Identification and conservation of novel Long Noncoding RNAs in cattle using RNASeq data

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lncRNA prediction strategies

- RNASeq based
- Homology based (Human Gencode 2 cow)
- Homology based (cow RNASeq filtered predictions vs several mamalian genomes)

Bovine data

- 30 Liver samples (@CRG) 2x 96bp, 2 500Mr
- 28 liver, small intestine, skeletal muscle samples 80bp x 1/2 (Jerry Taylor)
- 15 ovary samples 51bp x1 (Milano)
- 84 udder/muscle samples (Denmark)
- 1.2M bovine ESTs from NCBI (complementary to RNASeq)

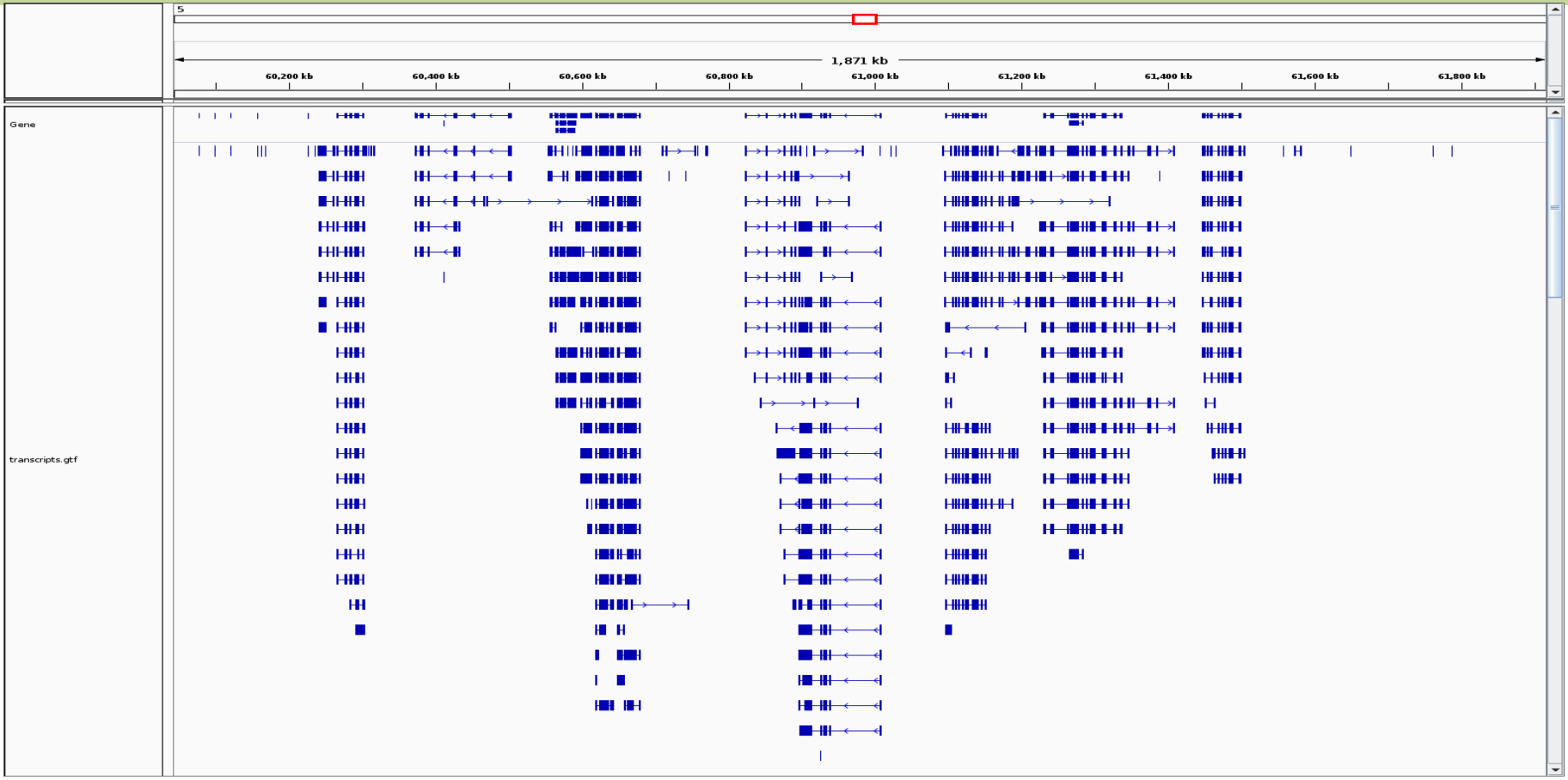
Bos strategy 1

- mapping both with in house RNASeq mapping pipeline GRAPe
- GSNAP
- GMAP for ESTs (filtered by seqclean from PASA)
- cufflinks gene models
- merge models
- remove genes overlapping with known genes from ENSEMBL 72

SEVENTH FRAMEWORK PROGRAMME

Quantomics

Bos results merged cufflinks



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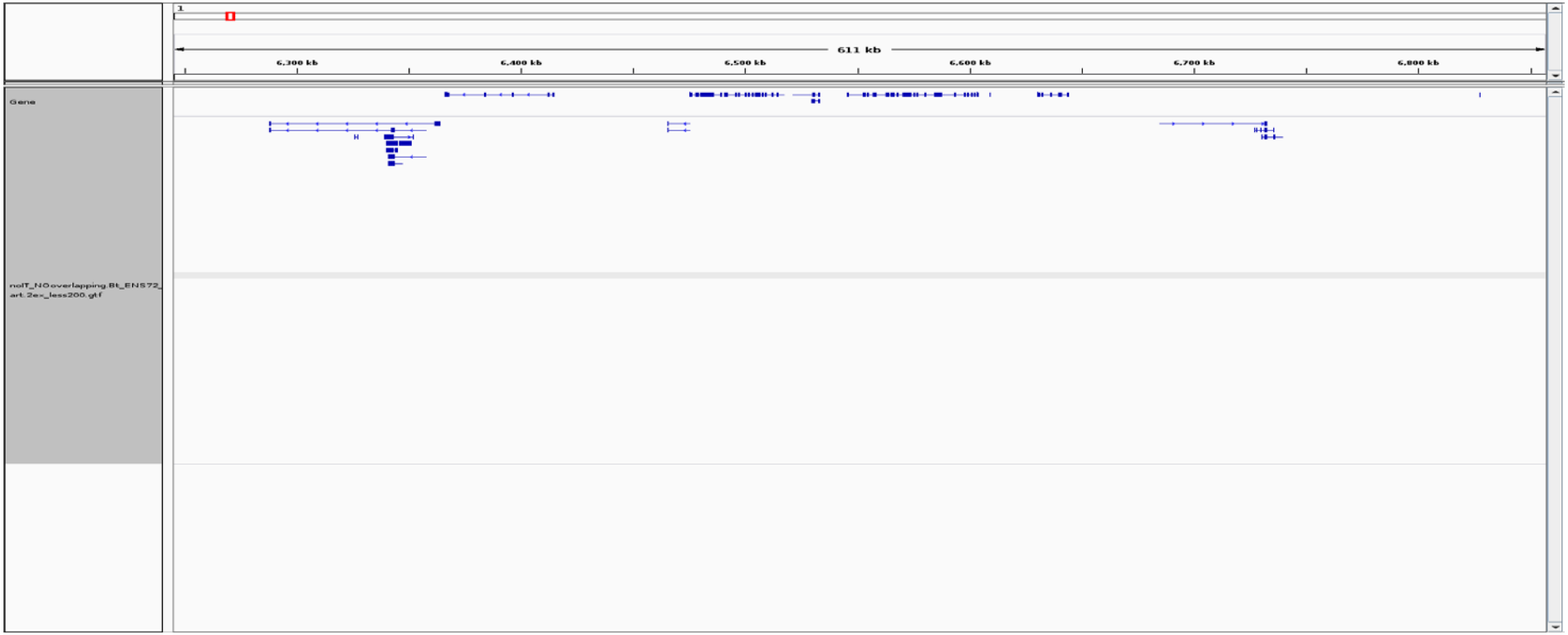
Remove genes overlapping with ENSEMBL 72



Bos strategy 2

- remove 1 exon genes and transcripts shorter than 150bp
- extract transcript sequences
- cluster transcripts at 90% identity (usearch)
- RESULT 1:
  - 15 356 transcripts from 9775 genes
- check for repeats, ORFs, sizes etc.
- find putative non-coding transcripts

Sanity check

- blastn all transcripts against human GENCODE 17 lncRNAs
- top hits (e= 0.0) include MALAT1, KCNQ1, KLHL7, MMP24 etc.

Repeat Masking

- 40% of all our transcript sequence was masked by RepeatMasker (*)
- selected transcripts with no more 20% of the repetitive sequence
  - RESULT: 4541 transcripts from 3255 genes
  - Sanity check: still got MALAT1 etc.

➢ * Gencode 17 human: 25% repetitive

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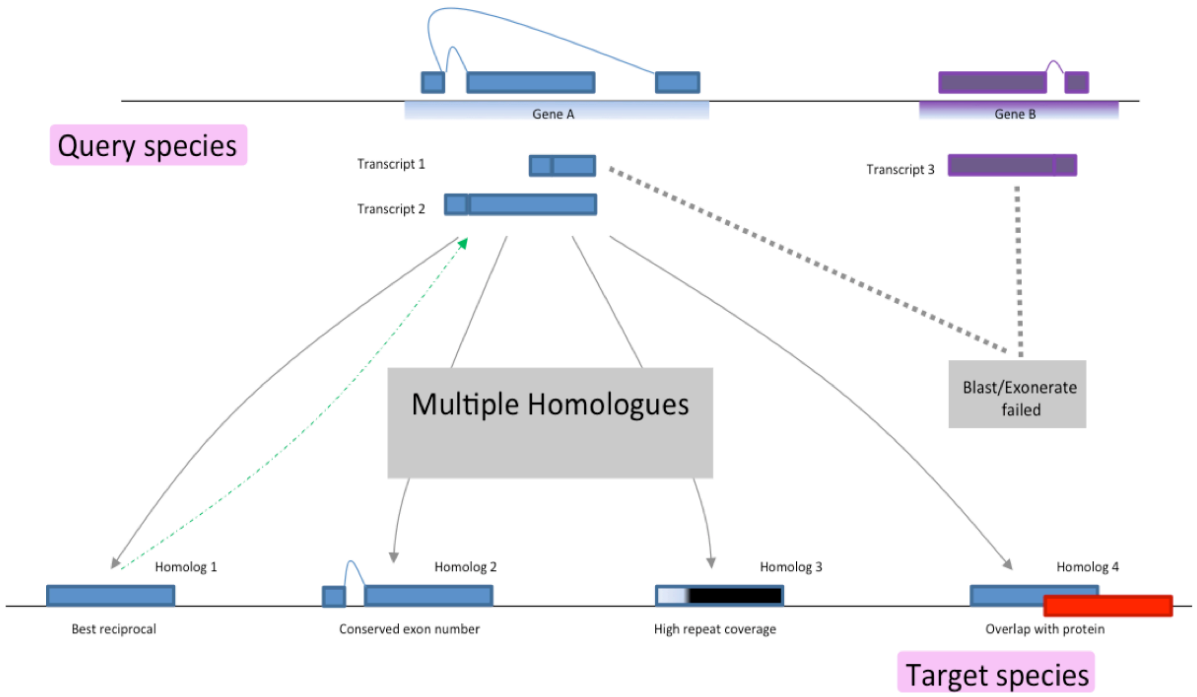
Homology prediction: PipeR

Mapping overview



Human Gencode 2 cow: homology

- Take all Gencode 17 lncRNA transcripts
- Run PipeR using cow genome as target
- RESULT:
  - 4210 human transcripts have 4758 homologues from 3195 genes in cow

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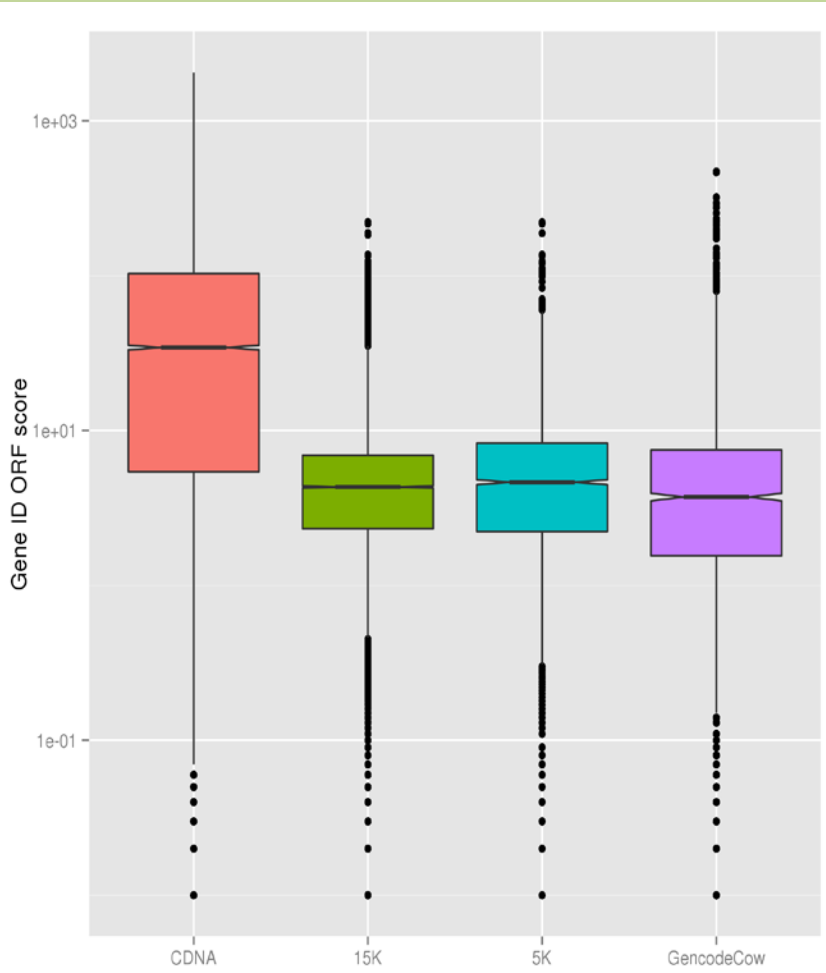
Results comparison: geneid coding potential



Homology vs RNASeq

- Little overlap:
  - 3195 genes have just 295(!) overlaps with strict non-repetitive RNASeq transcripts
  - But 1423 of them have overlap with all cufflinks 2 exons or more RNASeq based transcripts (at 10% or more)

- Probably in both approaches we do not get complete gene models but just gene fragments

Cow lncRNA (RNASeq) vs other species

- So far 874 cow queries queries produced:
  - 384 human
  - 404 pig
  - 211 mouse
  - Give it another week or so…

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Thank you for your attention!

And everybody involved during the study!

- Centre de Regulacio Genomica (CRG), Spain,
  - Bussotti, G., Prieto, P. Guigo, R. , Notredame, C
- Aarhus University, Denmark,
  - Sørensen, P.
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