

High Throughput Screening Facility

ICAMB AWAY DAY 2016

PETER BANKS

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Outline

Introduction- **who, what and where**

Reagents

- **siRNA library**
- **MRCT drug library**

Experiments

- **siRNA Screening**
- **Drug Screening**

Data Analysis

Acknowledgments and Contact Details



Introduction

David Lydall David.Lydall@ncl.ac.uk

Peter Banks Peter.Banks@ncl.ac.uk

Adrian Blackburn Adrian.Blackburn@ncl.ac.uk

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

Colony Pinning

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

Liquid handling

- Beckman FX within a sterile enclosure – **Mammalian**
- Beckman FX - **Microbial**

Ancillary

- Agar plate imaging systems
- Agar plate pouring
- Multi well plate filling



Dharmacon siRNA Library

Dharmacon siGENOME SMARTpool siRNA library

- ~7500 genes over 28 plates
- 1nmol in four separate 384 well plates
- Screen 400-2000 plates
- siGENOME – **type of siRNA**
- 4 siRNAs to a single gene in one well – **SMARTpool**
- 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

MRCT Drug Library

MRCT drug libraries – **60 plates**

MRCT Index library

- ~12,000 compounds representative of 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

- ~1,000 compounds

Free to access the libraries

- MRCT need to approve the screen

Libraries supplied blind

- Results must be fed back to MRCT to deconvolute

20ul of 10mM in DMSO

- 50-200 plates

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



**Thermo Scientific™
PLATE LIBRARY**

Optimise your experiment
with in-house stocks of
FREE PLATE SAMPLES!

LOCATION AND CONTACTS:
Queen's Medical Research Institute
Room E2.26
Contact: Dahlia
D.Shenton@ed.ac.uk
Scottish Centre for
Regenerative Medicine
Room G.45
Contact: Eoghan
Eoghan.Odulhir@ed.ac.uk

Give feedback at
www.surveymonkey.com/r/8BX6PWG

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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**

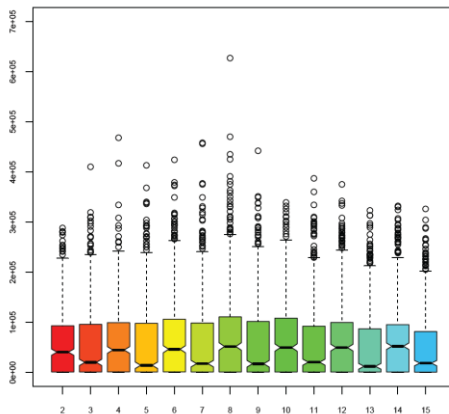
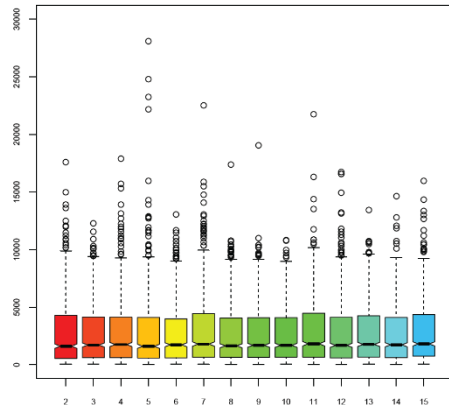
Plates – **£10**

Total **£300-£500** depending on your assay

Biolmaging Unit

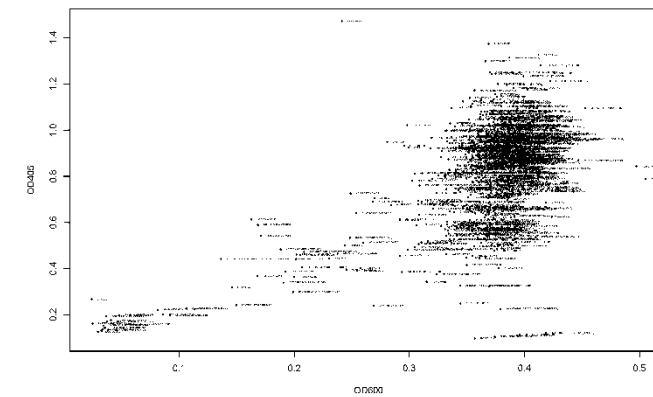
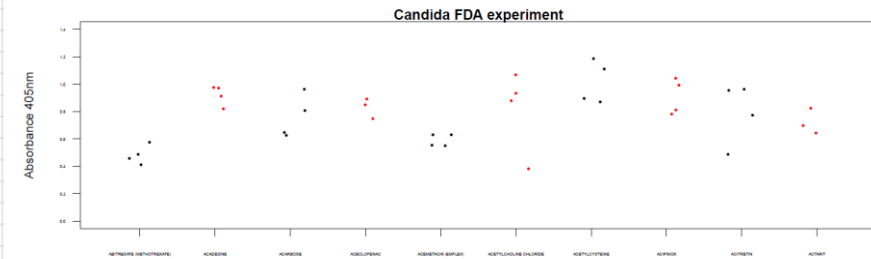
- **Alex Laude**
- <http://www.ncl.ac.uk/bioimaging/>

Data Analysis



R package version: 0.0-19
 Treatment: 37
 Medium: YE55_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: YE55_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

R courses

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

Bioinformatics Support Unit

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



Scientific Facilities Showcase

Thursday 17 November 2016, 9.00-12.00
Baddiley-Clark Seminar Room

go.ncl.ac.uk/showcase2016



BiImaging

High Throughput Screening

Electron Microscopy

Infectious Diseases

New Genomics

Bioinformatics

Pre Clinical In Vivo Imaging

Flow Cytometry

Protein Production & Analysis

Acknowledgements

Dave Lydall

Neil Perkins

Jan Quinn

Martin Cox

Conor Lawless

Darren Wilkinson

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HTSF Website

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