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Promoter sequence analysis of genes, differentially expressed in sheep, following a nematode parasite challenge

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Poster 69



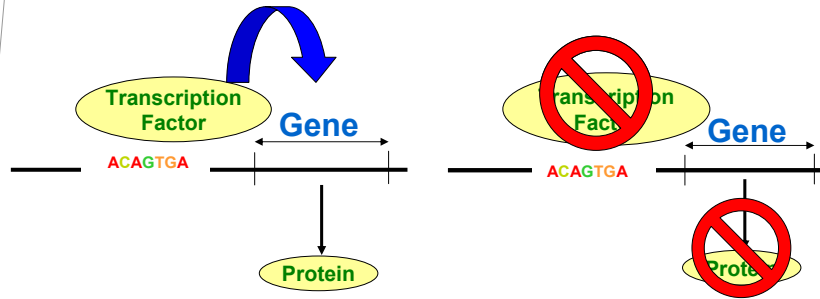
Outline

- **Background**
- **Previous experimental work**
- **Goals**
- **Results**
- **Future directions**



Regulation of Transcription

- Regulatory proteins, binding to the up-stream region of a gene act as either promoters or suppressors of transcription



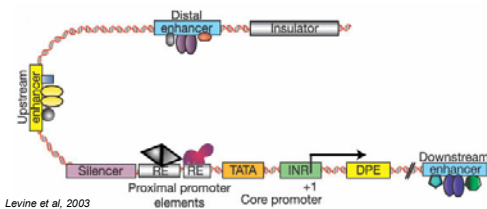
- Regulation of transcription is time, cell and tissue specific

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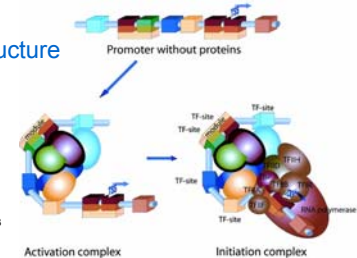
Promoter architecture

- Organization of promoter motifs represents a "footprint" of the transcriptional regulatory mechanisms
- Complex transcriptional control modules



Active promoters have a unique 3-dimensional structure

Changing the order or spacing of transcription factor binding sites (TFBS) can change the overall structure of the promoter and thus affect transcription

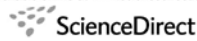


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Previous work



Available online at www.sciencedirect.com



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www.elsevier.com/locate/ijpara

Gastrointestinal nematode challenge induces some conserved gene expression changes in the gut mucosa of genetically resistant sheep

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Abstract

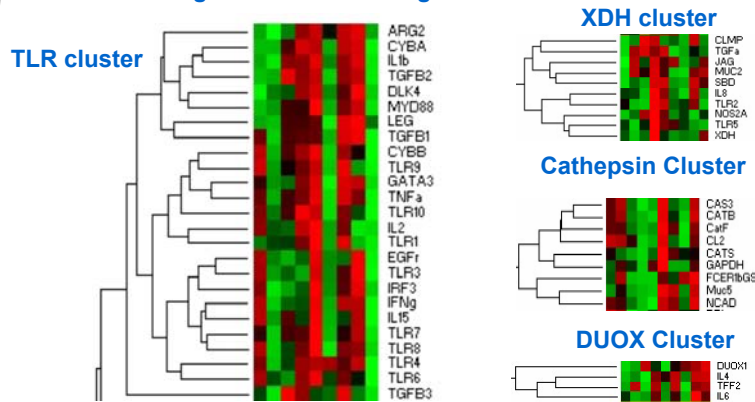
Sheep have a varying ability to resist infection with gastrointestinal nematodes. This ability is due in part to genetic differences that exist between individuals. In order to define these differences we have used real-time PCR to quantify gene expression responses in the gut mucosal surface of genetically resistant and susceptible sheep, following a nematode challenge. Expression profiles were determined in response to two different nematode species, *Haemonchus contortus* and *Trichostrongylus colubriformis*, and in divergent sheep originating from two different genetic backgrounds. Results show that the response generated differs between resistant and susceptible animals and is further impacted by the origin of the sheep and nematode species used for challenge. However, some conserved features of a response mounted by a resistant or a susceptible animal were identified. Genes found to be more abundantly expressed in resistant animals include markers of an early inflammatory response, several Toll-like receptors (*TLR2*, *4*, *9*) and free radical producing genes (*DUOX1* and *NOS2A*). Conversely, genes differentiating susceptible animals indicate a prolonged response and development of a chronic inflammatory state, characterised by elevated expression of members of the NF- κ B signalling pathway (*IKK β* and *NFKB1A*) together with delayed expression of regulatory markers such as *IL2RA* (CD25), *IL10* and *TGF β 2*. While multiple nematode response pathways were identified, the identification of conserved aspects of the response which associate with resistance provides evidence that alternative nematode control strategies, such as breeding for resistant animals, may be feasible.

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Data

Sets of differentially expressed (DE) genes in resistant and susceptible animals following nematode challenge



Co-expression → Transcriptional co-regulation → Common regulatory elements

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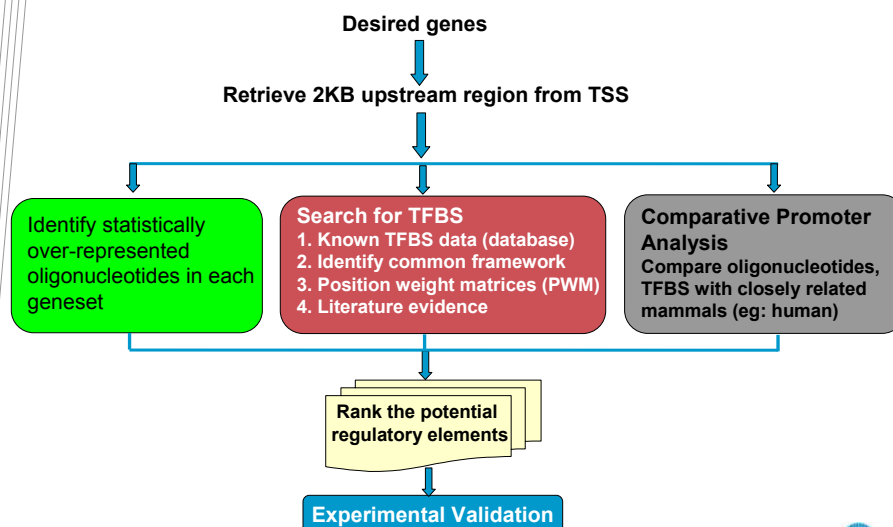


Goals

- To conduct a comprehensive analysis of promoter sequences for DE genes
- To identify potential TF binding sites involved in regulation
 - * To find common motifs shared by the majority of TFs
 - * Are there any global regulators (TFs) that turns on DE genes in sheep?
- To benchmark currently available methods and establish a broad protocol for regulatory sequence analysis
- To extensively use human promoter sequence data (well studied) using comparative genomics approaches
- To apply this protocol to other related projects at CSIRO as a starting point to understand transcription regulation



Promoter sequence analysis - schema



Over-represented consensus binding sites discovered in 22 Toll Like Receptor (TLR) pathway genes



Motif 1: TCAGAA

P-value : 3.5e-05



Motif 2: AGAGAAA

P-value : 3.5e-06



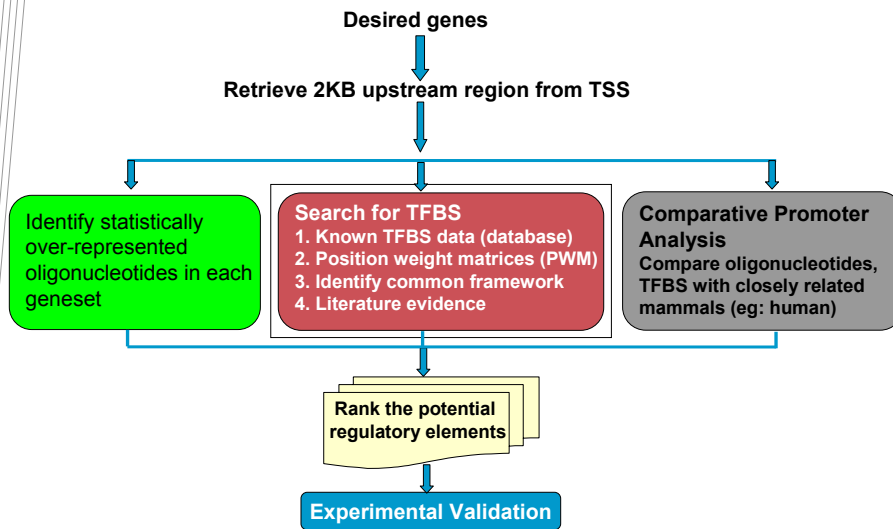
Motif 3: GGGAGGA

P-value : 2.1e-05

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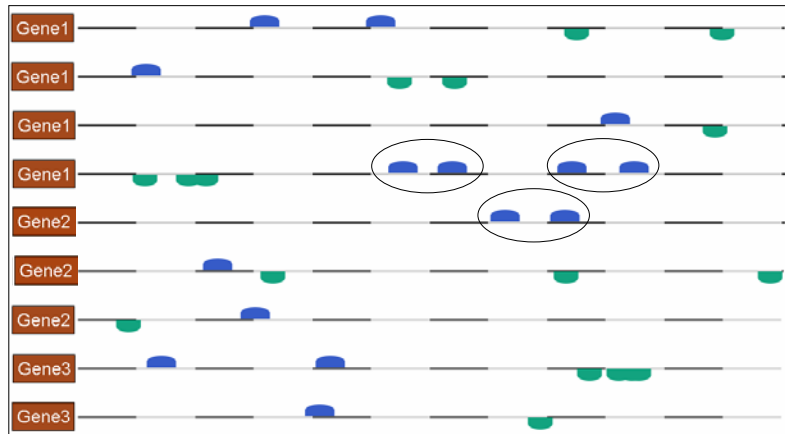
Promoter sequence analysis - schema



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Common patterns (frameworks) of TFBS identified in promoters from a set of three differentially expressed genes in TLR pathway



TFBS common to all sequences in a set of three promoters from TLR pathway



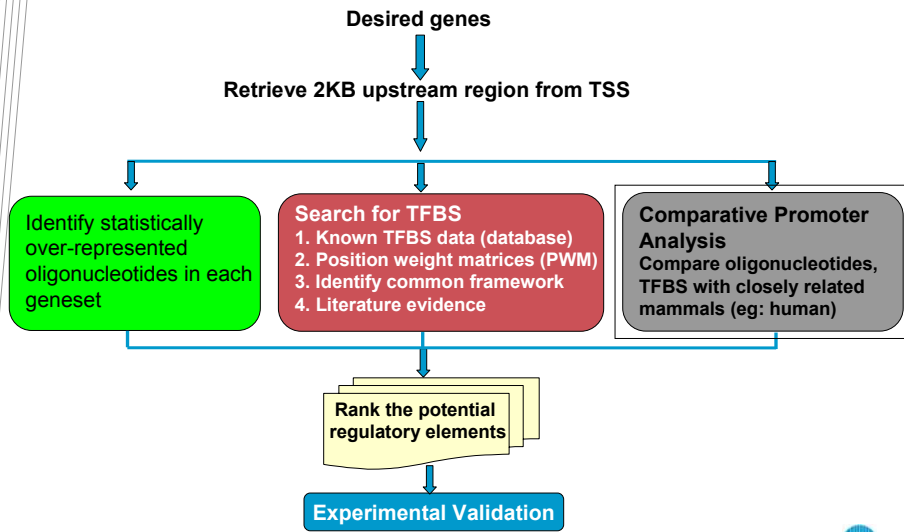
Selected hits from matrix search for transcription factor binding sites

Query Gene	Position (strand)	Sequence	Transcription Factor Name
Resistant: TLR2	150 (-)	aCACTTga	Nkx2-5 (homeobox gene)
Resistant: DUOX1	948 (-)	acaAACAaac	FOXD3(fork head)
Susceptible: IKBKB	686 (-)	atGGAAAttcc c	NF-kappaB
Susceptible: TGFB2	121 (+)	gatAACGGtc	v-Myb

Literature evidence in human →



Promoter sequence analysis - schema



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Promoter region- conserved in mammalian species

List of TFs

V\$AHRR V\$NKXH V\$HOXF V\$STAT O\$VTBP V\$SETF V\$EVI1 V\$DICE V\$EGRF V\$P53F V\$THAP V\$SP1F
 V\$MYT1 V\$FKHD V\$GKLF V\$BRNE V\$PIT1 V\$HNF1 V\$SORY V\$DMRT V\$E2FE V\$MYBL V\$GATA V\$NOLF
 V\$XBBF

Common TF matches located in aligned regions

TF	Species	Region	Sequence
TFBS1	Region_B_Human	1653	CCTGTACCTC ATCTACTCCC AGGTCCTCTT CAAGGGCCAA
	Region_B_Rhesus	1653	CCTGTACCTC ATCTACTCCC AGGTCCTCTT CAAGGGCCAA
	Region_B_Mouse	1546	GTTGTACCTT GTCTACTCCC AGGTTCTCTT CAAGGGACAA
	Region_B_Rat	1486	GCTGTACCTT ATCTACTCCC AGGTTCTCTT CAAGGGACAA
	Region_B_Cow	1653	GCTTTACCTC ATCTACTCAC AGGTCCTCTT CAGGGGCCAA
TFBS2	Region_B_Human	1903	CGGCCCGACT ATCTCGACT TTGCGGAGTCT GGGCAGGTCT ACTTTGGGAT
	Region_B_Rhesus	1903	CTGCCCGACT ATCTCGACT TTGCGGAGTCT GGGCAGGTCT ACTTTGGGAT
	Region_B_Mouse	1793	CTGCCCAAGT ACTTAGACT TTGCGGAGTCT GGGCAGGTCT ACTTTGGAGT
	Region_B_Rat	1733	CTGCCCAAGT ACTTAGACT caCGGAGTCT GGGCAGGTCT ACTTTGGAGT
	Region_B_Cow	1903	CTGCCCGACT ACCTGGACT A TGGCGAGTCT GGGCAGGTCT ACTTTGGGAT
TFBS3	Region_B_Human	2528	CAGACATGTT TTCCGTGAAA ACGGAGCTGA ACAATAG--
	Region_B_Rhesus	2541	CAGACATGTT TTCTGTGAAA ACGGAGCTGA ACAATAG--
	Region_B_Mouse	2394	CAGACATGTT TTCTGTGAAA ACGGAGCTGA GCTGtcc--
	Region_B_Rat	2377	CAAACATGTT TTCTGTGAAA ACGGAGCTGA ACTaccagct
	Region_B_Cow	2526	CAGATGTGTT TTCTGTGAAA ACGGAGCTGA ACTGCAG--

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Conclusions

- We believe that the promoter motifs identified in this study have regulatory potential
- Binding of transcription factors to these motifs might explain the differential expression observed following nematode challenge
- Functional variation in these motifs is therefore likely to contribute to an individual's ability to resist infection



Future directions

Computational

- To establish a roadmap for regulatory sequence analysis based on gene expression data

Experimental validation for initial results

- *In-vitro* experiments such as Electrophoretic Mobility Shift Assay (EMSA)
- *In-vivo* experiments (ChIP-Seq experiments)
- The second set of RT-PCR data spanning 102 genes



Acknowledgments



Antonio Reverter
CSIRO Livestock Industries



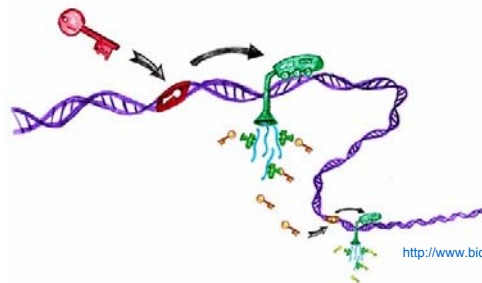
Aaron Ingham
CSIRO Livestock Industries

- ❖ OCE Post-doctoral fellowship
- ❖ SheepGenomics



Transcription Factors (TF) - Factsheet

- Found in all living organisms
- Approximately 10% of genes in the mammalian genome code for TFs
- Have affinity for short, degenerate DNA sequences (5-15 bp)
- Contain one or more DNA binding domains (DBDs)
- Mutated TF genes have been shown to cause numerous diseases (Eg: Haemophilia B Leyden)
- Potential Targets for several therapeutic drugs (Eg: breast cancer)



<http://www.biochem.arizona.edu/>

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Butt et al 1995, Latchman et al 2000



Challenges

- **Ovine genome incomplete, therefore we have chooses bovine as starting point**
- **Several physical binding sites comes up in predictions as a physical TFBS is found every 10 to 15 bps throughout any mammalian genome**
- **A single isolated TF binding site carries no function**
- **TFs work through complexes which are represented on sequence level through sets of TF binding sites in certain distance relationship and orientation ->promoter frameworks**
- **Therefore, we have to decipher gene anatomy landmarks , rather than just promoter motifs**
- **So wee are talking promoter model/ framework and not individual TFBS**

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Why are we doing what we are doing ?

- Promoter features provide clues to gene function that are not obvious from the protein sequence alone
- The organizational features of promoters derived from promoter sequences contain information about the spatial and temporal 'functional context' of expression.
- Genes having similar expression patterns contain common motifs in their promoter regions. Thus, a common set of TFs is likely to control these genes
- Understanding the gene regulation in livestock species is still in primitive stages , so Starting point .. **



Different tissues and families of genes differentially use distinct types of promoters.

Tamoxifen – drug for breast cancer



Gene Cluster	Number of genes per cluster	Sequence	observed occurrences	expected occurrences	P-value
TLR Cluster	25	TCAGAAA	25	9.81	3.5e-05
XDH cluster	10	AGAGAAA	16	3.88	3.3e-06
XDH cluster	10	ccgcg	18	5.29	1.1e-05
Cathepsin cluster	9	cccccg	17	4.42	4.2e-06

