

High Throughput Screening Facility: Update and Upgrades 2016

NORTH EAST FUNGAL FORUM

PETER BANKS

Introduction

David Lydall David.Lydall@ncl.ac.uk

Peter Banks Peter.Banks@ncl.ac.uk

Adrian Blackburn Adrian.Blackburn@ncl.ac.uk

2011- High Throughput Screening Facility

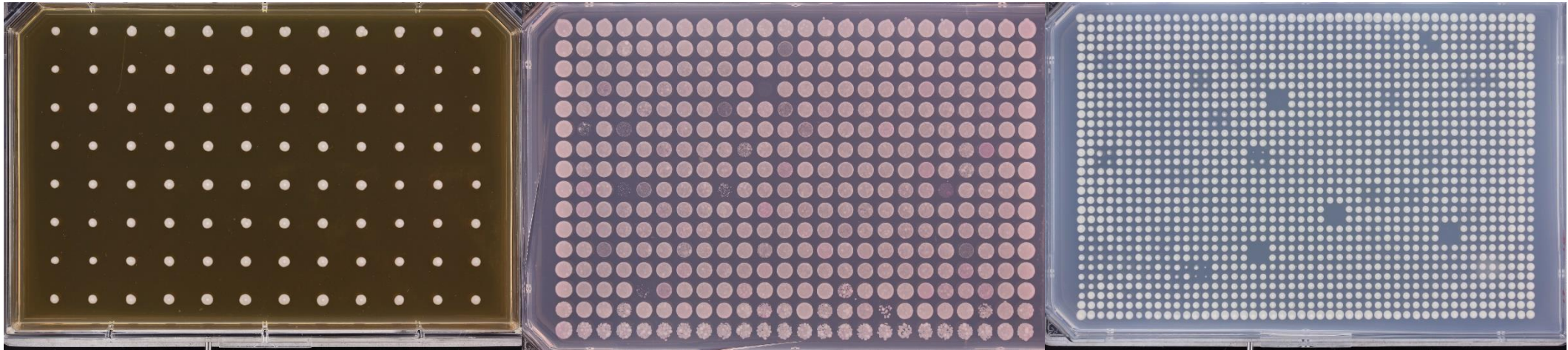
- Equipment
- Reagents
- Expertise

2016 – £400,000 from the Research Infrastructure Fund (RIF)



High Throughput Screening

Transfer manual bench top assays to automated robotic assays commonly in 96, 384 or 1536 format



96 well format

384 well format

1536 well format

S&P BM6 and BM3 colony pinning robots

Housed on 2nd floor Leech and 2nd floor Cookson

Uses metal 96, 384, 1536 and cherry pick pin tools – **low consumable costs**

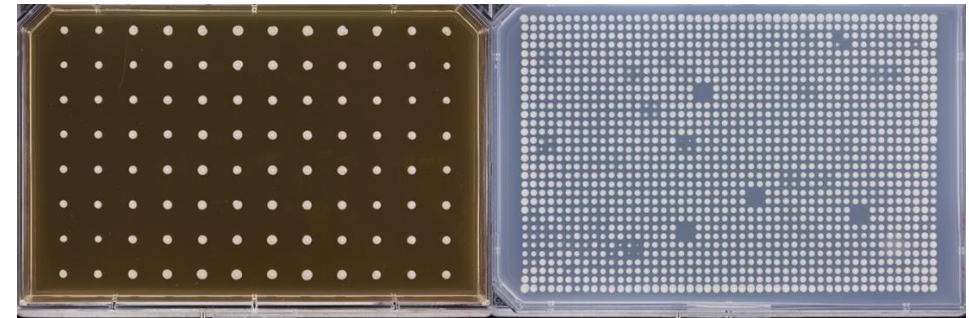
Replicates microbial colonies from agar to agar – **Synthetic Genetic Array**

Inoculate microbial colonies from agar into liquid – **Quantitative Fitness Analysis**

Pin microbial colonies from liquid onto solid – **Thawing frozen stocks**

High capacity – **180-240 plates in one run**

Slow but robust and easy to use



96 well format

1536 well format

Singer ROTOR Colony Pinning Robot

Housed on 2nd floor Cookson

Uses plastic 96, 384, 1536 disposable pin tools –
associated consumable costs

Replicates microbial colonies from agar to agar –
Synthetic Genetic Array

Inoculate microbial colonies from agar into
liquid – **Quantitative Fitness Analysis**

Pin microbial colonies from liquid onto solid –
Thawing frozen stocks

Low capacity – **5 plates in one run**

Very quick and easy to use



Biomek FXP Liquid Handling Robots

2 systems – 2nd floor Leech and 2nd floor Cookson

1- 96 disposable plastic tips

- Dilution of microbial cultures
- Inoculation of culture from frozen stocks
- Freezing down microbial culture in glycerol – 96 or 384
- Cherry picking

96 well pin tool with free floating pin

- Spotting 96 well plates cultures – Quantitative Fitness Analysis

Very quick and easy to use

High capacity

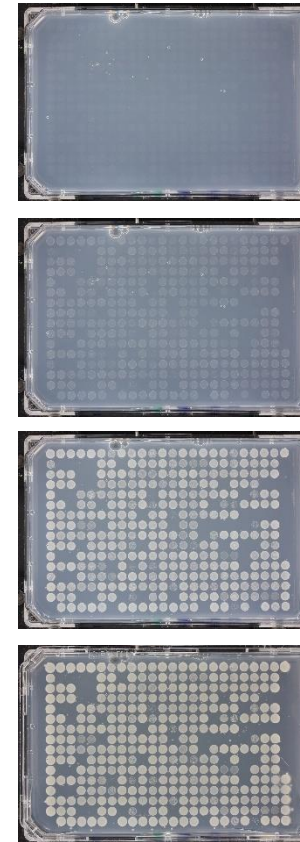
- Deck space for 16 plates
- Cytomat Plate hotel 190 plates in one run



S&P Automated Imaging Systems

Two temperature controlled automated imaging systems

- 20°C-37°C
- Capacity of 160 – 190 plates
- Cycle time of 2 minutes – 1 day
- Run for days
- High quality agar plate images



Ancillary Items

S&P Manual Imager

- High resolution images of round or rectangular agar plates

Singer Serial Filler Plate Pourer

- Round or rectangular plates
- Aseptically dispense up to 2L of media in one run

Matrix Plate Filler

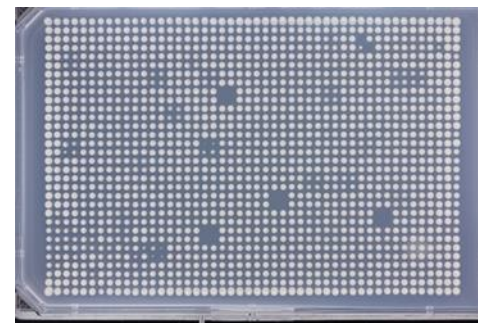
- Dispense 1-2000ul
- 96 or 384 well plates

GreenLab rectangular plates

- Washable and autoclavable plates
- Huge cost saving – Nunc plates are £2+

Consumable discounts

- Sigma media and antibiotics



S. cerevisiae Synthetic Genetic Array

Robotically create double mutant strains

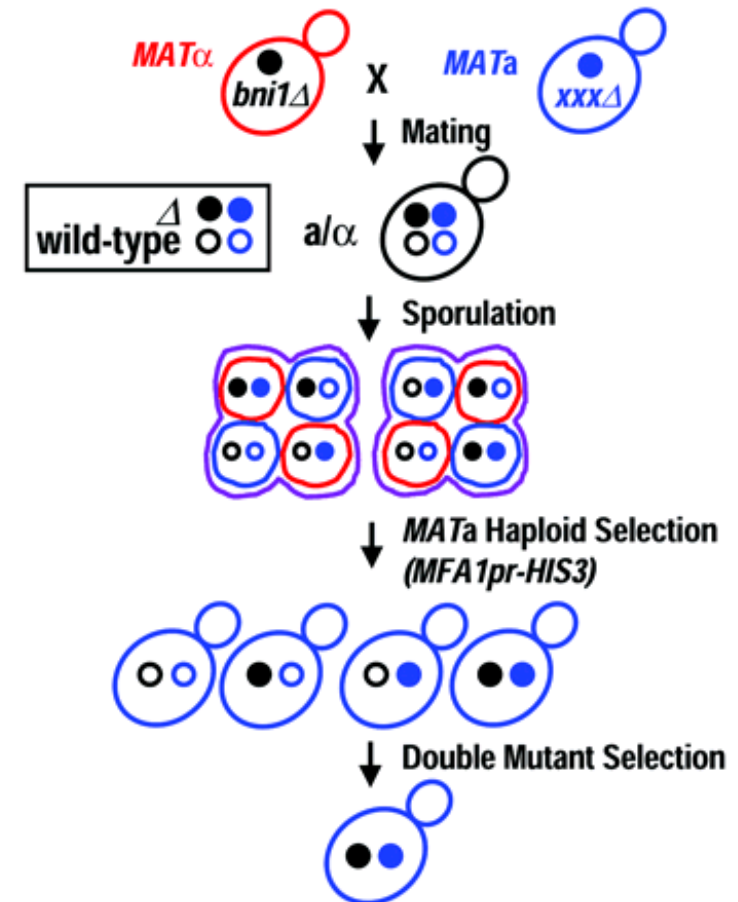
- One query strain
- Thousands of library strains
- Takes a month
- Synthetic lethality
- Library of double mutant strains

Start with many different types of query mutant

- Single gene deletion – *rad9Δ*
- Point mutant – *cdc13-1*
- Point mutant with a gene deletion – *cdc13-1 rad9Δ*
- Two/three/four gene deletions *cdc13Δ exo1Δ rad9Δ*
- Covering plasmids – essential genes *dna2Δ*

Start with many different types of library

- Deletion library ~4500 strains – KANMX
- Overexpression library ~6000 strains – URA
- DaMP Library ~800 strains – KANMX
- Histone point mutant library ~ 400 strains – NATMX



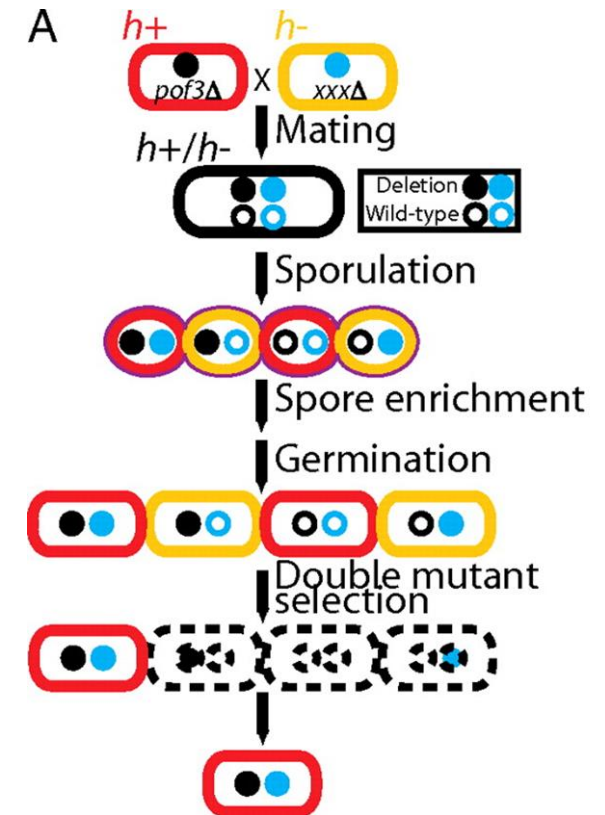
S. pombe Synthetic Genetic Array

Robotically create double mutant strains

- One query strain
- One library of ~3300 of strains - Bioneer
- Takes a month
- Synthetic lethality
- Library of double mutant strains

Start with different types of query mutant

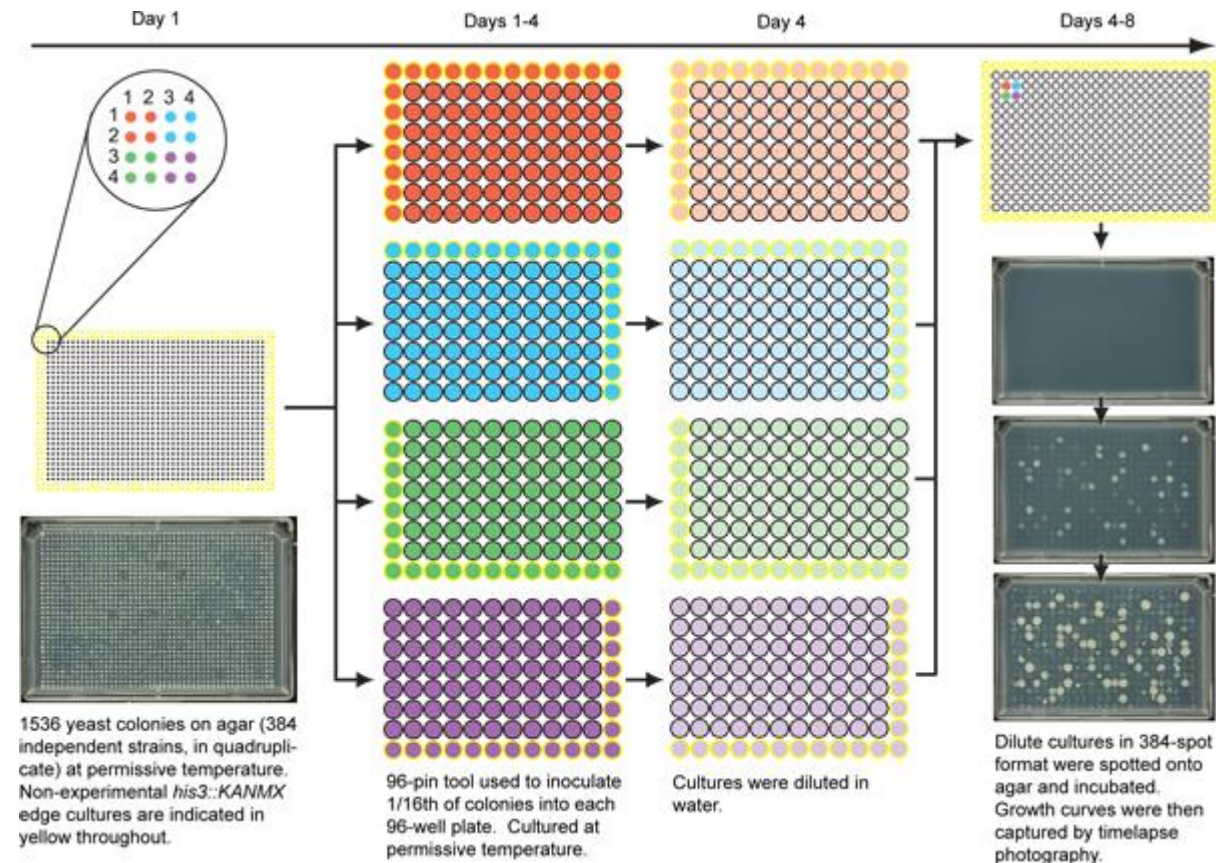
- Single gene deletion – *exo1Δ*
- Point mutant – *pot1-1*
- Two gene deletions – *exo1Δ rad9Δ*
- Complexity inhibited by the lack of markers



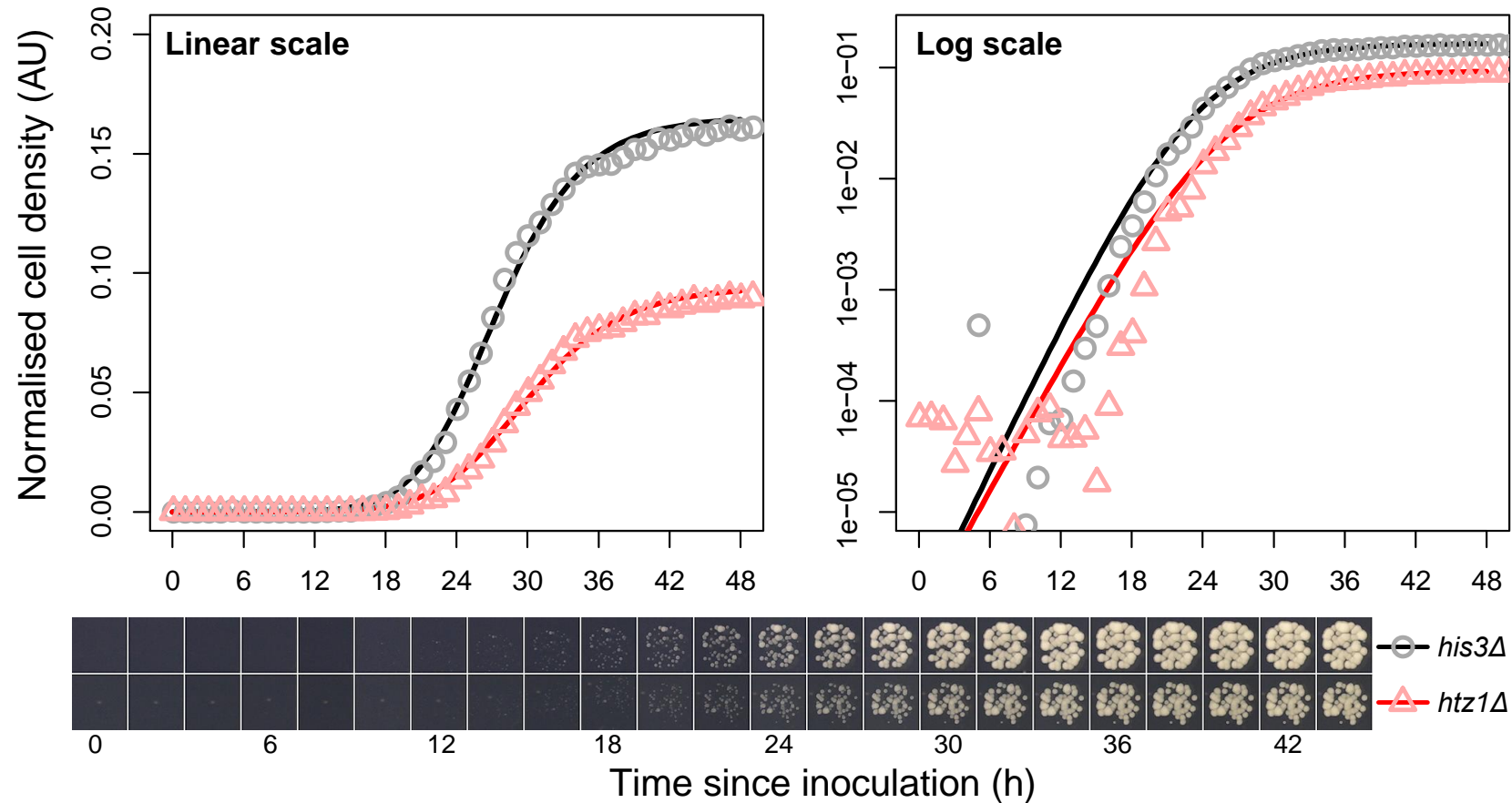
S. cerevisiae Quantitative Fitness Analysis

Quantitative Fitness Analysis (QFA) is a complementary series of experimental and computational methods for estimating microbial culture fitnesses

<http://www.jove.com/video/4018/a-quantitative-fitness-analysis-workflow>

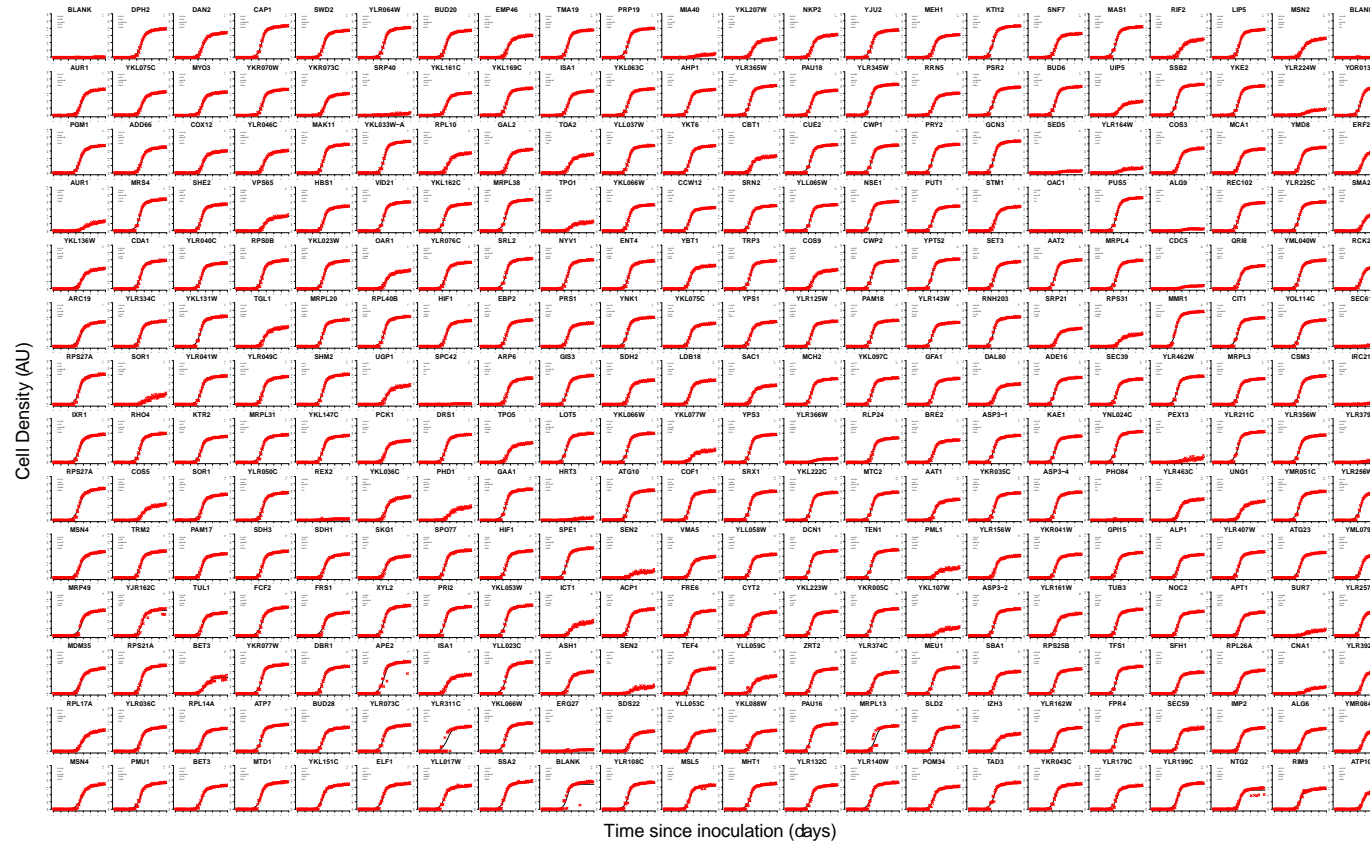


Quantitative Fitness Analysis



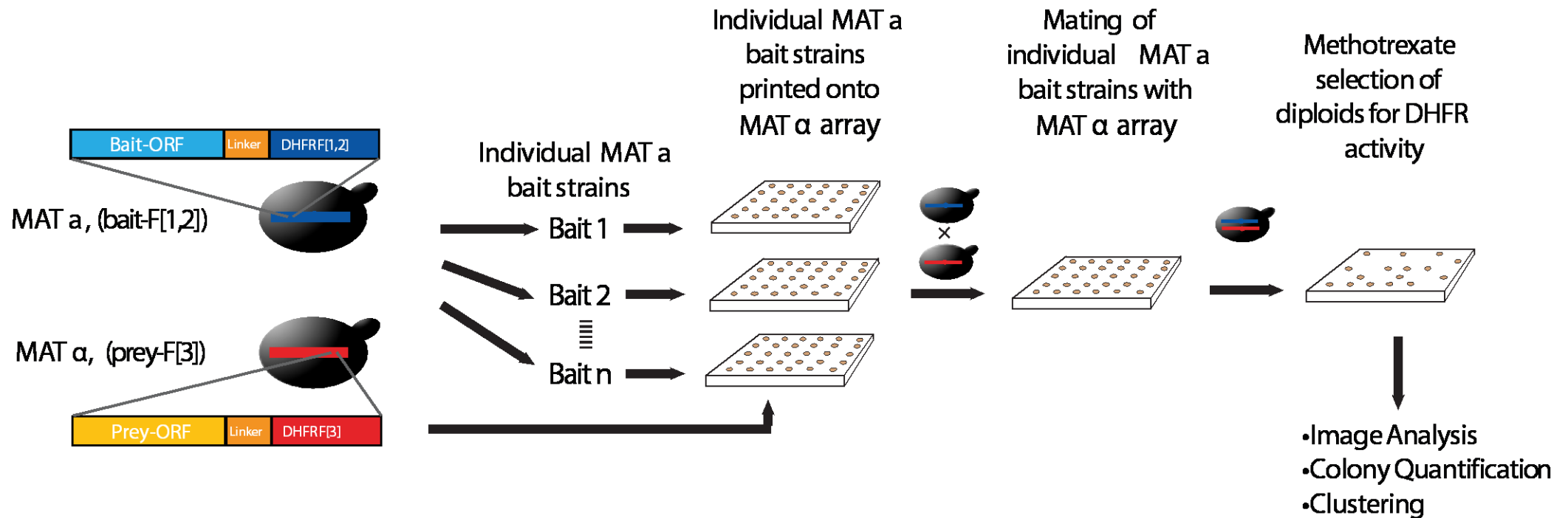
Quantitative Fitness Analysis

DLR00030513 Treatment: gal20 Medium: gal Plate: 9



Quantitative Fitness Analysis

Protein-fragment Complementation Assay (PCA)



Tarassov et al; Science 2008

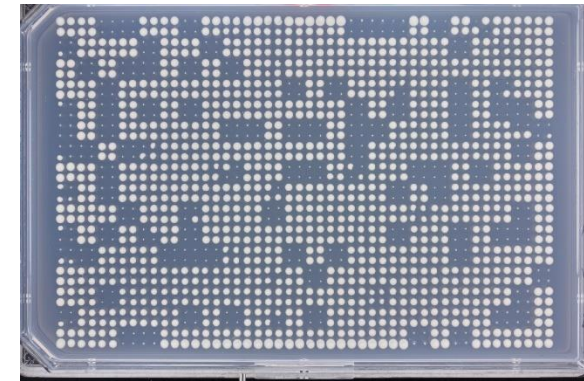
Protein-fragment Complementation Assay (PCA)

Genome wide protein interaction screening

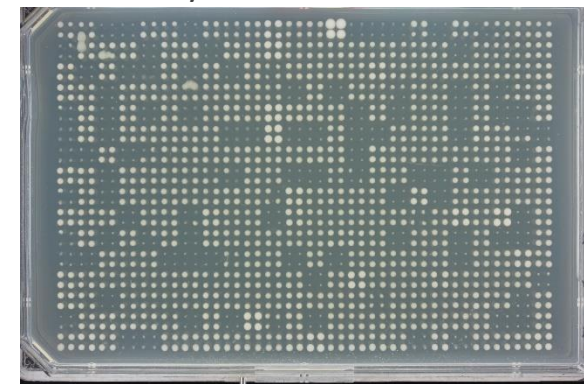
- Two libraries each ~5000 strains
- Run the experiment in both directions
- Two weeks per experiment
- Multiple experiments at once

Increase screen complexity

- Screen protein interactions in the presence/absence of drugs
- Protein interactions in combination with deletion mutations

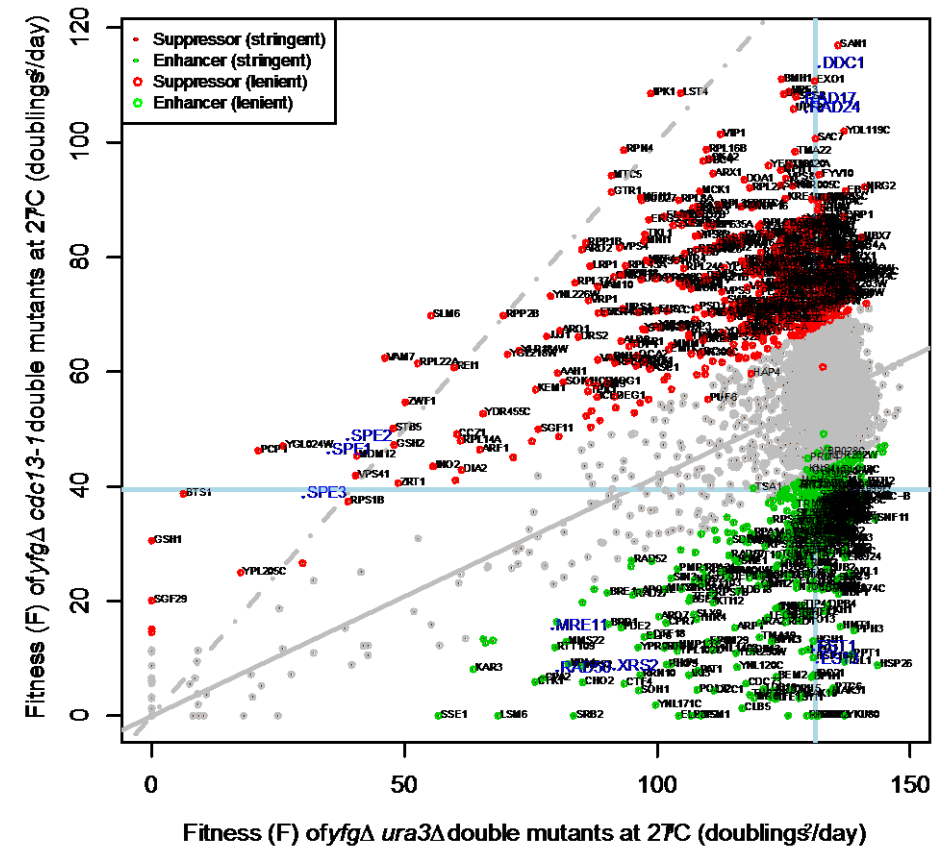
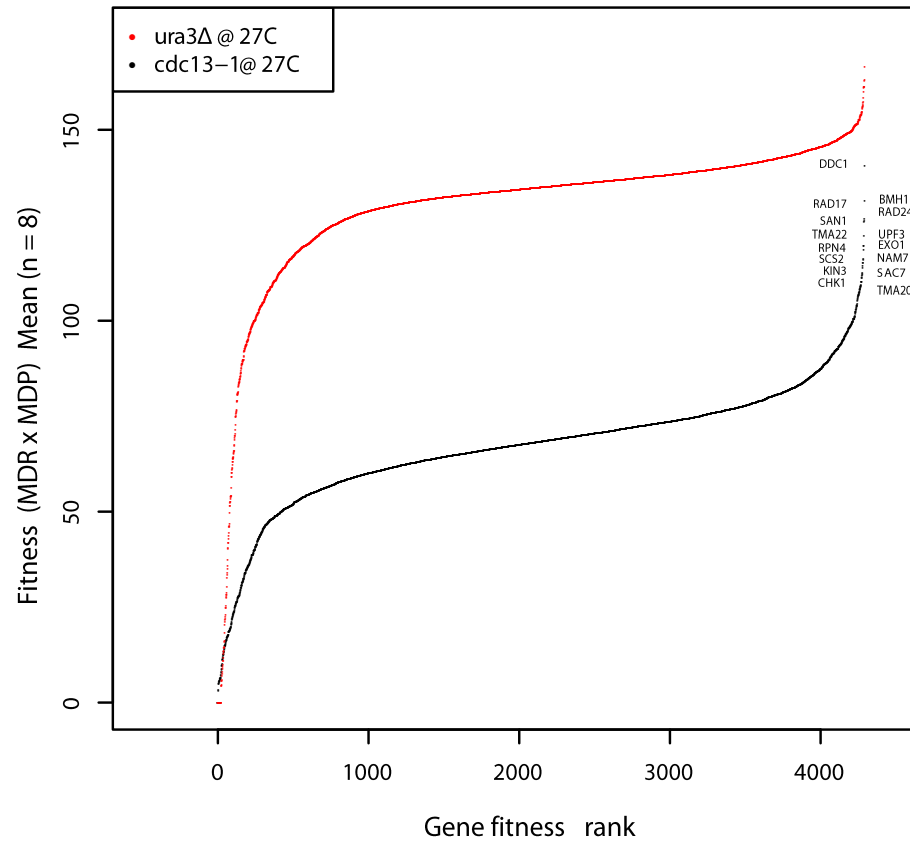


-Lysine +Methionine



-Lysine +Methionine +Methotrexate

Data Analysis



Random Colony Picking

Genome wide protein interaction screening

- Two libraries each ~5000 strains
- Run the experiment in both directions
- Two weeks per experiment
- Multiple experiments at once

Increase screen complexity

- Screen protein interactions in the presence/absence of drugs
- Protein interactions in combination with deletion mutations

MRC Technology Drug Library



MRCT Index library

- ~12,000 compounds selected as representatives of the full MRCT collection

MRCT kinase library

- ~6,700 compounds predicted to inhibit kinases

MRCT natural product library

- ~4,000 purified novel natural products from plants or fungi

FDA approved drugs library

- ~1,400 compounds

ABOUT US

MRC Technology is an independent life science medical research charity committed to drastically improving positive patient outcomes everywhere.

As a champion for human health, MRC Technology partners with academic, biotechnology, pharmaceutical, and charity organisations to move promising medical research forward into viable and accessible patient treatments.

Our people combine commercialisation and technology transfer/IP management skills with diagnostic and drug discovery expertise, specialising in small molecule and therapeutic antibodies. MRC Technology projects have led to the approved drugs Tysabri®, Actemra®, Entyvio® and Keytruda®, changing the lives of countless patients by harnessing the potential of science.

Newcastle University Single Cell Unit

Provide experimental scale

- Process samples in multiwell plates

Coordinate with Genomic facility

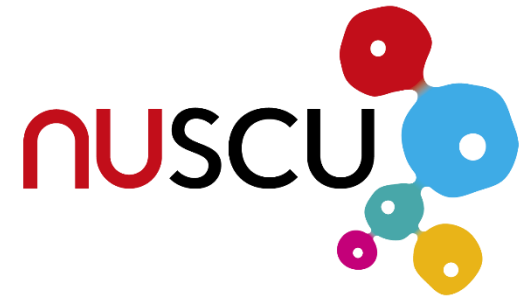
- Beckman Coulter robotics

Longstanding connection to Beckman technical staff

- Write procedures
- Repair robots
- Consult on the most up to date methods

Beckman DART software

- Sample tracking across robotic systems



Acknowledgements

Dave Lydall

Neil Perkins

Martin Cox

Conor Lawless

Darren Wilkinson

Peter.Banks@ncl.ac.uk

Adrian.Blackburn@ncl.ac.uk

HTSF Website

<http://research.ncl.ac.uk/bioHT/>

	Cost £/Hour Standard Robotic Systems	Cost £/Day Automated Imaging systems
With technical assistance	35.81	24.25
Without technical assistance	10.99	7.20