

# **Rumen Metaproteomics**

‘...the large scale characterization of the entire protein complement of environmental microbiota at a given point in time...’

- Part of a multi -‘omic’ approach to characterise complex environmental microbial communities

**Microbiome** Amplicon sequencing (Taxonomic composition)

**Metagenome** (Taxonomic composition and potential function)

**Metatranscriptome** (Transcriptional activity at a point in time)

**Metaproteome** (Translational activity at a point in time)

**Environmental Metabolome** (Reactions)

# Background

- Gel based methods (2D SDS PAGE)
  - 'Metaproteomics'
    - Wilmes and Bond, 2004
    - Wilmes *et al.*, 2008
- Shotgun metaproteomics
  - Human gut microbiota
    - VerBerkmoes *et al.*, 2009
    - Kolmeder *et al.*, 2012
- Metaproteomics Data Analysis
  - Rooijers *et al.*, 2011
- Reviews
  - Muth *et al.* 2013

# Workflow

- **Extraction**

- Sample preparation
- Buffer
- Lysis method

- **Separation**

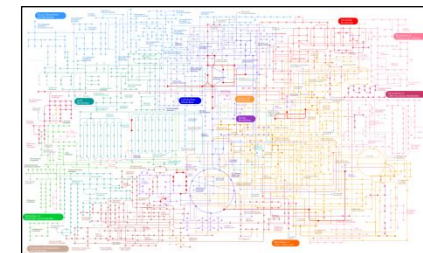
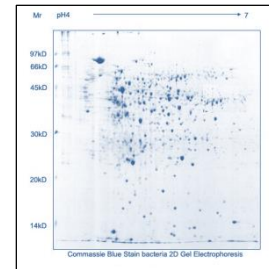
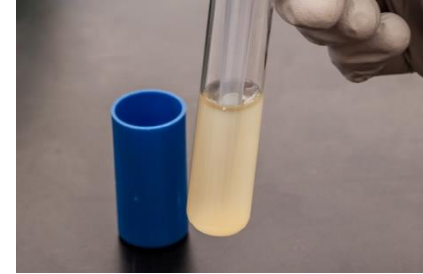
- By size (1D SDS PAGE)
- By size and isoelectric point (2D SDS PAGE)

- **Identification**

- Digestion (Trypsin)
- LC MS/MS
- Protein Identification (MASCOT)

- **Data Analysis**

- Function summaries (GO terms, SEED classification)
- Map to functional pathways (KEGG)



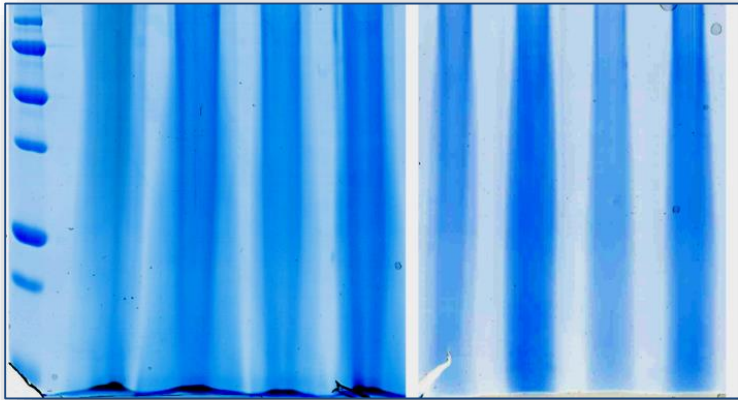
# Methodological challenges

- Complexity
- Heterogeneity
- Metaproteome Bioinformatics

# Sample quality

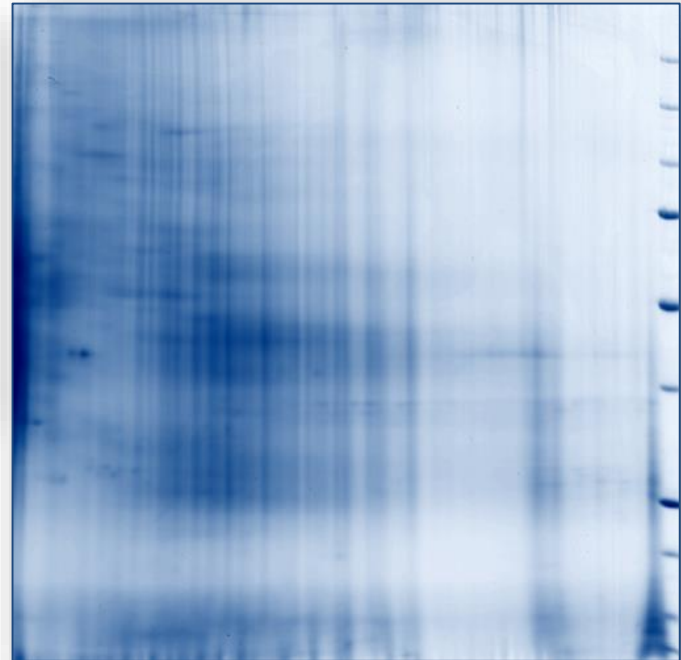
- Humic compounds
  - Co-precipitate with protein extract
  - Very difficult to remove
    - (Wu *et al.*, 2014) TCA/Acetone/Phenol method
  - Visualised by stains (eg. Coomassie Blue)
    - Affects assays
    - Causes background staining on SDS PAGE gels
      - (Benndorf *et al.*, 2007)
      - (Heyer *et al.*, 2013)
  - Humic compounds degrade proteins
    - (Chourey *et al.*, 2010)

**1D SDS PAGE**



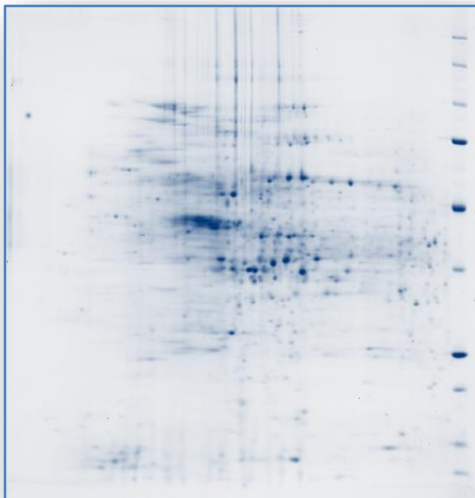
Schneider *et al.*, 2012

**2D SDS PAGE**

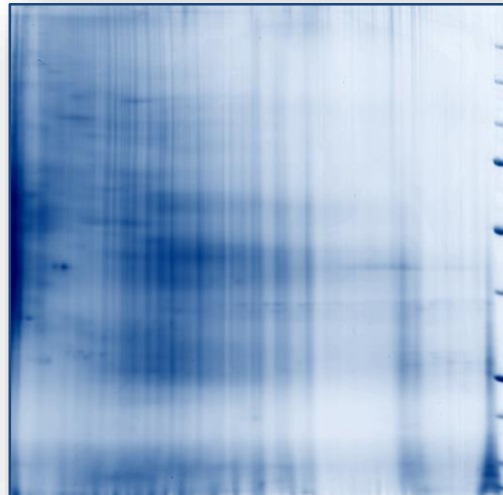


# Rumen digesta sample types

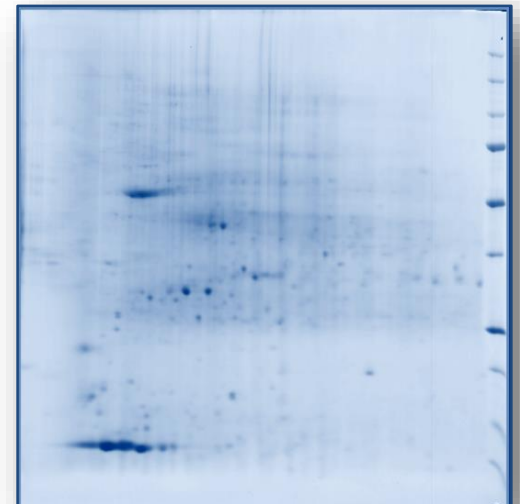
- Fresh rumen content taken at slaughter
- Ruminal cannula (Stored at -20 °C)
- Naso-gastric tube (NGT) (Stored at -20 °C)



Fresh rumen digesta



'Grab' sample from cannulated animal



Sample taken by NGT



# Lysis and Buffers

- Mechanical Lysis
  - Repeated bead beating (Yu and Morrison, 2004)
- Buffer
  - Aqueous
  - Surfactant/detergent
  - Chaotropic agent

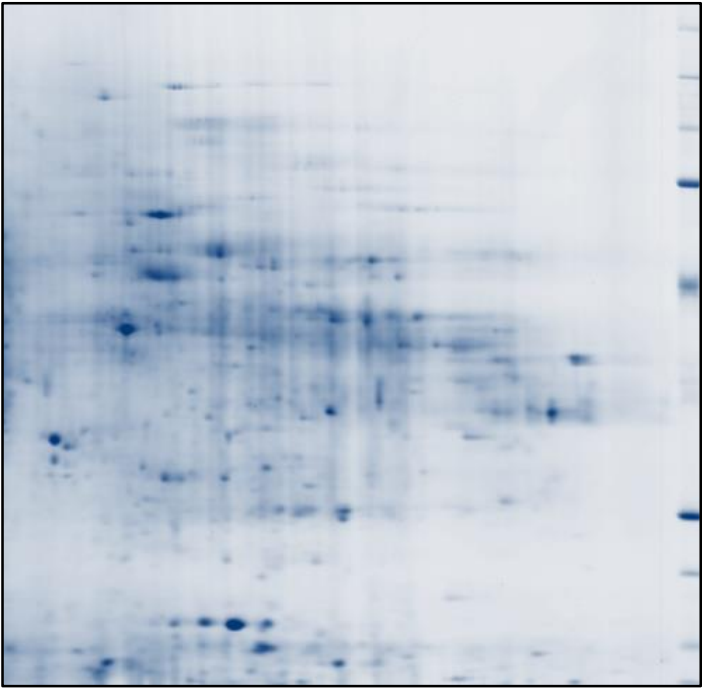


Urea 7M  
Thiourea 2M  
CHAPS 4%  
DTT 0.1%

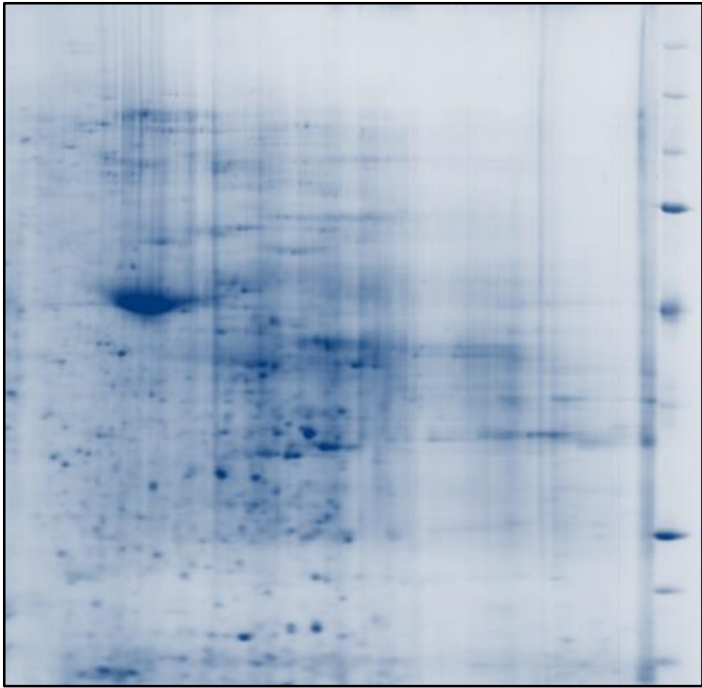
Ampholytes 3/10  
Protease Inhibitor Cocktail

Rabilloud *et al.*, 1997

Beat beating in PBS buffer

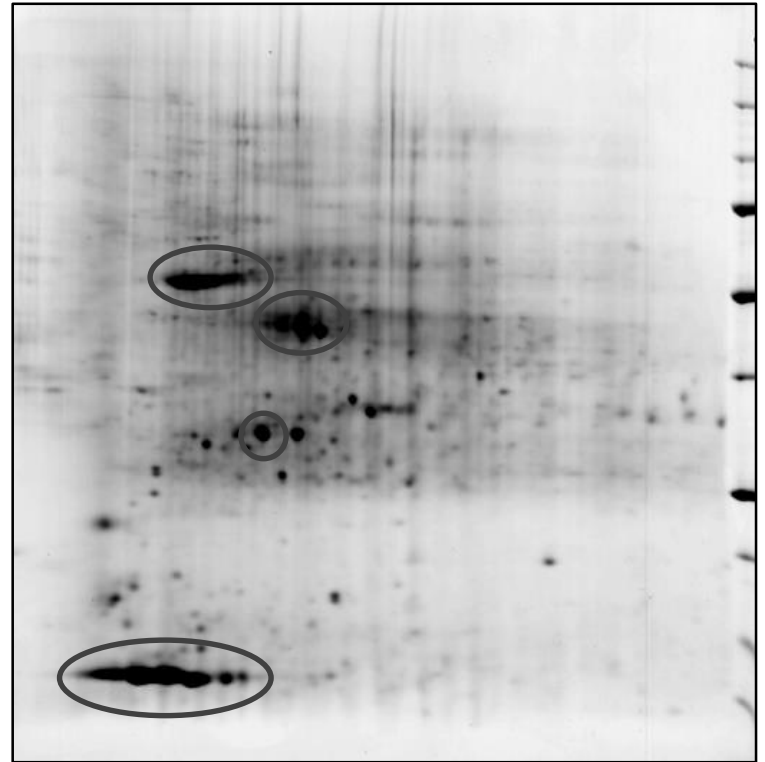
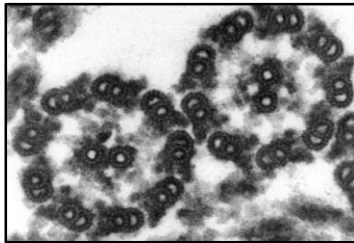


Beat beating in Rabilloud buffer



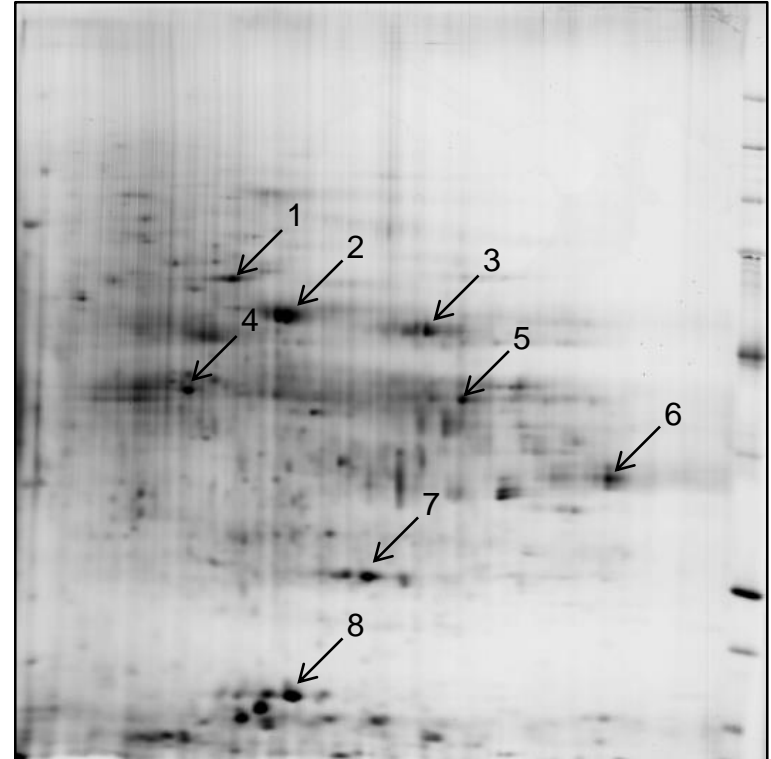
# Dairy Cow (NGT sample)

- Entodinium sp. (Ciliate)
  - Actin
  - Alpha Tubulin
  - Beta Tubulin
  - Dynein Light Chain
    - Axonemal Isoforms



# Beef steer rumen (NGT sample)

- Sample centrifuged to remove ciliates
- MASCOT search against NCBI nr 20140323
  - 1. Methyl-coenzyme M reductase
    - *Methanobrevibacter smithii*
  - 2. Chaperonin GroEL
    - *Prevotella ruminicola*
  - 3. Phosphoenolpyruvate carboxykinase
    - *Prevotella ruminicola*
  - 4. Methyl-coenzyme M reductase
    - *Methanobrevibacter smithii*
  - 5. Phosphoglycerate kinase
    - *Prevotella ruminicola*
  - 6. Glyceraldehyde-3-phosphate dehydrogenase
    - *Prevotella sp.*
  - 7. Triose-phosphate isomerase
    - *Prevotella ruminicola*
  - 8. Chain A, Complex Of Bovine Odorant Binding Protein (obp)
    - *Bos taurus*



# Shotgun rumen metaproteomics

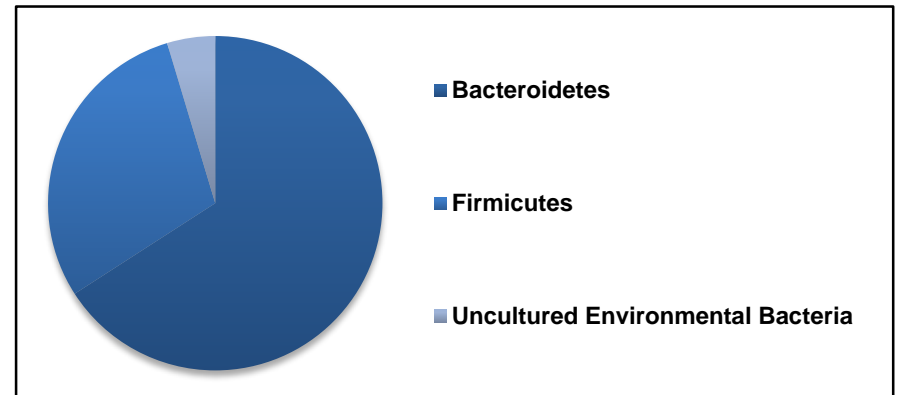
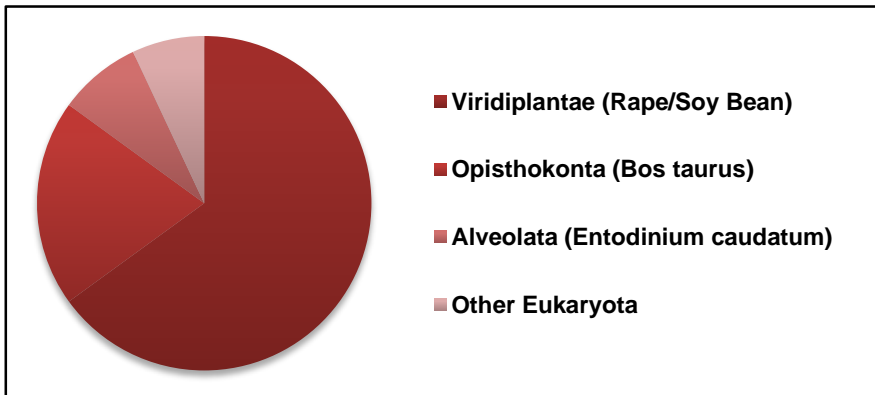
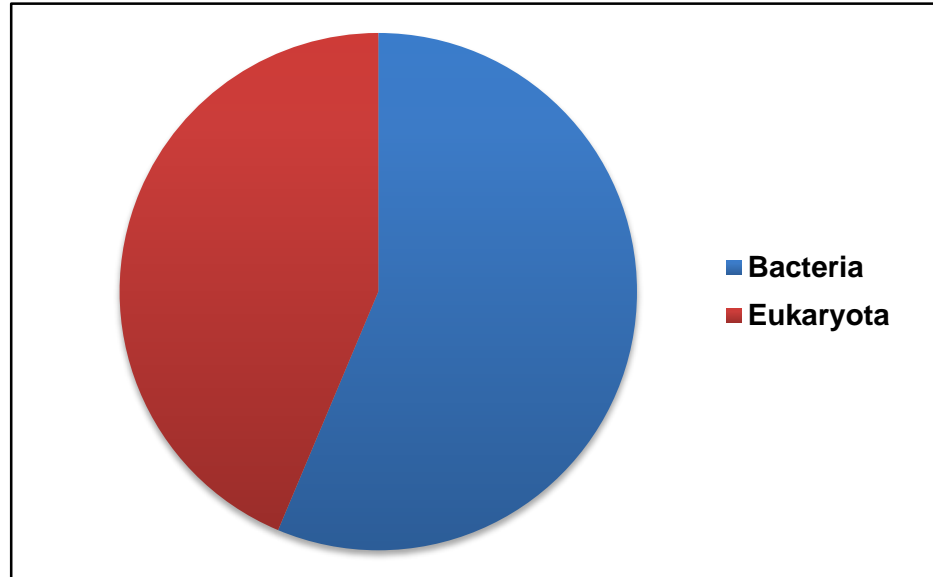
- Rumen digesta samples (NGT)
  - Four dairy cows (University of Nottingham)
- Centrifuge to remove (reduce) ciliates
- Protein extracted
- Run to 2cm on 10% Bis-Tris gel
- Trypsinised
- LC MS/MS
  - Ultimate 3000 RS LC nano system
  - LQT Orbitrap Velos
  - Proteome Discoverer



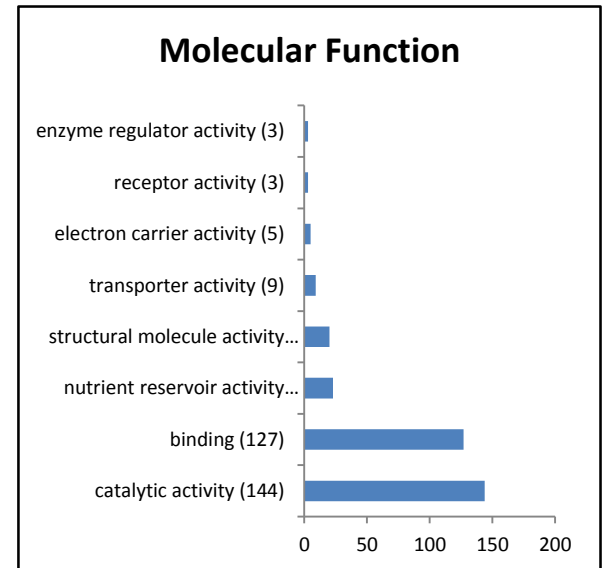
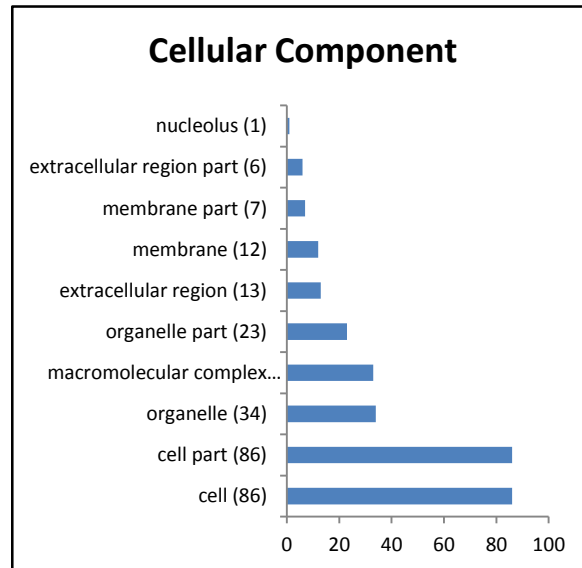
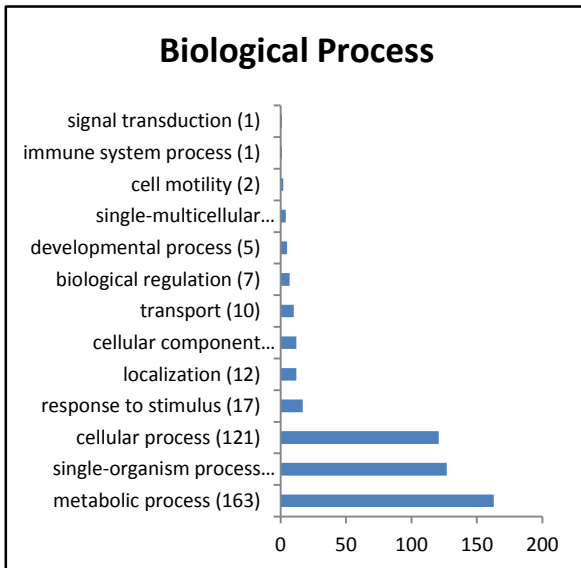
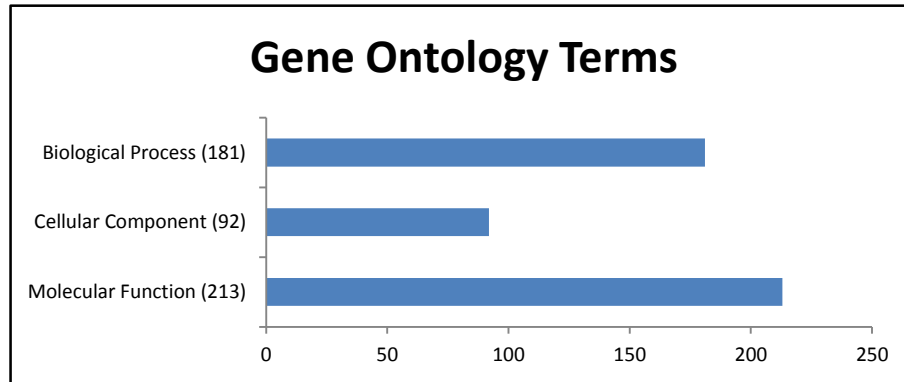
# Analysis pipeline

- Protein list (368 from NCBI nr database search)
- Pool Protein hit GI numbers (229 unique proteins)
- Map to UniProt KB ID numbers
  - Summarise by taxa
  - Summarise by function using Gene Ontology (GO) terms
- Map to KEGG orthologues (KO)
  - Draw pathway maps

# Taxonomic Summary



# Functional summary







- Validation of methods
  - Repeatability
  - Representativeness
  - Reliability of data analysis
    - Tanca *et al.*, 2013
- Increase depth of coverage
  - Improved sensitivity of mass spectrometer
  - Reduce complexity of each MS run (gel pixelation)
- Statistical comparison between samples
- Integration with metagenomic data





# Acknowledgements

- Rowett Institute, University of Aberdeen
  - Nichol Fergus
  - Martin Reid
  - Louise Cantlay
- Institute of Medical Sciences, University of Aberdeen
  - David Stead
  - Ian Davidson
- FingerPrints Proteomics, University of Dundee
  - Douglas Lamont
- RuminOmics
  - [www.ruminomics.eu](http://www.ruminomics.eu)
  - @ruminomics

