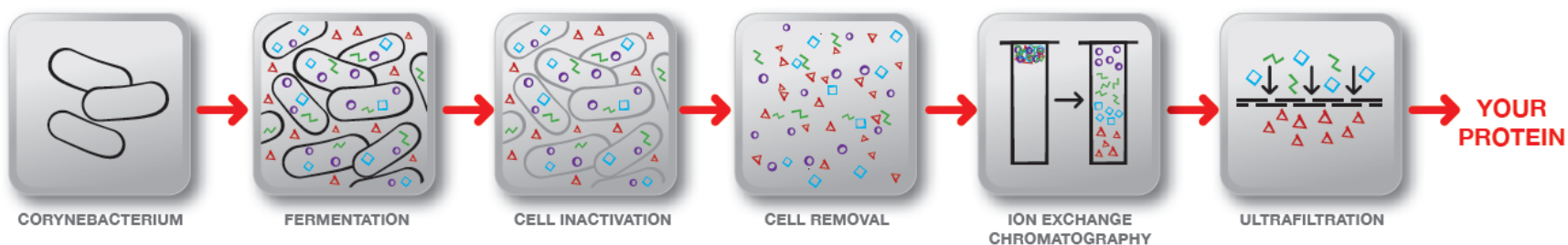


# Need Protein Expression?

## Don't Make It Complicated.



## Corporate Data and Financial Highlights

<b>Establishment</b>	<b>May 20, 1909</b>
<b>Employees</b>	<b>30,000</b>
<b>Net sales</b>	<b>¥1,106,807 Mil (\$9,460 Mil)</b>
<b>Operating income</b>	<b>¥60,322 Mil (\$516 Mil)</b>
<b>Net (loss) income</b>	<b>¥34,912 Mil (\$298 Mil)</b>
<b>Shareholders' equity</b>	<b>¥528,762 Mil (\$4,519 Mil)</b>

(As of March 31, 2006)



## Ajinomoto Global Share

AJINOMOTO®

60%



Everyone else

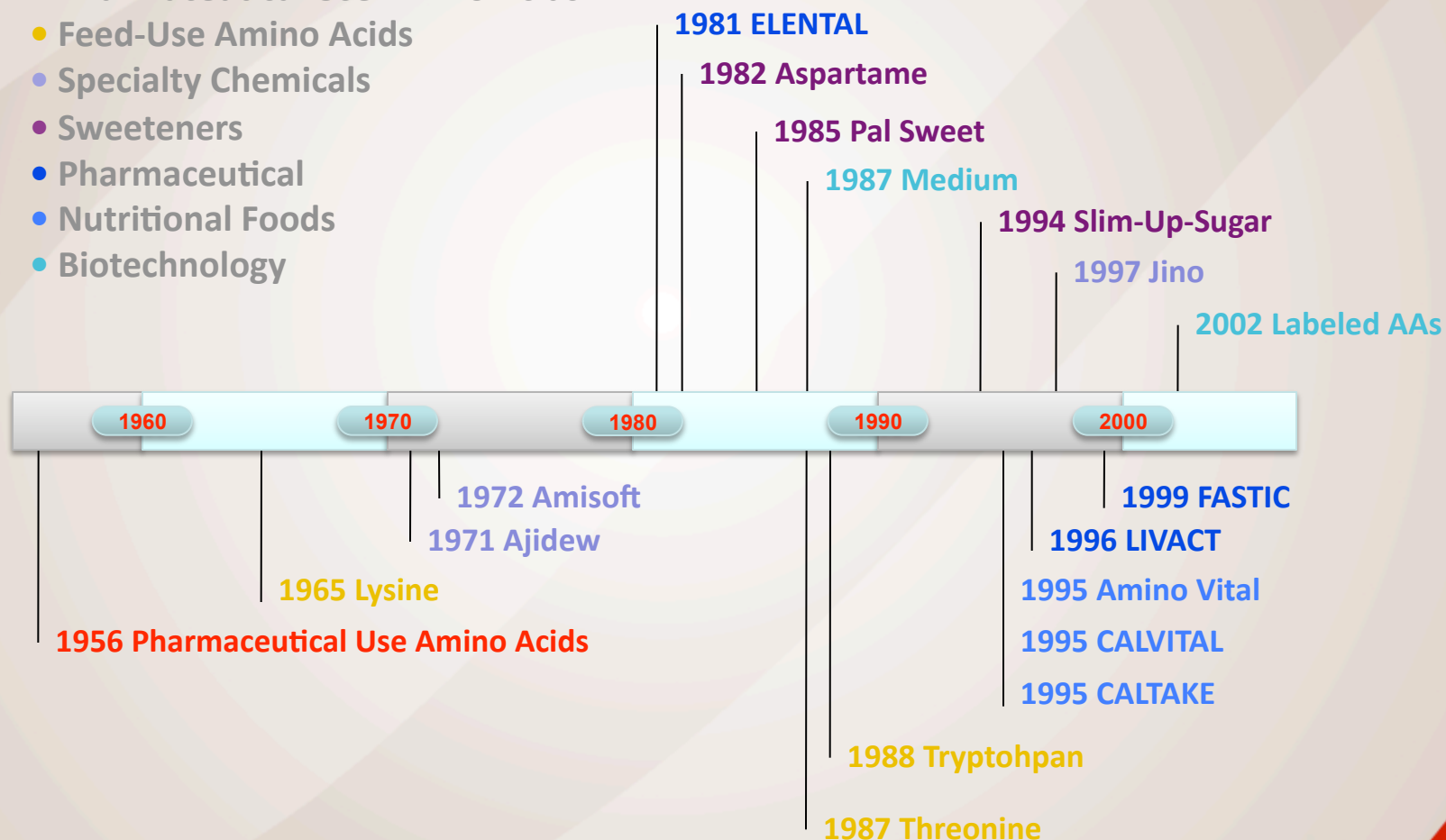
40%

- Pioneer in Amino Acids production since 1956
- No. 1 Market Share
- More than 130 patent applications on Amino Acids during last 3 years



# 50 Years of Fermentation Experience

- Pharmaceutical Use Amino Acids
- Feed-Use Amino Acids
- Specialty Chemicals
- Sweeteners
- Pharmaceutical
- Nutritional Foods
- Biotechnology



## Ajinomoto BIO Technologies

- Bacterial Fermentation
  - Amino Acids
  - Nucleic Acids
- Bacterial Enzymation
  - L-Alanyl-L-Glutamine
- Bacterial Expression (CORYNEX™)
  - Transglutaminase
  - IGF-1
  - Custom protein expression



## Commitment to Quality

- cGMP production
- FDA approved facility
- Conformity to JP/USP/EP
- High Standard of Quality – Corporate Compliance Program (ASQA)
- Complete Animal Component Free Facility
- Accommodate quality audits / quality questionnaires
- Kosher and Halal Certified
- Microbiological Controlled
- Drug Master Files for all Amino Acids Produced



## Corynex Expression System

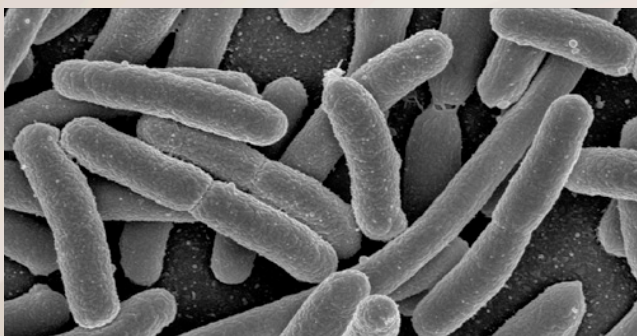
### Breakthrough technology from Ajinomoto



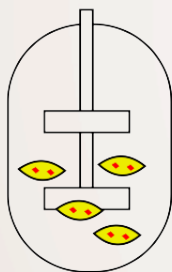
- Gram-positive, fast growing soil bacterium
- Non-sporulating
- Nonpathogenic
- Genome-clarified
- Patented



## Production Using *e-coli*



Cultivation



Cell  
Harvest



Cell  
Disruption



Partial  
Purification



Unwanted  
Refolding



Complex  
Purification



**Complex process with many purification steps.**

**Produces unwanted refolding and many impurities.**



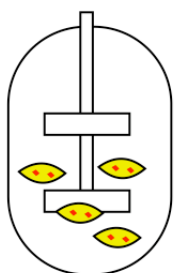


## Corynebacterium glutamicum



**Ajinomoto's unique production process using specially developed expression system**  
*Corynebacterium glutamicum* - an amino acid producing bacterium

**Cultivation**



**Cell  
Removal**



**High yield of  
r-protein**



**Simple  
Purification**



**Purified  
Active r-protein**

**Simple process  
Few impurities  
Well suited for  
high volume production,  
with minimal contaminants**



## Advantages of Corynex

Expression system	Protein Accumulation	Protein Extraction	Refolding	Purification process
E.coli	Intra-Cell	Additional Steps Required	Additional Steps Required	Complicated
Yeast	Extra-Cell	Not Required	Additional Steps Required	Complicated
CORYNEX	Extra-Cell	Not Required	Not Required	Simple



## Advantages of Corynex Expression

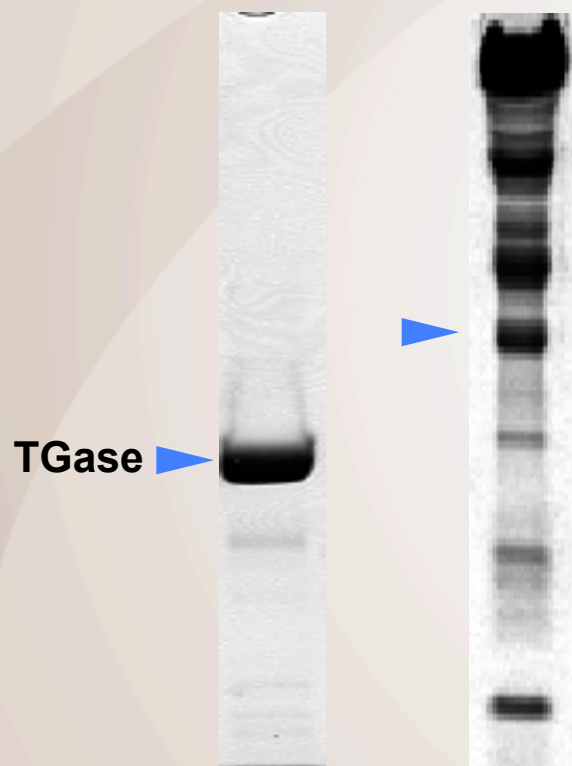
- Simplified expression process
  - Quick strain development
  - Developmental tool box
- Secretes active proteins
- Engineered to eliminate host protein secretion
  - Fewer purification steps
  - Fewer unwanted byproducts (proteases)
- High efficiency/no capacity constraints
- Wide range of target proteins capabilities: including complicated r-Proteins.
- Continuous R&D development – published.



# Secretes Fewer Host Proteins

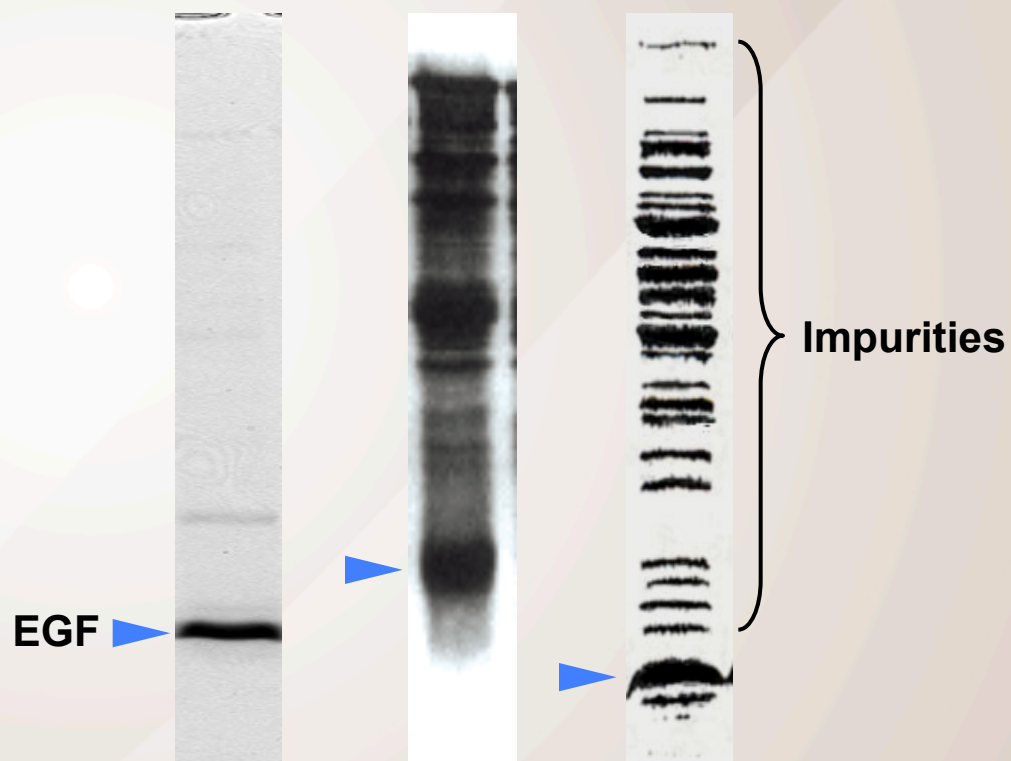
## Transglutaminase

*C. glutamicum* *B. brevis*



## Human Epidermal Growth Factor

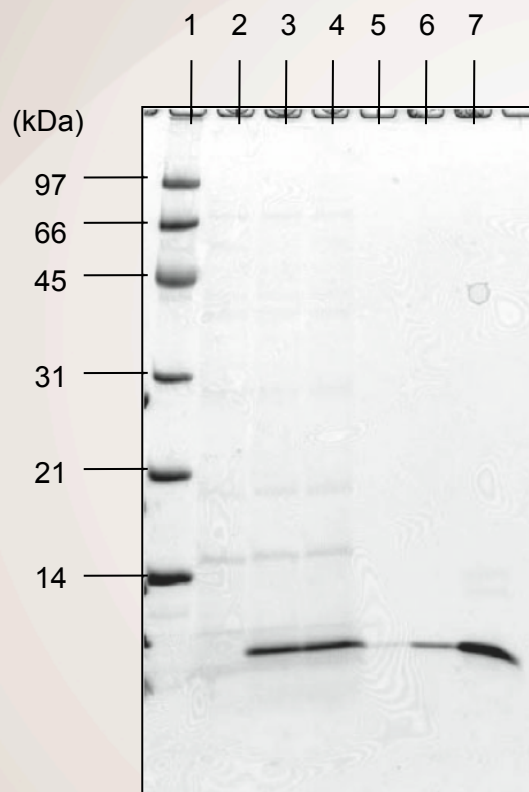
*C. glutamicum* *B. brevis* *E. coli*



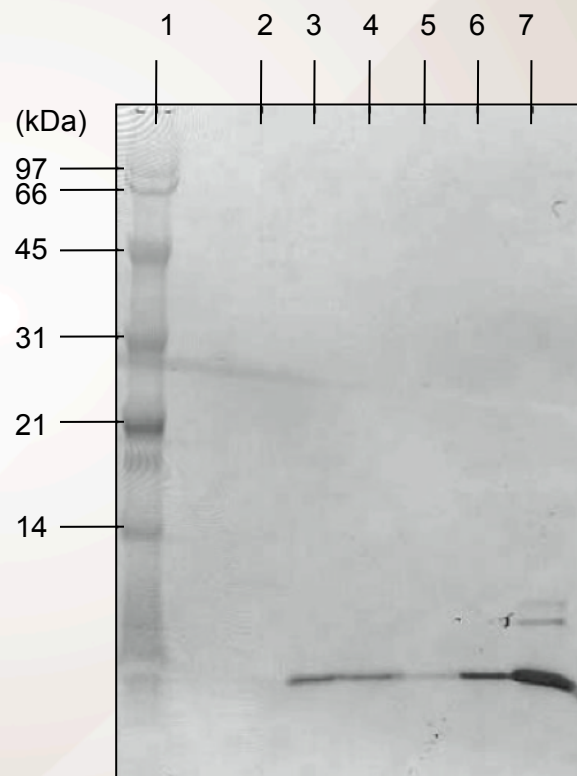
These gels represent the fermentation broth



# Expressing Directly into the Medium with *Folded* Structure



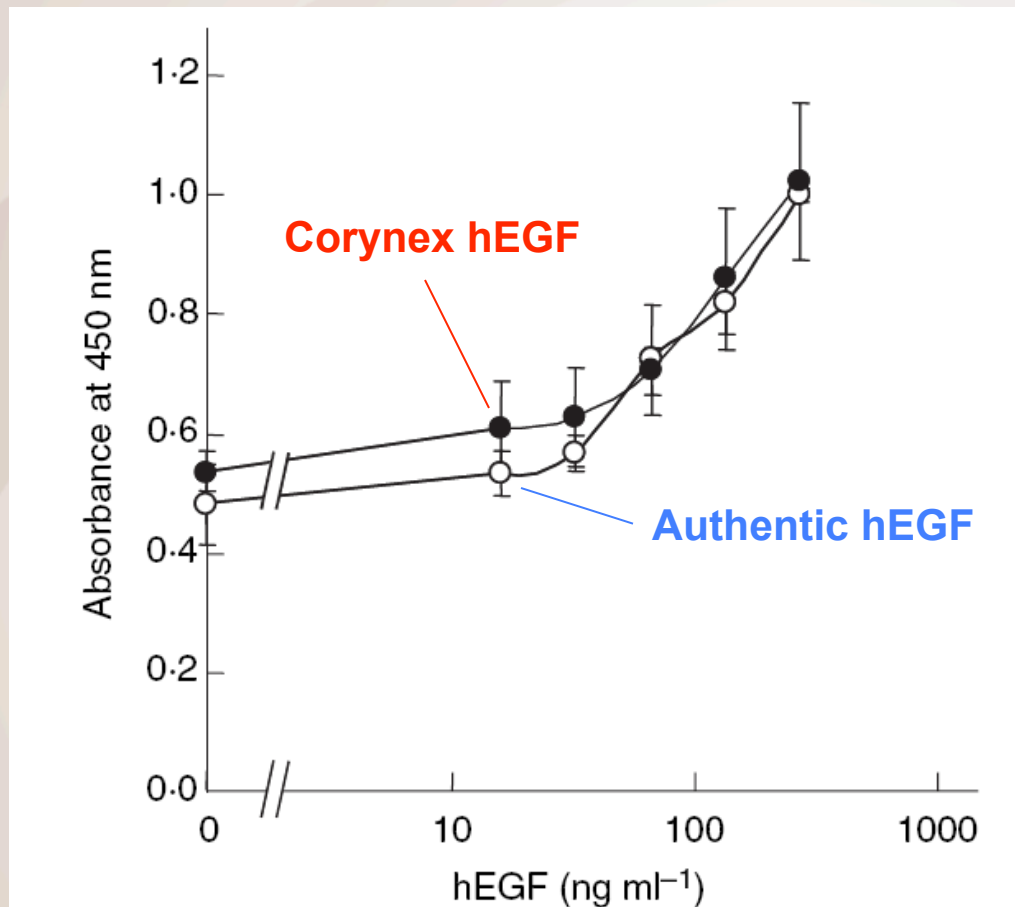
**Gel**



**Western**



## The Protein is *Active*



**Figure 2** Stimulation of the growth of MCF-7 cells by human epidermal growth factor (hEGF). Authentic hEGF (open circle) or hEGF secreted by *Corynebacterium glutamicum* (closed circle) was added to the cultures at various concentrations (0, 15-625, 31-25, 62-5, 125 and 250 ng ml<sup>-1</sup>). After 4 days, the numbers of living cells were measured using the WST-1 assay method.



# Production Process for IGF-1 using Corynex

## GroPep

E.Coli

Fermentation

Cell harvest

Cell disruption

Centrifuge

Refolding

Ion Ex. Chrom.

Rev.-phase Chrom.

Ion Ex. Chrom.

Gel filtration

Ultrafiltration

Diafiltration

Freeze dry

## Ajinomoto

Corynebacterium

Fermentation

pH adjustment

MF filtration (Cell removal)

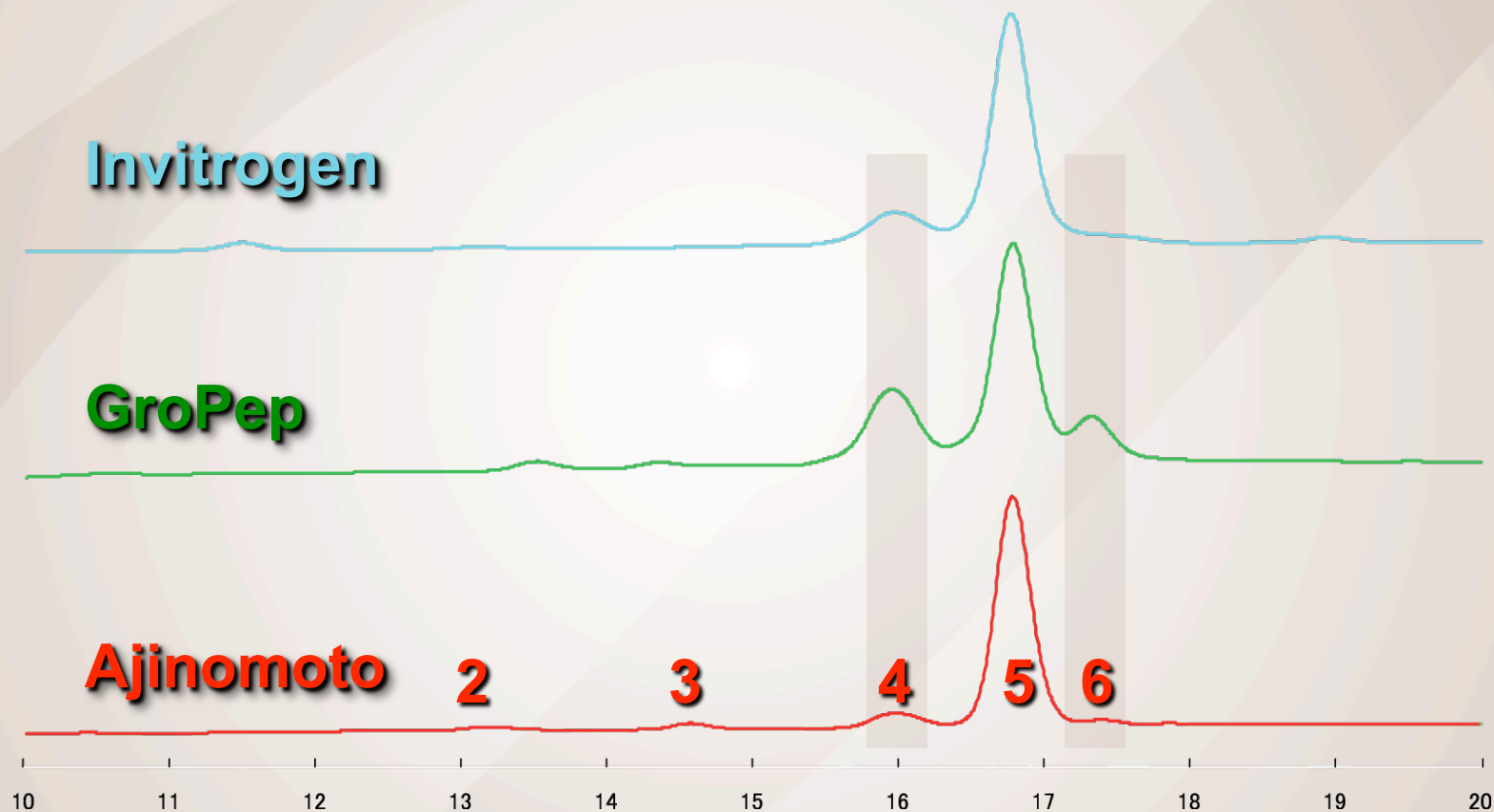
Ion Ex. Chrom.

0.22  $\mu$  m filtration





# Comparison of IGF-1 quality



# A Wide Range of Proteins

Heterologous protein production by CORYNEX in test-tube culture

Protein	Organism
<u>Prokaryote</u>	
Transglutaminase	<i>Streptomyces mobaraensis</i>
Prolyl-endo peptidase	<i>Streptomyces mobaraensis</i>
Serine protease	<i>Streptomyces alboqriseolus</i>
Phytase	<i>Bacillus subtilis</i>
Phytase	<i>Bacillus licheniformis</i>
Phytase	<i>Escherichia coli</i>
Isomalto dextranase	<i>Arthrobacter</i>
Dextranase	<i>Arthrobacter</i>
Protein glutaminase	<i>Chryseobacterium proteolyticum</i>
<u>Eukaryote</u>	
EGF	Human
Insulin	Human
IGF-I	Human
Serum albumin	Human
LIF	Human
scFv	Mouse
GFP	Jellyfish



# Corynex Expression Toolbox

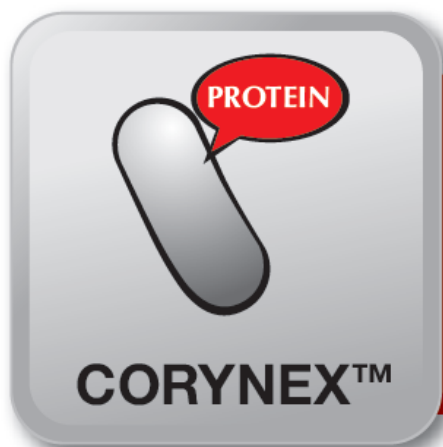
- **Codon Usage**
- **Signal Peptides**
- **Secretion Pathways**
- **Fusion Partners/co-expression products**



# Ajinomoto Corynex Offerings

- Strain Development
- Fermentation Services
- Purification Services
- Protein Characterization
- Analytical Services





**Advanced Science That Delivers Better Results.**  
Visit [www.corynex.com](http://www.corynex.com), e-mail [corynex@ajiusa.com](mailto:corynex@ajiusa.com)  
or call 877-526-7963 for details.

**EXPRESSION SYSTEM**

