Metatranscriptomics

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science foundation ireland

Agriculture and Food Development Authority

The Irish Agriculture and Food Development Authority

Why do Metatranscriptomics?

- Primer-free 16S rRNA community analysis
- Measure gene expression
- Look at metabolically active organisms
- Enrich genes for specific organisms

Design

Morning x3

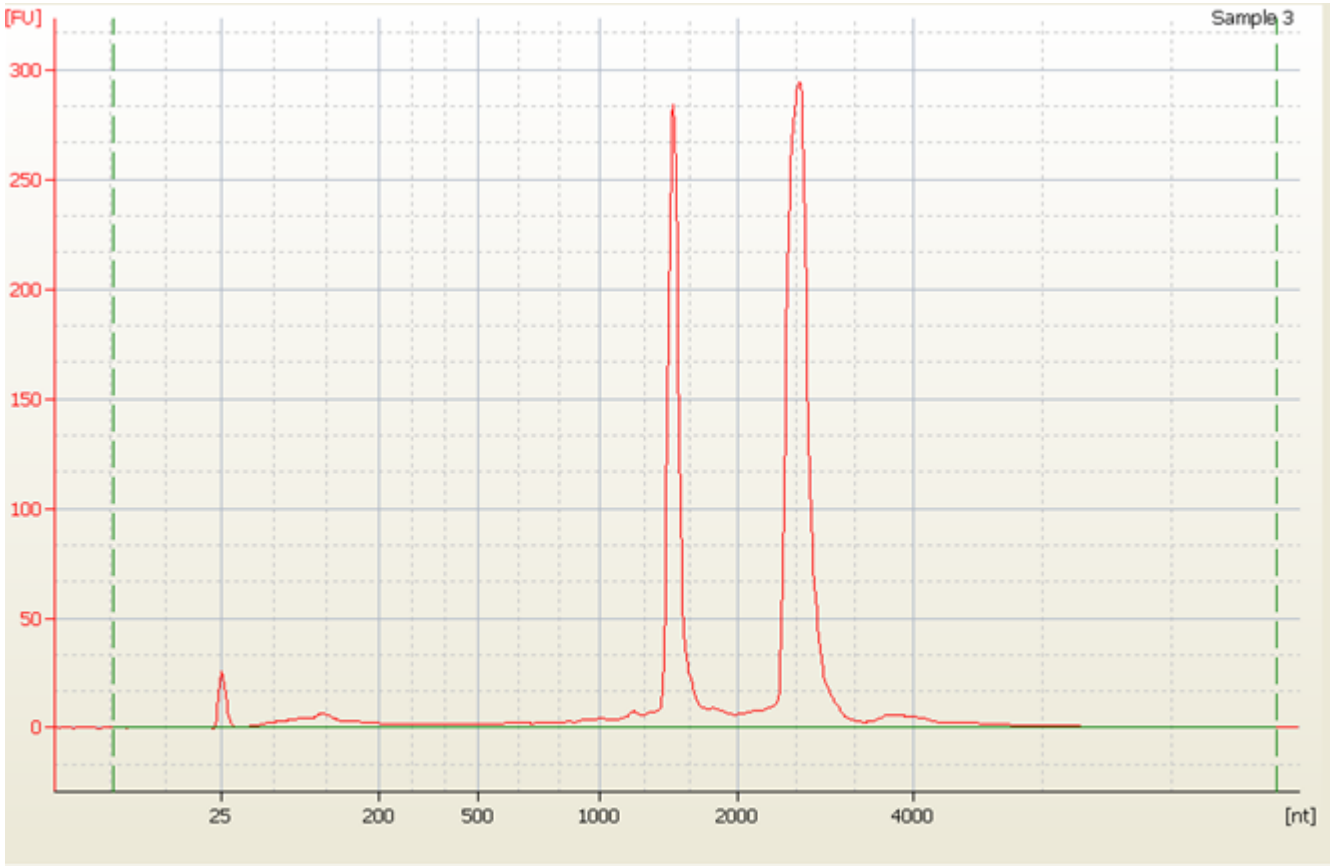
Evening x3

Laboratory protocol

Liquid Nitrogen
Grinding

RNA purification
(RNAeasy)

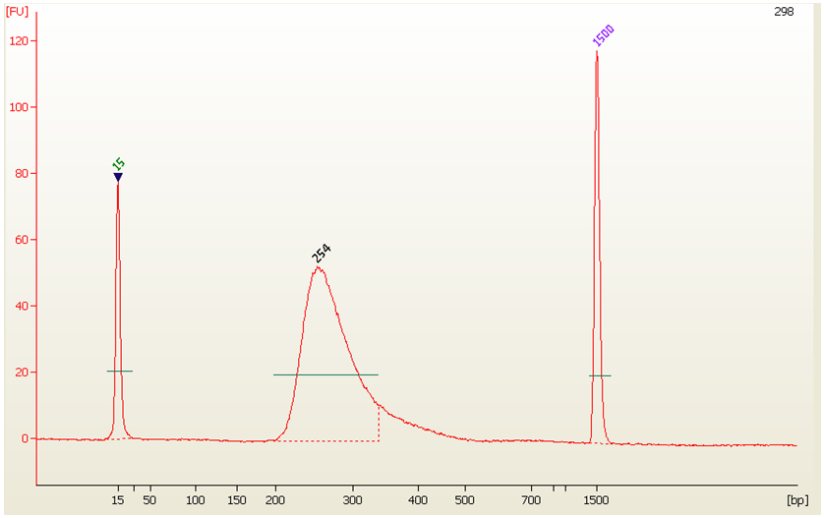
Bead beating



RNA Area:	1,272.6		rRNA Ratio [23s / 16s]:	1.8	
RNA Concentration:	735	ng/µl	RNA Integrity Number (RIN):	10	(B.02.08)

Eukaryotic mRNA

illumina TruSeq



Invitrogen life technologies

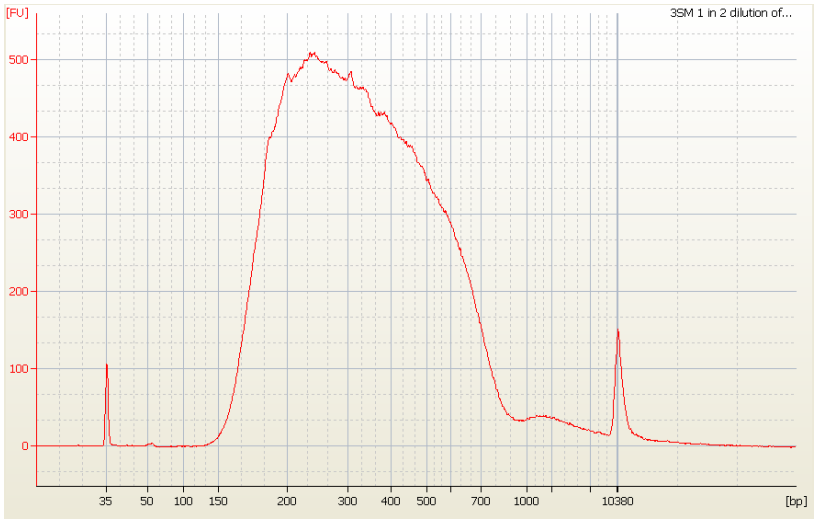
Genomic DNA degrataion (Turbo DNase)

epicentre an Illumina company

Ribo-Zero™

ScriptSeq™

Prokaryotic mRNA



Illumina MiSeq

250bp Paired-end

Eukaryotic mRNA

Trimmed to 150bp

Morning 1	1,594,735
Morning 2	1,455,903
Morning 3	1,274,027
Evening 1	1,908,175
Evening 2	1,220,750
Evening 3	787,169

Prokaryotic mRNA

Trimmed to 140bp

Morning 1	1,157,429
Morning 2	1,647,889
Morning 3	2,082,328
Evening 1	2,085,877
Evening 2	1,920,904
Evening 3	1,744,960

Contig Assembly

http://trinityrnaseq.sourceforge.net/

Read Quantification

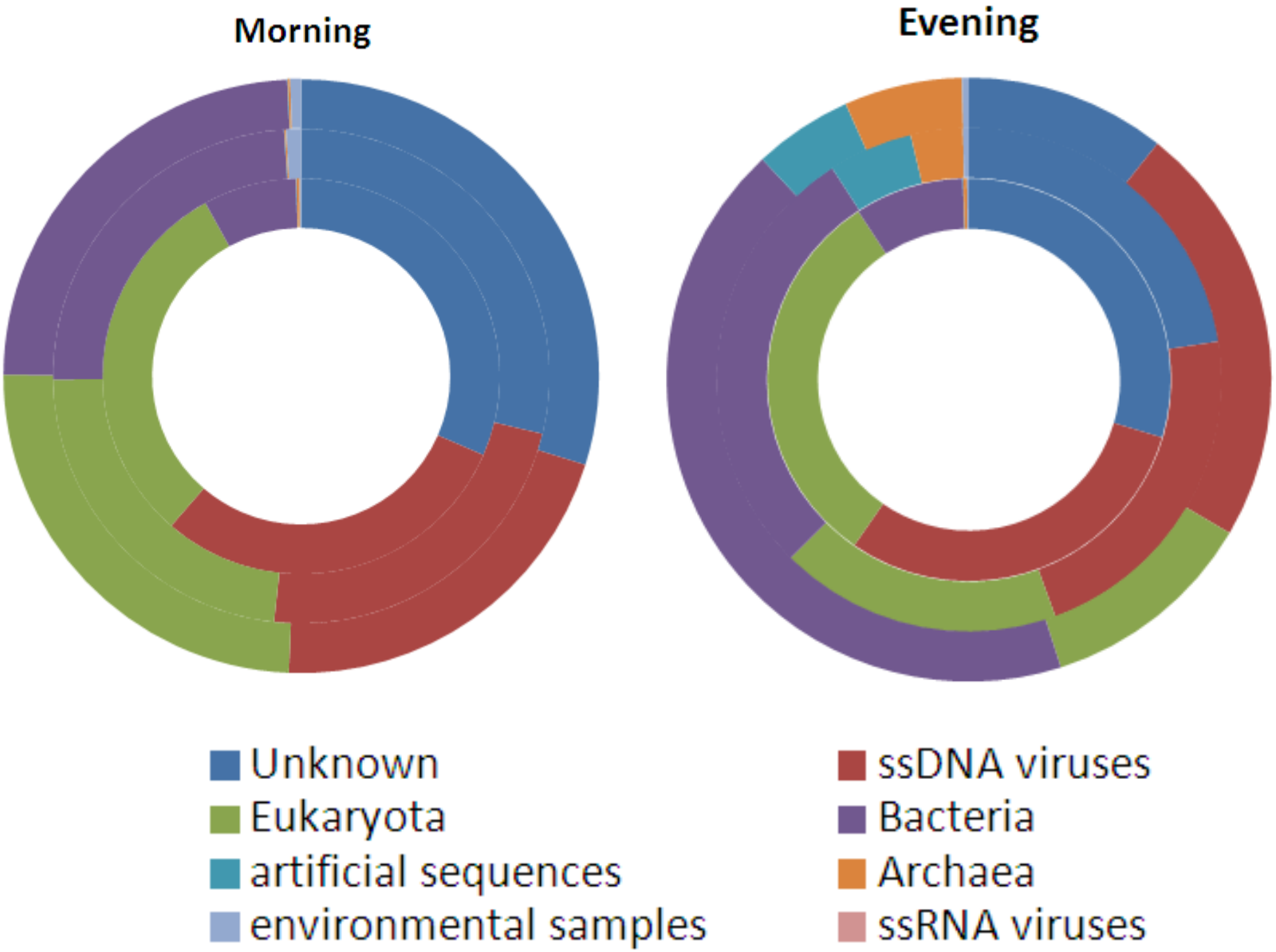
http://bowtie-bio.sourceforge.net/index.shtml

Taxonomic and functional assignment

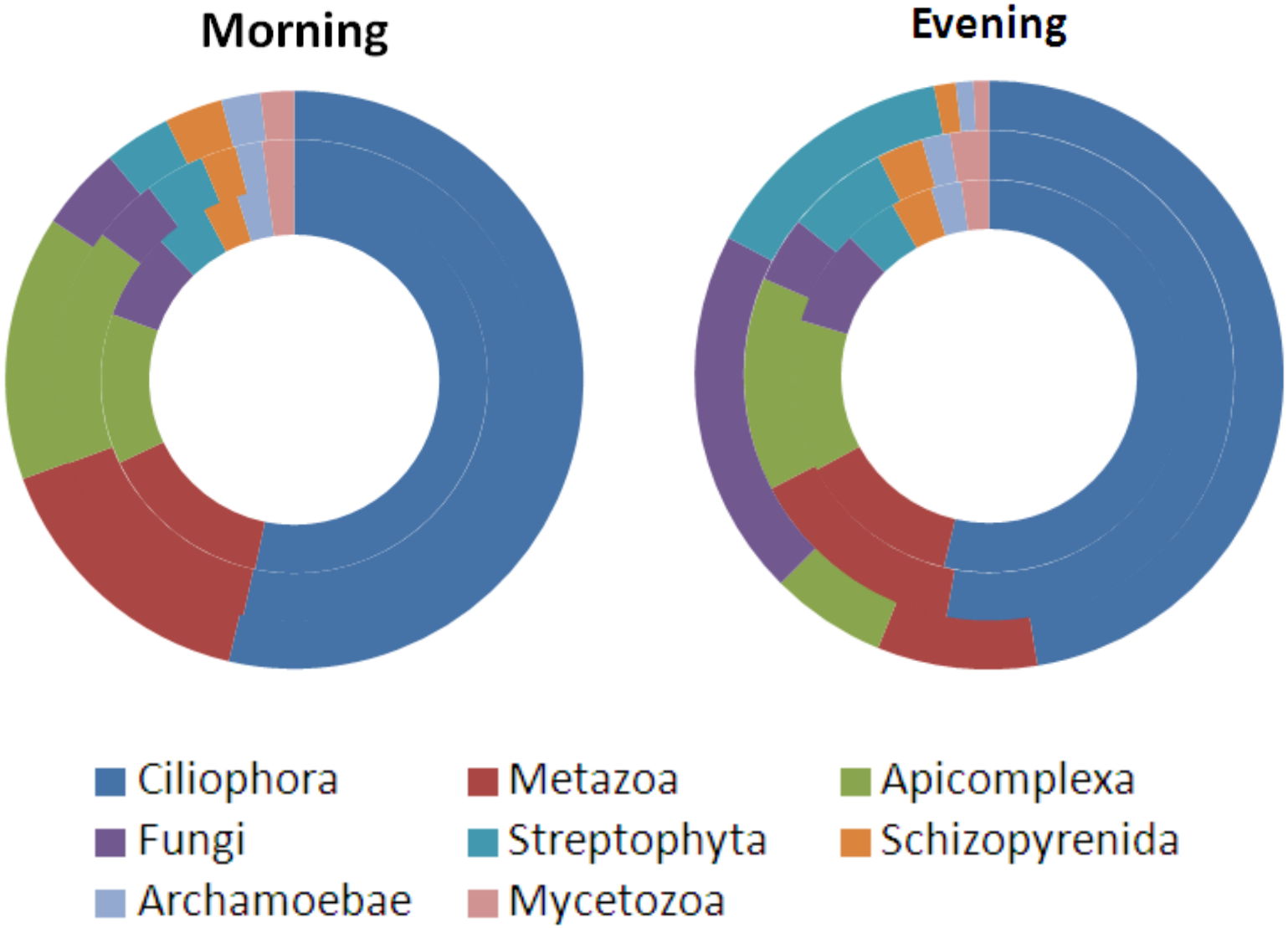
nt/nr

http://eggnog.embl.de/version_3.0/

Taxonomy PolyA mRNA



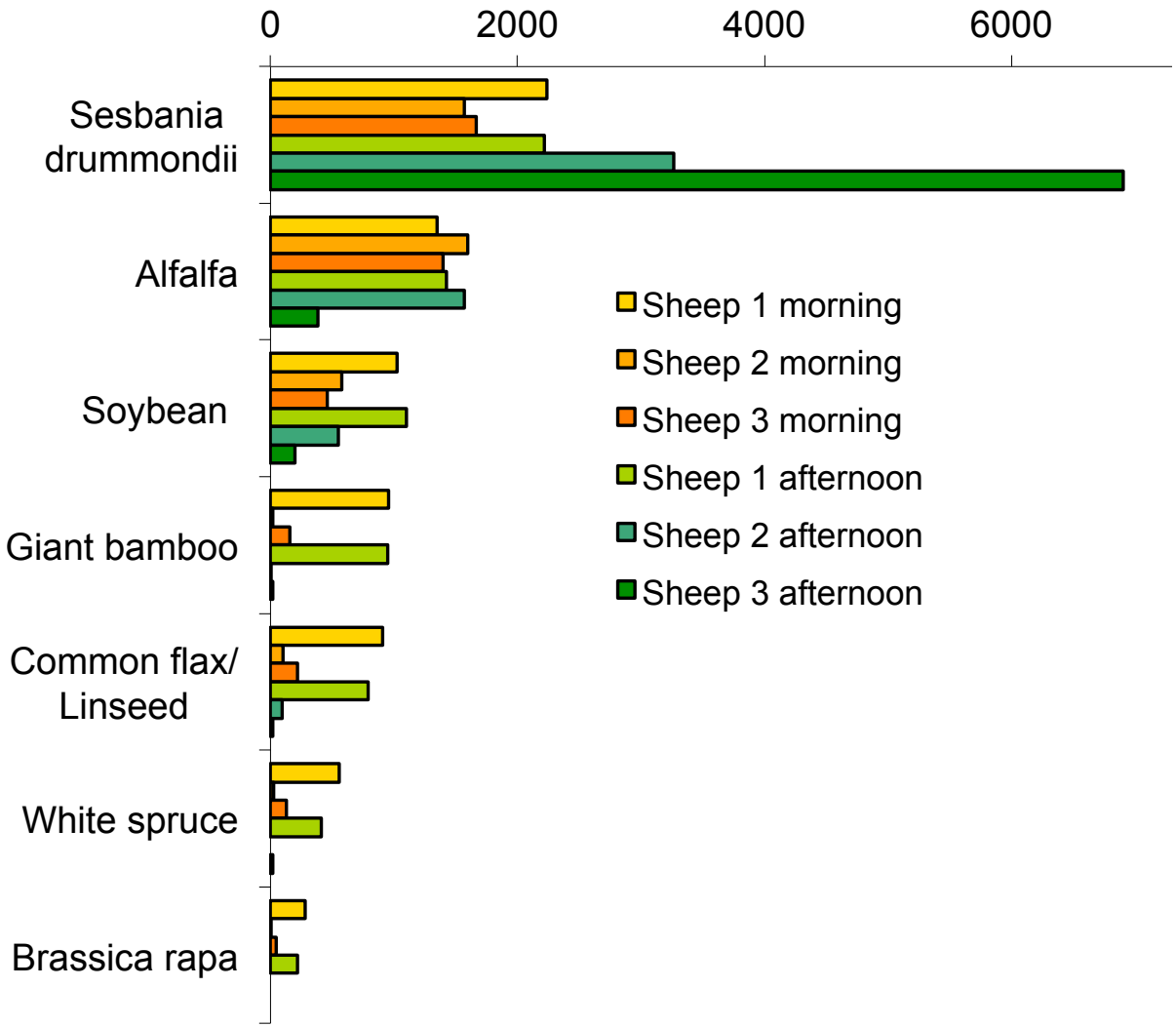
Taxonomy Eukaryotic mRNA



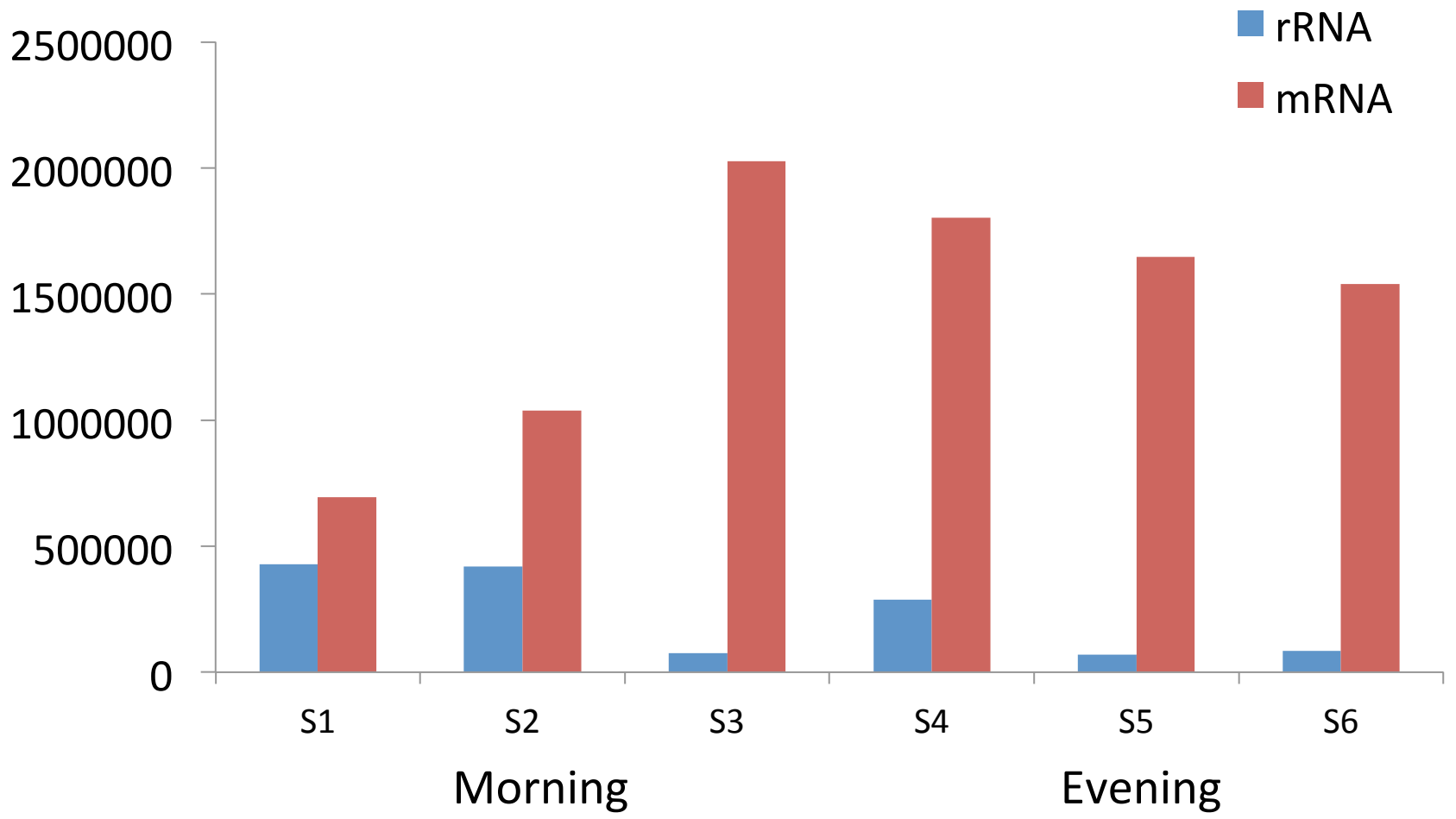
Plant mRNA abundance

Feed pellet composoition

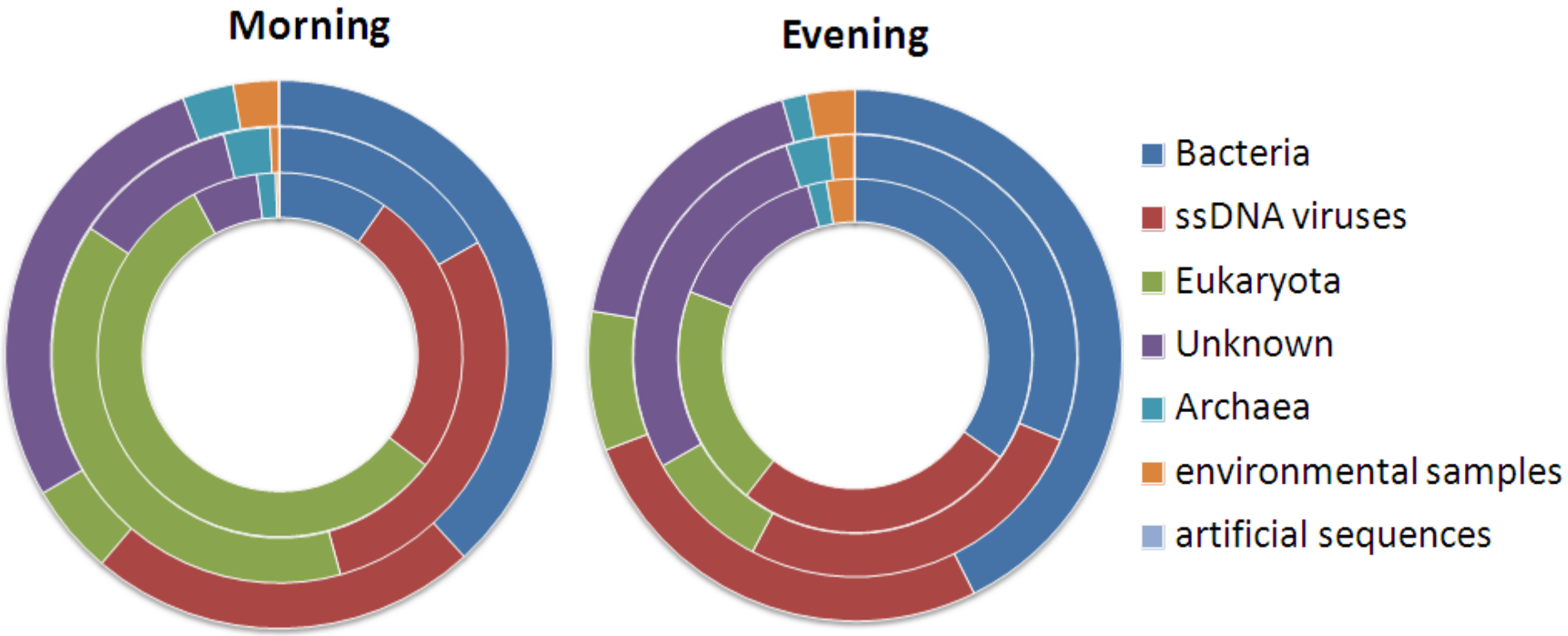
Barley 26%
Maize gluten feed 22%
Sugar Beet molassed 14%
Soya Hull 14%
Cane Molasses 8.5%
Soya Extract 7%
Distillers grains 6%

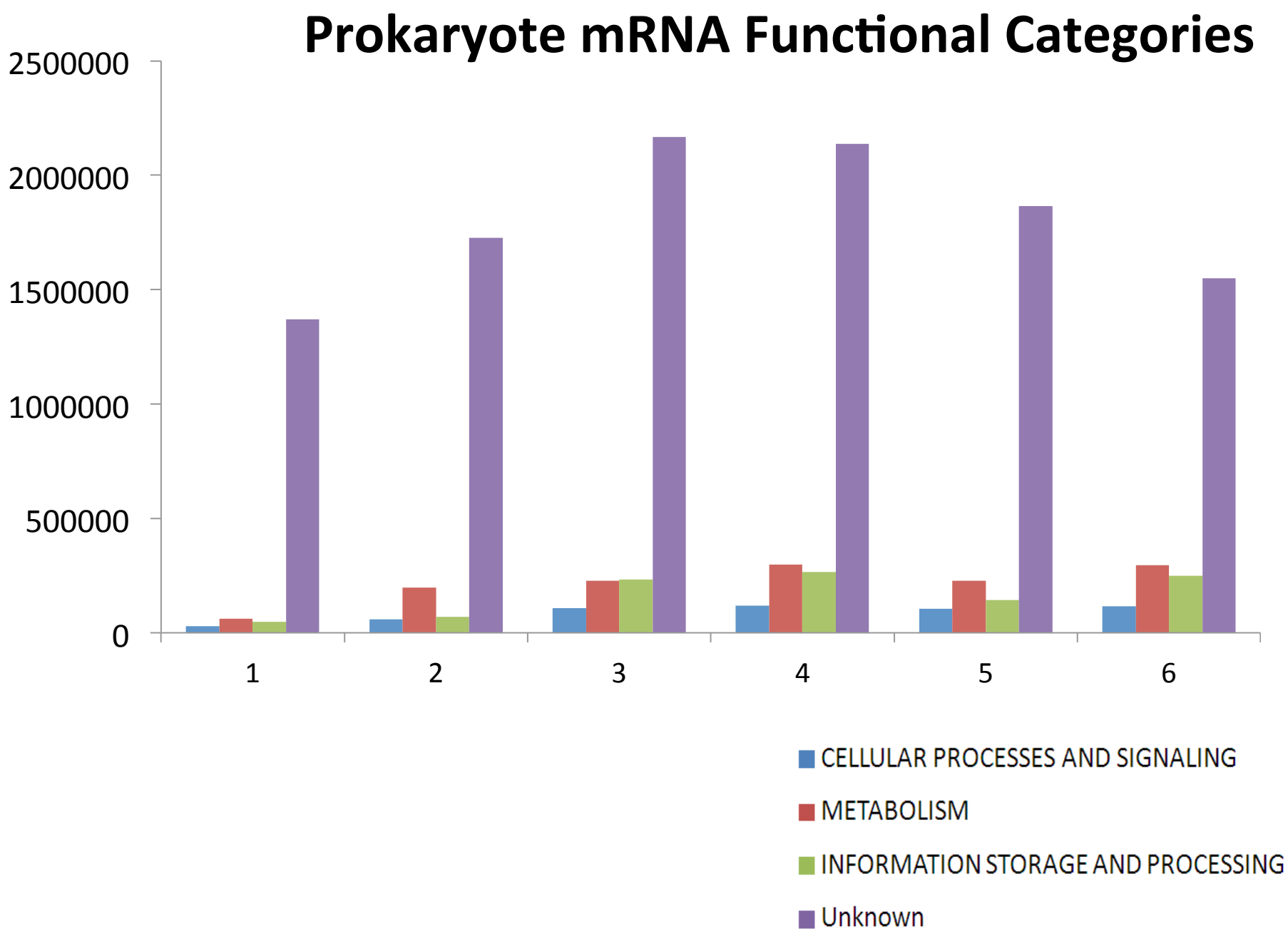


rRNA versus mRNA

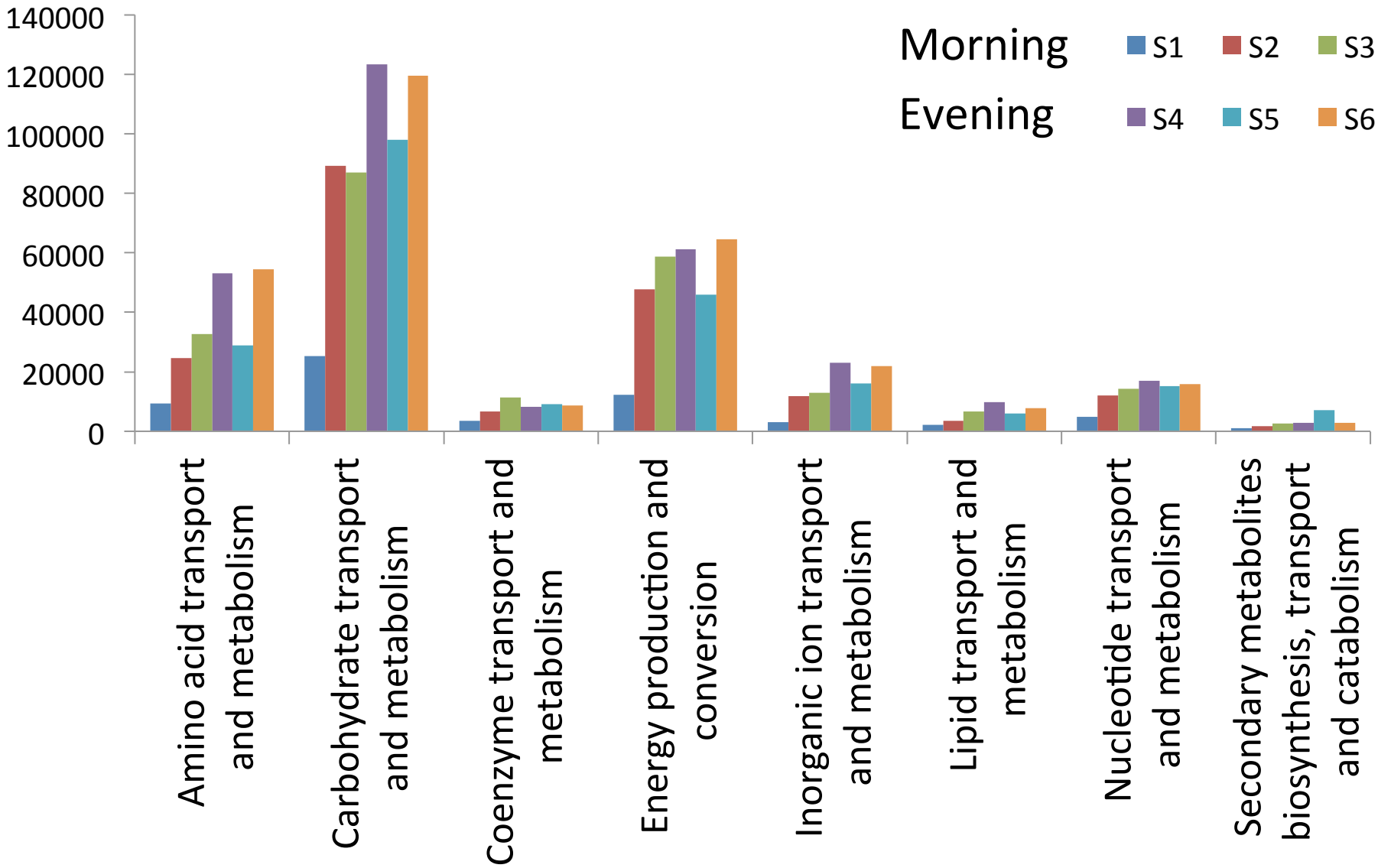


Taxonomy RiboZero mRNA




Prokaryote mRNA Functional Categories
2500000
2000000
1500000
1000000
500000
0
1
2
3
4
5
6
CELLULAR PROCESSES AND SIGNALING
METABOLISM
INFORMATION STORAGE AND PROCESSING
Unknown

Prokaryote mRNA Metabolic functions



Conclusions

- rRNA over-representation can be dealt with in the lab
  - However bioinformatic approaches are still not optimal
- Species and Functional annotation continues to be a huge problem
  - Requires more genomic information from rumen Microbes
- Even with low coverage sequencing a good snapshot of metabolic functions is possible
  - 800 Euro miSeq run for 6 samples
  - 80 Euro per sample, library prep.
- Unknown genes are going to continue to be a problem and demonstrate how little we really know about the genes expressed in rumen microbial communities.

Acknowledgements

Matt McCabe	Samples preparation and analysis
Padraig O'Kiely	Fistulated sheep
Paddy Blount	Sample collection
Jess McCabe	Sample collection & RNA extraction
Francesco Rubino	Bioinformatics
ICHEC	Computional server access
Teagasc	RMIS funding