region that should have no specific interactions

Switch over to the primer pool tab by clicking the **3. primer pool** button from the my5C.primer menu

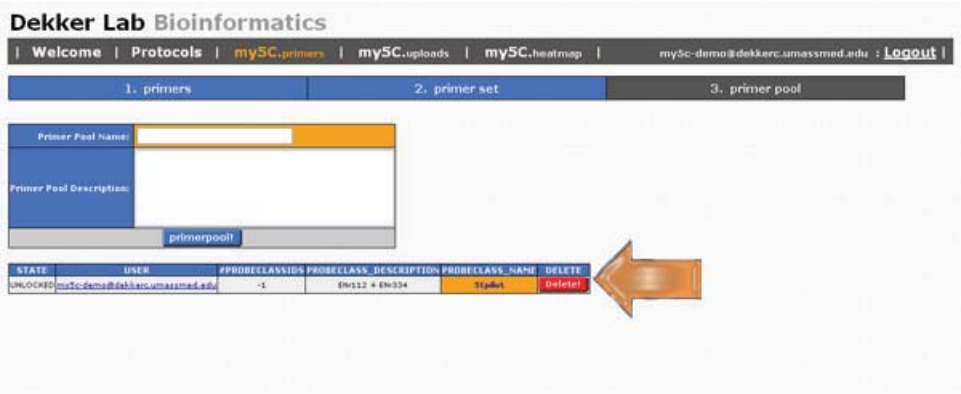


You should now be here

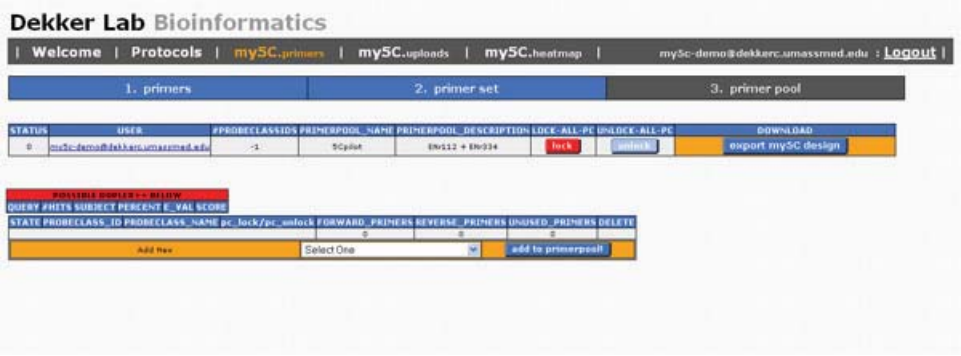


Enter a Primer Pool Name and a Primer pool description, then press **primerpool!**

Your newly created primerpool will be displayed below.
Click on the orange box containing the primer pool name to edit the primer pool, and add multiple primer sets to the pool.
Be aware that only primer sets with the same forward and reverse PCR tails can be added to one primer pool.

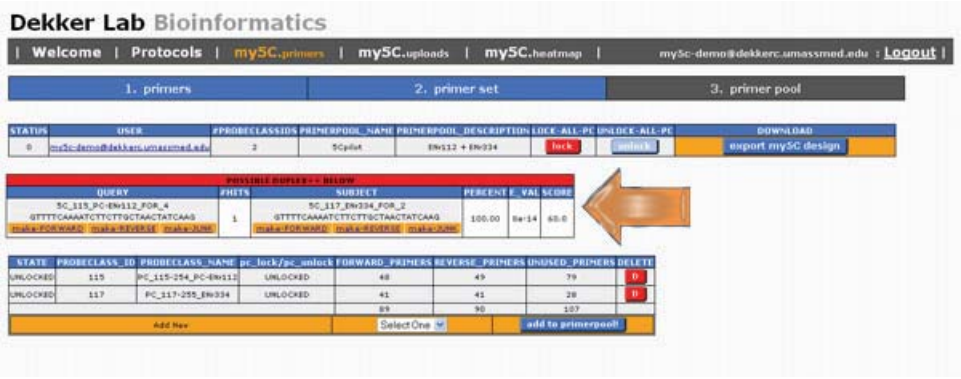


You will then be taken here:



By using the **Add New** dropdown, select the primer sets you wish to add to this primer pool.

Select each primerset, then press **add to primerpool!** The result should be similar to:

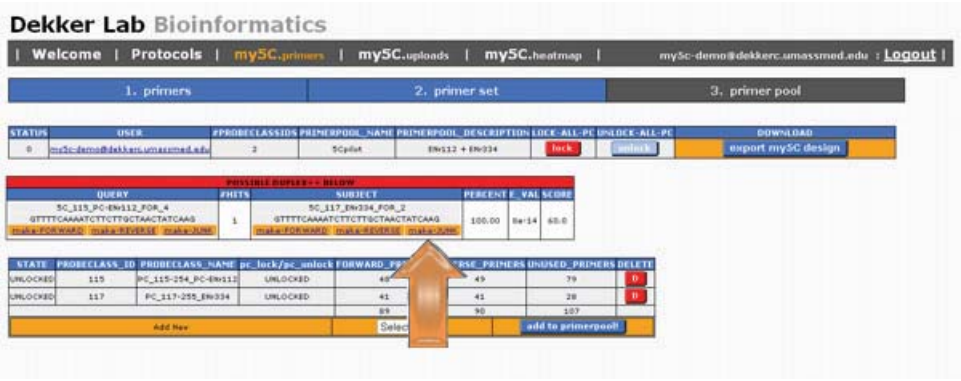


Duplex Filtering

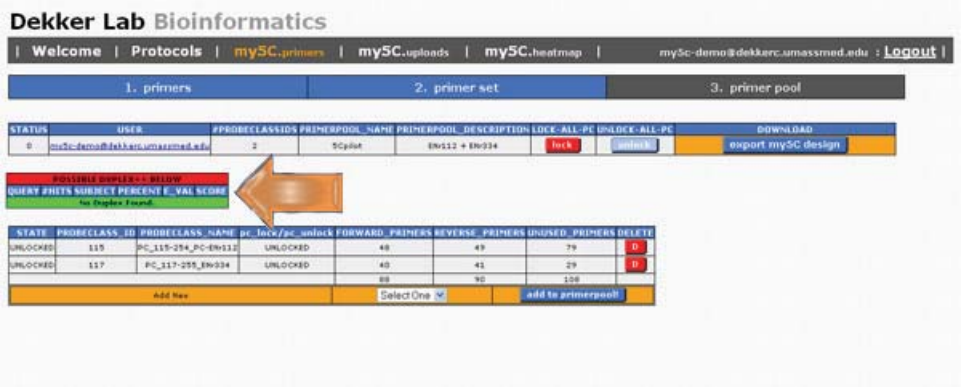
As you add new primer sets to the primerpool, the primerpool is checked for any duplexes that may exist between any primers in the pool.
If any are found, they will be displayed in the **POSSIBLE DUPLEX++** table.
You then have the option to either:

- Make one of the culprits *forward*
- Make one of the culprits *reverse*
- Make one of the culprits *junk*

It is not mandatory to resolved the duplexes found, however it is strongly advised to free the pool of these duplexes
Clicking here, will make this specific primer junk, thus purging the pool of this duplex.

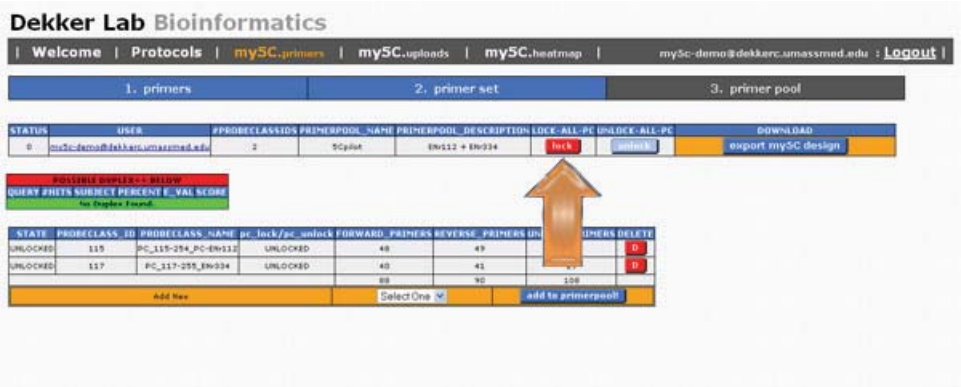


The duplex scan will then be re-run, and should yield no new duplexes.

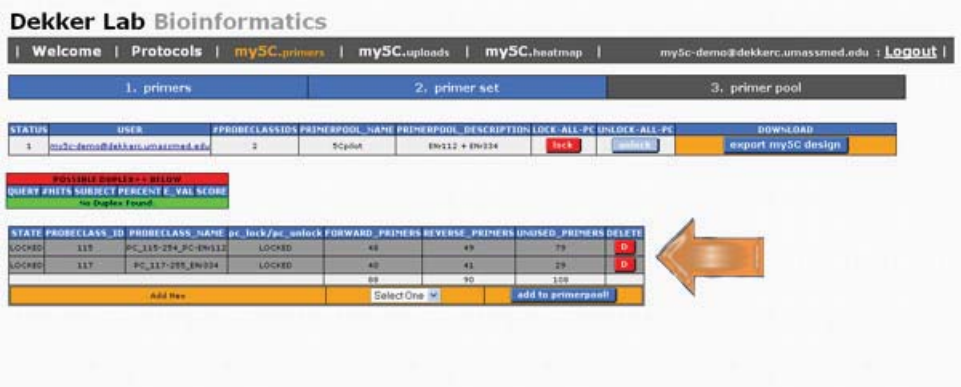


If no duplexes are found, you should get the green light, saying **No Duplex Found**.
At this point, the entire 5C design is almost complete.
In order to **LOCK** your primer sets from any further changes, you must click the lock button.
Doing so will prevent any accidental changes to the design.

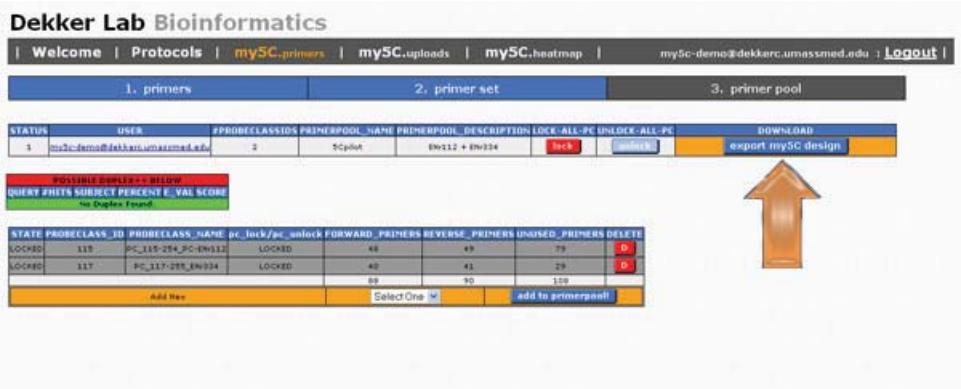
Click lock :



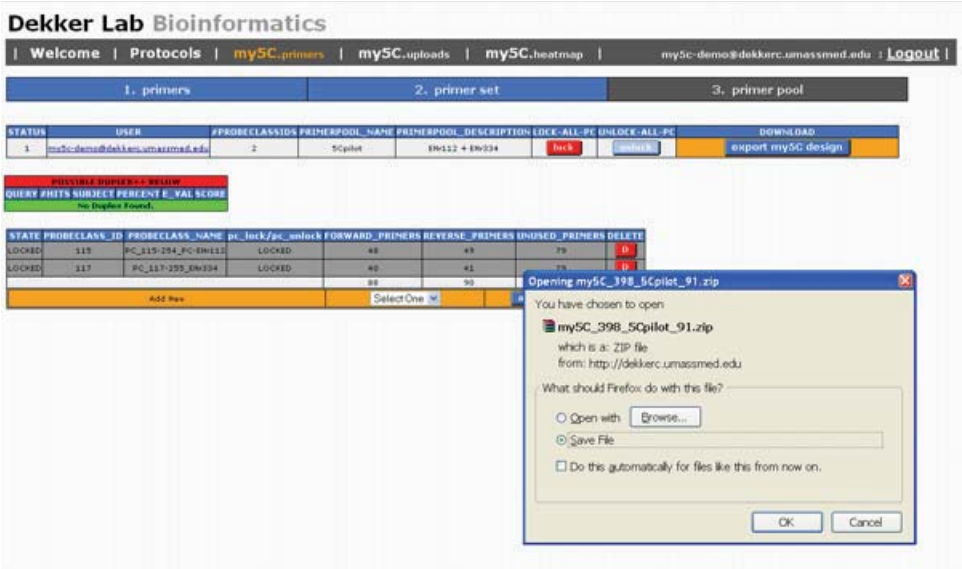
The Primer Set rows will then be turned a shade of gray to indicate they are locked.



The final step is to click the **export my5C design** button to download your 5C design in zip format.



It may take up to 1 minute to organize and zip all of the necessary files.



Save the zip file and unzip the archive.

my5C ZIP file

You should find 7 different folders inside of the archive.

- elements - containing all relevant files related to any elements files being intersected with your design.
- images - containing all primer layout images of all primer sets within your primer pool.
- microarray - custom microarray design files for all forward-reverse interactions within your 5C design.
    - 3 probe sizes are calculated.
        - 19 - 19 bases from each primer are used to make the microarray probe (38 total).
        - 20 - 20 bases from each primer are used to make the microarray probe (40 total).
        - 21 - 21 bases from each primer are used to make the microarray probe (42 total).
- primers - text files for all primer sets within your 5C design. All relevant information for each primer used.
- sequencing - necessary FASTA files used to map reads back to the primer pool for deep sequencing applications.
- to_order - a text file containing only necessary information to order primers.
- ucsc_bed - UCSC bed file containing each used primer in the 5C design.

And that's it! You are now ready to proceed to the 5C experiment!

Supplemental Data 3
A tutorial describing in detail how to use my5C.uploads. This tutorial can also be directly accessed through:
http://my5c.umassmed.edu/welcome/welcome.php?tab=uploads

Dekker Lab Bioinformatics

my5C.uploads manual

my5C.uploads

my5C.uploads is a tool to allow for the upload of interaction data of various formats. All data uploaded to my5C.uploads are made available in the my5C.heatmap tool for visualization.

Table of Contents

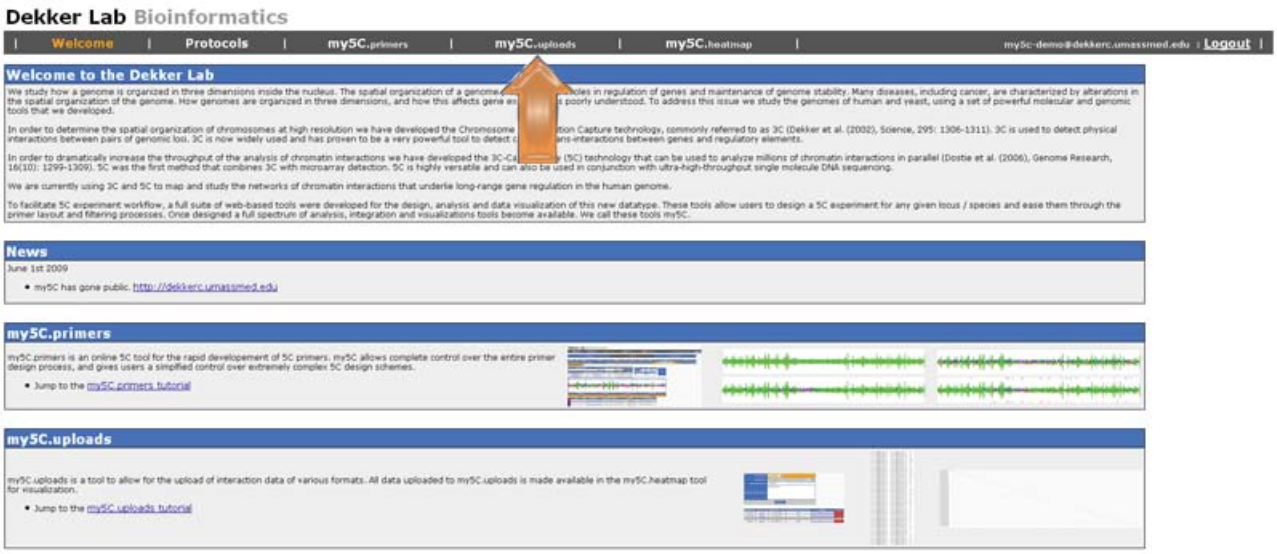
Getting Started

There are 2 main steps to my5c.uploads.

- 1. Choosing whether or not to link interaction data to a primer pool.
- 2. Uploading correct format of data.

Here we will walk you through a simplified upload process.

To start, click my5C.uploads on the main menu of the website.



You should now see this screen.

Linking interaction data to a primer pool

You should use this option if you have a dataset generated by using a primerpool designed with the my5C.primers tool.
This option links your interaction data directly to the primer pool, so all my5C.primers data can be combined with your interaction data.

List Format

The file format is as follows (tabbed delimited).

FORWARD_PRIMER_NAME REVERSE_PRIMER_NAME INTERACTION_COUNT

5C_115_ENr112_FOR_73	5C_115_ENr112_REV_72	6171
5C_115_ENr112_FOR_62	5C_115_ENr112_REV_63	5233
5C_115_ENr112_FOR_26	5C_115_ENr112_REV_27	4629
5C_115_ENr112_FOR_141	5C_115_ENr112_REV_140	4471
5C_115_ENr112_FOR_104	5C_115_ENr112_REV_105	4388
5C_115_ENr112_FOR_31	5C_115_ENr112_REV_30	4139
5C_115_ENr112_FOR_20	5C_115_ENr112_REV_19	4014
5C_115_ENr112_FOR_109	5C_115_ENr112_REV_108	3894
5C_115_ENr112_FOR_5	5C_115_ENr112_REV_6	3615
5C_115_ENr112_FOR_31	5C_115_ENr112_REV_32	3407
5C_115_ENr112_FOR_107	5C_115_ENr112_REV_105	3323
5C_115_ENr112_FOR_176	5C_115_ENr112_REV_175	3276
5C_115_ENr112_FOR_132	5C_115_ENr112_REV_131	3117
5C_115_ENr112_FOR_168	5C_115_ENr112_REV_166	3070
5C_115_ENr112_FOR_20	5C_115_ENr112_REV_21	3031

The primer names should match exactly to the names of the primers listed in the primer pool supplied in the my5C.primers zip file.

```
>5C_115_ENr112_FOR_4
TAATACGACTCACTATAGCCGTTTTCAAAATCTTCTTGCTAACTATCAAG
>5C_115_ENr112_FOR_6
TAATACGACTCACTATAGCCGCCAGAAGAACTGGCAGTACTTTTCCAAAG
>5C_115_ENr112_FOR_8
TAATACGACTCACTATAGCCATGAAGACGGAGGGTTATGAAAGGCAGAAG
>5C_115_ENr112_FOR_10
TAATACGACTCACTATAGCCAGGGAAGAAGCCAAAACGTACAAATAAAAG
>5C_115_ENr112_FOR_15
TAATACGACTCACTATAGCCTGAATTGAAACTATGGCATGAAATTTGAAG
>5C_115_ENr112_FOR_19
TAATACGACTCACTATAGCCTTTTTCTGGTAACCAGTCTCCATGCTGAAG
```

Sequencing reads can be mapped back to this primerpool FASTA file, yielding the correct primer names associated with each interaction pair.

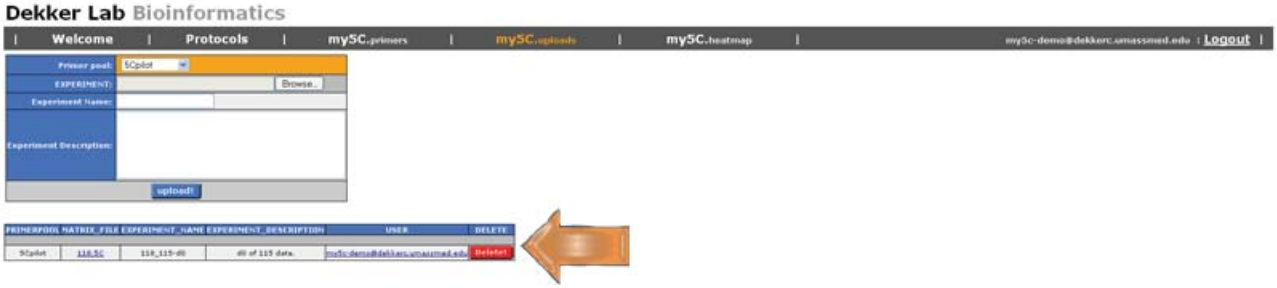
Fill in the necessary data to the my5C.uploads form.

- 1. Primer Pool - choose the primer pool you are uploading interaction data to.
- 2. EXPERIMENT - specify the file you are uploading (must be correct format)
- 3. Experiment Name - choose an experiment name to name your dataset.
- 4. Experiment Description - fill in the optional experiment description section.

The primer names in your experiment file, MUST match the primer names in your 5C design found in my5C.primers.
If they do not, the upload will fail and output an error message.

Once all of the information is filled in, press **upload!**

Depending on the size of your interaction file, it may take up up to a few minutes to complete the upload.
Once the upload is complete, your experiment will be available below.



You may now proceed to the my5C.heatmap tool to visualize your data.

Uploading custom interaction data (no primer pool)

If you have:

- A. 5C Interaction data produced from a design not created from the my5C.primers tool.
  or
- B. Any interaction data in the correct format

You may upload this data to the my5C.heatmap tool for visualization / analysis.

Matrix Format

The accepted matrix format is of the following format.
Headers must be included.
They are of the format.

NAME|ASSEMBLY|CHROMOSOME:STARTPOS-ENDPOS

i.e. gene1|hg18|chr2:51517722-51527793

5C_115_ENr112_FOR_29|hg18|chr2:51589990-51590633
5C_115_ENr112_FOR_31|hg18|chr2:51592097-51594618
5C_115_ENr112_FOR_34|hg18|chr2:51596572-51598771
5C_115_ENr112_FOR_37|hg18|chr2:51608630-51618243
5C_115_ENr112_FOR_39|hg18|chr2:51623052-51626805
5C_115_ENr112_FOR_41|hg18|chr2:51628358-51629331
5C_115_ENr112_FOR_44|hg18|chr2:51639631-51640555
5C_115_ENr112_FOR_46|hg18|chr2:51641891-51642390
5C_115_ENr112_FOR_48|hg18|chr2:51642577-51643716
5C_115_ENr112_FOR_52|hg18|chr2:51655037-51656385
5C_115_ENr112_FOR_56|hg18|chr2:51664343-51667206
5C_115_ENr112_FOR_59|hg18|chr2:51668252-51672775

Each row and each column must have a valid header.
A sample matrix format can be found below.

myDATA	B1|hg18|chr2:51527935-51528740	B2|hg18|chr2:51533038-51535102	B3|hg18|chr2:51540236-51546080
gene1|hg18|chr2:51517722-51527793	432	54	32
gene2|hg18|chr2:51528741-51533037	94	245	82
gene3|hg18|chr2:51535103-51540235	25	65	361

Two sample matrix files can be found here.

- smile.matrix
- ENr112.matrix

In order for the BP windowing and smoothing features of the my5C.heatmap tool to work correctly, chromosome and position values must be included in the headers.
Custom Matrix experiments are uploaded to my5C.uploads in the same manner as experiments linked to primer pools.
However, you must now select ***CUSTOM*** from the primer pool dropdown.
Fill in the necessary values and click **upload!**

Your new custom dataset can be found below.

You may now proceed to the my5C.heatmap tool to visualize your data.

Supplemental Data 4
A tutorial describing in detail how to use my5C.heatmap. This tutorial can also be directly accessed through:
http://my5c.umassmed.edu/welcome/welcome.php?tab=heatmap

Dekker Lab Bioinformatics

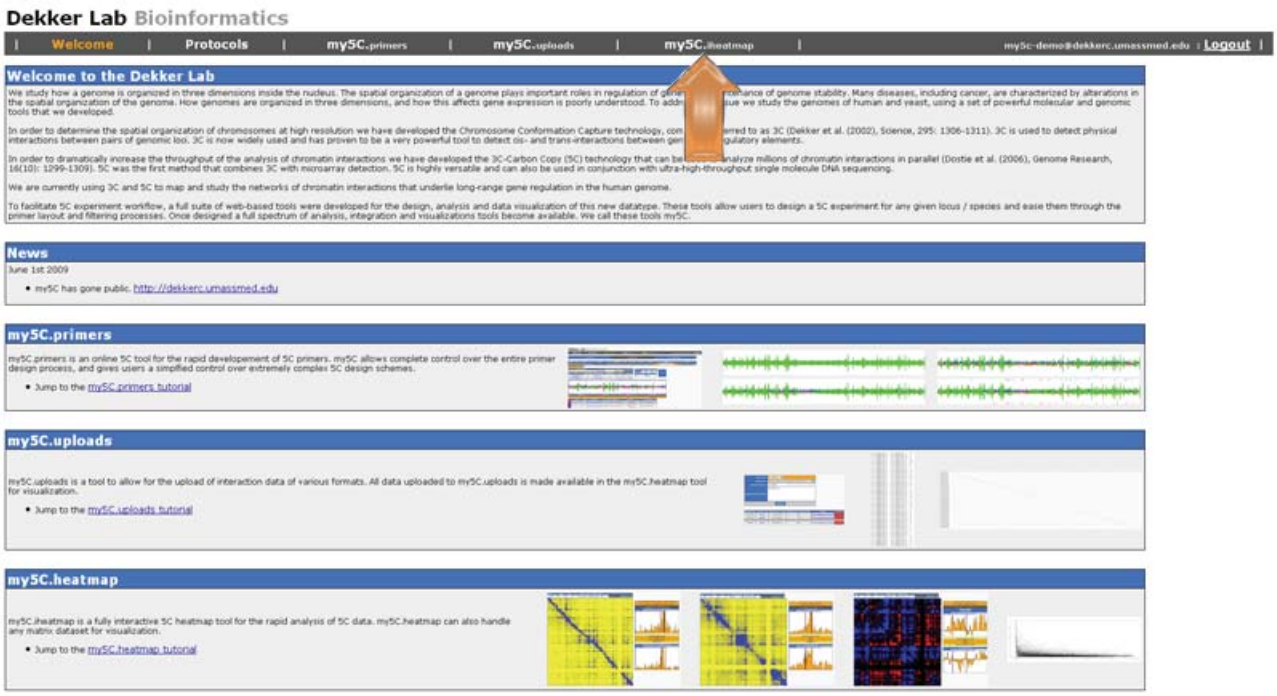
my5C.heatmap tutorial

my5C.heatmap

my5C.heatmap is an online 5C tool for the visualization and analysis of 5C interaction data.

Table of Contents

To start, click my5C.heatmap.



You should now see this screen.

UI Layout

The my5C.heatmap UI is broken into 4 parts.

- my5C.heatmap menu bar
- my5C.heatmap tool panel
- my5C.heatmap image panel
- my5C.heatmap click panel

my5C.heatmap menu bar

my5C.heatmap tool panel

my5C.heatmap image panel

my5C.heatmap click panel

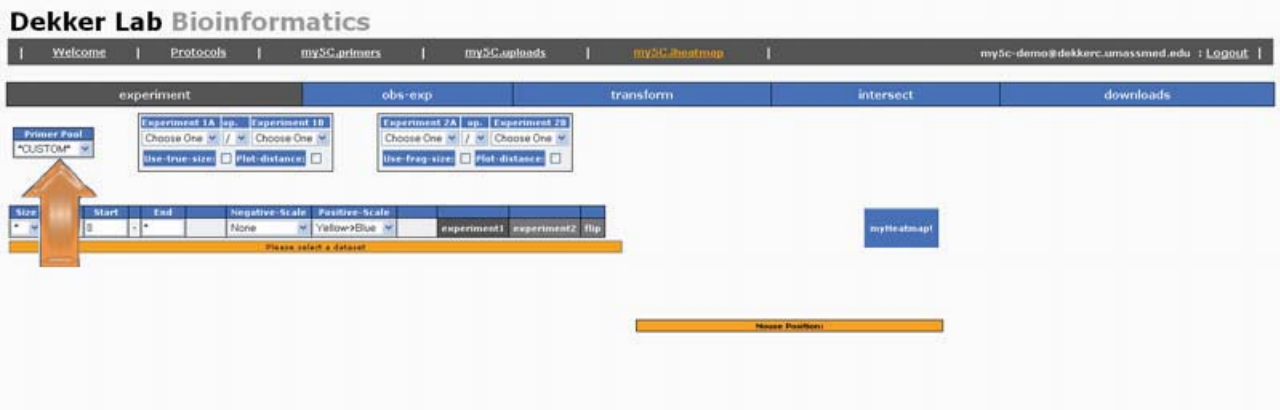
Quick Draw

If you have not loaded in a data set via my5C.uploads, please do so now.

- Uploading to a primer pool
- Uploading a custom matrix

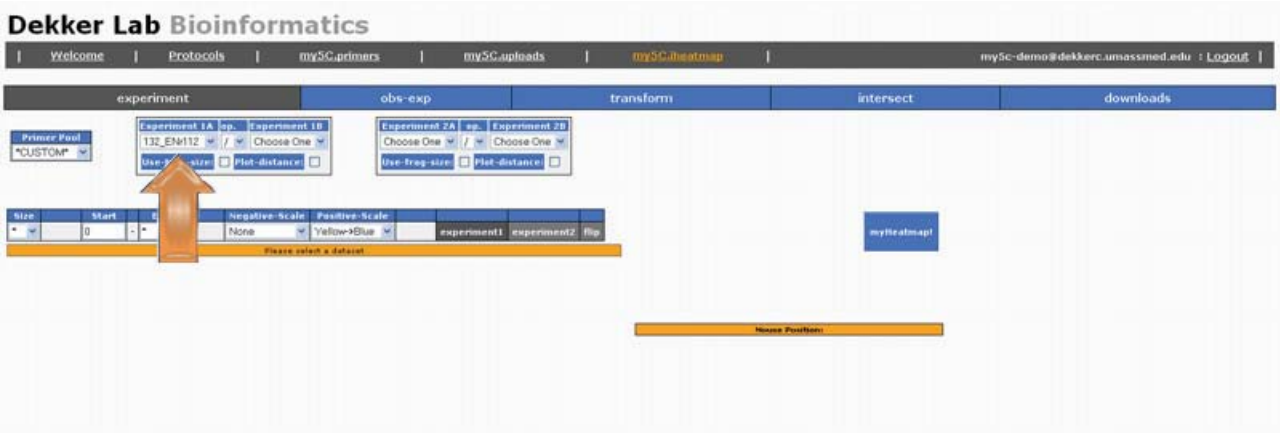
For this example, we will upload a CUSTOM matrix
The file used can be found here ENr112.matrix
Once the file has been correctly upload please proceed below.

Firstly, select *CUSTOM* as your primer pool.



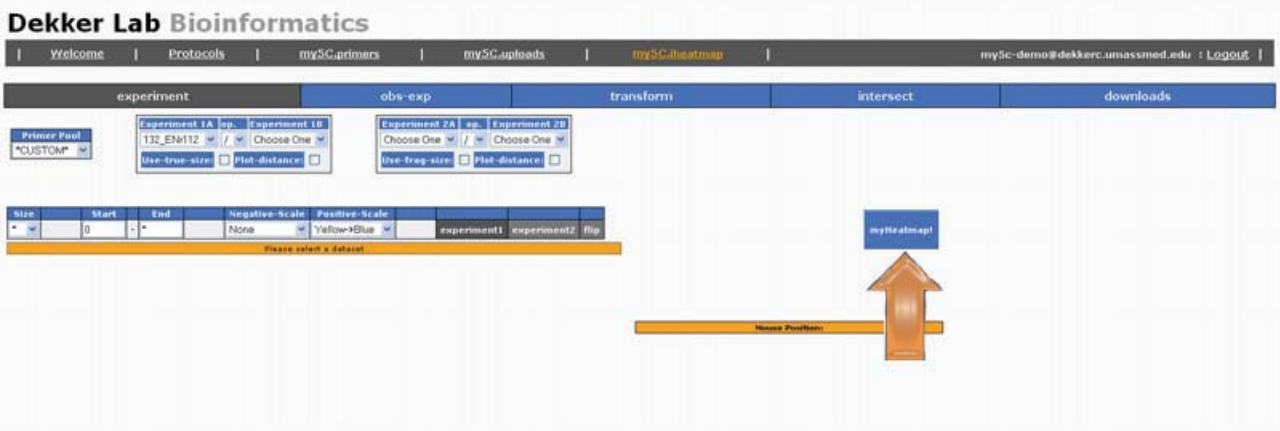
The page will then reload and pull in any experiments connected to your selected primer pool
In this case, the experiment you just loaded should be available in the dropdown.

Select the experiment...

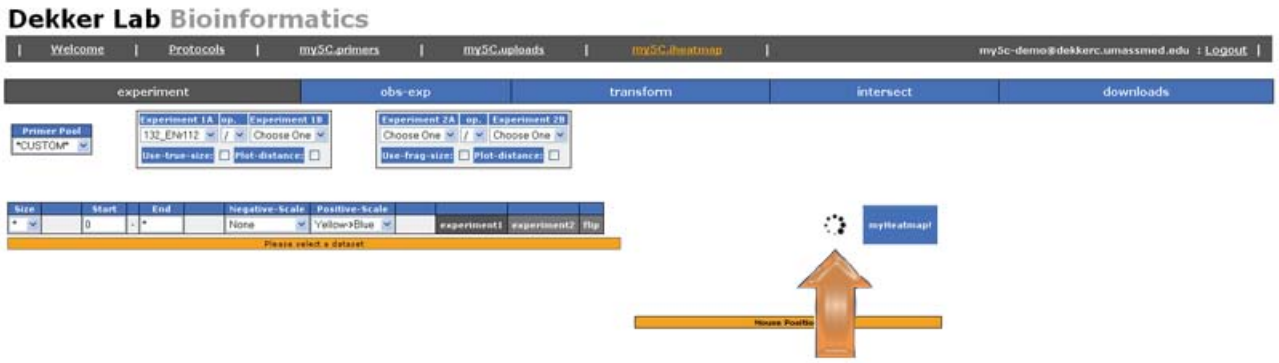


Thats it! You are now able to draw the heatmap!

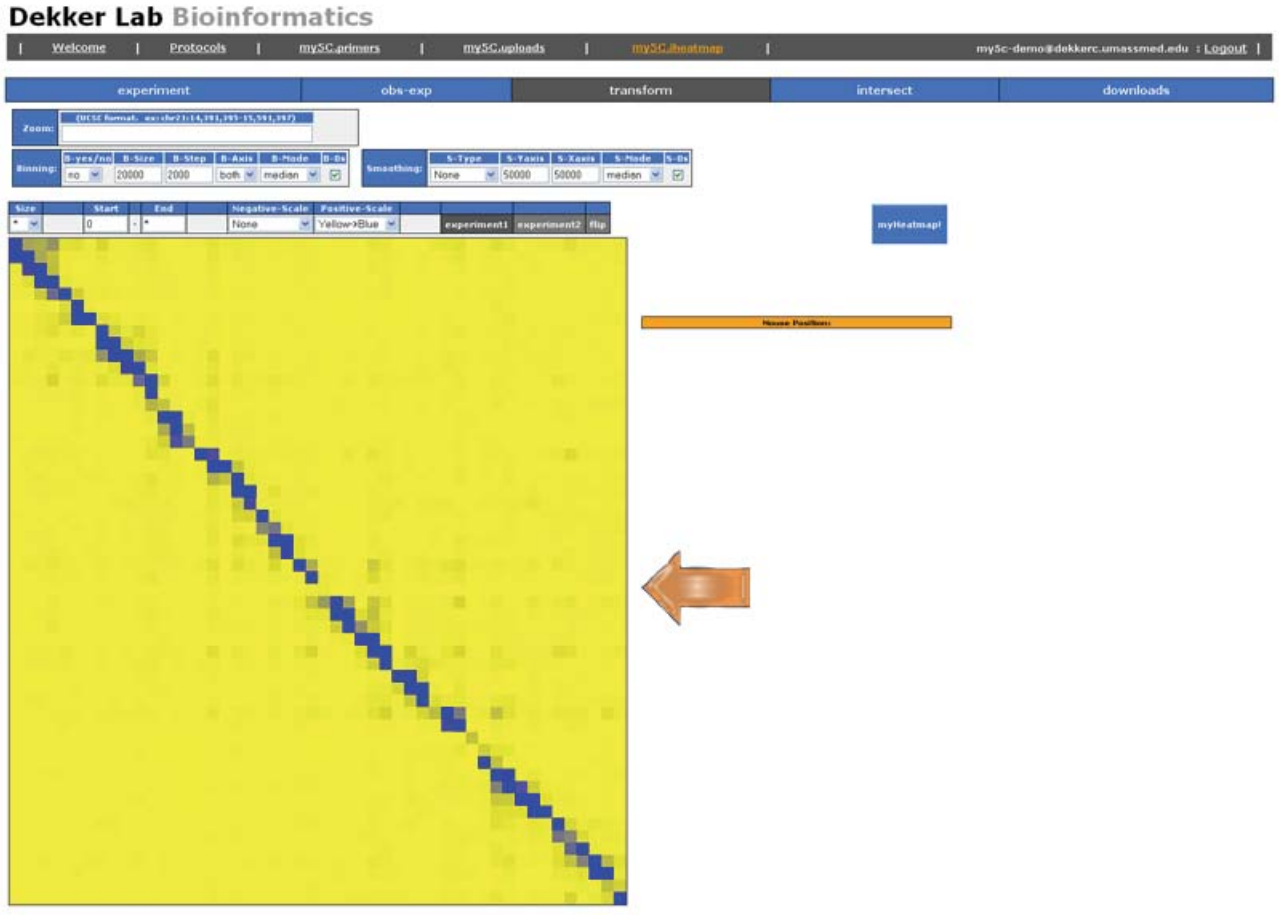
Click the **myheatmap!** button.



The heatmap can take anywhere from a few seconds to 10 minutes to draw, depending on the complexity of the options selected.
While the tool is working, you will see the *roller image animation*.



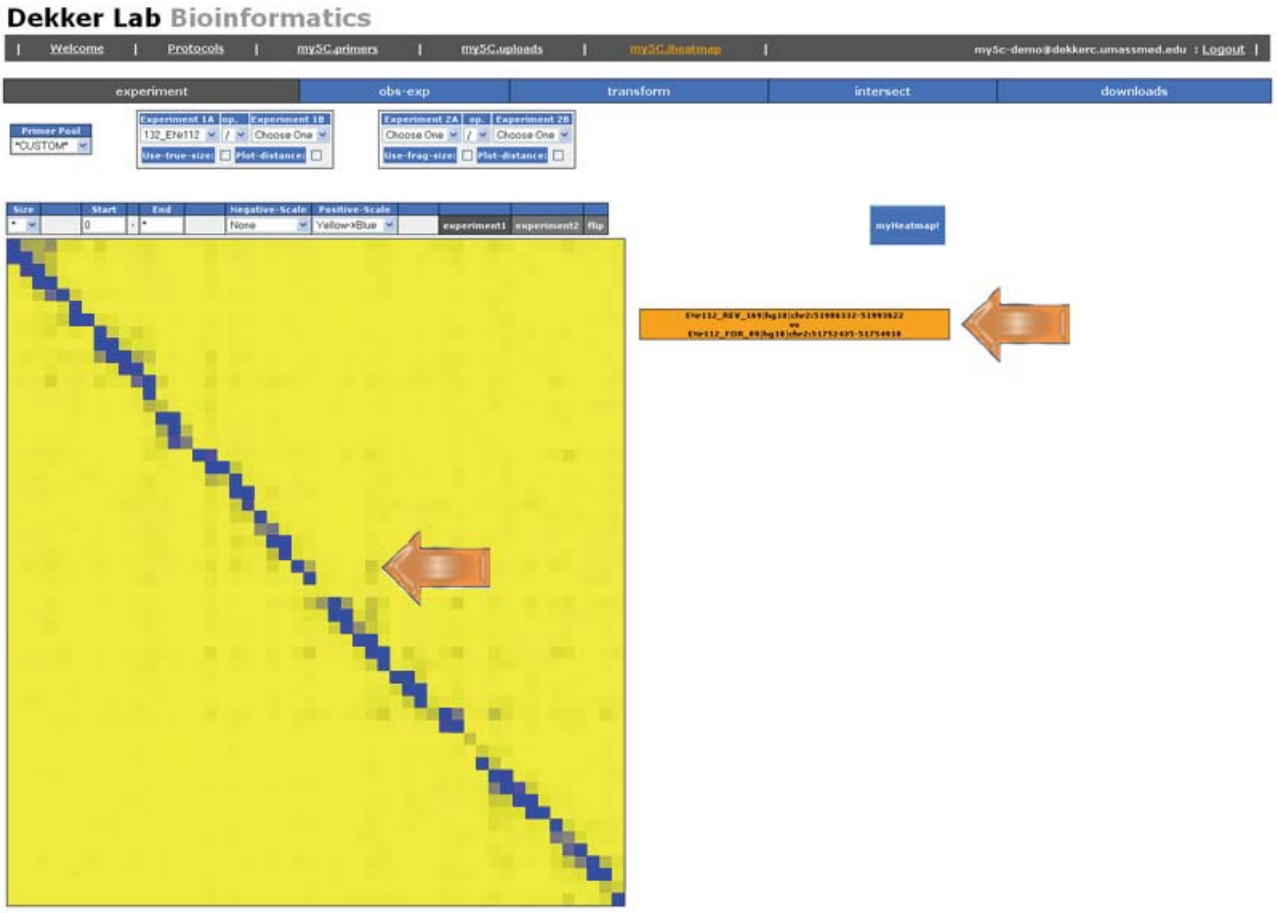
Once the heatmap has finished, the *roller image animation* will disappear and the heatmap will appear.



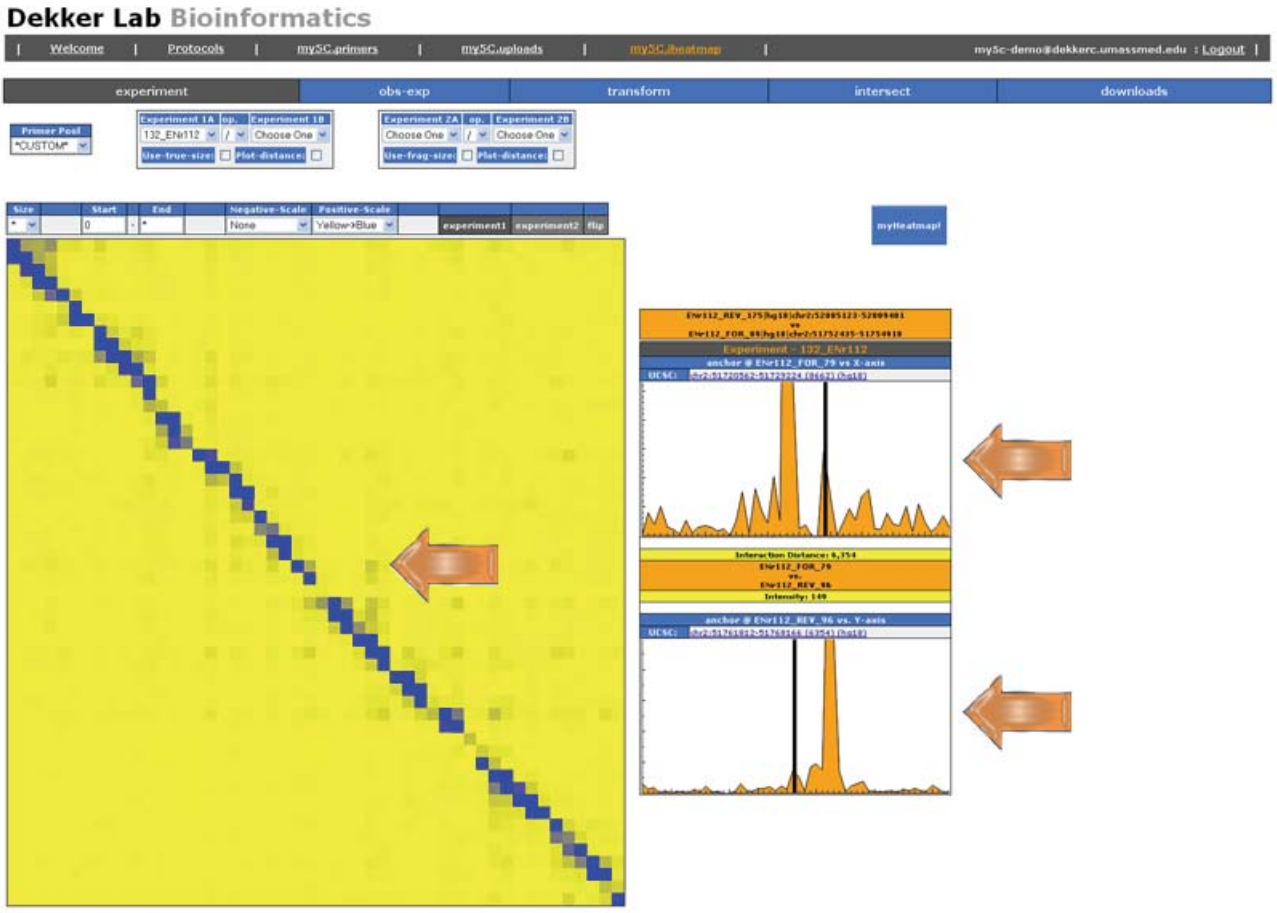
- All pixels are identical in size.
- Each pixel represents a unique interaction between a forward primer and a reverse primer.
- Forward primers can be found along the Y axis, sorted by the chromosomal position.
- Reverse primers can be found along the X axis, sorted by the chromosomal position.

Click

As you move your mouse around the heatmap, you will notice the screen is constantly updated with the current interaction the mouse is hovering over.



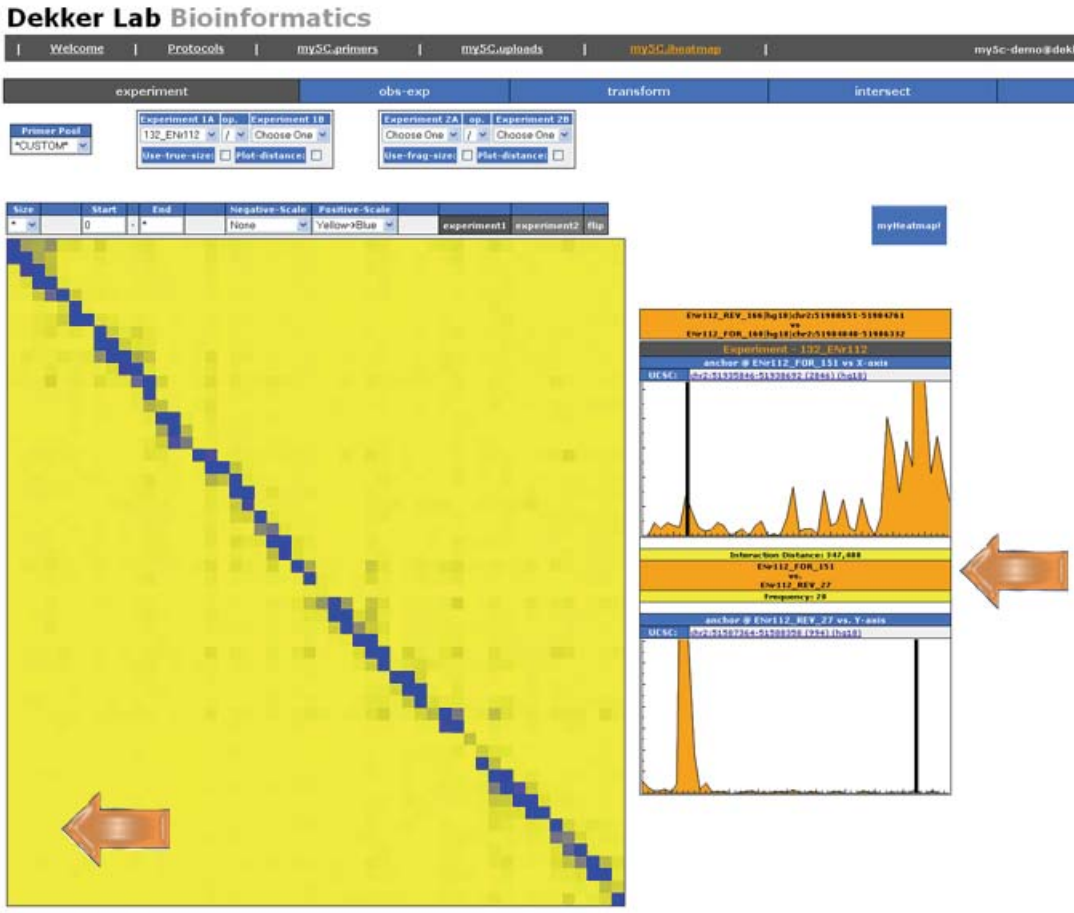
If you then click on a specific interaction, the *click panel* will appear to the right.



This panel contains information specific to both the forward and reverse primer/fragment you have clicked.

- Forward primer name
- Link to UCSC representing the forward primer fragment
- 3C style plot representing row interaction pattern (click for larger view).
- Reverse primer name
- Link to UCSC representing the reverse primer fragment
- 3C style plot representing column interaction pattern (click for larger view).
- Interaction distance
- Interaction frequency

You can use the UCSC genome browser links to investigate any 'elements' that lie within the fragments you are interrogating. As you click on new interactions, the *click panel* will automatically update itself.

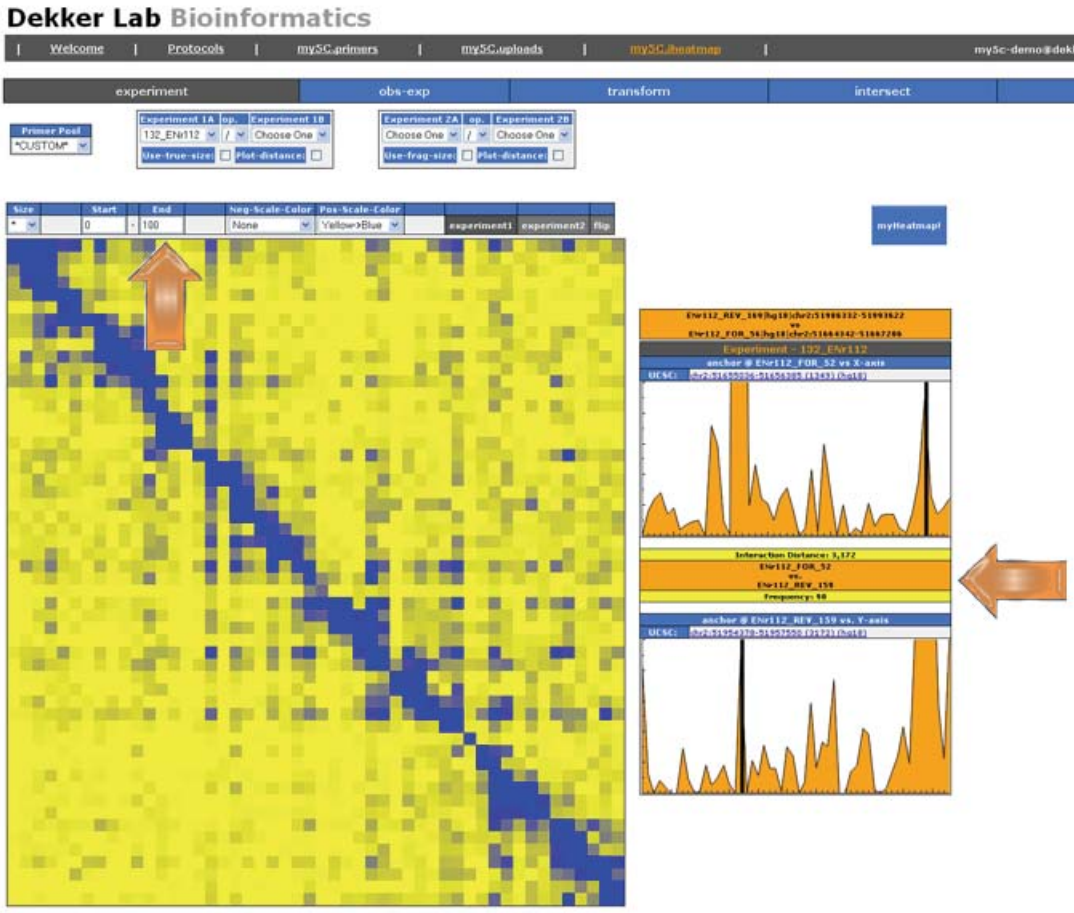


Main heatmap Controls

The Main heatmap Control has 6 main options.

- Size - the size in pixels of each interaction square (* = autoscale entire heatmap to 800 pixels wide).
- Start - specifies the minimum range of values to display.
- End - specifies the maximim range of values to display (* = autoscale to 97% saturation).
- Neg-Scale-Color - the negative scale color scheme to use.
- Pos-Scale-Color - the positive scale color scheme to use.
- Comparison - gives the ability to directly compare two experiments at once.
    - Expeirment1 - view selected experiment1 heatmap.
    - Expeirment2 - view selected experiment2 heatmap.
    - flip - flip between experiment1 and experiment2 heatmaps automatically at 1 second intervals.

i.e. changing end to 100 and then redrawing yields:



Any interactions with a frequency >= 100 will now be saturated at the maximum color (which is blue in this case).
Also note the **end** value also affects the 3C plots in the *click panel* by changing the Yaxis scale.
You can change the end value without redrawing the heatmap, and the 3C plots will correctly utilize the new end value.

Experiment

This tab controls which experiments (datasets) you are viewing. There are 3 main control blocks:

- Primer Pool & Primer Set - specifies the primer pool and primer set (FOR-REV combination to view).
- Experiment1 - experiment1 dataset to use.
- Experiment2 - expeiriment2 dataset to use.

Choosing a primer pool

Any primer pools you have created will be available within this dropdown.
The ***CUSTOM*** primer pool will always be available within this dropdown.
If you select a primer pool you have created, all possible FOR-REV combinations between all primers sets within that primer pool will appear below the primer pool dropdown.

i.e. Select the sample primer pool that was created in the my5C.primers tutorial

Choosing a primer set

Once you click a valid primer pool, a new drop down, *Primer Set* will appear listing all possible FOR-REV combinations between all primers sets within that primer pool.
Choose a FOR-REV primer set combination.

Choosing an experiment

Then choose an experiment that has been linked to the primer pool (Uploading to a primer pool).

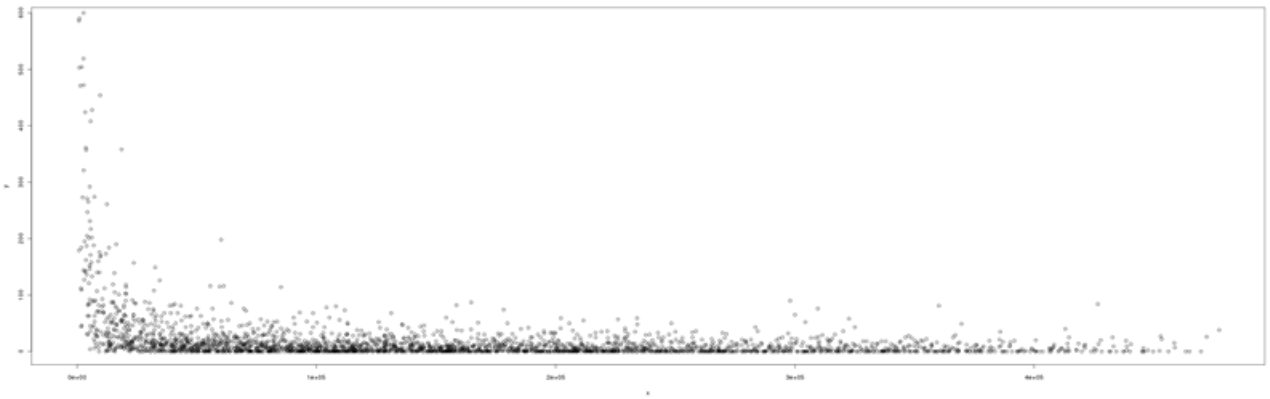
Obs-exp

Chromatin fragments that are close to each other in the linear genome will interact with each other more frequently than fragments that are further apart.
The interactions between close chromatin fragments are apparent in the heatmap as a diagonal.

Interaction frequencies can be normalized for distance by dividing the observed value by the expected value.
The obs-exp option has 4 options:

- Type - a yes/no dropdown to control exp-obs calculations
- alpha - alpha value for LOESS smoothing (which % of data to smooth by)
- plot - what to plot as result. (obs / exp , obs - exp, obs, exp)
- Log2 - Log2 transform the plot value.

We start with a basic scatter plot of the 5C data.



- Each point represents a unique interaction.
- Y-axis : interaction frequency
- X-axis : distance of interaction

The expected value is the interaction frequency between two fragments that is solely due to the proximity of these fragments in the linear genome.

The expected value is calculated by using a LOESS SMOOTHING model.

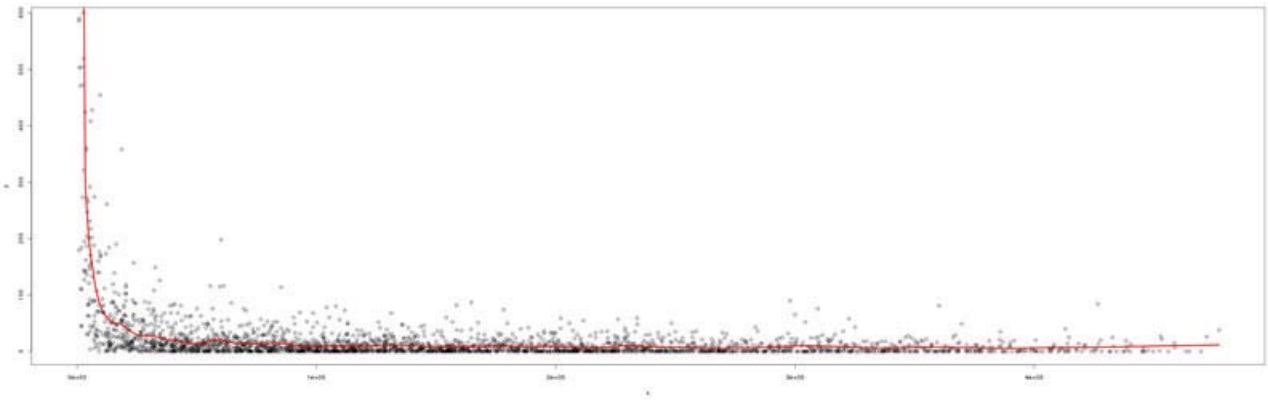
LOESS combines much of the simplicity of linear least squares regression with the flexibility of nonlinear regression.
It does this by fitting simple models to localized subsets of the data to build up a function that describes the deterministic part of the variation in the data, point by point.
http://www.itl.nist.gov/div898/handbook/pmd/section1/pmd144.htm

If the obs/exp ratio is >1, it means that the interaction frequency between the two fragments is higher than expected based on distance.
If the obs/exp ratio is <1, it indicates that two regions interact less frequent than expected based on distance.

After performing LOESS SMOOTHING, we can plot the function as a line, yielding the expected Y-value for each X-value.

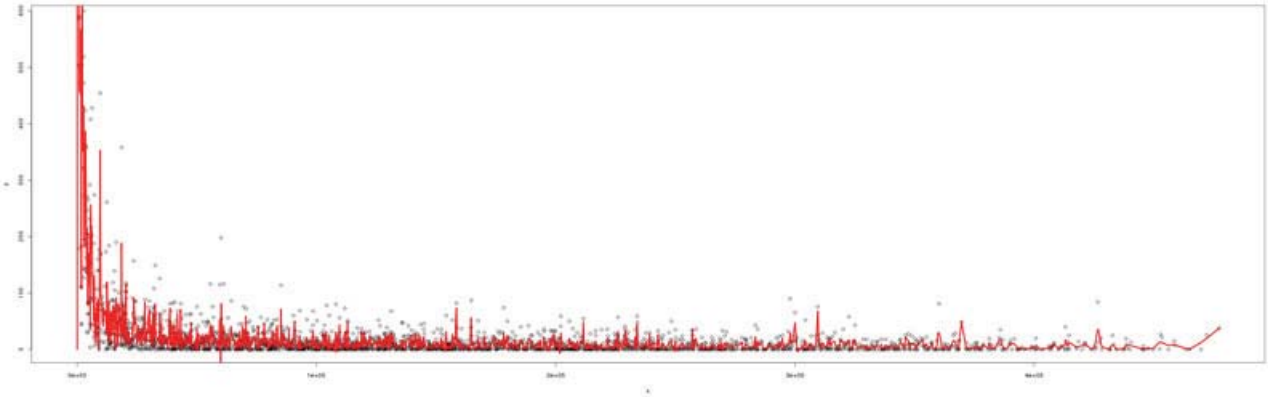
alpha

Default alpha=0.05



LOESS SMOOTHING has one main parameter, alpha.
alpha is called the smoothing parameter because it controls the flexibility of the LOESS regression function.
Large values of q produce the smoothest functions that wiggle the least in response to fluctuations in the data.
The smaller q is, the closer the regression function will conform to the data.
Using too small a value of the smoothing parameter is not desirable, however, since the regression function will eventually start to capture the random error in the data.
http://www.itl.nist.gov/div898/handbook/pmd/section1/pmd144.htm

Using alpha=0.0025



As you can see, using too small of an **alpha** value can produce non-optimal results.

Plot

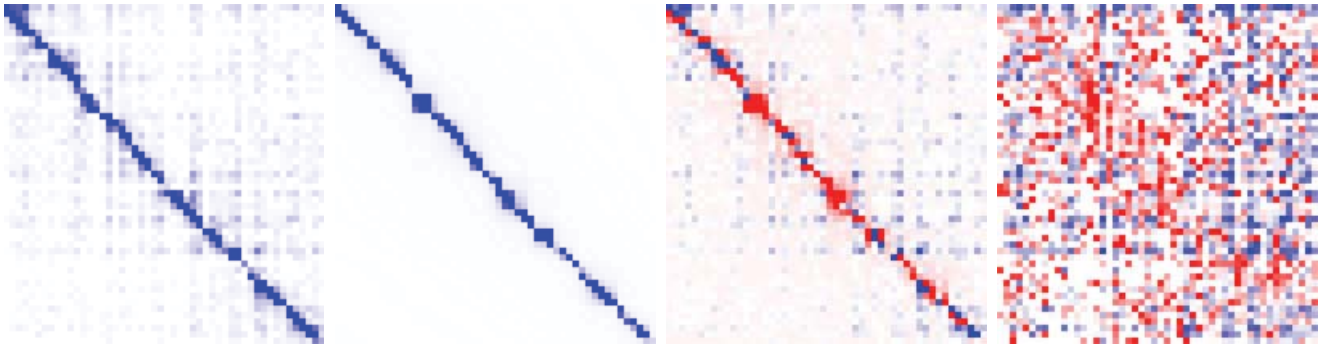
You can plot a variety of obs|exp combinations.
Each plot type has it strengths and weaknesses.
Experiment with each type.

Log2

This function transforms the data so that both higher and lower obs/exp ratios are on the same scale.
If this box is unchecked, observed values that are higher than expected result in obs/exp ratios ranging from 1 to infinity, whereas observed values that are lower than expected result in obs/exp ratios between 0 and 1.
By calculating the log2 value of the obs/exp ratios, the values will range between -1 and 1.
Values between 0 and 1 will indicate an interaction frequency that is higher than expected and values between -1 and 0 will represent a frequency that is lower than expected.

Now that the expected is calculated, you can perform a variety of obs | exp comparisons.

Obs **Exp** **Obs - Exp** **log2(Obs/Exp)**



Transform

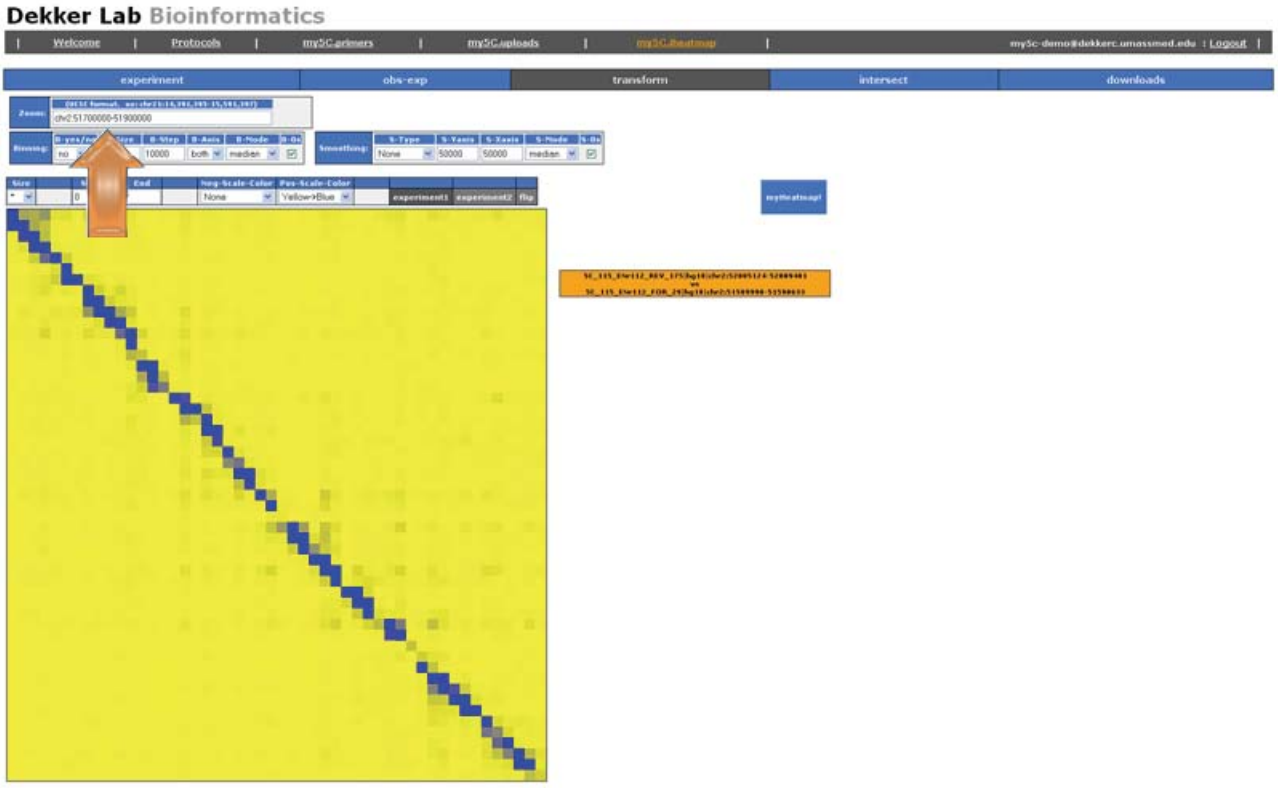
The transform menu controls various data transformations.

- Zoom - zoom in on a section of the heatmap by specifying genomic coordinates.
- Binning - bin the data in the heatmap to generate a heatmap spanning your full region of interest.
- Smoothing - smooth the data available on the heatmap.

Zoom

To zoom in on a subset of your 5C region, specify genomic coordinates in the zoom field.

i.e. chr2:51700000-51900000



This will zoom into the above listed coordinates, showing only those interactions that exist within the specified subset.

Binning

Binning is a way to transform the fragment based interaction maps to a kb specified segments of dna interaction map.

By doing so you can now estimate interaction between non-interrogated fragments of DNA.

It uses a sliding window approach.
Instead of looking at the interaction between a specific forward and reverse combination, you can view interactions of segments of DNA of a specified KB length.
There are 6 options to binning:

- B-yes/no : use binning option (yes/no).
- B-Size : size (in BP).
- B-Step : step size (in BP).
- B-Axis : which axis to bin data by.
- B-Mode : mode used to plot the data by.
- B-0s : Use 0s in binning, or ignore.

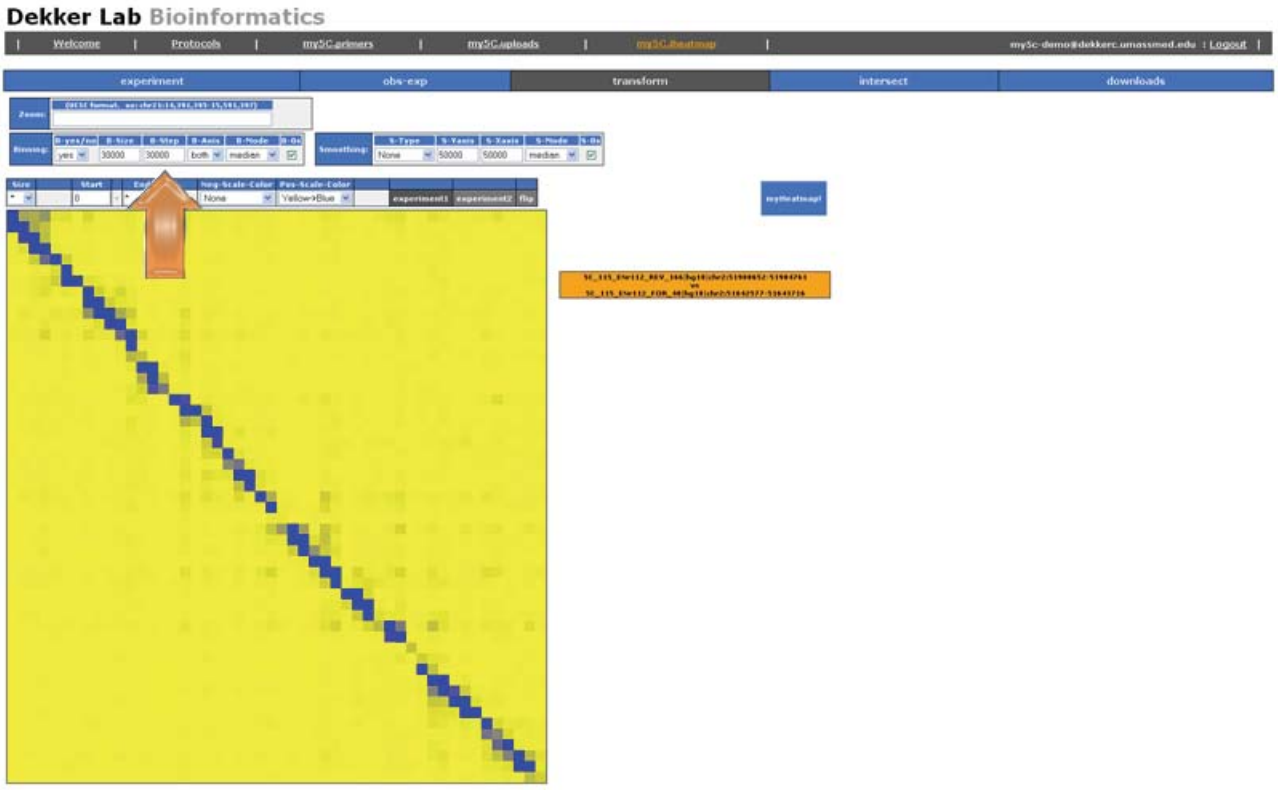
The B-Size value specifies the size of the segment of DNA to use.
The B-Step value specifies the step size in BP to slide the bin by.
All primer connections that exist between these two segments of DNA can then either be plotted as the :

B-Mode

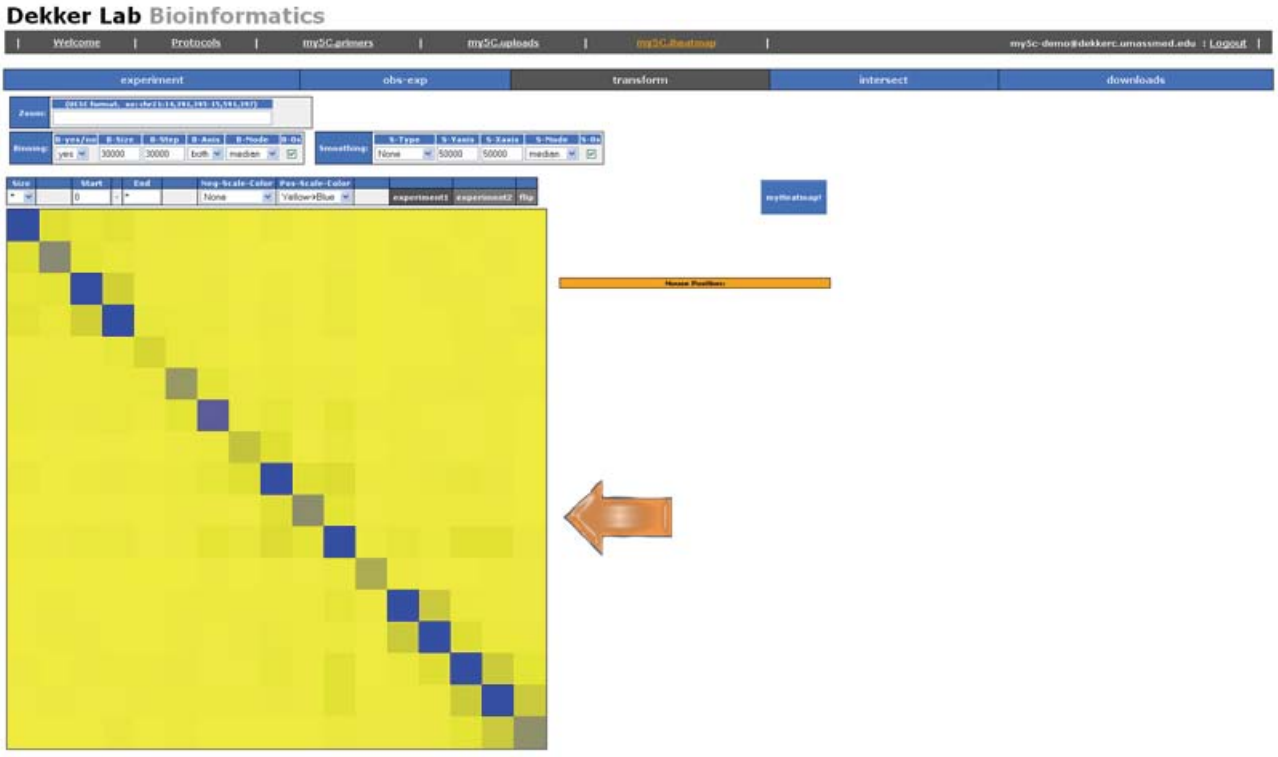
- Median - the median of all FOR-REV interactions
- Average - the average of all FOR-REV interactions
- Sum - the sum of all FOR-REV interactions
- Count - the total count of all FOR-REV interactions

Specify a set of values to use for the binning.

- B-yes/no : yes
- B-Size : 30000
- B-Step : 30000
- B-Axis : both
- B-Mode : median
- B-0s : checked



Then click **myHeatmap**



The total number of squares has now changed.
The total number of squares is now

(B-Step / Region Size) or (30000 / 499999) = ~17

So the new heatmap consists of 17x17 = (289) interactions, which now represent 30kb segments of DNA interactions with a step of 30kb (meaning no overlap).

If you change the values to

- B-yes/no : yes
- B-Size : 30000
- B-Step : 10000
- B-Axis : both
- B-Mode : median
- B-0s : checked

The total number of squares has now changed again.
The total number of squares is now

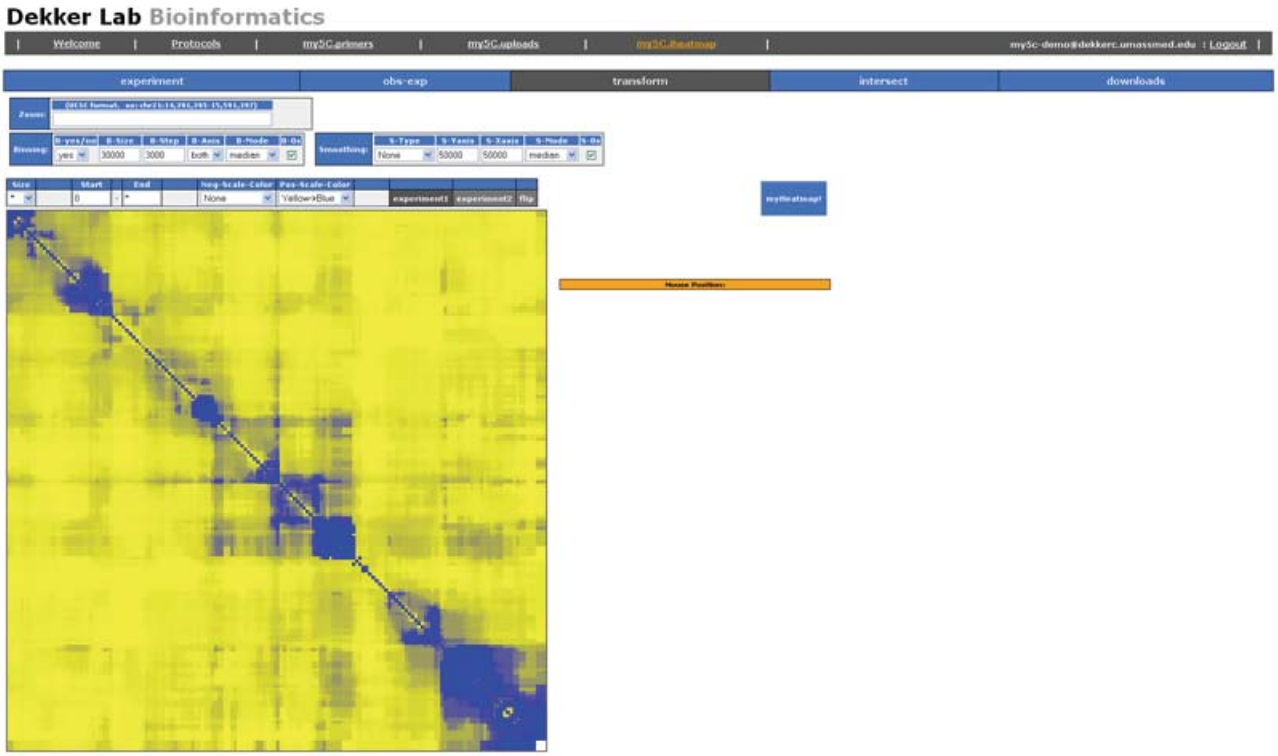
(B-Step / Region Size) or (10000 / 499999) = ~50

So the new heatmap consists of 50x50 = (2500) interactions, which now represent 30kb segments of DNA interactions with a step of 10kb.

The windows now overlap by 20kb.
Each square from the first image is now broken into 9 smaller squares, yielding finer resolution of the data.

If you change the values yet again to

- B-yes/no : yes
- B-Size : 30000
- B-Step : 3000
- B-Axis : both
- B-Mode : median
- B-0s : checked



The total number of squares has now changed again.
The total number of squares is now

(B-Step / Region Size) or (3000 / 499999) = ~167

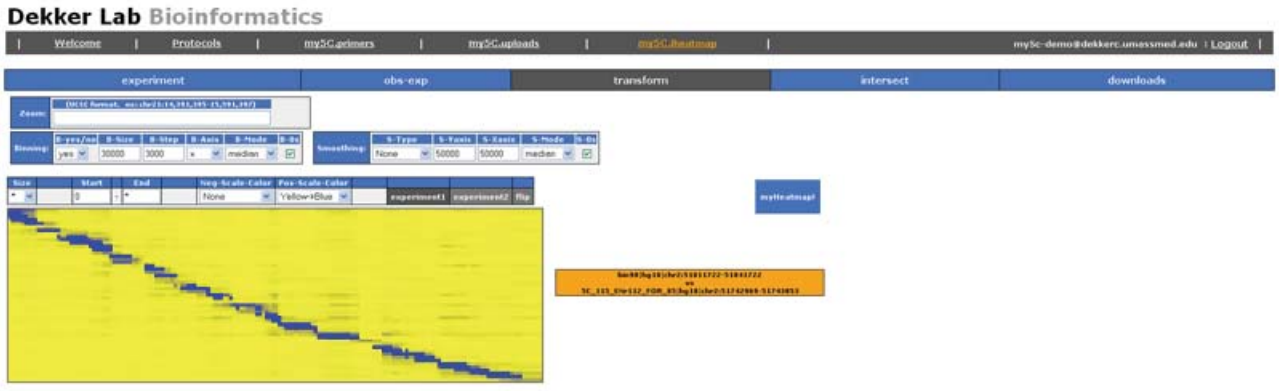
So the new heatmap consists of 167x167 = (27,889) interactions, which now represent 30kb segments of DNA interactions with a step of 3kb.

The windows now overlap by 27kb.

You can experiment with different values of B-Size and B-Step to obtain the best visualization of your data.

You can also specify the axis by which to bin the data.
Binning the data by only axis can be useful for some designs.
If you change the values to:

- B-yes/no : yes
- B-Size : 30000
- B-Step : 3000
- B-Axis : x
- B-Mode : median
- B-0s : checked



Only the X axis is now binned, and the Y axis is kept as a normal square pixel representing a single forward primer/fragment.

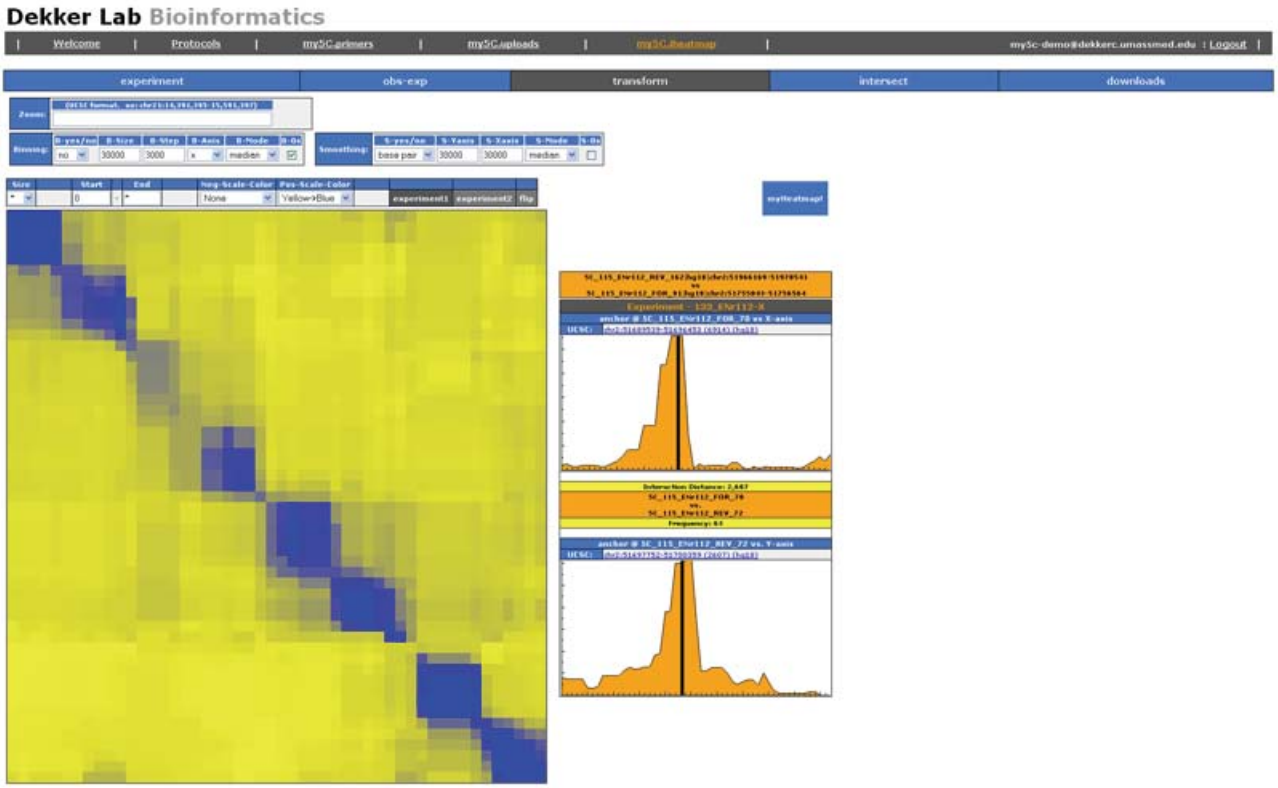
Smoothing

Smoothing is calculated by positioning at a single interaction, then looking Xkb up/down the Y axis and Xbk up/down the X axis. Smoothing has 5 options.

- S-Type : Which method to smooth by (interaction or base pair).
- S-Yaxis : Yaxis smooth parameter
- S-Xaxis : Xaxis smooth parameter
- S-Mode : mode used to plot the data by.
- S-0s : Use 0s in smoothing, or ignore.

Specify a set of values to use for the smoothing.

- S-Type : base pair
- S-Yaxis : 30000
- S-Xaxis : 30000
- S-Mode : median
- S-0s : checked



You can experiment with different smoothing types and sizes on your data.

Intersect

The intersection tool allows you to analyze whether a 5C fragment that forms a specific interaction, harbors a specific feature (e.g., a particular histone modification).
You can simply upload a list with names and coordinates of your elements of interest and this tool will intersect this list with the fragments or windows in the heatmap.

Element file

Upload your file containing your elements of interest.

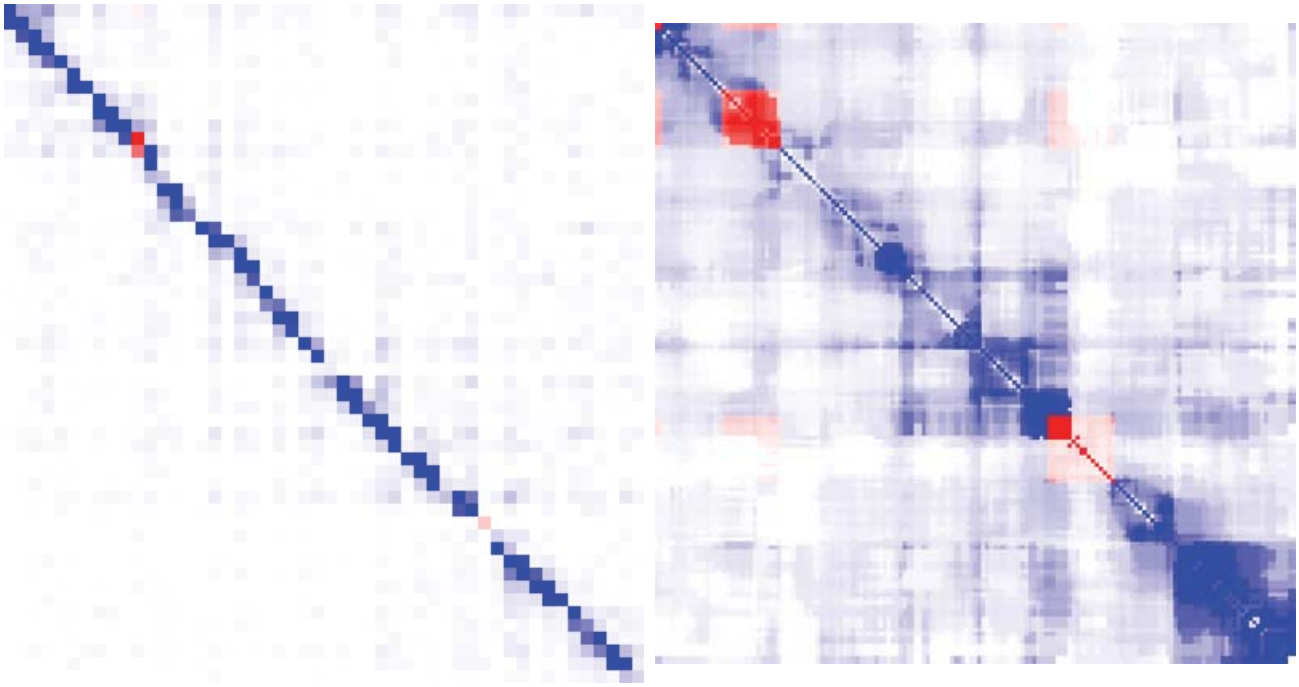
Format for this file is 4 columns, tab delimited.
chromosome - start_position - end_position - name
Sample file can be found here: sample element file

```
chr2    51512209        51522208        fake_gene1
chr2    51845055        51866223        fake_gene2
chr2    51597610        51612209        fake_gene3
```

And upon upload with default settings...

Normal

Windowed



You can specify the color schemes of each intersection type below.

Exten. (Extension)

Elements of interest can be located close to a fragment, but not close enough to overlap with a fragment.
In that case, basepairs can be added on both sides of the element to find an overlap.
When 100 is entered, 100 base-pairs are added on both the 5' and 3' end of all the elements in the "element file" before starting the intersection process.

Collapse

This option allows fragments that do not intersect with an element to be excluded from visualization.
No = show all fragments.
Yes = show only fragments that intersect with an element.

Collapse By

Choose which on which axis you want to collapse the heatmap:

- Both
- Y
- X

Color scheme (Y x X)

You can pick a separate color scheme for each of the following options:

- 0x0 = Both Y and X fragments do not intersect with an element.
- 0x1 = Only the fragment on the X-axis overlaps with an element.
- 1x0 = Only the fragment on the Y-axis overlaps with an element.
- 1x1 = Both X and Y fragments intersect with an element.

Press MyHeatmap to see your results.

Downloads

The results of every kind of analysis can be downloaded here.

Matrix w/ headers

This file contains the data in the same format as the heatmap: a matrix. Headers are included on both X and Y axes.

	5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740	5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102	5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080
5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793	2739	292	261
5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037	1305	3615	274
5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235	43	679	1071

Matrix w/o headers

Same as above, except there are no headers.

2739	292	261
1305	3615	274
43	679	1071

Pairwise

The downloaded file contains 3 columns representing the two interacting fragments and the interaction frequency.
This file can be used to visualize the data as a network in Cytoscape (http://www.cytoscape.org/).
Interacting fragments will be visualized as nodes and the interaction frequency as an edge.

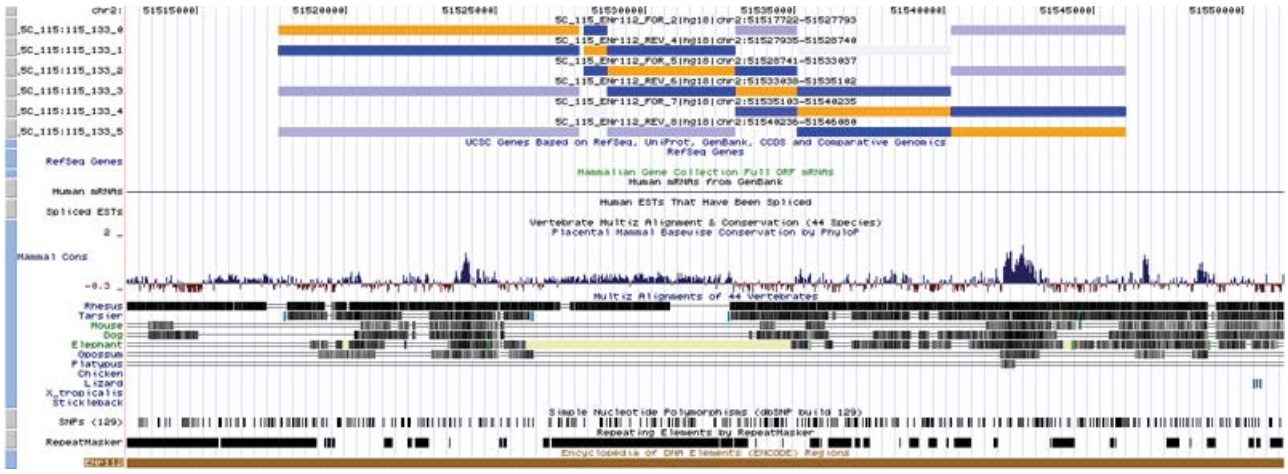
5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793	2739	5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740
5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793	292	5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102
5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793	261	5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080
5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037	1305	5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740
5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037	3615	5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102
5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037	274	5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080
5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235	43	5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740
5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235	679	5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102
5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235	1071	5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080

5C.bed

This file can be used to visualize the data in the UCSC genome browser. Simply download the 5C.bed file and upload it as a custom track in the genome browser.

```
track name=DEKKER_5C_115:115_133_0 description=5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793 visibility=dense autoScale=off priority=0 itemRgb=On
chr2    51517722    51527793    5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793 1000    +    51517722    51527793    255,165,0
chr2    51527935    51528740    5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740 2739    +    51527935    51528740    2,2,255
chr2    51533038    51535102    5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102 292 +    51533038    51535102    170,170,255
chr2    51540236    51546080    5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080 261 +    51540236    51546080    179,179,255
track name=DEKKER_5C_115:115_133_2 description=5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037 visibility=dense autoScale=off priority=2 itemRgb=On
chr2    51528741    51533037    5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037 1000    +    51528741    51533037    255,165,0
chr2    51527935    51528740    5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740 1305    +    51527935    51528740    2,2,255
chr2    51533038    51535102    5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102 3615    +    51533038    51535102    2,2,255
chr2    51540236    51546080    5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080 274 +    51540236    51546080    175,175,255
track name=DEKKER_5C_115:115_133_4 description=5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235 visibility=dense autoScale=off priority=4 itemRgb=On
chr2    51535103    51540235    5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235 1000    +    51535103    51540235    255,165,0
chr2    51527935    51528740    5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740 43  +    51527935    51528740    243,243,255
chr2    51533038    51535102    5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102 679 +    51533038    51535102    57,57,255
chr2    51540236    51546080    5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080 1071    +    51540236    51546080    2,2,255
track name=DEKKER_5C_115:115_133_1 description=5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740 visibility=dense autoScale=off priority=1 itemRgb=On
chr2    51527935    51528740    5C_115_ENr112_REV_4|hg18|chr2:51527935-51528740 1000    +    51527935    51528740    255,165,0
chr2    51517722    51527793    5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793 2739    +    51517722    51527793    2,2,255
chr2    51528741    51533037    5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037 1305    +    51528741    51533037    2,2,255
chr2    51535103    51540235    5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235 43  +    51535103    51540235    243,243,255
track name=DEKKER_5C_115:115_133_3 description=5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102 visibility=dense autoScale=off priority=3 itemRgb=On
chr2    51533038    51535102    5C_115_ENr112_REV_6|hg18|chr2:51533038-51535102 1000    +    51533038    51535102    255,165,0
chr2    51517722    51527793    5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793 292 +    51517722    51527793    170,170,255
chr2    51528741    51533037    5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037 3615    +    51528741    51533037    2,2,255
chr2    51535103    51540235    5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235 679 +    51535103    51540235    57,57,255
track name=DEKKER_5C_115:115_133_5 description=5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080 visibility=dense autoScale=off priority=5 itemRgb=On
chr2    51540236    51546080    5C_115_ENr112_REV_8|hg18|chr2:51540236-51546080 1000    +    51540236    51546080    255,165,0
chr2    51517722    51527793    5C_115_ENr112_FOR_2|hg18|chr2:51517722-51527793 261 +    51517722    51527793    179,179,255
chr2    51528741    51533037    5C_115_ENr112_FOR_5|hg18|chr2:51528741-51533037 274 +    51528741    51533037    175,175,255
chr2    51535103    51540235    5C_115_ENr112_FOR_7|hg18|chr2:51535103-51540235 1071    +    51535103    51540235    2,2,255
```

Upon upload to the UCSC genome browser, the bed file looks like:



Supplemental Data 5
An example file in the format of a FASTA file that contains the DNA sequence and genomic information of a genomic region that users can upload to My5C.primers. The genomic region of this file corresponds to Enr112.
See Supplemental File 1 for more details on this file format.
#
>hg18_dna range=chr2:51512209-52012208 5'pad=0 3'pad=0 strand=+ repeatMasking=none
AAAGAATACCCAGAAATGTGGAAGCAACCTTGGAACTGGGTAACAGACAGAGGTTGGAGGAGTT
TGGAGGCTTAGAAGAAGACAGAAAAATGTGGGAGAGTTTGGAATTCCCTAAAGACTTGTTGAAT
GGATTTGGCCAAAATGCTAATAATGATATGGACAATGAAATTCAGGCAGAGGTGGTCTCAGATG
GAGATGAGAAACTTGTTTGGAATTAGAGCAAGGTGACTTTTGTTAGTTTTGGCAAAAAGACTGG
CAGCGTTTTGCCCCTGCCCTGGAGATGTGTGAAACTTTGAACTTGAGAGAGATGATATAGGATA
TCTGGTGGGAGAAATTTCTAAGCAGCAATGCATTTAAGAGGTGACTTGTGTGTTGTTAAAGGCA
TTCAGTTTTATAAGGGAAACAGAGCATAGAAGTTTGGAAACTGCAGCCTGACCATGTGGTAGAA
AAGGAAATCCTATTTTCTGAGGAGAAATTCAAGCCAGATGCAGAAATTTGCATAAGTACTGAGG
AGCCAAATGTTAATCCCTAAGAAAATGGGAAAAAAGGTCTCCAGGACATATCAGAGGTGTTCAT
GGCAGCCCCTCTCAACACAGGCCCTGAGGCCTAGGAGGTAAAAGTGGTTTCATGGGC

Supplemental Data 6
An example file for defining variable step sizes of alternating design schemes (arbitrarily chosen for Enr122). See Supplemental File 1 for more details on this file format. As an example this file can be directly uploaded to my5C.
#

chr2	51512209	52012208	GLOBAL_40kb_spacing	20000
chr2	51612209	51912208	SEMI_20kb_spacing	10000
chr2	51712209	51852208	SPECIFIC_0kb_spacing	0

Supplemental Data 7
An example file with a list of genomic elements (arbitrarily chosen in Enr112) in the format required for upload in My5C.primers. Users can upload similar files describing elements of interest in order to design 5C primers for the overlapping restriction fragments. See Supplemental File 1 for details on on the format of this file. As an example his file can be directly uploaded to my5C.
#

CHROMOSOME	START_POSITION	END_POSITION	ELEMENT_NAME

An example is:

chr2	51512209	51522208	fake_gene1
chr2	51845055	51866223	fake_gene2
chr2	51597610	51612209	fake_gene3

Supplemental Data 8
An example file for uploading interaction data to My5C.uploads linked to a primer pool. You should use this option if you have a dataset generated by using a primerpool designed with the my5C.primers tool. This is the DEFAULT method of uploading data for most users. NOTE: this file only serves as an example of this particular format and cannot be uploaded as there is no corresponding primer design in my5C.primers.
#

5C_123_ENr112_FOR_66	5C_123_ENr112_REV_82	14
5C_123_ENr112_FOR_147	5C_123_ENr112_REV_41	1
5C_123_ENr112_FOR_118	5C_123_ENr112_REV_134	3
5C_123_ENr112_FOR_49	5C_123_ENr112_REV_81	19
5C_123_ENr112_FOR_94	5C_123_ENr112_REV_95	640
5C_123_ENr112_FOR_76	5C_123_ENr112_REV_130	1
5C_123_ENr112_FOR_33	5C_123_ENr112_REV_129	17
5C_123_ENr112_FOR_35	5C_123_ENr112_REV_75	1
5C_123_ENr112_FOR_70	5C_123_ENr112_REV_24	1
5C_123_ENr112_FOR_11	5C_123_ENr112_REV_141	33
5C_123_ENr112_FOR_23	5C_123_ENr112_REV_38	60
5C_123_ENr112_FOR_47	5C_123_ENr112_REV_79	1
5C_123_ENr112_FOR_142	5C_123_ENr112_REV_101	2
5C_123_ENr112_FOR_131	5C_123_ENr112_REV_146	6
5C_123_ENr112_FOR_5	5C_123_ENr112_REV_152	1

Supplemental Data 9
An example file for uploading interaction data to My5C.uploads linked to a *CUSTOM* primer pool. This is NOT the default method for uploading data. This method can be used for interaction data not created from a 5C design using my5C.primers. Any sort of interaction data can be used in this specified format, not just 5C data but also interaction data obtained with other methods. This file contains 5C data we obtained for ENr112 in K562 cells, as described in the supplemental material. This file can be directly uploaded to my5C. See Supplemental Data 1 for more details.
#

	ENr112_REV_4|hg18|chr2:51527935-51528740	ENr112_REV_6|hg18|chr2:51533038-51535102	ENr112_REV_8|hg18|chr2:51540236-51546080	ENr112_REV_10|hg18|chr2:51546312-51552998	ENr112_REV_15|hg18|chr2:51559484-51560996	ENr112_REV_19|hg18|chr2:51567795-51570297	ENr112_REV_21|hg18|chr2:51572373-51573197	ENr112_REV_27|hg18|chr2:51587365-51588358	ENr112_REV_30|hg18|chr2:51590634-51592096	ENr112_REV_32|hg18|chr2:51594619-51596246	ENr112_REV_35|hg18|chr2:51598772-51607737	ENr112_REV_38|hg18|chr2:51618244-51623051	ENr112_REV_40|hg18|chr2:51626806-51628357
ENr112_FOR_2|hg18|chr2:51517722-51527793	2739	292	261	358	75	82	12	115	21	15	47	60	12
ENr112_FOR_5|hg18|chr2:51528741-51533037	1305	3615	274	184	34	24	10	49	21	16	25	41	13
ENr112_FOR_7|hg18|chr2:51535103-51540235	43	679	1071	202	28	6	7	20	2	10	13	29	3
ENr112_FOR_9|hg18|chr2:51546081-51546311	26	82	2033	1900	61	15	10	21	9	0	19	34	0
ENr112_FOR_14|hg18|chr2:51554892-51559483	28	26	160	504	2581	140	26	38	15	10	14	17	10
ENr112_FOR_18|hg18|chr2:51567623-51567794	0	0	28	12	90	1108	30	6	5	0	5	0	0
ENr112_FOR_20|hg18|chr2:51570298-51572372	17	22	54	72	140	4014	3031	88	55	8	35	17	9
ENr112_FOR_26|hg18|chr2:51581328-51587364	16	17	53	88	32	68	28	4629	141	53	112	49	11
ENr112_FOR_29|hg18|chr2:51589990-51590633	0	2	30	16	1	5	7	1416	2619	136	70	55	1
ENr112_FOR_31|hg18|chr2:51592097-51594618	7	57	24	29	66	38	57	357	4139	3407	271	157	15
ENr112_FOR_34|hg18|chr2:51596572-51598771	16	30	6	55	21	25	20	37	82	695	1381	78	20
ENr112_FOR_37|hg18|chr2:51608630-51618243	31	33	25	116	16	43	11	90	48	58	503	1642	61
ENr112_FOR_39|hg18|chr2:51623052-51626805	2	10	11	11	7	14	0	12	5	10	51	1281	665
ENr112_FOR_41|hg18|chr2:51628358-51629331	0	0	10	17	0	31	9	11	10	5	36	231	33
ENr112_FOR_44|hg18|chr2:51639631-51640555	1	0	0	4	8	13	1	13	10	6	10	48	15
ENr112_FOR_46|hg18|chr2:51641891-51642390	0	1	0	0	0	16	0	0	0	0	3	12	1
ENr112_FOR_48|hg18|chr2:51642577-51643716	11	0	13	9	19	1	1	0	0	5	14	53	12
ENr112_FOR_52|hg18|chr2:51655037-51656385	0	16	24	28	14	18	4	7	9	10	0	72	15
ENr112_FOR_56|hg18|chr2:51664343-51667206	6	21	3	22	0	11	6	11	0	3	10	0	28
ENr112_FOR_59|hg18|chr2:51668252-51672775	0	9	32	8	10	8	11	18	0	10	18	62	8
ENr112_FOR_62|hg18|chr2:51677993-51680020	2	12	26	23	13	8	1	1	5	1	24	47	1
ENr112_FOR_65|hg18|chr2:51682518-51682986	0	6	5	9	0	2	0	6	17	35	15	14	26
ENr112_FOR_68|hg18|chr2:51685513-51688426	11	3	12	0	15	5	5	3	1	0	10	13	11
ENr112_FOR_70|hg18|chr2:51689539-51696453	5	11	4	14	4	13	0	11	0	13	4	26	0
ENr112_FOR_73|hg18|chr2:51700360-51701401	0	9	11	8	3	2	7	17	0	13	1	28	1
ENr112_FOR_75|hg18|chr2:51703448-51707009	9	10	11	36	14	0	13	9	9	0	9	36	14
ENr112_FOR_79|hg18|chr2:51720563-51729224	0	40	19	51	15	11	2	26	5	14	17	14	13
ENr112_FOR_83|hg18|chr2:51736099-51741376	0	3	12	24	7	11	4	9	3	3	17	49	4
ENr112_FOR_85|hg18|chr2:51742969-51743053	1	2	0	3	5	1	0	0	0	0	0	0	0
ENr112_FOR_87|hg18|chr2:51748093-51752202	1	1	5	36	0	10	0	13	8	4	12	11	3
ENr112_FOR_89|hg18|chr2:51752436-51754918	0	0	14	14	6	1	9	8	4	6	12	39	4
ENr112_FOR_91|hg18|chr2:51755043-51756564	0	0	6	41	1	9	0	0	0	5	2	21	1
ENr112_FOR_95|hg18|chr2:51760459-51761812	0	7	0	7	0	0	0	0	1	0	8	0	0
ENr112_FOR_97|hg18|chr2:51768167-51776128	8	14	16	8	37	8	2	17	1	12	5	28	9
ENr112_FOR_99|hg18|chr2:51783704-51787756	0	16	29	16	14	4	0	11	0	10	32	37	9
ENr112_FOR_104|hg18|chr2:51795919-51796489	0	3	2	5	9	0	0	0	0	0	11	16	2
ENr112_FOR_107|hg18|chr2:51797488-51798251	0	0	0	9	6	11	0	1	0	0	2	16	0
ENr112_FOR_109|hg18|chr2:51803280-51804404	0	11	25	1	9	3	6	0	12	0	12	0	0
ENr112_FOR_114|hg18|chr2:51818218-51827804	5	5	10	13	15	17	4	18	21	16	12	32	6
ENr112_FOR_117|hg18|chr2:51836323-51837378	0	0	8	4	14	5	0	8	2	0	4	8	3
ENr112_FOR_125|hg18|chr2:51859909-51860450	1164	145	246	347	96	194	33	184	74	95	124	168	37
ENr112_FOR_128|hg18|chr2:51864108-51864732	0	0	6	3	0	2	0	8	0	0	0	0	0
ENr112_FOR_132|hg18|chr2:51876281-51877086	1	0	0	0	5	0	0	0	0	1	19	0	1
ENr112_FOR_136|hg18|chr2:51883792-51884035	0	0	1	1	0	4	0	3	0	0	1	0	0
ENr112_FOR_139|hg18|chr2:51897129-51897413	0	1	8	18	0	10	3	6	0	0	7	0	4
ENr112_FOR_141|hg18|chr2:51903330-51903877	0	3	9	1	4	0	4	1	0	0	0	10	8
ENr112_FOR_144|hg18|chr2:51906989-51909043	0	2	2	5	7	1	6	7	0	5	14	0	1
ENr112_FOR_146|hg18|chr2:51912298-51913618	0	3	5	16	0	0	0	1	0	0	0	9	3
ENr112_FOR_151|hg18|chr2:51935847-51938692	0	0	9	5	9	7	6	28	16	6	3	4	7
ENr112_FOR_156|hg18|chr2:51946365-51948772	3	6	1	0	8	0	0	0	7	0	3	0	5
ENr112_FOR_161|hg18|chr2:51960177-51966168	0	5	6	4	4	10	0	0	0	0	8	0	0
ENr112_FOR_165|hg18|chr2:51973862-51980651	0	9	20	10	1	0	20	21	0	0	25	0	5
ENr112_FOR_168|hg18|chr2:51984841-51986332	0	0	2	0	0	25	5	1	0	0	0	0	1
ENr112_FOR_172|hg18|chr2:51998465-51998931	0	0	1	0	0	0	0	0	0	0	0	0	0

	ENr112_REV_43|hg18|chr2:51638642-51639630	ENr112_REV_45|hg18|chr2:51640556-51641890	ENr112_REV_47|hg18|chr2:51642391-51642576	ENr112_REV_51|hg18|chr2:51647742-51655036	ENr112_REV_54|hg18|chr2:51659035-51663983	ENr112_REV_57|hg18|chr2:51667207-51667476	ENr112_REV_60|hg18|chr2:51672776-51677281	ENr112_REV_63|hg18|chr2:51680021-51680634	ENr112_REV_67|hg18|chr2:51684894-51685512	ENr112_REV_69|hg18|chr2:51688427-51689538	ENr112_REV_72|hg18|chr2:51697752-51700359	ENr112_REV_74|hg18|chr2:51701402-51703447	ENr112_REV_78|hg18|chr2:51717363-51720562	ENr112_REV_82|hg18|chr2:51733841-51736098	ENr112_REV_84|hg18|chr2:51741377-51742968
ENr112_FOR_2|hg18|chr2:51517722-51527793	9	0	14	16	68	11	46	36	16	6	20	23	25	12	66
ENr112_FOR_5|hg18|chr2:51528741-51533037	8	0	0	2	52	4	34	11	22	10	24	5	14	11	0
ENr112_FOR_7|hg18|chr2:51535103-51540235	21	0	1	7	37	0	10	12	7	2	9	3	11	12	17
ENr112_FOR_9|hg18|chr2:51546081-51546311	12	7	8	4	30	0	1	0	4	0	6	3	21	3	5
ENr112_FOR_14|hg18|chr2:51554892-51559483	16	8	0	6	20	0	17	30	13	2	7	2	4	22	4
ENr112_FOR_18|hg18|chr2:51567623-51567794	0	0	0	5	0	0	0	0	0	3	0	0	0	0	0
ENr112_FOR_20|hg18|chr2:51570298-51572372	0	12	6	1	18	0	17	17	20	3	1	12	0	9	0
ENr112_FOR_26|hg18|chr2:51581328-51587364	9	6	0	4	18	0	20	3	18	0	9	3	13	1	13
ENr112_FOR_29|hg18|chr2:51589990-51590633	6	10	2	12	10	0	17	16	3	0	1	5	4	1	2
ENr112_FOR_31|hg18|chr2:51592097-51594618	3	28	0	12	86	1	46	27	14	5	17	6	3	11	0
ENr112_FOR_34|hg18|chr2:51596572-51598771	8	5	7	8	54	0	34	0	2	2	12	5	12	14	5
ENr112_FOR_37|hg18|chr2:51608630-51618243	114	17	7	30	84	8	50	20	19	14	53	26	15	6	12
ENr112_FOR_39|hg18|chr2:51623052-51626805	34	11	2	8	38	6	20	14	5	3	17	6	5	3	14
ENr112_FOR_41|hg18|chr2:51628358-51629331	176	13	1	32	75	1	9	0	55	0	0	8	0	0	2
ENr112_FOR_44|hg18|chr2:51639631-51640555	2886	1483	46	6	52	0	11	11	4	6	17	0	0	10	56
ENr112_FOR_46|hg18|chr2:51641891-51642390	273	1113	204	4	37	7	14	16	1	0	4	0	0	0	0
ENr112_FOR_48|hg18|chr2:51642577-51643716	127	586	448	31	39	4	16	13	33	5	16	18	0	10	24
ENr112_FOR_52|hg18|chr2:51655037-51656385	59	9	1	900	600	19	46	24	215	21	10	24	31	17	12
ENr112_FOR_56|hg18|chr2:51664343-51667206	26	18	4	15	1771	1236	171	41	25	11	0	1	14	9	10
ENr112_FOR_59|hg18|chr2:51668252-51672775	7	19	3	16	247	179	1148	63	0	26	21	1	8	10	13
ENr112_FOR_62|hg18|chr2:51677993-51680020	8	23	0	9	65	13	1704	5233	122	26	72	12	14	42	45
ENr112_FOR_65|hg18|chr2:51682518-51682986	32	0	0	5	54	16	152	632	688	36	28	7	15	5	5
ENr112_FOR_68|hg18|chr2:51685513-51688426	13	17	2	16	38	8	107	121	951	1926	70	17	22	23	31
ENr112_FOR_70|hg18|chr2:51689539-51696453	11	5	3	8	57	0	31	7	82	398	471	63	22	19	27
ENr112_FOR_73|hg18|chr2:51700360-51701401	34	3	0	10	68	6	91	65	91	70	6171	1847	80	46	13
ENr112_FOR_75|hg18|chr2:51703448-51707009	34	7	0	0	34	4	51	18	12	13	195	793	79	33	1
ENr112_FOR_79|hg18|chr2:51720563-51729224	8	12	0	25	76	4	81	44	10	21	103	25	1296	265	77
ENr112_FOR_83|hg18|chr2:51736099-51741376	4	8	1	5	50	3	28	18	2	15	37	11	38	1443	2250
ENr112_FOR_85|hg18|chr2:51742969-51743053	0	0	0	2	15	0	6	0	0	0	0	2	3	46	389
ENr112_FOR_87|hg18|chr2:51748093-51752202	5	0	0	0	14	0	13	5	5	2	35	11	56	173	530
ENr112_FOR_89|hg18|chr2:51752436-51754918	14	0	0	4	36	7	5	0	0	4	12	6	32	67	88
ENr112_FOR_91|hg18|chr2:51755043-51756564	0	0	0	0	33	9	30	22	24	5	23	8	31	24	47
ENr112_FOR_95|hg18|chr2:51760459-51761812	14	0	0	0	5	0	3	1	1	5	16	5	4	24	15
ENr112_FOR_97|hg18|chr2:51768167-51776128	29	22	3	0	78	0	49	18	7	6	45	8	63	108	47
ENr112_FOR_99|hg18|chr2:51783704-51787756	6	16	1	14	30	0	32	23	23	0	35	4	27	32	24
ENr112_FOR_104|hg18|chr2:51795919-51796489	0	1	0	0	13	0	10	0	1	0	4	0	6	24	20
ENr112_FOR_107|hg18|chr2:51797488-51798251	20	0	0	1	6	0	4	1	1	0	10	0	15	0	14
ENr112_FOR_109|hg18|chr2:51803280-51804404	0	4	0	0	16	0	1	1	23	6	24	7	4	25	26
ENr112_FOR_114|hg18|chr2:51818218-51827804	3	13	0	4	60	0	26	17	10	1	29	6	33	52	35
ENr112_FOR_117|hg18|chr2:51836323-51837378	0	0	0	8	10	0	19	0	0	0	0	8	13	6	10
ENr112_FOR_125|hg18|chr2:51859909-51860450	116	59	23	22	265	25	133	73	2291	40	221	48	99	118	195
ENr112_FOR_128|hg18|chr2:51864108-51864732	0	0	0	0	5	0	0	0	6	2	0	0	5	0	0
ENr112_FOR_132|hg18|chr2:51876281-51877086	0	0	0	0	0	0	25	0	2	0	10	0	10	0	10
ENr112_FOR_136|hg18|chr2:51883792-51884035	0	0	0	0	0	6	3	0	7	0	0	0	3	0	6
ENr112_FOR_139|hg18|chr2:51897129-51897413	0	0	3	1	7	7	1	4	8	0	0	0	7	8	0
ENr112_FOR_141|hg18|chr2:51903330-51903877	0	0	0	0	15	0	26	0	0	5	11	4	16	13	8
ENr112_FOR_144|hg18|chr2:51906989-51909043	8	3	0	0	0	0	6	1	1	0	0	0	2	5	4
ENr112_FOR_146|hg18|chr2:51912298-51913618	7	0	0	0	0	0	3	4	0	4	3	2	7	6	0
ENr112_FOR_151|hg18|chr2:51935847-51938692	9	7	0	2	5	0	7	10	12	0	1	0	12	33	0
ENr112_FOR_156|hg18|chr2:51946365-51948772	8	0	0	7	12	3	16	9	8	5	0	1	3	3	9
ENr112_FOR_161|hg18|chr2:51960177-51966168	7	0	0	9	6	0	4	0	11	0	0	3	1	3	18
ENr112_FOR_165|hg18|chr2:51973862-51980651	13	9	0	0	14	7	2	13	34	0	17	0	16	16	7
ENr112_FOR_168|hg18|chr2:51984841-51986332	8	0	0	7	9	0	3	0	2	0	0	0	3	5	0
ENr112_FOR_172|hg18|chr2:51998465-51998931	0	3	0	1	14	0	3	0	0	2	1	0	0	11	10

	ENr112_REV_86|hg18|chr2:51743054-51748092	ENr112_REV_88|hg18|chr2:51752203-51752435	ENr112_REV_90|hg18|chr2:51754919-51755042	ENr112_REV_94|hg18|chr2:51760158-51760458	ENr112_REV_96|hg18|chr2:51761813-51768166	ENr112_REV_98|hg18|chr2:51776129-51783703	ENr112_REV_103|hg18|chr2:51795875-51795918	ENr112_REV_105|hg18|chr2:51796490-51797369	ENr112_REV_108|hg18|chr2:51798252-51803279	ENr112_REV_112|hg18|chr2:51811271-51814071	ENr112_REV_115|hg18|chr2:51827805-51832838	ENr112_REV_118|hg18|chr2:51837379-51843515	ENr112_REV_126|hg18|chr2:51860451-51862390	ENr112_REV_131|hg18|chr2:51875055-51876280
ENr112_FOR_2|hg18|chr2:51517722-51527793	0	0	14	0	59	50	2	0	0	43	65	76	0	8
ENr112_FOR_5|hg18|chr2:51528741-51533037	3	0	6	2	24	25	12	0	22	0	25	52	0	5
ENr112_FOR_7|hg18|chr2:51535103-51540235	2	0	0	1	36	13	1	6	10	4	0	32	3	13
ENr112_FOR_9|hg18|chr2:51546081-51546311	0	0	0	0	0	26	0	0	0	4	0	1	2	5
ENr112_FOR_14|hg18|chr2:51554892-51559483	3	6	0	0	13	33	1	0	4	7	6	21	0	2
ENr112_FOR_18|hg18|chr2:51567623-51567794	0	0	0	0	0	0	0	1	0	1	0	0	4	13
ENr112_FOR_20|hg18|chr2:51570298-51572372	0	2	0	0	9	0	0	0	6	3	12	0	2	22
ENr112_FOR_26|hg18|chr2:51581328-51587364	5	0	0	0	11	2	0	0	14	3	20	35	0	3
ENr112_FOR_29|hg18|chr2:51589990-51590633	0	1	0	0	0	11	1	1	6	0	7	0	0	6
ENr112_FOR_31|hg18|chr2:51592097-51594618	0	0	17	9	23	16	1	0	9	8	35	33	1	14
ENr112_FOR_34|hg18|chr2:51596572-51598771	0	0	0	6	7	4	0	0	32	0	36	12	0	2
ENr112_FOR_37|hg18|chr2:51608630-51618243	4	0	24	1	44	19	14	11	22	14	9	35	0	25
ENr112_FOR_39|hg18|chr2:51623052-51626805	3	0	0	2	5	10	0	0	1	3	7	19	6	5
ENr112_FOR_41|hg18|chr2:51628358-51629331	3	19	0	0	10	15	0	1	12	2	19	5	5	10
ENr112_FOR_44|hg18|chr2:51639631-51640555	0	8	0	0	1	4	1	3	5	0	0	16	11	13
ENr112_FOR_46|hg18|chr2:51641891-51642390	0	0	0	10	0	5	0	1	0	0	2	19	0	0
ENr112_FOR_48|hg18|chr2:51642577-51643716	0	1	5	8	9	11	0	8	9	3	0	25	0	7
ENr112_FOR_52|hg18|chr2:51655037-51656385	0	5	43	2	60	27	0	20	0	5	2	21	6	36
ENr112_FOR_56|hg18|chr2:51664343-51667206	0	0	5	0	15	19	0	19	3	8	0	30	0	10
ENr112_FOR_59|hg18|chr2:51668252-51672775	4	9	8	5	10	43	9	2	11	3	2	39	3	6
ENr112_FOR_62|hg18|chr2:51677993-51680020	2	1	7	0	29	14	1	7	4	3	0	3	0	31
ENr112_FOR_65|hg18|chr2:51682518-51682986	0	1	0	9	30	31	31	0	28	27	36	0	1	55
ENr112_FOR_68|hg18|chr2:51685513-51688426	0	0	0	0	40	39	0	4	10	5	4	30	0	4
ENr112_FOR_70|hg18|chr2:51689539-51696453	4	0	0	2	17	0	0	6	9	11	8	10	0	76
ENr112_FOR_73|hg18|chr2:51700360-51701401	2	7	0	0	43	1	0	1	2	17	2	47	9	26
ENr112_FOR_75|hg18|chr2:51703448-51707009	8	4	7	0	27	24	1	7	16	4	6	30	0	6
ENr112_FOR_79|hg18|chr2:51720563-51729224	12	18	0	0	149	62	0	23	46	26	68	80	12	2
ENr112_FOR_83|hg18|chr2:51736099-51741376	44	29	5	21	102	30	3	10	32	13	31	53	0	1
ENr112_FOR_85|hg18|chr2:51742969-51743053	58	0	0	12	9	16	0	0	3	4	4	7	0	0
ENr112_FOR_87|hg18|chr2:51748093-51752202	337	1072	321	11	168	86	0	30	18	10	40	114	1	10
ENr112_FOR_89|hg18|chr2:51752436-51754918	63	913	864	92	188	51	0	22	17	20	19	13	0	6
ENr112_FOR_91|hg18|chr2:51755043-51756564	22	144	1811	361	152	82	0	14	16	14	30	40	10	8
ENr112_FOR_95|hg18|chr2:51760459-51761812	3	29	45	1674	1971	55	0	12	24	12	29	28	0	13
ENr112_FOR_97|hg18|chr2:51768167-51776128	17	0	11	90	992	1511	10	118	46	13	63	116	8	23
ENr112_FOR_99|hg18|chr2:51783704-51787756	3	9	18	46	139	1899	39	72	93	64	39	37	19	9
ENr112_FOR_104|hg18|chr2:51795919-51796489	0	0	3	1	12	27	1112	4388	109	2	17	29	0	11
ENr112_FOR_107|hg18|chr2:51797488-51798251	0	0	8	0	46	51	112	3323	1214	34	1	68	0	14
ENr112_FOR_109|hg18|chr2:51803280-51804404	0	0	2	23	62	64	17	158	3894	89	58	64	12	1
ENr112_FOR_114|hg18|chr2:51818218-51827804	32	0	14	28	76	126	11	44	119	205	1812	454	12	9
ENr112_FOR_117|hg18|chr2:51836323-51837378	3	0	6	0	17	20	9	0	18	14	647	1396	5	64
ENr112_FOR_125|hg18|chr2:51859909-51860450	36	49	76	75	148	115	152	152	51	83	233	337	1239	950
ENr112_FOR_128|hg18|chr2:51864108-51864732	0	0	0	5	8	1	2	0	3	4	7	11	184	110
ENr112_FOR_132|hg18|chr2:51876281-51877086	0	0	0	0	9	0	0	7	12	15	13	54	8	3117
ENr112_FOR_136|hg18|chr2:51883792-51884035	0	5	0	0	11	3	1	20	5	11	16	0	2	24
ENr112_FOR_139|hg18|chr2:51897129-51897413	1	7	0	0	17	5	0	10	0	0	18	21	2	16
ENr112_FOR_141|hg18|chr2:51903330-51903877	3	5	0	6	3	5	0	14	3	13	28	20	13	40
ENr112_FOR_144|hg18|chr2:51906989-51909043	0	3	1	0	23	19	1	30	15	10	16	19	9	22
ENr112_FOR_146|hg18|chr2:51912298-51913618	0	1	0	5	13	10	0	5	19	7	8	2	3	12
ENr112_FOR_151|hg18|chr2:51935847-51938692	3	5	5	1	31	7	9	25	6	3	26	8	0	15
ENr112_FOR_156|hg18|chr2:51946365-51948772	0	0	0	11	17	1	0	5	2	6	20	33	12	10
ENr112_FOR_161|hg18|chr2:51960177-51966168	0	2	0	1	9	28	0	8	3	0	17	16	5	0
ENr112_FOR_165|hg18|chr2:51973862-51980651	0	15	6	1	50	25	1	6	10	0	25	23	0	26
ENr112_FOR_168|hg18|chr2:51984841-51986332	3	13	0	1	25	18	18	0	9	6	29	30	24	16
ENr112_FOR_172|hg18|chr2:51998465-51998931	0	2	0	0	1	0	0	0	8	0	10	10	0	4

	ENr112_REV_135|hg18|chr2:51880508-51883791	ENr112_REV_138|hg18|chr2:51887965-51897128	ENr112_REV_140|hg18|chr2:51897414-51903329	ENr112_REV_143|hg18|chr2:51904789-51906988	ENr112_REV_145|hg18|chr2:51909044-51912297	ENr112_REV_147|hg18|chr2:51913619-51915640	ENr112_REV_153|hg18|chr2:51940807-51943686	ENr112_REV_159|hg18|chr2:51954379-51957550	ENr112_REV_162|hg18|chr2:51966169-51970541	ENr112_REV_166|hg18|chr2:51980652-51984761	ENr112_REV_169|hg18|chr2:51986333-51993622	ENr112_REV_175|hg18|chr2:52005124-52009401
ENr112_FOR_2|hg18|chr2:51517722-51527793	20	81	49	2	19	35	40	84	19	27	15	38
ENr112_FOR_5|hg18|chr2:51528741-51533037	9	22	0	12	11	0	6	12	9	2	22	26
ENr112_FOR_7|hg18|chr2:51535103-51540235	13	11	13	3	16	6	0	0	3	13	0	0
ENr112_FOR_9|hg18|chr2:51546081-51546311	0	1	15	0	0	0	1	8	0	0	0	7
ENr112_FOR_14|hg18|chr2:51554892-51559483	11	18	18	4	10	0	5	4	4	6	13	6
ENr112_FOR_18|hg18|chr2:51567623-51567794	0	36	0	0	10	1	0	0	0	0	14	0
ENr112_FOR_20|hg18|chr2:51570298-51572372	0	23	43	6	1	3	1	0	1	12	14	25
ENr112_FOR_26|hg18|chr2:51581328-51587364	9	17	18	3	12	0	18	29	9	0	5	2
ENr112_FOR_29|hg18|chr2:51589990-51590633	0	1	24	11	2	0	19	9	4	16	0	1
ENr112_FOR_31|hg18|chr2:51592097-51594618	15	23	10	10	19	18	25	0	1	10	19	2
ENr112_FOR_34|hg18|chr2:51596572-51598771	0	8	23	0	7	19	9	1	4	8	0	6
ENr112_FOR_37|hg18|chr2:51608630-51618243	5	33	0	0	1	0	58	18	0	10	14	17
ENr112_FOR_39|hg18|chr2:51623052-51626805	5	21	24	1	1	0	1	5	3	5	0	6
ENr112_FOR_41|hg18|chr2:51628358-51629331	0	36	1	0	17	0	29	10	0	15	11	12
ENr112_FOR_44|hg18|chr2:51639631-51640555	4	0	10	0	1	0	14	18	0	1	0	1
ENr112_FOR_46|hg18|chr2:51641891-51642390	4	0	8	0	0	1	0	6	0	0	0	0
ENr112_FOR_48|hg18|chr2:51642577-51643716	19	20	0	6	0	0	0	0	8	1	6	0
ENr112_FOR_52|hg18|chr2:51655037-51656385	13	14	14	5	2	15	36	90	26	14	18	24
ENr112_FOR_56|hg18|chr2:51664343-51667206	14	1	31	4	0	5	0	0	6	0	15	6
ENr112_FOR_59|hg18|chr2:51668252-51672775	10	6	28	6	9	11	4	21	7	2	22	14
ENr112_FOR_62|hg18|chr2:51677993-51680020	6	23	0	0	24	0	8	11	13	22	7	11
ENr112_FOR_65|hg18|chr2:51682518-51682986	17	14	28	24	57	7	3	31	0	5	16	0
ENr112_FOR_68|hg18|chr2:51685513-51688426	0	10	30	1	4	15	18	16	11	20	15	30
ENr112_FOR_70|hg18|chr2:51689539-51696453	4	5	20	6	6	7	17	16	1	10	13	24
ENr112_FOR_73|hg18|chr2:51700360-51701401	20	18	11	2	5	8	10	1	8	19	8	0
ENr112_FOR_75|hg18|chr2:51703448-51707009	6	6	6	8	0	9	8	30	8	8	16	8
ENr112_FOR_79|hg18|chr2:51720563-51729224	11	38	20	17	50	6	55	24	6	16	34	13
ENr112_FOR_83|hg18|chr2:51736099-51741376	7	15	6	16	6	10	1	0	16	30	25	0
ENr112_FOR_85|hg18|chr2:51742969-51743053	0	7	0	0	3	9	0	7	1	6	0	0
ENr112_FOR_87|hg18|chr2:51748093-51752202	11	48	7	2	52	14	42	59	0	18	48	24
ENr112_FOR_89|hg18|chr2:51752436-51754918	0	18	16	0	18	1	14	16	11	12	1	21
ENr112_FOR_91|hg18|chr2:51755043-51756564	19	31	15	0	0	0	6	33	5	8	11	0
ENr112_FOR_95|hg18|chr2:51760459-51761812	3	17	14	8	9	8	13	30	6	5	4	0
ENr112_FOR_97|hg18|chr2:51768167-51776128	7	73	13	8	13	17	87	74	14	1	24	41
ENr112_FOR_99|hg18|chr2:51783704-51787756	21	51	56	21	13	20	25	0	10	0	33	5
ENr112_FOR_104|hg18|chr2:51795919-51796489	0	24	0	0	30	4	4	0	16	0	6	0
ENr112_FOR_107|hg18|chr2:51797488-51798251	8	3	1	0	16	1	27	14	4	6	0	0
ENr112_FOR_109|hg18|chr2:51803280-51804404	11	53	69	14	18	1	23	18	2	12	31	12
ENr112_FOR_114|hg18|chr2:51818218-51827804	49	198	76	14	45	8	50	42	42	0	82	31
ENr112_FOR_117|hg18|chr2:51836323-51837378	4	42	13	0	25	2	2	38	27	8	6	0
ENr112_FOR_125|hg18|chr2:51859909-51860450	60	294	186	64	191	64	224	206	124	155	200	122
ENr112_FOR_128|hg18|chr2:51864108-51864732	5	13	0	0	5	3	0	2	9	2	10	5
ENr112_FOR_132|hg18|chr2:51876281-51877086	143	70	63	0	20	1	0	0	5	0	18	5
ENr112_FOR_136|hg18|chr2:51883792-51884035	1168	187	17	16	3	6	5	3	0	17	9	5
ENr112_FOR_139|hg18|chr2:51897129-51897413	32	2138	783	31	52	7	6	13	16	4	11	12
ENr112_FOR_141|hg18|chr2:51903330-51903877	41	428	4471	590	146	35	12	24	0	0	20	6
ENr112_FOR_144|hg18|chr2:51906989-51909043	16	170	162	1001	1816	84	29	43	12	6	12	0
ENr112_FOR_146|hg18|chr2:51912298-51913618	17	86	82	85	1990	1449	23	19	12	7	10	2
ENr112_FOR_151|hg18|chr2:51935847-51938692	13	81	60	29	65	47	1343	105	42	68	45	23
ENr112_FOR_156|hg18|chr2:51946365-51948772	14	12	30	11	44	32	472	408	101	14	51	39
ENr112_FOR_161|hg18|chr2:51960177-51966168	8	56	24	0	22	21	77	519	932	51	18	21
ENr112_FOR_165|hg18|chr2:51973862-51980651	0	57	72	7	26	7	86	190	424	2217	217	85
ENr112_FOR_168|hg18|chr2:51984841-51986332	0	7	45	6	1	10	50	56	87	3070	2588	57
ENr112_FOR_172|hg18|chr2:51998465-51998931	0	14	19	0	23	10	20	22	49	23	201	133

Supplemental Data 10

An example file for uploading interaction data to My5C.uploads linked to a *CUSTOM* primer pool. This is NOT the default method for uploading data. This method can be used for interaction data not created from a 5C design using my5C.primers. Any sort of interaction data can be used in this specified format, not just 5C data but also interaction data obtained with other methods. This file contains 5C data we obtained for ENr132 in K562 cells, as described in the supplemental material. This file can be directly uploaded to my5C. See Supplemental Data 1 for more details.
#

	5C_1717_ENr132_REV_58|hg18|chr13:12599593-112606712	5C_1717_ENr132_REV_66|hg18|chr13:12666558-112671812	5C_1717_ENr132_REV_70|hg18|chr13:12679772-112685198	5C_1717_ENr132_REV_72|hg18|chr13:12686986-112694962	5C_1717_ENr132_REV_73|hg18|chr13:12694962-112710020	5C_1717_ENr132_REV_79|hg18|chr13:12726636-112734690	5C_1717_ENr132_REV_80|hg18|chr13:12734690-112762651	5C_1717_ENr132_REV_81|hg18|chr13:12762651-112775903	5C_1717_ENr132_REV_84|hg18|chr13:12782804-112792148	5C_1717_ENr132_REV_85|hg18|chr13:12792148-112801701
5C_1717_ENr132_FOR_55|hg18|chr13:112586352-112590823	8	0	0	24	6	19	9	7	0	9
5C_1717_ENr132_FOR_56|hg18|chr13:112590823-112594321	339	1	38	47	21	50	40	31	21	3
5C_1717_ENr132_FOR_57|hg18|chr13:112594321-112599593	126	0	0	2	0	1	2	4	0	0
5C_1717_ENr132_FOR_59|hg18|chr13:112606712-112609250	2096	0	0	41	0	108	41	77	0	61
5C_1717_ENr132_FOR_60|hg18|chr13:112609250-112612436	919	0	71	122	57	196	153	32	36	23
5C_1717_ENr132_FOR_61|hg18|chr13:112612436-112615712	249	3	114	131	27	155	68	28	0	9
5C_1717_ENr132_FOR_62|hg18|chr13:112615712-112634863	224	30	328	380	223	685	168	249	98	136
5C_1717_ENr132_FOR_63|hg18|chr13:112634863-112647248	161	8	100	33	148	569	273	53	184	14
5C_1717_ENr132_FOR_64|hg18|chr13:112647248-112663512	25	27	221	247	102	457	390	151	70	43
5C_1717_ENr132_FOR_65|hg18|chr13:112663512-112666558	10	390	217	462	191	425	470	111	53	40
5C_1717_ENr132_FOR_67|hg18|chr13:112671812-112672333	0	187	252	109	0	47	27	35	0	0
5C_1717_ENr132_FOR_68|hg18|chr13:112672333-112672849	0	25	111	17	7	6	0	8	0	0
5C_1717_ENr132_FOR_69|hg18|chr13:112672849-112679772	10	31	1523	400	100	171	105	66	38	8
5C_1717_ENr132_FOR_71|hg18|chr13:112685198-112686986	45	0	9108	5606	641	319	199	60	114	1
5C_1717_ENr132_FOR_74|hg18|chr13:112710020-112710122	17	28	105	510	1332	322	178	31	60	0
5C_1717_ENr132_FOR_76|hg18|chr13:112711617-112712622	17	14	82	257	1130	729	222	19	0	31
5C_1717_ENr132_FOR_77|hg18|chr13:112712622-112724841	0	0	86	143	139	1337	153	92	61	79
5C_1717_ENr132_FOR_78|hg18|chr13:112724841-112726636	16	0	119	401	387	5993	509	142	101	79
5C_1717_ENr132_FOR_82|hg18|chr13:112775903-112777978	0	0	26	40	28	259	884	3049	619	86
5C_1717_ENr132_FOR_83|hg18|chr13:112777978-112782804	1	0	51	65	48	169	579	661	1804	473
5C_1717_ENr132_FOR_87|hg18|chr13:112816824-112824287	0	21	18	65	27	88	512	152	217	296

Supplemental Data 11
An example of data display in the UCSC genome browser. This is a TIFF file. The 5C data is for Enr112.


RefSeq Genes
Human mRNAs
Spliced ESTs
Mammal Cons
Multiz Alignments of 44 Vertebrates
Rhesus
Mouse
Dog
Elephant
Platypus
Chicken
Lizard
Stickleback
SNPs (129)
RepeatMasker

Supplemental Data 12
DNA sequences of 5C primers used for analysis of the conformation of ENr112. This is the standard output of My5C.primers. This is a Tab-delimited text file. See supplemental Data 1 for details.
#

PRIMER NAME	REGION	TYPE	ASSEMBLY	CHROMOSOME	FRAGMENT_ID	PRIMER ID	P_STARTPOS	P_ENDPOS	P_SPECIFIC	P_SPECIFIC SIZE	P_FILLER	P_FILLER SIZE	P_TM	P_GC	F_STARTPOS	F_ENDPOS	F_SIZE	ELEMENTID	INTERSECTION ID	E_NAME	P_MER	P_UBLAST	P_SBLAST	TAIL	BARCODE_SEQ	BARCODE_NUM	PRIMER_SEQUENCE
5C_123_ENr112_FOR_2	ENr112	FOR	hg18_dna	chr2	2	1	51527754	51527793	TAAGTGGAAACAGTCTGAACATTGTATCAACTCAGGTAAG	40		0	64.03	37.5	51517722	51527793	10071	223	156		212	1	7	CCTCTCTATGGGCAGTCGGTGAT	CGACCA	898	CCTCTCTATGGGCAGTCGGTGATCGACCATAAGTGGAAACAGTCTGAACATTGTATCAACTCAGGTAAG
5C_123_ENr112_FOR_5	ENr112	FOR	hg18_dna	chr2	5	1	51532998	51533037	TAAACTAGTCAGGCTCTTCTGAATCCTCTTCCCAACTAAG	40		0	64.68	48.48	51528741	51533037	4296	223	168		470	1	28	CCTCTCTATGGGCAGTCGGTGAT	TTGTGC	1500	CCTCTCTATGGGCAGTCGGTGATTTGTGCTAAACTAGTCAGGCTCTTCTGAATCCTCTTCCCAACTAAG
5C_123_ENr112_FOR_7	ENr112	FOR	hg18_dna	chr2	7	1	51540196	51540235	ACAAGGAATAGACTACTTAATTTTATTCACTAGGACAAAG	40		0	60.19	30	51535103	51540235	5132	223	175		590	1	17	CCTCTCTATGGGCAGTCGGTGAT	ATGAAT	2537	CCTCTCTATGGGCAGTCGGTGATATGAATACAAGGAATAGACTACTTAATTTTATTCACTAGGACAAAG
5C_123_ENr112_FOR_9	ENr112	FOR	hg18_dna	chr2	9	1	51546276	51546311	CAATCCTCTCTGACTTGTCCCACCAGCATCATAAAG	36	TGCG	4	63.76	48.39	51546081	51546311	230	223	184		264	1	18	CCTCTCTATGGGCAGTCGGTGAT	GGTAAG	3947	CCTCTCTATGGGCAGTCGGTGATGGTAAGTGCGCAATCCTCTCTGACTTGTCCCACCAGCATCATAAAG
5C_123_ENr112_FOR_14	ENr112	FOR	hg18_dna	chr2	14	1	51559444	51559483	ATGTGTATCTATGACCATCTGATGTTAAAGACTTCGAAAG	40		0	62.7	35	51554892	51559483	4591	223	144		498	1	4	CCTCTCTATGGGCAGTCGGTGAT	TTTCCT	1345	CCTCTCTATGGGCAGTCGGTGATTTTCCTATGTGTATCTATGACCATCTGATGTTAAAGACTTCGAAAG
5C_123_ENr112_FOR_18	ENr112	FOR	hg18_dna	chr2	18	1	51567755	51567794	TTCCTTTGGGGATGAGTATCCAGTATTCCTACTCTCTAAG	40		0	64.47	44.74	51567623	51567794	171	223	155		200	1	15	CCTCTCTATGGGCAGTCGGTGAT	AAACTC	2692	CCTCTCTATGGGCAGTCGGTGATAAACTCTTCCTTTGGGGATGAGTATCCAGTATTCCTACTCTCTAAG
5C_123_ENr112_FOR_20	ENr112	FOR	hg18_dna	chr2	20	1	51572334	51572372	CTGAGACAAGAAACAGGGGTCGATAGATAGGATACTAAG	39	G	1	64.35	43.59	51570298	51572372	2074	223	157		163	1	4	CCTCTCTATGGGCAGTCGGTGAT	CGATTA	918	CCTCTCTATGGGCAGTCGGTGATCGATTAGCTGAGACAAGAAACAGGGGTCGATAGATAGGATACTAAG
5C_123_ENr112_FOR_26	ENr112	FOR	hg18_dna	chr2	26	1	51587325	51587364	CCCAGCCTTTCCAAAGATTTAGCCATCAAAATGCAAGAAG	40		0	64.8	37.84	51581328	51587364	6036	223	158		403	1	43	CCTCTCTATGGGCAGTCGGTGAT	TTAGAC	1464	CCTCTCTATGGGCAGTCGGTGATTTAGACCCCAGCCTTTCCAAAGATTTAGCCATCAAAATGCAAGAAG
5C_123_ENr112_FOR_29	ENr112	FOR	hg18_dna	chr2	29	1	51590595	51590633	CTCATTCTCTCTGGAGGCTAGTTGTTCCATTGTTGAAAG	39	G	1	64.78	41.67	51589990	51590633	643	223	159		325	1	27	CCTCTCTATGGGCAGTCGGTGAT	CGCGCT	817	CCTCTCTATGGGCAGTCGGTGATCGCGCTGCTCATTCTCTCTGGAGGCTAGTTGTTCCATTGTTGAAAG
5C_123_ENr112_FOR_31	ENr112	FOR	hg18_dna	chr2	31	1	51594579	51594618	CATGTTCCTATCCTCATCTCCATGTGATGATTGAGAGAAG	40		0	64.86	41.03	51592097	51594618	2521	223	160		445	1	14	CCTCTCTATGGGCAGTCGGTGAT	CAGGGG	767	CCTCTCTATGGGCAGTCGGTGATCAGGGGCATGTTCCTATCCTCATCTCCATGTGATGATTGAGAGAAG
5C_123_ENr112_FOR_34	ENr112	FOR	hg18_dna	chr2	34	1	51598732	51598771	ATTATTATGCCTTATCTTAGGTTATGCCTGGACTCAAAAG	40		0	62.99	35	51596572	51598771	2199	223	161		208	1	22	CCTCTCTATGGGCAGTCGGTGAT	CACAAC	552	CCTCTCTATGGGCAGTCGGTGATCACAACATTATTATGCCTTATCTTAGGTTATGCCTGGACTCAAAAG
5C_123_ENr112_FOR_37	ENr112	FOR	hg18_dna	chr2	37	1	51618204	51618243	TTTCCAGTTACATAATTCGTAGCACTTCCAGATACCAAG	39		0	64.35	40	51608630	51618243	9613	223	162		156	1	15	CCTCTCTATGGGCAGTCGGTGAT	AGACCC	2944	CCTCTCTATGGGCAGTCGGTGATAGACCCCTTTCCAGTTACATAATTCGTAGCACTTCCAGATACCAAG
5C_123_ENr112_FOR_39	ENr112	FOR	hg18_dna	chr2	39	1	51626766	51626805	TGGTCTGTACAATATTTTATAGACCCATTAATTTAAAAAG	40		0	58.46	25	51623052	51626805	3753	223	163		735	1	112	CCTCTCTATGGGCAGTCGGTGAT	TCTAAA	1130	CCTCTCTATGGGCAGTCGGTGATTCTAAATGGTCTGTACAATATTTTATAGACCCATTAATTTAAAAAG
5C_123_ENr112_FOR_41	ENr112	FOR	hg18_dna	chr2	41	1	51629292	51629331	CATATGTATGTTATGTTAGTGATTAACATATTTCAAAAAG	40		0	56.6	22.5	51628358	51629331	973	223	164		699	2	91	CCTCTCTATGGGCAGTCGGTGAT	TAAGGG	1727	CCTCTCTATGGGCAGTCGGTGATTAAGGGCATATGTATGTTATGTTAGTGATTAACATATTTCAAAAAG
5C_123_ENr112_FOR_44	ENr112	FOR	hg18_dna	chr2	44	1	51640516	51640555	TTAGGATAATTATTTGGCCTTCATTCTTTAGAAAAAGAAG	40		0	59.91	27.5	51639631	51640555	924	223	165		860	1	81	CCTCTCTATGGGCAGTCGGTGAT	GTGGGC	3580	CCTCTCTATGGGCAGTCGGTGATGTGGGCTTAGGATAATTATTTGGCCTTCATTCTTTAGAAAAAGAAG
5C_123_ENr112_FOR_46	ENr112	FOR	hg18_dna	chr2	46	1	51642351	51642390	TTAAAAATGCTGTTGATTCAAACATGAAACTGGAAAAAAG	40		0	61.7	27.5	51641891	51642390	499	223	166		646	1	114	CCTCTCTATGGGCAGTCGGTGAT	CCCACA	34	CCTCTCTATGGGCAGTCGGTGATCCCACATTAAAAATGCTGTTGATTCAAACATGAAACTGGAAAAAAG
5C_123_ENr112_FOR_48	ENr112	FOR	hg18_dna	chr2	48	1	51643677	51643716	TTTCTTCACTCTGGTAGTTGTTTCTTATGCTATTCAGAAG	40		0	62.93	35	51642577	51643716	1139	223	167		325	1	40	CCTCTCTATGGGCAGTCGGTGAT	GTTCGA	3406	CCTCTCTATGGGCAGTCGGTGATGTTCGATTTCTTCACTCTGGTAGTTGTTTCTTATGCTATTCAGAAG
5C_123_ENr112_FOR_52	ENr112	FOR	hg18_dna	chr2	52	1	51656352	51656385	TGCCAGCATCTACTTCTGTGAGCATCTCAGGAAG	34	CAAAAC	6	64.89	50	51655037	51656385	1348	223	169		441	1	27	CCTCTCTATGGGCAGTCGGTGAT	TGTACG	1891	CCTCTCTATGGGCAGTCGGTGATTGTACGCAAAACTGCCAGCATCTACTTCTGTGAGCATCTCAGGAAG
5C_123_ENr112_FOR_56	ENr112	FOR	hg18_dna	chr2	56	1	51667167	51667206	GAAACATTTCAAAGAAATGAATAGCTAGTATCCCACTAAG	40		0	61.08	32.5	51664343	51667206	2863	223	170		963	1	4	CCTCTCTATGGGCAGTCGGTGAT	ATGCTT	2501	CCTCTCTATGGGCAGTCGGTGATATGCTTGAAACATTTCAAAGAAATGAATAGCTAGTATCCCACTAAG
5C_123_ENr112_FOR_59	ENr112	FOR	hg18_dna	chr2	59	1	51672736	51672775	ATTTTTATAACATCATAAACGAACTTGACACCTTAAAAAG	40		0	59.31	25	51668252	51672775	4523	223	171		354	1	15	CCTCTCTATGGGCAGTCGGTGAT	CACTCA	530	CCTCTCTATGGGCAGTCGGTGATCACTCAATTTTTATAACATCATAAACGAACTTGACACCTTAAAAAG
5C_123_ENr112_FOR_62	ENr112	FOR	hg18_dna	chr2	62	1	51679981	51680020	TATTGCCATTAGAGGGAAATAGAGGAGCCACAAAACCAAG	40		0	63.87	44.12	51677993	51680020	2027	223	172		312	1	28	CCTCTCTATGGGCAGTCGGTGAT	TTGTGT	1501	CCTCTCTATGGGCAGTCGGTGATTTGTGTTATTGCCATTAGAGGGAAATAGAGGAGCCACAAAACCAAG
5C_123_ENr112_FOR_65	ENr112	FOR	hg18_dna	chr2	65	1	51682953	51682986	GGATGAACCTGACTTGAAGGGGATGGTGGATAAG	34	CACGAC	6	64.88	46.88	51682518	51682986	468	223	173		215	1	43	CCTCTCTATGGGCAGTCGGTGAT	CCTTAG	91	CCTCTCTATGGGCAGTCGGTGATCCTTAGCACGACGGATGAACCTGACTTGAAGGGGATGGTGGATAAG
5C_123_ENr112_FOR_68	ENr112	FOR	hg18_dna	chr2	68	1	51688387	51688426	TGGTCTTATAAAATATTATCACAGCCATTAAGATGAAAAG	40		0	59.64	27.5	51685513	51688426	2913	223	174		820	2	34	CCTCTCTATGGGCAGTCGGTGAT	CGGTCT	977	CCTCTCTATGGGCAGTCGGTGATCGGTCTTGGTCTTATAAAATATTATCACAGCCATTAAGATGAAAAG
5C_123_ENr112_FOR_70	ENr112	FOR	hg18_dna	chr2	70	1	51696416	51696453	TGAATGCTTTTAAGAGCACCCAAGGCACCTCTTGAAAG	38	AT	2	64.73	46.88	51689539	51696453	6914	223	176		606	1	44	CCTCTCTATGGGCAGTCGGTGAT	GGGCCA	4034	CCTCTCTATGGGCAGTCGGTGATGGGCCAATTGAATGCTTTTAAGAGCACCCAAGGCACCTCTTGAAAG
5C_123_ENr112_FOR_73	ENr112	FOR	hg18_dna	chr2	73	1	51701362	51701401	ACTACCTTGTTCAGGTAATCAGCATTTCTTGGGCACAAAG	40		0	64.94	42.86	51700360	51701401	1041	223	177		271	1	10	CCTCTCTATGGGCAGTCGGTGAT	GAGTGC	3804	CCTCTCTATGGGCAGTCGGTGATGAGTGCACTACCTTGTTCAGGTAATCAGCATTTCTTGGGCACAAAG
5C_123_ENr112_FOR_75	ENr112	FOR	hg18_dna	chr2	75	1	51706976	51707009	CCTGGTAACACAGGTGTAGGAAGTTTCCCTGAAG	34	CGAGAC	6	64.94	48.48	51703448	51707009	3561	223	178		190	1	30	CCTCTCTATGGGCAGTCGGTGAT	CCTCGG	79	CCTCTCTATGGGCAGTCGGTGATCCTCGGCGAGACCCTGGTAACACAGGTGTAGGAAGTTTCCCTGAAG
5C_123_ENr112_FOR_79	ENr112	FOR	hg18_dna	chr2	79	1	51729185	51729224	TACAAGTCTGTCTGAGCCTTTACTTCTGATTTCCACAAAG	40		0	64.72	40.54	51720563	51729224	8661	223	179		419	1	25	CCTCTCTATGGGCAGTCGGTGAT	CCGATC	228	CCTCTCTATGGGCAGTCGGTGATCCGATCTACAAGTCTGTCTGAGCCTTTACTTCTGATTTCCACAAAG
5C_123_ENr112_FOR_83	ENr112	FOR	hg18_dna	chr2	83	1	51741337	51741376	AAGTAGTTAATTTCTATAGACAAATGTTAAAGATTAGAAG	40		0	56.63	22.5	51736099	51741376	5277	223	180		493	1	33	CCTCTCTATGGGCAGTCGGTGAT	CCCTCT	17	CCTCTCTATGGGCAGTCGGTGATCCCTCTAAGTAGTTAATTTCTATAGACAAATGTTAAAGATTAGAAG
5C_123_ENr112_FOR_85	ENr112	FOR	hg18_dna	chr2	85	1	51743014	51743053	CCCCAAACCCTCTTTCCTTTAACCTAAAACATTATGTAAG	40		0	63.73	37.5	51742969	51743053	84	223	181		774	1	28	CCTCTCTATGGGCAGTCGGTGAT	CCGAAC	232	CCTCTCTATGGGCAGTCGGTGATCCGAACCCCCAAACCCTCTTTCCTTTAACCTAAAACATTATGTAAG
5C_123_ENr112_FOR_87	ENr112	FOR	hg18_dna	chr2	87	1	51752163	51752202	GAGGATGTTTTTGGGTGAGATTTGCTTTTGCAATGGCAAG	40		0	64.72	39.39	51748093	51752202	4109	223	182		376	1	16	CCTCTCTATGGGCAGTCGGTGAT	ACAACG	2211	CCTCTCTATGGGCAGTCGGTGATACAACGGAGGATGTTTTTGGGTGAGATTTGCTTTTGCAATGGCAAG
5C_123_ENr112_FOR_89	ENr112	FOR	hg18_dna	chr2	89	1	51754879	51754918	CTATTCCATATAGGACTGTAATATAACATGTGCTTCAAAG	40		0	60.45	32.5	51752436	51754918	2482	223	183		278	1	27	CCTCTCTATGGGCAGTCGGTGAT	TAATCA	1682	CCTCTCTATGGGCAGTCGGTGATTAATCACTATTCCATATAGGACTGTAATATAACATGTGCTTCAAAG
5C_123_ENr112_FOR_91	ENr112	FOR	hg18_dna	chr2	91	1	51756525	51756564	GGCCTGATAGACTAAAGAGACTGTGTTGATGCATTAAAAG	40		0	64.66	40	51755043	51756564	1521	223	185		203	1	19	CCTCTCTATGGGCAGTCGGTGAT	CATGCC	624	CCTCTCTATGGGCAGTCGGTGATCATGCCGGCCTGATAGACTAAAGAGACTGTGTTGATGCATTAAAAG
5C_123_ENr112_FOR_95	ENr112	FOR	hg18_dna	chr2	95	1	51761773	51761812	GTCTTCTGATACTACTGTTAGAATTTTCTGCCTGTGAAAG	40		0	63.16	37.5	51760459	51761812	1353	223	186		500	1	33	CCTCTCTATGGGCAGTCGGTGAT	GGGGTC	4084	CCTCTCTATGGGCAGTCGGTGATGGGGTCGTCTTCTGATACTACTGTTAGAATTTTCTGCCTGTGAAAG
5C_123_ENr112_FOR_97	ENr112	FOR	hg18_dna	chr2	97	1	51776089	51776128	TGTTCAGAGTAATATACCAGAGTAAGTACCATTTTATAAG	40		0	59.5	30	51768167	51776128	7961	223	187		306	1	45	CCTCTCTATGGGCAGTCGGTGAT	CGATGT	925	CCTCTCTATGGGCAGTCGGTGATCGATGTTGTTCAGAGTAATATACCAGAGTAAGTACCATTTTATAAG
5C_123_ENr112_FOR_99	ENr112	FOR	hg18_dna	chr2	99	1	51787717	51787756	AAGGACATGTGGAACATCATGTATTAAGTATTAAATAAAG	40		0	59.6	27.5	51783704	51787756	4052	223	188		703	1	99	CCTCTCTATGGGCAGTCGGTGAT	TTGTCG	1491	CCTCTCTATGGGCAGTCGGTGATTTGTCGAAGGACATGTGGAACATCATGTATTAAGTATTAAATAAAG
5C_123_ENr112_FOR_104	ENr112	FOR	hg18_dna	chr2	104	1	51796450	51796489	TCAAGGAGGCATGATTAGATTCTGATTGTGACTAAGAAAG	40		0	64.07	37.5	51795919	51796489	570	223	134		250	1	12	CCTCTCTATGGGCAGTCGGTGAT	ACGGCT	2289	CCTCTCTATGGGCAGTCGGTGATACGGCTTCAAGGAGGCATGATTAGATTCTGATTGTGACTAAGAAAG
5C_123_ENr112_FOR_107	ENr112	FOR	hg18_dna	chr2	107	1	51798212	51798251	AGAAACAATGACTCATGAGAAGCTAGTAGATGTGAACAAG	40		0	64.04	37.5	51797488	51798251	763	223	135		219	1	14	CCTCTCTATGGGCAGTCGGTGAT	ACGGCG	2291	CCTCTCTATGGGCAGTCGGTGATACGGCGAGAAACAATGACTCATGAGAAGCTAGTAGATGTGAACAAG
5C_123_ENr112_FOR_109	ENr112	FOR	hg18_dna	chr2	109	1	51804365	51804404	TTATACAGGGTGATTAGGGAATCTCTTGCTGATATTTAAG	40		0	62.39	35	51803280	51804404	1124	223	136		275	1	23	CCTCTCTATGGGCAGTCGGTGAT	CGACAG	907	CCTCTCTATGGGCAGTCGGTGATCGACAGTTATACAGGGTGATTAGGGAATCTCTTGCTGATATTTAAG
5C_123_ENr112_FOR_114	ENr112	FOR	hg18_dna	chr2	114	1	51827765	51827804	CTCAGTAATATTTGCTTAATATGCTGATAGGCTTATCAAG	40		0	60.77	32.5	51818218	51827804	9586	223	137		620	1	8	CCTCTCTATGGGCAGTCGGTGAT	TTGGTA	1526	CCTCTCTATGGGCAGTCGGTGATTTGGTACTCAGTAATATTTGCTTAATATGCTGATAGGCTTATCAAG
5C_123_ENr112_FOR_117	ENr112	FOR	hg18_dna	chr2	117	1	51837339	51837378	TTACAAGACATTTATAAGAACAACTGAATTCTAAGGAAAG	40		0	59.58	27.5	51836323	51837378	1055	223	138		690	1	94	CCTCTCTATGGGCAGTCGGTGAT	GAAAAG	3755	CCTCTCTATGGGCAGTCGGTGATGAAAAGTTACAAGACATTTATAAGAACAACTGAATTCTAAGGAAAG
5C_123_ENr112_FOR_128	ENr112	FOR	hg18_dna	chr2	128	1	51864693	51864732	TAATTAAATGCAGTTGATGGGACAGTGGTTTCCACGCAAG	40		0	64.96	53.85	51864108	51864732	624	223	140		134	1	2	CCTCTCTATGGGCAGTCGGTGAT	AATAGT	2669	CCTCTCTATGGGCAGTCGGTGATAATAGTTAATTAAATGCAGTTGATGGGACAGTGGTTTCCACGCAAG
5C_123_ENr112_FOR_132	ENr112	FOR	hg18_dna	chr2	132	1	51877047	51877086	AAACAAGTAGAGAAGAGAACTTAAAATCTCAAAGACAAAG	40		0	60.88	30	51876281	51877086	805	223	141		800	1	93	CCTCTCTATGGGCAGTCGGTGAT	GCGACT	3297	CCTCTCTATGGGCAGTCGGTGATGCGACTAAACAAGTAGAGAAGAGAACTTAAAATCTCAAAGACAAAG
5C_123_ENr112_FOR_136	ENr112	FOR	hg18_dna	chr2	136	1	51883996	51884035	AAATTACATATTTTTCATCAATGGCTCTTACTTTAGGAAG	40		0	60.04	27.5	51883792	51884035	243	223	142		938	1	11	CCTCTCTATGGGCAGTCGGTGAT	ACGAAC	2280	CCTCTCTATGGGCAGTCGGTGATACGAACAAATTACATATTTTTCATCAATGGCTCTTACTTTAGGAAG
5C_123_ENr112_FOR_139	ENr112	FOR	hg18_dna	chr2	139	1	51897374	51897413	TCATCCATTTATTTTTCGATTATTTAAGTATTCACACAAG	40		0	58.97	25	51897129	51897413	284	223	143		571	1	20	CCTCTCTATGGGCAGTCGGTGAT	TAAAGG	1711	CCTCTCTATGGGCAGTCGGTGATTAAAGGTCATCCATTTATTTTTCGATTATTTAAGTATTCACACAAG
5C_123_ENr112_FOR_141	ENr112	FOR	hg18_dna	chr2	141	1	51903838	51903877	TATAATCAAGAGTCTGCTAACTGATGACTGAAAATAGAAG	40		0	61.16	32.5	51903330	51903877	547	223	145		345	1	41	CCTCTCTATGGGCAGTCGGTGAT	TCTGCA	1138	CCTCTCTATGGGCAGTCGGTGATTCTGCATATAATCAAGAGTCTGCTAACTGATGACTGAAAATAGAAG
5C_123_ENr112_FOR_144	ENr112	FOR	hg18_dna	chr2	144	1	51909004	51909043	ATAAATATAGAGTGATTCACATAAAATCTGAATCTGAAAG	40		0	58.04	25	51906989	51909043	2054	223	146		624	1	68	CCTCTCTATGGGCAGTCGGTGAT	ATGGTG	2551	CCTCTCTATGGGCAGTCGGTGATATGGTGATAAATATAGAGTGATTCACATAAAATCTGAATCTGAAAG
5C_123_ENr112_FOR_146	ENr112	FOR	hg18_dna	chr2	146	1	51913579	51913618	CCCCTTCATTAATCATTTATATACTAGGGAAATTTTTAAG	40		0	58.46	27.5	51912298	51913618	1320	223	147		674	1	42	CCTCTCTATGGGCAGTCGGTGAT	CATTTA	598	CCTCTCTATGGGCAGTCGGTGATCATTTACCCCTTCATTAATCATTTATATACTAGGGAAATTTTTAAG
5C_123_ENr112_FOR_151	ENr112	FOR	hg18_dna	chr2	151	1	51938653	51938692	TGCTCATAATTTTTAGGACAGACCACTAGAGAAAAAGAAG	40		0	62.99	35	51935847	51938692	2845	223	148		523	1	61	CCTCTCTATGGGCAGTCGGTGAT	TCCCAG	1035	CCTCTCTATGGGCAGTCGGTGATTCCCAGTGCTCATAATTTTTAGGACAGACCACTAGAGAAAAAGAAG
5C_123_ENr112_FOR_156	ENr112	FOR	hg18_dna	chr2	156	1	51948733	51948772	TTAGACATATACAATGTCTTCATGGGTCATCAGTTCAAAG	40		0	62.92	35	51946365	51948772	2407	223	149		268	1	13	CCTCTCTATGGGCAGTCGGTGAT	TCCGTT	1077	CCTCTCTATGGGCAGTCGGTGATTCCGTTTTAGACATATACAATGTCTTCATGGGTCATCAGTTCAAAG
5C_123_ENr112_FOR_161	ENr112	FOR	hg18_dna	chr2	161	1	51966130	51966168	AAGACATGTGTCATGGCTTTCAGTAGGGGACTTGAAAAG	39	A	1	64.37	46.88	51960177	51966168	5991	223	150		170	1	17	CCTCTCTATGGGCAGTCGGTGAT	CAAAAA	682	CCTCTCTATGGGCAGTCGGTGATCAAAAAAAAGACATGTGTCATGGCTTTCAGTAGGGGACTTGAAAAG
5C_123_ENr112_FOR_165	ENr112	FOR	hg18_dna	chr2	165	1	51980616	51980651	GTGATGCTGGCAATTCACATATGCCAAAGAGGGAAG	36	TAAA	4	63.45	46.67	51973862	51980651	6789	223	151		320	1	18	CCTCTCTATGGGCAGTCGGTGAT	AACGAG	2619	CCTCTCTATGGGCAGTCGGTGATAACGAGTAAAGTGATGCTGGCAATTCACATATGCCAAAGAGGGAAG
5C_123_ENr112_FOR_168	ENr112	FOR	hg18_dna	chr2	168	1	51986293	51986332	ATACGTCATGAACAAAAGGTAATGAGGAGGGAATGAAAAG	40		0	64.64	37.5	51984841	51986332	1491	223	152		380	1	45	CCTCTCTATGGGCAGTCGGTGAT	ATGCGA	2510	CCTCTCTATGGGCAGTCGGTGATATGCGAATACGTCATGAACAAAAGGTAATGAGGAGGGAATGAAAAG
5C_123_ENr112_FOR_172	ENr112	FOR	hg18_dna	chr2	172	1	51998892	51998931	TTACCACTTCCTACAATAAAAAAAAGTGTGAGTAAGAAG	40		0	61.06	30	51998465	51998931	466	223	153		1093	1	23	CCTCTCTATGGGCAGTCGGTGAT	AGATGC	2972	CCTCTCTATGGGCAGTCGGTGATAGATGCTTACCACTTCCTACAATAAAAAAAAGTGTGAGTAAGAAG
5C_123_ENr112_FOR_176	ENr112	FOR	hg18_dna	chr2	176	1	52011584	52011623	TTCAGTCGTTCAGATGATACTTAAATCTTTGTCAGCAAAG	40		0	63.41	35	52009402	52012208	2806	223	154		364	1	23	CCTCTCTATGGGCAGTCGGTGAT	CTAACC	416	CCTCTCTATGGGCAGTCGGTGATCTAACCTTCAGTCGTTCAGATGATACTTAAATCTTTGTCAGCAAAG
5C_123_ENr112_REV_4	ENr112	REV	hg18_dna	chr2	4	2	51528701	51528740	CTTGATAGTTAGCAAGAAGATTTTGAAAACTGTTTTTGGG	40		0	62.24	32.5	51527935	51528740	805	224	32		1000	1	12	AGAGAATGAGGAACCCGGGGCAG	AACATT	2677	CTTGATAGTTAGCAAGAAGATTTTGAAAACTGTTTTTGGGAACATTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_6	ENr112	REV	hg18_dna	chr2	6	2	51535063	51535102	CTTTGGAAAAGTACTGCCAGTTCTTCTGGCTTTCGAATTT	40		0	64.62	44.12	51533038	51535102	2064	224	40		394	1	16	AGAGAATGAGGAACCCGGGGCAG	CCCTGC	3263	CTTTGGAAAAGTACTGCCAGTTCTTCTGGCTTTCGAATTTCCCTGCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_8	ENr112	REV	hg18_dna	chr2	8	2	51546041	51546080	CTTCTGCCTTTCATAACCCTCCGTCTTCATATAAGAAAGA	40		0	64.38	41.03	51540236	51546080	5844	224	48		199	1	24	AGAGAATGAGGAACCCGGGGCAG	TCTGCT	2862	CTTCTGCCTTTCATAACCCTCCGTCTTCATATAAGAAAGATCTGCTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_10	ENr112	REV	hg18_dna	chr2	10	2	51552959	51552998	CTTTTATTTGTACGTTTTGGCTTCTTCCCTCAGCCCAATT	40		0	64.73	42.86	51546312	51552998	6686	224	3		304	1	19	AGAGAATGAGGAACCCGGGGCAG	CGGTGC	3203	CTTTTATTTGTACGTTTTGGCTTCTTCCCTCAGCCCAATTCGGTGCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_15	ENr112	REV	hg18_dna	chr2	15	2	51560957	51560996	CTTCAAATTTCATGCCATAGTTTCAATTCATTTTGAGTTG	40		0	61.63	30	51559484	51560996	1512	224	18		1319	1	27	AGAGAATGAGGAACCCGGGGCAG	GCGCCC	4044	CTTCAAATTTCATGCCATAGTTTCAATTCATTTTGAGTTGGCGCCCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_19	ENr112	REV	hg18_dna	chr2	19	2	51570259	51570297	CTTCAGCATGGAGACTGGTTACCAGAAAAACTAGTGTGT	39	A	1	64.93	44.44	51567795	51570297	2502	224	25		308	1	21	AGAGAATGAGGAACCCGGGGCAG	AGTTTC	3745	CTTCAGCATGGAGACTGGTTACCAGAAAAACTAGTGTGTAAGTTTCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_21	ENr112	REV	hg18_dna	chr2	21	2	51573158	51573197	CTTTTTACCAGTTTAAGGATTCTTATATGCTCTCTCTTT	40		0	60.67	30	51572373	51573197	824	224	26		512	1	23	AGAGAATGAGGAACCCGGGGCAG	GGGTTA	1664	CTTTTTACCAGTTTAAGGATTCTTATATGCTCTCTCTTTGGGTTAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_27	ENr112	REV	hg18_dna	chr2	27	2	51588319	51588358	CTTAACTACTTGCTCTCTGATTGTCAGTTTTTACAGTGTC	40		0	63.31	37.5	51587365	51588358	993	224	27		368	1	10	AGAGAATGAGGAACCCGGGGCAG	TAGCCG	966	CTTAACTACTTGCTCTCTGATTGTCAGTTTTTACAGTGTCTAGCCGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_30	ENr112	REV	hg18_dna	chr2	30	2	51592057	51592096	CTTTCCTATCAATGTAAAATATTCTGTTTGGGGAAGTGGA	40		0	63.34	35	51590634	51592096	1462	224	28		918	1	28	AGAGAATGAGGAACCCGGGGCAG	CTCTAA	1467	CTTTCCTATCAATGTAAAATATTCTGTTTGGGGAAGTGGACTCTAAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_32	ENr112	REV	hg18_dna	chr2	32	2	51596207	51596246	CTTTGTTTTTGTCTGGAAATACCAACTTCTACTTCCCAAA	40		0	63.97	35	51594619	51596246	1627	224	29		640	1	89	AGAGAATGAGGAACCCGGGGCAG	AAATGT	2197	CTTTGTTTTTGTCTGGAAATACCAACTTCTACTTCCCAAAAAATGTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_35	ENr112	REV	hg18_dna	chr2	35	2	51607698	51607737	CTTCTTAGTCTGCCCAGTCCTACCTTCACTTTCTTAAAAT	40		0	64.6	48.48	51598772	51607737	8965	224	30		345	1	7	AGAGAATGAGGAACCCGGGGCAG	CGAGGA	1043	CTTCTTAGTCTGCCCAGTCCTACCTTCACTTTCTTAAAATCGAGGAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_38	ENr112	REV	hg18_dna	chr2	38	2	51623017	51623051	CTTACTGCAGGTTGGCGCAGCTATAAGACTGAAGT	35	TCACA	5	64.53	51.72	51618244	51623051	4807	224	31		90	1	4	AGAGAATGAGGAACCCGGGGCAG	TCGACA	1870	CTTACTGCAGGTTGGCGCAGCTATAAGACTGAAGTTCACATCGACAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_43	ENr112	REV	hg18_dna	chr2	43	2	51639591	51639630	CTTATTTGATTAATGTGATAAATCATAATGGTTTAGCGCA	40		0	60.33	27.5	51638642	51639630	988	224	34		627	1	47	AGAGAATGAGGAACCCGGGGCAG	GTAGAG	280	CTTATTTGATTAATGTGATAAATCATAATGGTTTAGCGCAGTAGAGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_45	ENr112	REV	hg18_dna	chr2	45	2	51641851	51641890	CTTCATATTAGTAGAAAATTGAGCTTTCTCTATGAACAAT	40		0	59.4	27.5	51640556	51641890	1334	224	35		358	1	22	AGAGAATGAGGAACCCGGGGCAG	GAATGT	2196	CTTCATATTAGTAGAAAATTGAGCTTTCTCTATGAACAATGAATGTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_47	ENr112	REV	hg18_dna	chr2	47	2	51642537	51642576	CTTATCAAATTGTACAGTTTAAACATGTGCAATATATTTT	40		0	58.29	22.5	51642391	51642576	185	224	36		964	1	129	AGAGAATGAGGAACCCGGGGCAG	CGCAGA	1139	CTTATCAAATTGTACAGTTTAAACATGTGCAATATATTTTCGCAGAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_51	ENr112	REV	hg18_dna	chr2	51	2	51654999	51655036	CTTCTGCTTTTTCTAGGGCAGCCTGCCCTGAAATTTAA	38	GA	2	63.76	53.85	51647742	51655036	7294	224	37		238	1	50	AGAGAATGAGGAACCCGGGGCAG	ATATCC	3993	CTTCTGCTTTTTCTAGGGCAGCCTGCCCTGAAATTTAAGAATATCCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_54	ENr112	REV	hg18_dna	chr2	54	2	51663944	51663983	CTTCTCAACTGTTTCCCTCACTAGATTGTGGGTCATTAGA	40		0	64.84	47.06	51659035	51663983	4948	224	38		294	1	26	AGAGAATGAGGAACCCGGGGCAG	GGGTGT	2176	CTTCTCAACTGTTTCCCTCACTAGATTGTGGGTCATTAGAGGGTGTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_57	ENr112	REV	hg18_dna	chr2	57	2	51667437	51667476	CTTGGAAACTTTTTGCAAATTTGGATTCAGACTTCTAGGG	40		0	64.58	37.5	51667207	51667476	269	224	39		540	1	36	AGAGAATGAGGAACCCGGGGCAG	GAGCAA	1476	CTTGGAAACTTTTTGCAAATTTGGATTCAGACTTCTAGGGGAGCAAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_60	ENr112	REV	hg18_dna	chr2	60	2	51677242	51677281	CTTCGAATTGTTGTTTGTGCTATTTTTGAAACTCTCACAC	40		0	63.69	35	51672776	51677281	4505	224	41		605	1	3	AGAGAATGAGGAACCCGGGGCAG	AATTCC	4005	CTTCGAATTGTTGTTTGTGCTATTTTTGAAACTCTCACACAATTCCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_63	ENr112	REV	hg18_dna	chr2	63	2	51680601	51680634	CTTCCACCAGGTGTCATAACCAACAGCTGGGTAT	34	ACACTT	6	64.64	51.72	51680021	51680634	613	224	42		120	1	10	AGAGAATGAGGAACCCGGGGCAG	GTCCCA	2040	CTTCCACCAGGTGTCATAACCAACAGCTGGGTATACACTTGTCCCAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_69	ENr112	REV	hg18_dna	chr2	69	2	51689499	51689538	CTTGAAAAGAGGAAATAATTACGGTACTTTAATAATTTTT	40		0	57.82	22.5	51688427	51689538	1111	224	44		920	1	125	AGAGAATGAGGAACCCGGGGCAG	GGTTGG	160	CTTGAAAAGAGGAAATAATTACGGTACTTTAATAATTTTTGGTTGGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_72	ENr112	REV	hg18_dna	chr2	72	2	51700323	51700359	CTTCCTTGACTCTCATGACAGAAATTCCCTGAAGCTG	37	ATG	3	63.56	44.12	51697752	51700359	2607	224	45		366	1	32	AGAGAATGAGGAACCCGGGGCAG	GCGGGA	1036	CTTCCTTGACTCTCATGACAGAAATTCCCTGAAGCTGATGGCGGGAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_74	ENr112	REV	hg18_dna	chr2	74	2	51703415	51703447	CTTGTCCTGAGGTGGGTTTTTGACCTTGGGGTT	33	AGAGCCC	7	64.91	51.72	51701402	51703447	2045	224	46		317	1	12	AGAGAATGAGGAACCCGGGGCAG	GGAAAA	1360	CTTGTCCTGAGGTGGGTTTTTGACCTTGGGGTTAGAGCCCGGAAAAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_78	ENr112	REV	hg18_dna	chr2	78	2	51720523	51720562	CTTCGTTATACAATATGCTTCTTTTTCTGGTTGCTTTTTA	40		0	61.69	30	51717363	51720562	3199	224	47		1050	1	6	AGAGAATGAGGAACCCGGGGCAG	AGACTG	721	CTTCGTTATACAATATGCTTCTTTTTCTGGTTGCTTTTTAAGACTGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_82	ENr112	REV	hg18_dna	chr2	82	2	51736059	51736098	CTTGGTAATTATTCACTTGTTAGTGTCTTTGTTTCAACCT	40		0	62.51	32.5	51733841	51736098	2257	224	49		408	2	21	AGAGAATGAGGAACCCGGGGCAG	TGTTAG	418	CTTGGTAATTATTCACTTGTTAGTGTCTTTGTTTCAACCTTGTTAGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_86	ENr112	REV	hg18_dna	chr2	86	2	51748053	51748092	CTTTCCATGAAAAATAAGTTTCATGGATTATTTCAAAGGT	40		0	60.76	27.5	51743054	51748092	5038	224	51		1048	1	95	AGAGAATGAGGAACCCGGGGCAG	CCTTTT	2735	CTTTCCATGAAAAATAAGTTTCATGGATTATTTCAAAGGTCCTTTTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_88	ENr112	REV	hg18_dna	chr2	88	2	51752401	51752435	CTTTTGACTGCAAAATTTGATGGGGGAGGGTGGCA	35	CGATG	5	64.05	46.67	51752203	51752435	232	224	52		278	1	16	AGAGAATGAGGAACCCGGGGCAG	GCTCTA	1772	CTTTTGACTGCAAAATTTGATGGGGGAGGGTGGCACGATGGCTCTAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_90	ENr112	REV	hg18_dna	chr2	90	2	51755003	51755042	CTTCTCTTCTAATGAGCTCCCACACTAGTGTGAATAATAA	40		0	63.45	37.5	51754919	51755042	123	224	53		277	1	16	AGAGAATGAGGAACCCGGGGCAG	TGGGGC	3074	CTTCTCTTCTAATGAGCTCCCACACTAGTGTGAATAATAATGGGGCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_94	ENr112	REV	hg18_dna	chr2	94	2	51760419	51760458	CTTTTGGATTCTTCTCTTTGTGCCTAGCATTCTGGTATTT	40		0	64.95	37.5	51760158	51760458	300	224	54		459	1	48	AGAGAATGAGGAACCCGGGGCAG	CCCGAC	3391	CTTTTGGATTCTTCTCTTTGTGCCTAGCATTCTGGTATTTCCCGACAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_96	ENr112	REV	hg18_dna	chr2	96	2	51768127	51768166	CTTAAAAACTCATACAACTTTAGTTTACCCTCATATTTGC	40		0	60.32	30	51761813	51768166	6353	224	55		423	1	53	AGAGAATGAGGAACCCGGGGCAG	CCGTCC	3983	CTTAAAAACTCATACAACTTTAGTTTACCCTCATATTTGCCCGTCCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_98	ENr112	REV	hg18_dna	chr2	98	2	51783664	51783703	CTTCTTTGAGACGTACCTCTGGCATTTTGCAAACTATCCA	40		0	64.83	44.12	51776129	51783703	7574	224	56		337	1	5	AGAGAATGAGGAACCCGGGGCAG	GTAAGA	1112	CTTCTTTGAGACGTACCTCTGGCATTTTGCAAACTATCCAGTAAGAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_103	ENr112	REV	hg18_dna	chr2	103	2	51795879	51795918	CTTACTGTGAACCAGAACTTCTGTTATGCATAAGAAATTG	40		0	62.64	35	51795875	51795918	43	224	4		365	1	18	AGAGAATGAGGAACCCGGGGCAG	ACCAAC	3453	CTTACTGTGAACCAGAACTTCTGTTATGCATAAGAAATTGACCAACAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_105	ENr112	REV	hg18_dna	chr2	105	2	51797342	51797369	CTTACCCAAATAGCCAGGCTGGCTGCCT	28	TCCACCGAG	12	63.37	56	51796490	51797369	879	224	5		438	1	13	AGAGAATGAGGAACCCGGGGCAG	GAGCAT	2500	CTTACCCAAATAGCCAGGCTGGCTGCCTTCCACCGAGTTAGAGCATAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_108	ENr112	REV	hg18_dna	chr2	108	2	51803240	51803279	CTTTTTATATAACTCTTGCTAACAGCTCTCTCAGATTATT	40		0	60.23	30	51798252	51803279	5027	224	6		278	1	37	AGAGAATGAGGAACCCGGGGCAG	TCTGCG	814	CTTTTTATATAACTCTTGCTAACAGCTCTCTCAGATTATTTCTGCGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_112	ENr112	REV	hg18_dna	chr2	112	2	51814032	51814071	CTTTTATAAAGTGCTCTTTTAAGGTAATTAGAAAATTATT	40		0	56.44	20	51811271	51814071	2800	224	7		763	1	41	AGAGAATGAGGAACCCGGGGCAG	CATCCG	999	CTTTTATAAAGTGCTCTTTTAAGGTAATTAGAAAATTATTCATCCGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_115	ENr112	REV	hg18_dna	chr2	115	2	51832799	51832838	CTTCTAGAGAAATCAAGCTATCAGAGAAGATGGATCTCAC	40		0	63.44	40	51827805	51832838	5033	224	8		342	1	22	AGAGAATGAGGAACCCGGGGCAG	GGATTC	3728	CTTCTAGAGAAATCAAGCTATCAGAGAAGATGGATCTCACGGATTCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_118	ENr112	REV	hg18_dna	chr2	118	2	51843476	51843515	CTTAACTAAATCATTACCATCAATATGTCAGTTGCCTAAC	40		0	61.1	32.5	51837379	51843515	6136	224	9		297	1	25	AGAGAATGAGGAACCCGGGGCAG	CGTGTA	1571	CTTAACTAAATCATTACCATCAATATGTCAGTTGCCTAACCGTGTAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_126	ENr112	REV	hg18_dna	chr2	126	2	51862355	51862390	CTTCTCGCCTTATCTTGATGAAGTGAGGAGGAGACA	36	AACC	4	64.45	48.48	51860451	51862390	1939	224	10		243	1	2	AGAGAATGAGGAACCCGGGGCAG	CCTTCA	1967	CTTCTCGCCTTATCTTGATGAAGTGAGGAGGAGACAAACCCCTTCAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_135	ENr112	REV	hg18_dna	chr2	135	2	51883752	51883791	CTTTTGTATTATGTTAAATCTTTGGATTCCTTGAAAAGTG	40		0	59.66	27.5	51880508	51883791	3283	224	12		626	2	52	AGAGAATGAGGAACCCGGGGCAG	GGCGTT	2608	CTTTTGTATTATGTTAAATCTTTGGATTCCTTGAAAAGTGGGCGTTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_138	ENr112	REV	hg18_dna	chr2	138	2	51897099	51897128	CTTCTCTCATCCCCTGGTGGGCTTATTGCT	30	GAAGTAGAC	10	64.06	53.57	51887965	51897128	9163	224	13		206	1	37	AGAGAATGAGGAACCCGGGGCAG	TGTCAC	3554	CTTCTCTCATCCCCTGGTGGGCTTATTGCTGAAGTAGACATGTCACAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_140	ENr112	REV	hg18_dna	chr2	140	2	51903290	51903329	CTTATTGTGTCATGTGTGACATTCTTATAGTTTGACCTTG	40		0	62.49	35	51897414	51903329	5915	224	14		626	1	14	AGAGAATGAGGAACCCGGGGCAG	GAGTTA	1668	CTTATTGTGTCATGTGTGACATTCTTATAGTTTGACCTTGGAGTTAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_143	ENr112	REV	hg18_dna	chr2	143	2	51906949	51906988	CTTCACTAGAATAATAAGGTTGCAGTTAACATTTAAATCA	40		0	59.78	27.5	51904789	51906988	2199	224	15		917	1	16	AGAGAATGAGGAACCCGGGGCAG	AACATA	1653	CTTCACTAGAATAATAAGGTTGCAGTTAACATTTAAATCAAACATAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_145	ENr112	REV	hg18_dna	chr2	145	2	51912258	51912297	CTTACTCAAACTTTAGTGGAACCACGGAAAATGAGGATAA	40		0	64.38	37.5	51909044	51912297	3253	224	16		225	1	19	AGAGAATGAGGAACCCGGGGCAG	GGTGCG	800	CTTACTCAAACTTTAGTGGAACCACGGAAAATGAGGATAAGGTGCGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_147	ENr112	REV	hg18_dna	chr2	147	2	51915601	51915640	CTTATGTTATAATGTGGAATTTGGAACTAAATAGTGTTTT	40		0	58.86	25	51913619	51915640	2021	224	17		475	1	29	AGAGAATGAGGAACCCGGGGCAG	CTTATG	619	CTTATGTTATAATGTGGAATTTGGAACTAAATAGTGTTTTCTTATGAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_153	ENr112	REV	hg18_dna	chr2	153	2	51943647	51943686	CTTCACACACATAGAACAGACAGCTAGAAGACCTAGCAAA	40		0	63.89	44.44	51940807	51943686	2879	224	19		312	1	26	AGAGAATGAGGAACCCGGGGCAG	GTGCAA	1480	CTTCACACACATAGAACAGACAGCTAGAAGACCTAGCAAAGTGCAAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_159	ENr112	REV	hg18_dna	chr2	159	2	51957511	51957550	CTTGGTCTCTGATACTTTATCTAGCTTCATTGCTGAGGAT	40		0	64.82	40	51954379	51957550	3171	224	20		247	1	21	AGAGAATGAGGAACCCGGGGCAG	GTGGCC	3848	CTTGGTCTCTGATACTTTATCTAGCTTCATTGCTGAGGATGTGGCCAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_162	ENr112	REV	hg18_dna	chr2	162	2	51970502	51970541	CTTTTGTGTTTGGTTTATTGTCTGATAGTACAACTCTAAA	40		0	61.05	30	51966169	51970541	4372	224	21		787	1	123	AGAGAATGAGGAACCCGGGGCAG	GTTGTA	1576	CTTTTGTGTTTGGTTTATTGTCTGATAGTACAACTCTAAAGTTGTAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_166	ENr112	REV	hg18_dna	chr2	166	2	51984722	51984761	CTTTAGATCTTTTGAGGGCCTTTTTAAGCCTGTGACTATC	40		0	64.76	40	51980652	51984761	4109	224	22		274	1	6	AGAGAATGAGGAACCCGGGGCAG	CAAACT	2903	CTTTAGATCTTTTGAGGGCCTTTTTAAGCCTGTGACTATCCAAACTAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_169	ENr112	REV	hg18_dna	chr2	169	2	51993583	51993622	CTTTCAATATGGAACTGGTAAAATAATCTGTCTCTGTAGT	40		0	61.5	32.5	51986333	51993622	7289	224	23		529	1	15	AGAGAATGAGGAACCCGGGGCAG	ACGCGA	1229	CTTTCAATATGGAACTGGTAAAATAATCTGTCTCTGTAGTACGCGAAGAGAATGAGGAACCCGGGGCAG
5C_123_ENr112_REV_175	ENr112	REV	hg18_dna	chr2	175	2	52009362	52009401	CTTCTCAACTTTCCAAGAAAAATCTTGAACGCTTGTTCAA	40		0	64.48	35	52005124	52009401	4277	224	24		542	1	25	AGAGAATGAGGAACCCGGGGCAG	TGTTGG	162	CTTCTCAACTTTCCAAGAAAAATCTTGAACGCTTGTTCAATGTTGGAGAGAATGAGGAACCCGGGGCAG

Supplemental Data 13
DNA sequences of 5C primers used for analysis of the conformation of ENr132. This is the standard output of my5C.primers. This is a Tab-delimited text file. See Supplemental Data 1 for details.
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PRIMER_NAME	REGION	TYPE	ASSEMBLY	CHROMOSOME	FRAGMENT_ID	PRIMER_ID	P_STARTPOS	P_ENDPOS	P_SPECIFIC	P_SPECIFIC_SIZE
5C_1717_ENr132_FOR_55	ENr132	FOR	hg18_dna	chr13	55	1	112590784	112590823	GACTCTGGGGAAGCCAGAGGGGGATGCAAG	30
5C_1717_ENr132_FOR_56	ENr132	FOR	hg18_dna	chr13	56	1	112594282	112594321	CTAACTGTATTTTCGCTGCATTGGCTGCCAATGCGGAAAG	40
5C_1717_ENr132_FOR_57	ENr132	FOR	hg18_dna	chr13	57	1	112599554	112599593	TTCGAGGGGGGGACACGGTATGGCCACCGAGAAG	34
5C_1717_ENr132_FOR_59	ENr132	FOR	hg18_dna	chr13	59	1	112609211	112609250	CTAAAGTTTGAGCATCACACTGTTCAGGTGCTGGGCAAAG	40
5C_1717_ENr132_FOR_60	ENr132	FOR	hg18_dna	chr13	60	1	112612397	112612436	GTGAAAAGGAGGCACACGTTCATAGAAAGTGAATATAAAG	40
5C_1717_ENr132_FOR_61	ENr132	FOR	hg18_dna	chr13	61	1	112615673	112615712	ACAGAACATTATCACTCACCTCACCTGGCAAAACAAAAAG	40
5C_1717_ENr132_FOR_62	ENr132	FOR	hg18_dna	chr13	62	1	112634824	112634863	ACCAATATTGGCTCATTAATTGCAACAAAGGCACCATAAG	40
5C_1717_ENr132_FOR_63	ENr132	FOR	hg18_dna	chr13	63	1	112647209	112647248	AAACTTGGGCCCAGTGTTCATGGTGTCCTAATGGAAG	37
5C_1717_ENr132_FOR_64	ENr132	FOR	hg18_dna	chr13	64	1	112663473	112663512	ACAGCGCCTGAAGACTTCTGAAGATGCGGGCTGCTAAG	38
5C_1717_ENr132_FOR_65	ENr132	FOR	hg18_dna	chr13	65	1	112666519	112666558	TGAGACCAGGTTCTACAGTTGCCCACGTTGATGTCAAG	38
5C_1717_ENr132_FOR_67	ENr132	FOR	hg18_dna	chr13	67	1	112672294	112672333	GTTCATTTTCAGTATTAATCGAAATATTACTTCAAATAAG	40
5C_1717_ENr132_FOR_68	ENr132	FOR	hg18_dna	chr13	68	1	112672810	112672849	CTGCTCCCTGATTCTTAGAGAATCAGCTCTTTTTCTGAAG	40
5C_1717_ENr132_FOR_69	ENr132	FOR	hg18_dna	chr13	69	1	112679733	112679772	CGAAGCGTTTTCACGATGGTCGCTGAAGCCAAAG	34
5C_1717_ENr132_FOR_71	ENr132	FOR	hg18_dna	chr13	71	1	112686947	112686986	TGATAAGGGTGAGTGATCTCAGCGGAATTCCCCTTTAAG	39
5C_1717_ENr132_FOR_74	ENr132	FOR	hg18_dna	chr13	74	1	112710083	112710122	GATCAGTTTCACATTAGGTGATTGCCGGACTTACATCAAG	40
5C_1717_ENr132_FOR_76	ENr132	FOR	hg18_dna	chr13	76	1	112712583	112712622	TCCTGGTTTTCAGGATGAAATTGTCCTCAAATAAAGCAAG	40
5C_1717_ENr132_FOR_77	ENr132	FOR	hg18_dna	chr13	77	1	112724802	112724841	GAATTCCACATAGGCTTCTGGGCGGTGCTGGAAG	34
5C_1717_ENr132_FOR_78	ENr132	FOR	hg18_dna	chr13	78	1	112726597	112726636	TTGGCGATTCTCAGTGTCAGGAAGGAGGCAGGAGACAAG	39
5C_1717_ENr132_FOR_82	ENr132	FOR	hg18_dna	chr13	82	1	112777939	112777978	CCTGCCCAGCCAATAATGATTTCTTTAAAAGATCCAGAAG	40
5C_1717_ENr132_FOR_83	ENr132	FOR	hg18_dna	chr13	83	1	112782765	112782804	CTTTTTGCGGGCCGACAGCTGTCTTAGAACAAG	33
5C_1717_ENr132_FOR_87	ENr132	FOR	hg18_dna	chr13	87	1	112824248	112824287	CGTCTTCAGGCAGTGATCGCCACACTGGCCAAG	33
5C_1717_ENr132_REV_58	ENr132	REV	hg18_dna	chr13	58	2	112606673	112606712	CTTGTGGGGTCAGCAGGGGCAGGAAGGATCTAA	33
5C_1717_ENr132_REV_66	ENr132	REV	hg18_dna	chr13	66	2	112671773	112671812	CTTAAGCACAGATCACTGACGGCCAGCAGTCTCAAATG	38
5C_1717_ENr132_REV_70	ENr132	REV	hg18_dna	chr13	70	2	112685159	112685198	CTTCCATCCTCGCCCTTGTTCTCTGCAATTTCCTTTGAAT	40
5C_1717_ENr132_REV_72	ENr132	REV	hg18_dna	chr13	72	2	112694923	112694962	CTTCCACATCATTATTCAGTGGGCCCAGCTCACTCTCTGG	40
5C_1717_ENr132_REV_73	ENr132	REV	hg18_dna	chr13	73	2	112709981	112710020	CTTACAAAGGACAGTGACTGAGGCCGCCCTTTAC	34
5C_1717_ENr132_REV_79	ENr132	REV	hg18_dna	chr13	79	2	112734651	112734690	CTTCACTTTTTAATGCACACGTATTAAATTGTTTAGCACG	40
5C_1717_ENr132_REV_80	ENr132	REV	hg18_dna	chr13	80	2	112762612	112762651	CTTACGACAGTTGTTAGGGGCTGAGCTGTGTCACG	35
5C_1717_ENr132_REV_81	ENr132	REV	hg18_dna	chr13	81	2	112775864	112775903	CTTTTAATTTTTGTTATTGGAAATGATCCTGAGAATGGGT	40
5C_1717_ENr132_REV_84	ENr132	REV	hg18_dna	chr13	84	2	112792109	112792148	CTTCTTCACACTGCAGGAAGCGCAGGTTTCTCAACAGGC	39
5C_1717_ENr132_REV_85	ENr132	REV	hg18_dna	chr13	85	2	112801662	112801701	CTTTTAAATTGCCAAACAGTGTTTCAATGATCTTCCCATT	40

PRIMER_NAME	P_FILLER	P_FILLER_SIZE	P_TM	P_GC	F_STARTPOS	F_ENDPOS	F_SIZE	ELEMENTID	INTERSECTIONID	F_NAME	P_MFR	P_UBLAST	P_SBLAST
5C_1717_ENr132_FOR_55	GGGAGCTCCC	10	64.73624	66.67	112586352	112590823	4471	928	828		570	1	15
5C_1717_ENr132_FOR_56		0	63.5279	50	112590823	112594321	3498	928	829		84	1	2
5C_1717_ENr132_FOR_57	ACTACT	6	64.16761	58.82	112594321	112599593	5272	928	830		28	1	4
5C_1717_ENr132_FOR_59		0	63.5279	50	112606712	112609250	2538	928	831		316	1	26
5C_1717_ENr132_FOR_60		0	58.4029	37.5	112609250	112612436	3186	928	832		330	1	59
5C_1717_ENr132_FOR_61		0	59.4279	40	112612436	112615712	3276	928	833		446	1	51
5C_1717_ENr132_FOR_62		0	58.4029	37.5	112615712	112634863	19151	928	834		843	1	20
5C_1717_ENr132_FOR_63	CGC	3	64.92993	56.76	112634863	112647248	12385	928	835		191	1	9
5C_1717_ENr132_FOR_64	CC	2	64.79764	55.26	112647248	112663512	16264	928	836		281	1	2
5C_1717_ENr132_FOR_65	GA	2	64.79764	55.26	112663512	112666558	3046	928	837		126	1	3
5C_1717_ENr132_FOR_67		0	52.2529	22.5	112671812	112672333	521	928	838		552	1	82
5C_1717_ENr132_FOR_68		0	60.4529	42.5	112672333	112672849	516	928	839		382	1	40
5C_1717_ENr132_FOR_69	TTGACC	6	64.16761	58.82	112672849	112679772	6923	928	840		42	1	11
5C_1717_ENr132_FOR_71	T	1	64.67213	53.85	112685198	112686986	1788	928	841		192	1	8
5C_1717_ENr132_FOR_74		0	60.4529	42.5	112710020	112710122	102	928	842		99	1	4
5C_1717_ENr132_FOR_76		0	58.4029	37.5	112711617	112712622	1005	928	843		598	1	30
5C_1717_ENr132_FOR_77	AAATTC	6	64.16761	58.82	112712622	112724841	12219	928	844		192	1	9
5C_1717_ENr132_FOR_78	C	1	64.67213	53.85	112724841	112726636	1795	928	845		964	2	88
5C_1717_ENr132_FOR_82		0	59.4279	40	112775903	112777978	2075	928	847		1201	1	28
5C_1717_ENr132_FOR_83	GGGTAAA	7	64.29684	60.61	112777978	112782804	4826	928	848		70	1	9
5C_1717_ENr132_FOR_87	GACTAAT	7	64.29684	60.61	112816824	112824287	7463	928	849		97	1	15
5C_1717_ENr132_REV_58	ATCAAAT	7	64.29684	60.61	112599593	112606712	7119	929	167		451	1	27
5C_1717_ENr132_REV_66	AA	2	64.79764	55.26	112666558	112671812	5254	929	168		330	1	17
5C_1717_ENr132_REV_70		0	63.5279	50	112679772	112685198	5426	929	169		290	1	6
5C_1717_ENr132_REV_72		0	63.5279	50	112686986	112694962	7976	929	170		380	1	33
5C_1717_ENr132_REV_73	GGGGAG	6	64.16761	58.82	112694962	112710020	15058	929	171		170	1	18
5C_1717_ENr132_REV_79		0	56.3529	32.5	112726636	112734690	8054	929	172		352	1	44
5C_1717_ENr132_REV_80	ACGGT	5	64.04576	57.14	112734690	112762651	27961	929	173		259	1	1
5C_1717_ENr132_REV_81		0	55.3279	30	112762651	112775903	13252	929	174		1275	1	274
5C_1717_ENr132_REV_84	T	1	64.67213	53.85	112782804	112792148	9344	929	175		232	1	28
5C_1717_ENr132_REV_85		0	56.3529	32.5	112792148	112801701	9553	929	176		488	1	44

PRIMER_NAME	TAIL	BARCODE_SEQ	BARCODE_NUM	PRIMER_SEQUENCE
5C_1717_ENr132_FOR_55	CCTCTCTATGGGCAGTCGGTGAT	CTCGTC	308	CCTCTCTATGGGCAGTCGGTGATCTCGTCGGGAGCTCCGGACTCTGGGGAAGCCAGAGGGGGATGCAAG
5C_1717_ENr132_FOR_56	CCTCTCTATGGGCAGTCGGTGAT	ACCGCC	2096	CCTCTCTATGGGCAGTCGGTGATACCGCCCTAACTGTATTTTCGCTGCATTGGCTGCCAATGCGGAAAG
5C_1717_ENr132_FOR_57	CCTCTCTATGGGCAGTCGGTGAT	TGCCAA	1802	CCTCTCTATGGGCAGTCGGTGATTGCCAAACTACTTTCGAGGGGGGGACACGGTATGGCCACCGAGAAG
5C_1717_ENr132_FOR_59	CCTCTCTATGGGCAGTCGGTGAT	CTCGAA	314	CCTCTCTATGGGCAGTCGGTGATCTCGAACTAAAGTTTGAGCATCACACTGTTCAGGTGCTGGGCAAAG
5C_1717_ENr132_FOR_60	CCTCTCTATGGGCAGTCGGTGAT	GCGGAT	3321	CCTCTCTATGGGCAGTCGGTGATGCGGATGTGAAAAGGAGGCACACGTTCATAGAAAGTGAATATAAAG
5C_1717_ENr132_FOR_61	CCTCTCTATGGGCAGTCGGTGAT	TGTATA	1894	CCTCTCTATGGGCAGTCGGTGATTGTATAACAGAACATTATCACTCACCTCACCTGGCAAAACAAAAAG
5C_1717_ENr132_FOR_62	CCTCTCTATGGGCAGTCGGTGAT	CAGCTC	708	CCTCTCTATGGGCAGTCGGTGATCAGCTCACCAATATTGGCTCATTAATTGCAACAAAGGCACCATAAG
5C_1717_ENr132_FOR_63	CCTCTCTATGGGCAGTCGGTGAT	ACCATC	2084	CCTCTCTATGGGCAGTCGGTGATACCATCCGCAAACTTGGGCCCAGTGTTCATGGTGTCCTAATGGAAG
5C_1717_ENr132_FOR_64	CCTCTCTATGGGCAGTCGGTGAT	GTTCGG	3407	CCTCTCTATGGGCAGTCGGTGATGTTCGGCCACAGCGCCTGAAGACTTCTGAAGATGCGGGCTGCTAAG
5C_1717_ENr132_FOR_65	CCTCTCTATGGGCAGTCGGTGAT	GTTGAA	3450	CCTCTCTATGGGCAGTCGGTGATGTTGAAGATGAGACCAGGTTCTACAGTTGCCCACGTTGATGTCAAG
5C_1717_ENr132_FOR_67	CCTCTCTATGGGCAGTCGGTGAT	GGTAAA	3946	CCTCTCTATGGGCAGTCGGTGATGGTAAAGTTCATTTTCAGTATTAATCGAAATATTACTTCAAATAAG
5C_1717_ENr132_FOR_68	CCTCTCTATGGGCAGTCGGTGAT	GACCGA	3598	CCTCTCTATGGGCAGTCGGTGATGACCGACTGCTCCCTGATTCTTAGAGAATCAGCTCTTTTTCTGAAG
5C_1717_ENr132_FOR_69	CCTCTCTATGGGCAGTCGGTGAT	GTCTTT	3349	CCTCTCTATGGGCAGTCGGTGATGTCTTTTTGACCCGAAGCGTTTTCACGATGGTCGCTGAAGCCAAAG
5C_1717_ENr132_FOR_71	CCTCTCTATGGGCAGTCGGTGAT	GTACTG	3463	CCTCTCTATGGGCAGTCGGTGATGTACTGTTGATAAGGGTGAGTGATCTCAGCGGAATTCCCCTTTAAG
5C_1717_ENr132_FOR_74	CCTCTCTATGGGCAGTCGGTGAT	CCGTAC	216	CCTCTCTATGGGCAGTCGGTGATCCGTACGATCAGTTTCACATTAGGTGATTGCCGGACTTACATCAAG
5C_1717_ENr132_FOR_76	CCTCTCTATGGGCAGTCGGTGAT	GTCGTC	3380	CCTCTCTATGGGCAGTCGGTGATGTCGTCTCCTGGTTTTCAGGATGAAATTGTCCTCAAATAAAGCAAG
5C_1717_ENr132_FOR_77	CCTCTCTATGGGCAGTCGGTGAT	TTGTTT	1493	CCTCTCTATGGGCAGTCGGTGATTTGTTTAAATTCGAATTCCACATAGGCTTCTGGGCGGTGCTGGAAG
5C_1717_ENr132_FOR_78	CCTCTCTATGGGCAGTCGGTGAT	TCACAG	1163	CCTCTCTATGGGCAGTCGGTGATTCACAGCTTGGCGATTCTCAGTGTCAGGAAGGAGGCAGGAGACAAG
5C_1717_ENr132_FOR_82	CCTCTCTATGGGCAGTCGGTGAT	CCACTT	133	CCTCTCTATGGGCAGTCGGTGATCCACTTCCTGCCCAGCCAATAATGATTTCTTTAAAAGATCCAGAAG
5C_1717_ENr132_FOR_83	CCTCTCTATGGGCAGTCGGTGAT	GCTCGT	3149	CCTCTCTATGGGCAGTCGGTGATGCTCGTGGGTAAACTTTTTGCGGGCCGACAGCTGTCTTAGAACAAG
5C_1717_ENr132_FOR_87	CCTCTCTATGGGCAGTCGGTGAT	AGGTTC	3028	CCTCTCTATGGGCAGTCGGTGATAGGTTCGACTAATCGTCTTCAGGCAGTGATCGCCACACTGGCCAAG
5C_1717_ENr132_REV_58	AGAGAATGAGGAACCCGGGGCAG	TACTGC	3254	CTTGTGGGGTCAGCAGGGGCAGGAAGGATCTAAATCAAATTACTGCAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_66	AGAGAATGAGGAACCCGGGGCAG	AAATAG	405	CTTAAGCACAGATCACTGACGGCCAGCAGTCTCAAATGAAAAATAGAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_70	AGAGAATGAGGAACCCGGGGCAG	TTGAGA	1098	CTTCCATCCTCGCCCTTGTTCTCTGCAATTTCCTTTGAATTTGAGAAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_72	AGAGAATGAGGAACCCGGGGCAG	GAAAGA	1108	CTTCCACATCATTATTCAGTGGGCCCAGCTCACTCTCTGGGAAAGAAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_73	AGAGAATGAGGAACCCGGGGCAG	GTAACA	1880	CTTACAAAGGACAGTGACTGAGGCCGCCCTTTACGGGGAGGTAACAAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_79	AGAGAATGAGGAACCCGGGGCAG	TCCCAC	3582	CTTCACTTTTTAATGCACACGTATTAAATTGTTTAGCACGTCCCACAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_80	AGAGAATGAGGAACCCGGGGCAG	CGGACA	1859	CTTACGACAGTTGTTAGGGGCTGAGCTGTGTCACGACGGTCGGACAAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_81	AGAGAATGAGGAACCCGGGGCAG	TTTAAC	3434	CTTTTAATTTTTGTTATTGGAAATGATCCTGAGAATGGGTTTTAACAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_84	AGAGAATGAGGAACCCGGGGCAG	GTTTCC	4008	CTTCTTCACACTGCAGGAAGCGCAGGTTTCTCAACAGGCTGTTTCCAGAGAATGAGGAACCCGGGGCAG
5C_1717_ENr132_REV_85	AGAGAATGAGGAACCCGGGGCAG	CATGAA	1319	CTTTTAAATTGCCAAACAGTGTTTCAATGATCTTCCCATTCATGAAAGAGAATGAGGAACCCGGGGCAG