

EADGENE **SABRE**
 CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

EADGENE and SABRE Post-Analyses Workshop
 12-14th November 2008, Animal Sciences Group, Wageningen UR, Lelystad

Combining QTL & Microarray Data

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If x marks the spot how deep is the treasure?

Suzanne Rowe

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DATA

- Differentially expressed genes
 - Hazard et al., 2008
- QTL for ACTH and Cortisol
 - Desautes et al., 2002.

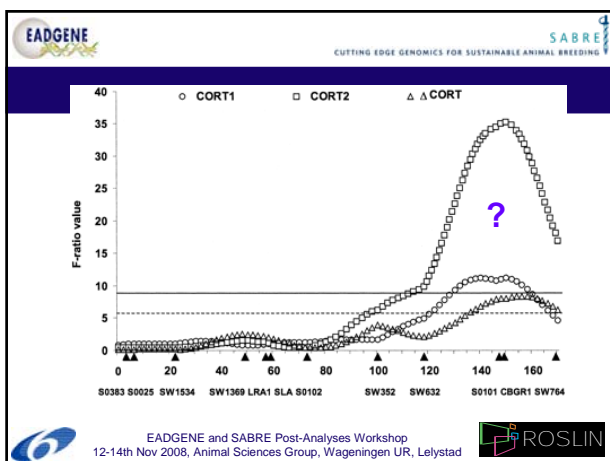
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QTL Data - Neuroendocrine response to novel environment, Meishan vs. Large White.

- Whole genome scan with 123 microsatellite markers (3-12 per chromosome)
- ACTH, Cortisol, and Glucose
 - Poststress cortisol level ($P < 0.001$) Chr 7
 - Basal cortisol level ($P < 0.05$) Chr 7
 - Poststress ACTH Chr1 ($P < 0.05$)
- Meishan Higher Cortisol, Lower ACTH

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Questions

- Candidate genes under peak
- Diff expressed genes under peak
- Shared Pathways
 - Diff expressed set and QTL regions
 - across QTL regions

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Where do the differentially expressed genes map on the genome?

?

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Download the latest genome!

- ftp://ftp.sanger.ac.uk/pub/S_scrofa/assemblies/PreEnsembl_Sscrofa7/

Tip to higher level directory

```
07/29/2008 12:49:26 2,561 EADGENE
07/29/2008 10:17:06 450,457,049 Ssp_sscrofa_sscrofa7_47_dna_chromosome_fa.gz
07/29/2008 01:23:06 283,460,328 Ssp_sscrofa_sscrofa7_47_masked_dna_chromosome_fa.gz
07/29/2008 02:19:06 10,728,429 Ssp_sscrofa_gpg
07/29/2008 07:14:06 4,488,037 Ssp_sscrofa_MSI.gz
```

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To Blast or not to blast, whether tis nobler.....

- `formatdb -i sscrofa.fa -p F`
- `seqret genbank:BX12345 -outseq BX12345.fasta`
- `seqret list:accession.txt -outseq allseq.fasta`
- `blastall -p blastn -d sscrofa.fa -F T -W 20 -b 2 -m 8 -i allseq.fasta -o allseqblast.out`

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Blastn Greatest Hits

- ~8000 hits
- Put in R and ask for top unique identifiers bit score and e-values
- 238 values

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Where do the genes map?

Chr	DE Genes	Chr	DE Genes
1 *	15	11 *	3
2	19	12	11
3 *	6	13	22
4	22	14	25
5 *	14	15	10
6	6	16	8
7 **	21	17 *	10
8 *	13	18 *	6
9	10	X	11
10	6		

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Chromosome 1

Up Meishan	Down Meishan	Down Meishan + ACTH
Hik1	Ssr2	Whsc11
Mrsp6	Rpl36	
Rnf2		
Txn		
Ppap2d		
Notch4		
Cd99		
Star		
unknown		
Unknown		

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Combining QTL & Microarray Data
 Suzanne Rowe
 The Roslin Institute and R(D)SVS, University of Edinburgh, UK

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Chromosome 7

Up Meishan	Up Meishan + ACTH	Down Meishan	Down Meishan + ACTH	Up ACTH	Down ACTH	Up Meishan down ACTH	Down Meishan up ACTH
C14orf2	Cd83	unknown	Ephx1	DDx3x	Cntf	Anpep	C14orf4
Col21a1	B3gnt3	Tmem14c	Nfat5	Ler3	Mapk14		
Hint1	Znf512	Tmem14c	Nr2f1		Mxra7		
Hist2h2aa	Znf259	Bphl					
Mrps10	Homer2	Tll12					
Notch4	Maob	unknown					
Nup50	unknown	H2afj					
Pgk1							
Suc1g2							

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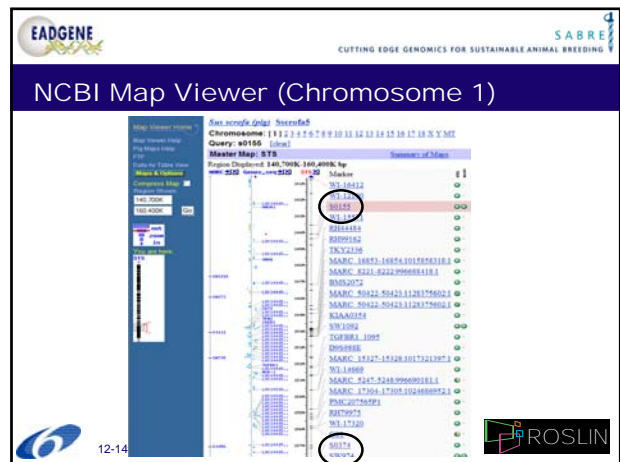
Hmm.....

What are the other genes under the QTL Peaks

?

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- ### Check marker positions to define region
- More Blasting (Appropriate?)
 - NCBI
 - Map Viewer
 - Blast facility
 - Chromosome 1
 - S0155 to S0374
 - 140 – 160 Mb
 - Chromosome 7
 - S0101 SW764 SW2446
 - 112 – 125 Mb
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- | | |
|---|---|
| <p>Chromosome 1 (131)</p> <p>CD72 molecule
 guanine nucleotide binding protein
 similar to cOR13F4 olfactory receptor
 Similar to glucosaminyl (N-acetyl) transferase 1,
 similar to glutamate receptor, ionotropic, N-methyl-D-aspartate 3A
 similar to Glyoxylate reductase/hydroxyypyruvate reductase
 similar to N-acetylneuraminic acid phosphate synthase
 similar to plasticity related gene 3
 similar to Signaling threshold-regulating transmembrane
 similar to STAR-related lipid transfer protein transforming growth factor, beta receptor 1</p> | <p>Chromosome 7 (130)</p> <p>alpha-1-antichymotrypsin 2
 ataxin 3
 serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1
 similar to cytochrome P450, family 46, subfamily A, polypeptide 1
 similar to DEAD (Asp-Glu-Ala-Asp) box polypeptide 24
 similar to glutathione S-transferase
 similar to goosecoid
 similar to Olfactory receptor 4K1
 similar to putative membrane steroid receptor
 similar to thyroid hormone receptor interactor 11</p> |
|---|---|
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Ok
 Now for the pig pathways
 ?

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KEGG Putative pathways

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Access Database

- ftp://ftp.genome.jp/pub/kegg/genes/organisms/ssc/ssc_ncbi-geneid.list Get Kegg I'd's
- ftp://ftp.genome.jp/pub/kegg/genes/organisms/ssc/ssc_pathway.list Get Kegg pathway I'd's
- ftp://ftp.genome.jp/pub/kegg/pathway/map_title.tab Map to Kegg pathways
- <ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/gene2go.gz> Get go terms

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Pig Pathways Chr 1 (2/131 genes)

TGFBR1	TGF-beta signaling pathway
Transforming growth factor beta 1	Chronic myeloid leukemia
	Pancreatic cancer
	Adherens junction
	Cytokine-cytokine receptor interaction
	MAPK signaling pathway
	Colorectal cancer
	Calcium signaling pathway
GNAQ	Melanogenesis
Guanine nucleotide binding protein	GnRH signaling pathway
	Long-term depression
	Gap junction
	Long-term potentiation

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Pig Pathways Chr 7 (2/130 genes)

LOC 396850 glutathione S-transferase [Sus scrofa] → Metabolism of xenobiotics by cytochrome P450
 → Drug metabolism - cytochrome P450
 → Glutathione metabolism

Serpina 1 "serpin peptidase inhibitor, clade A (alpha-1 antitrypsin), member 1 [Sus scrofa] → Complement and coagulation cascades

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Next Step

- Combine and compare gene lists
- Blast pig genes against human/model genome for pathway analysis?
- Need relevant gene universe to test for enrichment
- That's for the pathway people.....!!!



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Conclusion



- Used a few simple steps to combine QTL and microarray data – much more to do
- Explore differences and similarities between genes/pathways underlying phenotypic traits
- Still dependent on model species
- Is it merely verification (*feel good*) or is there useful information to be had?

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Thoughts on digging for treasure



- These maps are all different
- Is this quicksand – how should I test it - can I tell just by looking ?
- What tools should I use (shiny ones don't seem to work) ?
- What clues are damaged/lost by digging too hard ?
- When should I stop ?
- What does their map say ?

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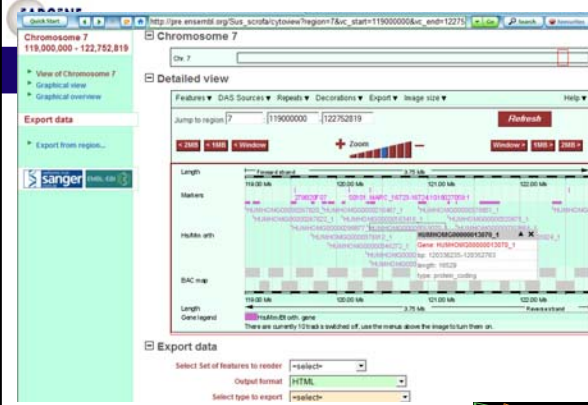
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http://gen.enscm.fr/Sus_scaffoldviewer/region?&uc_start=119000000&uc_end=12271

Chromosome 7
 119,000,000 - 122,752,819

View of Chromosome 7
 Graphical view
 Graphical overview

Export data
 Export from region...



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