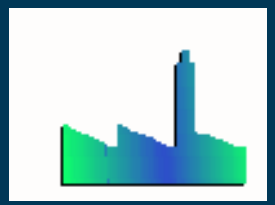
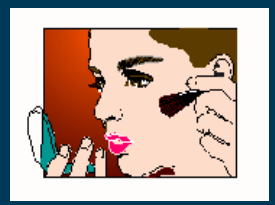
DYADIC®

THE *Chrysosporium lucknowense* EXPRESSION SYSTEM FOR THE PRODUCTION OF THERAPEUTIC PROTEINS

SIM Annual Meeting – August 1, 2006

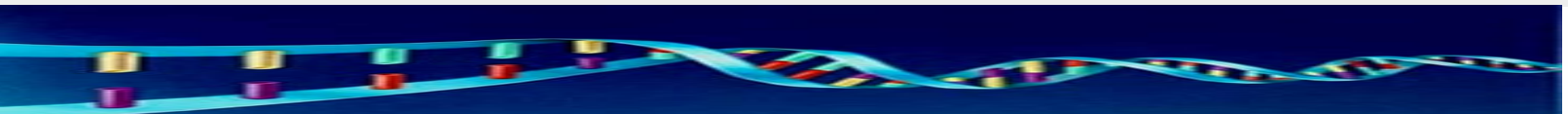




Forward Looking Statements

This Presentation contains forward-looking statements that are subject to various risks and uncertainties. Such forward-looking statements are made pursuant to the "safe-harbor" provisions of the Private Securities Litigation Reform Act of 1995 and are made based on management's current expectations or beliefs as well as assumptions made by, and information currently available to, management. A variety of factors could cause actual results, performance or achievements of Dyadic International, Inc. to be materially different from those that may be expressed or implied by these forward-looking statements and they are included in Dyadic's filings with the Securities and Exchange Commission, specifically in the "Description of Business--Risk Factors that May Affect Future Results" section of Dyadic's Annual Report on Form 10-KSB for the fiscal year ended December 31, 2005, and in subsequent filings with the SEC.



DYADIC®

Presentation Outline

Company description

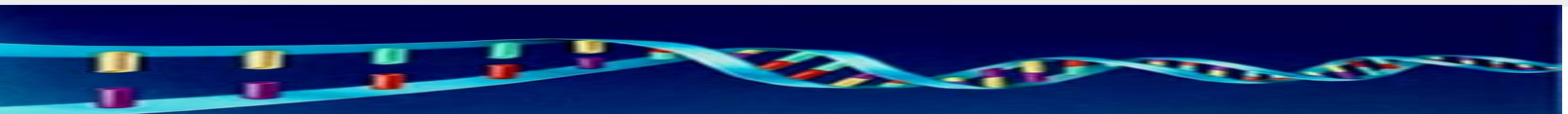
- Motivation for the work

Advantages of fungal expression systems

Description of C1 expression technology

Production of human proteins in C1

Next steps



DYADIC®

Dyadic – A Global ~$16 million (FY'05) Biotechnology Company (AMEX:DIL)



Enzyme

Develop, manufacture and market enzymes and other biological products for a variety of industrial uses




Textiles | Food | Animal Feed | Pulp & Paper

Biorefineries

Develop and manufacture fuels & chemicals from agricultural feedstocks


Ethanol | Chemicals

BioSciences

Focus on developing and producing antibodies and other therapeutic proteins


Pharmaceutical | Biotech

DYADIC®

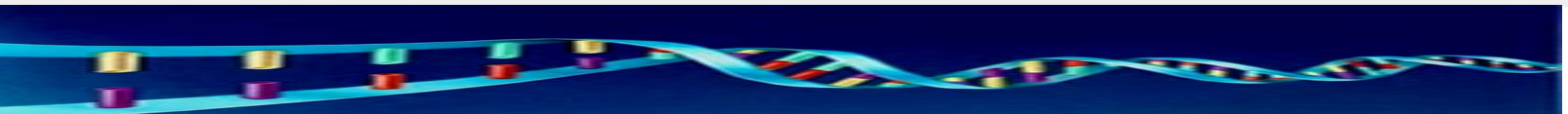
Dyadic's Integrated Technology Platform

- Based on *Chrysosporium lucknowense* (C1), a non-pathogenic fungal organism
  - Gene Discovery – HTS of expressed gene libraries
  - Gene Expression – high level protein production
  - Protein Production – fermentation up to 150,000 liters

- Proprietary and patent-protected technology

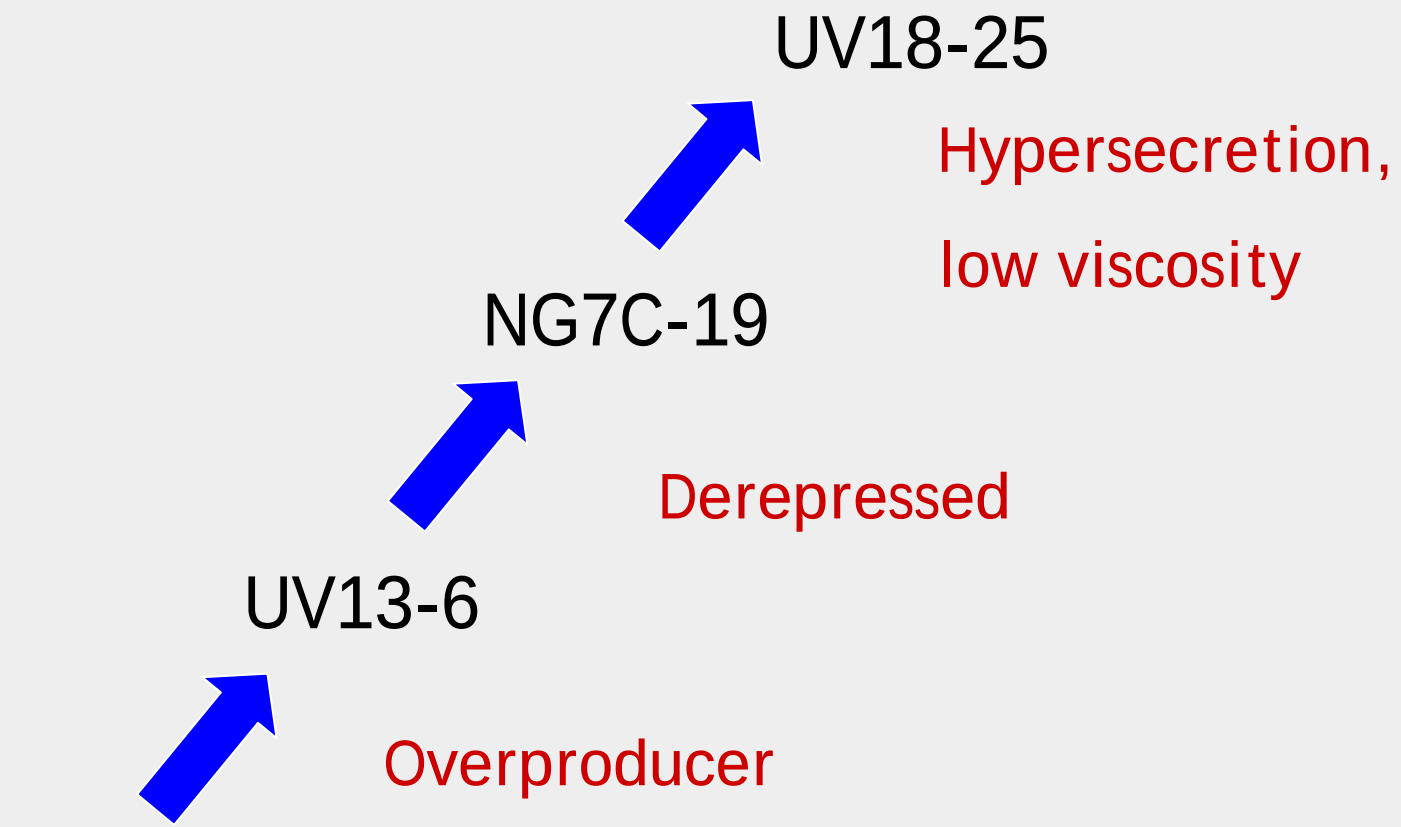
- Molecular genetics tools in place for strain development

- Key enabling feature of this technology platform is *morphology* characterized by *hyphal fragmentation* and *low viscosity*

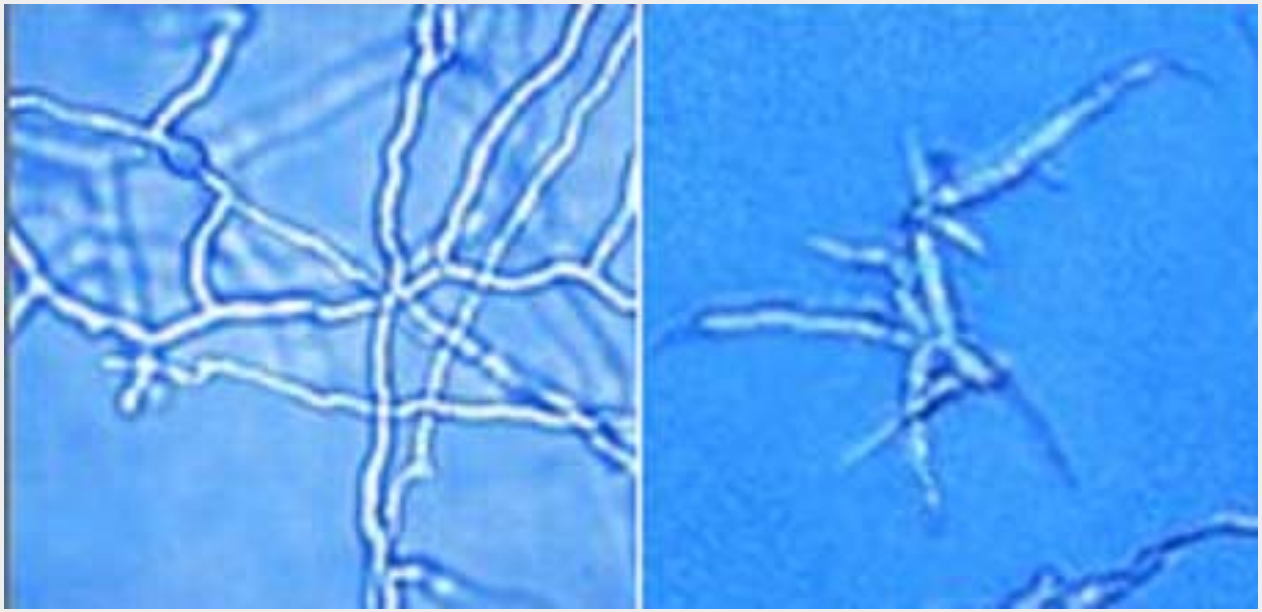


DYADIC®

C1 Strain Lineage

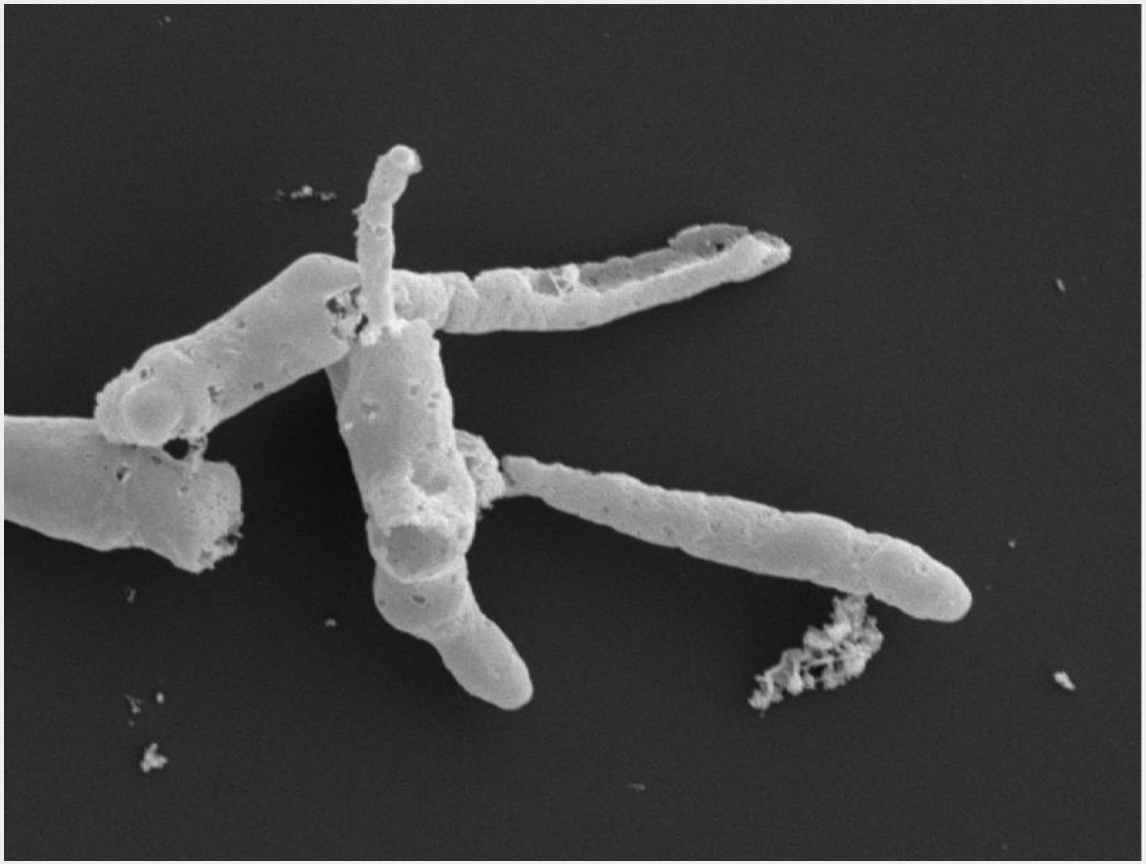


Wild Type C1 Evolved C1



Fungal morphology

Electron Micrograph of Hyphal Fragment



Advantages of Culture Morphology

Ultra high expression of proteins

- High secretion rates
- Low viscosity allows use of richer medium and higher aeration rates

Amenable to laboratory automation

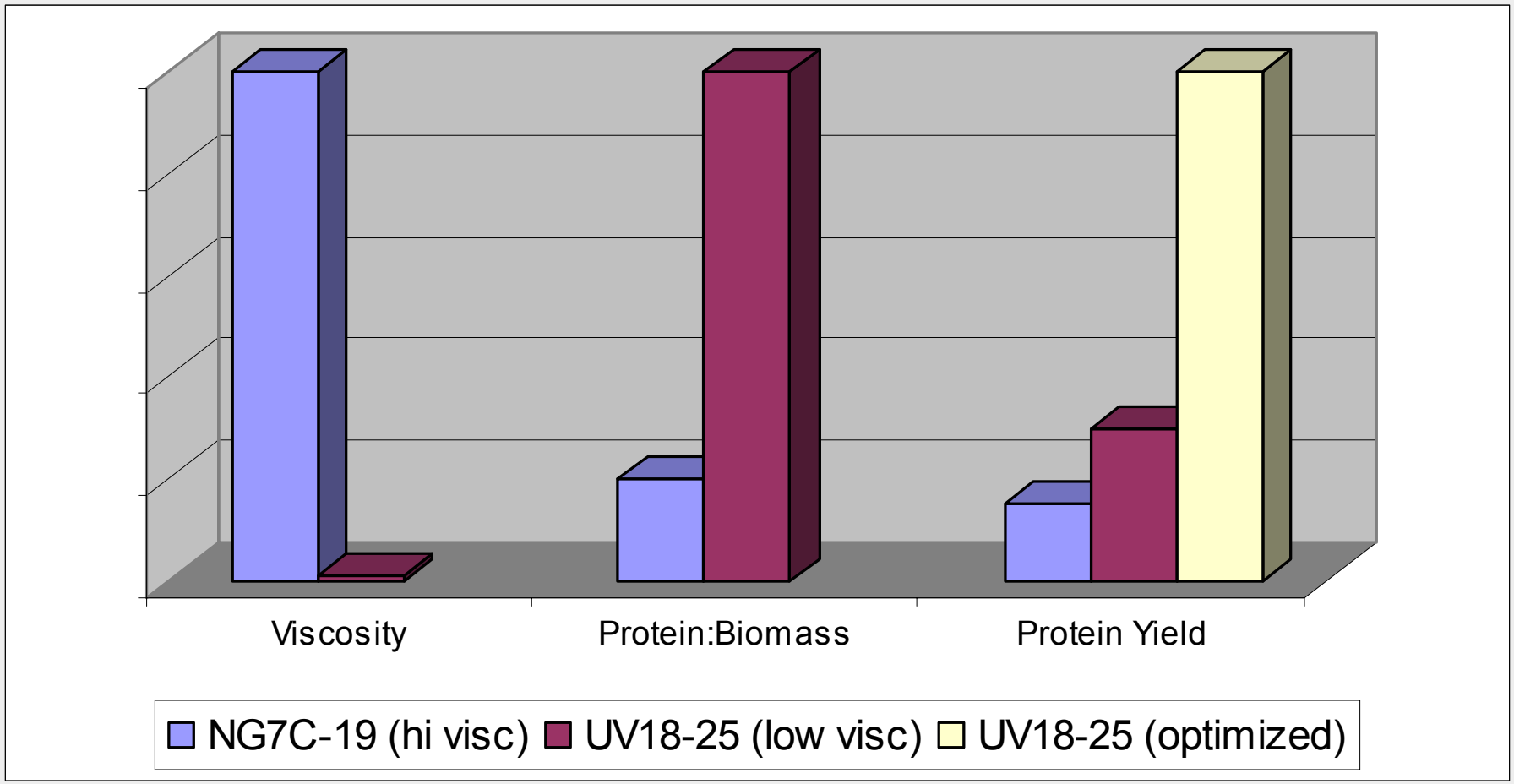
- No canula tip clogging
- Lack of well-to-well contamination

Influence of Morphology on C1 Technology

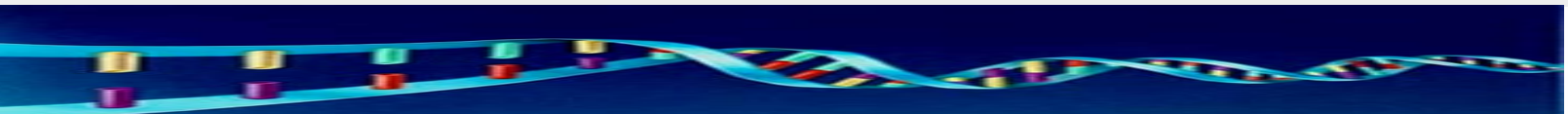
Morphology has enabling influence on several fungal technologies

- Commercial enzyme production in C1
- High-Throughput Screening in C1
- Recombinant gene expression in C1

Influence of Morphology on C1 Cellulase Fermentation



C1 Gene Expression System



DYADIC®

Gene Expression Tools Fully Developed

High transformation frequency

Several markers

- Auxotrophic and antibiotic resistance

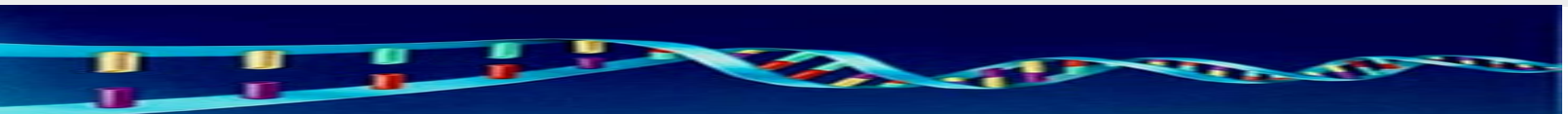
Ability to construct strains containing no foreign DNA

Multiple promoters available

- Constitutive and inducible

Several secretion signals

Knock out technology

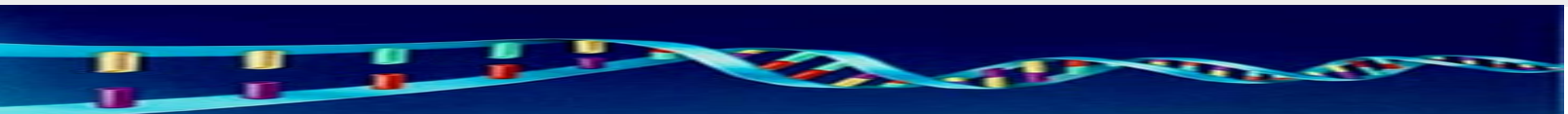


DYADIC®

Improved Hosts for Gene Expression

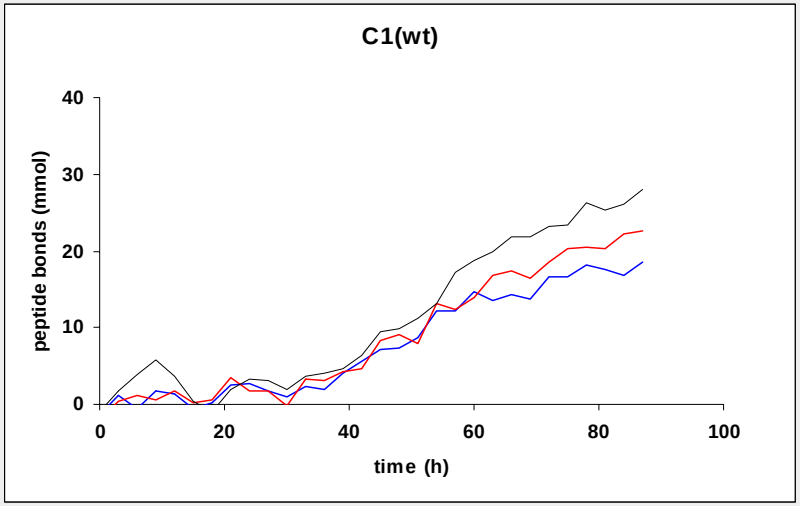
Reduced protease strains

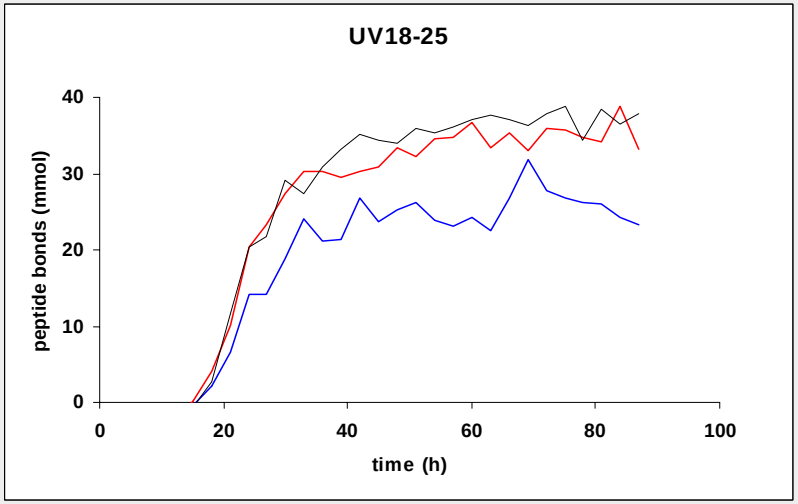
“Clean” background strains

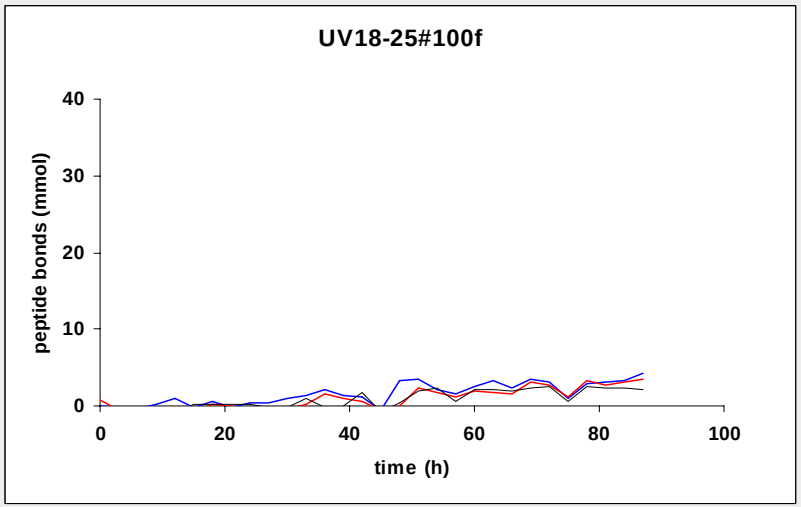


DYADIC®

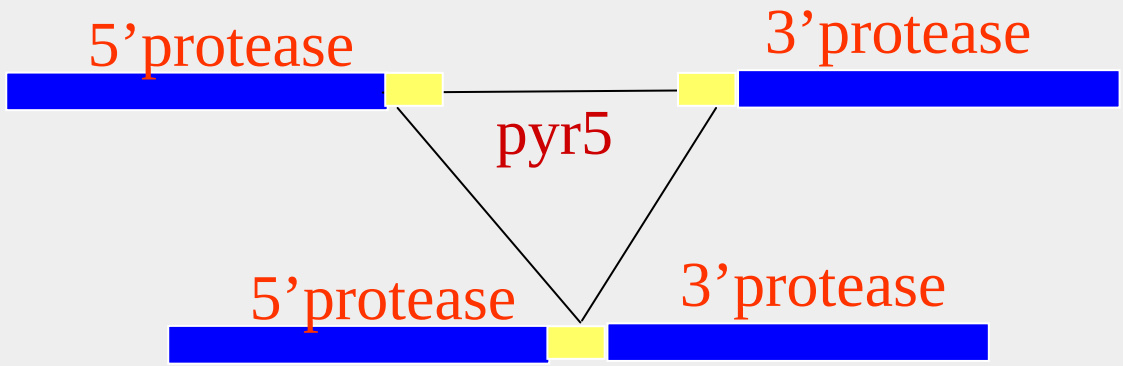
Protease mutants







Construction of disruption mutants for further reduced protease

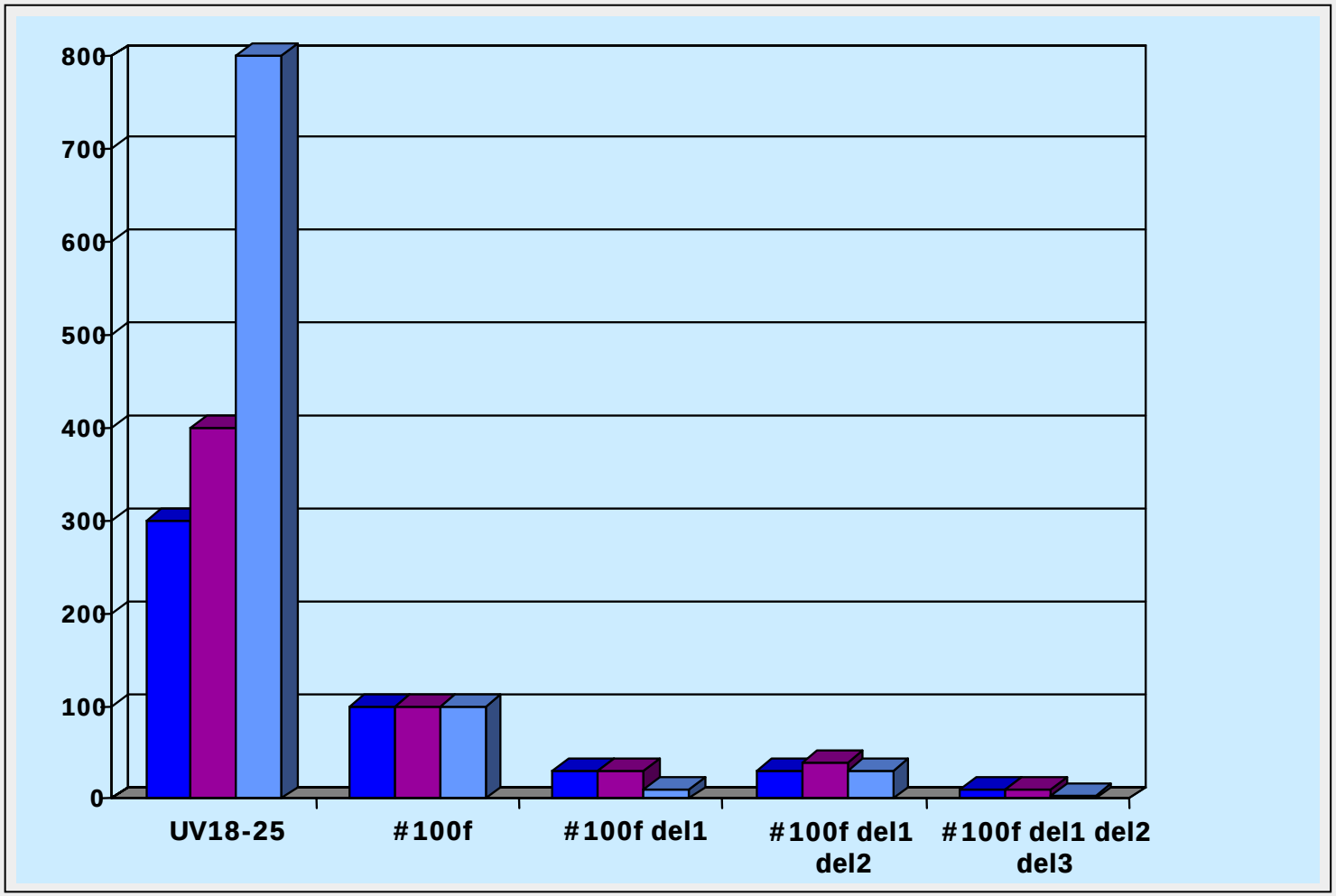


Protease deletions in C1

Three key proteases were identified

- Biochemical characterization, isolation, and identification of residual proteases and conditions under which they are produced
- Single, double and triple disruptants isolated
- Evaluation of these new hosts for heterologous gene expression and protein production

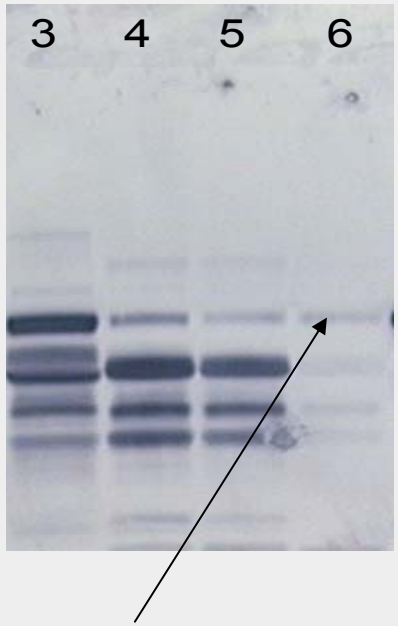
Reduced Protease Activities in Host Strains



In vitro Degradation of Antibody

#100f del1

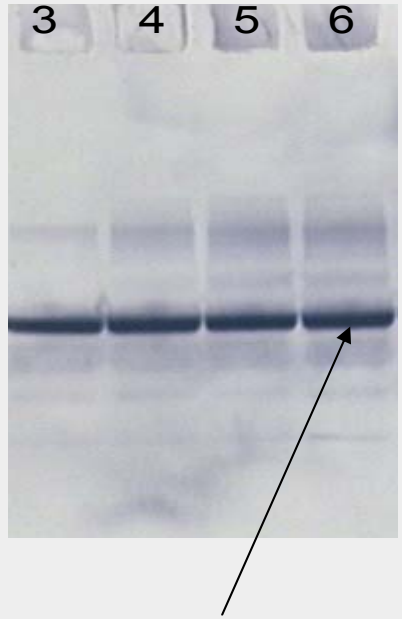
hrs: 0 4 8 24



Significant degradation after 24 hrs incubation

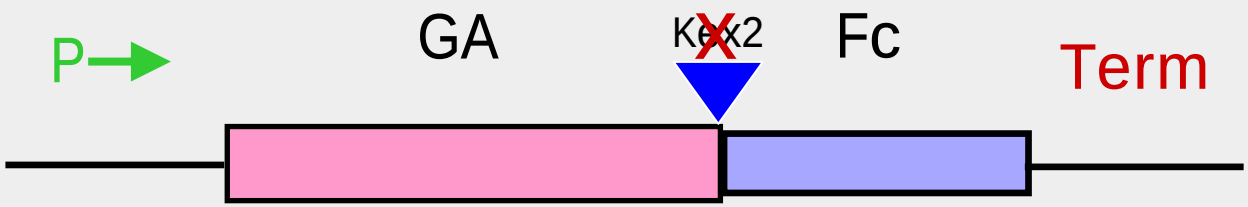
#100f del1 del2 del3

hrs: 0 4 8 24

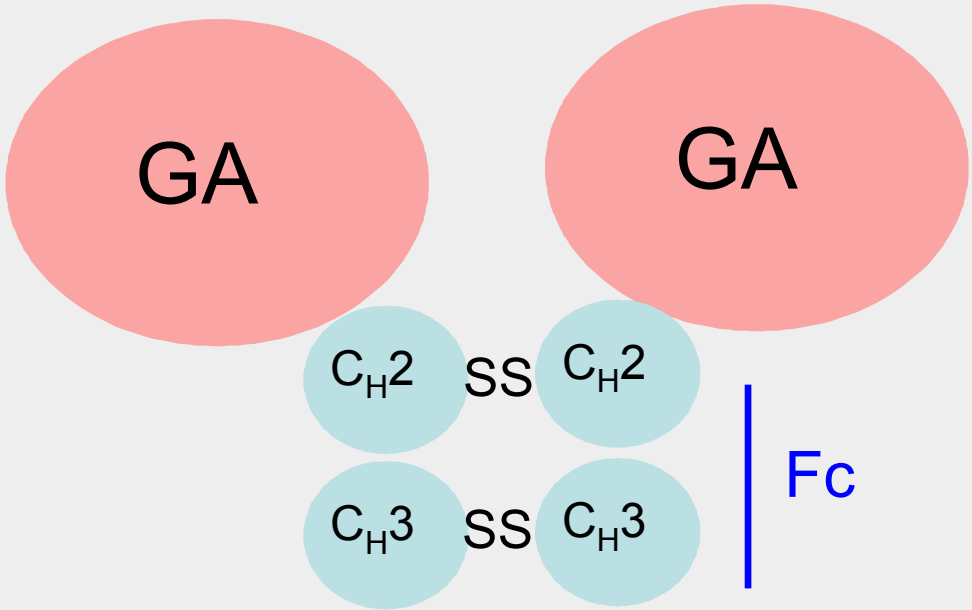


No degradation after 24 hrs

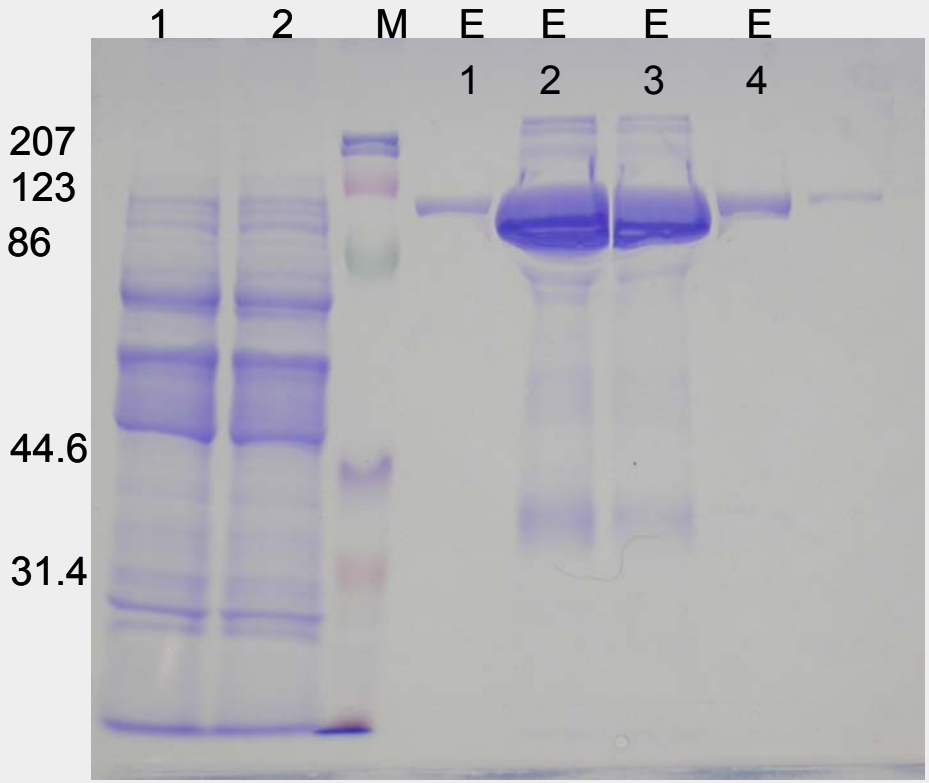
Expression of Glucoamylase-Fc Chimera



Gla-Fc Expressed Protein



Purification of Glucoamylase-Fc by Protein A



Yield >0.5 g/L

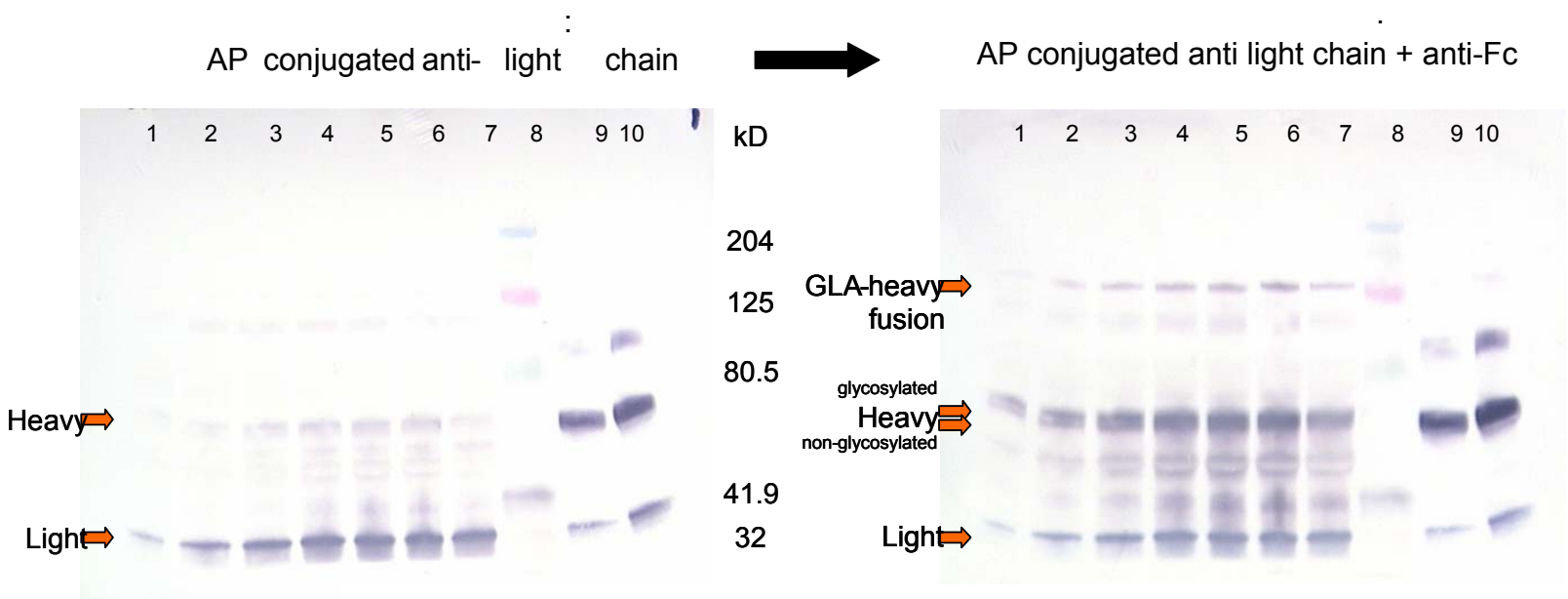
Purity ~90%

Expression of Full-length Antibodies in C1

Expression of several full-length IgG's in C1

- Several heavy chain variants
- κ and λ light chains

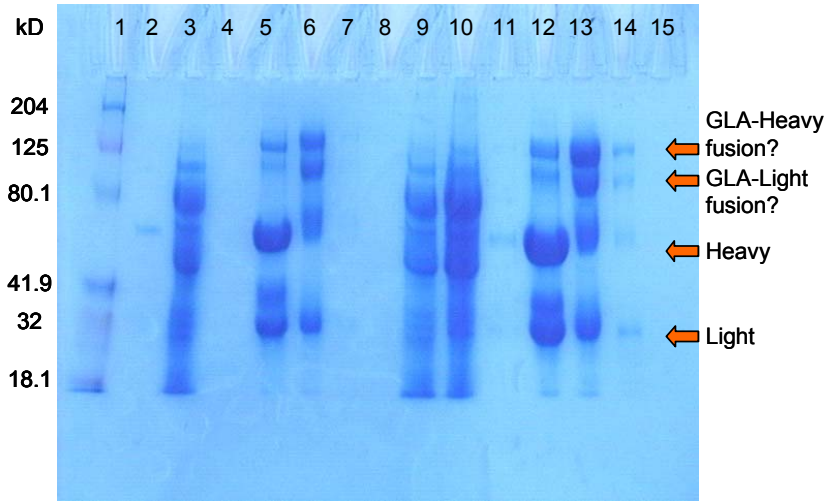
Full-length Antibody Expression in C1



Lanes 1-7 Fermentation samples
Lane 8 Marker
Lanes 9-10 Human IgG

Protein A purification of Fermentation Samples

SDS-PAGE:
Coomassie staining



Summary of Antibody Expression in C1

Results:

- Heavy and light chains both expressed
- Protein A binding
- Bio-activity observed in cell-based assay
- At least 400 mg/L observed in fermentors

Next steps

Biochemical Characterization

- N-terminal sequencing
- Mass spectrometry
- Glycan analysis

Activity Assays

- Bioassay
- BIACore

Next steps

Yield Improvement

- Fermentation optimization
- Additional transformants

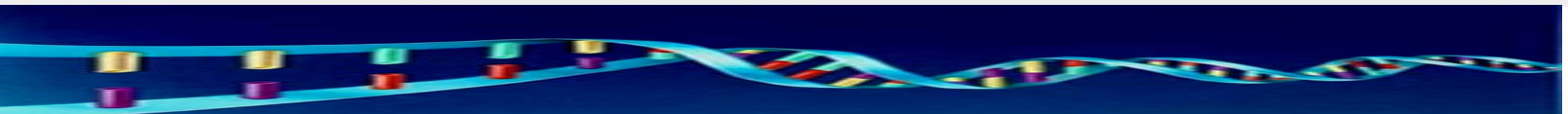
Further improved host strains using *Genomics* tools

- C1 genome sequenced in 2005
- Annotation project with Scripps-Florida will identify genes involved in:
  - Degradation
  - Secretion
  - Post-translational modification

C1 Gene Expression System Summary

- Expression tools fully developed
- Multiple products on the market, others in development
- Ability to express full-length biologically-active antibodies
- Other biotherapeutics also expressed
- Reduced protease strains developed
- Reduced background strains developed
- Safe, nontoxic, nonhazardous
- Sequenced genome with annotation in progress

A viable alternative expression system for biotherapeutics



DYADIC®

Acknowledgments

- Subsidiary and Research Collaborators
  - Dyadic Nederland BV, Zeist, the Netherlands
  - TNO Quality of Life, Zeist, the Netherlands
  - Bio-Technical Resources, Manitowoc, Wisconsin
  - Russian Academy of Sciences, Moscow State University, Russia