



# Production of full length antibody chains in *Chrysosporium*

Production of functional full length human monoclonal antibodies has been accomplished using highly productive low protease mutant *Chrysosporium* host strains. High level expression was achieved using a glucoamylase-carrier approach, and recombinant strains expressing both heavy and light chains were obtained. Heterodimeric antibody molecules were formed efficiently, allowing simple purification of the protein from the culture fluid using Protein A. Cell-based bio-assays performed on the culture supernatant and the purified samples revealed almost complete bioactivity.

## Host strain selection

Protease deficient *Chrysosporium* C1 strains were constructed using the classical mutagenesis method followed by targeted gene disruption. The protease activity of the low protease mutants was decreased dramatically to 15% and less compared to the C1 production strain (Figure 1).

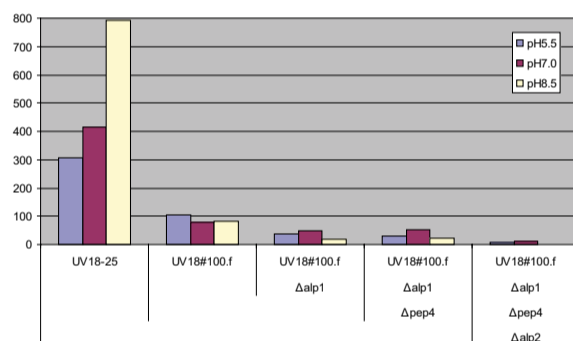


Figure 1. Protease activity in various protease deficient *Chrysosporium* C1 strains.

## Construction of the heavy and light chain expression vectors

The gene sequences of the light and heavy chains of human IgG1 were synthesized. The codons were optimized for the expression in C1 and potential dibasic processing sites (KR) were removed to avoid unwanted processing of the antibody chain in the secretion pathway. Plasmids expressing the light and heavy chains of a human IgG1 were separately constructed (Figure 2). Upstream of the *cbh1* promoter the *amdS* gene or the *pyr5* gene were inserted to be able to select transformants for the presence of both heavy and light chain fragments. For transformation the isolated expression cassettes were used.

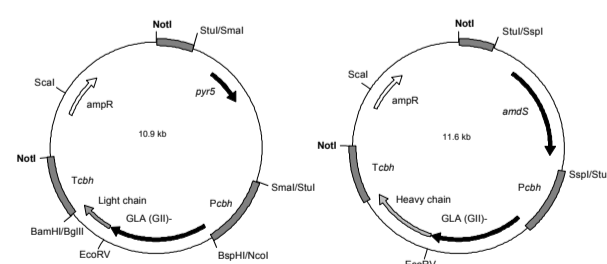


Figure 2. Schematic view of the plasmids expressing heavy and light chains of human IgG1 fused to *A. niger* glucoamylase including a *kex2* cleavage site.

## Construction of full length antibody producing strains

Co-transformation with both expression fragments to host strain UV18#100.f Δalp1 resulted in Pyr5<sup>+</sup> AmdS<sup>+</sup> transformants expressing both antibody chains. Transformants grown in microplate cultures under inducing conditions were screened for the highest level of antibody production in:

- Immuno spotblot analysis using AP-conjugated antibodies against heavy chain and light chain proteins
- ELISA screening method using Protein A coated microplates

## Production of full length antibody chains in fermenters

The best antibody producing transformants were selected for production in fermenters. One transformant produced up to **1 gram of full length human IgG1 per litre** culture filtrate measured by Western analysis (Figure 3). The antibody was isolated from the fermenter broth by Protein A purification showing that the antibodies were in their native dimerized form (Figure 4). A cell-based bioactivity assay for this specific antibody showed that the produced antibodies were completely active. Glycostructure analysis of the C1 produced heavy chain protein showed the presence of GlcNac2Man4-9 chains. As expected no glycostructures were observed for the C1 produced light chain protein.

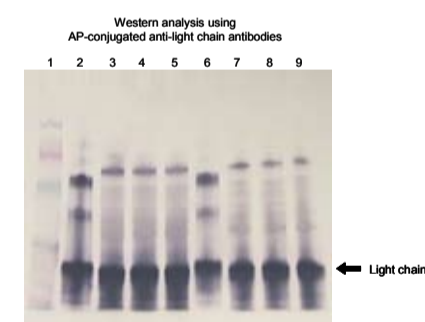


Figure 3. Western analysis of culture filtrate from a full length antibody expressing C1 strain. Lane 1: prestained protein marker. Lane 2: 2 μg IgG1 protein. Lane 3-5: 2 μl of culture filtrate. Lane 6: 1 μg IgG1 protein. Lane 7-9: 1 μl of culture filtrate.

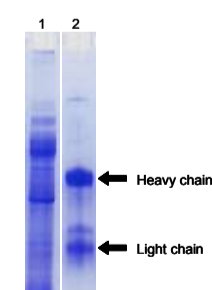


Figure 4. Polyacrylamide gel analysis of culture filtrate from a full length antibody expressing C1 strain. Lane 1 is unpurified culture filtrate. Lane 2 is Protein A purified culture filtrate.

## Strain improvement of the best antibody producing strain

A new plasmid was constructed containing both expression cassettes of the heavy and light chain. A DNA fragment containing both expression cassettes was co-transformed with the newly developed *nia1* selection marker to a *Nia1* mutant of the best antibody producing strain. Screening of the transformants was

carried out using the standard microplate screening set-up mentioned above. Introduction of the extra gene copies in the transformants resulted in a strain with a **two-fold increased level** of produced full length antibodies.

## Screening of libraries of libraries for antibody binding

*Chrysosporium* host strains can be utilized as a system for high throughput screening (HTS) of libraries (Verdoes *et al.*, 2007). Gene libraries can be generated containing variants of both heavy and light chains co-expressed in the same host strains. Using ELISA or other assays, the antibodies expressed by the separate clones can be assayed for any specific binding activity. To demonstrate a fungal high throughput robotic screening procedure for improved antibody variants in *Chrysosporium* a specific plasmid was constructed (Figure 5). Upon introduction into C1, this vector results in high transformation frequencies. Due to the presence of telomeric sequences (*hTel*), the vector is initially non-integrative. The use of doubly marked *pyr4-pyr5* mutants of C1 ensures that integrants contain the entire expression construct. Individual transformants are separated and screened for production of the desired binding activity in a high-throughput fashion.

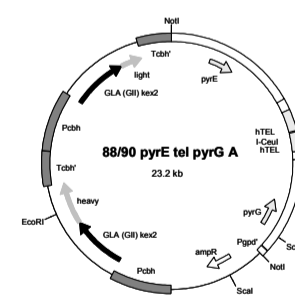


Figure 5. Schematic view of the plasmid expressing heavy and light chains of human IgG1 fused to *A. niger* glucoamylase including a *kex2* cleavage site, *pyrE*, *pyrG* and telomeric sequences.

## Conclusions

- Construction of low protease mutants resulted in an **85% reduction** in protease activity compared to the parent strain.
- Full length antibodies can be expressed up to **1-2 grams per litre** culture filtrate in a dimerized, properly folded, and active form in *Chrysosporium* C1 strains.

## References

- Verdoes J.C., Punt P.J., Burlingame R., Bartels J., van Dijk R., Slump E., Meens M., Joosten R., Emalfarb M. (2007). A dedicated vector for efficient library construction and high throughput screening in the hyphal fungus *Chrysosporium lucknowense*. *Ind. Biotechnol.* 3:48-57.
- Patent reference and patent application pending.