

Intergenic 70-mer Genome Tiling set

for *Saccharomyces cerevisiae*

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Introduction

We have designed a set of 70-mer oligos for tiling the intergenic regions of the *Saccharomyces cerevisiae* genome at an average resolution of 250 nucleotides. The set consists of 9,405 sequence optimized oligos chosen such that no intergenic region greater than 360 bases was left uncovered by a probe. The oligos were screened for uniqueness and melting temperature.

The set was designed for the production of yeast genome DNA microarrays and was printed on glass using conventional techniques. While all intergenic regions across the genome are covered at moderate resolution, the Open Reading Frames (ORFs) are not represented, with the exception of chromosome 3 for which both the intergenic regions and open reading frames are tiled.

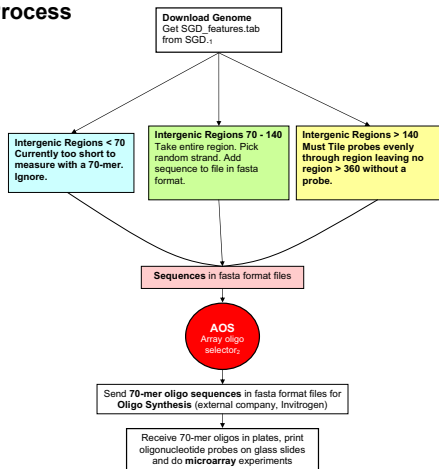
When used in conjunction with the commercially available expression oligo set from Operon Biotechnologies, this set offers nearly full genome coverage at higher resolution than was previously available, making it ideal for studies involving chromatin immunoprecipitation (ChIP), and comparative genomic hybridization (CGH).

Background

Groups printing their own yeast arrays have traditionally relied on PCR products to represent the genome as ORF and intergenic segments (1,2). A successful alternative to PCR products for construction of expression arrays has been the use of 70-mer oligonucleotides (3).

Long oligos adhere well to glass, can be bioinformatically optimized for high specificity to a target sequence with low cross hybridization to other sequences, and are not subject to the synthesis failures commonly encountered with PCR. Initial studies indicated that 70-mers work well as array reagents for ChIP chip based studies, and offers the advantage of generating higher resolution data than PCR products. Thus we sought to make an array consisting entirely of 70-mers to represent the yeast genome to replace the current PCR product based arrays.

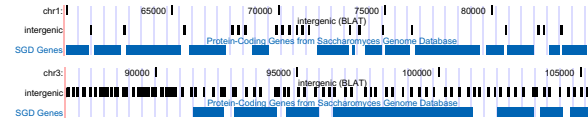
Design Process



1. SGD_features.tab file was downloaded from SGD (ftp.stanford.edu/pub/yeast/data_download/chromosomal_feature/) in December 2005. The file was parsed with a perl script and coordinates of the intergenic regions were determined.

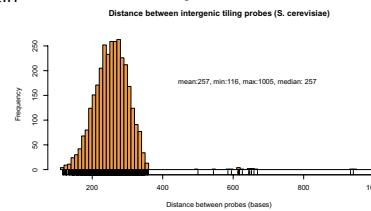
2. ArrayOligoSelector (AOS) is freely available open source software (<http://arrayoligosel.sourceforge.net>) designed by Zhu, Bozdech, and DeRisi [1] to select oligos from a given sequence based on uniqueness in genome, sequence complexity, self binding, and GC content.

UCSC Genome Browser Views of Probe Set

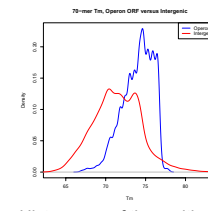


The probes are shown on the UCSC Genome Browser (<http://genome.ucsc.edu>). Curated genes and other features from SGD (<http://www.yeastgenome.org>) are shown in blue. The intergenic tiling probes are shown in black. Sections shown are from chromosome 1 and chromosome 3, which was tiled at ~250 bp resolution (ORF and intergenic regions).

Distance between probes



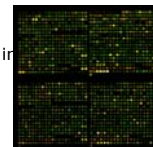
Histogram of the distances between probes. The probes with longer distances between them are regions where a suitable probe could not be found.



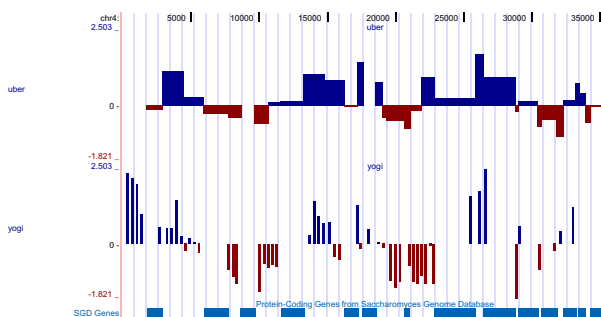
Histograms of the melting temperatures of the intergenic probe set and the Operon probe set (which has one probe per ORF).

Array Image

Shown at right is a typical microarray image from a Chromatin IP experiment using the intergenic tiling set in conjunction with the Operon Expression Set. The top 7 rows of each grid are the Operon ORF oligos, whereas the remaining rows consist of intergenic tiling oligos. Qualitatively, the sets are nearly indistinguishable.

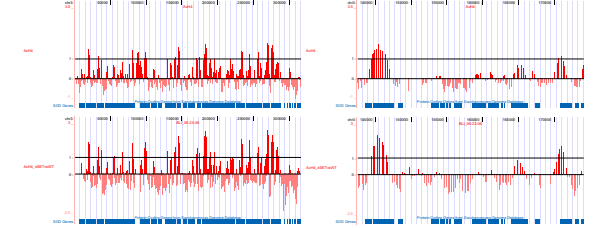


70-mer probes vs. PCR probes



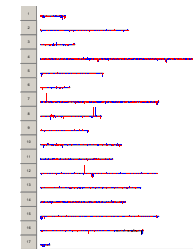
The data presented above represents log base 2 ratios (R/G) from a Chromatin IP experiment designed to map cohesin along the chromosome. The top browser track illustrates data obtained from PCR product arrays (one probe for each gene and intergenic region). The bottom track shows the same experiment using our YOGI arrays (intergenic tiling set + operon probes, one per ORF). The 70-mers offer higher resolution data. (data courtesy of Jen Gerton).

Probe set comparison with Agilent probes



The top two browser tracks represent data from Agilent Array4, in which an acetylated histone was mapped by Chromatin IP. The bottom browser track represents data from a parallel experiment using YOGI array (data courtesy of Bing Li, Workman Lab).

CGH with the Tiling array



A comparative genomic hybridization was performed using the tiling probes. Yeast strains SK1 and S288c were compared. A representation of the yeast genome is shown.

Conclusion

While commercial tiling arrays are becoming more common, they are still cost prohibitive to many groups. Decreasing oligo production costs have brought synthesis of custom genome-sized oligo sets within reach of organized groups choosing to their own microarrays. This set has proven to offer higher resolution data for CGH and ChIP based studies. The design is publicly available at <http://research.stowers-institute.org/microarray/>

Summary

-We created a design for an intergenic tiling set for yeast.
-In combination with the commercially available Operon Expression set, one can produce whole genome microarrays capable of collecting data at higher resolution than previously available.

Contact Information

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Acknowledgements

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References

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- (2) Reid JL, Iyer VR, Brown PO, Struhl K. (2000) Mol Cell. 6(6):1297-307.
- (3) Seidel, C., J.Ten Bosch, J., Batra, S., Lam, H., Tuason, N., Saljoughi, S., and Saul, R. (2000). 70-mer Oligonucleotides for DNA Microarrays. Abstract. Yeast Genetics and Molecular Biology Meeting, Seattle.
- (4) Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Keane Bell GW, Walker K, Rolfe PA, Herbolshaimer E, Zeitlinger J, Lewitter F, Kadonaga JD, Young RA. Genome-wide map of nucleosome acetylation and methylation in yeast. (2005) Cell. 122(4):517-27.