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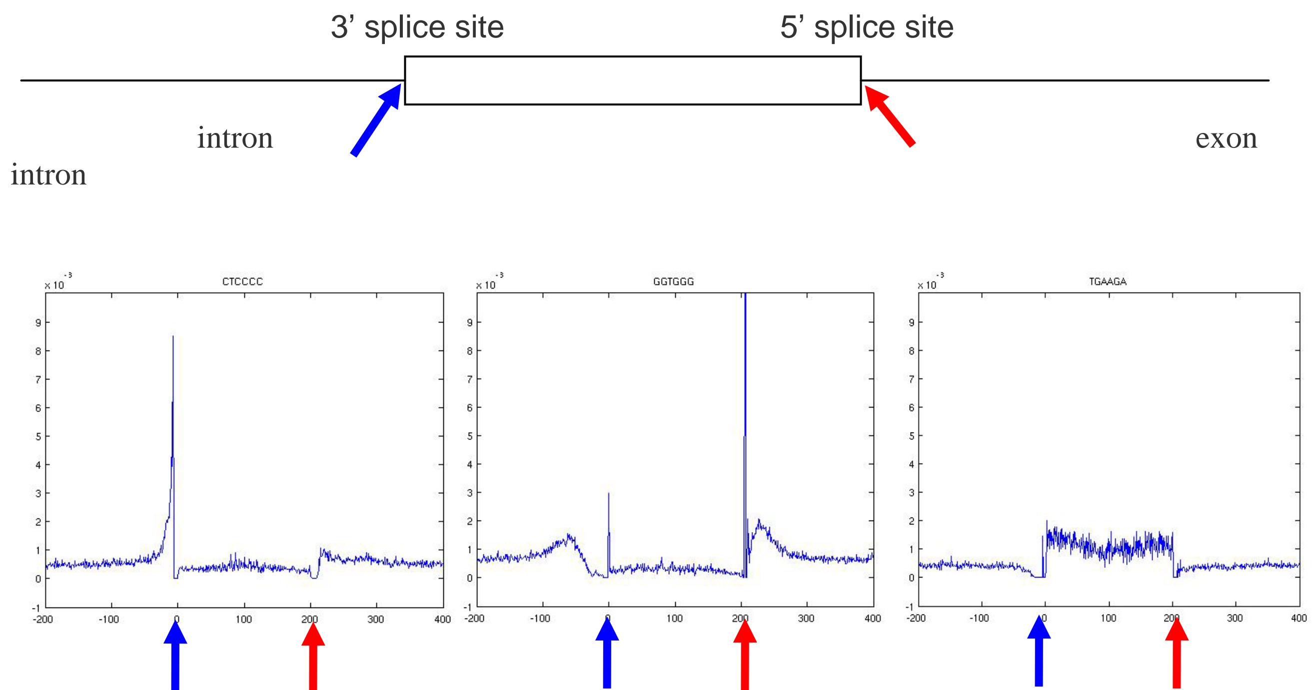
Fairbrother Lab Research Description:

My lab uses a combination of computational biology and high throughput genomics techniques to identify functional elements in the genome. I am particularly interested in sequence elements that regulate RNA splicing. Splicing is the process by which upwards of 90% of the pre-mRNA is removed from the RNA to make mRNA that can be translated into protein. A typical vertebrate gene contains an average of 10 introns and about ¾ of all genes are spliced in a tissue specific fashion creating multiple variant isoforms.

The basic method assumes that sequences important to splicing will have a signature distribution around splice sites. Enhancers will occur with increasing frequency. Silencers with decreasing frequency as you approach the site of splicing. Some enhancer may be specific to either the three or five prime splice site others may be specific to exons or introns. Below is the genomic distribtution of three words CTCCC, GGTGGG, or TGAAGA relative to splice junction sequences.



Computational Algorithm

Data mining

- extract all exons with 200 nucleotides of flanking sequence from the genome
- align above exon regions by splice sites
- for all possible hexamers, compute frequency at each position in alignment

Data analysis

- identify hexamers with non-random distributions
- cluster hexamers according to distribution
- compare distributions across species

Specific projects that move beyond identifying splicing signals include:

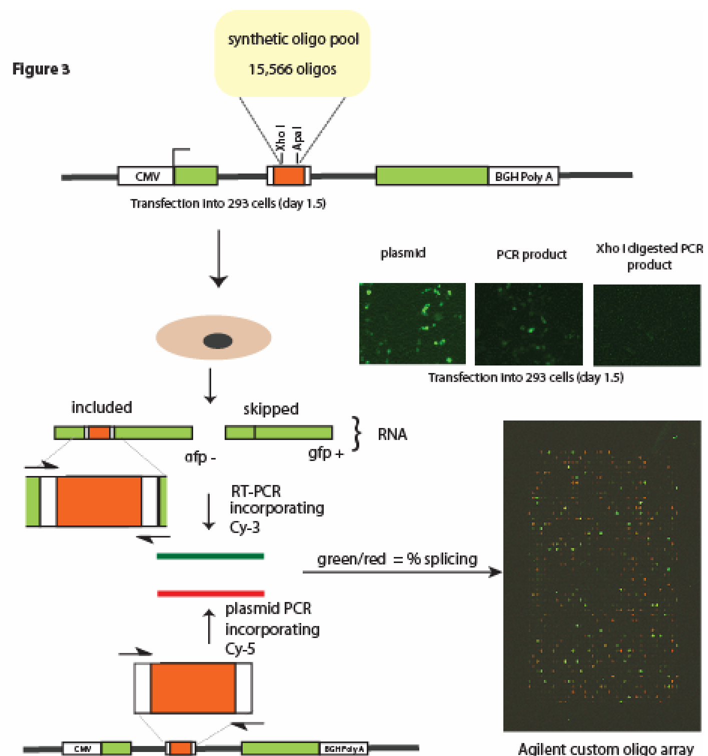
1) understanding how particular arrangements of sequence elements are read by the splicing machinery
2) identifying mutations/polymorphisms that disrupt splicing in the human population
3) investigating the evolution of gene expression signals.

Outside collaborations

We are currently involved in a collaboration with Parallele (now part of Affymetrix) to identify functional SNPs in the genome. We are also working with the Levine lab to identify a set of SNPs that 1) disrupt gene processing signals 2) are associated with an increased risk or earlier onset of cancer in defined patient groups.

Developing Tools to Validate Splicing Enhancers

To quantify the effect of a particular sequence on splicing it is necessary to use a reporter system. We have developed a high throughput system to test the effect of sequences on splicing. Using Agilent custom oligo arrays and a two color analysis scheme we assay the splicing efficiency of several thousand enhancers. Briefly, the reporter construct contains the candidate enhancer (orange box) in a test exon. The test exon is crippled by a mutation that impairs its recognition so its default is to be skipped and



omitted from the message. Exons that contain an enhancer will reverse this and be included in the message. In this fashion, candidate enhancers which are truly capable of enhancing splicing will become enriched in the pool of spliced products. This population of exon is amplified with incorporating Cy-3 label and by following the enrichment of Cy3 signal a splicing efficiency can be calculated for each candidate enhancer.

We are currently developing this method and applying it with various SELEX overexpression and chromatin IP approaches to define which sequences are enhancers and how they function.

A collaboration with the Tantin Lab (University of Utah) on binding sites for Oct4 also uses a similar method.

Recent Publications:

Nicole Pfarr, Dirk Prawitt, Michael Kirschfink, Claudia Schroff, Markus Knuf, Pirmin Habermehl, Wilma Mannhardt, Fred Zepp, William Fairbrother, Michael Loos, Christopher B. Burge and Joachim Pohlenz " Linking C5 deficiency to an exonic splicing enhancer mutation." J Immunol. 2005 Apr 1;174(7):4172-7.

Fairbrother WG, Holste D, Burge CB, Sharp PA. "Single nucleotide polymorphism-based validation of exonic splicing enhancers" PloS Biol.2004 Sep ;2(9):pg268.

Fairbrother WG, Yeo GW, Yeh R, Goldstein P, Mawson M, Sharp PA, Burge CB. "RESCUE-ESE identifies candidate exonic splicing enhancers in vertebrate exons."Nucleic Acids Res. 2004 Jul 1;32:pg187.

Fairbrother WG, Yeh RF, Sharp PA, Burge CB. "Predictive identification of exonic splicing enhancers in human genes." Science. 2002 Aug 9;297(5583):1007-13.