ASX Announcement

Lonza Poster detailing i-body expression in yeast

MELBOURNE Australia, 8 May 2017, AdAlta Limited (ASX: 1AD), the biotechnology Company advancing its lead i-body candidate towards clinical development, announces that contract manufacturer Lonza has presented data on the production of AdAlta's i-bodies in Lonza's XS™ Pichia (or yeast) Expression Technology. The data was presented at the 9th Conference on Recombinant Protein Production in Dubrovnik, Croatia.

As part of the process development collaboration entered into in December 2014, Lonza has optimized the expression efficiency of an i-body in its *XS™ Pichia* Expression System. The data presented demonstrates the scalability of this yeast system for the production of i-bodies and efficiency of expression levels. In particular, the Lonza team has been able to improve the host strains of Pichia to almost double the yield (to approximately 7 gm/L) in half the fermentation time.

AdAlta has already shown that its i-bodies can be produced in a number of expression systems. The work Lonza has done demonstrates the ability of AdAlta's i-body platform to be expressed in an alternative system to the current standard bacterial system (*Escherichia coli)*. As an alternative, yeast-based expression system, *Pichia* potentially provides high yields using a simple, fast fermentation method.

AdAlta's Chief Executive Officer Sam Cobb said, "This work by Lonza is a step towards confirming that i-bodies can be expressed at commercially scalable quantities, a core business objective that AdAlta has highlighted for the first half of 2017. This is vital as we progress towards human clinical trials. In addition, given the cost of biologic drugs, being able to reduce manufacturing costs should provide a market advantage."

Lonza's poster (RPP9), entitled *"Promoter and process engineering for recombinant protein production in Pichia pastoris towards simple, fast and methanol-free cultivation regimes and high product titers"* is attached and is available on the AdAlta website www.adalta.com.au.

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AdAlta Limited
15/2 Park Drive
Bundoora VIC 3083 Australia

T +61 3 9479 5159
E enquiries@adalta.com.au
ABN 92 120 332 925

adalta.com.au

Notes to Editors

About AdAlta

AdAlta Limited is an Australian based drug development company headquartered in Melbourne. The Company is focused on using its proprietary technology platform to generate i-bodies, a new class of protein therapeutics, with applications as therapeutic drugs to treat disease.

I-bodies are a promising, novel class of drugs that offer a new and more effective approach to treating a wide range of human diseases. They are identified and developed using our proprietary technology platform.

We have pioneered a technology that mimics the shape and stability of a crucial antigen-binding domain, that was discovered initially in sharks and then developed as a human protein. The result is a range of unique compounds, now known as i-bodies, for use in treating serious diseases.

AdAlta is developing its lead i-body candidate, AD-114, for the treatment of idiopathic pulmonary fibrosis (IPF) and other human fibrotic diseases, for which current therapies are sub-optimal and there is a high-unmet medical need.

The Company also plans to continue further drug discovery and development directed towards other drug targets and diseases with its i-body technology platform.

Further information can be found at: www.adalta.com.au.

About Lonza

Lonza is one of the world's leading and most-trusted suppliers to the pharmaceutical, biotech and specialty ingredients markets. We harness science and technology to create products that support safer and healthier living and that enhance the overall quality of life.

Not only are we a custom manufacturer and developer, Lonza also offers services and products ranging from active pharmaceutical ingredients and stem-cell therapies to drinking water sanitizers, from the vitamin B compounds and organic personal care ingredients to agricultural products, and from industrial preservatives to

microbial control solutions that combat dangerous viruses, bacteria and other pathogens.

Founded in 1897 in the Swiss Alps, Lonza today is a well-respected global company with more than 40 major manufacturing and R&D facilities and approximately 10,000 employees worldwide. The company generated sales of about CHF 3.6 billion in 2013 and is organized into two market-focused segments: Pharma&Biotech and Specialty Ingredients. Further information can be found atwww.lonza.com.

For more information, please contact:

AdAlta Limited
Sam Cobb, CEO
Tel: +61 (0)3 9479 5159
E: s.cobb@adalta.com.au

Media (Australia)
Andrew Geddes
Tel: +61 (0)408 677 734
E: adalta@instinctif.com

Media (International)
Sue Charles / Daniel Gooch
Tel: +44 (0)20 7866 7905
E: adalta@instinctif.com

Promoter and process engineering for recombinant protein production in *Pichia pastoris* towards simple, fast and methanol-free cultivation regimes and high product titers

Roland Prielhofer[1,§], Michaela Reichinger[2], Nina Wagner[2], Katrien Claes[2], Christoph Kiziak[2], Brigitte Gasser[1], Diethard Mattanovich[1]

[1]*Department of Biotechnology, University of Natural Resources and Life Sciences Vienna, Austria*
[§]*Current address: Austrian Centre of Industrial Biotechnology (ACIB), Vienna, Austria*
[2]Lonza AG, Rottenstraße 6, Visp 3930, Switzerland

P_{GTH1} Promoter Analysis

The gene encoding the *Pichia pastoris* high-affinity glucose transporter *GTH1* is repressed by excess glycerol and induced by limiting glucose [1]. Its promoter sequence P_{GTH1} was analyzed by Genomatix MatInspector to identify and select binding sites (TFBS) of important transcription factors (**Figure 1**). In addition, we cloned 8 shortened P_{GTH1} variants to identify its relevant regulatory region (red arrows, **Figure 1**). The shortened promoter variants were screened for eGFP expression in repressed (glycerol surplus) and induced (glucose limit) growth conditions (**Figure 2**). The induced expression capacity remained fully functional for the promoter variants down to a length of 328 bp, it was decreased for the 283 bp-variant and almost zero for the two shortest length variants (211 and 66 bp). This indicates that the region between position -328 and -211 contains important regulatory features (black dashed box, **Figure 1**).

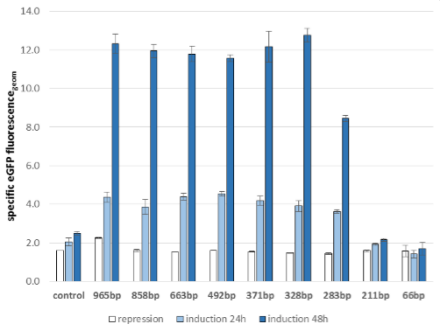

Figure 2: Screening data of the shortened P_{GTH1} promoter variants. Each 2 clones (selected in pre-screenings) were cultivated in triplicates and analysed for eGFP expression by flow cytometry.

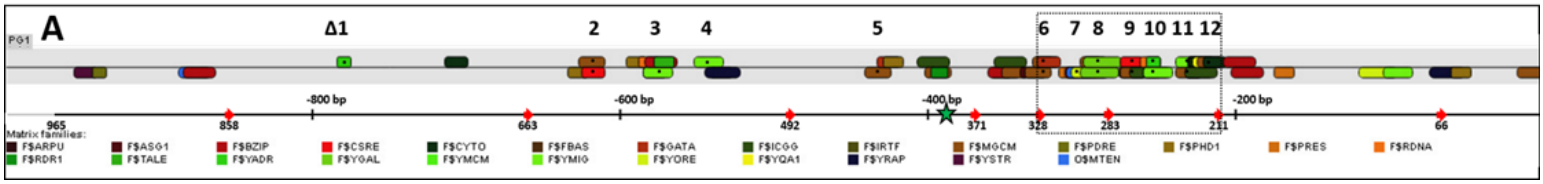

Figure 1: P_{GTH1} promoter sequence analysis for glucose-related TFBS with the MatInspector from Genomatix. Glucose related and directly adjacent matrix families are presented. Black dotted TFBS boxes and corresponding numbers indicate TFBS selected for deletion. The black dashed box indicates the most important promoter region. The green asterisk indicates the position of the prominent $TA(T)_{15}$ motif.

Deletion variants of the P_{GTH1} promoter

- TFBS indicated with numbers in **Figure 1** were deleted and the eGFP expression was analysed as described above (**Figure 3**). 5´-shortening of the promoter seems to be beneficial (stronger induction of Δ1,2,4)
- Deletions within the main regulatory region of Δ6 to Δ12 had different impacts on eGFP expression, but none showed increased induction without losing the repression properties
  > We assume that the main regulatory region of P_{GTH1} needs to be maintained for proper repression and induction
- Mig1 could play a bifunctional role in regulation (Δ3,4,10,11; *MIG1-1* and *MIG1-2* are regulated contrariwise upon glucose availability [2])

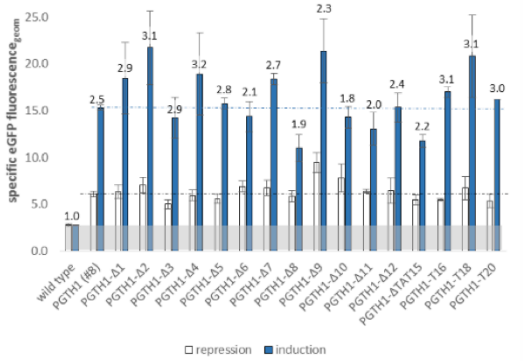

Figure 3: Screening data of the P_{GTH1} deletion variants. Triplicate Pool-screening of up to 9 clones were done. eGFP levels were analyzed by flow cytometry.

Improved P_{GTH1} by partial sequence duplication

Both duplication variants P_{GTH1}-d1240 and P_{GTH1}-d1427 showed more tight repression in excess glycerol and stronger induction upon limiting glucose (Figure 4 and 5).

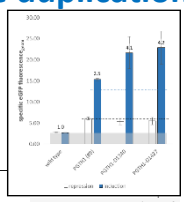

Figure 4: Screening data of P_{GTH1} duplicate variants.

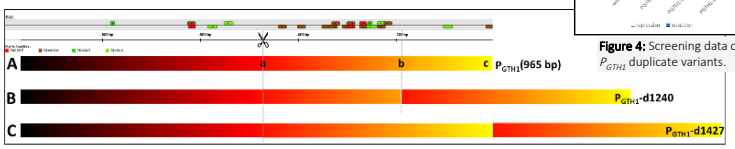

Figure 5: Illustration of P_{GTH1} promoter variants with duplicate fragments. PstI and BglII restriction sites (pos. 509-514 and 525-530, indicated with scissors) were used to generate P_{GTH1}-d1240 and P_{GTH1}-d1426 with duplicate fragments..

P_{GTH1}-d1240 speed fermentation highly outperforms P_{AOX1}

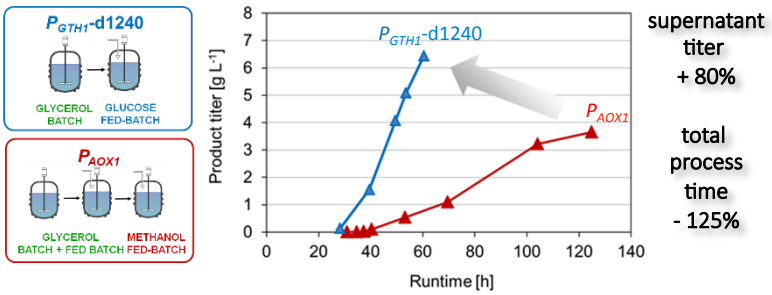

Figure 6: Fed batch fermentations of *P. pastoris* strains producing i-bodyTM (single domain antibody-like molecule of human origin, provided by AdAlta) under control of P_{GTH1}-d1240 and P_{AOX1}, respectively. P_{GTH1}-d1240 controlled production was speed fermented using a model-based [3] linear incremental feeding strategy. P_{AOX1} driven production was done in a typical three-stage process [4].

Summary

- Generation of P_{GTH1} promoter variants with shortened length to identify the main regulatory region of P_{GTH1}
- Analysis of TFBS roles by deletion variants of P_{GTH1}; Carbon source responsive elements play an essential role for its regulation.
- Clarification of the role of the TAT_{15} motif for promoter strength; Elongation of this motif to T_{16}, T_{18} and T_{20} had a positive effect.
- Generation of an improved version of P_{GTH1} - **P_{GTH1}-d1240** - which reached greatly enhanced expression capacities
- Speed fermentation strategy for P_{GTH1}-d1240, which highly outperformed the P_{AOX1}-driven production in a typical three stage methanol fed batch

References:
[1] Prielhofer et al. 2013, *Microb Cell Fact*, 12:5 [2] Prielhofer et al. 2015, *BMC Genomics*, 16:167 [3] Maurer et al. 2006, *Microb Cell Fact*, 5:37 [4] Looser et al. 2015, *Biotechnol Adv.*, 33:1177–1193

roland.prielhofer@boku.ac.at