

Functional (meta)genomics

*Harmonization of techniques associated with
ruminal microbiome and metagenome analysis*

Graeme Attwood

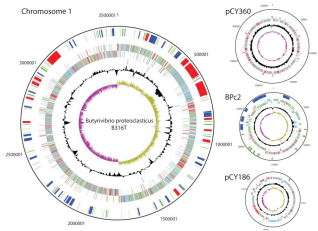
Joint RuminOmics/Rumen Microbial Genomics Network Workshop



Farming, Food and Health. **First**

Te Ahuwhenua, Te Kai me te Whai Ora. Tuatahi

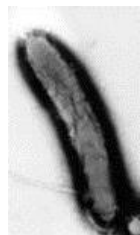
Functional (meta)genomics methods and techniques



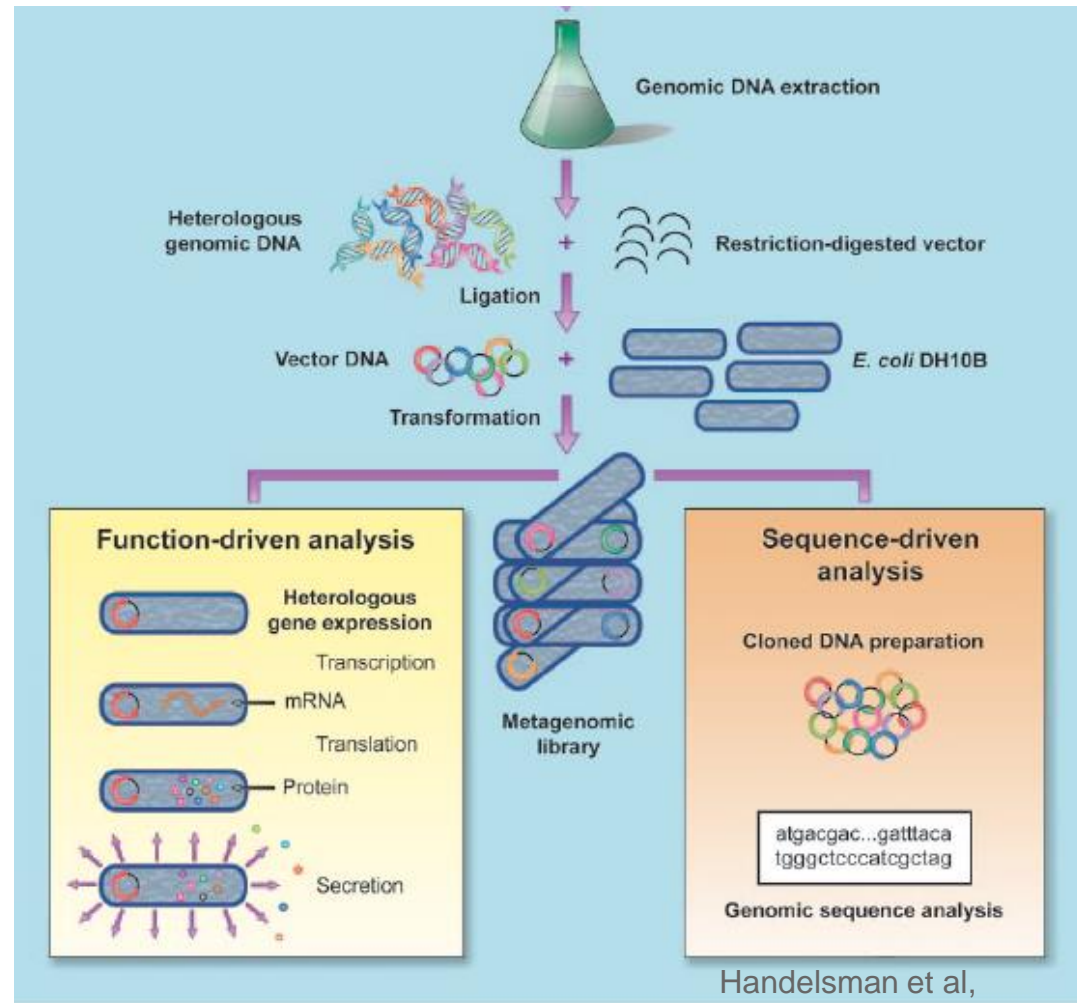
Genome/
metagenom
e
sequence
data

Functional genomics

Transcriptomics
Proteomics
Metabolomics
Mutagenesis

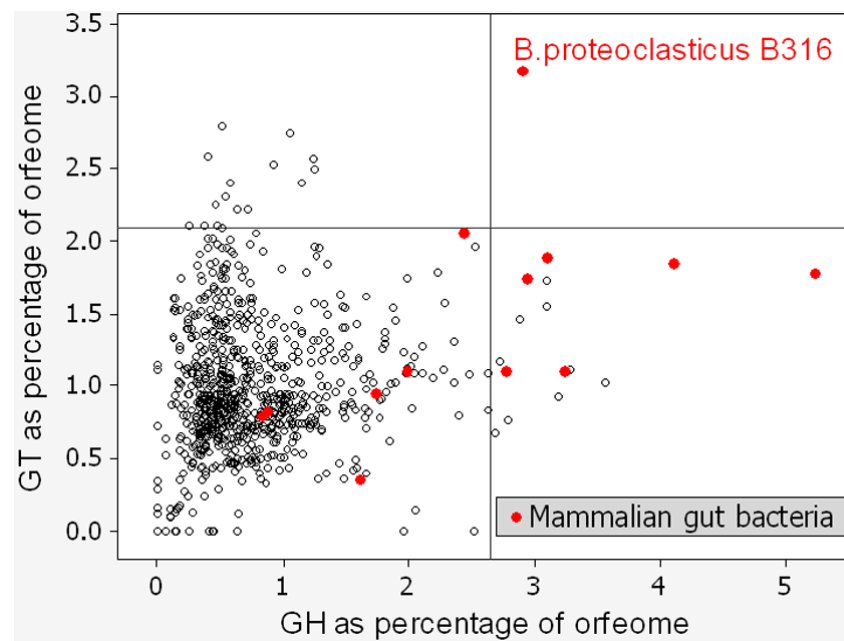
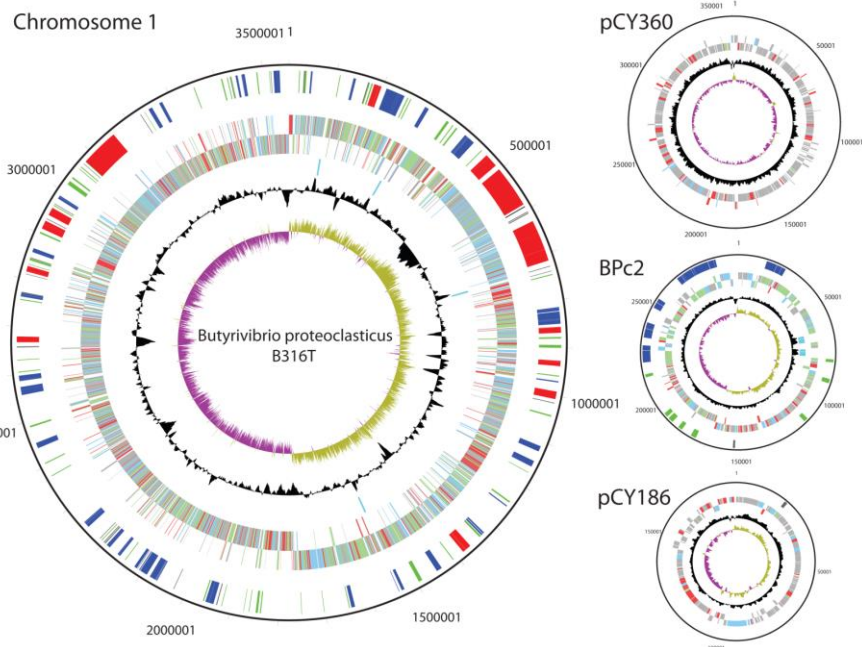


Phenotype
Gene/protein
functions

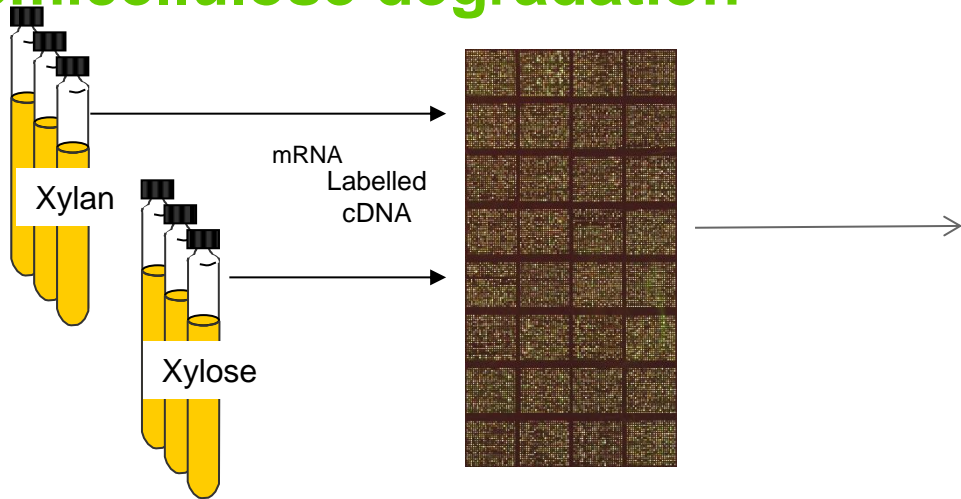


The Glycobiome of the Rumen Bacterium *Butyrivibrio proteoclasticus* B316^T Highlights Adaptation to a Polysaccharide-Rich Environment

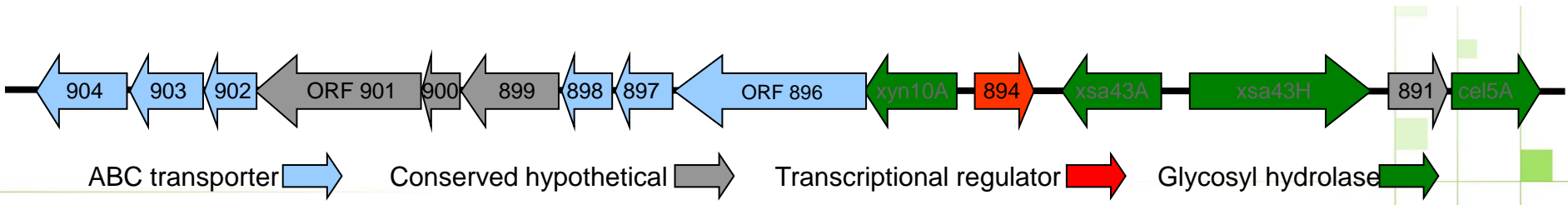
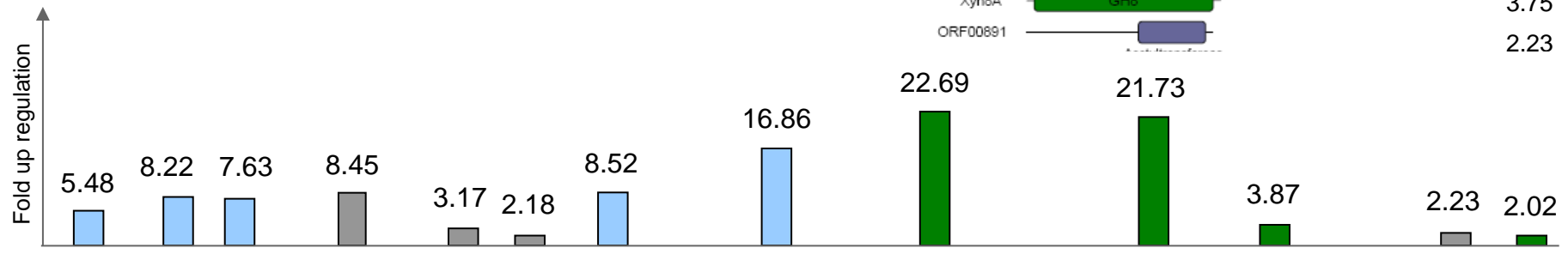
William J. Kelly¹, Sinead C. Leahy¹, Eric Altermann¹, Carl J. Yeoman^{1,2*}, Jonathan C. Dunne^{1,3}, Zhanhao Kong¹, Diana M. Pacheco¹, Dong Li¹, Samantha J. Noel¹, Christina D. Moon¹, Adrian L. Cookson¹, Graeme T. Attwood^{1*}



Microarray analysis of genes involved in hemicellulose degradation

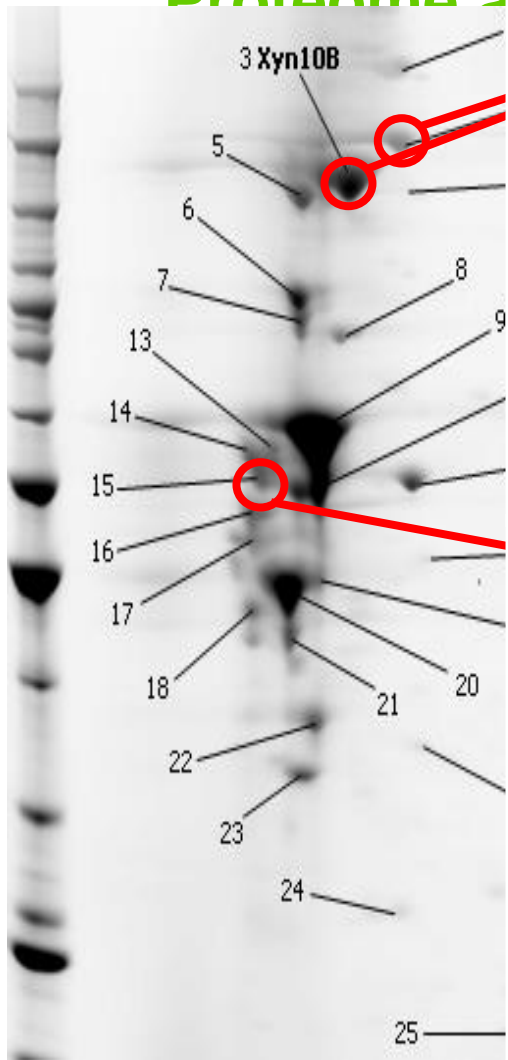


| Gene | Enzyme/Domain | Fold up-regulated |
|----------|-------------------------|-------------------|
| Aga27B | α -galactosidase | 3.06 |
| Agu47A | GH 67 | 2.82 |
| Arf51A | GH51 | 4.56 |
| Arf51B | GH51 | 9.25 |
| Cel5A | GH5 | 2.02 |
| Est1B | Esterase | 2.21 |
| Est1C | Esterase | 14.9 |
| Est1D | Lipase/Acylhydrolase | 3.71 |
| Gh2C | GH2 SBD, GH2, GH2, TBD | 2.98 |
| Gh3A | GH3 NTD, GH3 | 10.4 |
| Lic16B | GH16 | 4.60 |
| Xsa43A | GH43, CBM6 | 21.73 |
| Xsa43D | GH43 | 2.16 |
| Xsa43E | GH43 | 9.09 |
| Xsa43G | GH43 | 2.90 |
| Xsa43H | Carboxylesterase (GH43) | 3.87 |
| Xyl39A | GH39 | 3.46 |
| Xyn10A | GH10 | 22.69 |
| Xyn10B | GH10, CBM9 | 3.99 |
| Xyn10D | GH10, Esterase | 5.84 |
| Xyn10E | GH10 | 5.43 |
| Xyn8A | GH8 | 3.75 |
| ORF00891 | | 2.23 |



➡ ABC transporter
 ➡ Conserved hypothetical
 ➡ Transcriptional regulator
 ➡ Glycosyl hydrolase

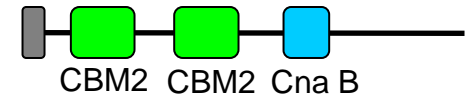
Proteome 2



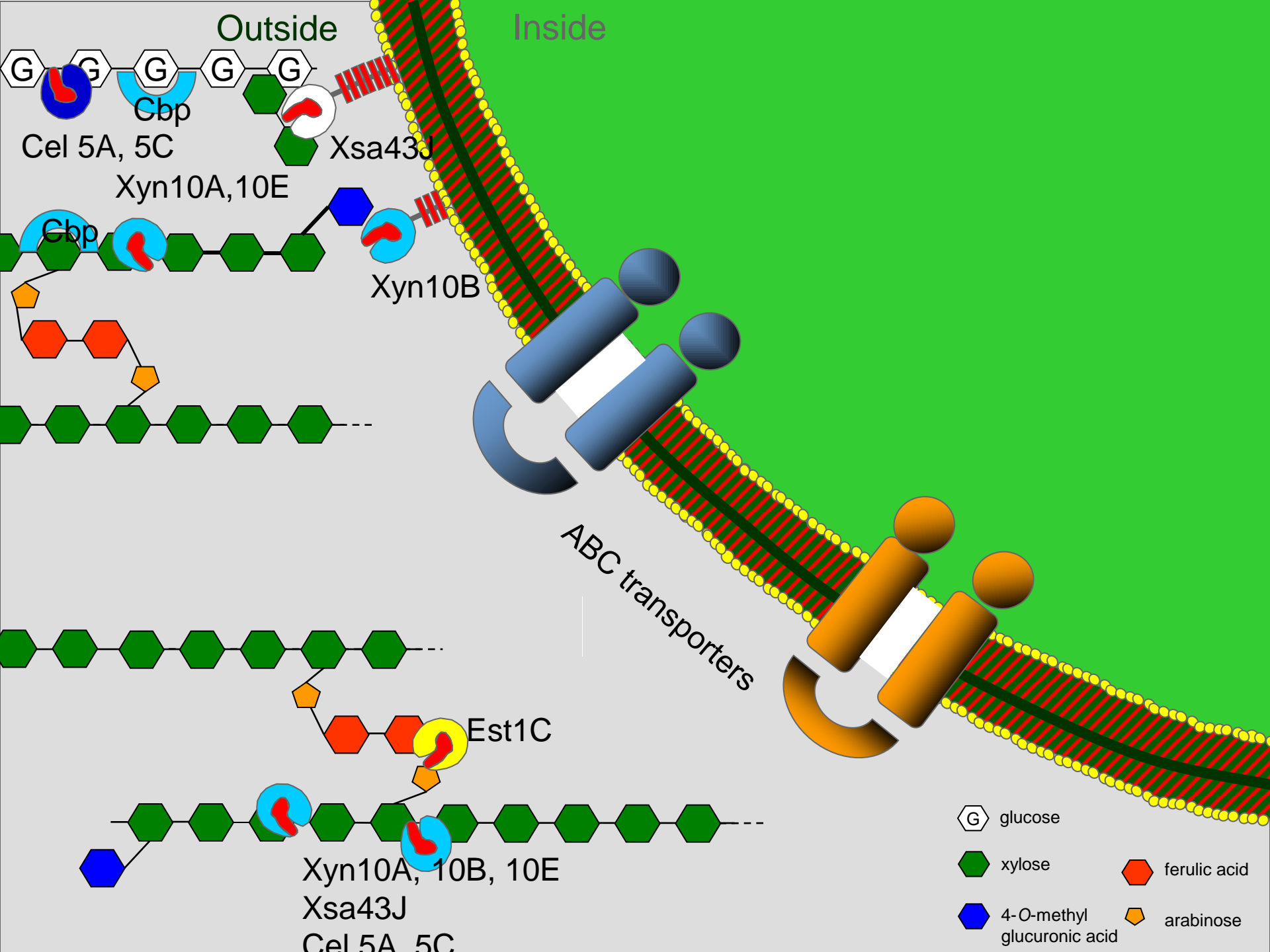
137 kDa
endoxylanase
Xyn10B (6.9%)

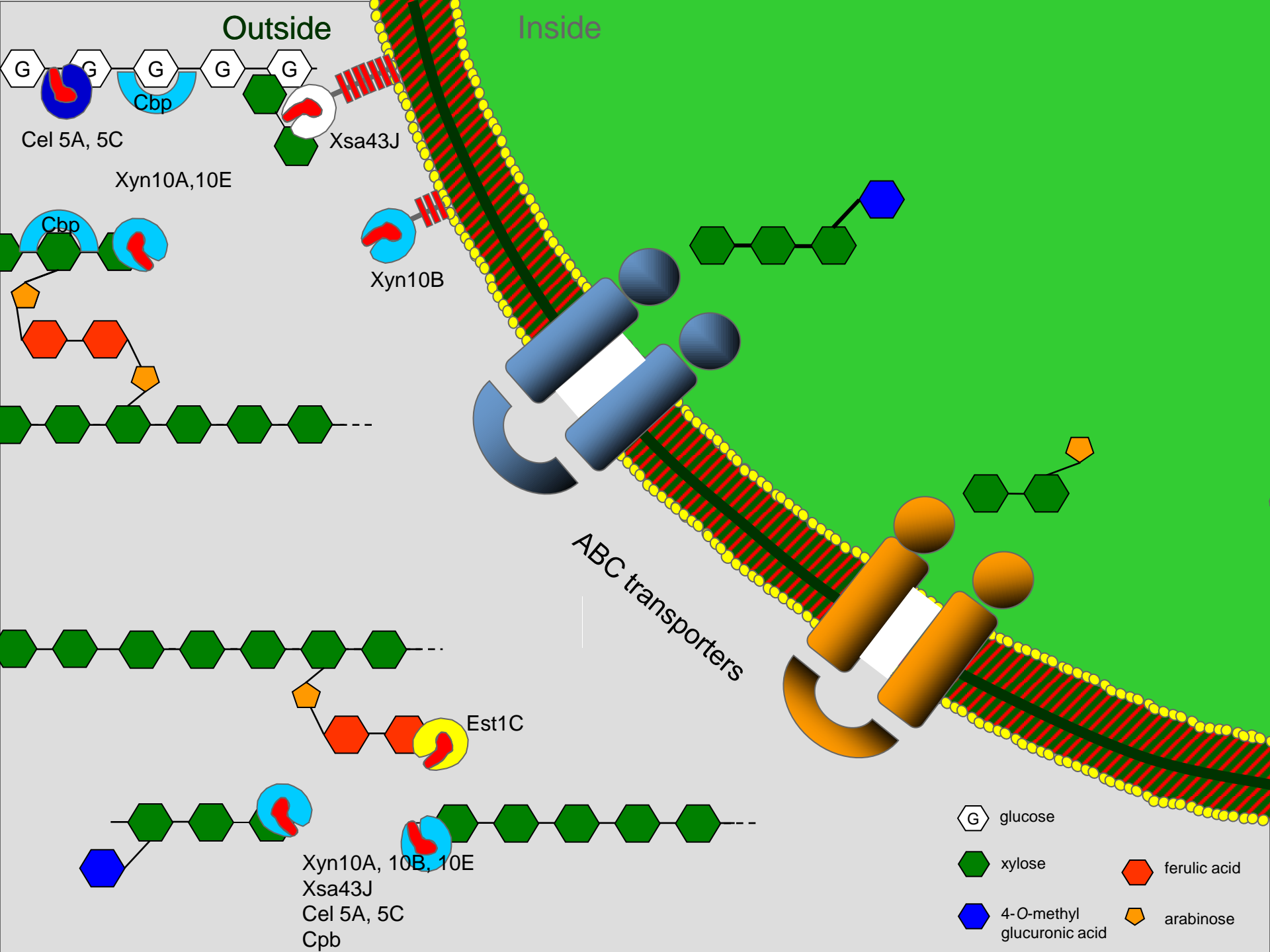


57 kDa carbohydrate
binding protein
ORF 0313



 Signal peptide  Cell wall binding repeat CBM = Carbohydrate binding module



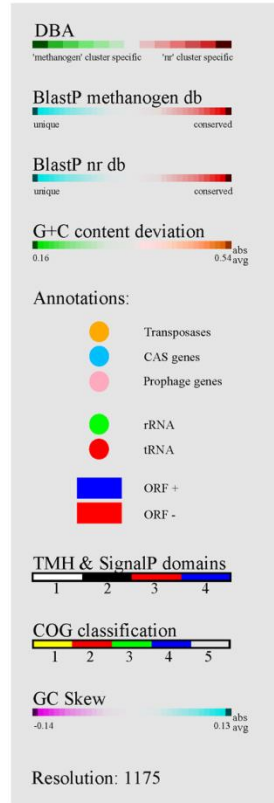
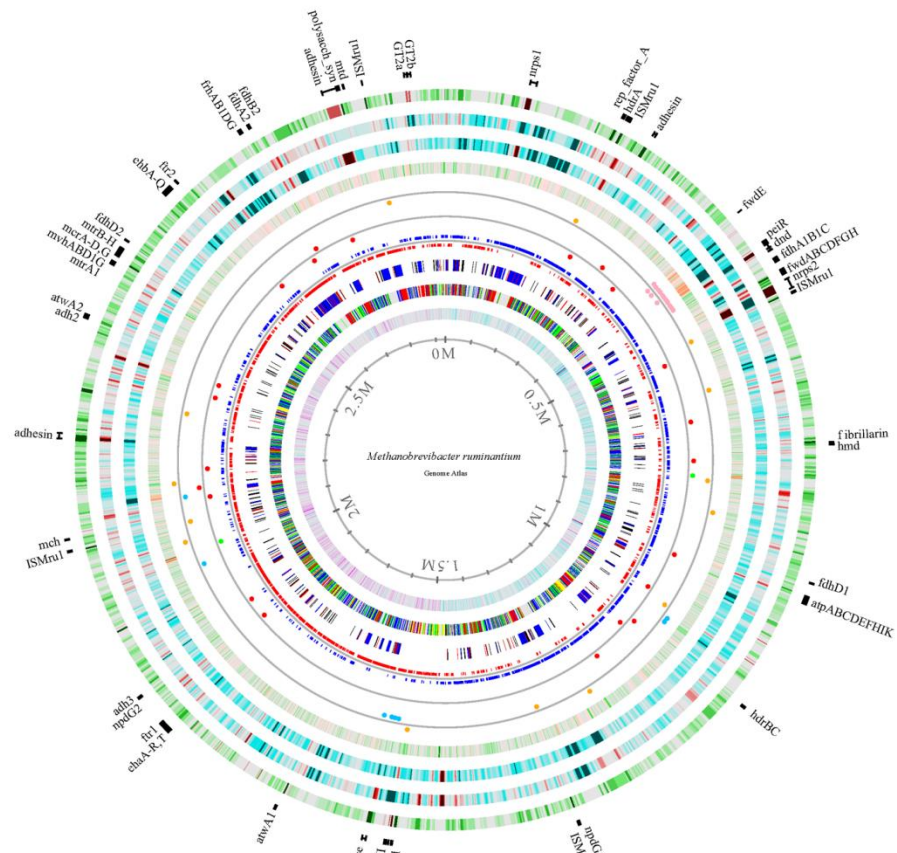
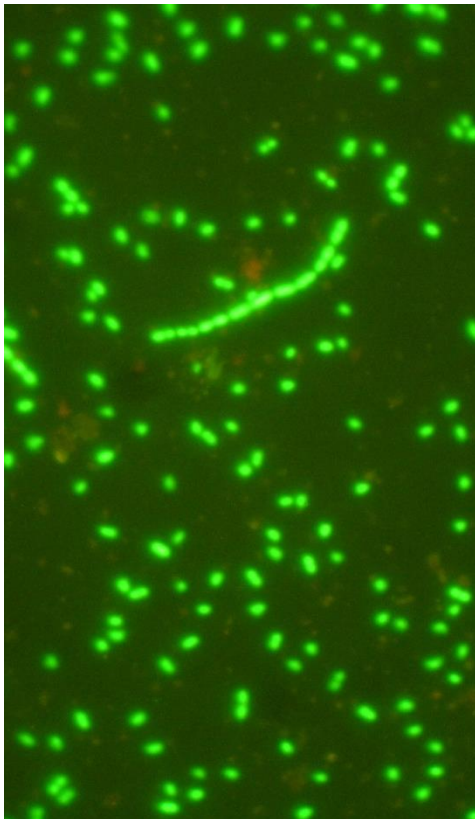




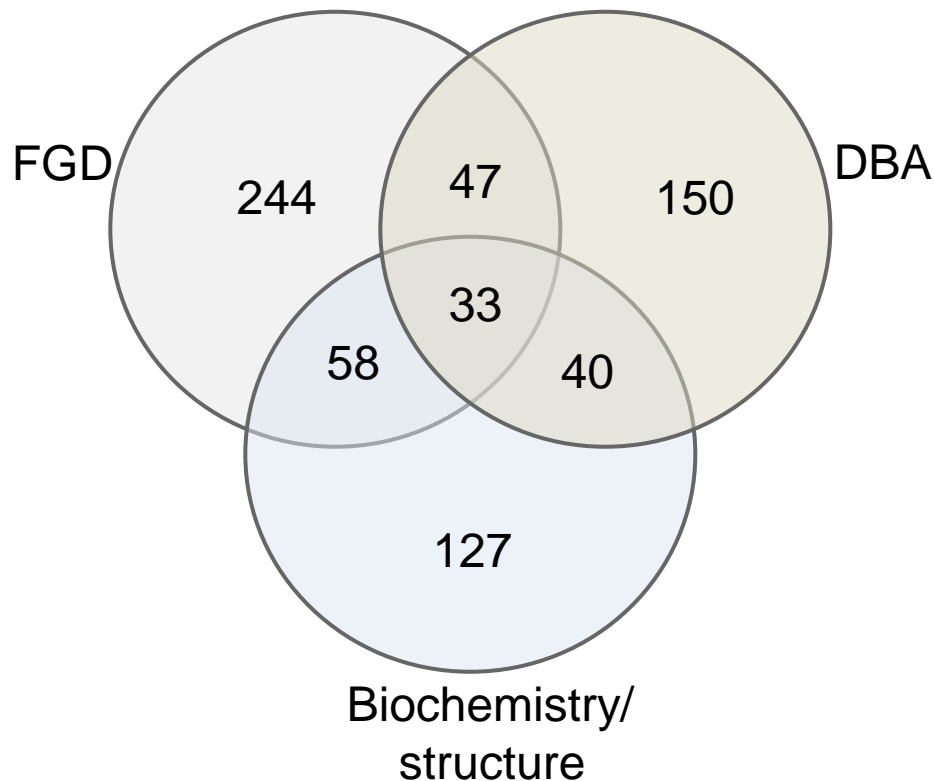
The Genome Sequence of the Rumen Methanogen *Methanobrevibacter ruminantium* Reveals New Possibilities for Controlling Ruminant Methane Emissions

Sinead C. Leahy, William J. Kelly, Eric Altermann, Ron S. Ronimus, Carl J. Yeoman, Diana M. Pacheco, Dong Li, Zhanhao Kong, Sharla McTavish, Carrie Sang, Suzanne C. Lambie, Peter H. Janssen, Debjit Dey, Graeme T. Attwood*

Rumen Microbial Genomics, Food Metabolism and Microbiology Section, Food and Textiles Group, AgResearch Limited, Grasslands Research Centre, Palmerston North, New Zealand



Comparison of methanogen genomes

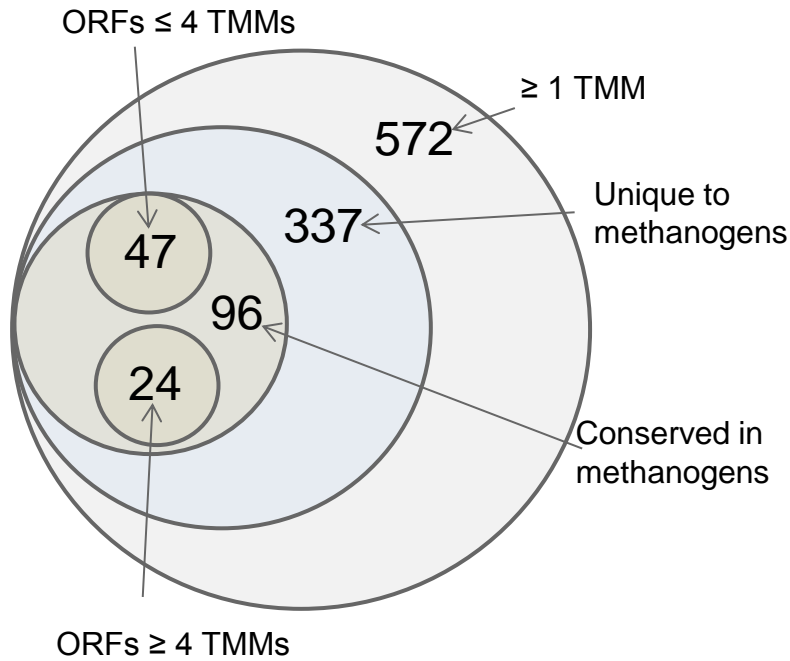


Chemogenomics targets

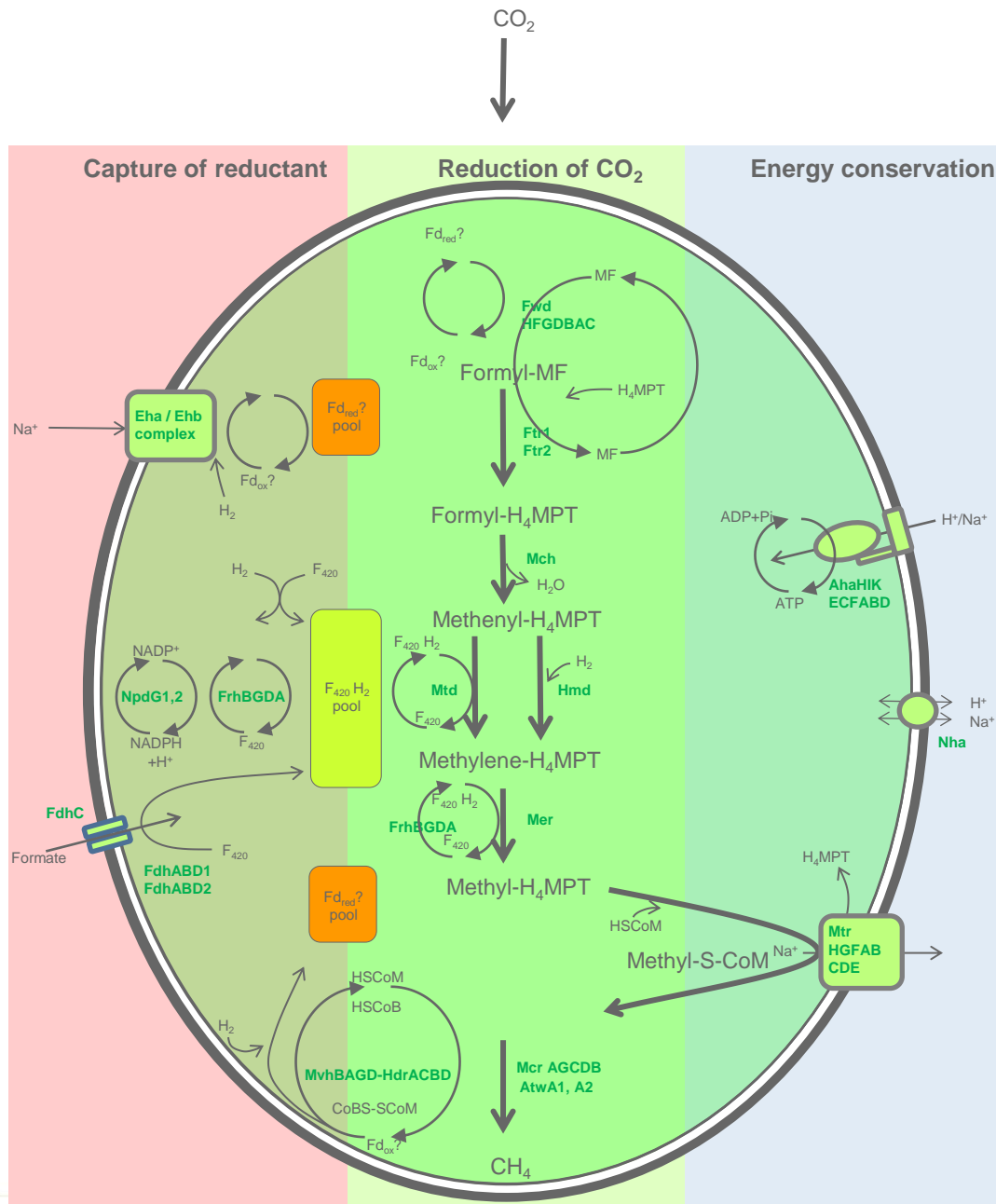
| Functional Category | Locus tag | Annotation |
|----------------------------------|---|--|
| Amino Acid metabolism | | |
| | mru0998, 1577 | AroBA |
| | mru0152 | LysA |
| Cell Cycle | | |
| | mru1864 | DNA topoisomerase VI A |
| Cell Envelope | | |
| | mru0456 | GlmU |
| Central Carbon Metabolism | | |
| | mru0655 | SdhB |
| Energy metabolism | | |
| | mru0696 | Ahal |
| | mru0569 | Mer |
| | mru1398 | EhaO |
| | mru2142 | Mtd |
| | mru1393 | Ftr1 |
| | mru1619 | Mch |
| | mru1695 | H ₄ MPT-linked C1 transfer pathway protein |
| | mru1262 | AtwA1 |
| | mru1916, 1923 | MtrEH |
| | mru0344, 0345 | FwdAC |
| | mru0668, 1778, 1971, 0436, 1929, 0097, 0181, 1915, 1771 | Methanogenesis marker proteins 1, 2, 3, 7, 8, 10, 11, 13, 14, 15 |
| Lipid Metabolism | | |
| | mru0924 | IdsA |
| | mru0922 | Fni |
| Protein Synthesis | | |
| | mru2169, 2069 | GatAB |
| Vitamins & Cofactors | | |
| | mru1559 | CitG |

Vaccine targets

Vaccine targets



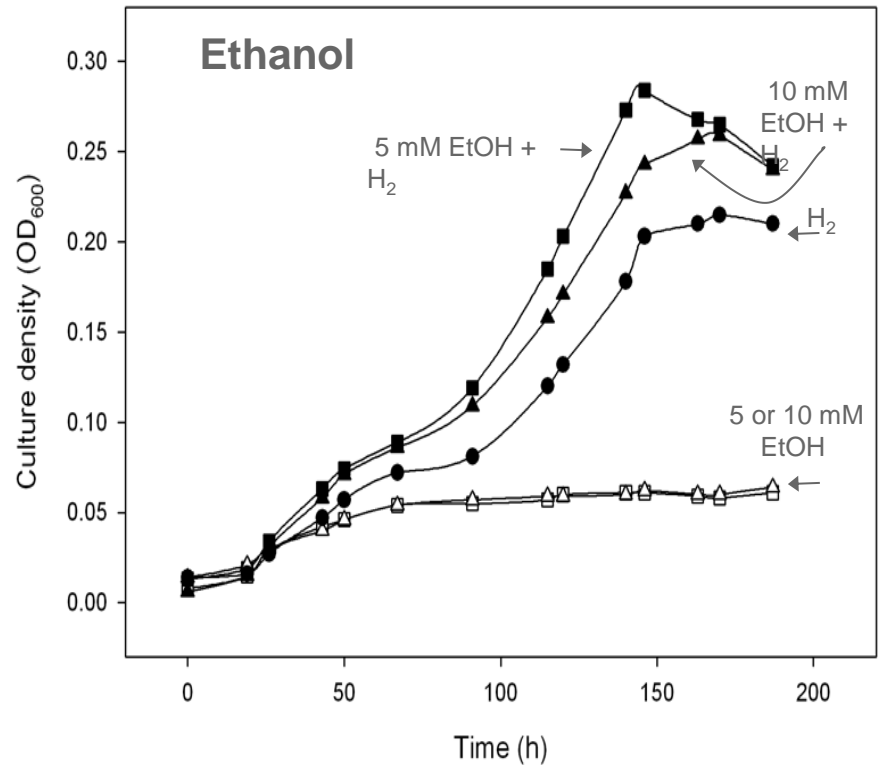
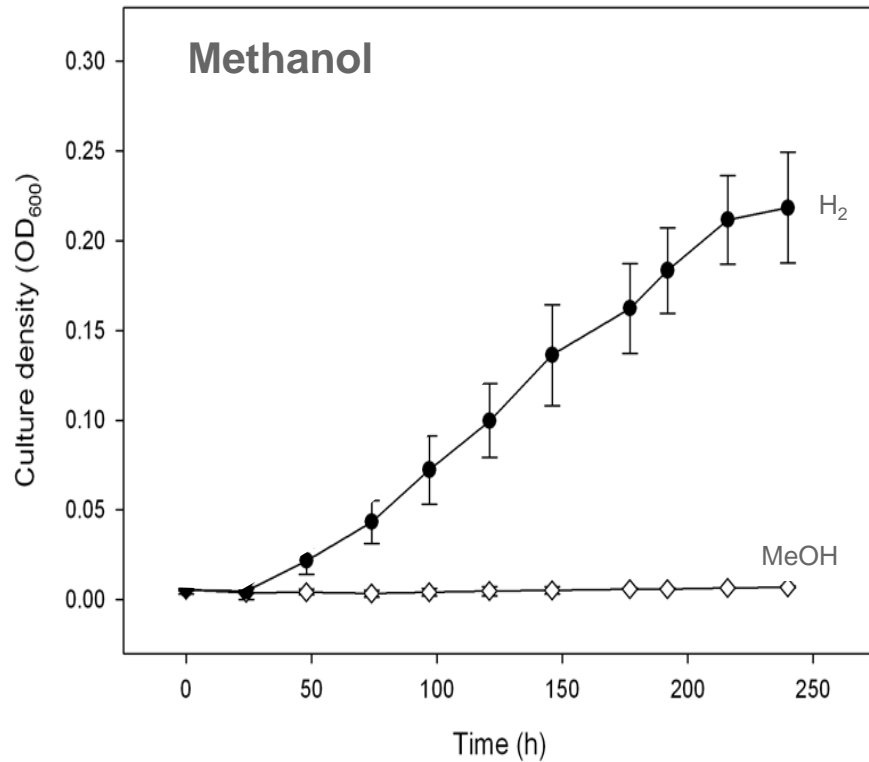
| Functional Category | | Locus tag | | Annotation | |
|---------------------------------|-----------|---------------------|------------|---|--|
| Energy Metabolism | | | | | |
| | mru0697 | | | AhaK | |
| | mru1405* | 1406*, 1407, 1408 | 1411, 1412 | EhaHGFEAB | |
| | mru2006, | 2007, 2008, 2010 | 2012, 2013 | EhbIHGECB | |
| | mru1917, | 1918, 1921*, 1922*, | 1923* | MtrGFCDE | |
| Protein Fate | | | | | |
| | mru0239 | | | SecG | |
| | mru0482 | | | SecE | |
| | mru1234* | | | type IV leader peptidase family protein | |
| Vitamins & Cofactors | | | | | |
| | mru0540 | | | CbiN1 | |
| Hypothetical | | | | | |
| | mru0542*, | 0840*, 1693, 2156* | | Hypothetical proteins | |
| | 0233, | 0234*, 0330, 1021, | | | |
| | 1144, | 1231, 1480*, 1585, | | | |
| | 1635*, | 1955, 2015*, 2046*, | | | |
| | 2056, | 2146* 0529 0081, | | | |
| | 0196, | 0225*, 0328*, 0412, | | | |
| | 0428*, | 0499, 0596, 0597, | | | |
| | 0693, | 0832, 1098, 1385, | | | |
| | 0147, | 0377*, 1375*, 1641, | | | |
| | 0543, | 0833, 1991, 0545*, | | | |
| | 0716*, | 0718*, 0838, 0968, | | | |
| | 1232, | 1550, 1884*, 2202, | | | |
| | 1694 | | | | |
| * ORFs \geq 5 TMMs | | | | | |

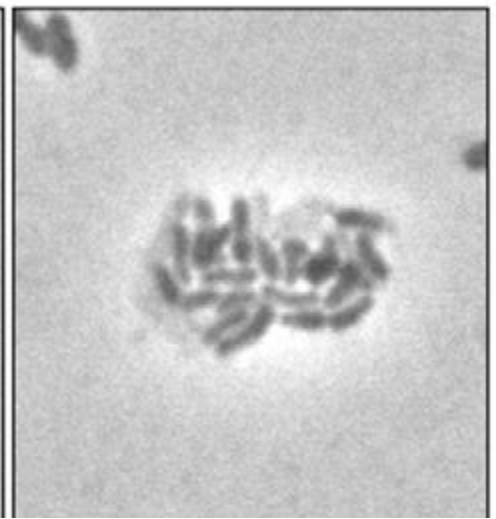
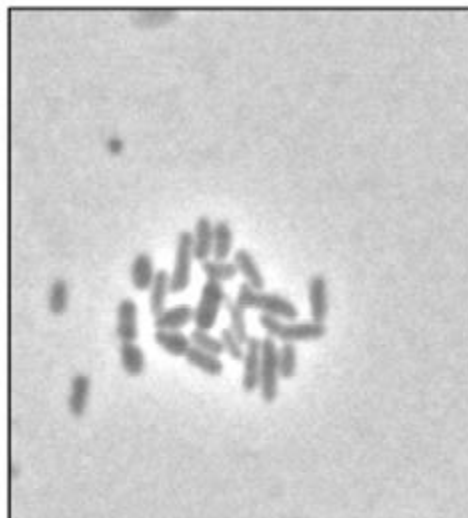
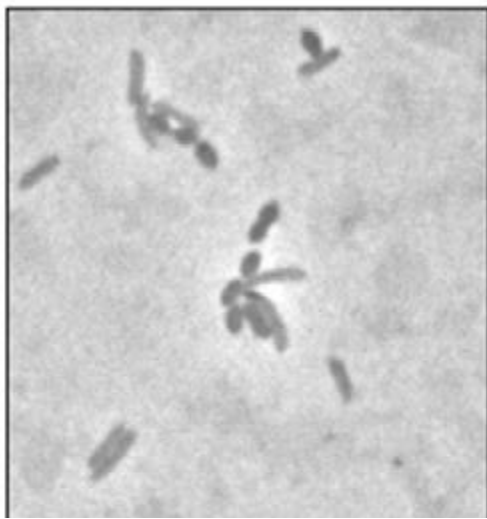


two NADPH-dependent F420 dehydrogenase (*npdG1, 2*) genes and three NAD-dependent alcohol dehydrogenase (*adh1, 2* and *3*) genes

does this allow growth on ethanol or isopropanol via NADP⁺-dependent oxidation of the alcohol coupled to F420 reduction of methenyl-H₄MPT to methyl-H₄MPT?

Stimulation of M1 growth by alcohols





| Locus tag | Annotation | Fold difference |
|---|--|------------------------|
| Energy Metabolism - Formate Metabolism | | |
| mru0333 | formate dehydrogenase alpha subunit FdhA | 3.46 |
| mru0334 | formate dehydrogenase beta subunit FdhB | 2.34 |
| Energy Metabolism - Methanogenesis | | |
| mru1928 | methyl-coenzyme M reductase beta subunit McrB | 2.26 |
| mru1926 | methyl-coenzyme M reductase C subunit McrC | 2.39 |
| mru1927 | methyl-coenzyme M reductase D subunit McrD | 2.23 |
| mru1925 | methyl-coenzyme M reductase gamma subunit McrG | 3.41 |
| mru1919 | tetrahydromethanopterin S-methyltransferase subunit A MtrA | 2.02 |
| mru1920 | tetrahydromethanopterin S-methyltransferase subunit B MtrB | 2.23 |
| mru1921 | tetrahydromethanopterin S-methyltransferase subunit C MtrC | 3.23 |
| mru1916 | tetrahydromethanopterin S-methyltransferase subunit H MtrH | 2.14 |
| mru1907 | methyl viologen-reducing hydrogenase gamma subunit MvhG | 2.25 |
| mru0344 | tungsten formylmethanofuran dehydrogenase subunit A FwdA | 2.12 |
| Cell envelope - Cell surface | | |
| mru1222 | adhesin-like protein | 2.31 |
| mru2173 | adhesin-like protein | 2.28 |
| mru2134 | adhesin-like protein | 4.08 |
| mru0076 | adhesin-like protein | 2.01 |
| mru0828 | adhesin-like protein with transglutaminase domain | 2.14 |
| mru1499 | adhesin-like protein with transglutaminase domain | 2.50 |

Significantly up-regulated (>2 fold) in co-culture vs monoculture

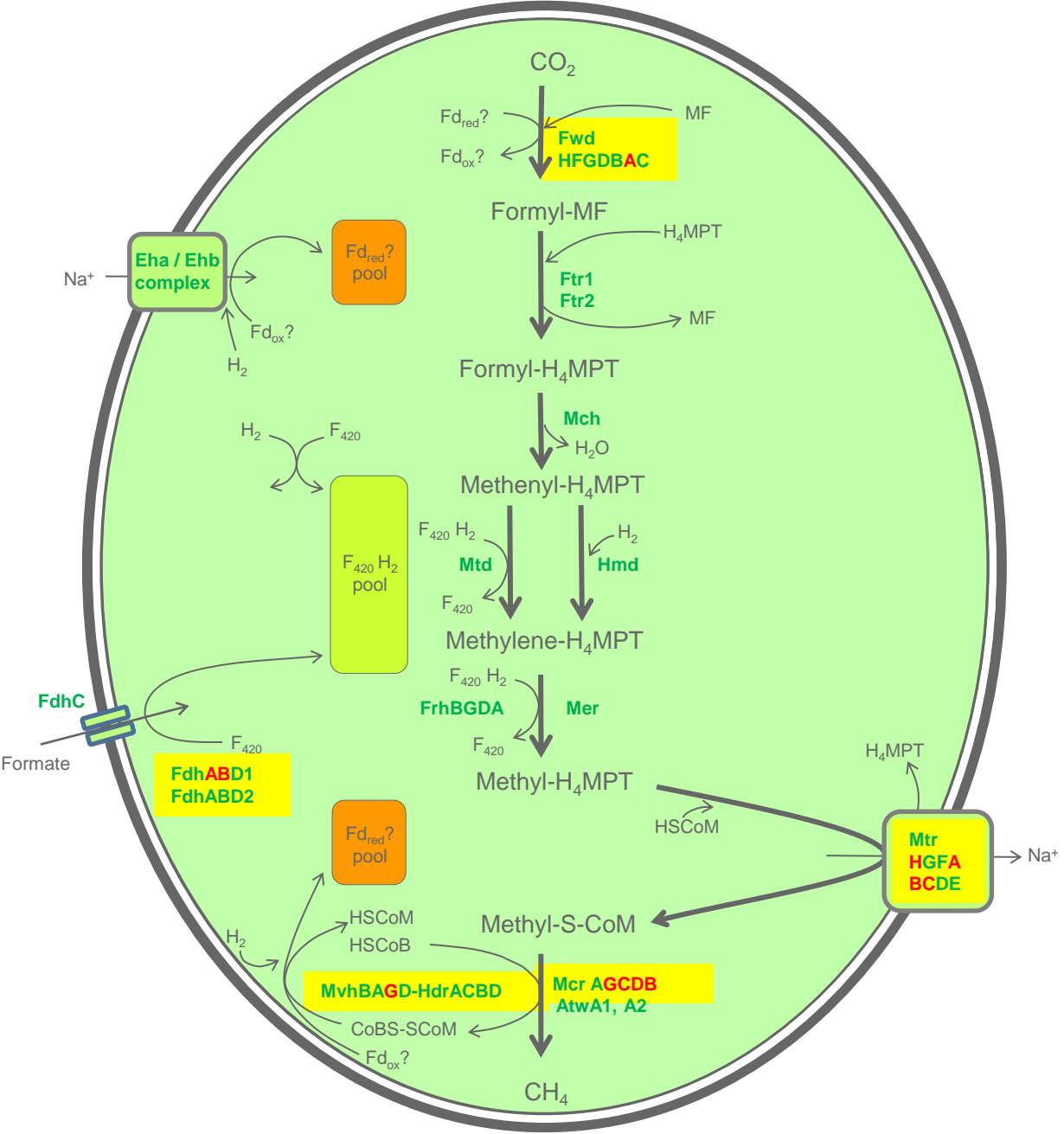
Formylmethanofuran dehydrogenase (fwdA),

H₄MPT methyltransferase (mtrABCH)

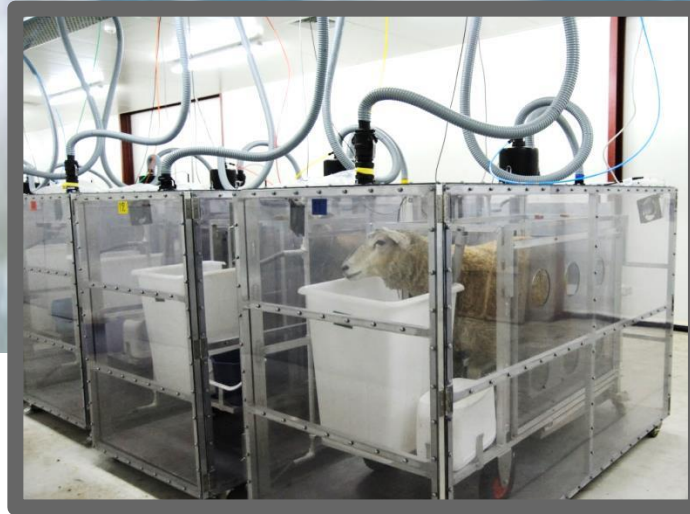
Methyl CoM reductase (mcrBCDG),

Methyl viologen-reducing hydrogenase (mvhG),

Formate utilisation (fdhAB)

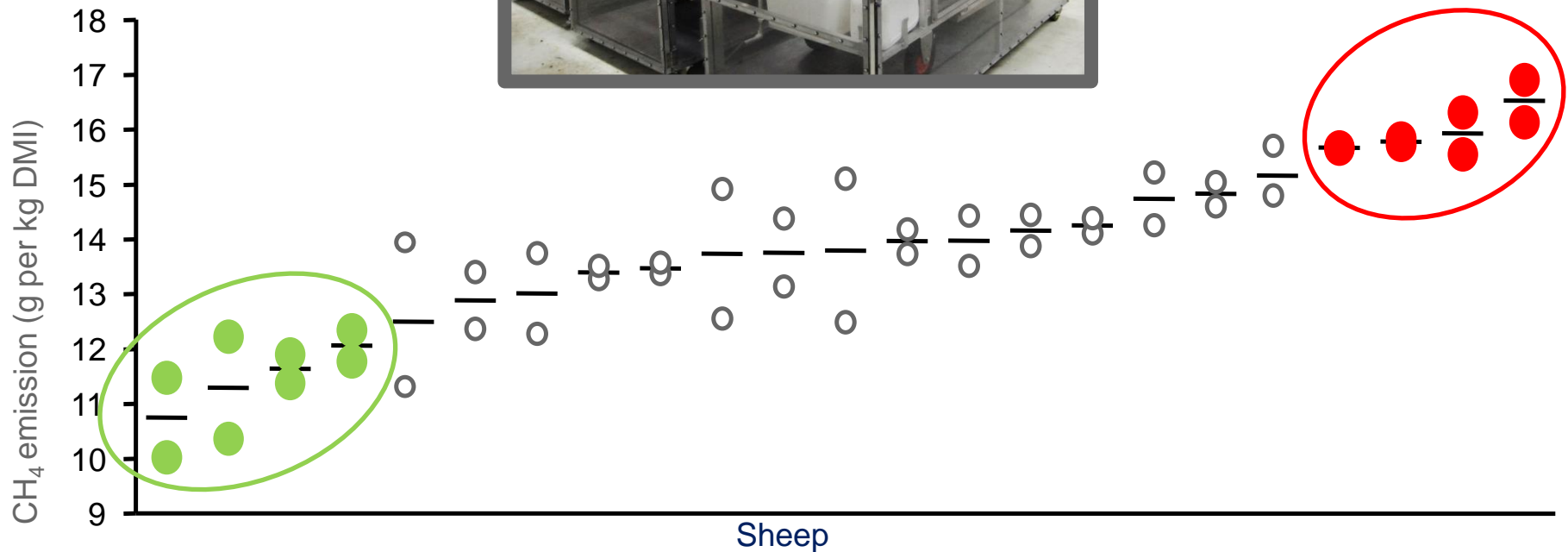


Sheep vary in their CH₄ emissions per unit of intake



Low CH₄ emitters

High CH₄ emitters



Sampling, nucleic acids extractions and analyses



4 highest and 4 lowest methane emitting sheep, stomach tubed rumen content x 2 time points

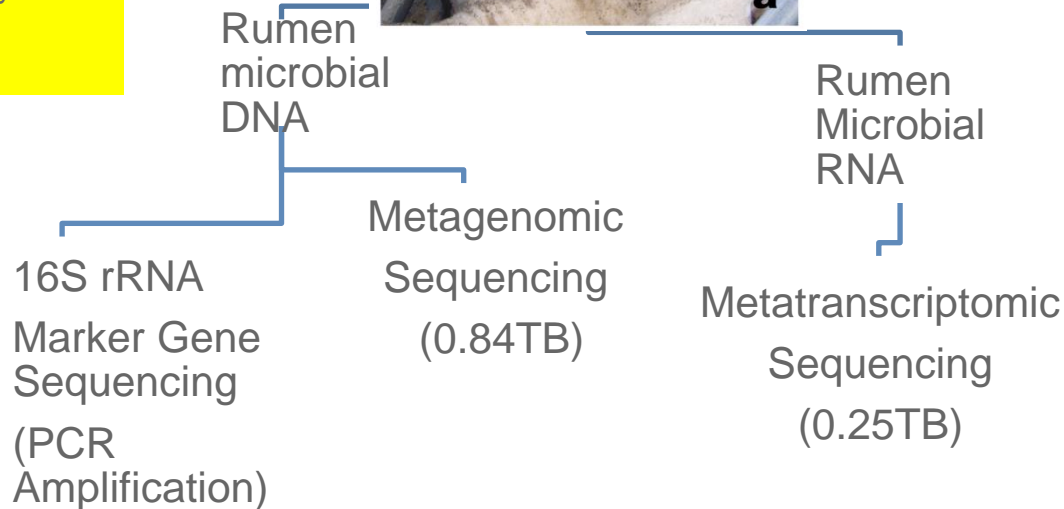
DNA extracted from frozen rumen contents using "Repeated Bead Beating and Column (RBB+C) purification" method (Yu & Morrison, 2004)

HMW DNA, for large paired-end insert libraries, prepared using non-mechanical lysis DNA extraction method (Rosewarne et al, 2011)



RNA extracted for transcript analysis using a hot lysis-acid phenol extraction method.

RNA concentration was determined using Qubit and quality checked via Bioanalyzer



DNA and RNA extractions



DNA extracted from frozen digesta using “Repeated Bead Beating and Column (RBB+C) purification” method (ref)

HMW DNA, for large paired-end insert libraries, prepared using a non-mechanical lysis DNA extraction method (*ref*)

RNA extracted for transcript analysis using a hot lysis-acid phenol extraction method.

RNA concentration was determined using Qubit and quality checked via Bioanalyzer

In vitro experimentation on methanogen co-cultures



- Methanogens in the rumen usually experience H_2 concentrations in the range 0.1 to 50 μM
- In the lab, methanogens grown by pumping with $H_2 + CO_2$ to 2 atm = $\sim 770 \mu M H_2$
- As methanogens grow, H_2 is used, so over time H_2 concentration falls in cultures, until cultures are re-pumped with H_2
- Controlling H_2 at a low concentration is technically not possible without a H_2 -producing partner
- Low H_2 concentrations achieved by co-inoculating with the slow growing, cellulose-degrading, H_2 -producing rumen bacterium *Ruminococcus flavefaciens* FD1
- In these co-cultures, H_2 is expected to be in the rumen relevant range ie 0.1 to 50 μM

Co-culture set up and sample collections



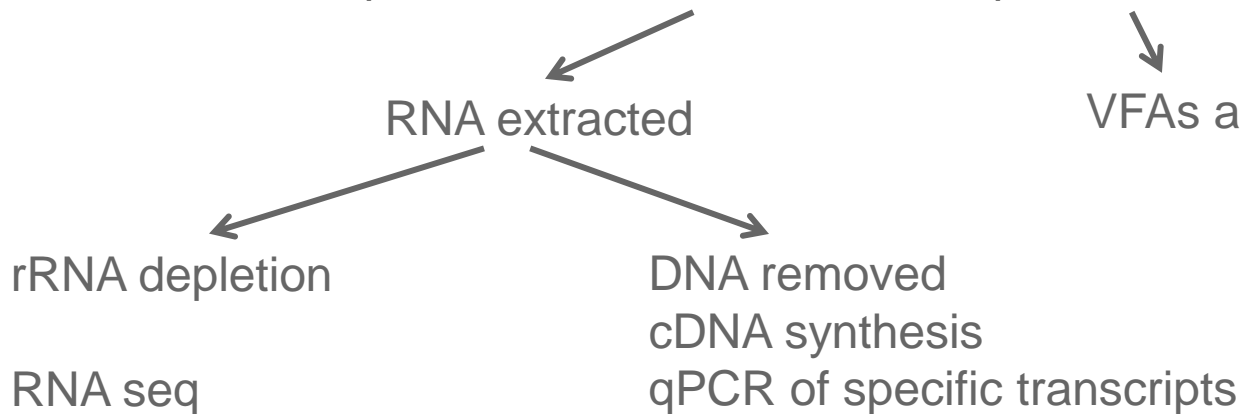
- *Methanobrevibacter ruminantium* M1

8 x 50 ml methanogen mono or co-cultures with *Ruminococcus flavefaciens* FD1 as hydrogen producer growing on cellulose pumped with H₂ + CO₂ or N₂ (FD1)

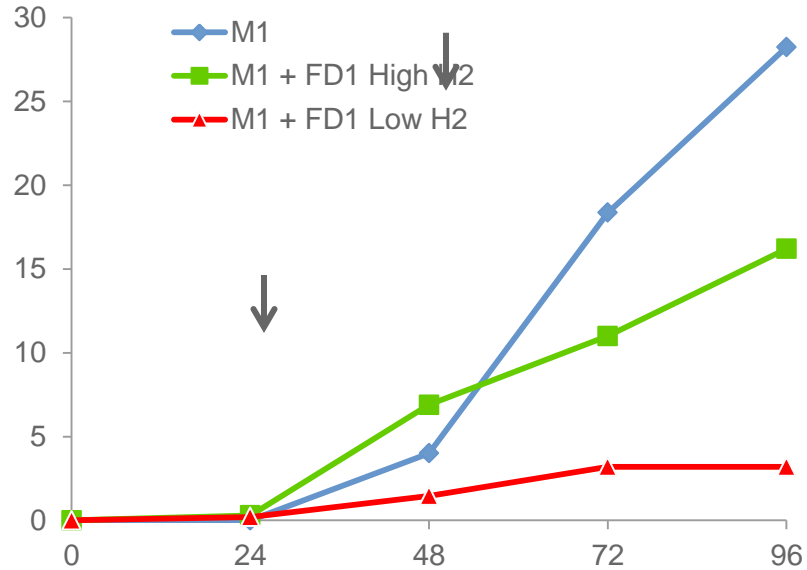
M1 grown on H₂ + CO₂ (hydrogenotrophic)

↓
38°C with shaking

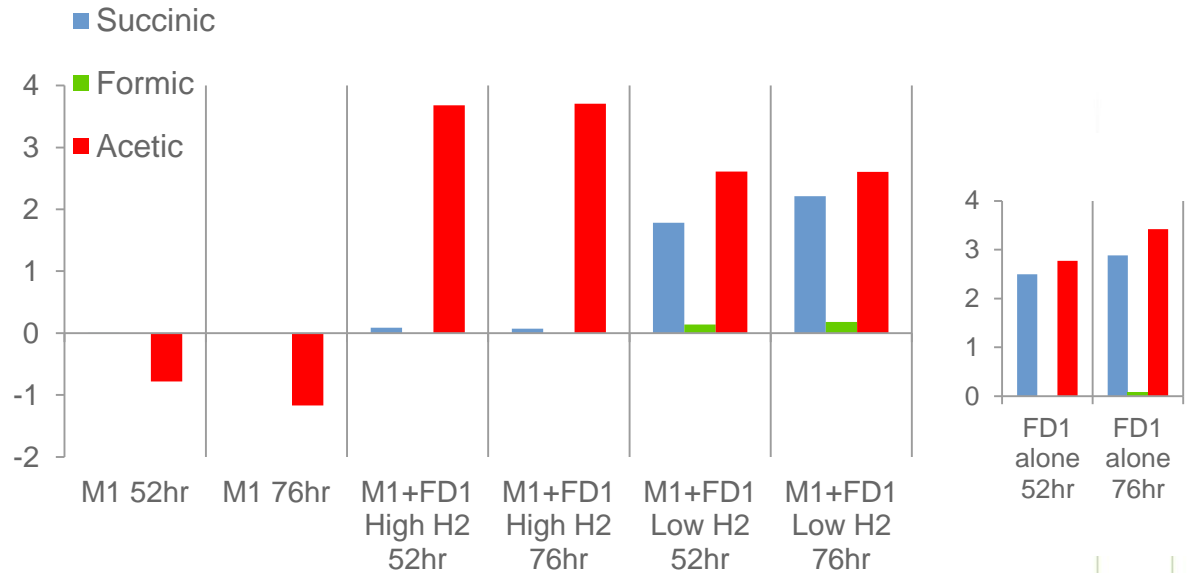
↓
CH₄, H₂ measurements made daily
4 x 50ml samples collected for RNA, DNA, protein and VFAs at 2 time points



Methanobrevibacter ruminantium M1 in co-culture with *Ruminococcus flavefaciens* FD-1



Volatile Fatty Acids



Cultured rumen methanogens available for *in vitro* testing

| Methanogen | Strain | Mode of methane formation |
|---|----------------|--|
| <i>Methanobrevibacter</i> spp. (ruminantium group) | M1, YLM1, NT7* | hydrogenotrophs |
| <i>Methanobrevibacter</i> spp. (gottschalkii group) | SM9, D5 | hydrogenotrophs |
| <i>Methanobrevibacter</i> sp. (wolunii clade) | AbM4 | hydrogenotroph |
| <i>Methanobacterium formicicum</i> | BRM9 | hydrogenotroph |
| <i>Methanosphaera</i> sp. | ISO3-F5 | methylotroph |
| <i>Methanosarcina</i> sp. | CM1 | acetoclastic |
| Rumen Cluster C | ISO4-H5 | methylotroph, co-culture with a <i>Succinivibrio</i> sp. |
| H ₂ producer | | Preferred substrate |
| <i>Ruminococcus flavefaciens</i> | FD-1 | Cellulose degrader |
| <i>Butyrivibrio proteoclasticus</i> | B316 | Xylan degrader |

Acknowledgements

agresearch



Funding provided through the NZAGRC science programme



Research was funded by and carried out under contract to the Pastoral Greenhouse Gas Research Consortium (PGgRc)

New Zealand Government

Funded by the New Zealand Government to support the objectives of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases



AgResearch Core funding

Bill Kelly

Sinead Leahy

Christina Moon

Eric Altermann

Adrian Cookson

Dragana Gagic

Suzanne Lambie

Jonathan Dunne

Zhanhao Kong

Carl Yeoman

Sandra Kittelmann

Henning Seedorf

Peter Janssen

Ron Ronimus

Neil Wedlock

John McEwan

Cesar Pinares-Patino

Joint Genome Institute

Edward Rubin

Weibing Shi

Christina Fan