NICB-Pfizer Collaboration: Improving Production of Biopharmaceuticals using Industrially-relevant CHO Cell Lines

Padraig Doolan
National Institute for Cellular Biotechnology (NICB)
NICB/Dublin City University

- National Centre of Expertise in Basic & Applied Molecular & Cell Biotechnology since 1987

- State of the art institute, dedicated to cell and tissue culture research

- New 3,200m² building (opened 2006)
Technology Platforms available at NICB

1. Cell Culture Suites
2. Transcriptomics (microarray)
3. Proteomics
4. miRNA screening
5. Bioinformatics
6. Cell Characterisation Suite
7. Functional Genomics
NICB-Pfizer Collaboration

- NICB/DCU’s ongoing collaboration with Pfizer (previously Wyeth Biopharma)
- Initially funded (€3.9M) in 2004 and successfully re-funded (€2.5M) for four additional years in 2008
- Bio-Manufacturing Sciences Group, Pfizer Inc., (Grange Castle, Clondalkin, Dublin) and Bioprocess R&D, Pfizer Inc. (Andover, MA, USA)

“Molecular Analysis and engineering of CHO cells for more efficient production of biopharmaceuticals”
Growth of Biologic Sales (US)

Figure 1 Total sales and sales growth trend in the US biotech market for biologic drugs (2003–2008).

NICB-Pfizer/Dublin City University Collaboration: Potential Project Outcomes

- **Project Scope:** Gain a deeper understanding of CHO cell biology
  - Integrated use of Microarray & Proteomics tools to interrogate selected CHO cell samples
  - Identification of gene & protein targets influencing phenotypes of interest

- **Project Outcome:** Move the CHO manufacturing platform to a new level (1-3g MAbs /L $\rightarrow$ >10g /L)
  - Potential targets for rational cell engineering strategies
  - Identify Biomarkers for cell line screening
  - Improved utilization of existing manufacturing capacity
  - Media or Process improvements
Limit Capital Expenditure by Innovation

Pfizer Bio-Manufacturing Sciences Group
(Grange Castle, Dublin)

Pfizer Bioprocess R&D
(Andover, MA, USA)

![Diagram of productivity over culture day for Process 1 and Process 2]
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7. Functional Genomics
1. Cell Culture Facilities

- Largest collection of clean rooms (built to Class C / class 10,000 standards) in any research facility in Ireland
Sample Matrix

Focus on Fed Batch Culture
- >500 biological samples examined
- >350 scans
- Largest collection of CHO transcriptomic data

<table>
<thead>
<tr>
<th>High Cell Density</th>
<th>Sustained High Cell Viability</th>
<th>High Max Qp</th>
<th>Sustained High Qp</th>
<th>Low Ammonia Producer</th>
<th>Low Lactate Producer</th>
<th>High Cell Growth Rate</th>
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<tbody>
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<td>Test</td>
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<td>MAb 3</td>
<td>Protein 1</td>
<td>Protein 1</td>
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<td>Fc-Fusion 1</td>
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13:15-14:45 03-06-2010 SFI/MMI Technology Platform Workshop
Four CHO MAb-secreting cell lines

Two cell lines with slow growth rates ("Slow") each paired with a separate cell line with a faster growth rate ("Fast")
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6. Cell Characterisation Suite

7. Functional Genomics
2. Transcriptomics (Microarray)

- Affymetrix Genechip system

- First Generation CHO Chip (WyeHamster2a)
  - 2835 library-derived CHO sequences
  - 732 public hamster sequences
  - 125 array quality control sequences
  - 22 product/process specific sequences
  - Total: 3,714 sequences
  - ~10-15% of CHO genome

- Oligo-Based microarray (Affymetrix)
  - 55 probesets per transcript
  - 18 μm feature size
### 118 DE Genes

#### Table 1

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<th>Gene Symbol (Upregulated)</th>
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<td>DDX36</td>
<td>DEAH (Asp-Glu-Ala-His) box polypeptide 36</td>
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<td>HNRNPC</td>
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<tr>
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<td>Heat shock 27kDa protein 1</td>
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<tr>
<td>IDE</td>
<td>Insulin-degrading enzyme</td>
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<td>MGAT5</td>
<td>Mannosyl (alpha-1,6-)glycoprotein beta,1,6-N-acetylglucosaminyltransferase</td>
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<td>MMP14</td>
<td>Matrix metalloproteinase 14 (membrane-inserted)</td>
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<td>RPS15</td>
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<td>Tmem16x</td>
<td>Thymosin, beta 4, X chromosome</td>
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<td>Amyloid beta (A4) precursor protein (peptidase A4 disease)</td>
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<td>CCS</td>
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<td>CORO1B</td>
<td>Coronin, actin binding protein, 1B</td>
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</table>
Technology Platforms available at NICB

1. Cell Culture Suites

2. Transcriptomics (microarray)

3. Proteomics

4. miRNA screening

5. Bioinformatics

6. Cell Characterisation Suite

7. Functional Genomics
3. Parallel Proteomics screening project

2-D DIGE
(Difference Gel Electrophoresis)

MALDI ToF-ToF 4800

LTQ ion trap

Ettan MALDI-TOF

Orbitrap XL

National Institute for Cellular Biotechnology
### 3. Parallel Proteomics screening project

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<tr>
<th>High Cell Density</th>
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<td>glycerol-3-phosphate dehydrogenase</td>
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<td>Copein 1</td>
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<td>gi27762594</td>
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<td>gi25742757</td>
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</table>

58 DE Proteins
Technology Platforms available at NICB

1. Cell Culture Suites

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3. Proteomics

4. miRNA screening

5. Bioinformatics

6. Cell Characterisation Suite

7. Functional Genomics
5. Bioinformatics@NICB

- Developed initially as essential component of microarray facility, but since extended into all research strands at NICB
- 3 full time bioinformaticians in dedicated laboratory
- Analysis of microarray/proteomic/miRNA data using combination of open source and commercial software
  - R/Bioconductor; Genespring; dChip
  - Mascot; Sequest, Progenesis LCMS, etc.
- Literature mining (PathwayStudio)
- Also provide medical statistics support
- Work with NICB wet-lab scientists from experimental design to publication
5. Bioinformatics@NICB

- Differential gene expression
- Significance analysis of microarrays
- Principal component analysis
- Hierarchical clustering analysis
- Artificial neural networks
- Support vector machines
- Partial least squares
- Genetic algorithms
- GSEA
- Meta analysis
- Coexpression network analysis

- Access to the Stokes ICHEC HPC for intensive computations
HCGR Experiment design+21 priority list

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Table III

<table>
<thead>
<tr>
<th>No.</th>
<th>Symbol</th>
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<th>Proteomics Results</th>
<th>Microarray Results</th>
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<td>1</td>
<td>ACTB</td>
<td>Actin, cytoplasmic 1 (Beta-actin)</td>
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<td>2</td>
<td>ACTR1A</td>
<td>Actin-related protein</td>
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<td>Enolase 1, alpha non-neuron</td>
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7. Functional Genomics

- 96/384w RT-PCR
- cDNA Overexpression
- siRNA, shRNA knockdown
- Impact of altering the expression of gene/protein/miR X on:
  - Growth, Qp, Viability, etc.
  - Cell Cycle
  - Apoptosis
  - Invasion
  - Drug Resistance
HCG R VCP-siRNA: Cell Density/Viability

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Cell Density

% Viability

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HCGR VCP-OE: Cell Density/Viability

Cell Density

% Viability

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HCGR Results of Functional Validation on VCP

- **INCREASED** at the protein level in association with high growth rate
- **DECREASED** at the gene level in association with high growth rate

- siRNA: Decreases growth & destroys viability
- OE: Increases growth, slight improvement in viability in one cell line

- FV results tally with proteomics result
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4. miRNA Screening

- TLDAs
- ~650 miRNAs
- Multiplex RT
- 2 x 384w plates with target-spec. PCR primers

- **Anti-mirs** and **Pre-mirs** for knockdown/knockup
- Transient transfection
- Cellular phenotype modified?
- miRNA target genes?
miRs as engineering targets?

First CHO miRNA

A representative graph of growth cycle

Consensus  

\[ \text{GUACCA UGUCGG AGCUUAUC GACUG UGUUG CUGU G} \]  

\[ ^\text{UAUGGU ACAGUC UCGGGUAG CUGAC ACAAC GGUA C} \]  

\[ ^\text{C^ UUU UG} \]

Initial identification of low temperature and culture stage induction of miRNA expression in suspension CHO-K1 cells

Patrick Gannell *, Niall Barron, Niraj Kumar, Martin Clynes  
National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland  
Received 2 February 2007; revised 2 April 2007; accepted 16 April 2007

Available online at www.sciencedirect.com

Short communication

Viable cells/mL (x10^5)

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<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>144</th>
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<tr>
<td>Viable Cells/mL</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>10</td>
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\[ \text{GUACCA UGUCGG AGCUUAUC GACUG UGUUG CUGU G} \]  

\[ ^\text{UAUGGU ACAGUC UCGGGUAG CUGAC ACAAC GGUA C} \]  

\[ ^\text{C^ UUU UG} \]

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\[ ^\text{UAUGGU ACAGUC UCGGGUAG CUGAC ACAAC GGUA C} \]  

\[ ^\text{C^ UUU UG} \]
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6. Cell Characterisation Core Facility

- A key element in studying the behavior of mammalian cells in different biological systems

- Interlinking with the Microarray and Proteomic core facilities to further validate and study results obtained

- Typical studies have included:
  - Drug localisation within cells
  - Location of receptors and target proteins
  - Location of fusion proteins within cells
6. Cell Characterisation Core Facility

- P.A.L.M. Laser Capture Microdissection microscope
- Time-lapse Fluorescent Microscope with CO₂ and N₂ environmental control
- Leica SP2 AOBS Confocal; capable of spectral scanning and unmixing
- The facility also has cytometry capability
  - Guava EasyCyte Plus platform
  - BD FACS Aria cell sorter
NICB Technology Platform Outputs: Publications

National Institute for Cellular Biotechnology

BMC Biotechnology

Differential protein expression following low temperature culture of suspension CHO-K1 cells
Niraj Kumar*, Patrick Gammell, Paula Mcleady, Michael Henry and Martin Clynes

Address: National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland
*Corresponding Author: niraj.kumar@dcu.ie

Prevalence and prognostic and predictive relevance of PRAME in breast cancer

VIII.

Molecular Cancer

Investigation of the molecular whole genome microarrays

Techniques for clustering gene expression data

SFI/MMI Technology Platform Workshop
# The Pfizer/DCU family Status Report 2010

## AM 102427 & AM 102587 - Differential Expression Profiling Analysis of Cell Culture Phenotypes and Uses Thereof

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The specifications focus on the time course analysis and the genes and proteins identified using the time course analysis of industrially relevant cell line phenotypes through the use of sequence similarity and homology analysis methods. The specification describes differential expression profiling analysis of differentially expressed genes and proteins regulating or indicative of cell culture productivity (such as maximum cellular productivity or a sustained high cell productivity), peak cell density, sustained cell viability, rate of ammonia production or consumption, or rate of lactate production. The specification further includes methods for manipulating the identified genes and proteins to engineer improved cell lines.

## AM 102912 - Pathway Analysis of Cell Culture Phenotypes and Uses Thereof

<table>
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<th>Country</th>
<th>Status</th>
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<td>United States</td>
<td>Filed: March 20, 2008, CHS#: (2004658-2219)</td>
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</table>

The specification describes pathway analysis of various differentially expressed genes and proteins identified using various differential profiling methods. The specification further includes methods for manipulating the identified genes and proteins to engineer improved cell lines.

## AM 102921 - Pathway Analysis of Cell Culture Phenotypes and Uses Thereof

<table>
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<tr>
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<td>Filed: March 20, 2008, CHS#: (2004658-2220)</td>
</tr>
</tbody>
</table>

The specification describes pathway analysis of differentially expressed genes and proteins identified using various differential profiling methods.
The intercourse on terms of equality between the representatives of these different estates of the nation [government and industry] is like a sensitive nervous mechanism endowing the community which possess it with capacities and potentialities realisable in no other way.

Sir Cyril Hinshelwood, President, Royal Society, 19th July 1960

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