



# BioScreening Technology Group

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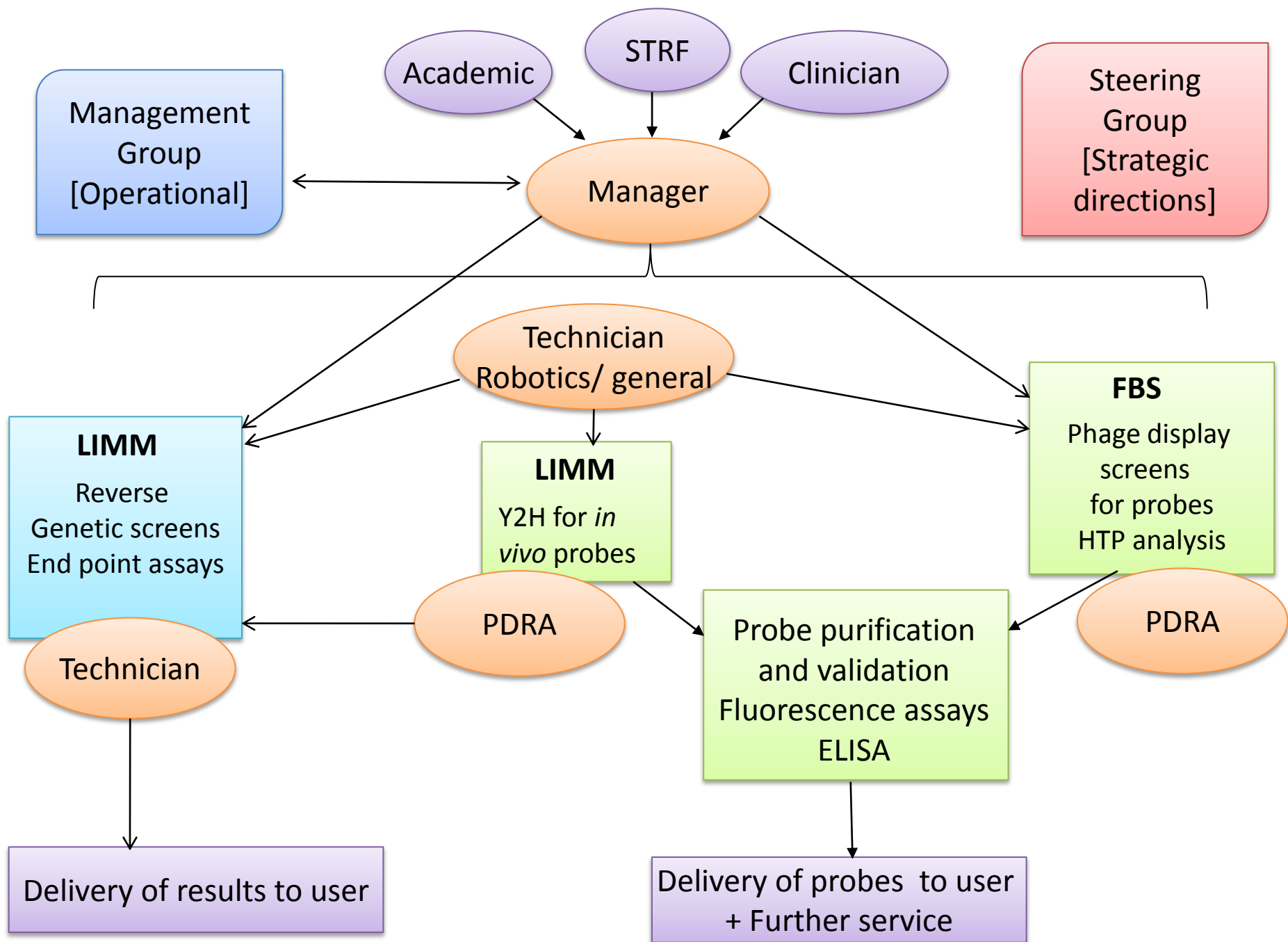
# What is **BSTG**?

New screening facility being established by the BHRC

To enable academic researchers and clinicians to obtain tools and information for translation of basic research to clinical application.

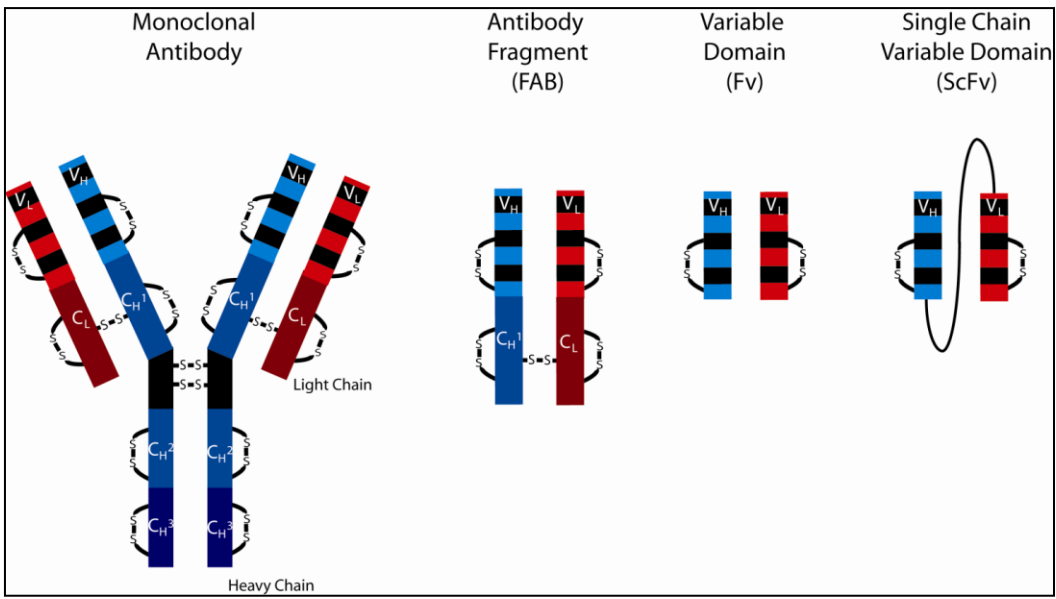
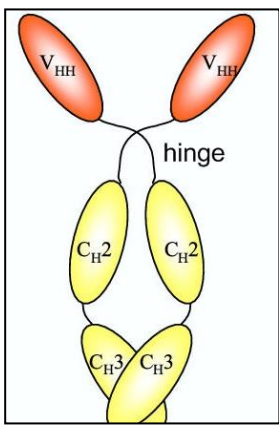
**We offer two complementary services:**

- **Probe Tools** will provide affinity reagents for molecules of clinical interest such as candidate drug targets or biomarkers for diagnostic, imaging and therapeutics
- **Reverse Genetics** will perform whole or partial-genome siRNA screens of mouse or human to identify novel genes important in cellular pathways and human disease.

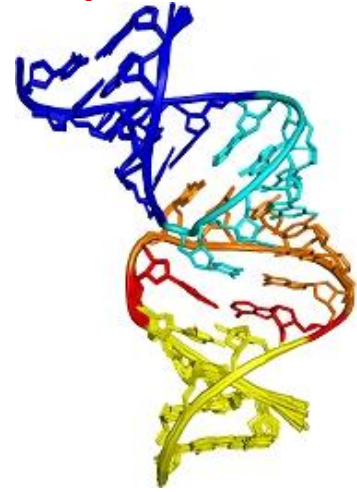


# Probe Tools:

## Binding Proteins



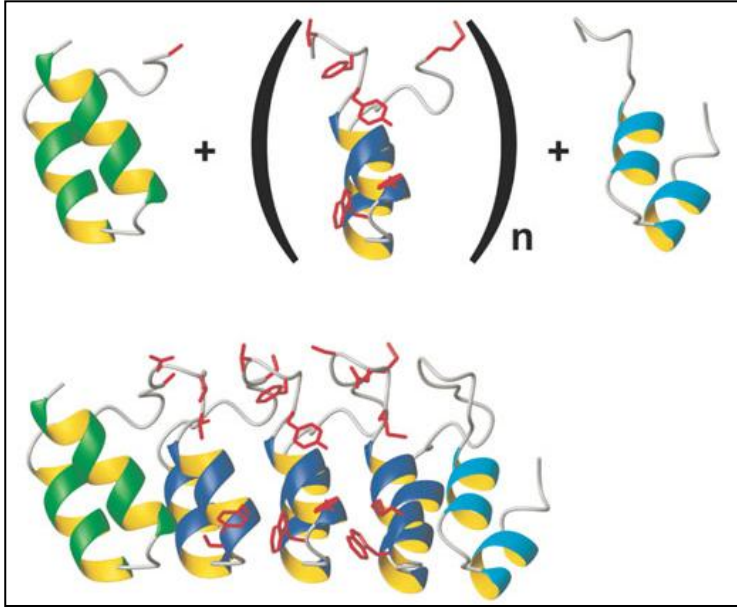
## RNA aptamers



<p><b>Loop randomization</b> (e.g. fibronectin)</p>	<p><b>Flat surface randomization</b> (e.g. protein Z)</p>	<p><b>Loop &amp; helix randomization</b> (e.g. ankyrin repeat protein)</p>	<p><b>Cavity randomization</b> (e.g. lipocalin)</p>
<p><b>Small, stable, highly expressed</b></p>			

# DARPin Consensus Repeat Library

• Derived from alignment of ~700 ankyrin domain



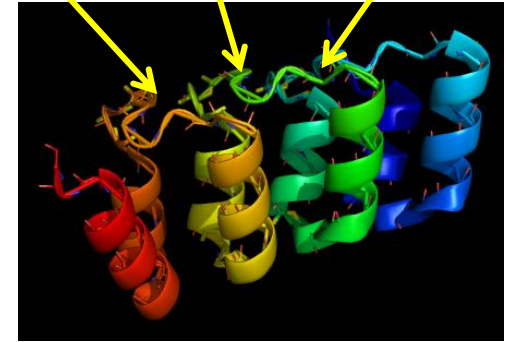
Leads library

Binding sites  
contains 5  
randomised  
sequences  
=  $1.5 \times 10^{19}$

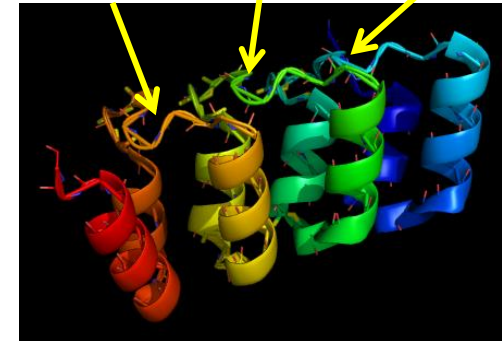
Library of  $10^{10}$   
built

Phage display  
selection

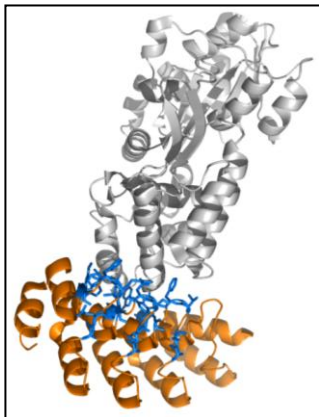
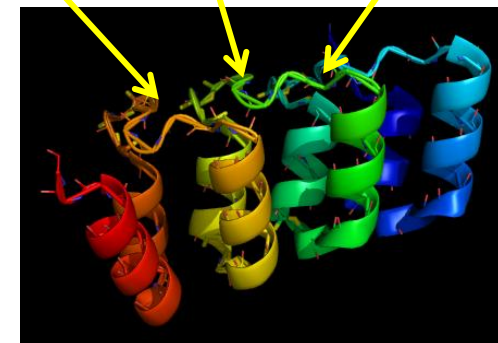
QRWEF VDVPE AKSDS



GAADY EFHNS IKGMG



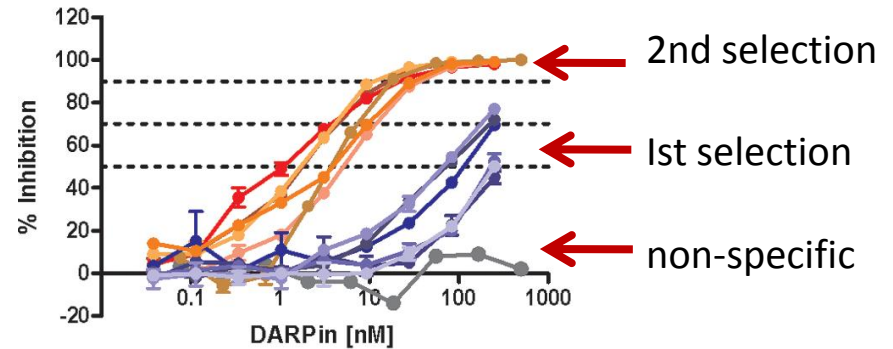
TDHGG LISEA RGFGI



# Applications of DARPins

## Inhibition of HIV cell entry - potential microbicides

Schweizer et al. 2008 PLOS PATHOGENS, 4 e1000109

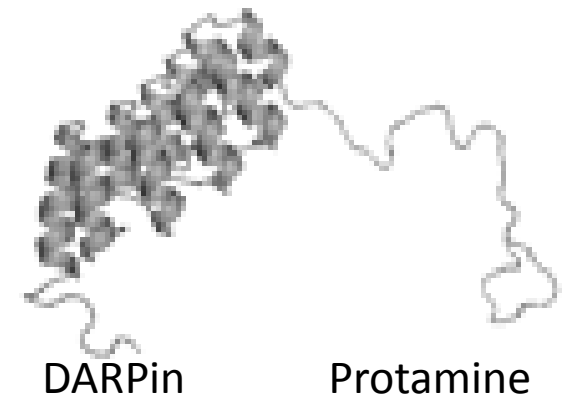


## Delivery of siRNA to tumour cells

DARPIn targeting to cell surface receptor

DARPIn fused to truncated protamine  
RSQSRSRYRQRQRSRRRRRRRSRS which  
binds the siRNA

Winkler J et al. Mol Cancer Ther 2009;8:2674-2683



# Applications of DARPins

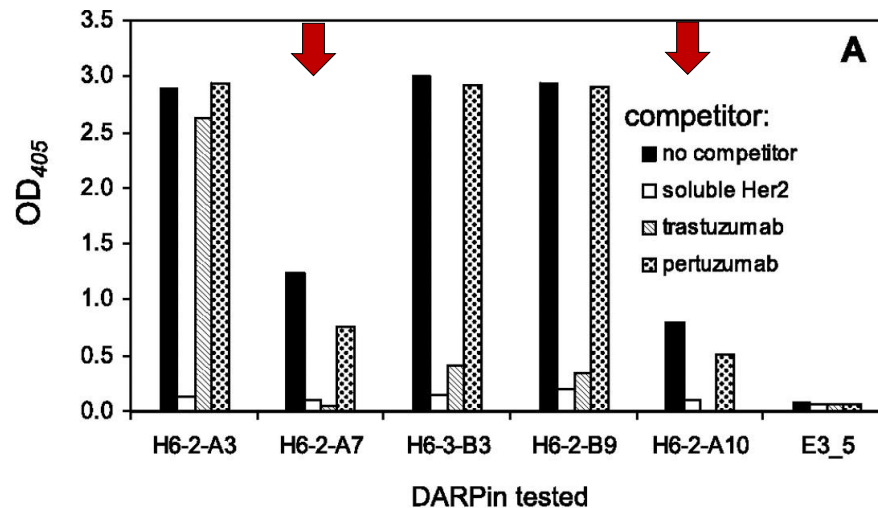
## Human epidermal growth factor receptor (Her2)

Her2-extracellular domain is target for Herceptin a humanized MAb

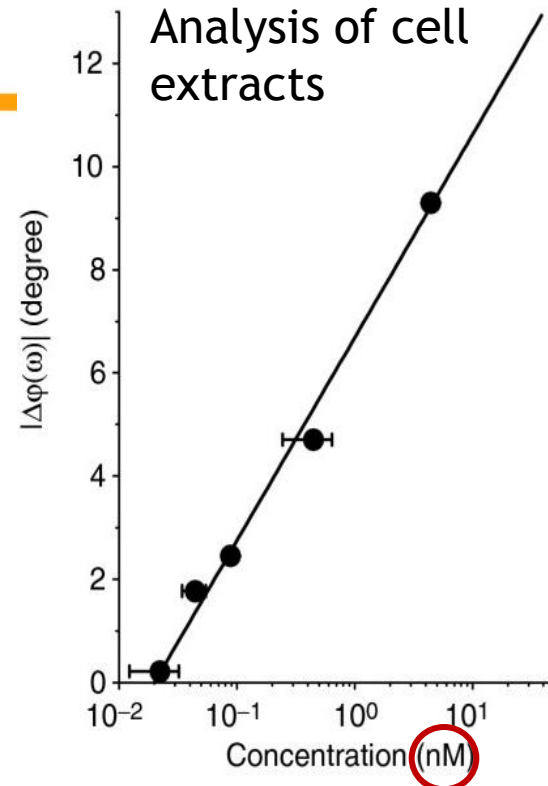
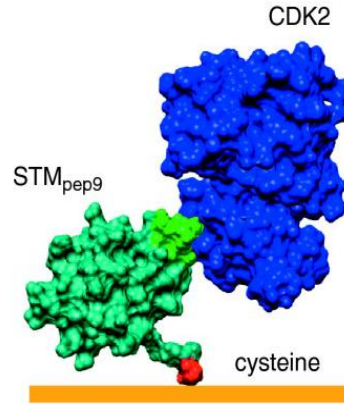
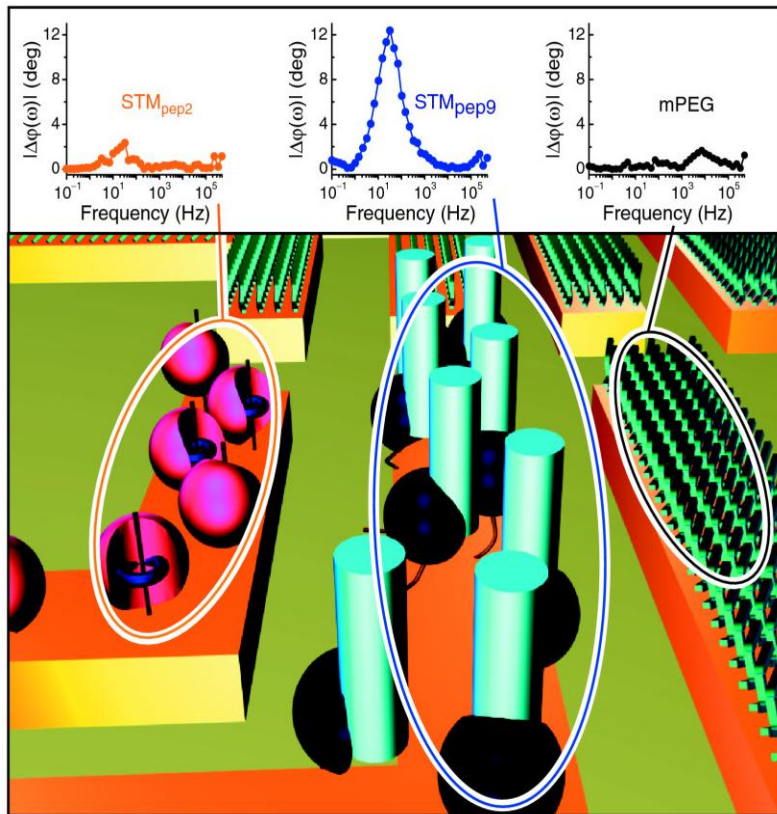
**Diagnosis:** Her2 over-expressed in 20-30% of breast cancer patients so detection is important

DARPins selected with nM binding efficacy and “affinity matured” by EP-PCR to pM

Some DARPins compete for same binding site as MABs



# Electronic label-free detection of binding



Single binding loop: increase with multiple loops

Challenge the array with complex biological mixture and detect specific binding

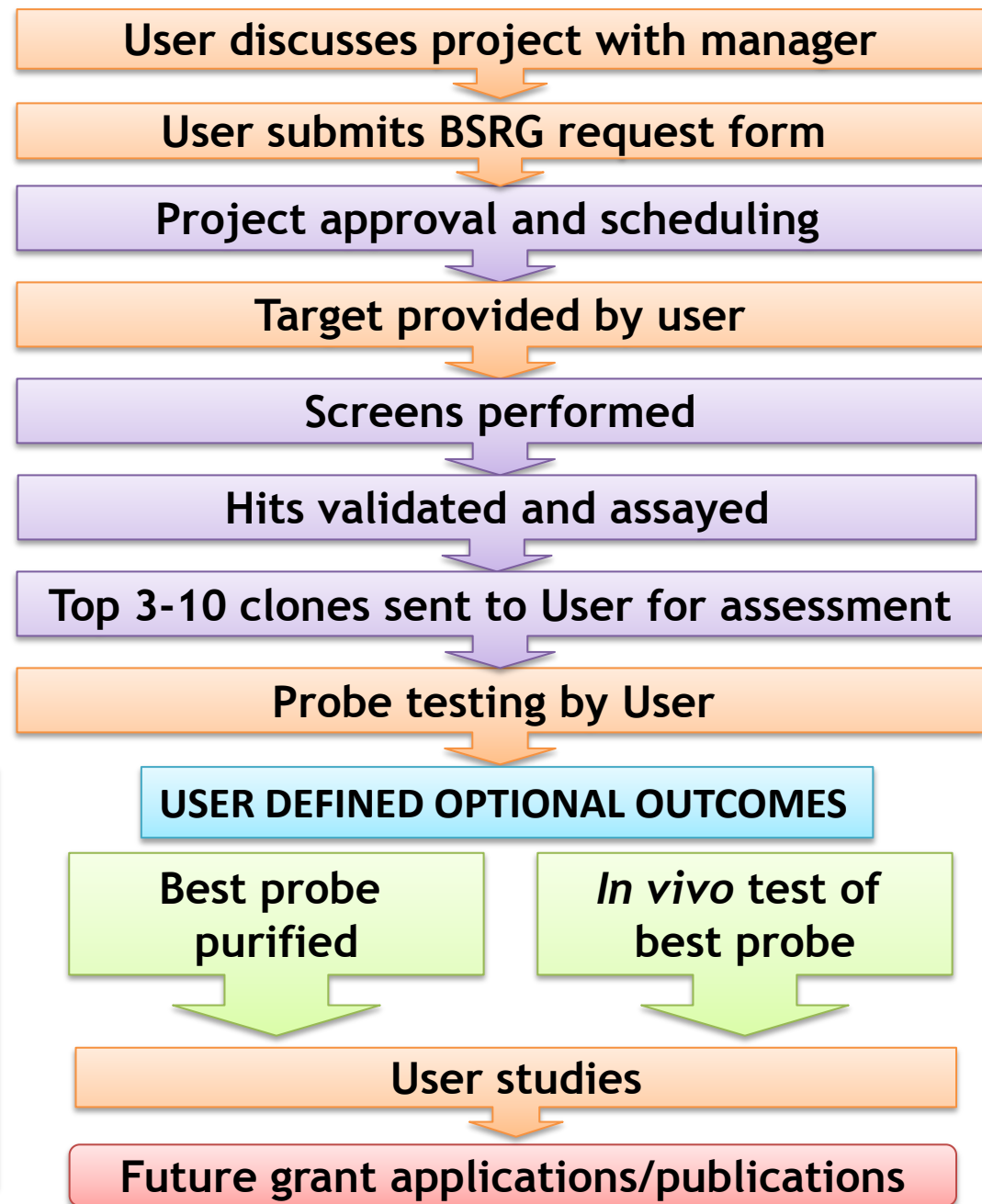
# Probe Tools Workflow

- non-antibody scaffolds (small , stable, highly expressed proteins)
- antibody fragments
- RNA SELEX

- Phage display
- Y2H

Other services available, including

- binding affinity by SPR,
- Large-scale purification of selected probe,
- testing probe *in vivo* in human cells (RG service)



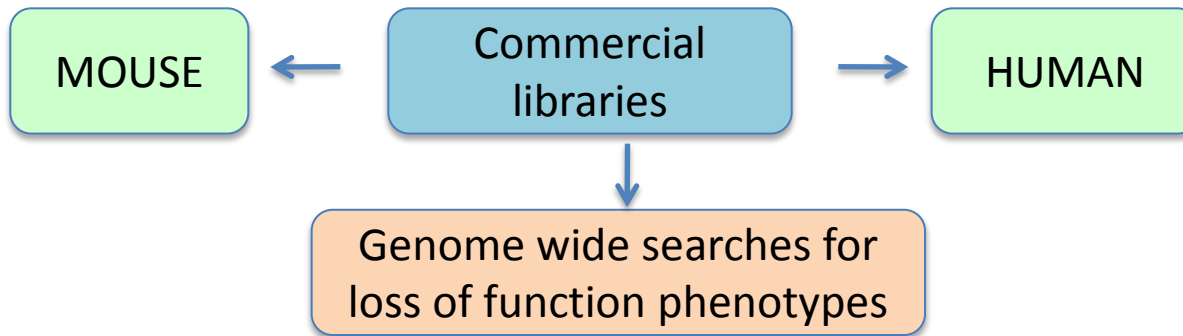
# Reverse Genetics Service (RG)

## Aim of the RG

- To enhance the reputation of the University of Leeds by providing a world-class **whole and partial genome siRNA screening service** to Leeds researchers
- Currently only a handful of UK Institutes offer an siRNA screening facility
- Established by Drs Jacquie Bond, Sandra Bell and Ewan Morrison, LIMM

# siRNA Technology

- RNA interference (RNAi)
  - basic cellular mechanism controlling gene expression,
  - induced *in vivo* by small interfering RNAs (siRNAs)
- *in vitro* siRNA-mediated knockdown of gene expression
  - powerful/widely used for loss of gene function studies in mammalian cells.



- Properly applied, genome wide siRNA knockdown approaches can lead to:
  - systems-level understanding of a process
  - comprehensive identification of the molecular components underlying a process

# Applications of siRNA whole and partial genome screens

Vol 464 | April 2010 | doi:10.1038/nature08869

nature

## ARTICLES

### Phenotypic profiling of the human genome by time-lapse microscopy reveals cell division genes

Beate Neumann<sup>1\*</sup>, Thomas Walter<sup>1\*</sup>, Jean-Karim Hériché<sup>2†</sup>, Jutta Bulkescher<sup>1</sup>, Holger Erfle<sup>1,3,†</sup>, Christian Conrad<sup>1,3</sup>, Phill Rogers<sup>1†</sup>, Ina Poser<sup>2</sup>, Michael Held<sup>1†</sup>, Urban Liebel<sup>1†</sup>, Cihan Cetin<sup>3</sup>, Frank Sieckmann<sup>8</sup>, Gregoire Pau<sup>9</sup>, Rolf Kabbe<sup>10</sup>, Annelie Wünsche<sup>2</sup>, Venkata Satagopam<sup>1</sup>, Michael H. A. Schmitz<sup>2</sup>, Catherine Chapuis<sup>3</sup>, Daniel W. Gerlich<sup>7</sup>, Reinhard Schneider<sup>4</sup>, Roland Eils<sup>10</sup>, Wolfgang Huber<sup>9</sup>, Jan-Michael Peters<sup>11</sup>, Anthony A. Hyman<sup>6</sup>, Richard Durbin<sup>5</sup>, Rainer Pepperkok<sup>2</sup> & Jan Ellenberg<sup>2</sup>

#### Integrated Systems and Technologies

### Genome-Wide siRNA Screen for Modulators of Cell Death Induced by Proteasome Inhibitor Bortezomib

Siquan Chen<sup>1</sup>, Jonathan L. Blank<sup>2</sup>, Theodore Peters<sup>1</sup>, Xiaozhen J. Liu<sup>2</sup>, David M. Rappoli<sup>1</sup>, Michael D. Pickard<sup>3</sup>, Saurabh Menon<sup>1</sup>, Jie Yu<sup>2</sup>, Denise L. Driscoll<sup>2</sup>, Trupti Lingaraj<sup>2</sup>, Anne L. Burkhardt<sup>2</sup>, Wei Chen<sup>2</sup>, Khristofer Garcia<sup>1</sup>, Darshan S. Sappal<sup>2</sup>, Jesse Gray<sup>1</sup>, Paul Hales<sup>1</sup>, Patrick J. Leroy<sup>2</sup>, John Ringeling<sup>1</sup>, Claudia Rabinov<sup>2</sup>, James J. Spelman<sup>2</sup>, Jay P. Morgenstern<sup>1</sup>, and Eric S. Lightcap<sup>2</sup>

Cancer Research

**BHRC** BIOMEDICAL HEALTH RESEARCH CENTRE

Promoting Excellence in Translational Research across Leeds

nature

Vol 464 | 15 April 2010 | doi:10.1038/nature08895

## LETTERS

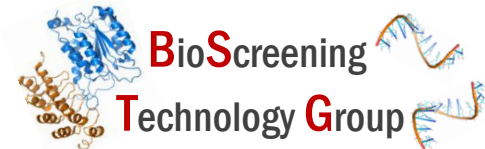
### Functional genomic screen for modulators of ciliogenesis and cilium length

Joon Kim<sup>1</sup>, Ji Eun Lee<sup>1</sup>, Susanne Heynen-Genel<sup>2</sup>, Eigo Suyama<sup>2</sup>, Keiichiro Ono<sup>3</sup>, KiYoung Lee<sup>3,4</sup>, Trey Ideker<sup>3</sup>, Pedro Aza-Blanc<sup>2</sup> & Joseph G. Gleeson<sup>1</sup>

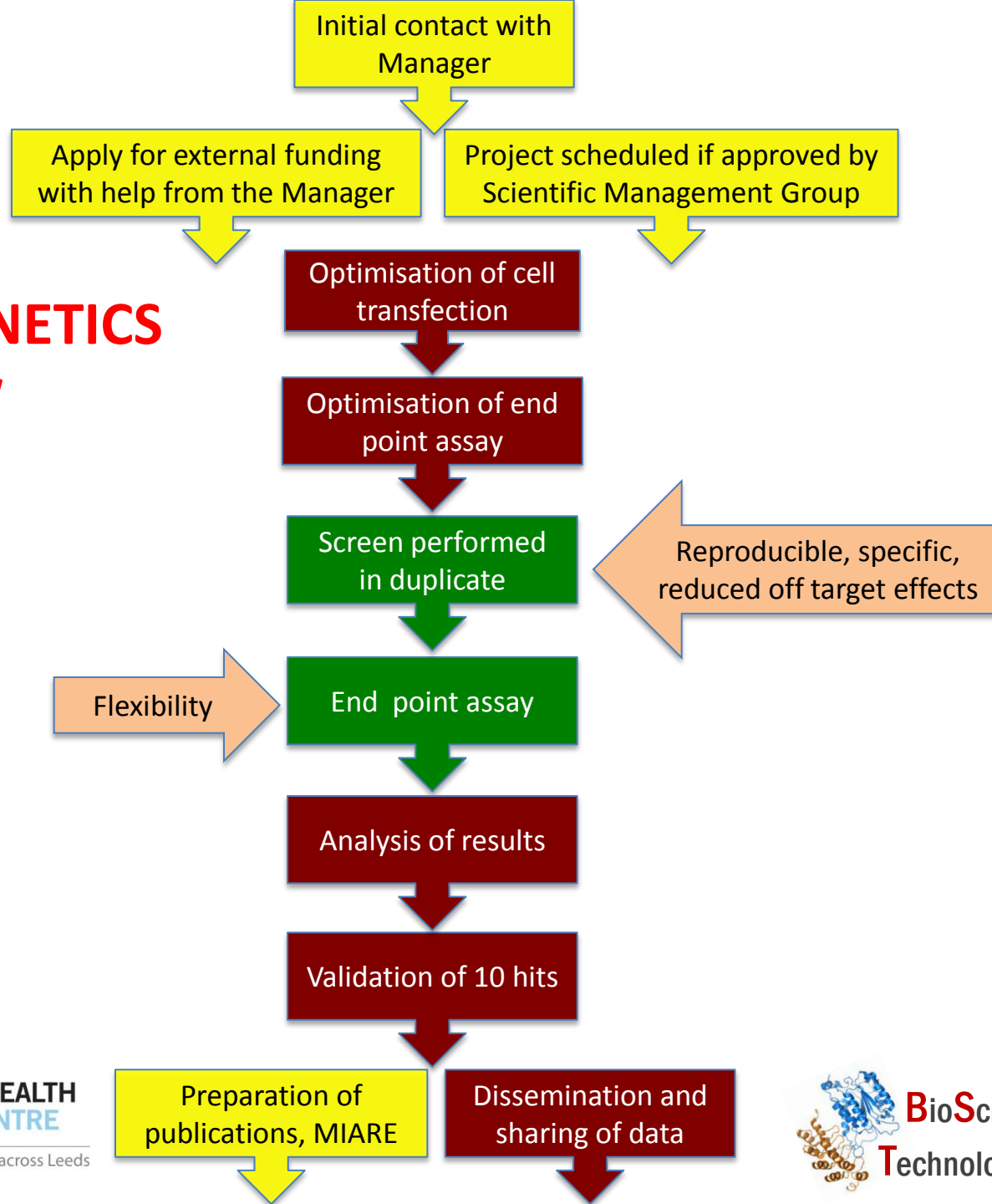
### Small interfering RNA library screen of human kinases and phosphatases identifies polo-like kinase 1 as a promising new target for the treatment of pediatric rhabdomyosarcomas

Kaiji Hu,<sup>1</sup> Cathy Lee,<sup>1</sup> Dexin Qiu,<sup>2</sup> Abbas Fotovati,<sup>1</sup> Alastair Davies,<sup>1</sup> Samah Abu-Ali,<sup>1</sup> Daniel Wai,<sup>3</sup> Elizabeth R. Lawlor,<sup>3</sup> Timothy J. Triche,<sup>3</sup> Catherine J. Pallen,<sup>2</sup> and Sandra E. Dunn<sup>1</sup>

B1. Increased expression of WEE 1 was also noted. The induction of apoptosis after PLK1 silencing was confirmed by increased p-H2AX, propidium iodide uptake, and chromatin condensation, as well as caspase-3 and poly(ADP-ribose) polymerase cleavage. Pediatric Ewing's sarcoma



# REVERSE GENETICS WORK FLOW



# Image Database

- Funding bodies require funded data to be freely available
- Encourage data sharing amongst Leeds researchers and clinicians
- Reanalyse screens to answer different research question
- Ultimately aim to make the datasets from completed screens
  - freely available to Leeds Researchers as part of a searchable online database
  - Data release negotiable.

# Leeds University CELL PHEnotype Resource

“LUCEPHER”

# Costs

## Funding- **Costs for service based on Research Council TRAC system** External

- Obtained in advance
- Project grants or pump-priming grants
- Grant writing input provided by service
- BSTG staff as named co-applicant

## BHRC subsidised

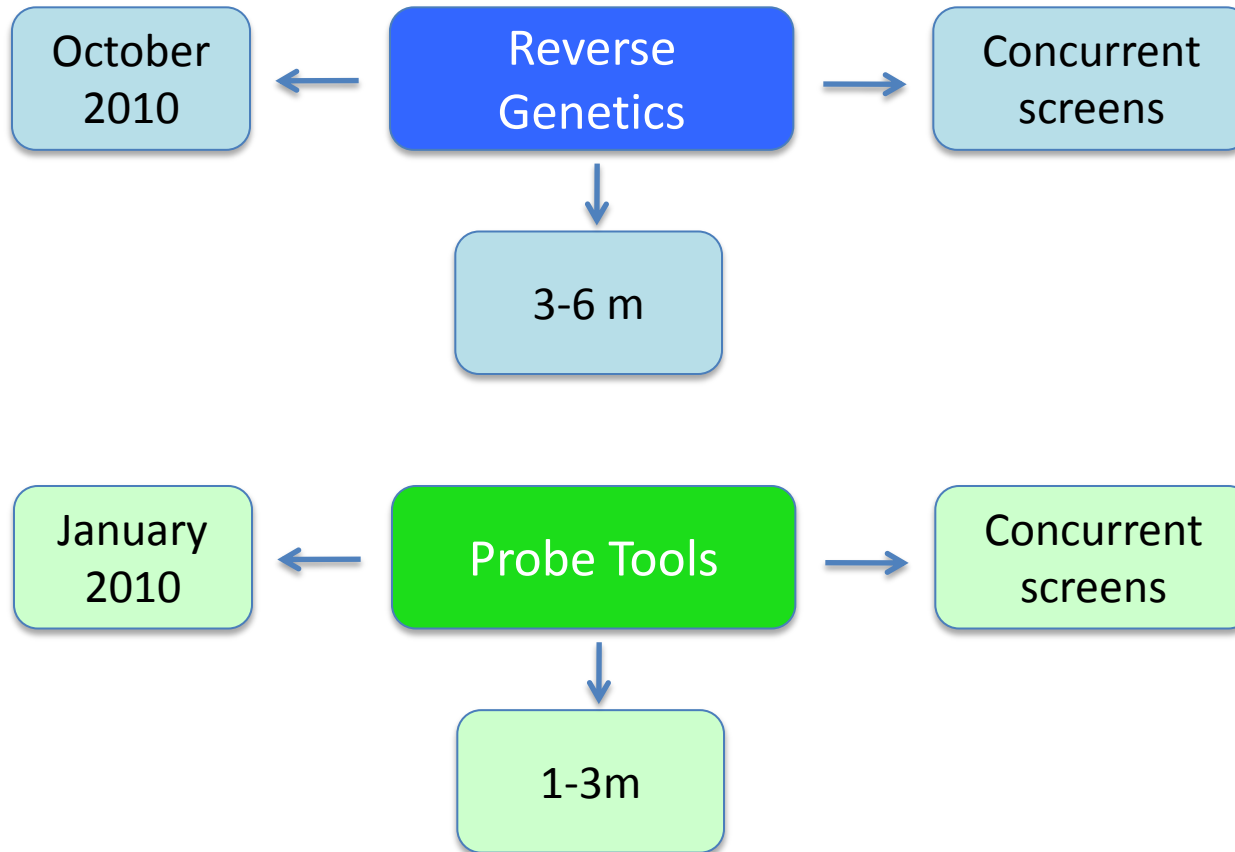
- STRF 100%
- Other Leeds researchers 90%
- External funding would be required for subsequent follow up studies

## Subsequent screens externally funded

## Publications

- Service not for profit thus BSTG staff as co-authors

# Time scales



# Summary

- **BioScreening Technology Group**  
<http://www.bhrc.ac.uk/initiatives/technology-groups/bioscreening-technology-group/> offers two services
  - Bio marker tools
    - Develop probes for biomarkers/drug targets
    - FBS & LIMM- Mike McPherson, Steve Baldwin, Paul KoFerrigno
  - Reverse Genetics
    - Whole and partial genome siRNA screening
    - LIMM- Drs Bond, Bell and Morrison
- BSTG has strong synergies with the MCCB and Bioinformatics Technology Groups