How might small ruminant industries utilize genomic tools?

RNAseq and juniper utilization

Penny K. Riggs

ANIMAL SCIENCE
TEXAS A&M UNIVERSITY

TEXAS A&M AGRILIFE RESEARCH

Mechanisms that enable juniper consumption by goats

- OBJECTIVE: Examine gene expression in liver of goats selected for EBV for differential consumption of juniper

- Approach: Liver samples from 8 goats for RNAseq analysis
  - 4 male, 4 female, selected for high- or low- juniper consumption
  - Not challenged with juniper in diet

USDA NSIIC

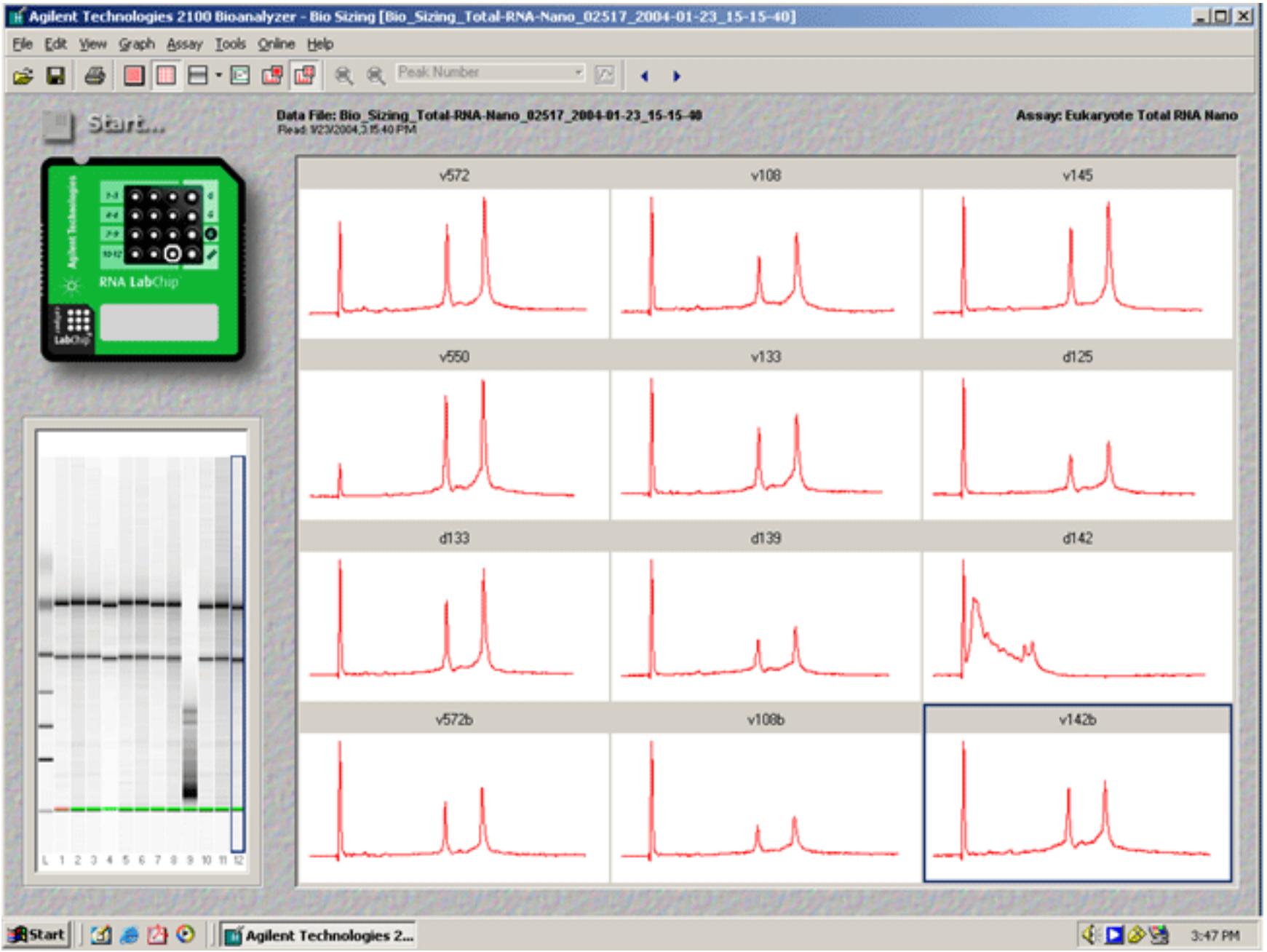
Initial approach

- Expression analysis by qRT-PCR of selected metabolism genes from liver biopsies

- Targets
  - Cytochrome p450 genes
  - Uridine glucoronosyltransferase genes
  - Glutathione transferase genes
  - Genes hypothesized to have some potential role in metabolism of monterpenes

Challenges

- Available genome sequence
- RNA quality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RNA LabChip
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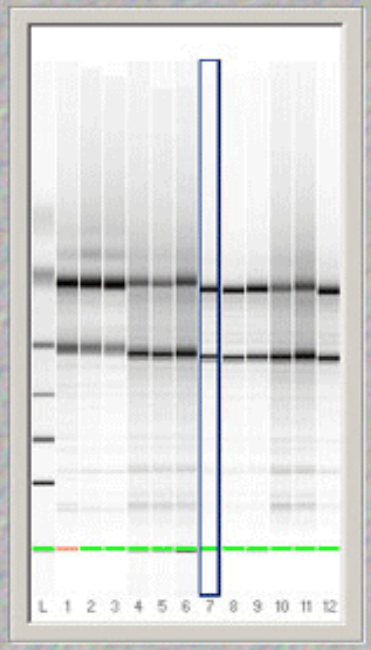
Assay: Eukaryote Total RNA Nano



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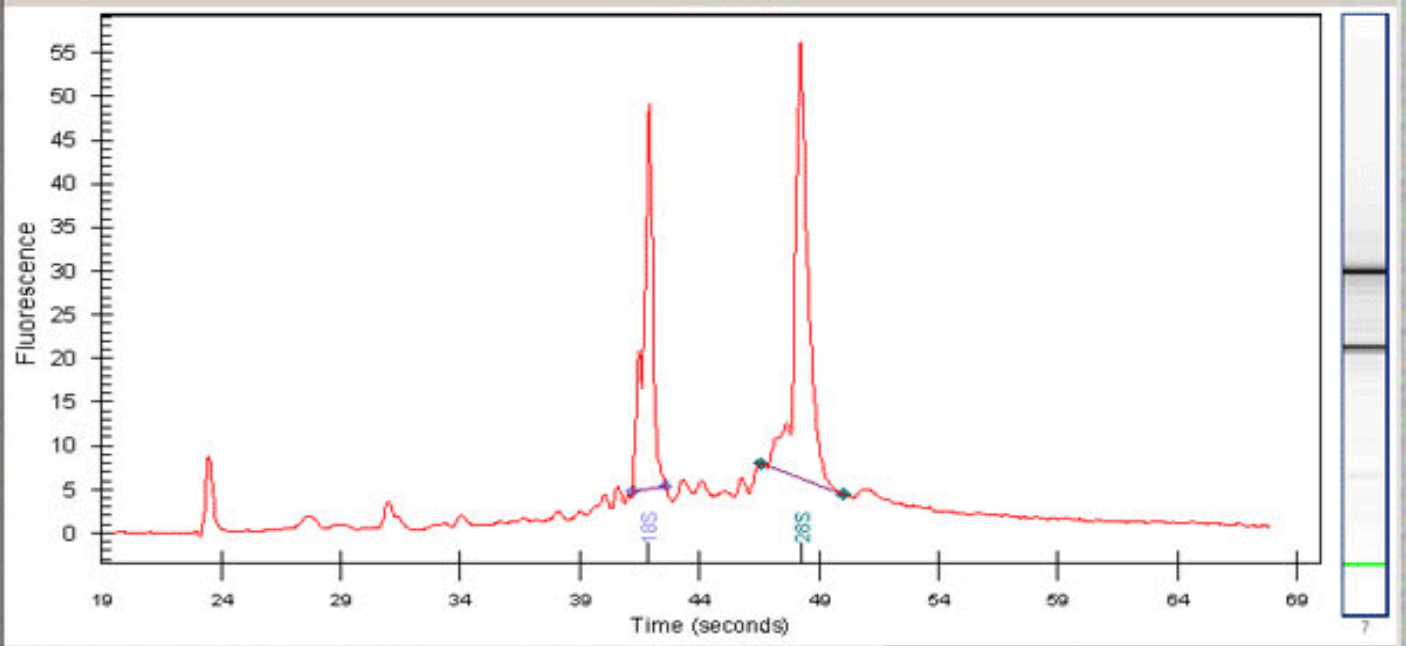

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1	18S	41.22	42.59	47.62	15.49
2	28S	46.57	50.02	67.46	21.94

Sample | Settings | RNA | Results | Errors

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RNA Concentration	193 ng/ul
rRNA Ratio [28S / 18S]	1.42

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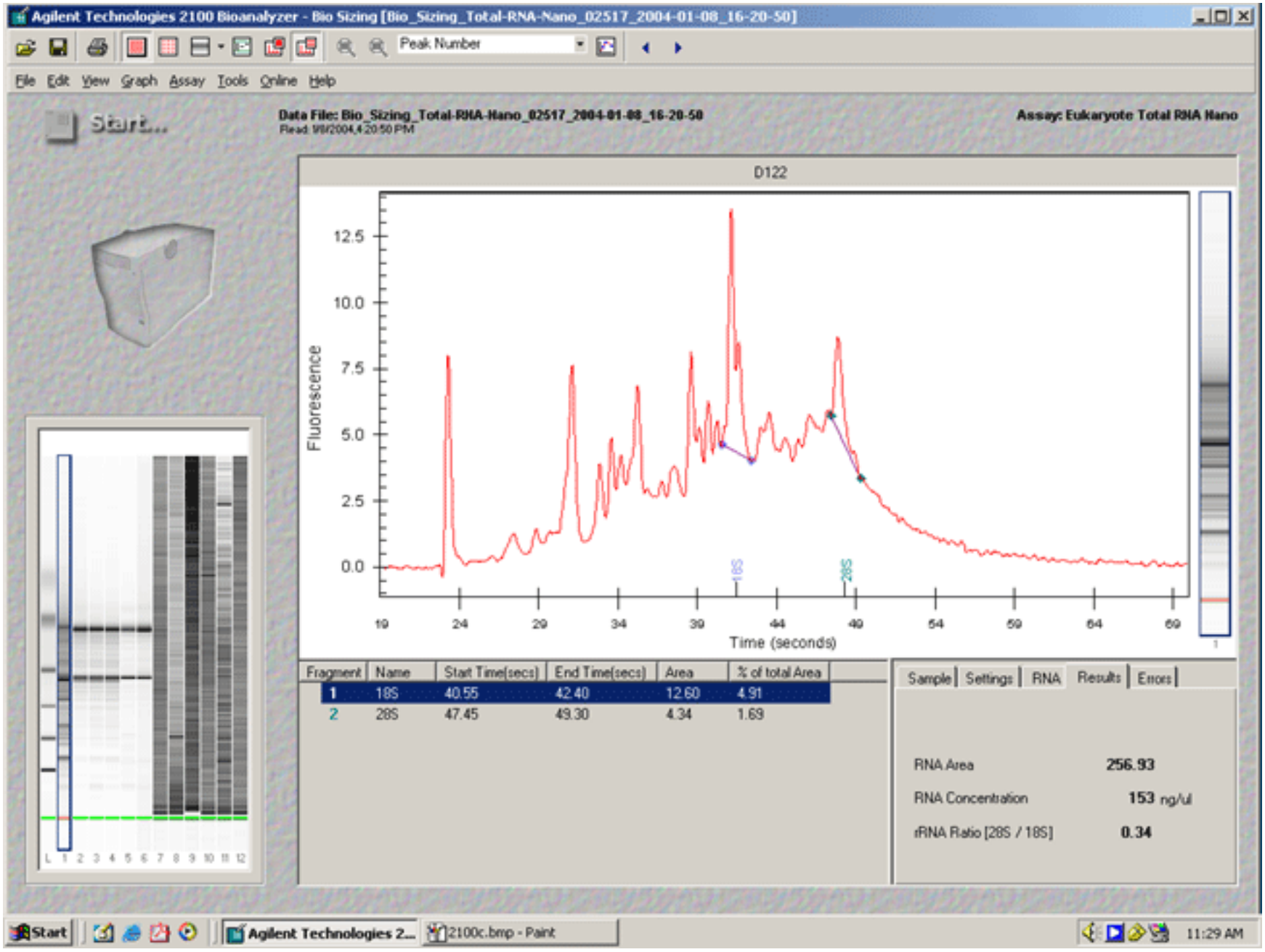
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Assay: Eukaryote Total RNA Nano
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7.5
5.0
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18S
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19
24
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34
39
44
49
54
59
64
69
Time (seconds)
Fragment | Name | Start Time(secs) | End Time(secs) | Area | % of total Area
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2 | 28S | 47.45 | 49.30 | 4.34 | 1.69
Sample | Settings | RNA | Results | Errors
RNA Area 256.93
RNA Concentration 153 ng/ul
rRNA Ratio [28S / 18S] 0.34
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Challenges

- RNA Quality
  - Goat liver – challenging!
  - Protocol modifications required
  - Able to obtain high quality RNA
  - Important to evaluate RNA
- Sequence
  - Highly variable genes – needed sequence from goat population

RNA Seq results

3.1 Raw data

	Total read pairs	Read length	Total read bases
4049goat	23,900,520	101	4,827,905,040
4330goat	25,908,855		5,233,588,710
4361goat	24,657,198		4,980,753,996
4722goat	25,013,997		5,052,827,394
4927goat	25,916,644		5,235,162,088
4941goat	24,208,812		4,890,180,024
5038goat	26,667,106		5,386,755,412
5152goat	24,775,686		5,004,688,572

Illumina TruSeq - MacroGen

RNA Seq Results

- Sequence was assembled and aligned to the draft goat genome sequence (chi_ref_CHIR_1.0 http://www.ncbi.nlm.nih.gov/genome/10731

Nat Biotechnol. 2013 Feb;31(2):135-41. doi: 10.1038/nbt.2478. Epub 2012 Dec 23.

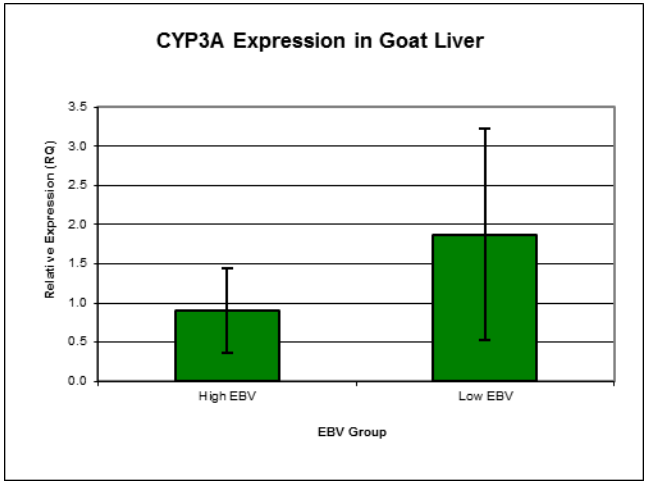
Sequencing and automated whole-genome optical mapping of the genome of a domestic goat (Capra hircus).

Dong Y[1], Xie M, Jiang Y, Xiao N, Du X, Zhang W, Tosser-Klopp G, Wang J, Yang S, Liang J, Chen W, Chen J, Zeng P, Hou Y, Bian C, Pan S, Li Y, Liu X, Wang W, Servin B, Sayre B, Zhu B, Sweeney D, Moore R, Nie W, Shen Y, Zhao R, Zhang G, Li J, Faraut T, Womack J, Zhang Y, Kijas J, Cockett N, Xu X, Zhao S, Wang J, Wang W.

- Yunnang black goat was donor
- Only about 50% of our sequence data could be aligned to existing genome assembly

RNA Seq Results

- Generated new draft liver transcriptome from our data.
- Assembled RNAseq data into contigs
  - Despite short read length, able to get long contigs up to nearly 5kb lengths.
  - More than 250,000 unique transcripts
  - Novel transcripts between groups
  - Identified goat-specific cytochrome genes for targeted expression analysis



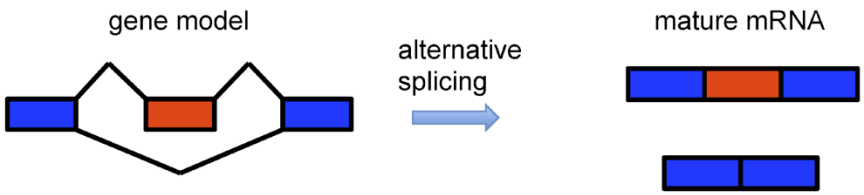
RNA Seq results

- Splicing variation with Dr Peng Yu
  - New software to analyze splice variants

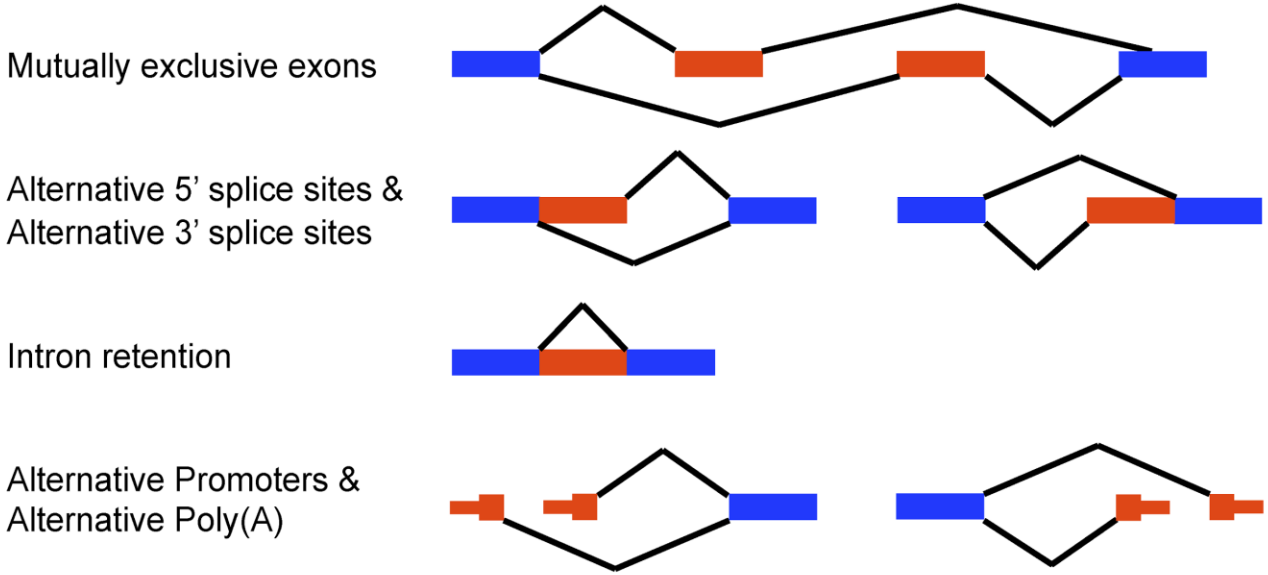
- Initial results
  - 54 significant splice variation events

Splice Variation

- Exon Skipping – can yield two isoforms

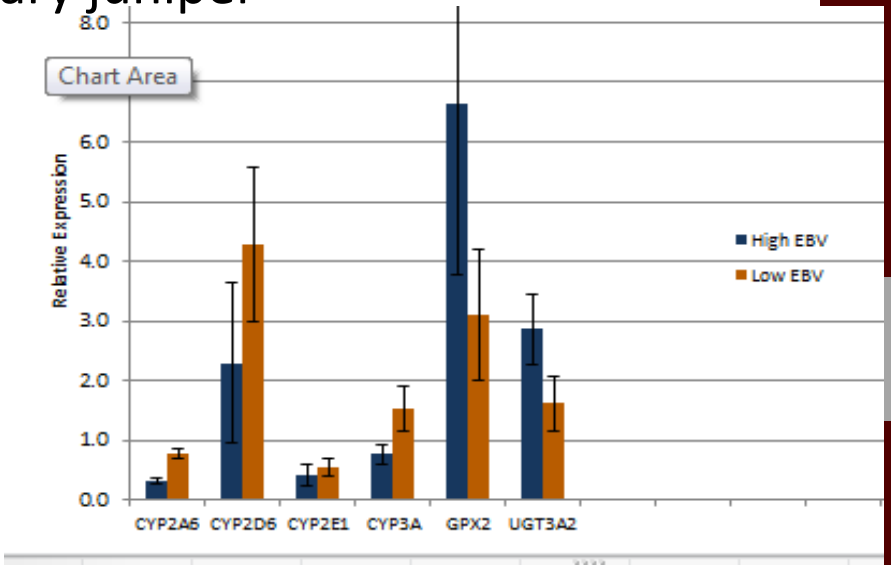


- Often more complicated



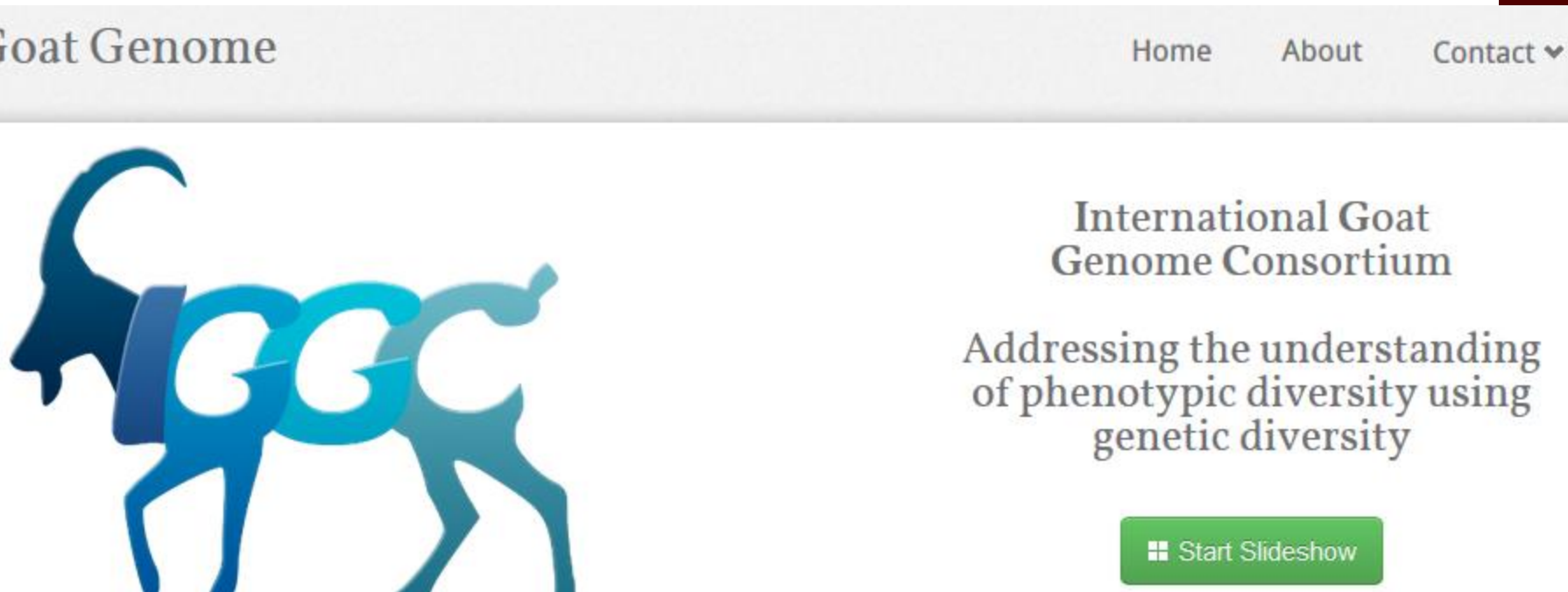
Directions

- Utilizing transcriptome data to examine specific sequences for expression analysis
  - E.g. Cyp, UPG, GST gene families
- Contribute new data for revised goat genome assembly
- Challenge experiments with dietary juniper



Resource outlook

- Much progress in past few years



CHI Genome v2.0 initial availability late summer??

ISGC

International Sheep Genomics Consortium

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International Sheep Genomics Consortium

The sheep genome has been published in Science

- The fully annotated genome (version 3.1) is available via
- ENSEMBL
- NCBI
- UCSC

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Science 6 June 2014:
Vol. 344 no. 6188 pp. 1168-1173
DOI: 10.1126/science.1252806

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REPORT

The sheep genome illuminates biology of the rumen and lipid metabolism

Yu Jiang[1,2,3,*], Min Xie[4,*], Wenbin Chen[4,*], Richard Talbot[5], Jillian F. Maddox[6,†], Thomas Faraut[7], Chunhua Wu[8,‡], Donna M. Muzny[9], Yuxiang Li[4], Wenguang Zhang[1,10,11], Jo-Ann Stanton[12], Rudiger Brauning[13], Wesley C. Barris[2,§], Thibaut Hourlier[14,21], Bronwen L. Aken[14,21], Stephen M. J. Searle[14], David L. Adelson[2,ll], Chao Bian[4], Graham R. Cam[2,¶], Yulin Chen[3], Shifeng Cheng[4], Udaya DeSilva[2,#], Karen Dixen[15], Yang Dong[1], Guangyi Fan[4], Ian R. Franklin[2,**], Shaoyin Fu[10], Pablo Fuentes-Utrilla[5], Rui Guan[4], Margaret A. Highland[16,17], Michael E. Holder[9],

www.goatgenome.org

Towards a 50K International Goat SNP chip

Home Contacts

As it was announced about a year ago, the International Goat Genome Consortium federates existing projects on SNP detection in order to produce a high density SNP chip. The objective is to have a 50K SNP chip available at the end of year 2011. This first effort to give access to a high density genotyping tool may be completed in the future by other contributions and the tool may evolve regarding the needs of the International Community. If you are interested in ordering this tool, please contact Gwenola Tosser-Klopp (IGGC co-coordinator, INRA), Wenguang Zhang (IGGC co-coordinator, IMAU/KIZ/BGI) and Cindy Lawley (Illumina Scientist/Agriculture Consortia Manager Illumina, Inc.)

http://snp.toulouse.inra.fr/~sigenae/50K_goat_snp_chip/index.html

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RESEARCH ARTICLE

Design and Characterization of a 52K SNP Chip for Goats

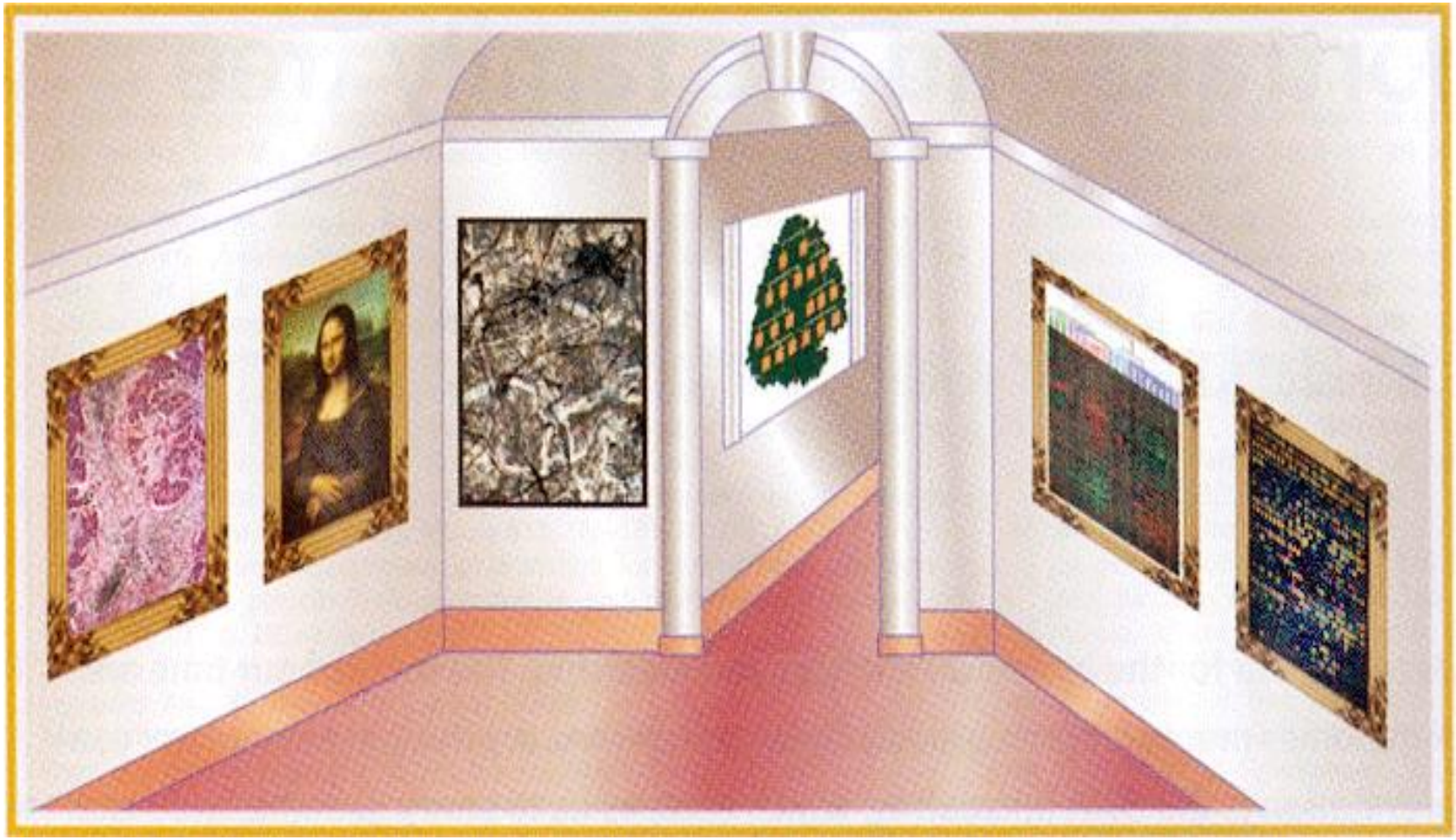
Gwenola Tosser-Klopp, Philippe Bardou, Olivier Bouchez, Cédric Cabau, Richard Crooijmans, Yang Dong, Cécile Donnadieu-Tonon, André Eggen, Henri C. M. Heuven, Saadiah Jamli, Abdullah Johari Jiken, Christophe Klopp, Cynthia T. Lawley, [...], and the International Goat Genome Consortium [view all]

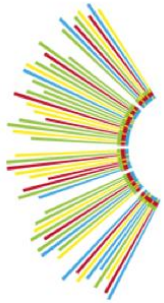
Published: January 22, 2014 • DOI: 10.1371/journal.pone.0086227

52k Chips available from illumina

Can add content

Many new SNP available (e.g. Reecy et al, others)





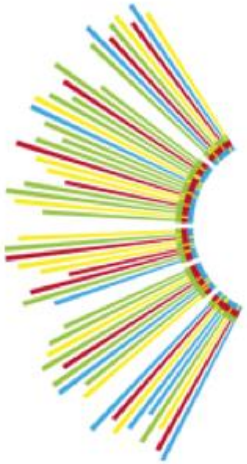
FAANG

Functional Annotation of Animal Genomes

The most significant challenge in the post-genomic era is connecting genotype to quantitative phenotype in basic and applied biology - the Genome to Phenome challenge.

There are new opportunities in animal genomics and phenomics, and we propose the organization of an international effort- the "Functional Annotation of ANimal Genomes" project to identify all functional elements in animal genomes. FAANG will:

- Coordinate international efforts to maximize efficiency and data quality.
- Deliver standardized datasets from a limited set of individuals representing species that have reference quality draft genome assemblies.
- Establish an infrastructure capable of efficiently analyzing genome-wide functional data for animal species.



FAANG

Functional Annotation of Animal Genomes

A coordinated international action to accelerate genome to phenome

FAANG aims to:

- Standardize core assays and experimental protocols
- Coordinate and facilitate data sharing
- Establish an infrastructure for analysis of these data
- Provide high quality functional annotation of animal genomes

More information: www.FAANG.org

Questions answered: faang@iastate.edu

Summary

- Genome and molecular tools are becoming more readily available
- Can start to utilize for selection and understanding of mechanisms, in conjunction with traditional methods

Acknowledgments

Dr. Dan Waldron
Kelli Kochan
Maisie Llewellyn
Kranti Konganti
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Gwenola Tosser

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USDA-NIFA, Texas Dept of Agriculture