The Buffalo Atlas
A RNAseq-based Gene Expression Atlas in Domestic Water buffalo
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Agrigenomics
- Exponential population growth, changing climate
- Feed the world’s growing population
- Help drive sustainable productivity and tackle the challenges facing world food supply
- Aid plant and animal breeders identify desirable traits, leading to healthier and more productive crops and livestock

Distribution of buffalo population

Increasing global buffalo population

Significant Indian buffalo population

The Buffalo Atlas Project

£13M India-UK collaboration in Farmed Animal Health and Disease
Transcriptome analysis in Indian water buffalo and the genetics of innate immunity
David Hume, The Roslin Institute, and Satish Kumar, Centre for Cellular and Molecular Biology
Objectives of the Atlas project
- define a comprehensive buffalo transcriptome based upon quantitative mRNA profiling of tissues and cell types
- prevalence and significance of expressed genetic variation and allelic imbalance within and between buffalo breeds
- compare the buffalo transcriptome to other ruminants and to other mammals and to gain insights into evolution of the mammalian transcriptome
- identify sets of coexpressed transcripts to annotate genes of unknown function within clusters by association with genes of known function
- provide an online resource for the ruminant genomics research community where free access to all data will be available together with the results of our analyses

Atlas outline
- Eighty tissue and cell types collected per animal from eight adult buffalo (4 male, 4 female)
  - Mediterranean, Padharpuri, Bnadawari breeds
  - Diverse collection of tissue and cell types
  - Shown to give the maximum coverage of the mammalian transcription in human and mouse FANTOM projects
  - Main organ systems, immune tissues and cells, GI tract
- RNA-seq based atlas of gene expression
  - Core atlas
    - RNA seq of 168 tissues from 4 Mediterranean buffalo
    - Supplemented with Indian buffalo samples
    - RNA seq of 58 tissues from 4 Indian buffalo

Sequencing and Analysis Approach
- Stranded Illumina TruSeq libraries were prepared and sequenced by Edinburgh Genomics
  - 125bp PE sequencing
  - 70 samples sequenced at high depth (100M reads) Total RNAseq
  - 156 samples sequenced at medium depth (25M reads) mRNAseq
  - Sequencing process ongoing
    - Progress – sequences for 80 samples received
    - Analysis ongoing
      - Bioinformatic pipeline developed by Steve Bush
      - FASTQC>Trimmomatic>HISAT2/Stringtie>Ballgown
      - Differential expression visualised in Biolayout Express3D

Example dataset in Biolayout Express3D

Genome resequencing
- Buffalo draft genome sequence submitted by A Zimin and J Williams in Sept 2013
  - Female Mediterranean buffalo, 70X coverage
- Collaboration with S Kumar, CCMB and S Gokhale, BAIF
  - Resequenced multiple representative individuals from diverse Indian buffalo breeds (SciGenom, India)
  - 36 buffalo from six Indian breeds, 10X coverage (Illumina)
  - Sequences to be analysed at Roslin by Prasun Dutta, postgraduate student
  - Improve assembly of the buffalo genome and to document the variome

Disease susceptibility in Indian buffalo
- Differing disease susceptibility in buffalo and cattle and other ruminants
  - Buffalo and cattle are generally afflicted by the same diseases, but often with different levels of susceptibility
- Tuberculosis and Brucellosis are both very prevalent diseases in Indian buffalo
  - No test and slaughter programme in India
  - Level of prevalence suggests uninfected animals may be resistant
  - Unique opportunity to identify genetic variation controlling the pathology of these important pathogens
Epidemiology study

• Investigate the underlying mechanism of disease resistance (or tolerance) in Indian buffalo to two important diseases bovine tuberculosis and brucellosis

• Genotyping of three groups of buffalo
  – definitively bTB infected
  – definitively brucella infected
  – negative for both diseases
  – 500 buffalo per group, identified by ELISA and retested after one and two years
  • Genotype by sequencing, looking to identify and validate polymorphisms in genes responsible for the immune response to understand the molecular mechanisms of susceptibility or resistance

Summary and conclusions

• Generation of an atlas of gene expression in water buffalo
• Resequencing of diverse Indian buffalo breeds
  • improve the assembly of the buffalo genome and to document the variome
• Provide an online resource for the ruminant genomics research community
• Investigate the underlying mechanism of disease resistance (or tolerance) in Indian buffalo

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