

Symposium Productivity and Operational Excellence in Biomanufacturing

6th of October 2008 - EVRY

« *Strain and Process engineering,
the key fo maximizing productivity* »

Eric Devic – GTP Technology – Toulouse (France)

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GTP'S OVERVIEW

- 100% private company, founded in 2000
- Located in Labege, France (near Toulouse)
- 20 people
- 1.2 M€ Turn-over (2007)

GTP'S MISSION

- GTP is a **Contract Research Organization** committed to providing **R&D solutions for recombinant protein expression**.
- We offer a comprehensive range of **custom-designed services** from « **Gene To Protein** » to provide proteins for multiple applications.

OVERVIEW

SERVICES

PRODUCTS



We help bio-pharmaceutical companies to save time during drug discovery pre-clinical phases.

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AREAS OF EXPERTISE

- **Production of protein for research:** Target proteins for rational drug design,...
- **Therapeutic protein production** for pre-clinical phases:
 - Cell line & strain engineering (Bacteria, Yeast and Mammalian cell)
 - USP and DSP development
 - QC development
 - Delivery of Bioequivalent batches
 - Technology transfer to CMO
- **Reagent protein production:** Process development, production of up to 50 gr batches

OVERVIEW

SERVICES

PRODUCTS



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RECOGNIZED EXPERTISE

- More than **500 protein production projects** performed to date
- Extensive experience with **multiple expression systems** (*E.coli*, *L. lactis* – *P. Pastoris* – Insect cells – Mammalian cells)
- Partnerships with **leading pharmaceutical, cosmetic and biotech companies**
- Over **60 customers**
- Long-term collaborations: **60% of our business is recurrent**

OVERVIEW

SERVICES

PRODUCTS



Many leading pharma, biotech companies put their trust in our expertise.

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PROVEN TRACK RECORD

- Batches of up to 100 mg for HTS: about **200 projects performed**
- Batches of up to 50 mg of protein for structural studies (NMR, crystallography): **18 projects performed**
- Recurrent delivery of 1-10 gr of protein for diagnostic or analytical use: **20 projects performed**
- Process development for therapeutic proteins: **10 projects performed** with batch production of grams of protein for preclinical trials

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Productivity and Operational Excellence in Biomanufacturing

« **Cell line and Process engineering,**
the key fo maximizing productivity »

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Products in Development

Products in clinical development in USA

Monoclonal Antibodies	160 ~76 in 2004	
Vaccines	62	
Recombinant hormones / proteins	43	
Antisense (oligonucleotides)	20	
Interferons	18	
Growth factors	16	
Interleukins	8	
Other	71	PhRMA Survey, 2006

➔ **Mammalian / Microbial ratio : 60/40**

Biomanufacturing project :

The first question : Mammalian OR Microbial ?

➔ **Microbial** : System of choice for most of non-glycosylated protein
More secure and much less expensive

The « star » system : *E. coli* { Big background, large track records
High yield
Free to operate
Many and large CMO capacities

Alternatives: *P. pastoris*, Filamentous fungi

+ **New systems** :

Pfenex™ – *P. fluorescens* (DOW Pharma)
LactXpress™ – *L. lactis* (INRA-GTP)

BUT for Antibodies

Antibody Production

	Mammalian	Fungus	Yeast	Bacteria
Mab	+++	++	++	-
Fab	+	++	++	+++
scFv	-	+	++	+++
Single domain	-	+	++	+++

➔ Mammalian expression system remains the main expression system to express MAb

Emerging systems : Transgenic systems, Avian cells, Yeast, Insect cells

Manufacturing Challenges for Mammalian cell products

Manufacturing costs, especially for large dose products such as antibodies

➔ Requirements can be 100s of kg/ per annum

Of the **957 kg** of proteins manufactured in 2002:

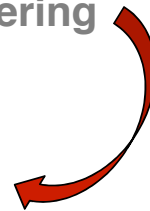
- cytokines, hormones and enzymes : 40 kg
- Mabs and fusion proteins : **917 kg**

(UBS Report - 2003)

Responding to the Challenge

- Increase in global capacity especially for mammalian cell culture
 - In 2006 ~ 2.2 million litres (more than doubling 2002 capacity)
- Improve mammalian cell culture process at large scale
 - Up to 20 000L suspension reactors
- Improve clone productivity : **Cell line Engineering**

Year	« Good cell line »
1995	0.5 g/L
2005	3 g/L
Now	Up to 30 g/L



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Drug discovery companies Challenge

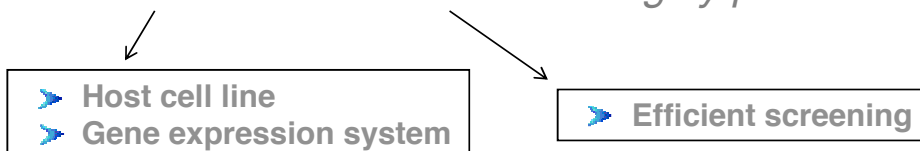
Get product with....

→ Good efficacy AND good productivity

CRUCIAL STEP = Cell Line Engineering

To get a producing clone with the best productivity

Creation and Selection of highly productive cell line



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Cell Line Engineering

➔ *Creation of highly productive cell line*

A- Host cell line choice

- ☑ High capacity for protein synthesis and secretion
- ☑ Ability to grow in suspension
- ☑ Adapted serum-free medium available
- ☑ Genetically stable

Commonly used host cell lines

CHO (« the standard »), Baby Hamster Kidney (**BHK**) cells
NS0, Sp2/0 (mouse myeloma), mouse hybridoma
PER.C6™ human cells (Crucell)

Cell Line Engineering

➔ *Creation of highly productive cell line*

B- Gene expression system

➤ Expression vector :

- Promotor
- Terminator
- 5' and 3' UTR, Introns

➤ Gene of interest :

- Codon usage
- Cryptic splice sites, poly A signals, etc
- Direct and inverted repeats
- GC content
- Undesirable mRNA folding

➤ Gene amplification :

- DHFR
- Glutamine synthase™

➤ Chromatin Opening element:

- Sellexis Genetic Elements™
- UCOE™ (Millopore)

Strategic choices

➔ Host cell line

Type	Asset	Caveat
CHO	« the standard » Free to operate	
NS0, Sp2/0	Trak records	
CHOK1- SV	High biomass Longer viability	Licence (Lonza)
PER.C6	Human type Glyc. High biomass	Licence (Crucell)

Type	Asset	Caveat
DHFR	High yield Free to operate	
GS	High yield	Licence (Lonza)
COE	High yield. Faster	Licence (Selexis, Milipore)

Gene expression system



Cell Line Engineering

➔ *Selection of highly productive cell line*

Transfection results in heterogeneous population

The challenge : Select the « **desirable** » cell line

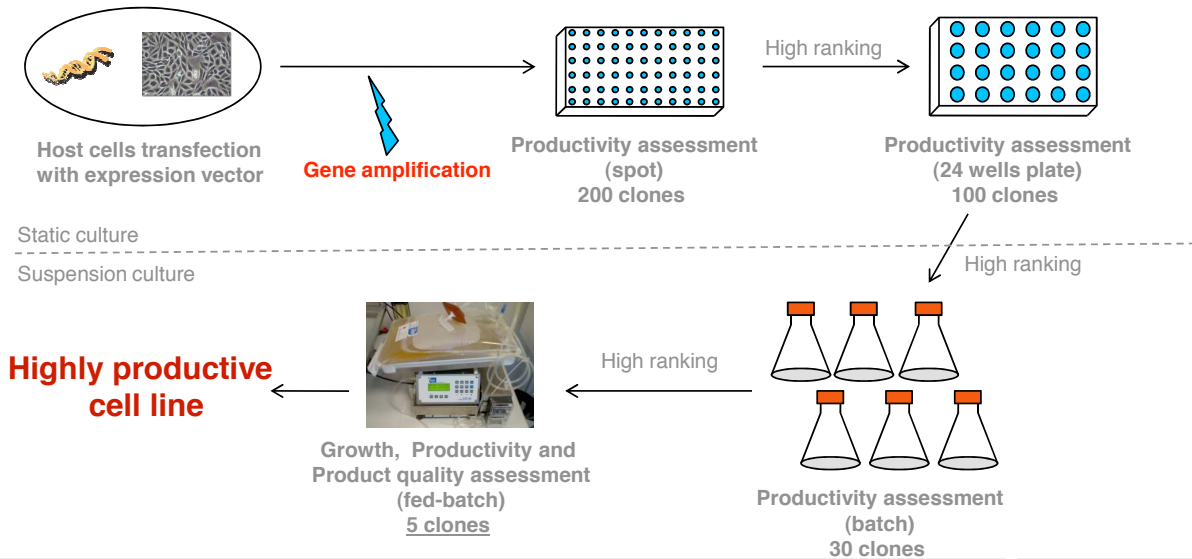
« Desirable » means:

- Production of desired quality product
- Good cell line growth
- High production rate in bioreactor

➔ The experiment shows that it is necessary to screen large number of clone in order to few good (« desirable ») cell lines.

Cell Line Engineering

➔ *Selection of highly productive cell line*



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Key learning

Cell line engineering = crucial step in Bioproduction project

Which means :

- ☑ Make the good technical choices
- ☑ Make the good strategic choices
- ☑ Make the good clone selection

➔ to get the BEST clone and maximize success for further steps

GTP Technology : your starting partner for Bioproduction projects

● GTP can help you in :

- Cell line and strain engineering
- Process development (USP – DSP)



● GTP's Strengths

- Costs
- Flexibility
- Project dedication
- Independence from CMO

Thank you for your attention

GTP TECHNOLOGY

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