High-throughput screening of yeast biodiversity for the production of high value biobased chemicals

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Biorefinery centre

- Aims to provide expertise from Biomass production to final chemical production
  - Biomass
  - Pre-treatment
  - Enzyme Hydrolysis
  - Fermentation – With the NCYC
  - Distillation / Purification

- Takes an integrated approach to the biorefinery concept
- Performs experimentation from small laboratory to small industrial scales

Background – Bio-refining

- **Energy**
  - Replacement of petroleum with a renewable source
  - High volume / low value product
- **Biobased chemicals**
  - Low volume / high value product
  - Provides a financial impetus towards bio-refining
  - Therefore secondary products of great importance

Energy – Aims

Aims

- Final ethanol yield above 10% v/v to enable viable distillation
  - This will require a high substrate concentration to be obtained
- Minimal enzyme addition

Energy – Methods

Methods

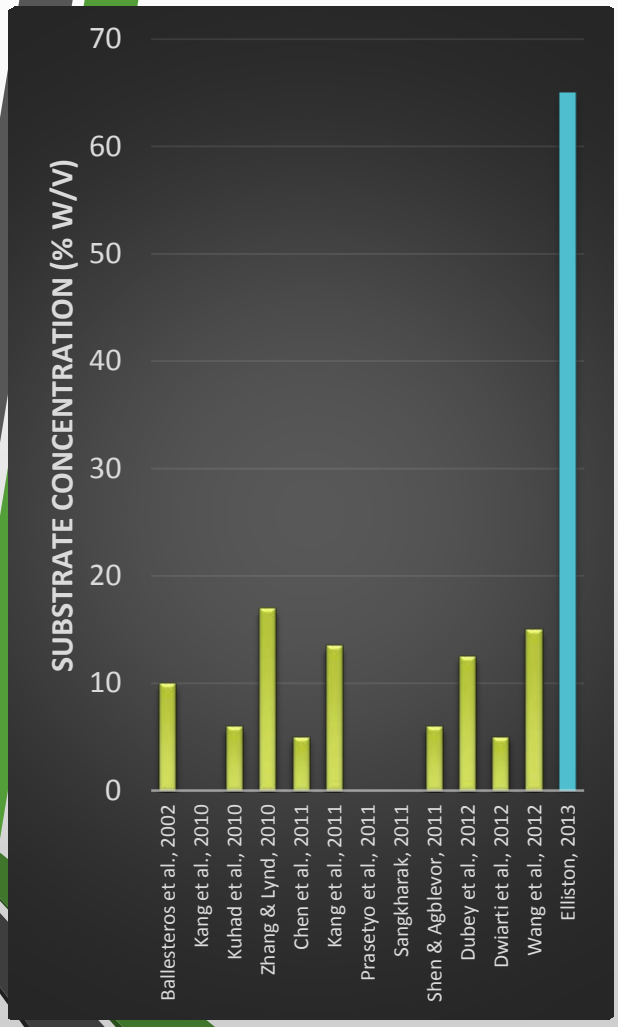
- Generally use Saccharomyces cerevisiae
- Office copier paper substrate
- Stepwise batch addition regime
- 5 L high torque reactor

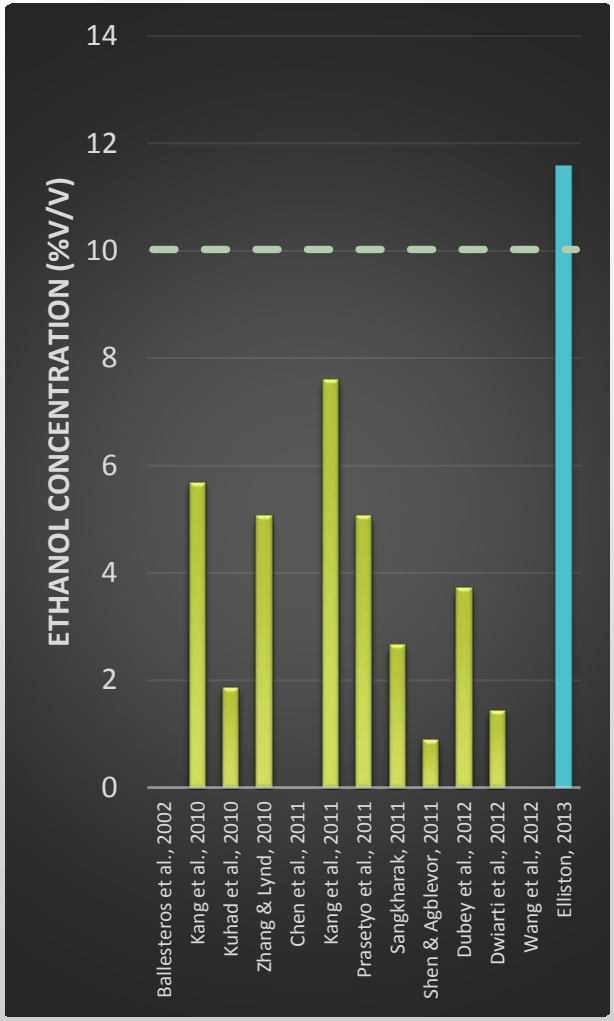
Energy – Results

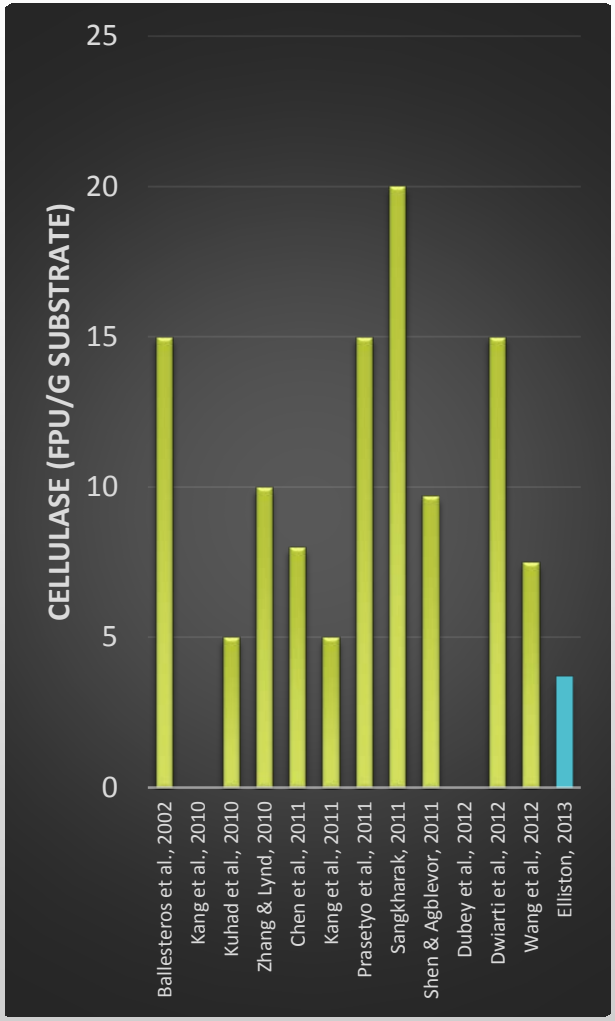
Results

- Substrate concentration 65% (w/v) equivalent
- Ethanol concentration 11.6% (v/v)
- Final enzyme concentration 3.7 FPU/g

Energy – Comparison to literature







A Elliston, S.R.A. Collins, D.R. Wilson, I.N. Roberts, K.W. Waldron (2013)
High concentrations of cellulosic ethanol achieved by fed batch semi simultaneous saccharification and fermentation of waste-paper
Bioresource Technology **134**(0): 117-126.

Bio-refining

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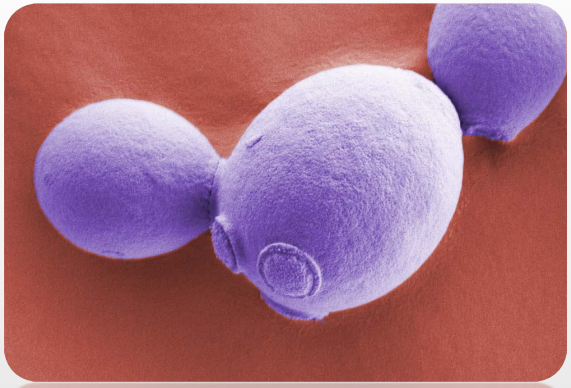
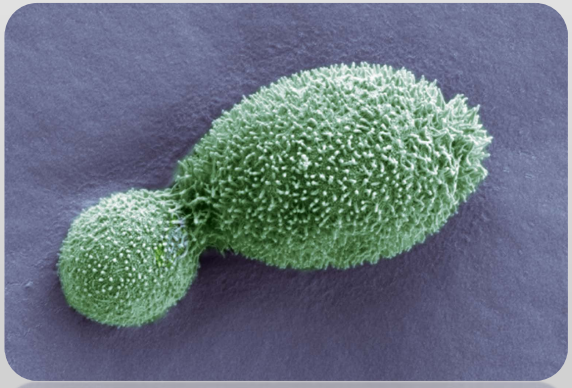
Biobased chemicals

- Screen the National Collection of Yeast Cultures (NCYC) for high value platform / speciality chemical production
  - Enable high-throughput screening of the whole collection
  - Explore metabolite production from the large range of yeast strains available
  - Screen yeast on both “model” and “real world” substrates
  - Select initial list of desirable chemicals to screen for

NCYC Collection

- The NCYC contains ~4000 individual strains of non-pathogenic yeast covering a large yeast phenology
- Top ten most abundant species in collection:
  - Saccharomyces cerevisiae (1035)
  - Schizosaccharomyces pombe (495)
  - Saccharomyces paradoxus (123)
  - Saccharomyces pastorianus (59)
  - Pichia angusta (48)
  - Zygosaccharomyces bailii (41)
  - Rhodotorula mucilaginosa (36)
  - Saccharomyces bayanus (33)
  - Pichia membranifaciens (28)
- Metabolites haven't been intimately studied



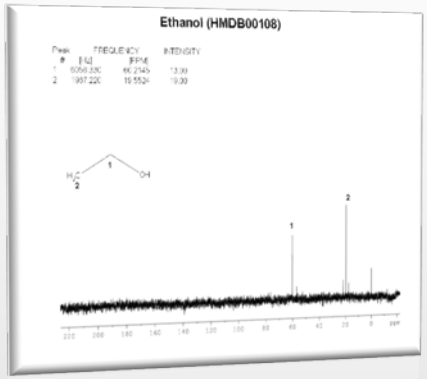


High-throughput screening

- The strains were originally stored either freeze dried in glass ampoules or frozen in liquid nitrogen this format doesn't allow for high-throughput
- In order to screen the collection has therefore been transferred to 96 well plate format which can be accessed via a liquid handling robot

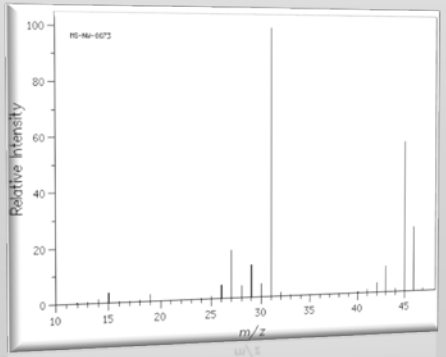
Initial screening

- 1st Phase general screen by NMR
- Targeted chemicals analysed by LC-MS / GC-MS / 2D NMR
- Genome sequencing in tandem

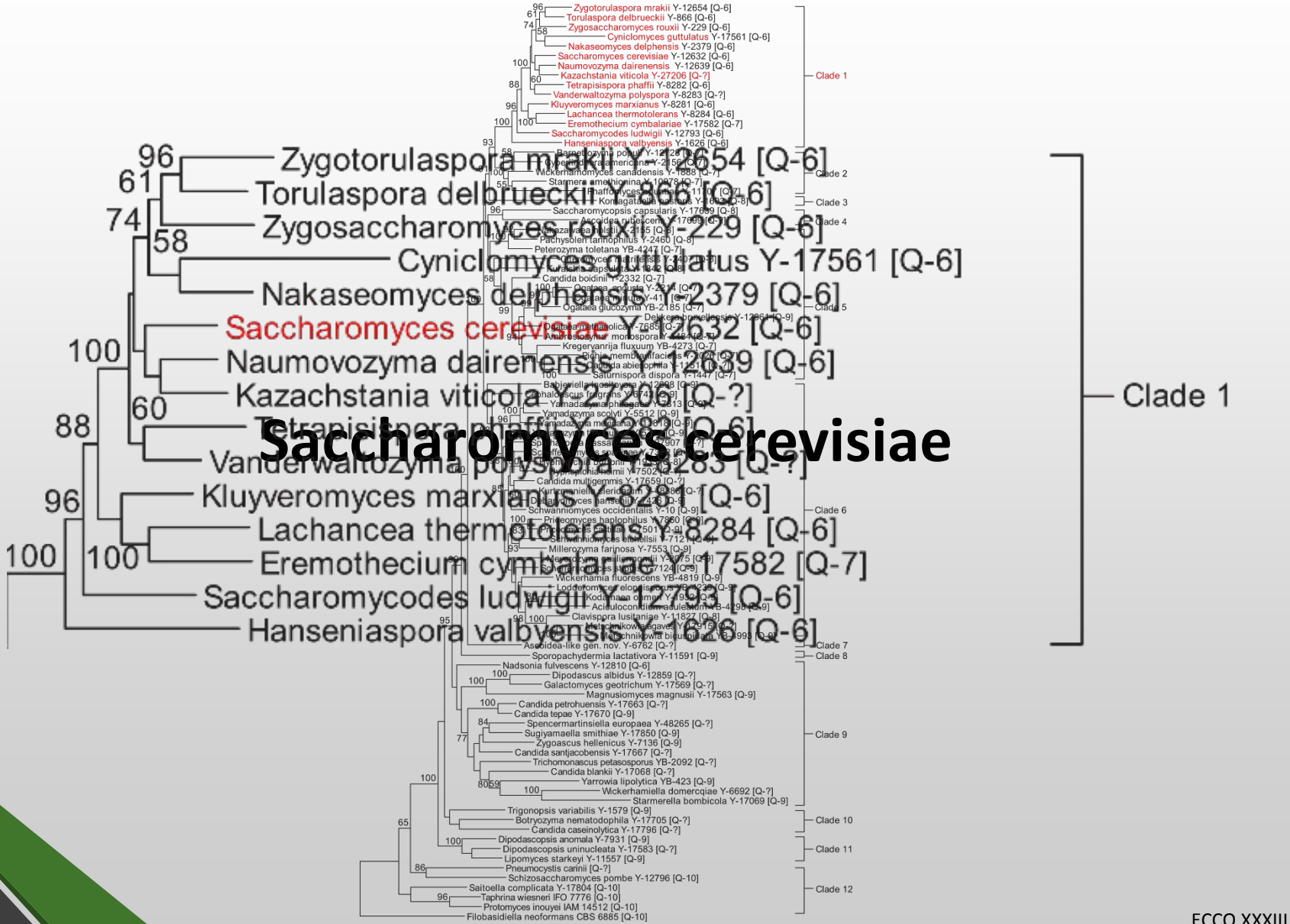


Method

- Minimal media
- ^{1}H NMR
- pH buffering



1st Screen - Selection

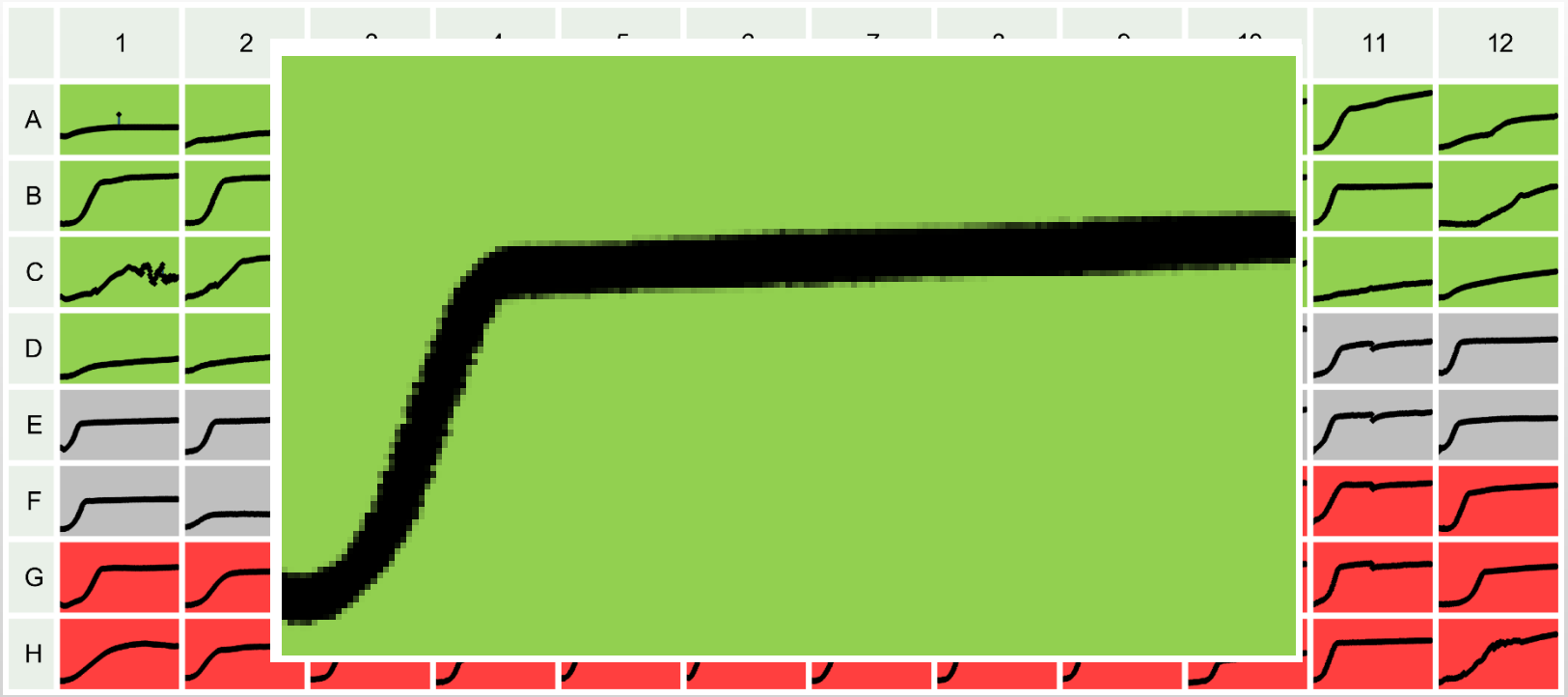


Metabolomics

Broad genetic diversity pilot

S.bayanus	1		*N.dairenensis*	1
C.glabrata	1		*Nadsonia fulvescens Var. fulvescens*	1
Candida catenulata	1		*Nk.Delphensis*	1
Candida famata var. famata	2		*Pichia anomala*	2
Candida kefyr	1		*Pichia membranifaciens*	6
Candida krusei	2		*Pichia subpelliculosa*	1
Candida tropicalis	1		*Rhodotorula glutinis var. glutinis*	3
D.hansenii	1		*Rhodotorula minuta var. minuta*	1
Debaryomyces hansenii	3		*Rhodotorula mucilaginosa*	4
Debaryomyces polymorphus	1		*S.cariocanus*	1
Dekkera anomala	1		*S.kudriavzevii*	1
Geotrichum candidum	1		*S.mikatae*	1
Guilliermondella selenospora	1		*S.paradoxus*	1
H.valbyensis	1		*S.uvarum*	1
Hanseniaspora valbyensis	1		*Saccharomyces cerevisiae*	33
Hanseniaspora vineae	1		*Saccharomyces pastorianus*	1
K.lactis	1		*S'my.Ludwigii*	1
K.marxianus	1		*T.delbrueckii*	1
Kaz.servazzii	1		*V.polyspora*	1
Kaz.viticola	1		*Williopsis saturnus var. saturnus*	3
Kloeckera africana	2		*Y.lipolytica*	1
Kloeckera corticis	1		*Z.rouxii*	1
L.thermotolerans	1		*Zt.mrakii*	1

Growth Curves



Top 12 chemical opportunities

1. ***Four Carbon 1,4-Diacids (Succinic, Fumaric, and Malic)***
2. ***2,5-Furan dicarboxylic acid (FDCA)***
3. ***3-Hydroxypropionic acid (3-HPA)***
4. ***Aspartic acid***
5. ***Glucaric acid***
6. ***Glutamic acid***
7. ***Itaconic acid***
8. ***Levulinic acid***
9. ***3-Hydroxybutyrolactone***
10. ***Glycerol***
11. ***Sorbitol***
12. ***Xylitol/arabinitol***

(Bozell and Petersen 2010)

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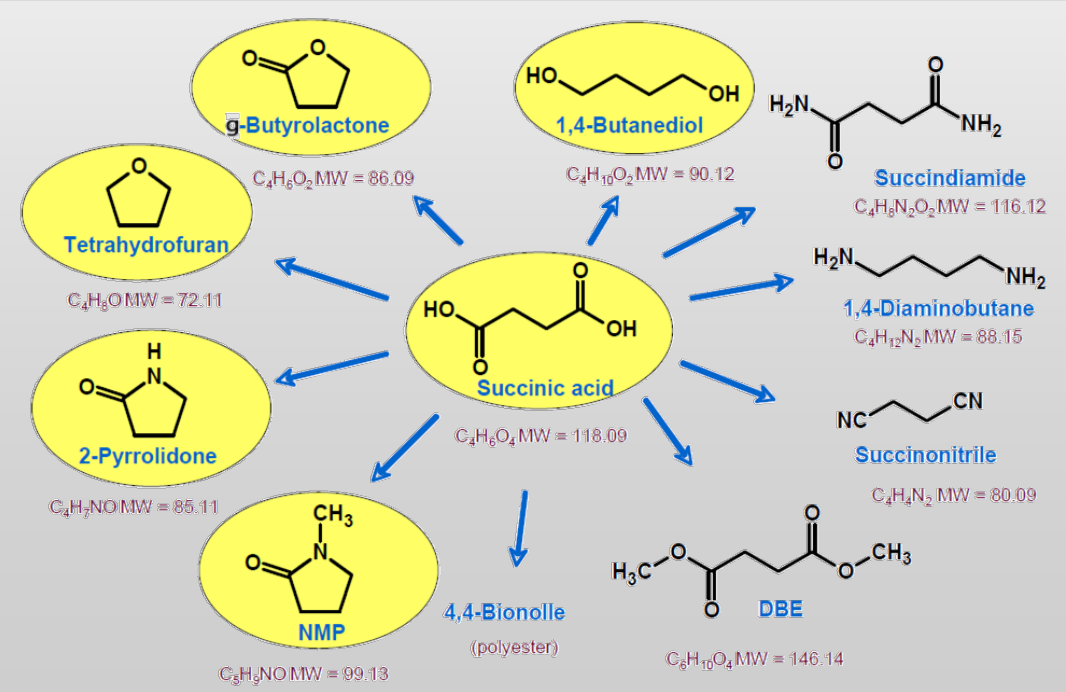
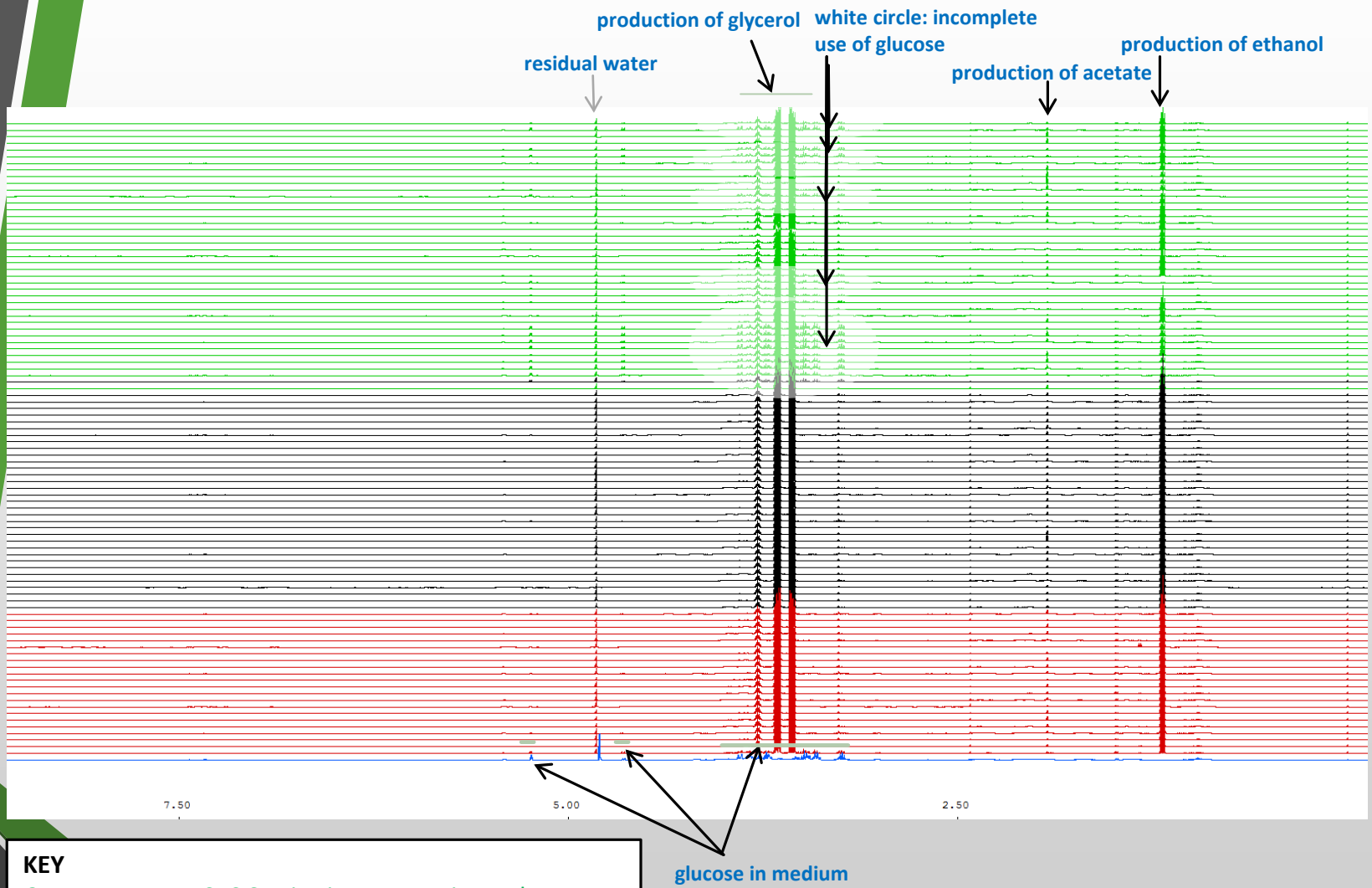
Top 12 chemicals

1. *Four Carbon 1,4-Diacids (Succinic, Fumaric, and Malic)*

- Green solvents
- Fibres such as nylon / polyester
- Fermentation by overproduction from Krebs cycle pathways



Results - Spectra

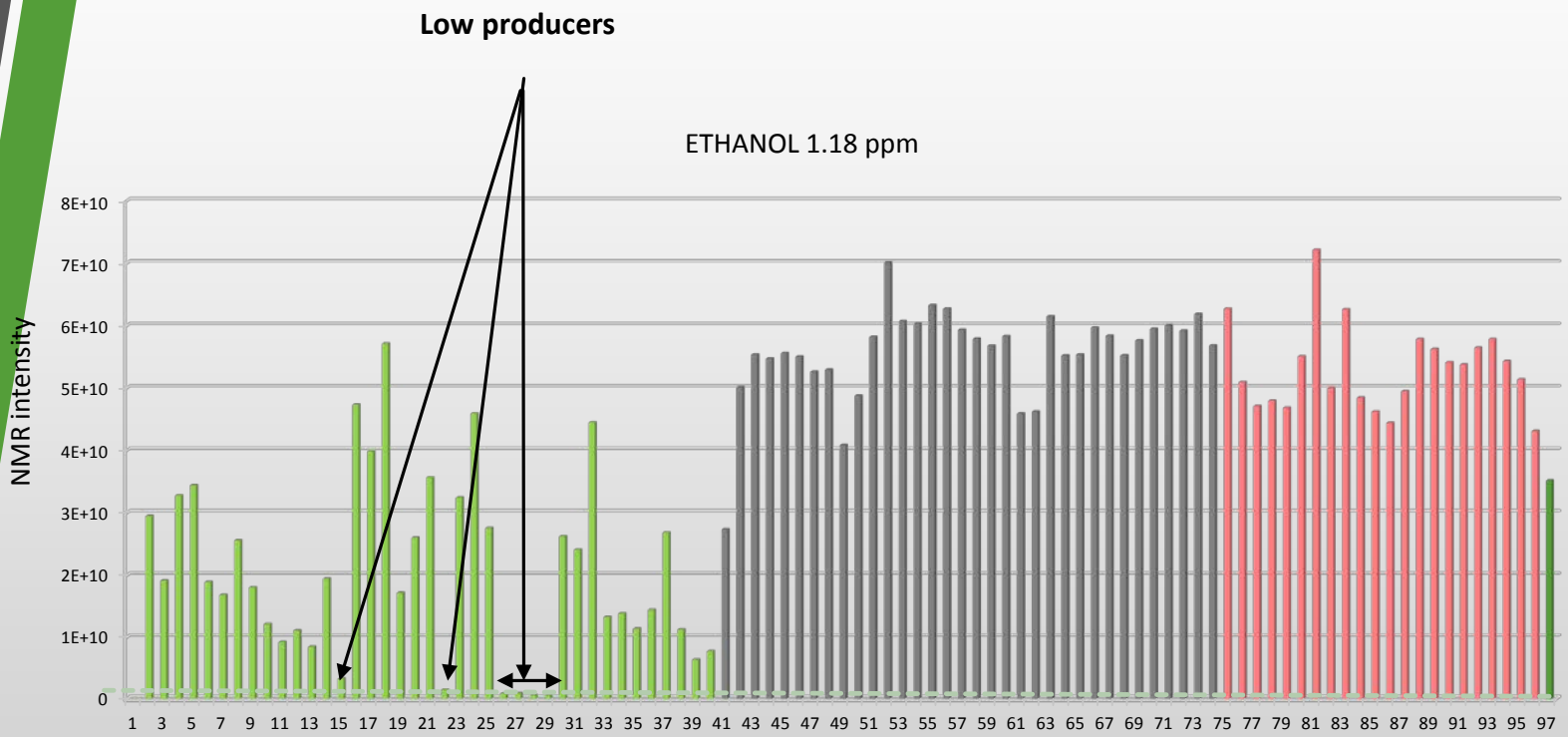


KEY

Green:	NCYC Strains in consecutive order
Black:	*Saccharomyces spp.*
Red:	Large variability in phylogeny set
Blue:	Medium

Energy goal

Ethanol



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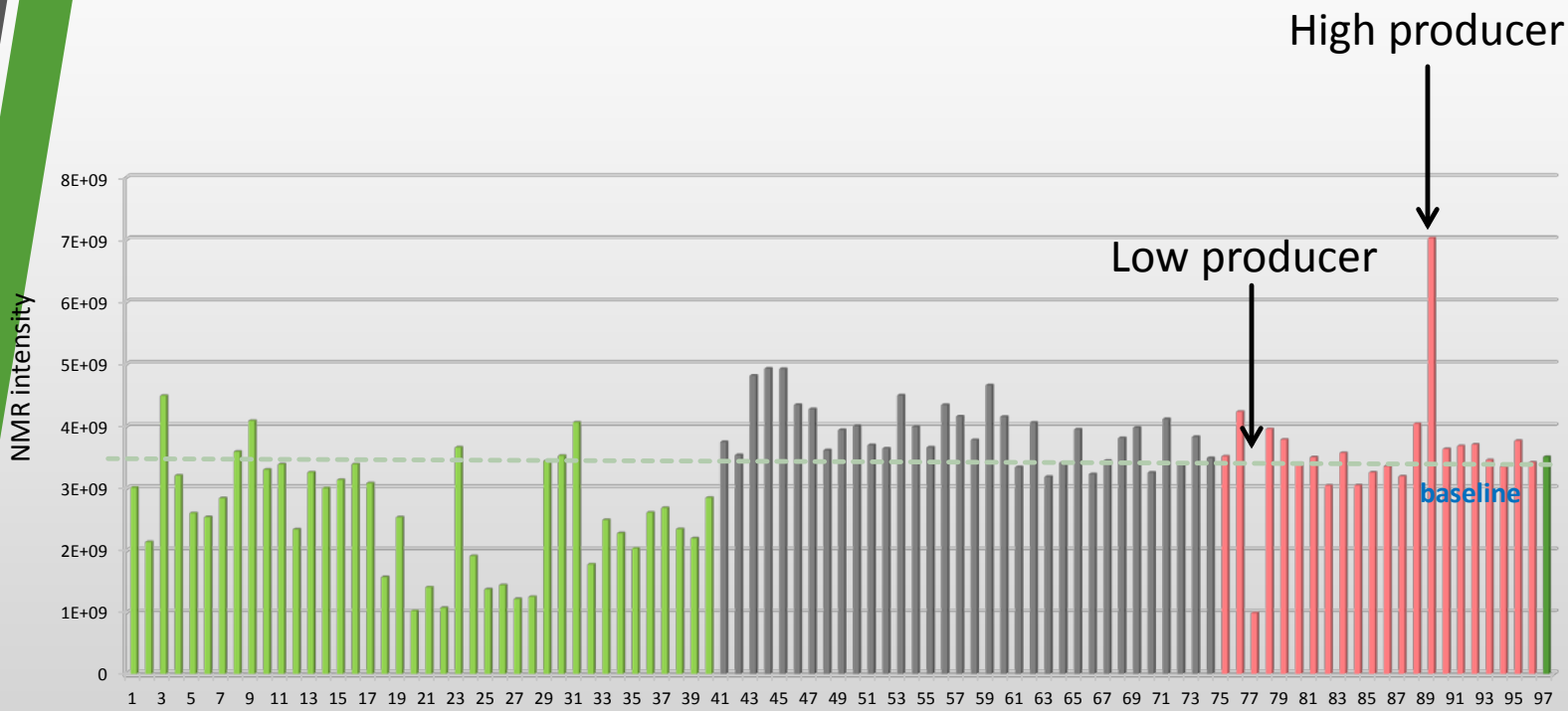
Economical goal

Succinic Acid



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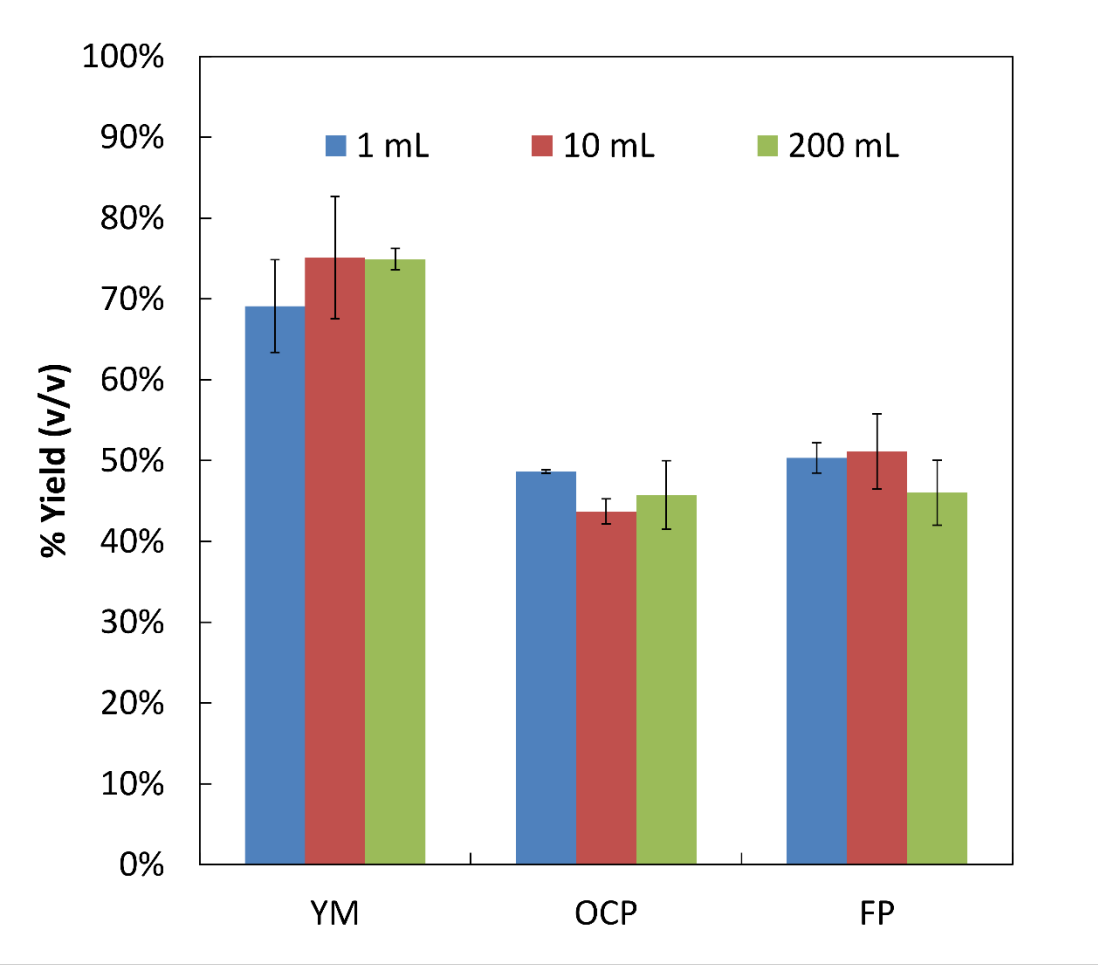
Main products

- **Main products**
  - Ethanol (Energy)
  - Acetate
  - Lactate (Platform)
  - Succinate (Platform)
- **Examples of other products**
  - Isobutyric
  - 2,3 butanediol
  - 3-hydroxymethylglutaric
  - Acetoin
  - Citric acid

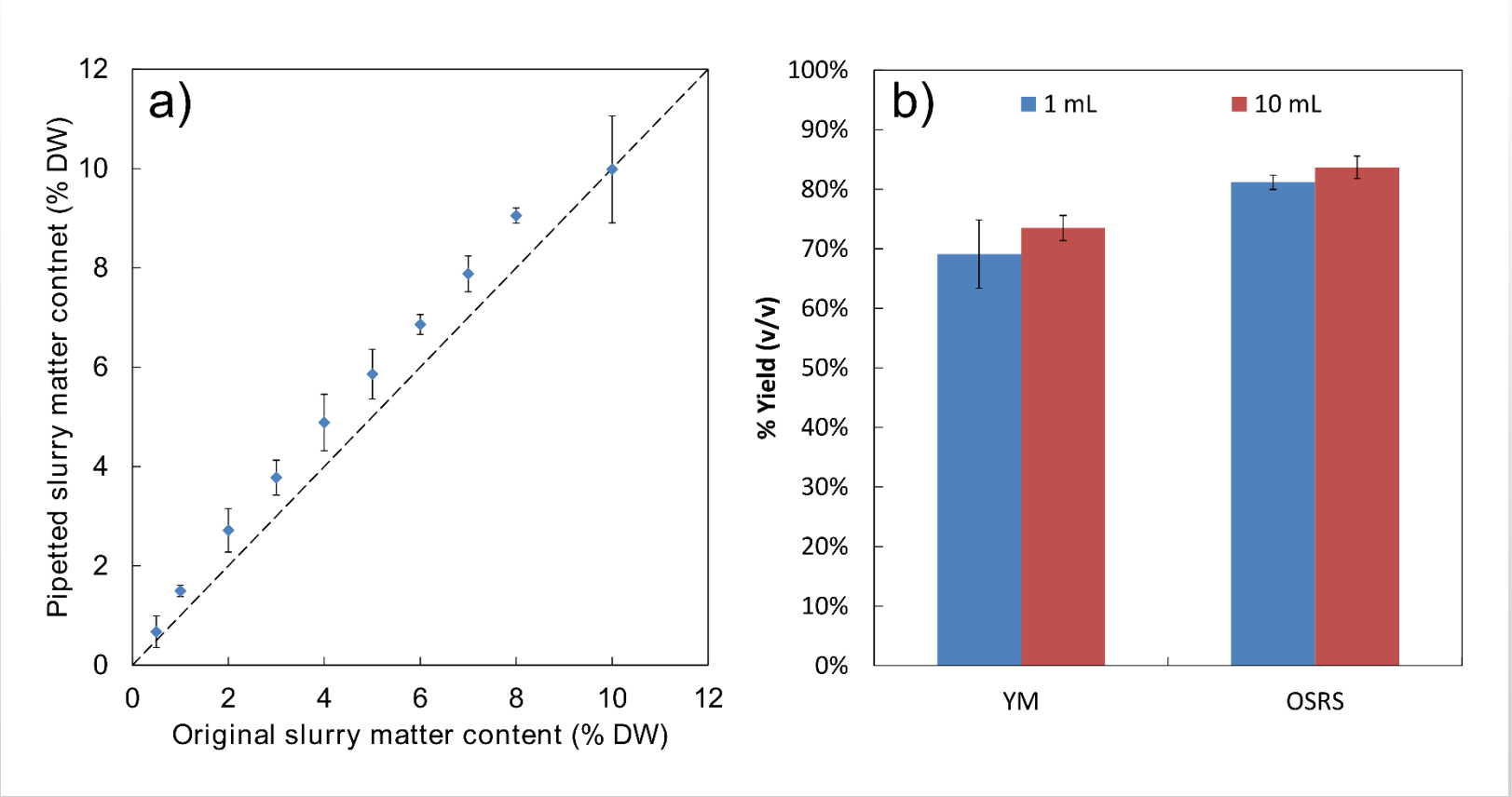
Solid "Real World" Biomass Substrate

Simultaneous Saccharification and Fermentation



YM ($F_{2,6}$ = 1.73, p = **0.288**)
OCP ($F_{2,6}$ = 0.49, p = **0.642**)
FP ($F_{2,6}$ = 1.56, p = **0.297**)

SSF Continued



YM ($F_{1,4}$ = 1.32, p = **0.314**)

BMS ($F_{1,4}$ = 1.46, p = **0.294**)

Conclusion

- High-throughput screening has been achieved on “model” liquid substrates utilising NMR analysis
  - US DOE “hit list” used for initial target chemicals
- First plate has already highlighted interesting high producer of Succinic Acid
- Solid “real world” substrates show potential for rapid assessment using a 96 well plate high-throughput methodology
  - This novel method is currently in preparation for publication
- The work highlights the importance of culture collections and the biodiversity that they contain

Further work

- Screening of NCYC will continue on liquid substrates
- Screening to continue on solid substrates
- Comparison of Metabolomics dataset against genetic data

Thank you

- **Keith Waldron**
- Julie Soucouri
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- Carmen Nueno Palop
- Chris Bond
- Jeanette Newman

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- Ian Colquhoun
- Mark Philo

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