Newcastle University

Faculty of Medical Sciences

Scientific Facilities

High Throughput Screening Facility

RESEARCH FACILITIES AND YOU 2017

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High Throughput Screening

The HTSF combines robotics, high throughput reagents and expertise to provide a bespoke cell based screening service to all researchers in FMS

- Laboratory Automation
- Biological screening services for a range of organisms
- Drug screening in collaboration with LifeArc

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Equipment

2 sites – 2nd floor Cookson and 2nd floor Leech

Liquid handling mammalian cells

- Beckman FX within a sterile enclosure – Class II
- Beckman FX – Class I

Colony Pinning microbes

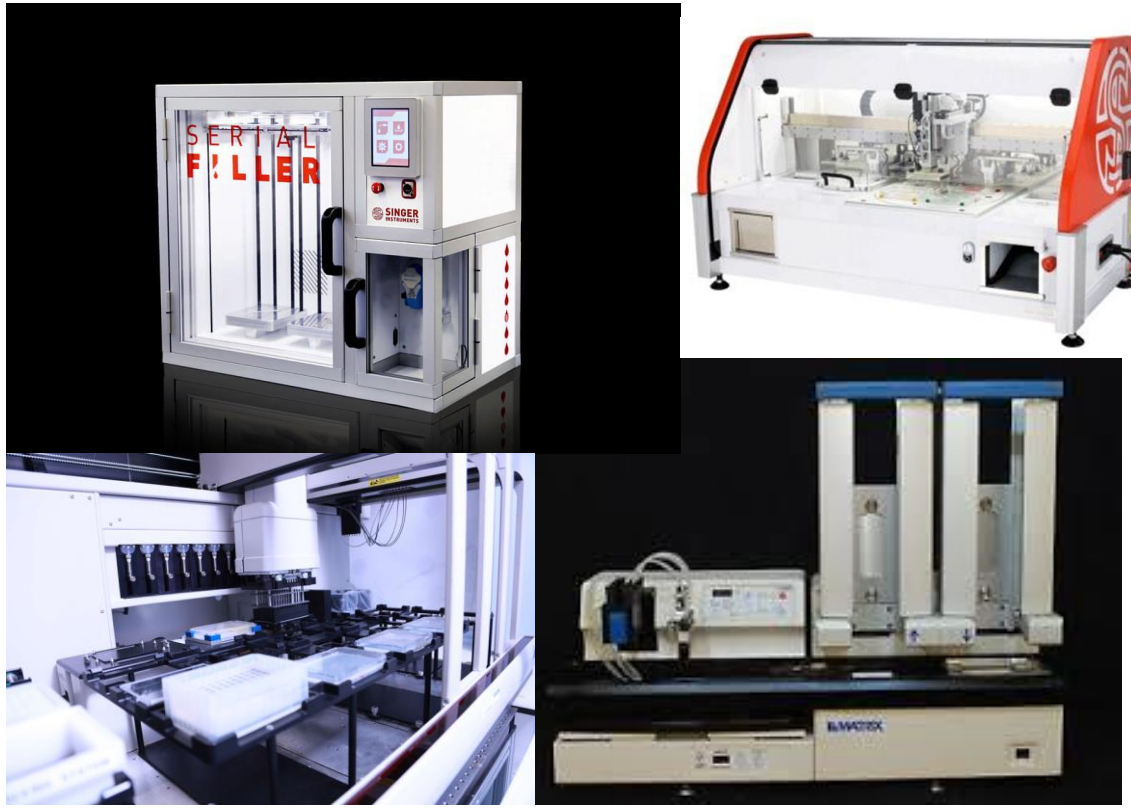
- S&P BM3
- S&P BM5
- Singer ROTOR

Data collection

- Agar plate imaging systems
- Automated plate readers
- High Content Imaging system – Bioimaging
- High Throughput Flow Cytometer – FACS

Ancillary

- Agar plate pouring
- Multi well plate filling



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Laboratory Automation

We can automate repetitive processes

Genome wide deletion collections of microorganisms (384 well plates)

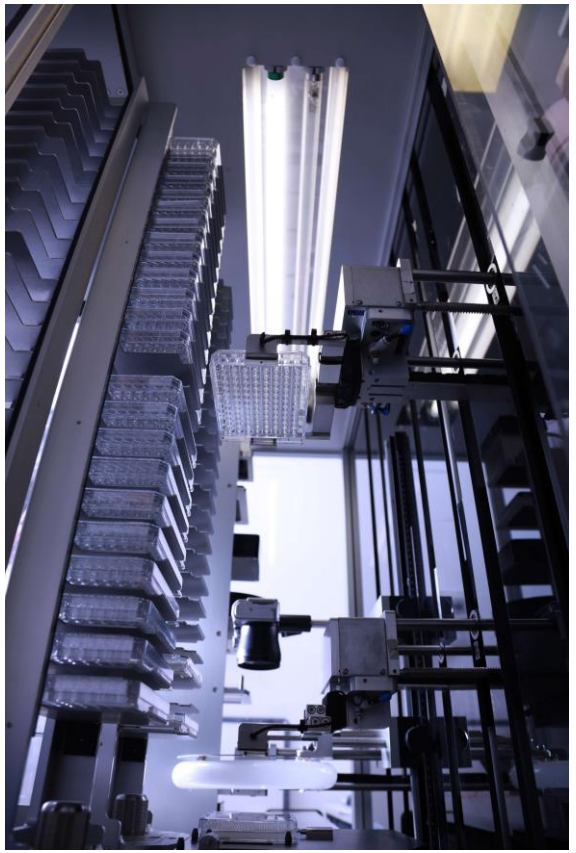
- *Saccharomyces cerevisiae* – 18 plates
- *Schizzosaccharomyces pombe* – 11 plates
- *Bacillus subtilis* – 11 plates

Reviving frozen glycerol stocks

- On solid agar – 1 hour
- Into liquid cultures – 2 hours

Adding glycerol to culture and freezing

- 2 hours



Biological Screening

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Cell based biological screening is the combination of laboratory automation with high throughput biological reagents.

- Identify new research directions
- Preliminary data generation for grant applications

High throughput reagents are specifically designed for automation

- Excellent coverage for large scale experiments
- Post screen validation is critical to success

Yeast collections

- Deletion library ~4500 strains
- Overexpression library ~6000 strains
- DaMP Library ~800 strains
- Histone point mutant library ~ 400 strains
- Temperature sensitive collections

Bacterial collections

- *Bacillus subtilis*
- *Escherichia coli*

Dharmacon Human siRNA library

Dharmacon siRNA Library

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Dharmacon siGENOME SMARTpool siRNA library

- siGENOME – type of siRNA
- 4 siRNAs to a single gene in one well – SMARTpool
- ~7500 genes over 28 plates
- 1nmol in four separate 384 well plates
- Screen 400-2000 plates
- Supply the gene list
- Add individual controls to experiments

Cherry pick individual 384 or 96 well plates

- G Protein Coupled Receptors
- Protein Kinases -3 plates
- Ion Channels
- Phosphatases – 1 plate
- Proteases
- Ubiquitin Conjugation 1 - Cullins, E1, E2, HECT E3 Ligases
- Ubiquitin Conjugation 2 - F-box, SOCS box E3 Ligases
- Ubiquitin Conjugation 3 - RING finger and RING finger-like E3 Ligases
- Drug Targets

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Drug Screening

High throughput screening of drug libraries has been a staple of high throughput screening for pharmaceutical companies for many years

- Identify novel therapeutic agents for specific conditions
- Screen 10^5 to 10^6 compounds
- Pharmaceutical companies have an extensive list of pre-screen requirements

The HTSF has a collaboration with the life science charity LifeArc to provide FMS researchers with drug screening

- LifeArc links basic research to industrial collaborators
- Expertise – medicinal chemistry, IP and screening
- Provided a 20,000 compound library to screen here

MRC/AZ Centre for Lead Discovery

Tolerability to DMSO (0-10%)

Signal to Background ratio >3 (but dependant on variability)

%CV for max activity/DMSO vehicle plates <10%

Robust Z' ≥ 0.5

Target XC50 pharmacology of non-specific compound

Data from a minimum of 10 independent, 384well plate runs.

Small scale Protein production and evidence that sufficient quantities for a full screen can be generated (Use of an external supplier is acceptable)

Stability data on reagents

Data on assay drift and lack of influence of well location

Secondary assays that can confirm any hits from the primary assay in a more physiological system

Selectivity assays – these will be specific to the target, but should focus on related targets that would be detrimental to the therapeutic index of your primary target. Assays should not be generic.

LifeArc Drug Library

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LifeArc drug libraries – 60 plates

Index library
- ~12,000 compounds representative of full MRCT collection

kinase library
- ~6,700 compounds predicted to inhibit kinases

Natural product library
- ~1000 purified novel natural products from plants or fungi

FDA approved drugs library
- ~1,000 compounds

Free to access the libraries
- LifeArc need to approve the screen

Libraries supplied blind
- Results must be fed back to LifeArc to deconvolute

20ul of drug at 10mM in DMSO
- 50-200 plates

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Screening – Optimisation

Bench top assay is adapted to high throughout robotic assay

- Very often the most time consuming part – a single plate robotic assay

Many choices to make

- Plate density – 384 vs 96
- Cell number per well
- Reagent types – standard vs high throughput
- Reagent volume/concentrations

Repeat numbers – 4

Cost

- Free Thermo plate library
- Free trial samples
- Robotic costs under £100

THE BioImaging Unit

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Screen

1 library plate – 4 repeats

Day 1 – Plate cells out
- 50 ml of cells at your appropriate density
- 15 mins for four plates

Day 2 – Transfection/drug addition
- User supplies media and any reagents
- 1 hour for four plates

Day 3/4/5
- Change Media
- Luciferase assay 2 hours
- Antibody staining 5 hours

siRNA – £50

Robot Time – £150-£300

Robot Tips – £50

Transfection reagent – £60

Plates – £10

Total £300-£500 depending on your assay

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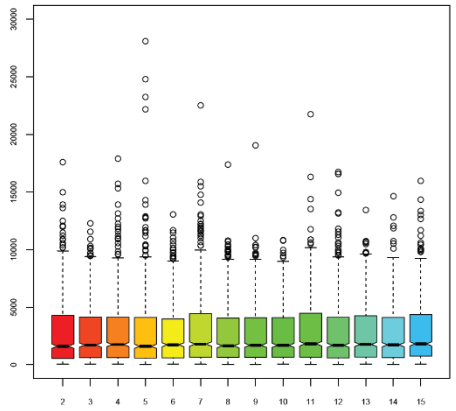
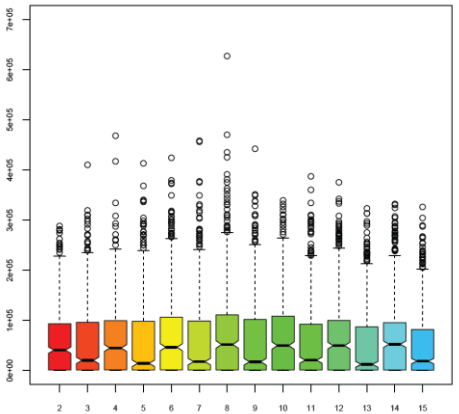
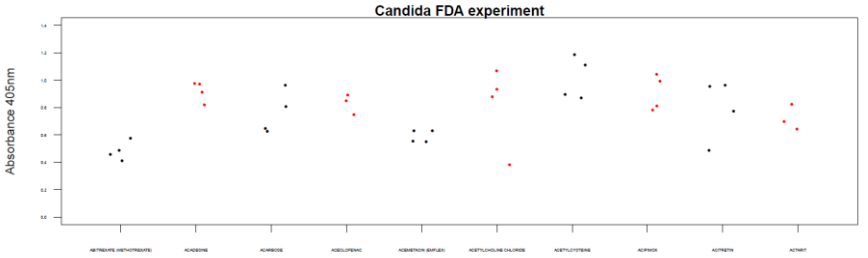
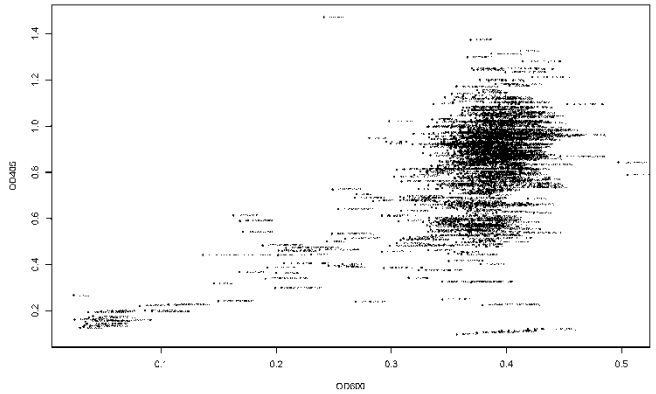
Data Analysis





R package version: 0.0-19						
Treatment: 37						
Medium: YE5S_GH						
Screen ID: QFA0089						
Screen name: pot1-1						
Libraries: pot1-1						
Client: SID						
User: SID						
PI: DAL						
Date: 2014/04/11						
Fitness definition: MDRMDP						
Condition: YE5S_GH						
##						
Gene	ORF	MedianFit	MeanFit	VarianceFit	NumRepeats	SEFit
SPAC1002.01	SPAC1002.01	45.67602071	45.55108874	136.5632446	4	5.843013876
pom34	SPAC1002.02	0	10.33812149	427.5070235	4	10.33812149
gls2	SPAC1002.03C	31.48131897	31.23084888	51.10339406	4	3.574331898
jmj2	SPAC1002.05C	39.17466695	36.10210929	180.6930891	4	6.721106478
bqt2	SPAC1002.06C	36.04907082	39.50708093	120.0299373	4	5.477908756
ats1	SPAC1002.07C	0	0	0	4	0
SPAC1002.12c	SPAC1002.12C	31.55007193	33.1592966	45.96544004	4	3.389890855
itt1	SPAC1002.14	26.91125138	24.27565593	114.9648816	4	5.361083883
urg2	SPAC1002.17C	18.81631727	19.47474406	4.967224907	4	1.114363597
urg3	SPAC1002.18	22.11026069	20.75929954	174.2771724	4	6.600703985
urg1	SPAC1002.19	46.23778821	45.37212432	3.520856264	4	0.938197243
SPAC1002.20	SPAC1002.20	35.8484572	33.2226362	54.75880496	4	3.699959627
psp3	SPAC1006.01	2.229726626	6.202746225	93.3917736	4	4.831970964
red1	SPAC1006.03C	0	0	0	4	0
mcp3	SPAC1006.04C	33.46108401	29.6629432	105.5012608	4	5.135690332
rgf2	SPAC1006.06	11.31532715	14.25079284	49.70679969	4	3.525152468
win1	SPAC1006.09	0	1.70233175	11.59173355	4	1.70233175
SPAC1039.02	SPAC1039.02	0	0	0	4	0
SPAC1039.03	SPAC1039.03	38.2425297	40.67746581	94.73005201	4	4.866468227
SPAC1039.04	SPAC1039.04	5.835202598	7.954314242	96.33651118	4	4.907558232
klf1	SPAC1039.05C	19.45426299	18.39563743	62.11796946	4	3.940747691
SPAC1039.06	SPAC1039.06	6.358457947	6.786677237	50.27796702	4	3.545347903
SPAC1039.08	SPAC1039.08	0	0	0	4	0





Data Analysis – Alternatives

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R courses

- Colin Gillespie
  http://www.jumpingrivers.com//

Bioinformatics Support Unit

- Simon Cockell
- http://bsu.ncl.ac.uk/support/

Acknowledgements

Dave Lydall

Neil Perkins

Jan Quinn

Martin Cox

Conor Lawless

Darren Wilkinson

Peter.Banks@ncl.ac.uk

HTSF Website

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