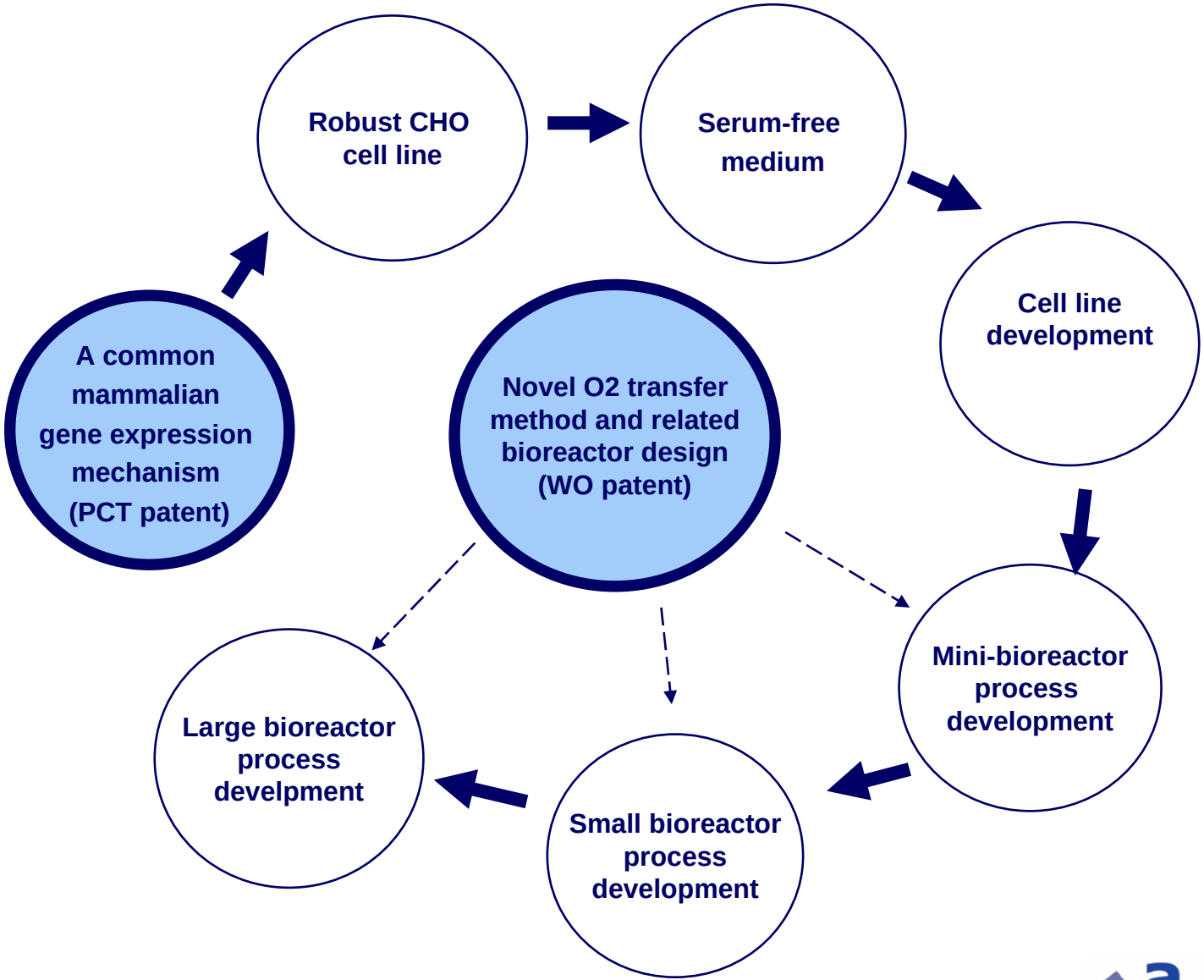
A Revolution in Mammalian Protein Production

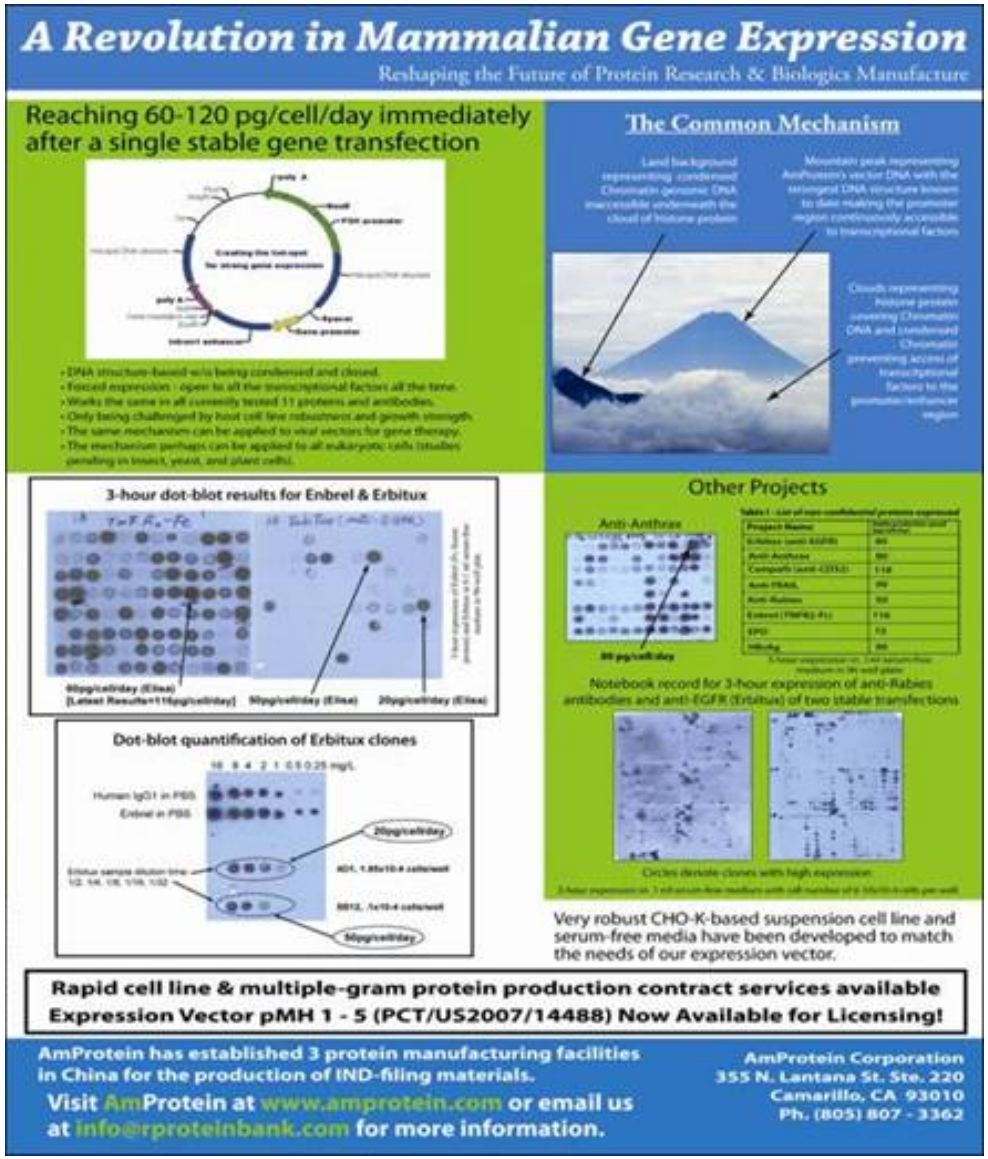
Matthew Hui, PhD M.D. & Jia Qian, PhD

amProtein™ Biotherapeutics

Introduce Standard Protein Production Platform



A Second Revolution – Gene Expression



Discovery of a physical DNA structure-based common mechanism for mammalian gene expression is a second revolution in mammalian cell expression since the discovery of DHFR.

PCT/US2007/14488
(Published in August)

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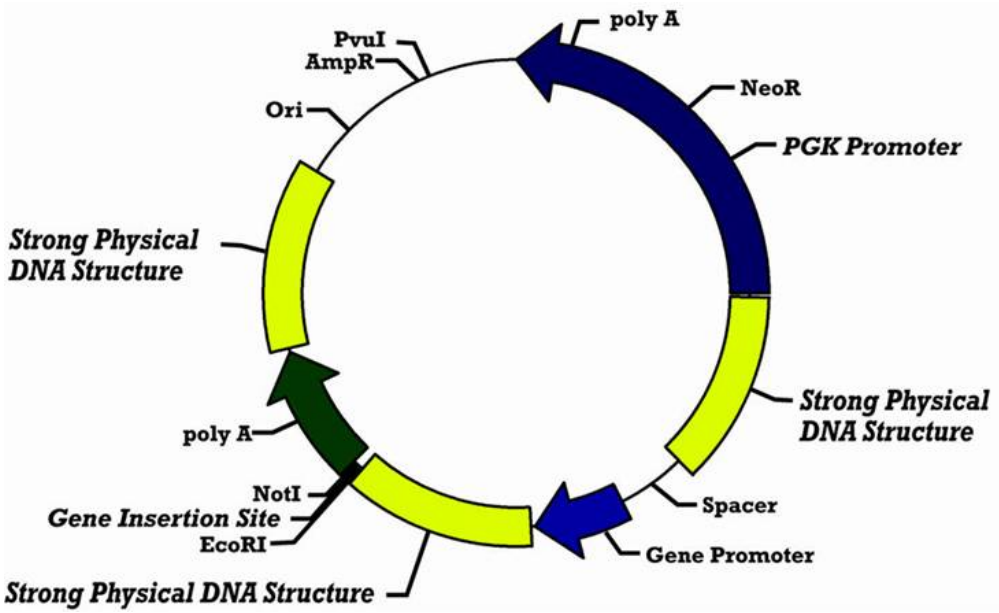
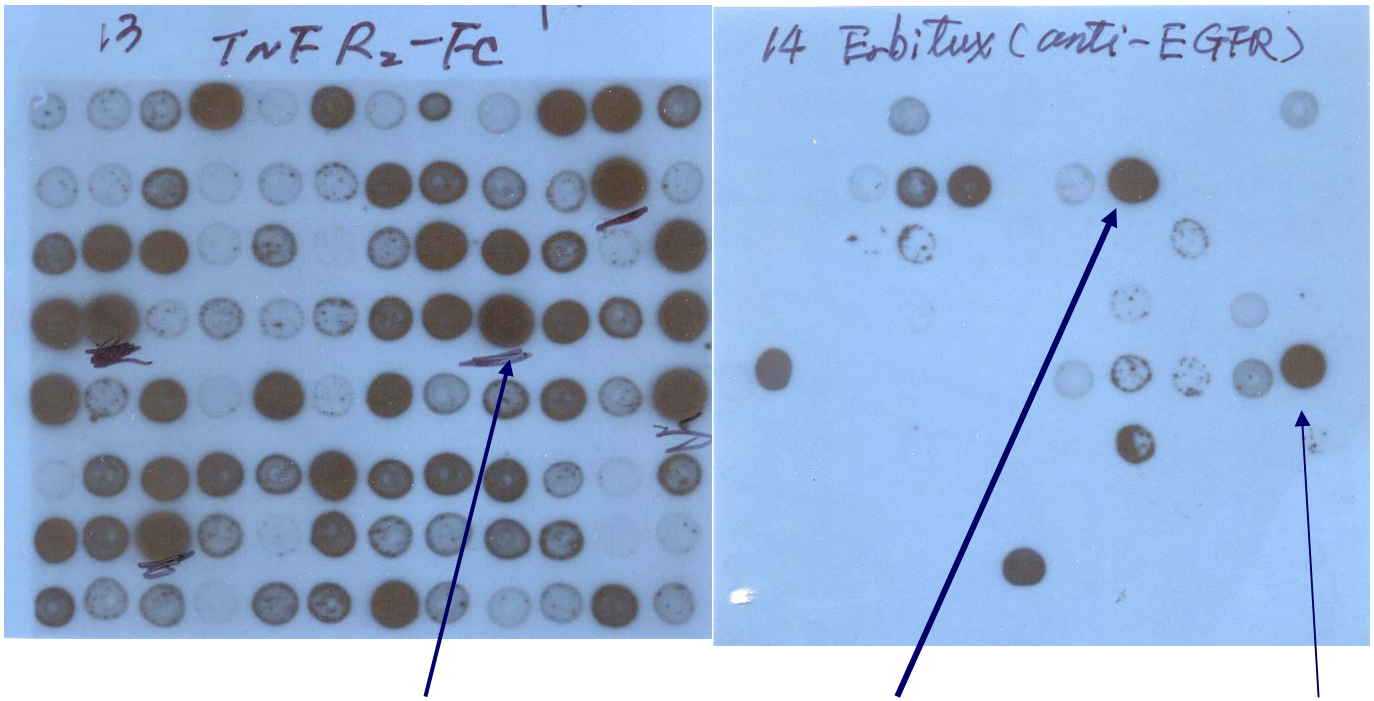
The Strongest Mammalian Gene Expression



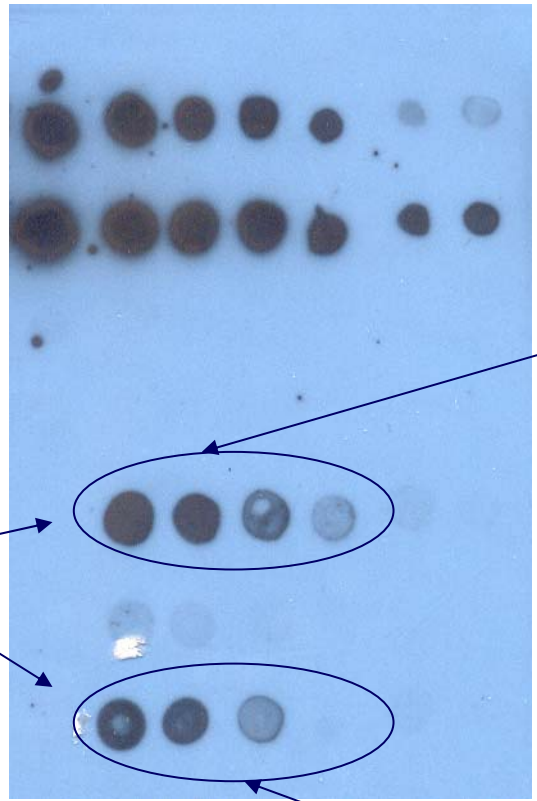
- The strongest mammalian vector known to date
- Initial production line immediately to reach 50-116pg/cell/day

Fc Fusion Protein Expression



3-hour expression of Enbrel (Fc fusion protein) and Erbitux in 0.1 ml serum-free medium in 96-well plate: >50pg/cell/day

Dot-blot Quantitation of Erbitux Clones



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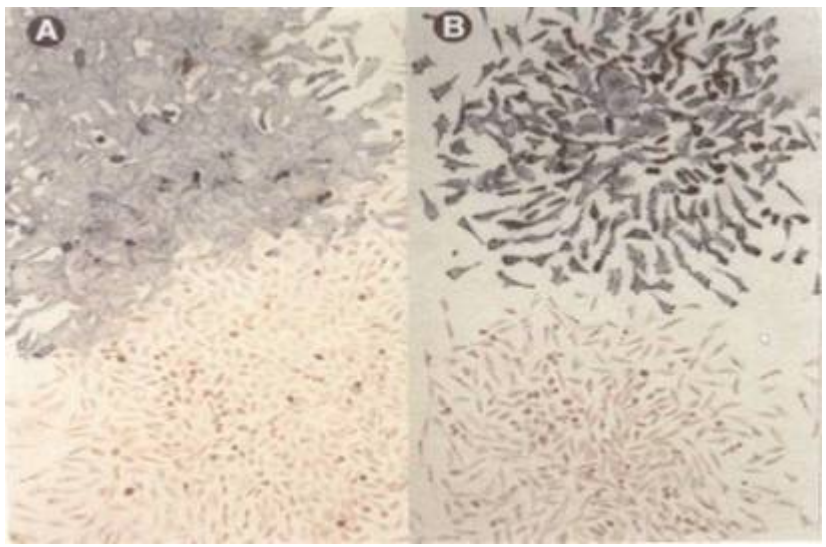
Industry Host Cell Line

The Cells: A robust CHOKS host was just developed to tolerate harsh Feed-batch cell culture condition

- Adherent for cell cloning
- Immediately suspension adaptable
- High density (5% to 7% of total culture volume)



Generic Drug Candidates Under Development

Cytokine Factors	IGF , KGF, TPA , GCSF, FSH , LH, interferon beta 1a, BNP, rhEPO, IL-1ra
Antibodies	Erbitux , anti CD20, Campath, anti CD22, anti Her2, anti IgE
Vaccine	HE vaccine , HBsAg vaccine
Fusion Protein	FC-endostatin , Fc-leptin , Fc-IL-1ra , Fc-leptin , Enbrel, VEGFtrap-Fc
Others	rHSA , Transferrin , factor VII , BMP2 , BMP7 , huPH20-N , GH

By taking advantage of this revolutionary platform a pharmaceutical company can lower their costs and greatly speed up development pipelines.

Non-Confidential Project Expression Level

Project Name	Expression Level (pg/cell/day)
Enbrel	116
Anti-EGFR	80
Anti-anthrax	90
HSA	271
Anti-CD52	118
Anti-Rabies	50
EPO	72
HBsAg	90
FSH	20
LH	10

Difficult-expressed Proteins

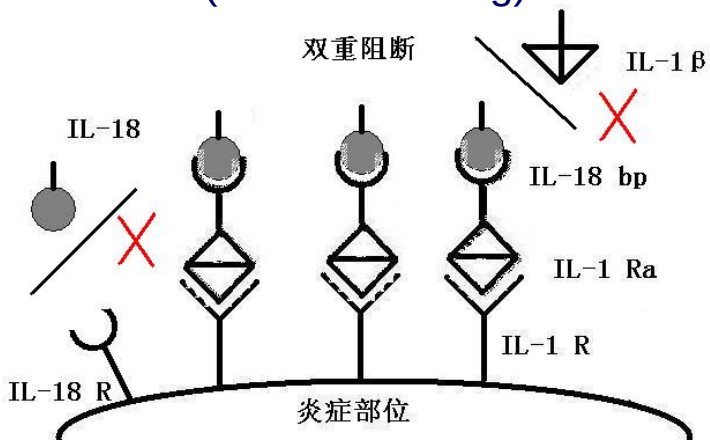
- **Internal projects (dual domain drugs)**

1. **IL-18bp-Fc-IL-1ra**
2. **IL-4R-Fc-IL-1ra**
3. **TNFR2-Fc-IL-1ra**
4. **Symlin-Fc-leptin**
5. **PYY-Fc-Leptin**
6. **GLP1-Fc-Leptin**

- **Contract Projects**

1. **Trail receptor activating Mb-1**
2. **Trail receptor activating Mb-2**
3. **Trail receptor activating Mb-3**

(double blocking)



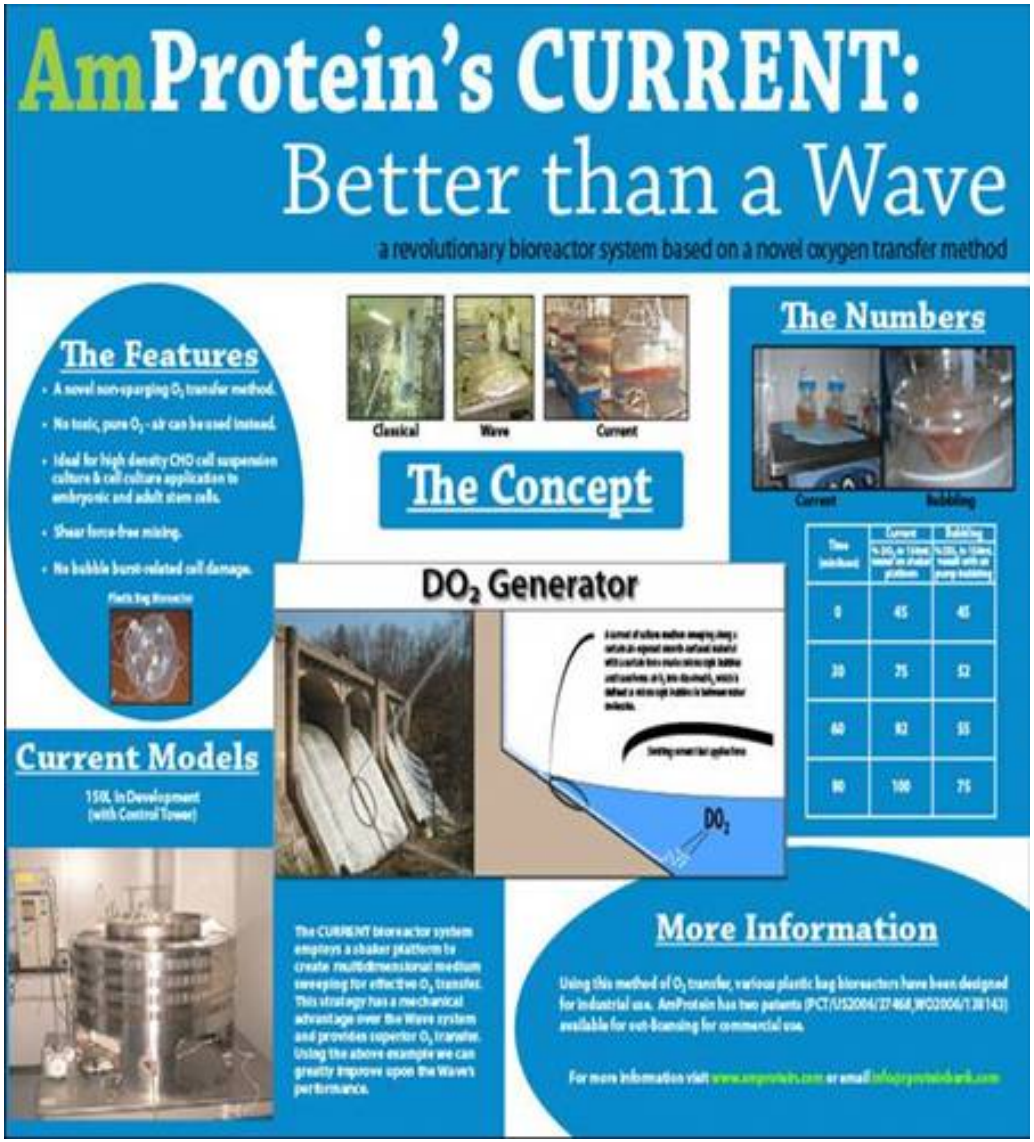
(Inflammation site)

- Dual domain drugs good for multiple factorial human diseases
- Difficult to express

High Yield Cell Line Quick Selection Process

Project	Normal	Ours
Cell transfection	Transfection 1 day	Transfection, 1 day
	DHFR selection 10 days	G418 selection, 5 days
Cell cloning growth	Growth and MTX gene copy amplification 4 months	Growth 7 days
Screening	ELISA 1 day	Dot-blot or ELISA 1 day
Expression Level	15-45 pg/cell/day	50-120 pg/cell/day
Time	4.5 months	14 days

Novel Oxygen Transfer Method for Bioreactors



Our advertisement has appeared in Science, Nature Biotechnology, Bioprocess International, and on the cover of China Biotechnology

Sparge-free Oxygen Transfer Method: dynamic intereaction between the culture media and bioreactor's wall

WO2007/142664 & WO2006/138143

amProtein™ Biotherapeutics

Disposable Plastic Bag Bioreactors

5–150 Liter Working Volume

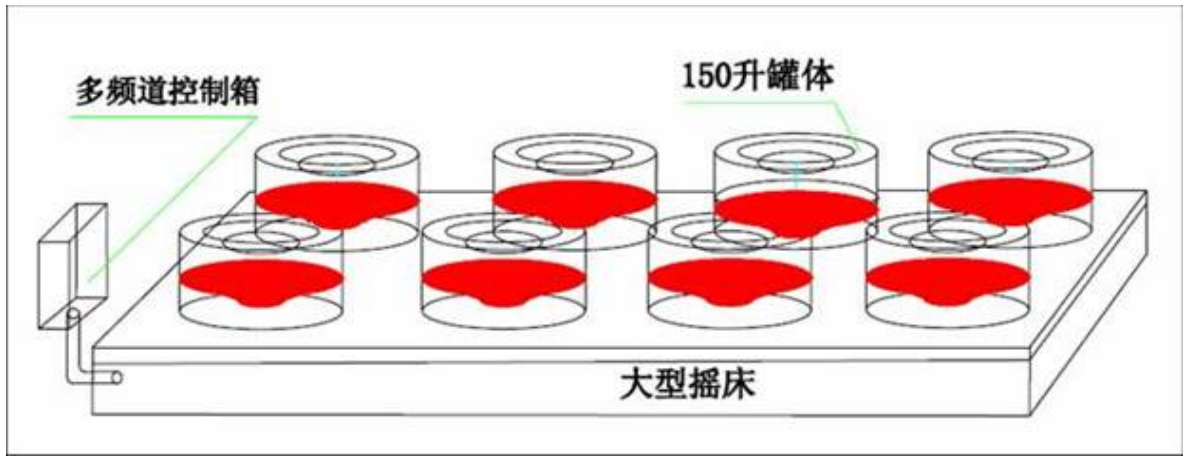
Testing Process

Finished Products

Cell growth density can reach
5~7% PCV without control tower

150L Single-Use Plastic Bag Bioreactors

1. Process Development
2. Seed Train
3. Parallel Scale-up for Single Batch Culture
   (10 × 150L =1500L/ 1 production phase in 3 weeks)



Large scale GMP production diagram

Advantage: 150L industrial process development is much simpler than 7000L large, stainless steel bioreactor process development, and there is no production down-time due to sterility examinations.

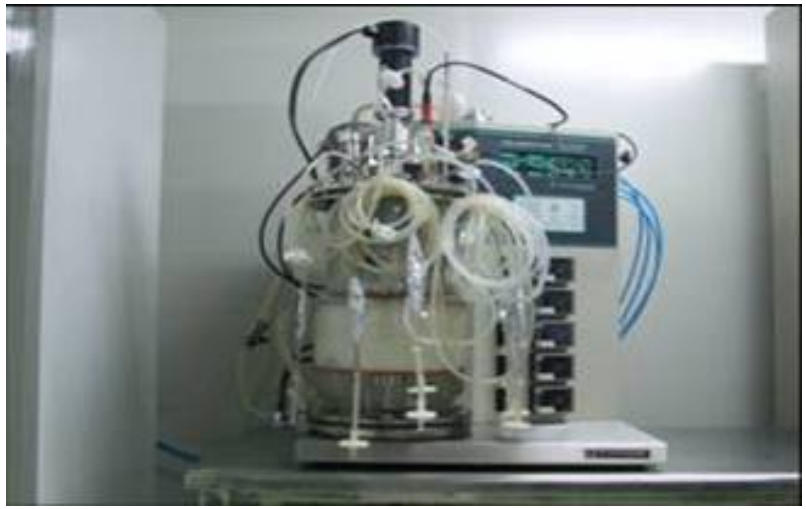
40 & 150 ML Mini-Bioreactors

Industrial Process Using

Newly developed mini-reactors with a unit price of 1.5 Million US Dollars

Newly developed mini-reactors costing less than $1000 per unit.

Hayaobio's Classical Bioreactor System




Cell density has reached 5~7%PCV, namely 5~7% culture volume is cell mass

High-speed Rotating Bioreactor

Specialized for micro-carrier cell culture and vaccine production

1. Scale up
2. Seed chain
3. Parallel scale up

100×=15000升/3 weeks

Advantage:：High O2 transfer and low mixing with limited damage on cells attached on micro-carriers, great for micro-carrier VERO, MDCK cell culture

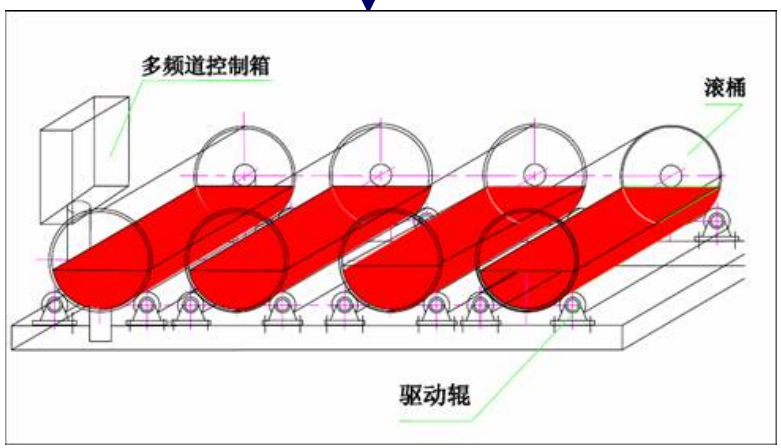
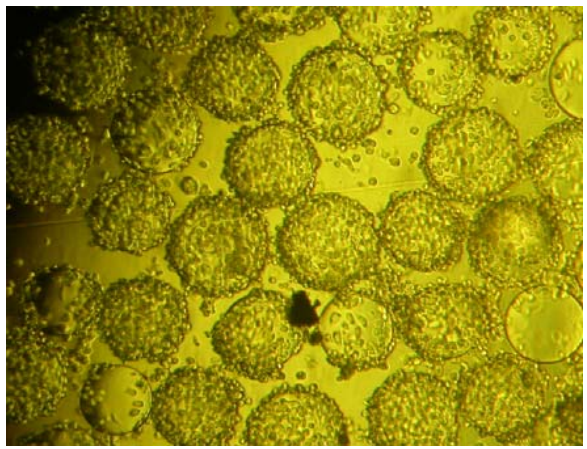
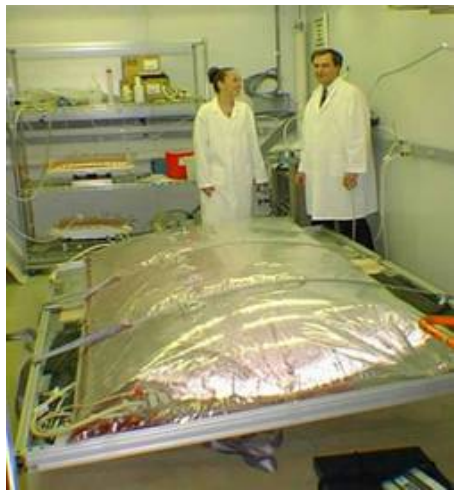


Illustration for scale up model



Comparison of AmProtein's Bioreactor to others

AmProtein's current



Wave

Classical

Mechanically less complicated
Sheer force free, no impeller
No bubbling or sparging
Superior novel O_2 transfer method

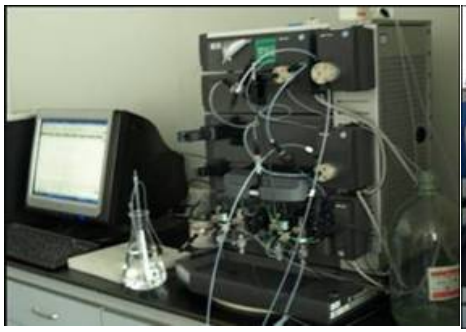
Future Single-use Bioprocess Bag

Commercialized Bioprocess bag、Media Mixing Equipment and complementary filtration system

Hangzhou China has the potential to become the future world manufacturing center for bio-process plastic bag

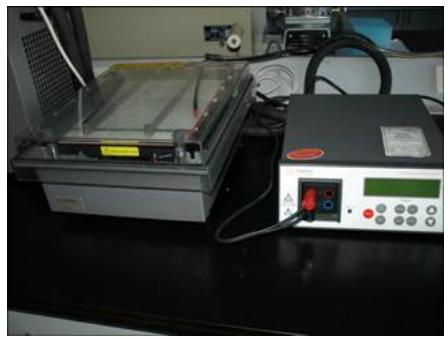
Bioassay






4 AKTA Protein Purification Sets



3 HPLC Sets for Protein Characterization

Quality Control System

Biochemical analysis

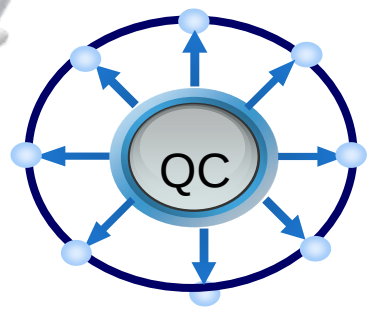
Protein binding

Cell-based

Host protein

Protein-A residue

Protein quantitation



Molecular Weight

Glycosylation

Disulfide bond

Peptide mapping

Protein sequencing

HPLC

Amino assay content

Purity analysis

cDNA residue analysis

amProtein™ Biotherapeutics

Our R&D Team

- 52 researchers, 6 PhD, 21 Master
- Management Team: M Hui, MD PhD, Qian Jia PhD, HC Li PhD, XQ Zhang PhD
- Consultants：Alan Herman (ex-Amgen）、Tsutomo Arakawa (ex-Amgen）、Beyon Chan (ex-Amgen）、Tom Dimer (ex-Amgen)

Management & Information System

Camara phone conference

High speed Internet

Cell phone 24 hours

Industrial Info

PCTwww.wipo.int/pctdb/en/

USPTO www.uspto.gov/patft/

US FDA www.fda.gov

Drug Bank www.drugbank.ca

EU patent office

http://ep.espacenet.com/advancedSearch?locale=en_EP

amProtein™ Biotherapeutics

Operation Manual for Staff Training


Amprotein Internal Use handbook(Ⅰ)

工业化哺乳动物细胞平台操作手册

编辑：惠觅宙 贾茜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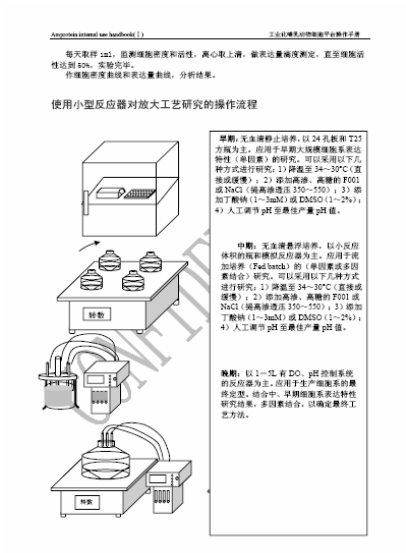
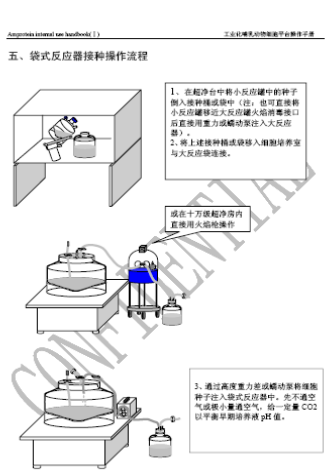

使用小型反应器对放大工艺研究的操作流程

早期，无血清静止培养，以 24 孔板和 T25 方瓶为主，应用于早期大规模细胞系表达特性（单因素）的研究，可以采用以下几种方式进行研究：1）降温至 34～30°C（直接或缓慢）；2）添加高渗、高糖的 F001 或 NaCl（提高渗透压 350～550），3）添加丁酸钠（1～3mM）或 DMSO（1～2%）；4）人工调节 pH 至最佳产量 pH 值。

中期，无血清悬浮培养，以小反应体积的摇和模拟反应器为主，应用于流加培养（Fed-batch）的（单因素或多因素结合）研究。可以采用以下几种方式进行研究：1）降温至 34～30°C（直接或缓慢）；2）添加高渗、高糖的 F001 或 NaCl（提高渗透压 350～550），3）添加丁酸钠（1～3mM）或 DMSO（1～2%）；4）人工调节 pH 至最佳产量 pH 值。

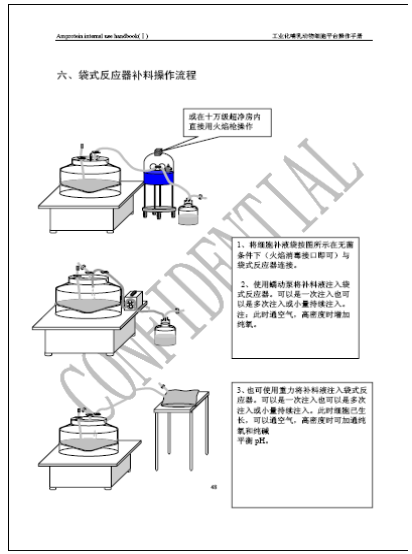
晚期，以 1～5L 有 DO、pH 控制系统的反应器为主，应用于生产细胞系的最终定型。结合中、早期细胞系表达特性研究结果，多因素结合，以确定最终工艺方法。


五、袋式反应器接种操作流程

1、在超净台中将小反应罐中的种子倒入接种瓶或袋中（注：也可直接将小反应罐移近大反应罐火焰消毒接口后直接用重力或蠕动泵注入大反应器）。
2、将上述接种瓶或袋移入细胞培养室与大反应袋连接。

或在十万级超净房内直接用火焰枪操作

3、通过高度重力差或蠕动泵将细胞种子注入袋式反应器中，先不通空气或极小量通空气，给一定量 CO2 以平衡早期培养的 pH 值。


六、袋式反应器补料操作流程

或在十万级超净房内直接用火焰枪操作

1、将细胞补液袋按图所示在无菌条件下（火焰消毒接口即可）与袋式反应器连接。

2、使用蠕动泵将补料通注入袋式反应器。可以是一次注入也可以是多次注入或小量持续注入。注，此时通空气，高密度时增加纯氧。

3、也可使用重力将补料通注入袋式反应器。可以是一次注入也可以是多次注入或小量持续注入。此时细胞已生长，可以通空气，高密度时可加通纯氧和纯碱
平衡 pH。

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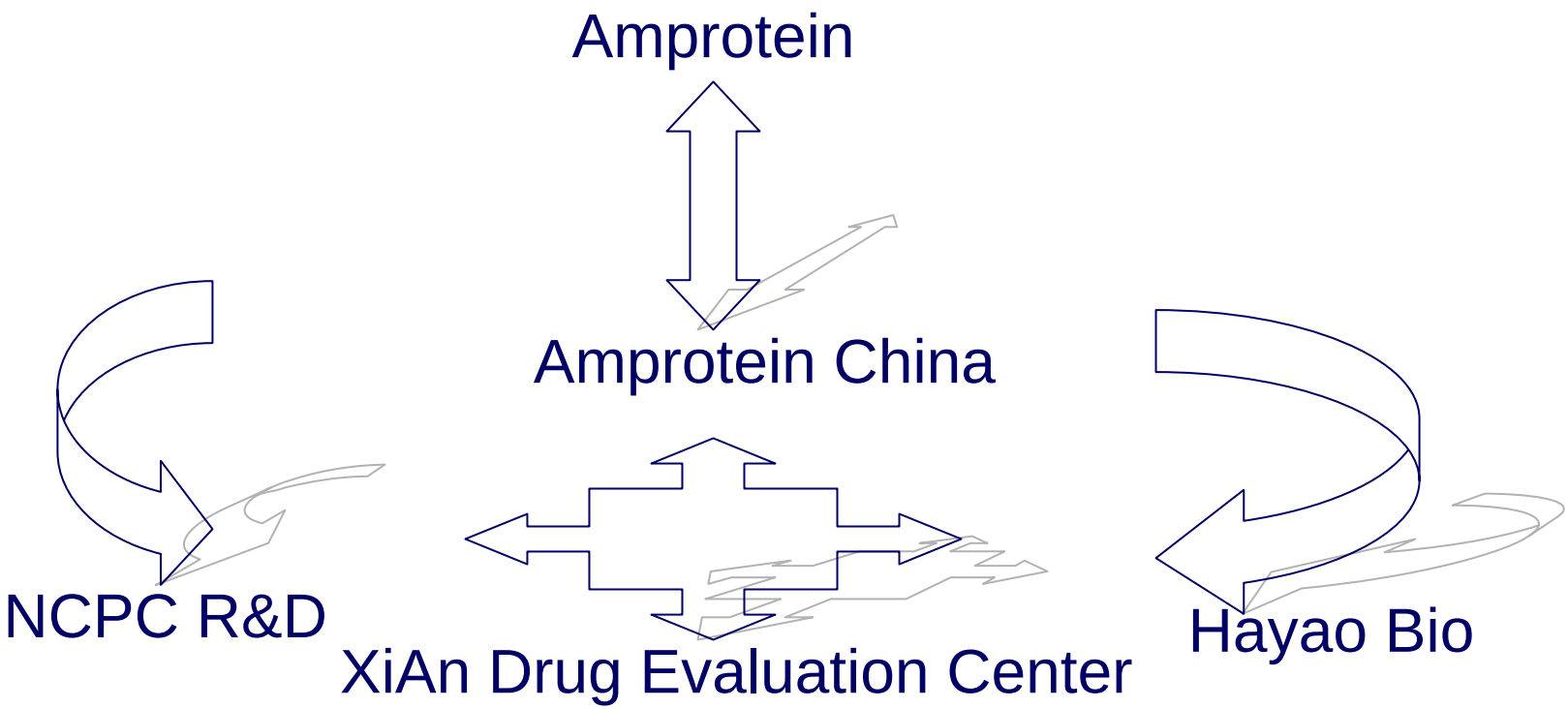
AmProtein Alliance Members



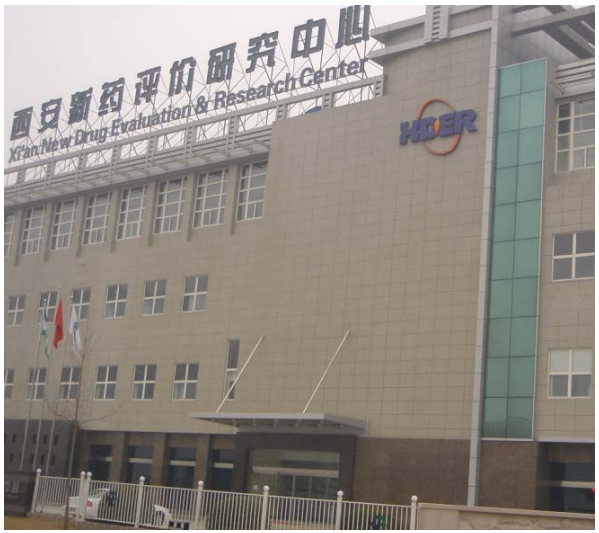
NCPC's Facility in Beijing

AmProtein alliance member

amProtein™ Biotherapeutics

Drug Evaluation Center for preclinical evaluation

Complete Downstream Bioassays available



- ✓Cell-based Assay
- ✓Live imaging
- ✓Tissue Distribution
- ✓Efficacy
- ✓PK
- ✓Toxicity

Our Business Focus

- Difficult protein expression
- Series Vector licensing out
- PD by using Disposable Bioreactor
- From DNA to Kg protein
- Pre clinical evaluation
- Bio similar and bio similar improve
- Co-development for Dual domain drug

AmProtein - China Operation

Welcome to visit our facilities in China

www.amprotein.com

www.amprotein-china.com

Thanks and Questions!

amProtein™ Biotherapeutics