



The New Standard For Protein Expression Addressing Speed, Quality and Cost of Goods

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Global Business Development Leader
***Pfēnex* Expression Technology™**

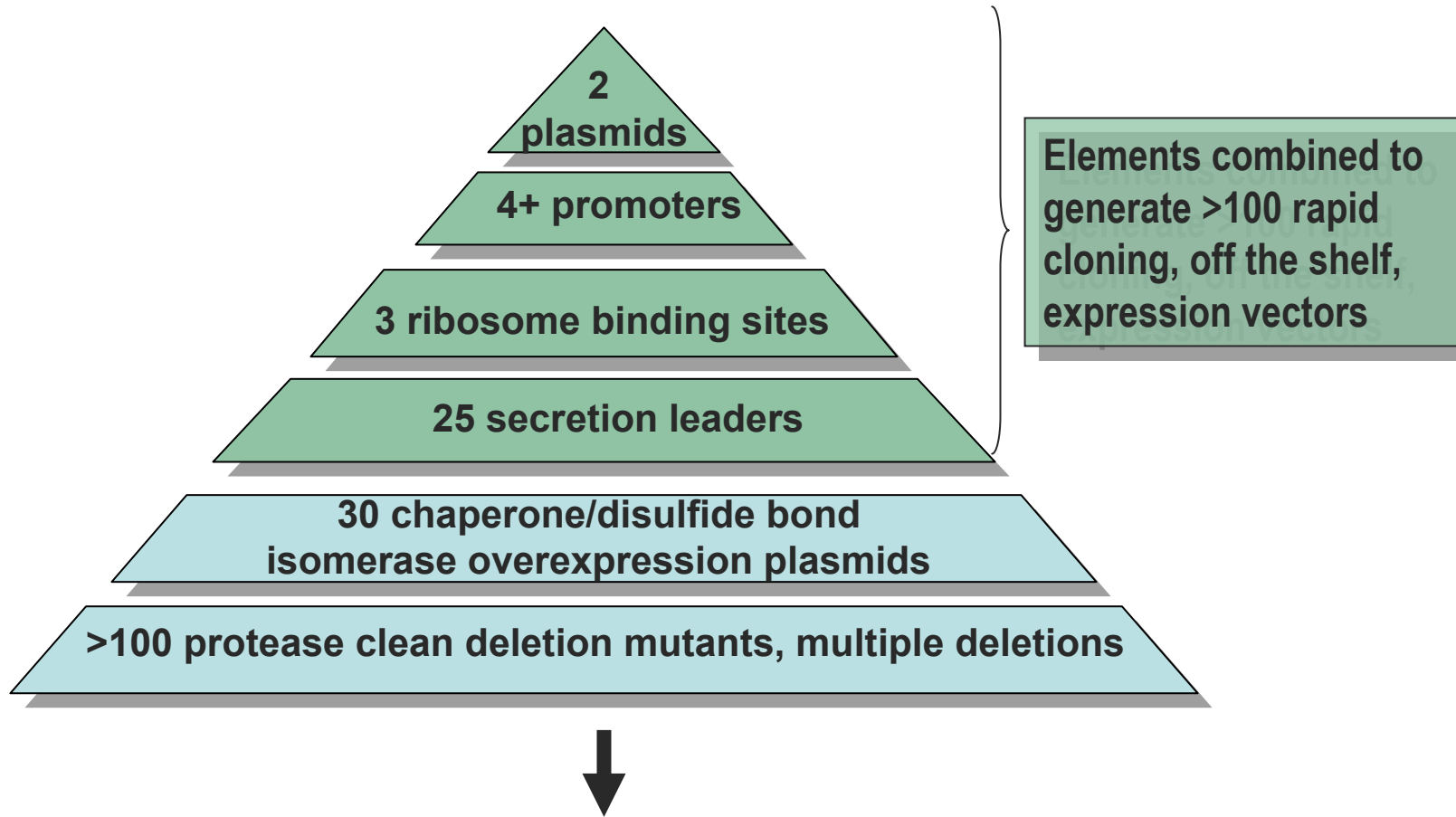
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- Located in San Diego, California
- 40 FTEs primarily in SoCal, with Business Development offices in St. Louis and Boston
- ~32,000 square feet of space in Sorrento Valley housing capabilities in molecular biology, analytical biochemistry, fermentation, downstream processing and product development
- Expertise in protein engine production and strain engineering using *Pseudomonas fluorescens*
 - Current partner programs span from discovery to clinical development stages
 - Pfēnex innovator programs in vaccines and biosimilars/biobetters

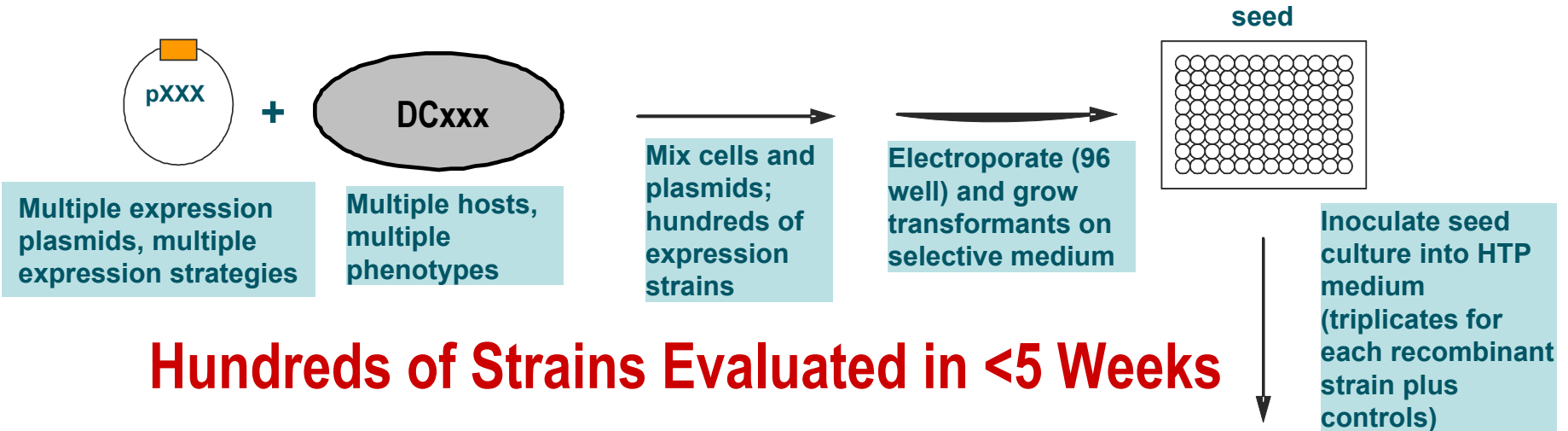


Pfenex Expression Technology™ Toolbox:

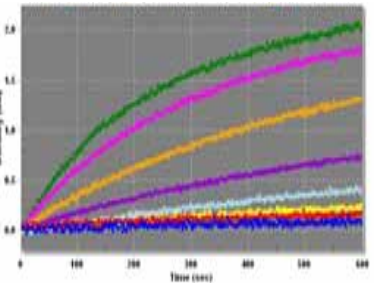


Hundreds of unique combinations enable strain discovery for optimal biopharmaceutical production

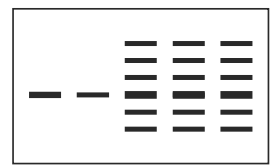
Robotically Enabled High Throughput Strain Development



Hundreds of Strains Evaluated in <5 Weeks



Biolayer Interferometry
Function



SDS-CGE
Titer



HTP Expression
30-50 OD Achieved

Normalize cultures to $OD_{600} = 20$; sonicate cultures



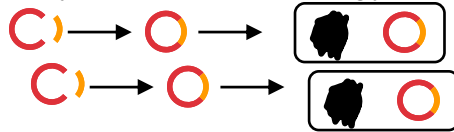
24 hours – induce, OD reading
48 hours – collect cells, OD reading

Analyze soluble / insoluble cellular fractions



Micro/Mini-Bioreactors- Rapid Protein Production

Multiple constructs
Vary expression strategy

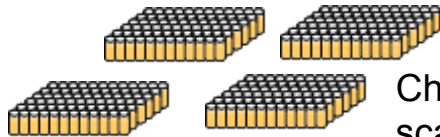


>300 host strains
Altered host cells
for maximal expression

Transformation



Grow-out, induction,
assay



Choose clones,
scale-up

Fermentation range finding; variable
induction conditions in one week
Purify **mgs** of protein

24-unit 5 mL



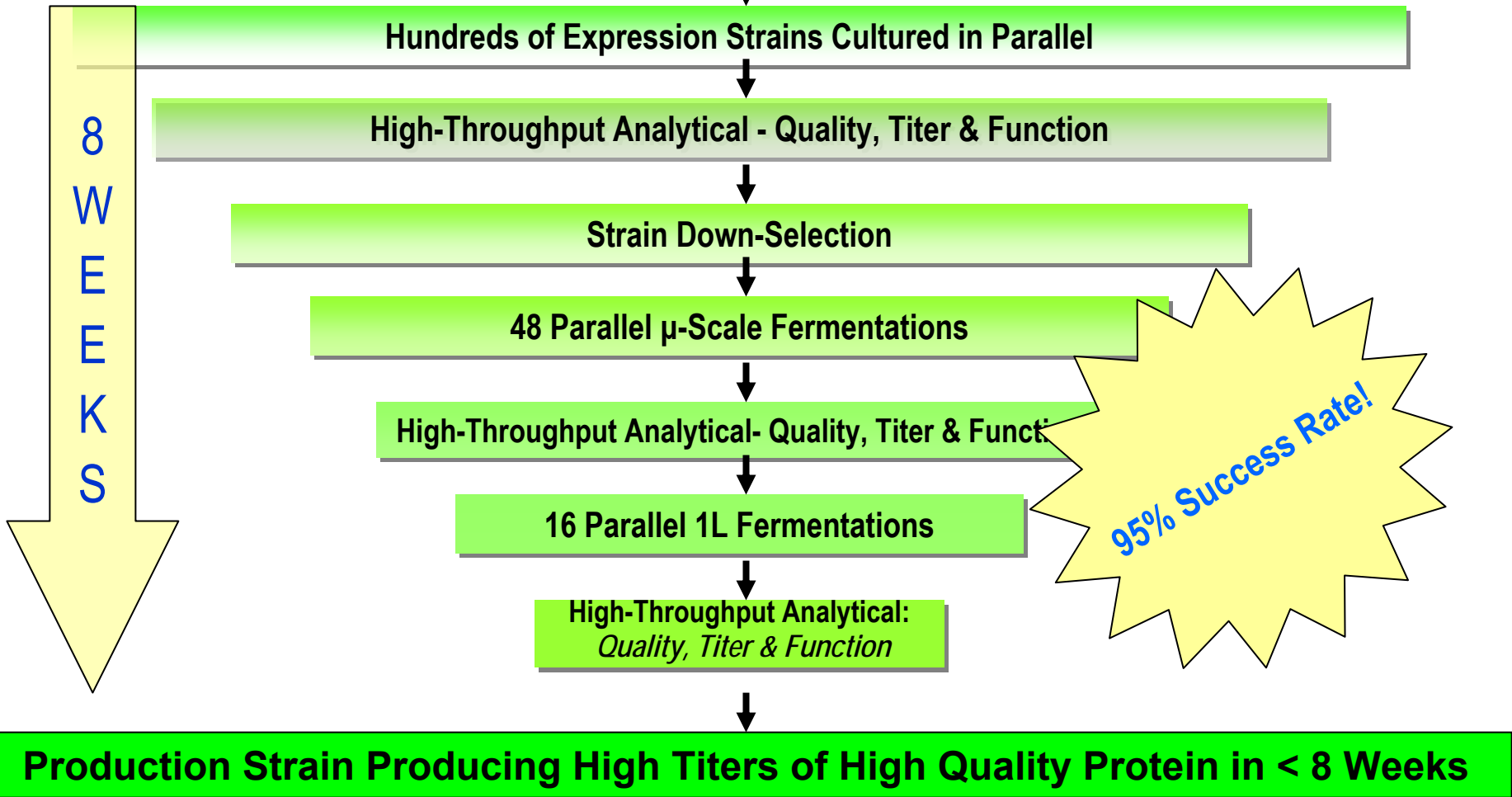
Confirmation in one week
Purify **grams** of protein

8-unit 1.0L



Enables *rapid* production of protein to
speed product progression through
development value chain

DNA + Pfenex Expression Technology Toolbox





Three Components to Overall Cost

Speed -

- 95% success rate in identifying a production strain within 5 weeks
- Rapid advancement of protein leads into proof-of-concept or clinical studies
- Scalability of the process is virtually guaranteed enabling the rapid scale up to support clinical and commercial requirements

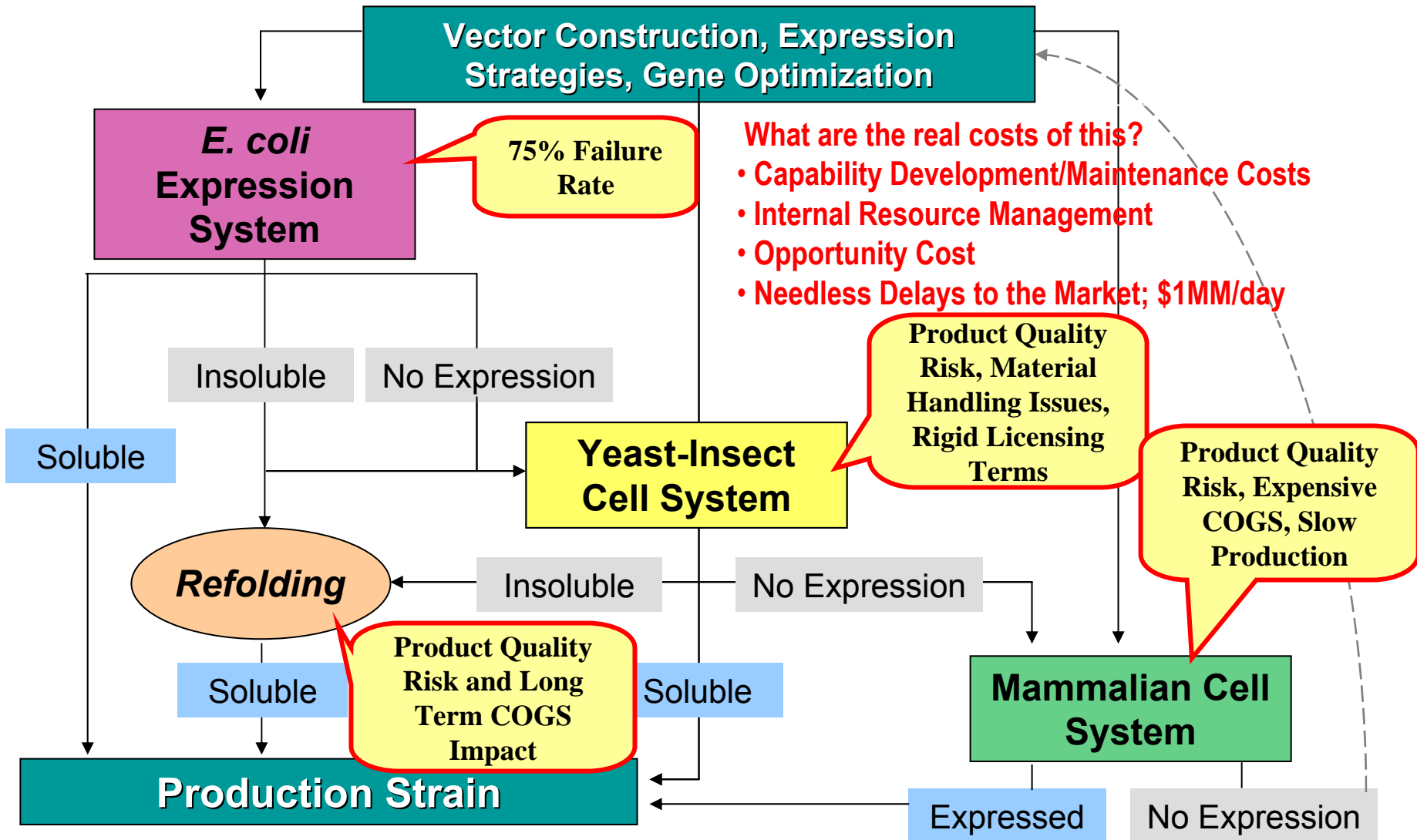
Quality -

- 95% success rate in achieving soluble active expression enabled through the over expression of folding modulator proteins
- Extensive library of protease knockout strains ensures the avoidance of proteolytic clipping of the target protein
- Lack of post translational modifications ensures homogeneity of the target protein

Overall Yield -

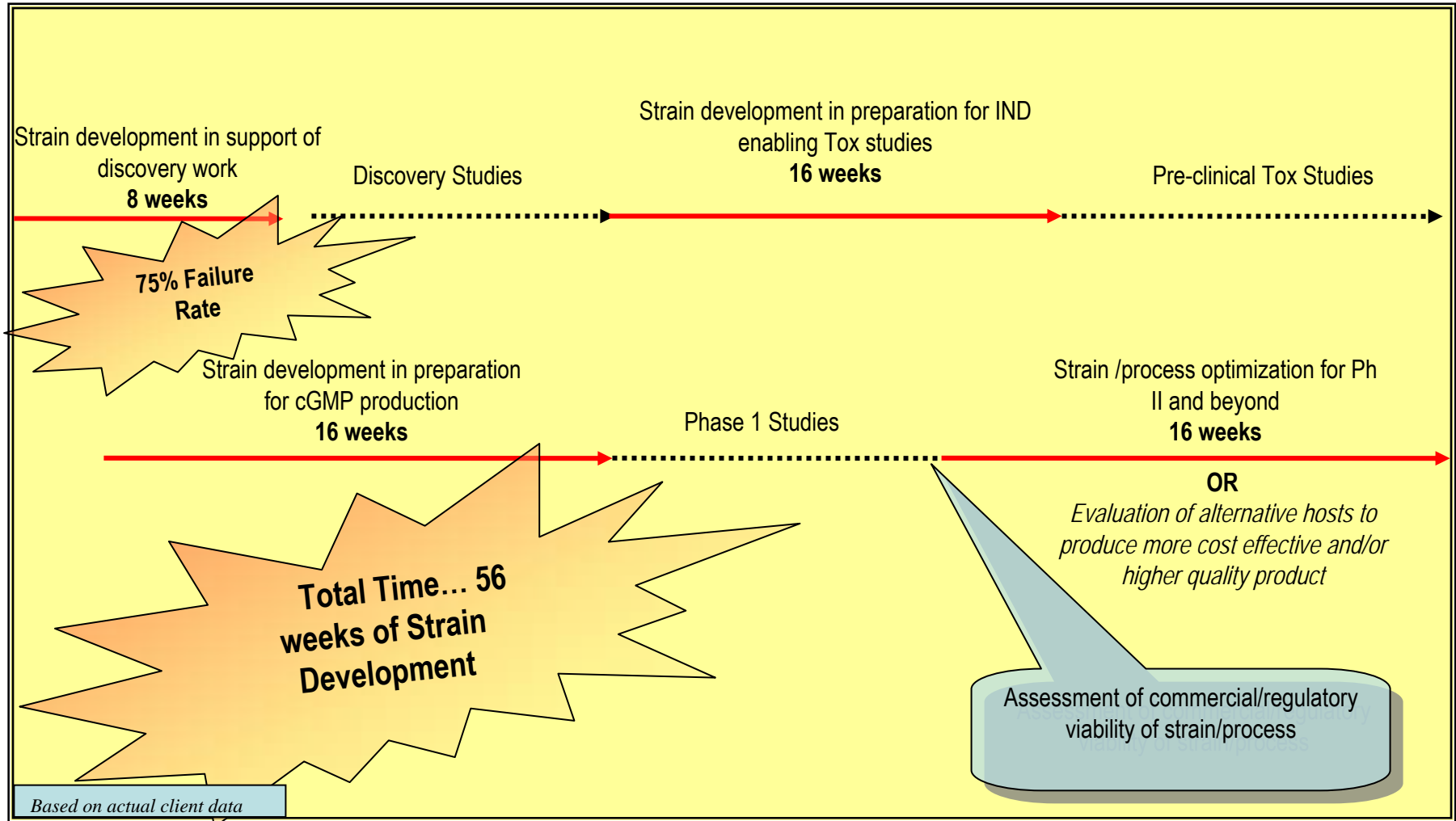
- 36 hour fermentation cycle time
- Low cost fully defined medium with no animal-derived components
- High specific and volumetric *soluble* expression
- Advantaged protein recovery technologies

Traditional Strain Engineering: Linear, Iterative, *Expensive*



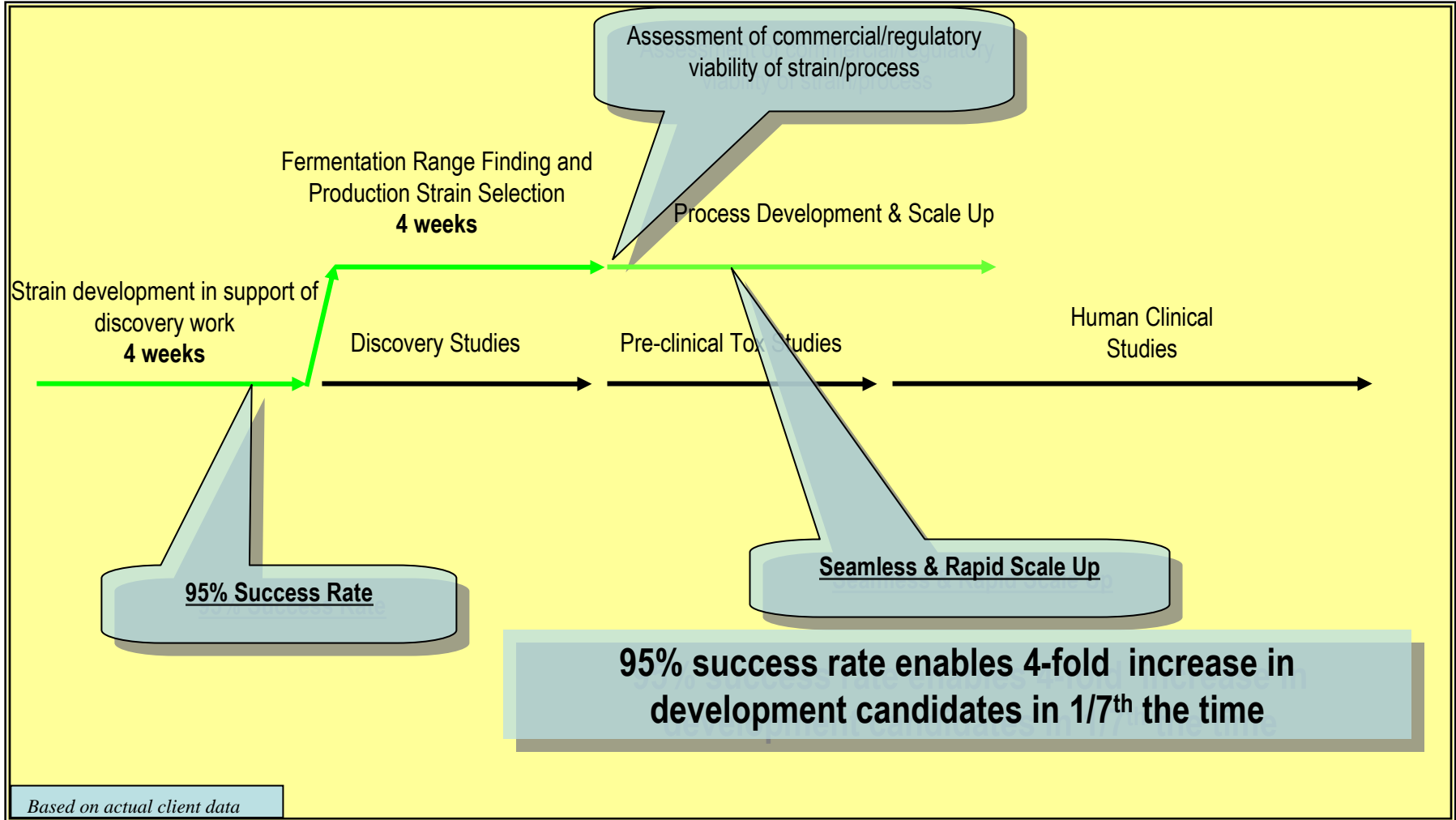


Typical Strain Development ...





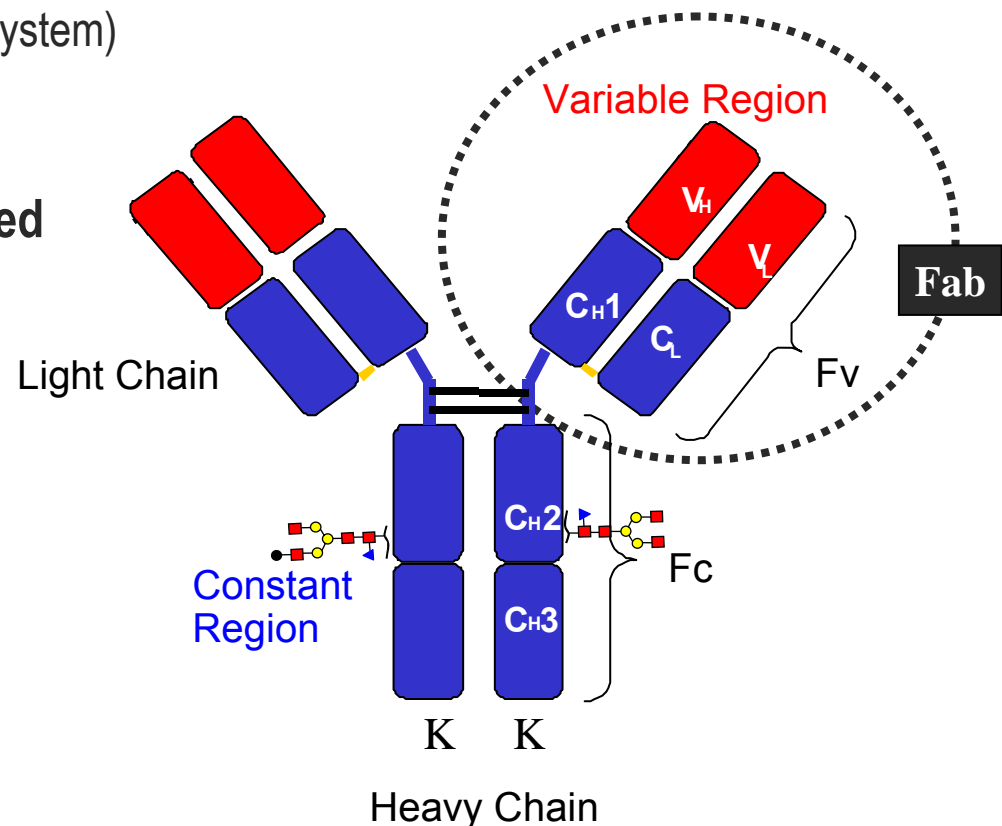
The Pfēnex Success Rate & Speed Advantage....





Speed Case Study...

- Fab-based therapeutic (no inter-chain disulfide)
- **Anticipated high-volume product**
- Multiple expression technologies evaluated
 - bacterial (*E. coli*-based secretion system)
 - yeast
 - mammalian
- Significant challenges encountered
 - **productivity**
 - product quality



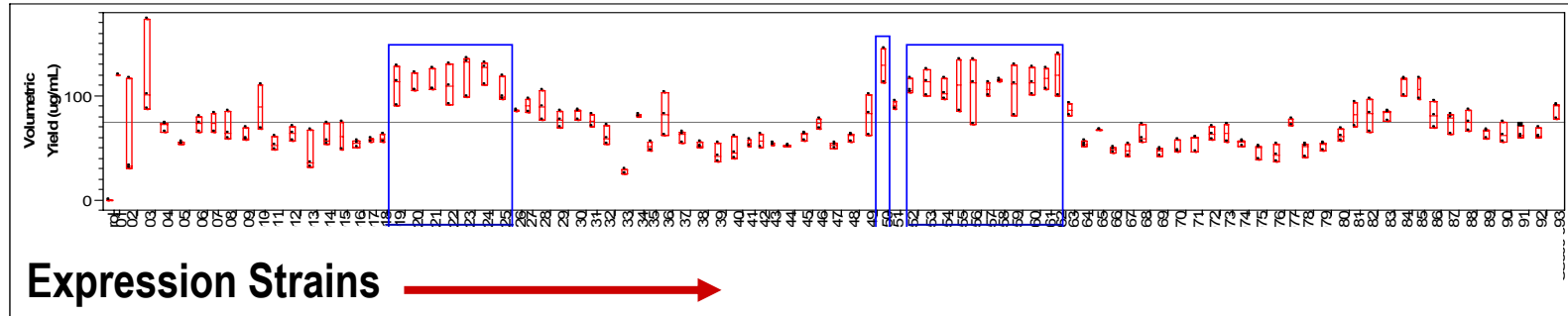


High Throughput Screening: Fab

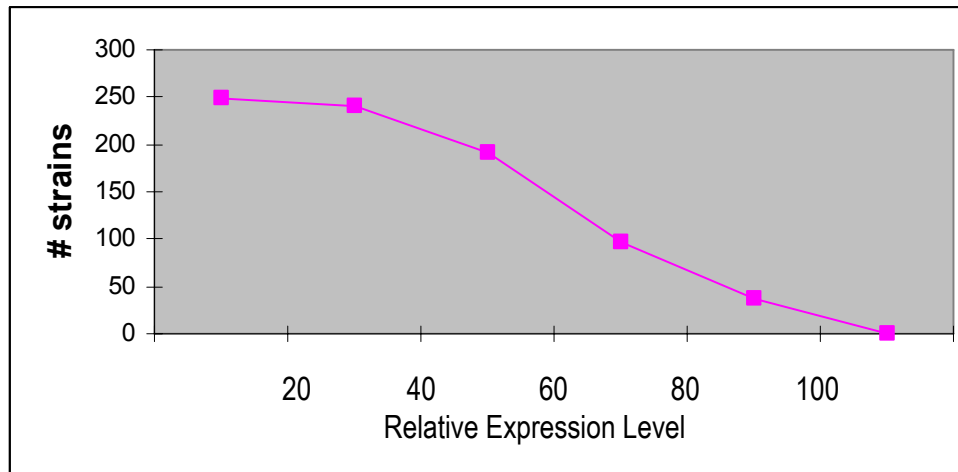
Periplasmic expression, multiple secretion leaders:
8 expression plasmids x 31 expression hosts=
248 strains tested in parallel
96 well format. 0.5 ml cultures

Maximum
100-200 mg/L

Relative
Expression



Secretion leader choice for each chain *and* host strain have impact on yield

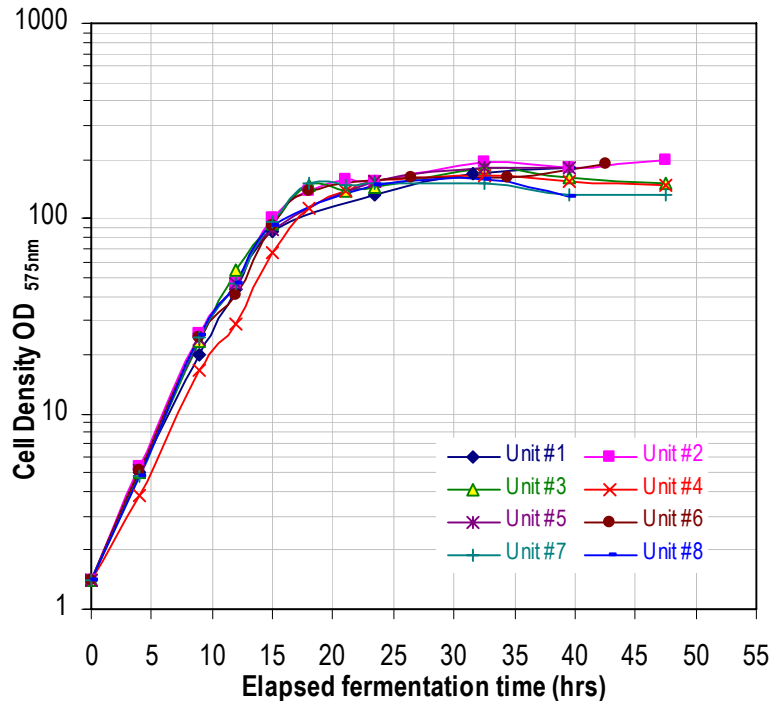


- Most strains express a small amount of active Fab
- Only a small subset exhibit high levels of active Fab

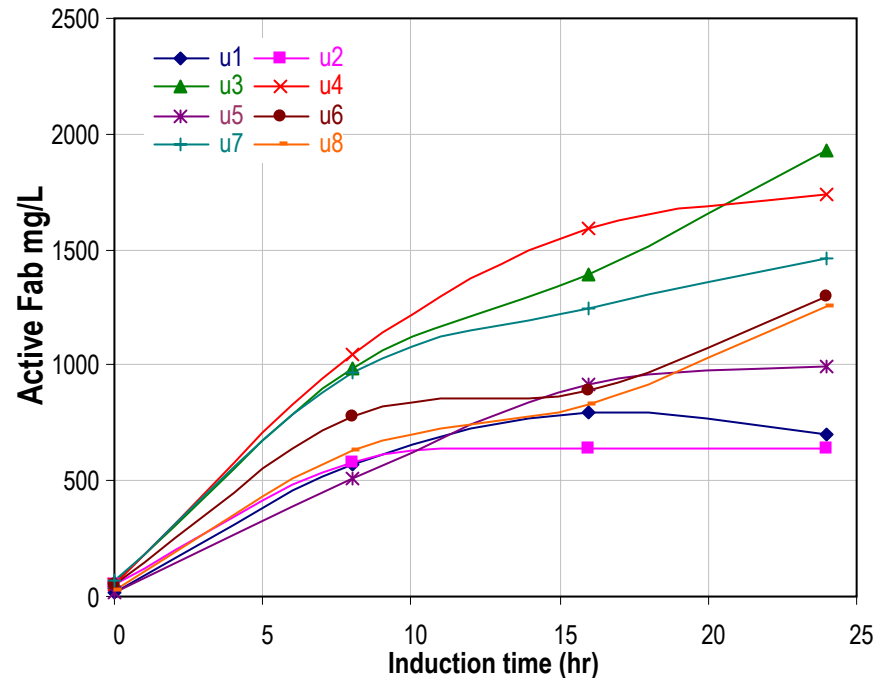
Fab Production in *Pfenex* Expression Technology™



Fermentation Range-Finding



Productivity of Active Fab Fragment



Eight 1L fermentations with variable induction parameters identified conditions producing soluble and active Fab fragment at a 10-20 fold improvement over 96 well scale, within one week



Fab Strain Engineering Results

- **Rapid Strain Engineering**
 - Six weeks from DNA sequence to data on Titer, Quality and Function
- **Very high productivity**
 - ~1000 mg/L/day, 5 to 20 fold over other tested systems
 - limited range-finding optimization points to potential further improvement
- **Superior product quality**
 - proper N-terminal processing of secretion signal (LC-MS)
 - limited proteolysis (LC-MS and RP-HPLC)
 - proper intra-chain disulfide formation (peptide mapping)
 - equivalent antigen binding characteristics (BIAcore)
 - no antigen contamination (ELISA)



Fab Expression Summary

| Technology | Harvestable Productivity (mg/L/day) | Product Quality |
|--------------------------------------|-------------------------------------|---------------------------------|
| <i>E. coli</i> | 200 | Secretion signal mis-processing |
| Yeast | 50 | Proteolysis, mannosylation |
| Mammalian | 150 | Antigen contamination |
| Pfēnex Expression Technology™ | 1000 | Superior Protein Quality |



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Quality -

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Overall Yield -

- 36 hour fermentation cycle time
- Low cost fully defined medium with no animal-derived components
- High specific and volumetric *soluble* expression
- Advantaged protein recovery technologies



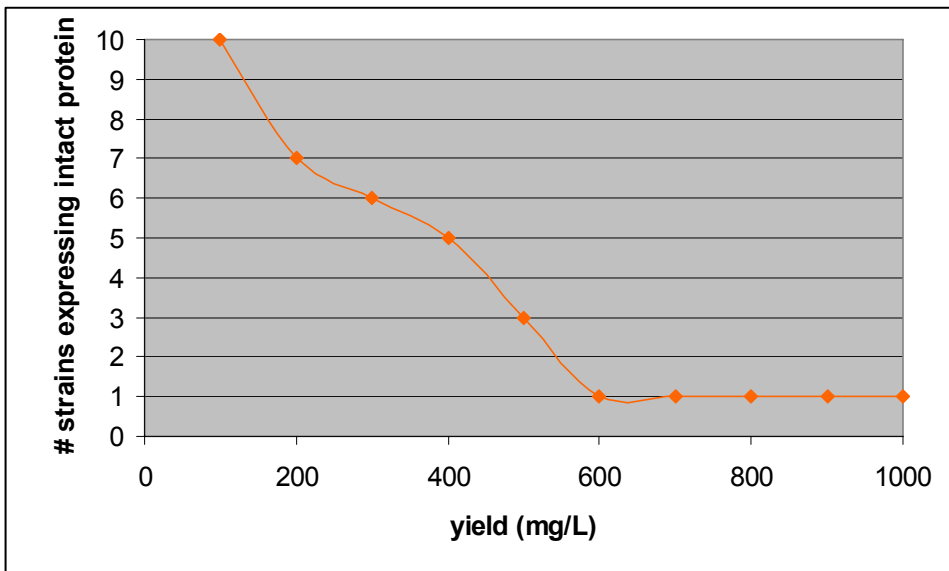
Quality Case Study

- **Growth factor variant**
- **Anticipated high volume product**
- **Multiple expression technologies evaluated**
 - yeast
 - mammalian
- **Significant challenges encountered**
 - productivity
 - **product quality**



Expression of Growth Factor Protein in *Pfēnex*

- Fifteen “Rapid Cloning” vectors constructed containing 15 unique *P. fluorescens* Sec-type secretion leaders
- Plasmids are transformed into 20 individual host strains having phenotypes designed to stabilize recombinant, expressed proteins (e.g. protease knockouts, chaperones over-expressed)
- 300 unique strains grown and expression of Growth Factor tested in parallel



Host strain and secretion leader have significant impact on yield

- Only 10 of 300 strains tested produced any full length protein
- 5 secretion leaders; 2 host strains represented

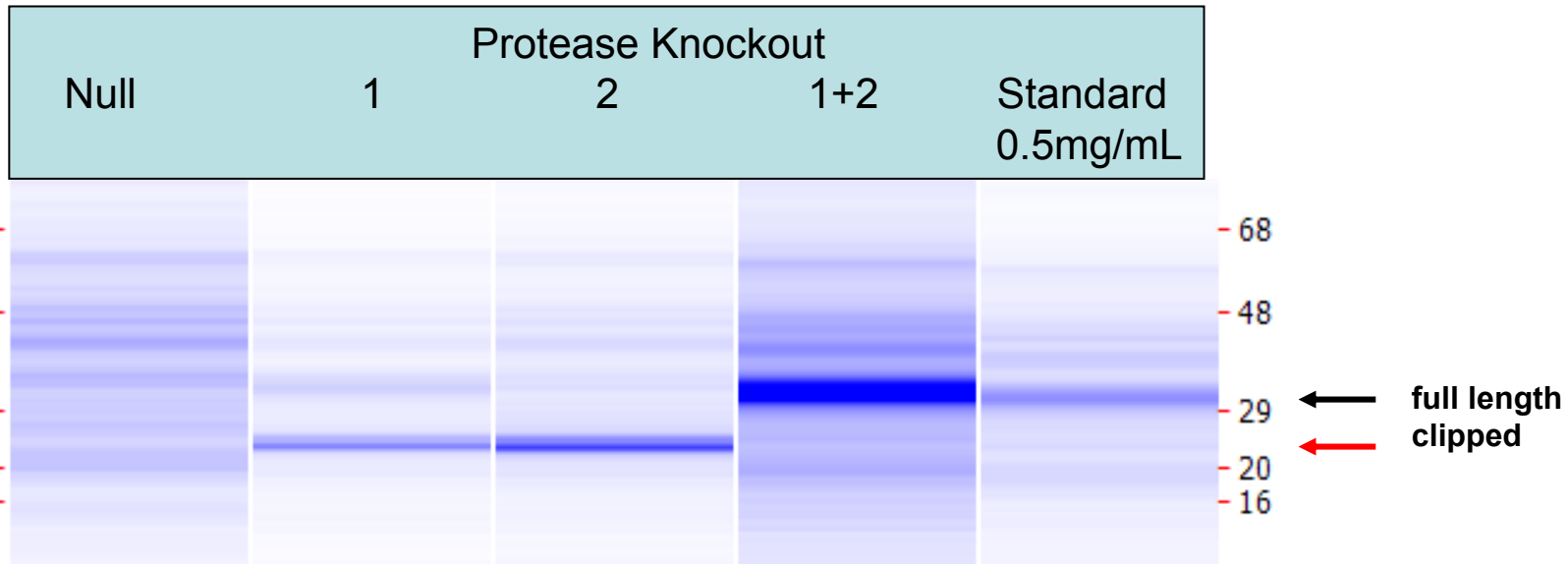


Expression of Growth Factor Protein in *Pfēnex*

Use of a dual protease knockout strain results in marked increase in expression of High Quality Protein

- Very high titer; >1 g/L in 96 well format
- Superior product quality, no degradation
- Soluble and active protein

Strain choice controls expression level and quality





Growth Factor Strain Engineering Results

- **Rapid Strain Engineering**
 - Six weeks from DNA sequence to data on Titer, Quality and Function
- **Extremely high productivity**
 - ~750 mg/L/day at 96-well scale
 - expect >3 fold increase at scale
- **Superior product quality**
 - proper N-terminal processing (LC-MS)
 - limited proteolysis – at site introduced to minimize mannosylation (LC-MS and RP-HPLC)
 - no mannosylation or other modifications (LC-MS)
 - proper disulfide formation (peptide mapping)
 - equivalent bioactivity (bioassay)



Growth Factor Expression Summary

| Technology | Harvestable Productivity (mg/L/day) | Product Quality | Comments |
|--------------------------------------|-------------------------------------|--------------------------------------|--|
| Yeast | 50-500 | extensive proteolysis, mannosylation | several systems evaluated |
| Mammalian | <50 | proteolysis, O-glycosylation | not preferred for high-volume products |
| Pfēnex Expression Technology™ | 750 (96-well scale) | Superior Product Quality | Meets commercialization target |



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Pfēnex Expression Technology Driving COGS Lower...

Examples of Stalled Development:

| Protein Type | Alternative Host | Pfēnex Results |
|----------------------------------|---|---|
| Fab | Yeast: quality issues, low yield | 10-20X yield improvement high quality at 1L scale |
| Microbial outer membrane protein | <i>E. coli</i> : no expression | Soluble active expression multiple g/L; 1.0L scale |
| Growth Factor | Yeast: low yield, degradation, glycosylation | 20X yield improvement at HTP scale , high quality, active |
| Therapeutic Enzyme | <i>E. coli</i> : undesirable isoforms, quality issues | 10X yield improvement ; no isoform issues |
| Human Cytokine | <i>E. coli</i> - inclusion bodies; no soluble expression | Soluble active expression ; elimination of refold step |
| Vaccine Antigen | <i>E. coli</i> : plasmid instability, very low expression | 26X yield improvement ; soluble active protein; plasmid stable |
| Vaccine Antigen | Native organism; cost issues | 10X yield improvement |

 ...Creating Success from Failure

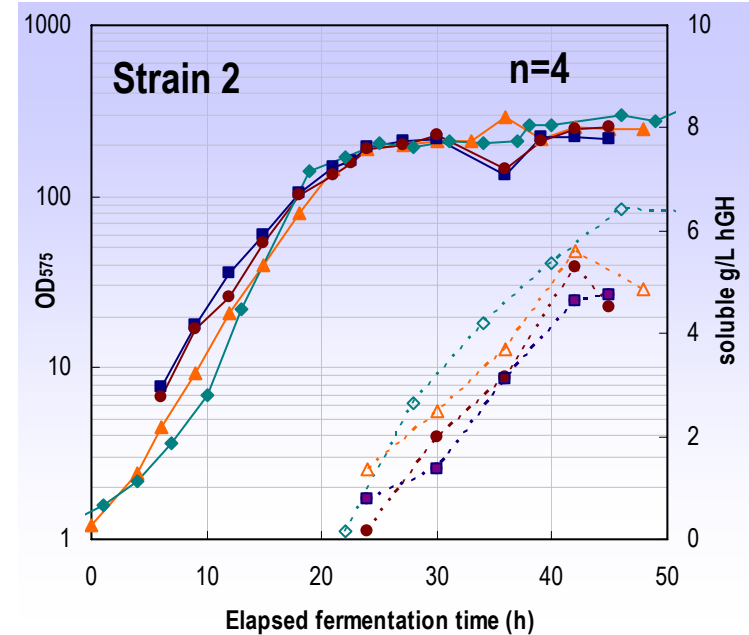




Soluble Protein Expression Decreases COGS

Innovator Molecule is cost challenged by insoluble expression and expensive refolding process

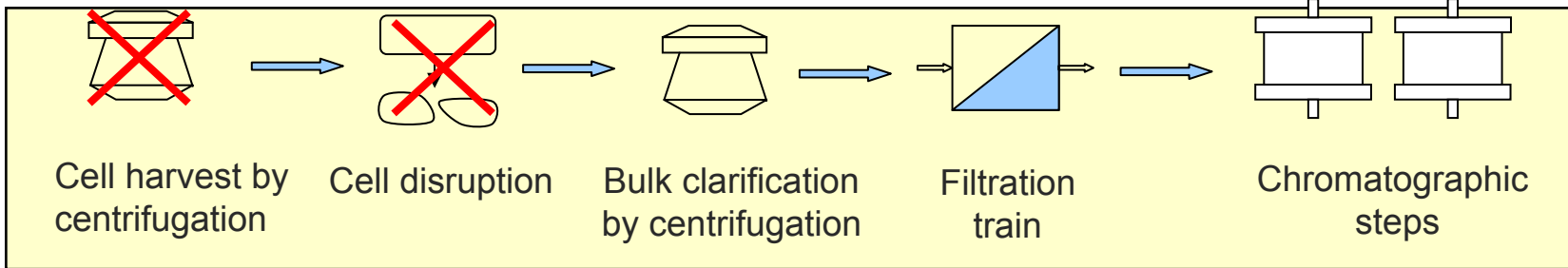
| hGH Strain | g/L insoluble hGH | g/L soluble hGH |
|----------------------------------|-------------------|-----------------|
| Strain 1, Cytoplasmic expression | 13.5 ± 1.5 | ND |
| Strain 2, Periplasmic expression | 2.7 ± 0.9 | 5.5 ± 0.7 |
| Strain 3, Periplasmic expression | 0.5 ± 0.1 | 4.4 ± 0.2 |



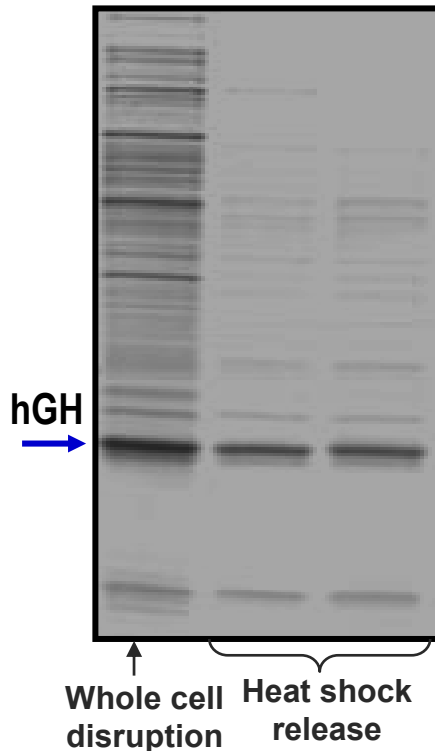
Soluble expression of active hGH in *Pf_{en}ex* confers a greater than 3-fold manufacturing cost advantage

In this commoditized market, lower COGS allows maintenance of margin, providing potential of development of differentiation strategies, and improved market share

Periplasmic Release Results in Further Reduction in COGS



SDS-PAGE analysis of cell extracts



Heat shock release from periplasm:

- Process demonstrated using scalable equipment
- Centrifuge throughput ↑ 2X
- Filtration capacity ↑ 3-5X
- Dynamic binding capacity of capture column ↑ 3X
- Reduced number of steps and process time

Heat shock release yield = 80%
 Centrifugation yield = 80%
 Depth filtration yield = >95%
 UF/DF yield = >95%
 Sterile filtration = >95%



Biopharm Services bioSolve Model

COGS Analysis Summary:

- ***Pfēnex* results in lower cost of goods than *E. coli*, *Pichia* and CHO**
 - In a representative scenario, at identical expression levels, *Pfēnex* COGS was 79%, 18% and 43% lower than *E. coli*, *Pichia pastoris* and CHO, respectively
- **Requirement for refolding from inclusion bodies results in the highest COGS for *E. coli***
 - For comparable COGS, *E. coli* insoluble expression needs to be about 4 to 5-fold higher than soluble, periplasmic expression in *Pfēnex*
- ***Pfēnex* increases capital efficiency**
 - At identical expression levels, *Pfēnex*-based process produces 1.6X, 3.2X and 4.8X product compared to processes based on *Pichia pastoris*, CHO and *E. coli*, respectively



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The *Pfēnex* Advantage over Traditional Technologies: Improvements in *Speed, Titer, Quality = Lower Cost of Goods Sold*



Traditional Technologies (*linear, iterative*)

Pfēnex Expression Technology™ (*high throughput, parallel process*)

E.coli

25% success rate
 Refold Step introduces product variability and increased costs
 Antibiotics required for plasmid maintenance
 Poor performance at small scale and scalability not predictive due to facultative anaerobic nature
 Limited toolbox

95% success rate

Routine Soluble Active Expression
 Complementation obviates the need or antibiotics
 Routinely achieve 30-50 OD₆₀₀ at 0.5 mL scale and has successfully scaled to thousands of liters due to obligate aerobic nature
 Extensive toolbox

Yeast

Heterogeneous Glycosylation
 96 hour upstream
 Material Handling issues (methanol and waste streams)
 Proteolytic degradation
 Limited toolbox

Homogeneous product
 36 hour cycle time on average
 Fully defined medium, no animal derived materials
 >100 unique protease knockout strains
 Extensive toolbox

Mammalian

Minimum 336 hr cycle time
 Expensive Media
 Capital Intensive

36 hour cycle time on average
 Inexpensive fully defined medium

Key Benefits Summary of *Pfenex* Expression Technology™



• Speed of Development

- ✓ Extensive expression toolbox: expression strategies and host strains
- ✓ Robust high throughput methods developed to identify best strains quickly
- ✓ Rapid fermentation optimization and reliable scale-up to thousands of liters
- ✓ More leads through development, allowing more & faster “go, no go” decisions

• Product Quality

- ✓ Correct disulfide bonding
- ✓ Secretion leader processing fidelity
- ✓ Very low product heterogeneity
- ✓ No glycosylation

• Cost of Goods

- ✓ High specific and volumetric expression of target protein
- ✓ High cell density, defined mineral salts medium
- ✓ Two day fermentation turnaround
- ✓ Simplified and scalable downstream processing methods, periplasmic release; no unique processing equipment required

• Safety

- ✓ *Pseudomonas fluorescens* – nonpathogenic, BSL-1 organism
- ✓ No antibiotics required; no animal-derived components
- ✓ Host cell protein kit for *P. fluorescens* available through Cygnus Technologies



The combination of technologies available through the **Pfēnex Expression Technology™** platform make it operationally the most complete, flexible and productive bacterial strain development and biopharmaceutical production platform available today.





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Hank Talbot, Ph.D.: Head of Research and Site Operations

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All of our Industry Partners!

