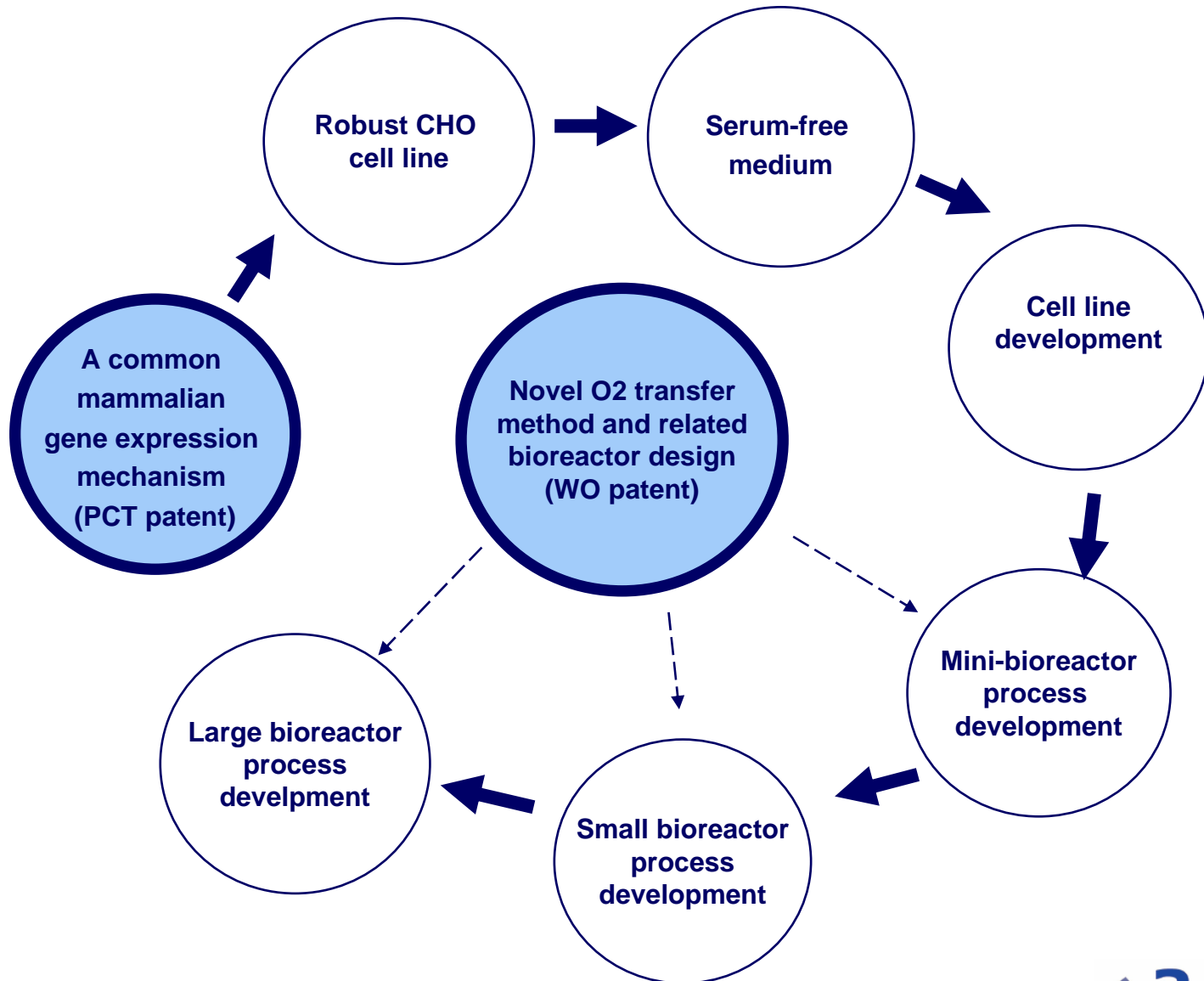


A Revolution in Mammalian Protein Production

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

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.

A Revolution in Mammalian Gene Expression

Reshaping the Future of Protein Research & Biologics Manufacture

Reaching 60-120 pg/cell/day immediately after a single stable gene transfection

Creating the full length for strong gene expression

- DNA structure-based cells being condensed and closed.
- Forced expression - open to all the transcriptional factors all the time.
- Works the same in all currently tested 51 proteins and antibodies.
- Only being challenged by host cell line robustness and growth strength.
- The same mechanism can be applied to viral vectors for gene therapy.
- The mechanism perhaps can be applied to all eukaryotic cells (studies pending in insect, yeast, and plant cells).

The Common Mechanism

Level background representing condensed chromatin genomic DNA inaccessible underneath the shield of histone protein

Mountain peak representing AmProtein's vector DNA with the strongest DNA structure known to date making the promoter region continuously accessible to transcriptional factors

Clouds representing histone proteins covering Chromatin DNA and Chromatin governing access of transcriptional factors to the promoter/enhancer region

3-hour dot-blot results for Enbrel & Erbitux

3-hour expression of Enbrel in 100% of wells (100% of clones) and Erbitux in 100% of wells (100% of clones)

3-hour expression of Enbrel in 100% of wells (100% of clones) and Erbitux in 100% of wells (100% of clones)

3-hour expression of Enbrel in 100% of wells (100% of clones) and Erbitux in 100% of wells (100% of clones)

Other Projects

Anti-Anthrax

Project Name	100% (100% of clones)
Anti-Anthrax	100%
Anti-Rabies	100%
Erbitux (100% of clones)	100%
Enbrel (100% of clones)	100%
EGFR	100%
IGG	100%

3-hour expression of 100% of clones (100% of clones) in 100% of wells (100% of clones)

Notebook record for 3-hour expression of anti-Rabies antibodies and anti-EGFR (Erbitux) of two stable transfections

Circles denote clones with high expression

3-hour expression in 100% of wells (100% of clones) with cell number of 2.5 x 10⁵ cells per well

Very robust CHO-K-based suspension cell line and serum-free media have been developed to match the needs of our expression vector.

Rapid cell line & multiple-gram protein production contract services available
Expression Vector pMH 1 - 5 (PCT/US2007/14488) Now Available for Licensing!

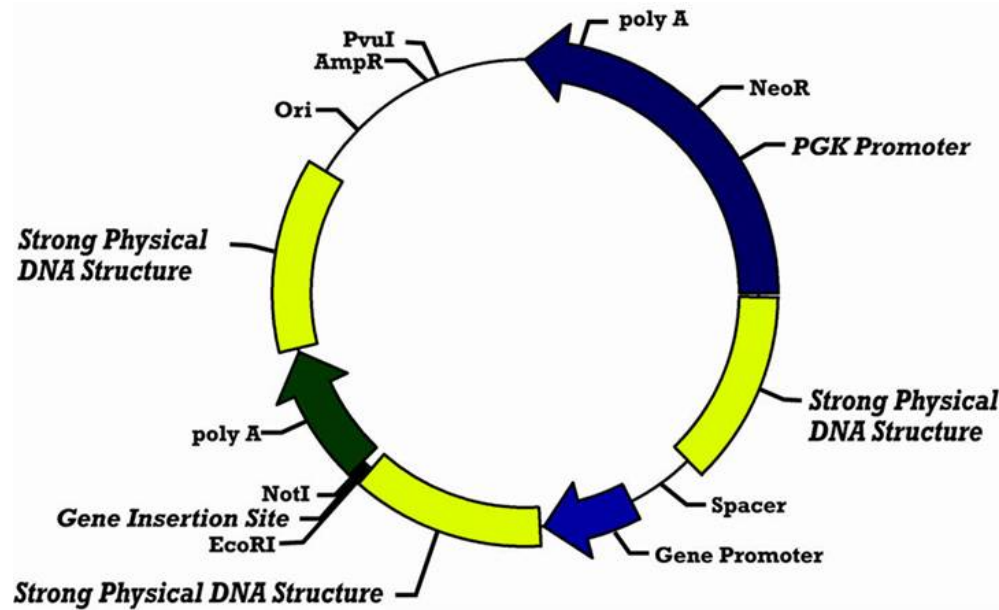
AmProtein has established 3 protein manufacturing facilities in China for the production of IND-filing materials.

Visit AmProtein at www.amprotein.com or email us at info@amprotein.com for more information.

AmProtein Corporation
 355 N. Lantana St. Ste. 220
 Camarillo, CA 93010
 Ph. (805) 807 - 3362

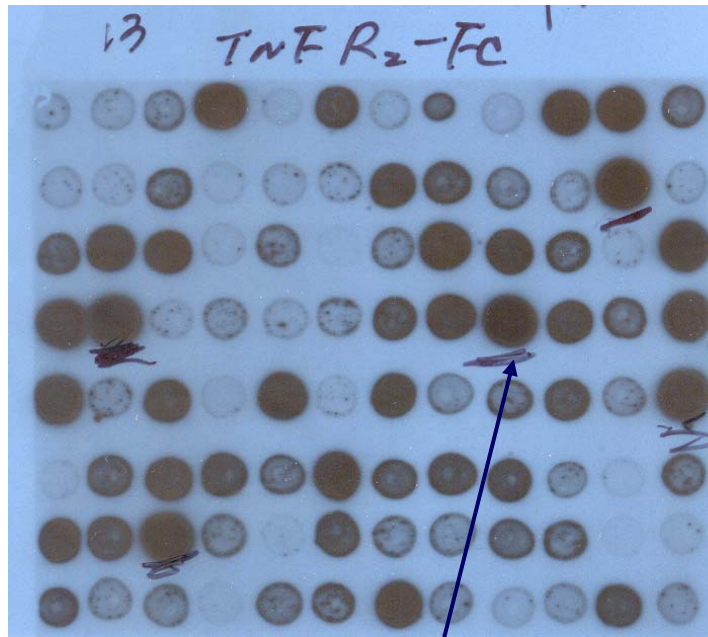
PCT/US2007/14488
(Published in August)

The Strongest Mammalian Gene Expression

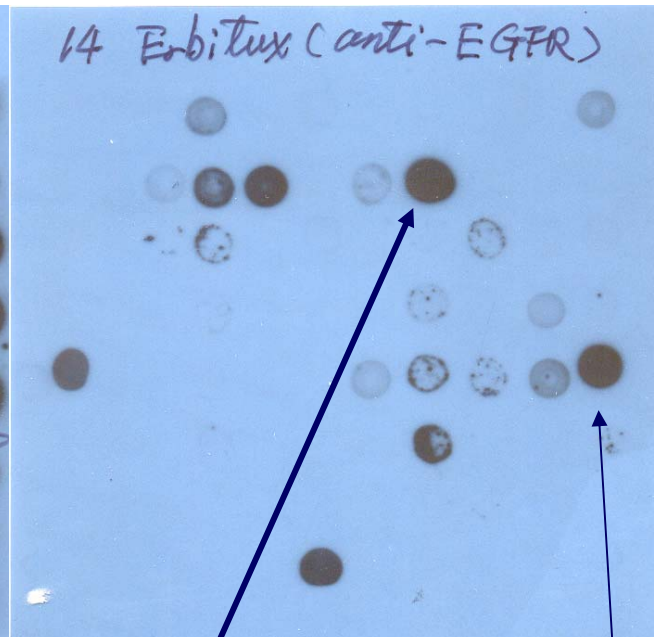


- The strongest mammalian vector known to date
- Initial production line immediately to reach 50-116pg/cell/day

Fc Fusion Protein Expression



60pg/cell/day (Elisa)

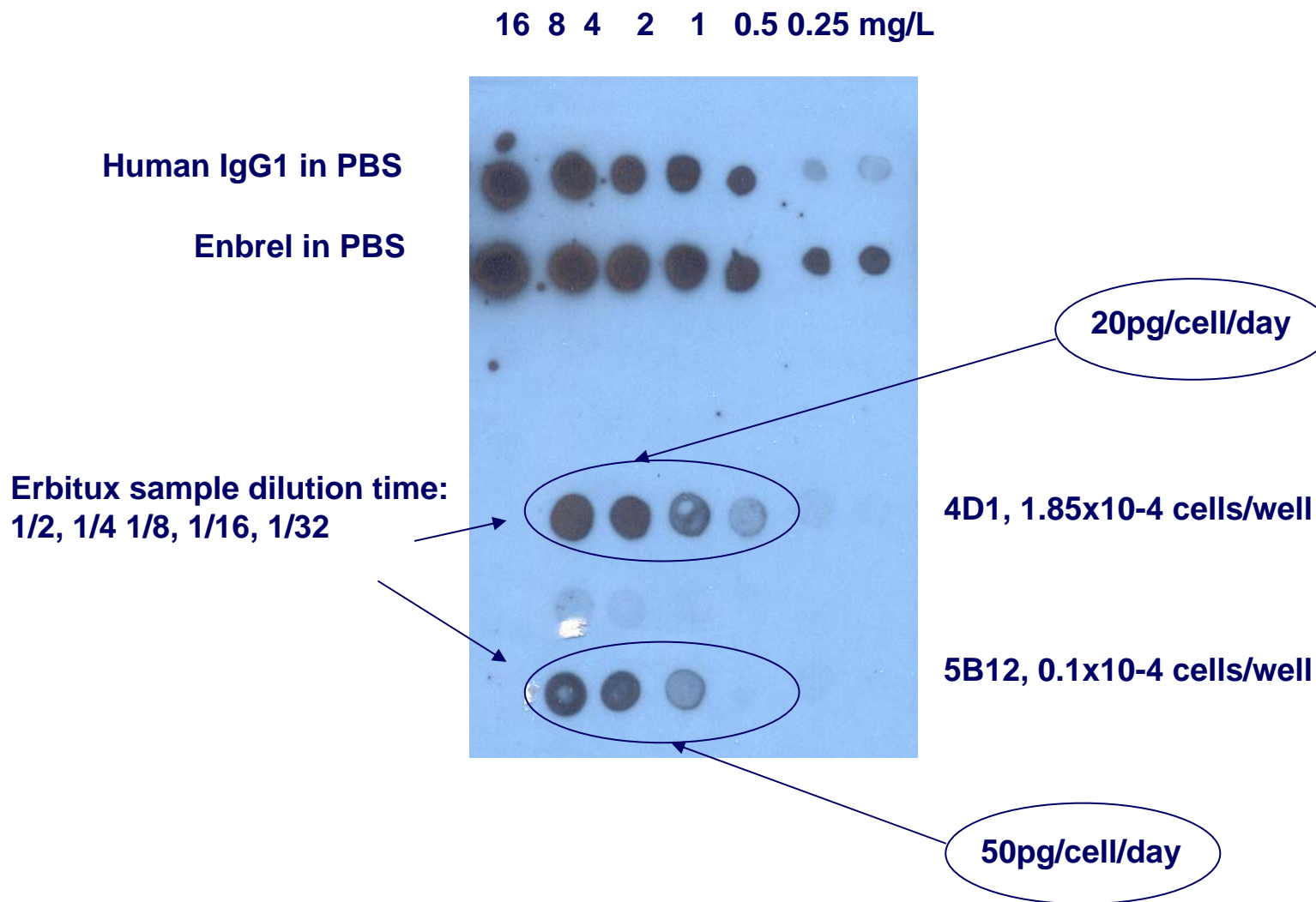


50pg/cell/day (Elisa)

20pg/cell/day (Elisa)

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

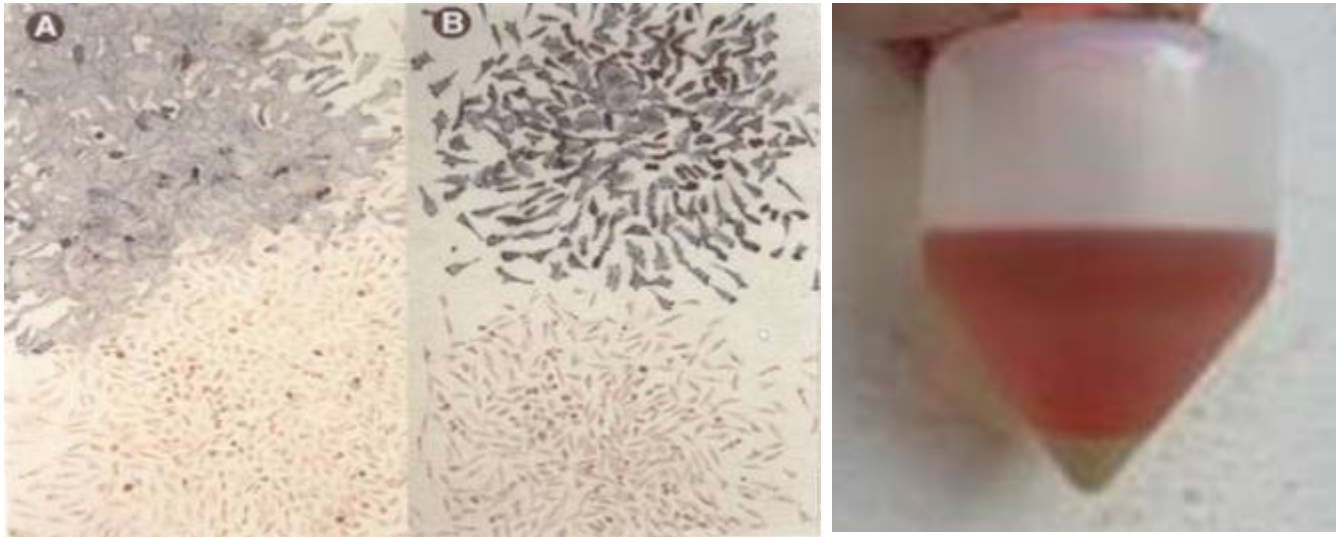
Dot-blot Quantitation of Erbitux Clones



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	Erbix , 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

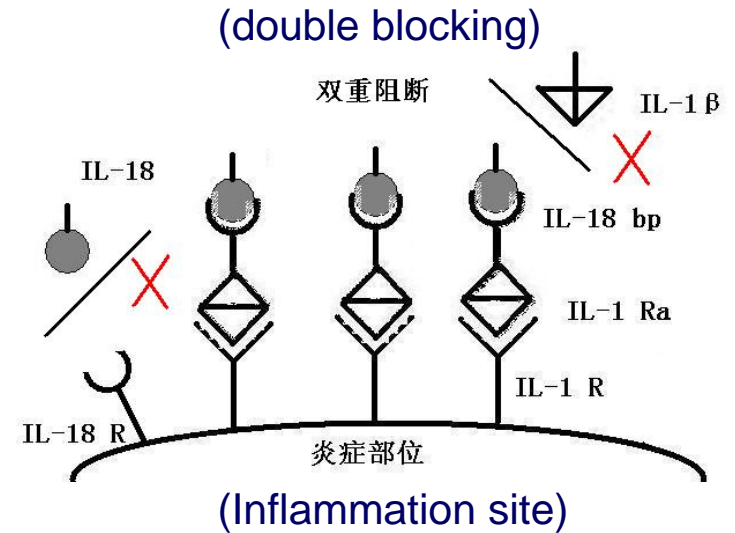
Difficult-expressed Proteins

■ 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



- ◆ 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

AmProtein's CURRENT: Better than a Wave

a revolutionary bioreactor system based on a novel oxygen transfer method

The Features

- A novel non-sparging O₂ transfer method.
- No toxic, pure O₂ - air can be used instead.
- Ideal for high density CHO cell suspension culture & cell culture application in embryonic and adult stem cells.
- Shear force free mixing.
- No bubble burst-related cell damage.

Fluid Bag Bioreactor





Classical Wave Current

The Concept

The Numbers



Current Bubbling

Flow (L/min)	Current % O ₂ in liquid (at 100% saturation)	Bubbling % O ₂ in liquid (at 100% saturation)
0	45	45
20	75	52
40	82	55
80	100	75

DO₂ Generator



A column of culture media sweeping along with an upward flow of air that creates a thin film of culture media on the surface of the column. The air bubbles rise through the column and are absorbed into the culture media.

Setting optimal oxygen flow

DO₂

Current Models

150L In Development (with Control Tower)



The CURRENT bioreactor system employs a shaker platform to create multidimensional medium sweeping for effective O₂ transfer. This strategy has a mechanical advantage over the Wave system and provides superior O₂ transfer. Using the above example we can greatly improve upon the Wave's performance.

More Information

Using this method of O₂ transfer, various plastic bag bioreactors have been designed for industrial use. AmProtein has two patents (PCT/US2006/27466, WO2006/138143) available for out-licensing for commercial use.

For more information visit www.amprotein.com or email info@amprotein.com

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

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

WO2007/142664 & WO2006/138143

Disposable Plastic Bag Bioreactors

5–150 Liter Working Volume

Testing Process



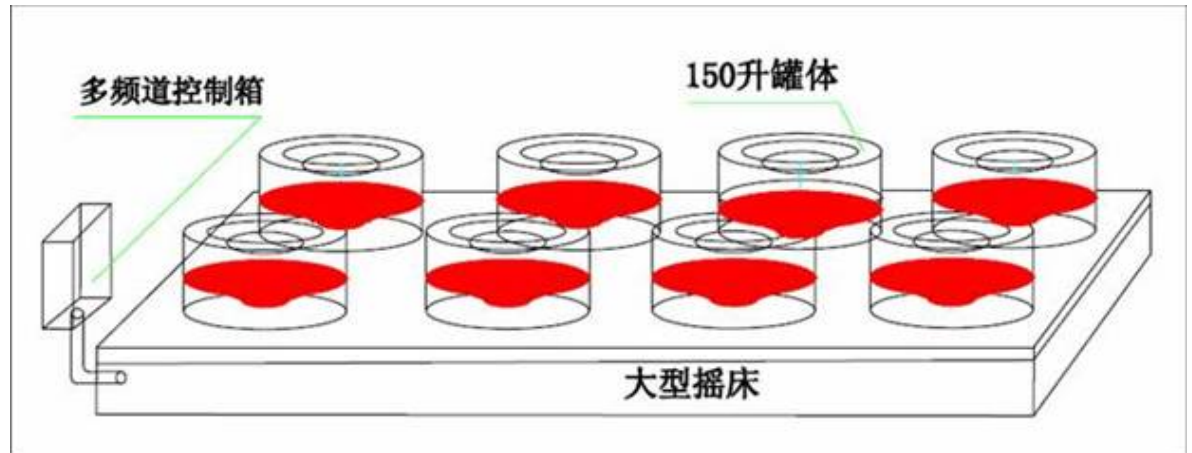
Finished Products



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
($10 \times 150\text{L} = 1500\text{L}$ / 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

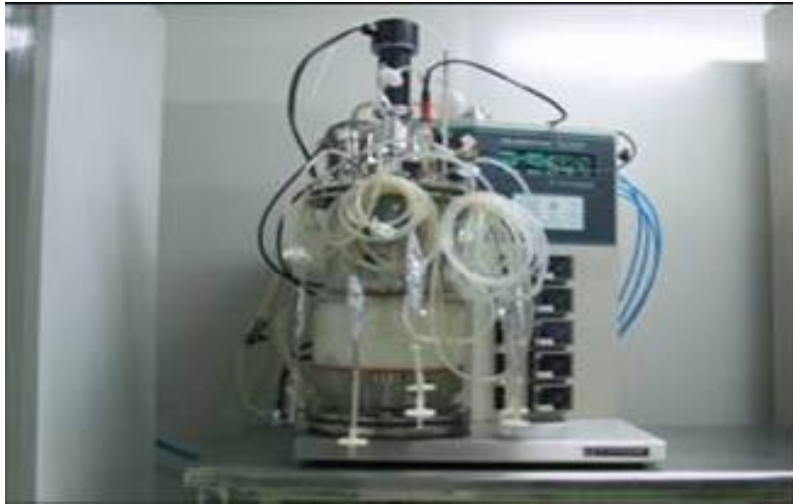


Newly developed mini-bioreactors with a unit price of 1.5 Million US Dollars



Newly developed mini-bioreactors 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
 $100\times=15000$ 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

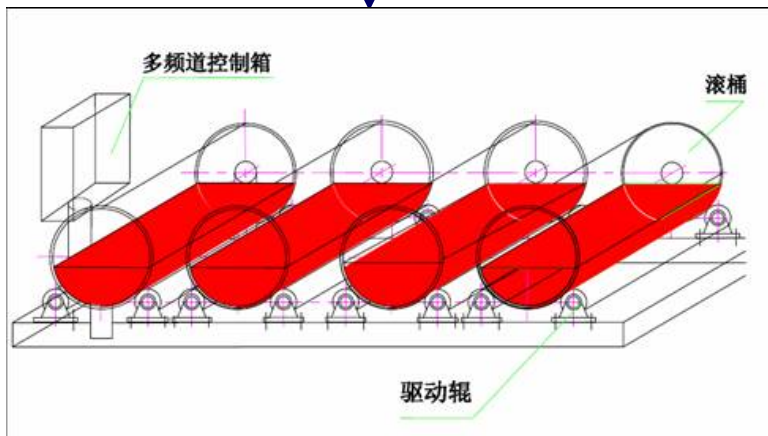
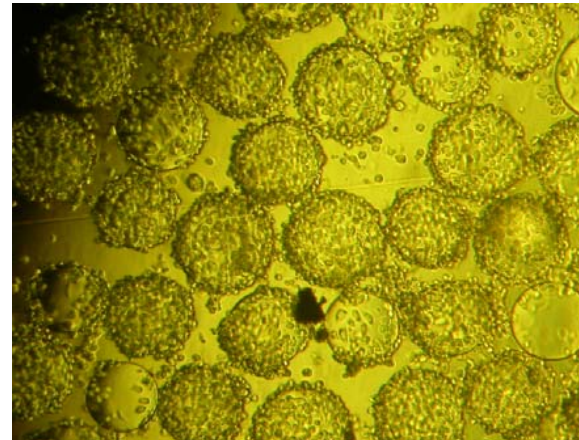


Illustration for scale up model



Comparison of AmProtein's Bioreactor to others



AmProtein's current

Mechanically less complicated
Sheer force free, no impeller
No bubbling or sparging
Superior novel O₂ transfer method



Wave



Classical

Future Single-use Bioprocess Bag



Commercialized Bioprocess bag, Media Mixing Equipment and complementary filtration system

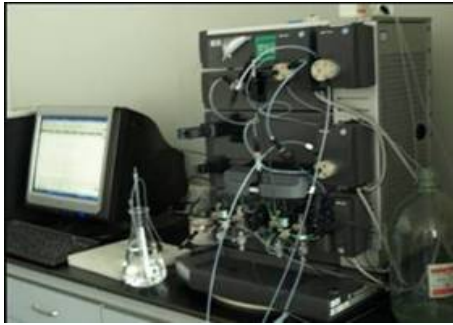


Hangzhou China has the potential to become the future world manufacturing center for bio-process plastic bag

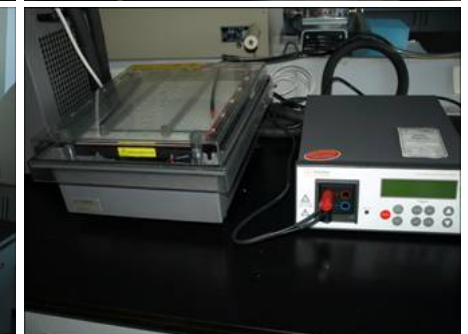
Bioassay



4 AKTA Protein Purification Sets



3 HPLC Sets for Protein Characterization



Quality Control System

Biochemical analysis

Protein binding

Cell-based

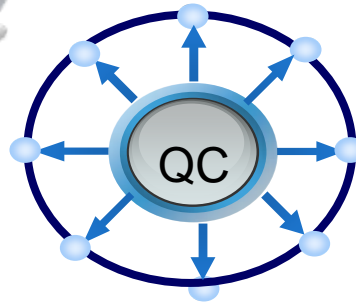


Host protein

Protein-A residue



Protein quantitation



Molecular Weight

Glycosylation

Disulfide bond

Peptide mapping

Protein sequencing



HPLC

Amino assay
content



Purity analysis



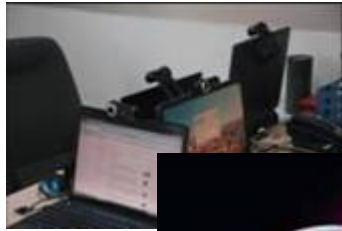
cDNA residue
analysis

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) 、 Tsutomu 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



The screenshot displays the USPTO website with the FDA section highlighted. The FDA section includes links for 'Consumer Health Information', 'FDA Licenses', 'FDA Proposes', 'Baxter's Multiple Allergic Reaction', 'FDA Notices', 'New Era Canning', and 'FDA Newsroom'. Below this is the PATENTSCOPE search interface, which includes a 'Structured Search' section with a table of search criteria:

AND	Field	Operator
<input type="checkbox"/>	Publication Number	<input type="checkbox"/>
<input type="checkbox"/>	Application Number	<input type="checkbox"/>
<input type="checkbox"/>	Publication Date	<input type="checkbox"/>
<input type="checkbox"/>	English Title	<input type="checkbox"/>
<input type="checkbox"/>	English Abstract	<input type="checkbox"/>
<input type="checkbox"/>	Applicant Name	<input type="checkbox"/>
<input type="checkbox"/>	Int. Class	<input type="checkbox"/>
<input type="checkbox"/>	Inventor Name	<input type="checkbox"/>
<input type="checkbox"/>	National Phase Country	<input type="checkbox"/>
<input type="checkbox"/>	Description	<input type="checkbox"/>

PCT www.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

Operation Manual for Staff Training

AmproteIn Internal Use handbook (1) 工业化哺乳动物细胞平台操作手册

AmproteIn Internal Use handbook (1) 工业化哺乳动物细胞平台操作手册

编辑: 惠觅宙 贾茜

AmproteIn Internal Use handbook (1) 工业化哺乳动物细胞平台操作手册

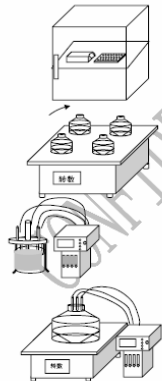
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AmproteIn Internal Use handbook (1) 工业化哺乳动物细胞平台操作手册

每天取样 1:1, 监测细胞密度和活性。离心取上清, 测定重量和吸光度, 直至细胞活性达到 80% 实验完毕。
作细胞密度曲线和表达量曲线, 分析结果。

使用小型反应器对放大工艺研究的操作流程



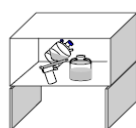
前期: 冻液冻存培养基, 以 24 孔板和 T25 为取型。应用于早期细胞培养活性特性 (单因素) 的研究。可以采用以下几种方式进行研究: 1) 冻液至 34-30°C (直接或缓慢); 2) 添加高糖、高糖的 P001 或 NaCl (冻液冻存至 370-350); 3) 添加丁酰胺 (1-3mM) 或 DMSO (1-2%); 4) 人工调节 pH 至最佳产量 pH 值。

中期: 冻液冻存培养基, 以小型反应器的瓶型反应型为主。应用于冻液冻存 (Fed batch) 的研究 (单因素或多因素组合) 研究。可以采用以下几种方式进行研究: 1) 冻液至 34-30°C (直接或缓慢); 2) 添加高糖、高糖的 P001 或 NaCl (冻液冻存至 370-350); 3) 添加丁酰胺 (1-3mM) 或 DMSO (1-2%); 4) 人工调节 pH 至最佳产量 pH 值。

晚期: 以 1-1L 有 DO₂ 控制系统的反应器为主, 应用于生产细胞系的最终类型。结合中、早期细胞系表达特性研究结果, 多因素组合, 以确定最佳工艺方法。

AmproteIn Internal Use handbook (1) 工业化哺乳动物细胞平台操作手册

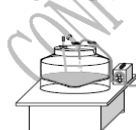
五、袋式反应器接种操作流程



1、在超净台中将小反应器中的种子加入接种袋中 (注: 也可使用小反应器将袋式反应器接种袋接口直接插入重力或蠕动泵注入反应器);
2、将上述接种液或袋式接种液加入反应器中。



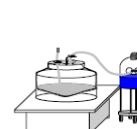
或在十万级超净室内
直接用火焰枪操作



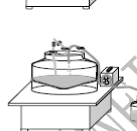
3、通过高重力泵或蠕动泵将细胞种子注入袋式反应器中, 先不通气或通小量空气, 待产气量 CO₂ 以平衡早期培养 pH 值。

AmproteIn Internal Use handbook (1) 工业化哺乳动物细胞平台操作手册

六、袋式反应器补料操作流程

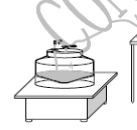


或在十万级超净室内
直接用火焰枪操作



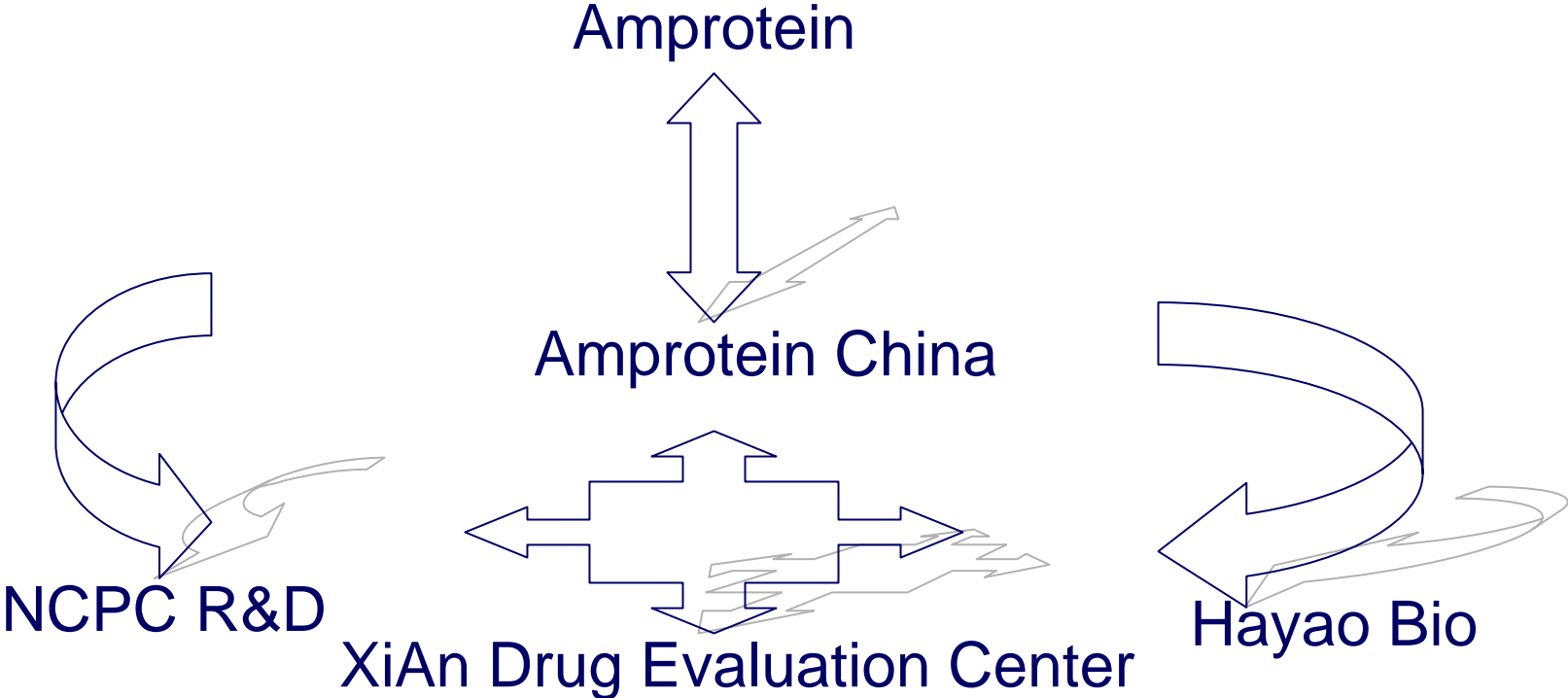
1、将细胞补料液按照要求在无菌条件下 (火焰枪接口即可) 与袋式反应器连接。

2、使用蠕动泵将补料液注入袋式反应器, 可以是一次注入也可以多次注入或小量持续注入。此时细胞已产气, 可以通空气, 高密度时可通纯氧和调 pH。



3、也可使用重力泵补料液注入袋式反应器, 可以是一次注入也可以多次注入或小量持续注入。此时细胞已产气, 可以通空气, 高密度时可通纯氧和调 pH。

AmProtein Alliance Members



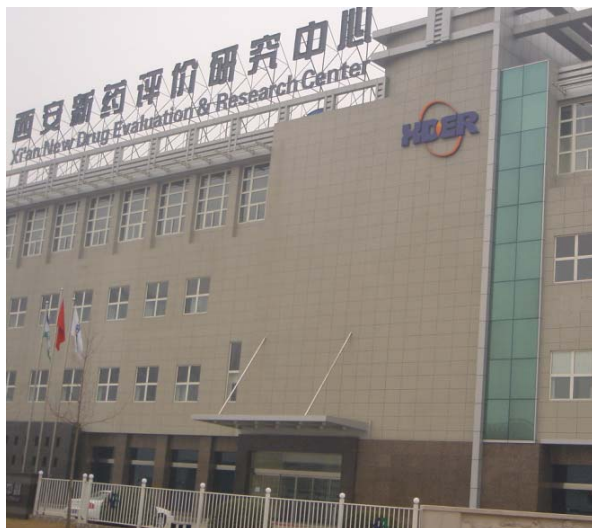
NCPC's Facility in Beijing



AmProtein alliance member

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!