High Throughput Screening Facility: Update and Upgrades 2016

NORTH EAST FUNGAL FORUM

PETER BANKS
Introduction

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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 2\textsuperscript{nd} floor Leech and 2\textsuperscript{nd} floor Cookson
Uses metal 96, 384, 1536 and cherry pick pin tools – \textit{low consumable costs}
Replicates microbial colonies from agar to agar – \textit{Synthetic Genetic Array}
Inoculate microbial colonies from agar into liquid – \textit{Quantitative Fitness Analysis}
Pin microbial colonies from liquid onto solid – \textit{Thawing frozen stocks}
High capacity – \textit{180-240 plates in one run}
Slow but robust and easy to use
Singer ROTOR Colony Pinning Robot

Housed on 2\textsuperscript{nd} 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 – 2\textsuperscript{nd} floor Leech and 2\textsuperscript{nd} 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
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

![Graph showing normalised cell density over time for different strains](image)

- **Linear scale**
  - Normalised cell density (AU)
  - Time since inoculation (h)

- **Log scale**
  - Normalised cell density (AU)
  - Time since inoculation (h)

**Strains:**
- his3Δ
- htz1Δ
Quantitative Fitness Analysis
Quantitative Fitness Analysis
Protein-fragment Complementation Assay (PCA)

Individual MAT a bait strains printed onto MAT α array

Mating of individual MAT a bait strains with MAT α array

Methotrexate selection of diploids for DHFR activity

• Image Analysis
• Colony Quantification
• Clustering

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
Data Analysis
Random Colony Picking

Genome wide protein interaction screening
- Two libraries each ~5000 strains
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- 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

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**HTSF Website**  
[http://research.ncl.ac.uk/bioHT/](http://research.ncl.ac.uk/bioHT/)

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<tr>
<th></th>
<th>Cost £/Hour</th>
<th>Cost £/Day</th>
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<td><strong>Standard Robotic Systems</strong></td>
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<tr>
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