



The EB66 cell line for the industrial production of high potency antibodies and analytical methods for low-fucosylated clones screening

PEGS, Boston
May 3rd 2012

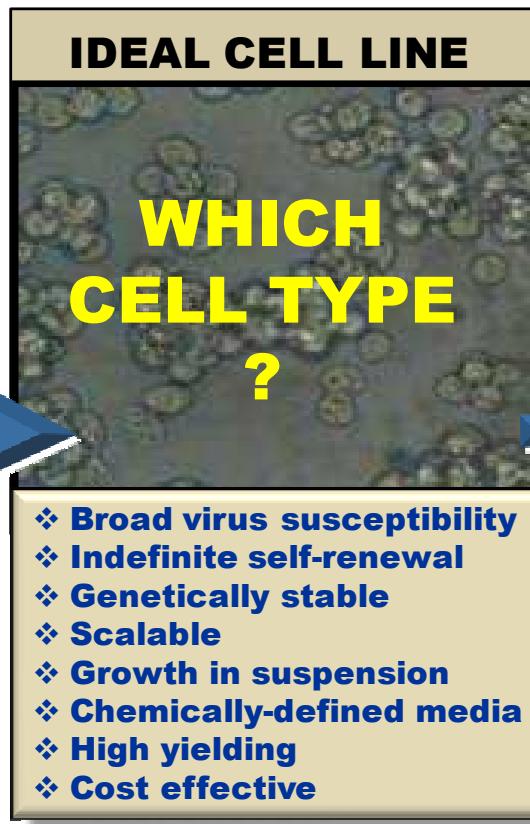
Cell substrates for vaccines production

A stem cell alternative to the embryonated chicken eggs



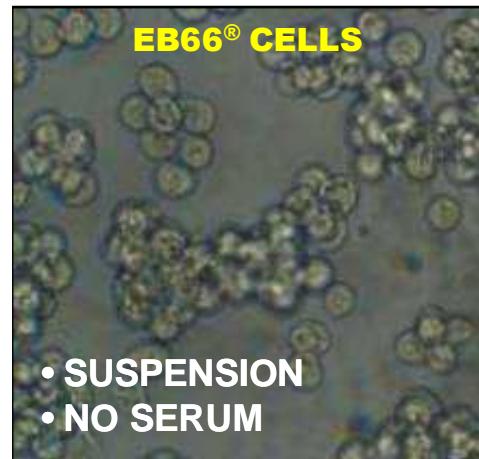
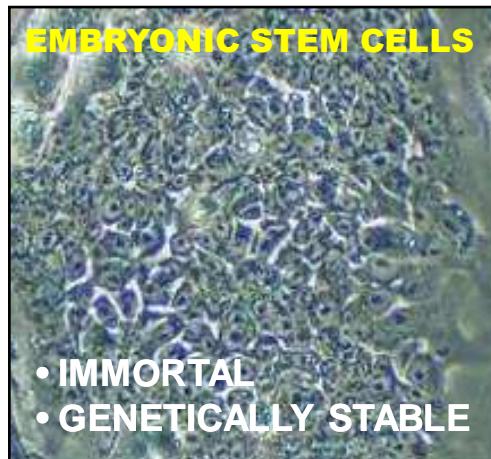
BUT BESET BY ISSUES

- Cumbersome manufacturing process
- Slow reactivity in pandemic crisis
- Exposure to risks of outbreak of bird diseases & eggs penury
- Egg-component allergies
- Quality concern
- Susceptible to contaminations
- (e.g. Shortage of Influenza vaccines in the US in 2004)



Derivation of Duck Embryonic Stem Cells

A new established cell line meeting industry and regulatory requirements



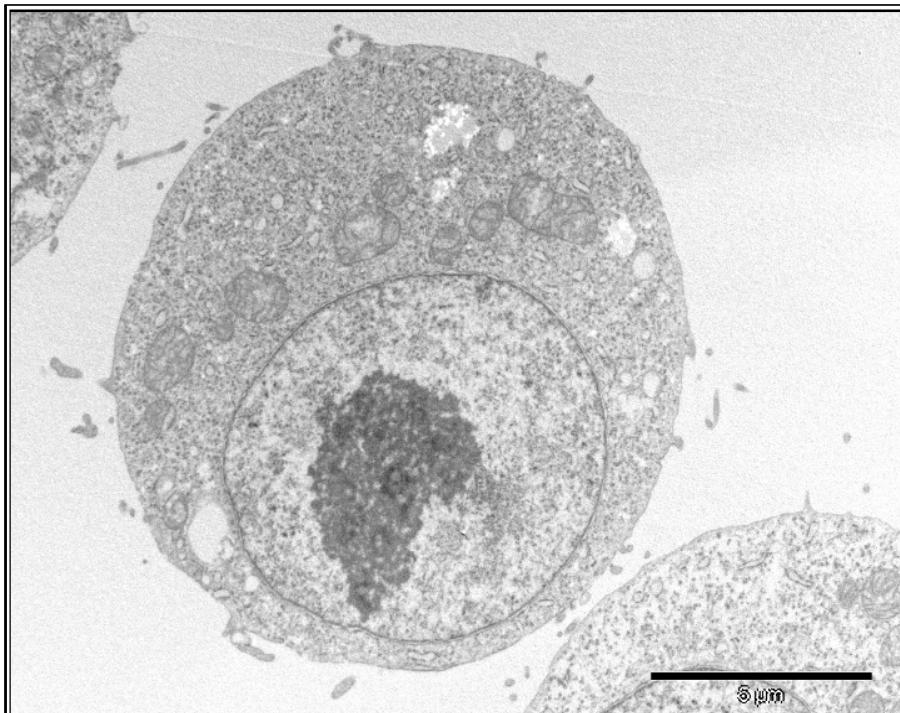
No genetic modifications and no viral nor chemical modifications

Fully documented from animal substrate to isolated EB66® Cells

The duck EB66® cell line

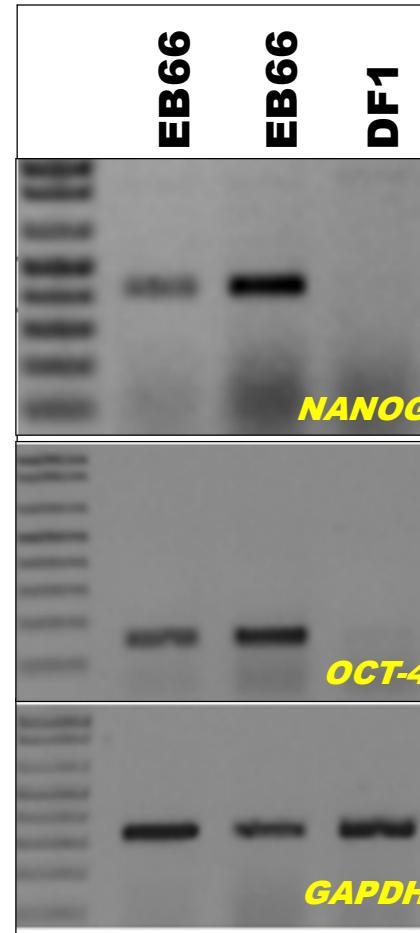
Maintenance of ES cells unique properties

ULTRASTRUCTURE SIMILAR TO EMBRYONIC STEM CELLS

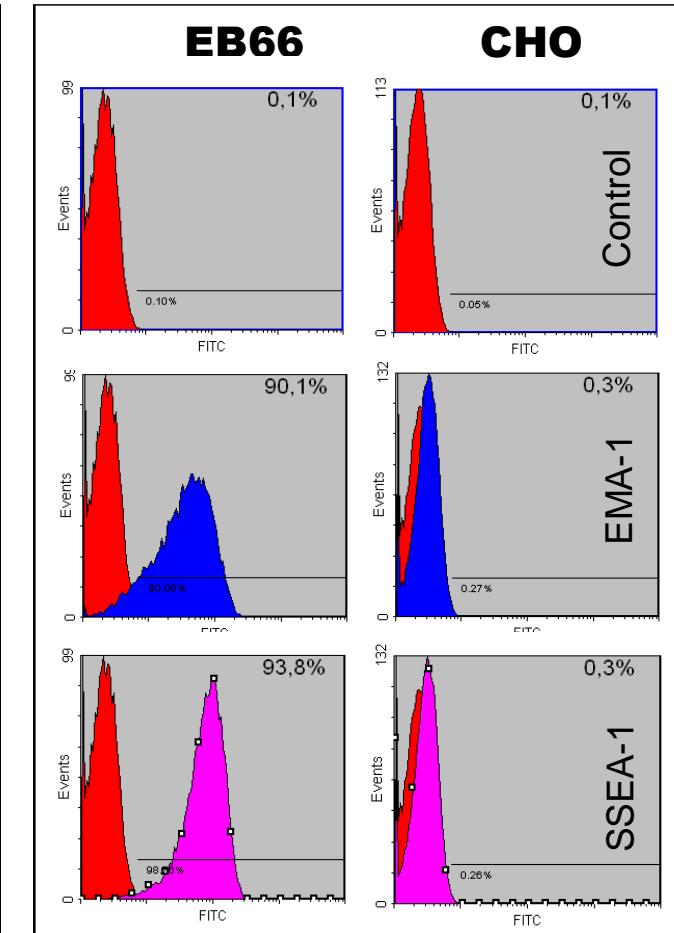


- Abundant mitochondria & ribosomes
- Large nucleus & nuclear bodies
- Small size (~8-10 μm)

EXPRESSION OF « STEMNESS » GENES



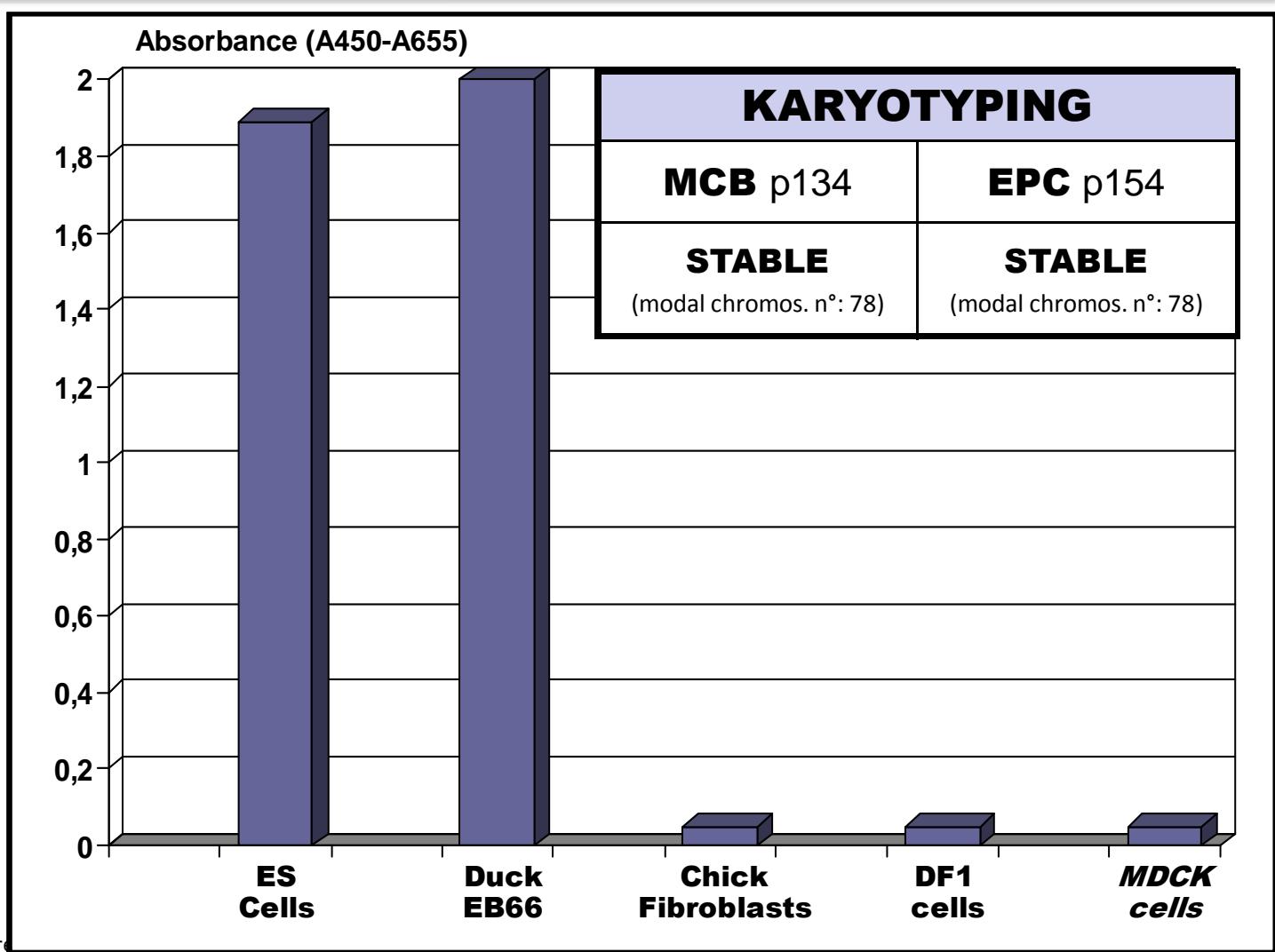
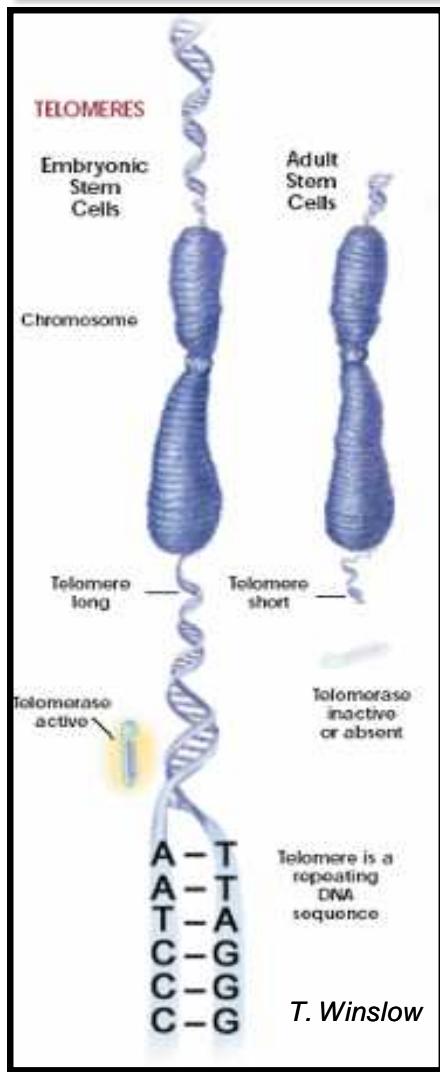
EXPRESSION OF ES CELL SURFACE MARKERS



The duck EB66® cell line

Maintenance of ES cells unique properties

STRONG EXPRESSION OF TELOMERASE, INVOLVED IN MAINTENANCE OF IMMORTALITY & GENETIC STABILITY



Cell growth in stirred tank bioreactors

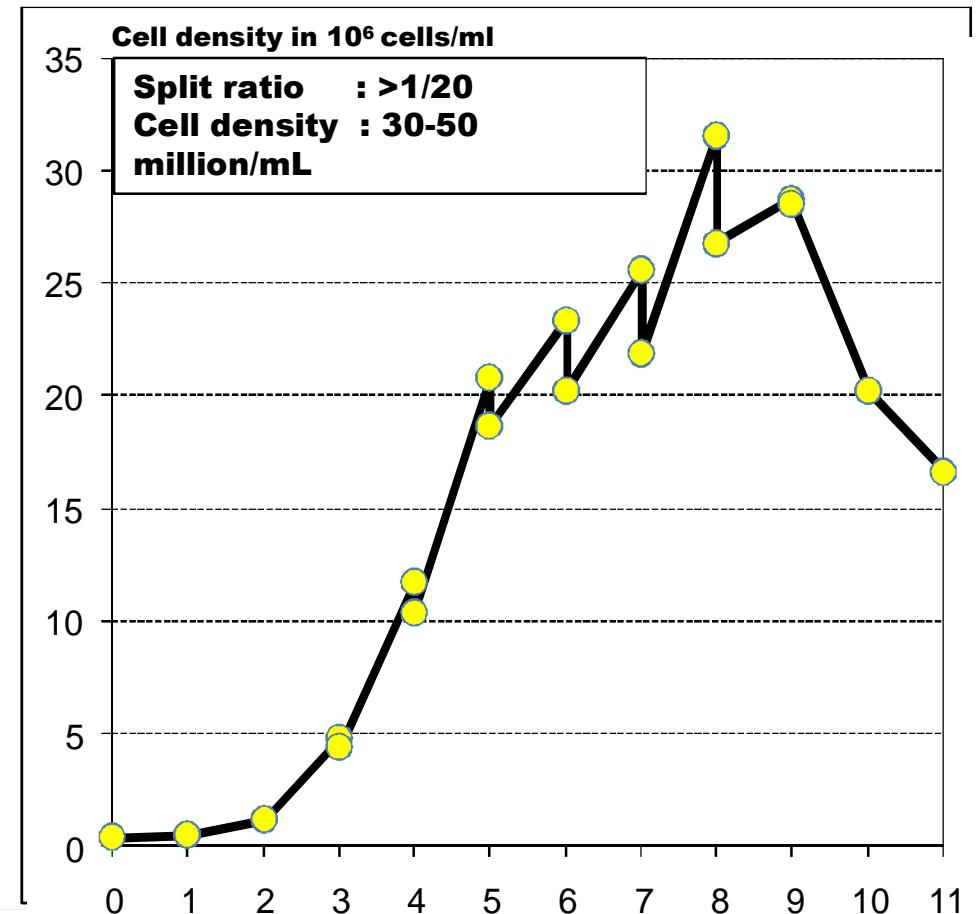
Cell culture characteristics; currently up to 1000L



**Single-Use
Bioreactor**



**Stainless Steel
Bioreactor**

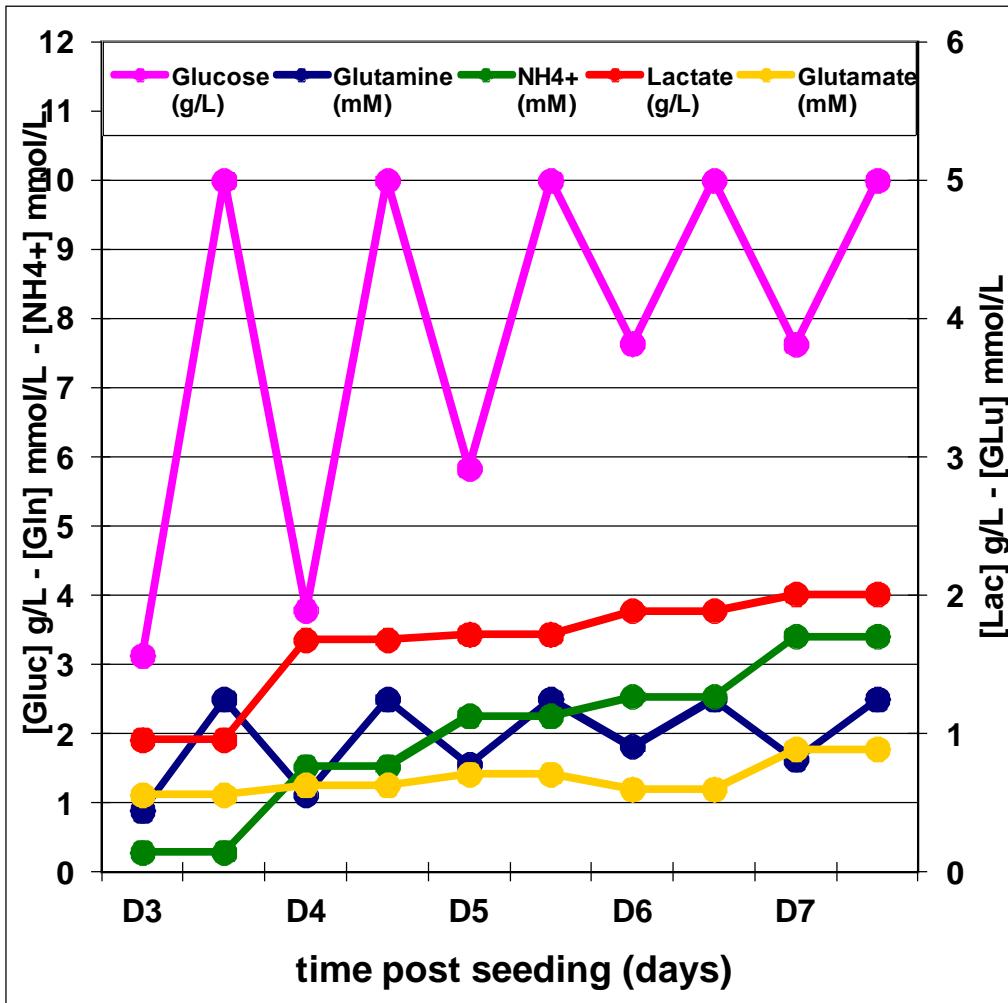


Custom serum-free medium:

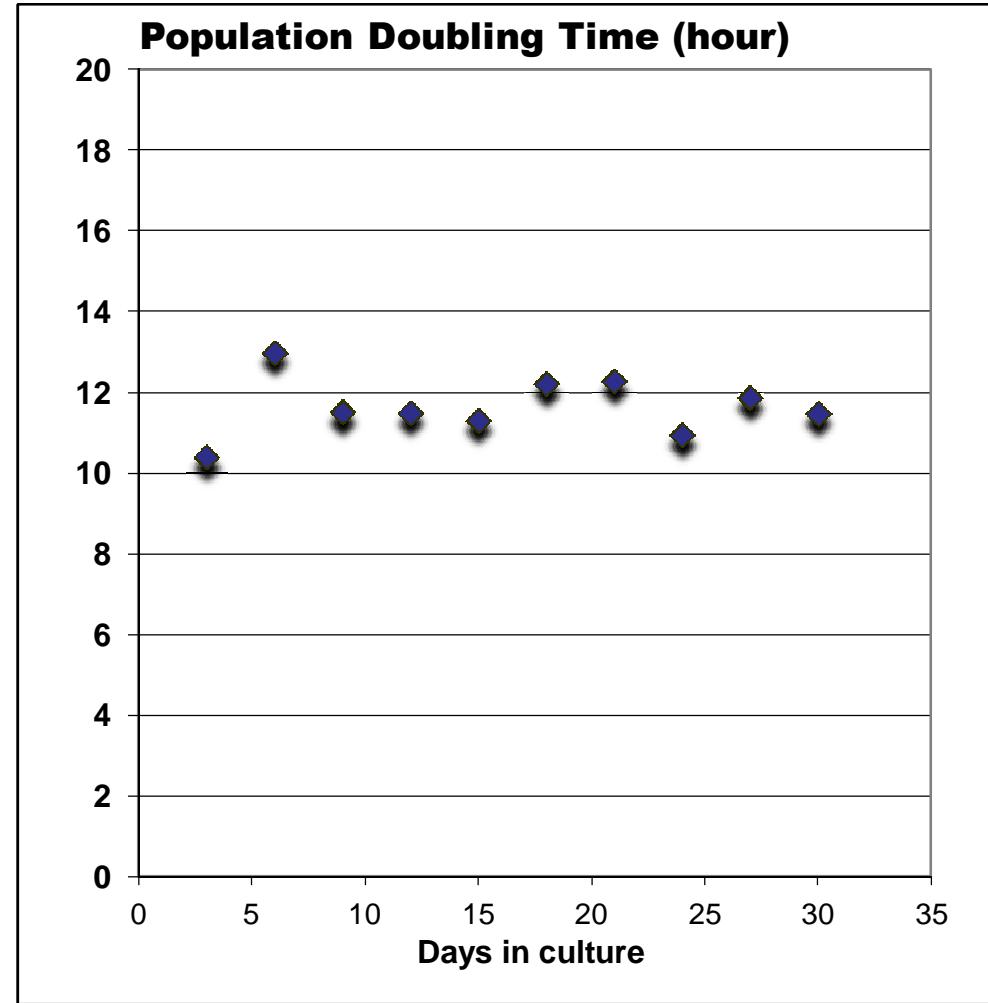
- ✓ Liquid & powder
- ✓ R&D grade & GMP grade
- ✓ Devoid of components of primary & secondary animal origin
- ✓ Cost-effective

Cell growth in stirred tank bioreactors

Cell culture characteristics; currently up to 1000L



**No accumulation of lactate or ammonium,
& limited consumption of glutamine**



Short Population Doubling Time
(~12 hours at 39 °C, ~15 hours at 37 °C)

The EB66® cell line

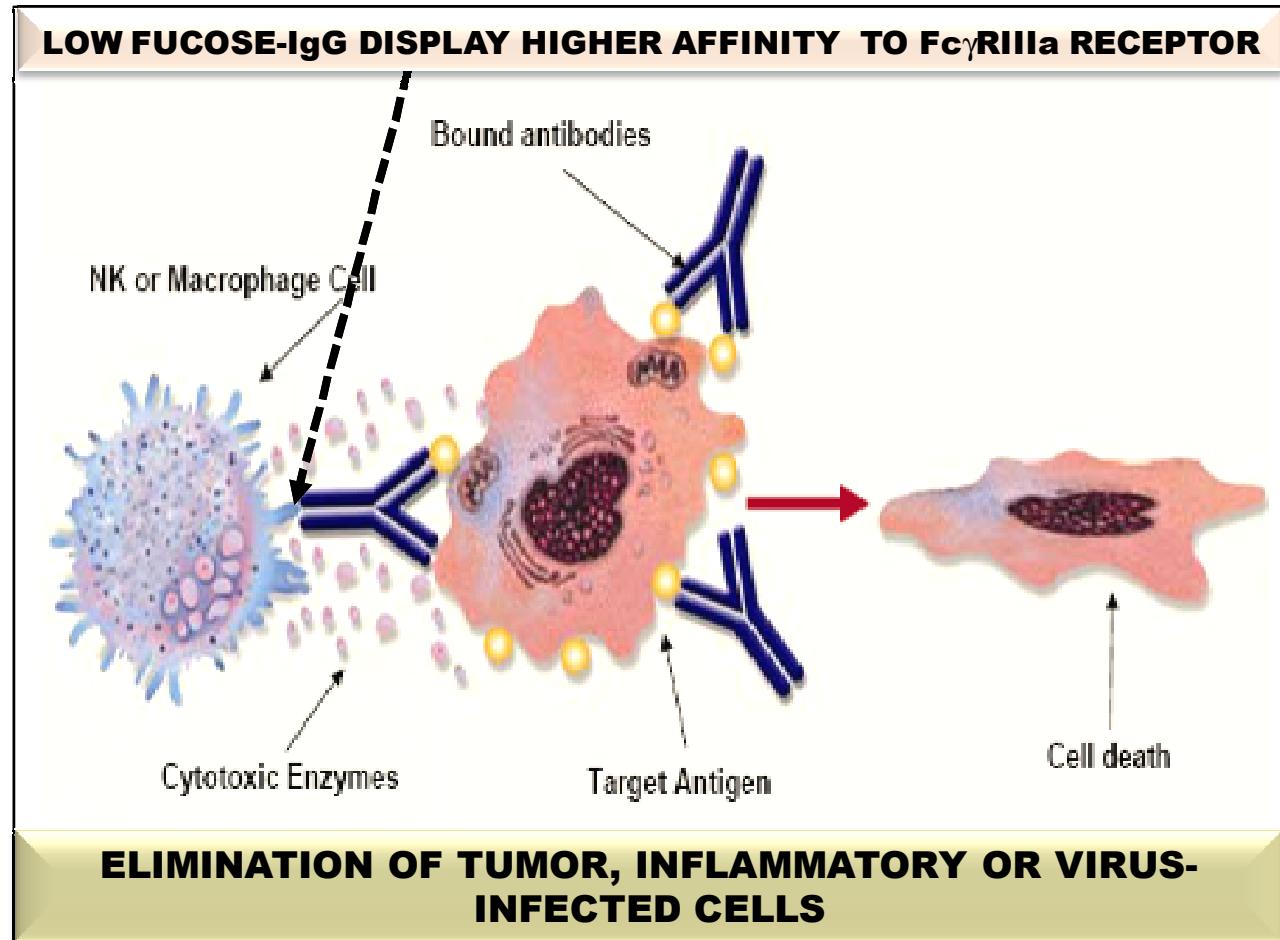
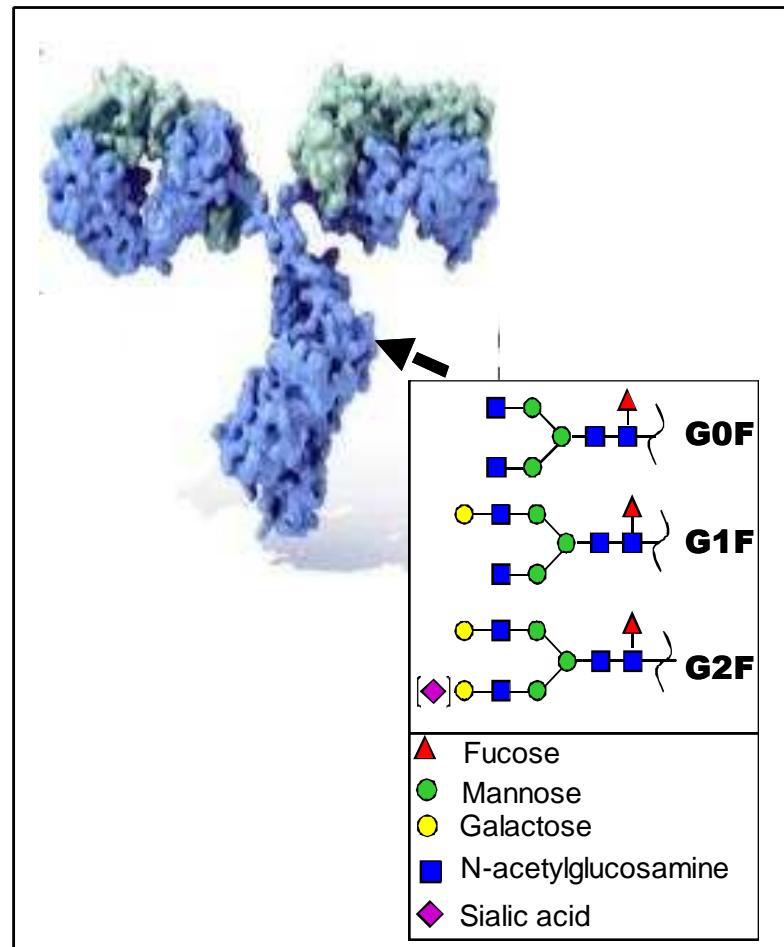
A new standard for the production of vaccines



- **18 Commercial licenses + ~12 research licenses**
- **2 Phase I clinical trials completed for flu vaccines in the USA & Japan**
- **A first veterinary EB66 vaccine marketed in 2013**

■ Antibody-Dependent Cytotoxicity Activity (ADCC)

IgG with low fucose display high affinity towards Fc γ RIIIa receptors



■ Biomanufacturing of Antibodies with enhanced ADCC

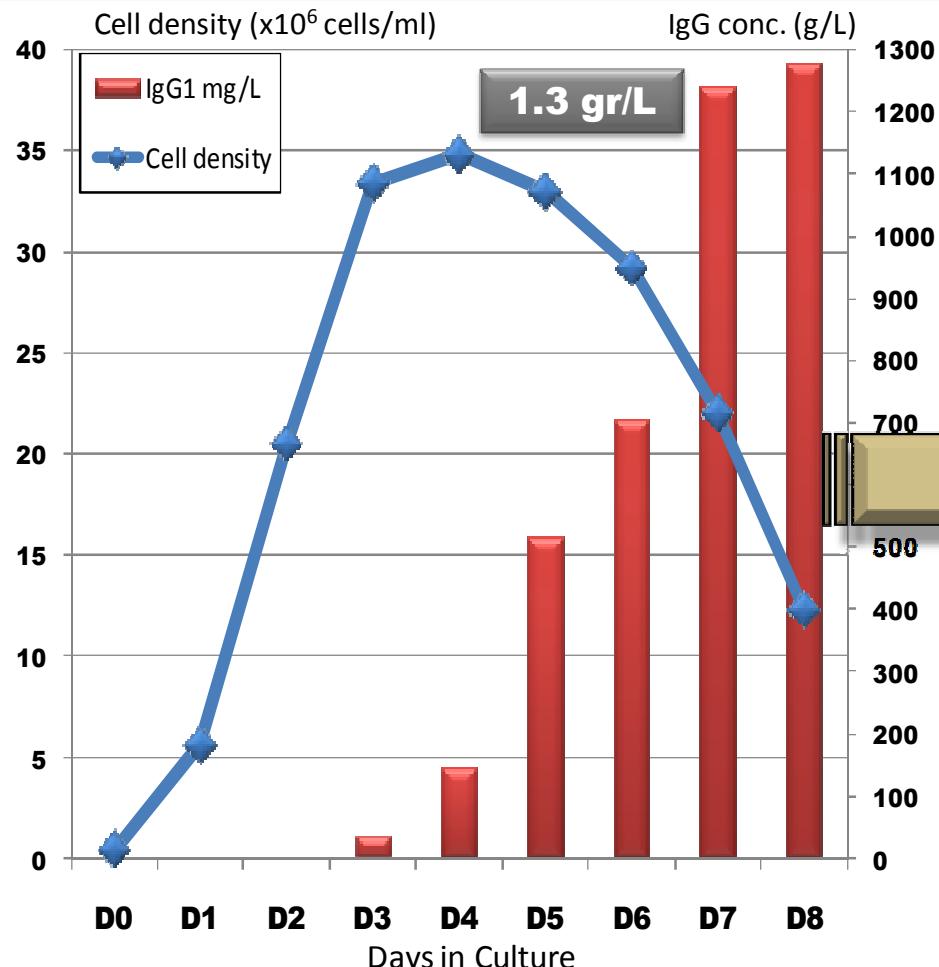
Development of a novel cell substrate for antibody production



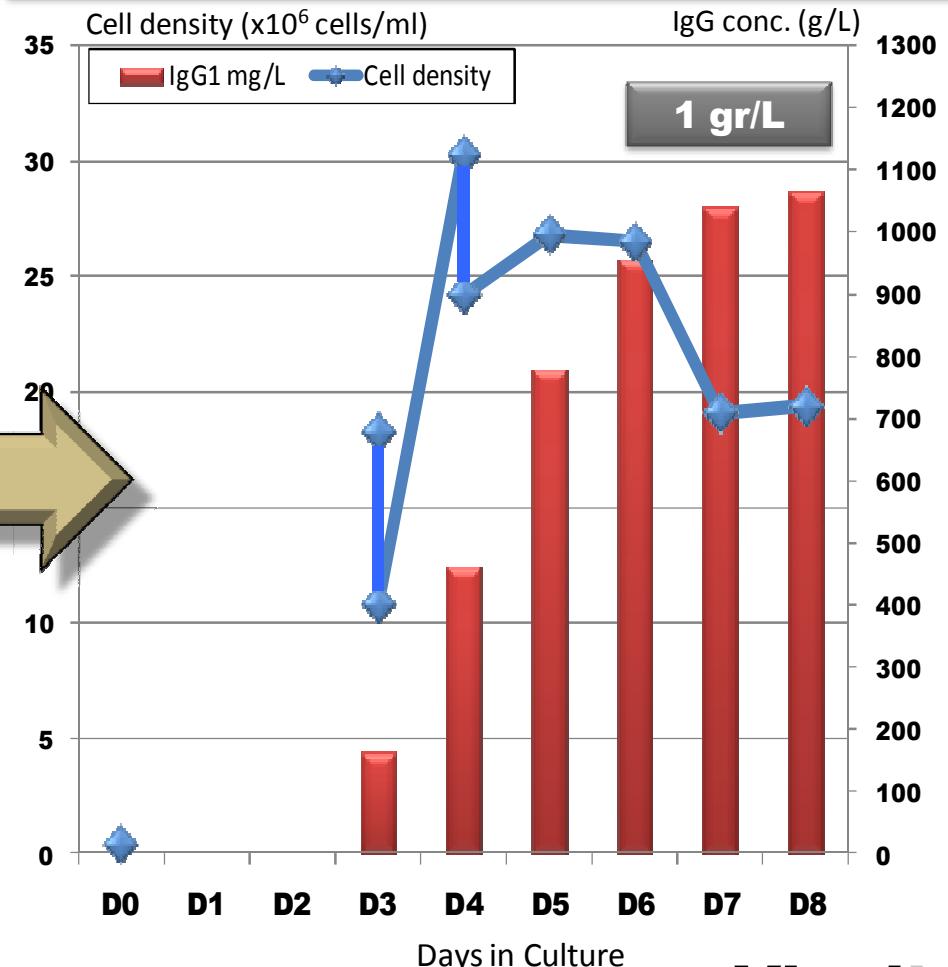
Cell Culture Media in fed-batch Culture

mAb production and upscaling

FEDBATCH PRODUCTION PROCESS IN SHAKE FLASK

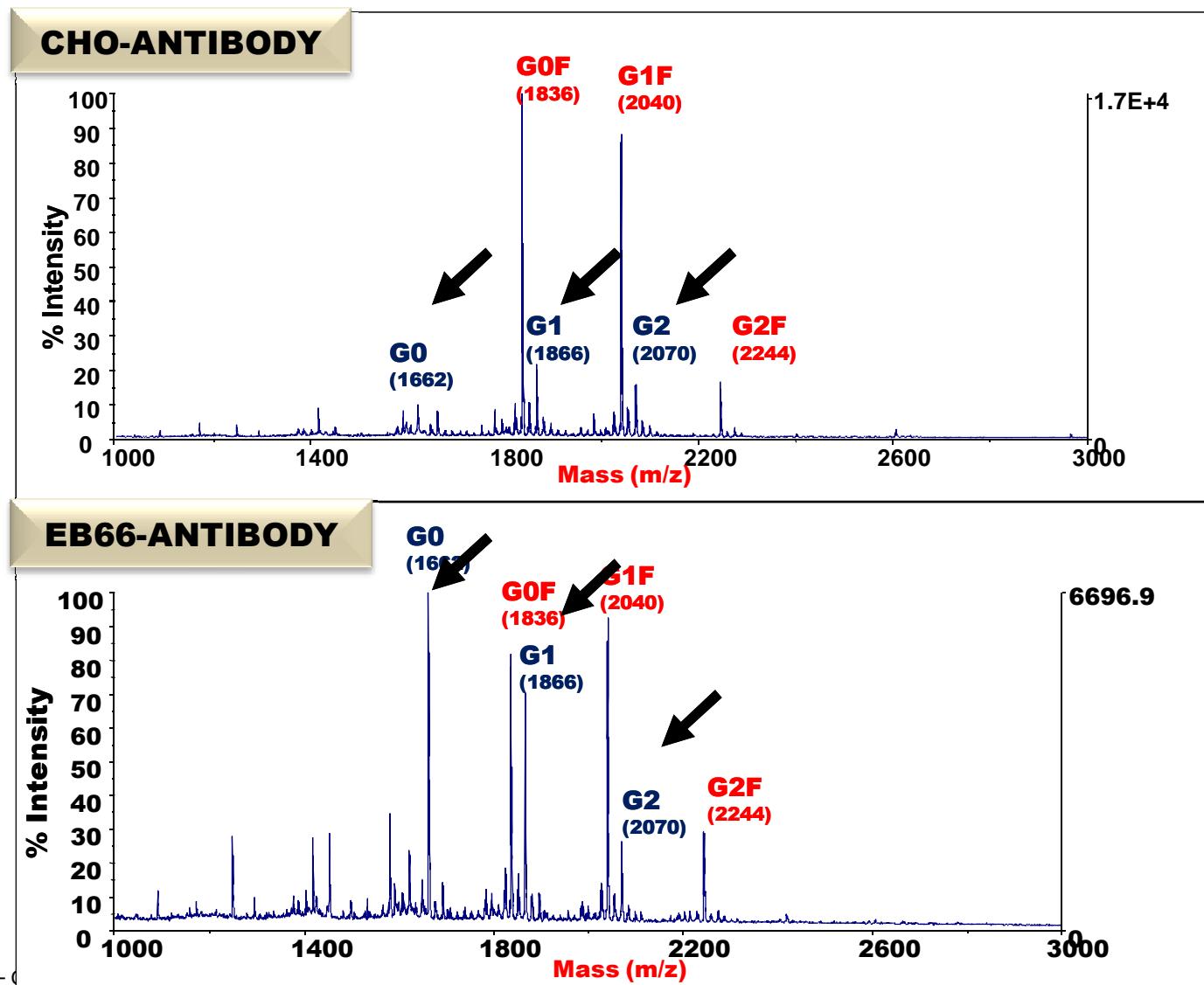
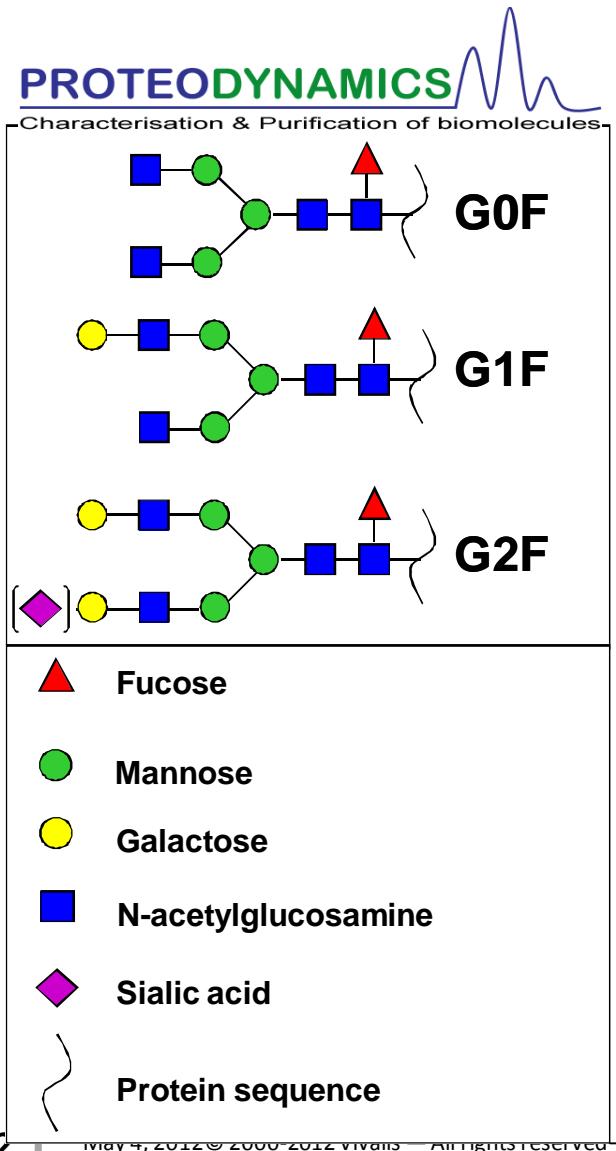


FEDBATCH PRODUCTION PROCESS IN 20L DISPOSABLE BIOREACTOR



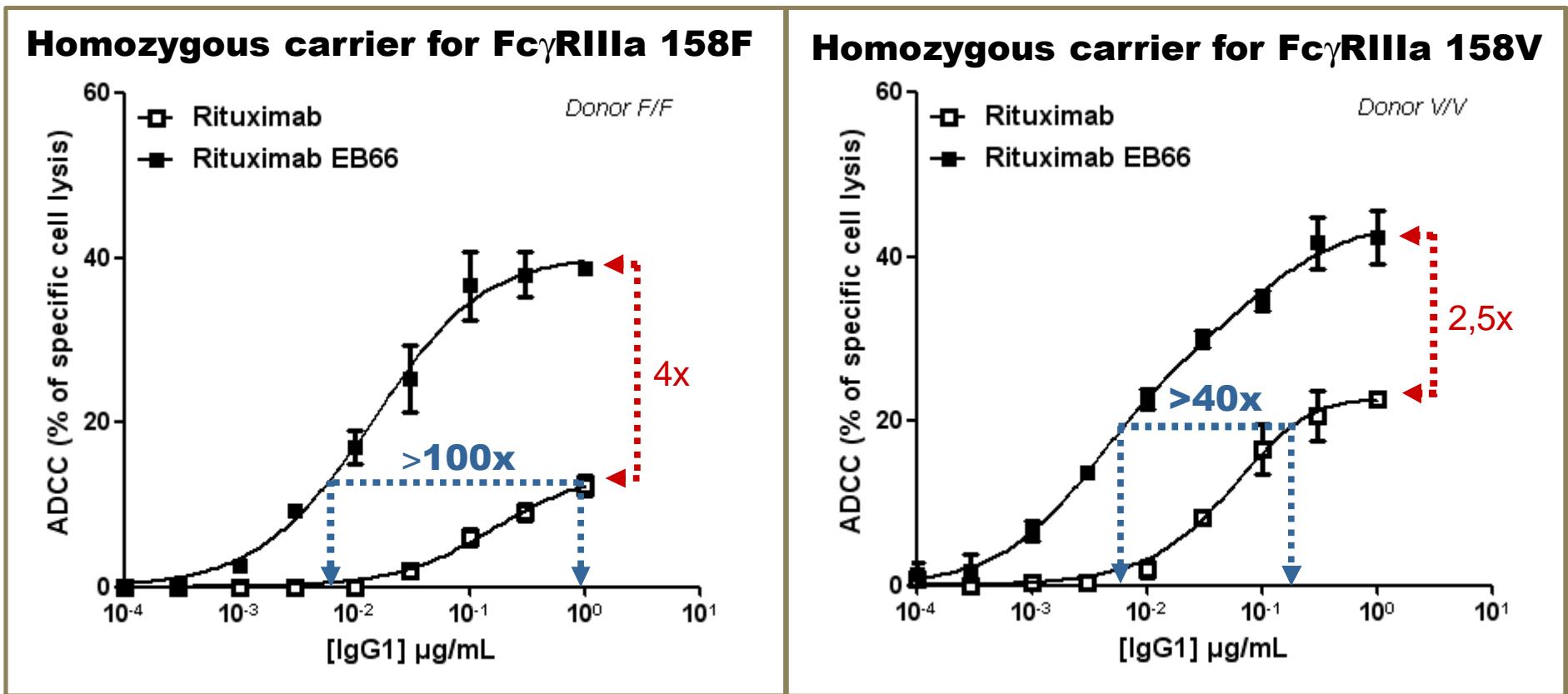
Glycosylation profile of EB66-IgGs

Increased percentage of G0/G1/G2 vs G0F/G1F/G2F populations (MALDI-TOF analysis)



■ ADCC activity of EB66-antibodies

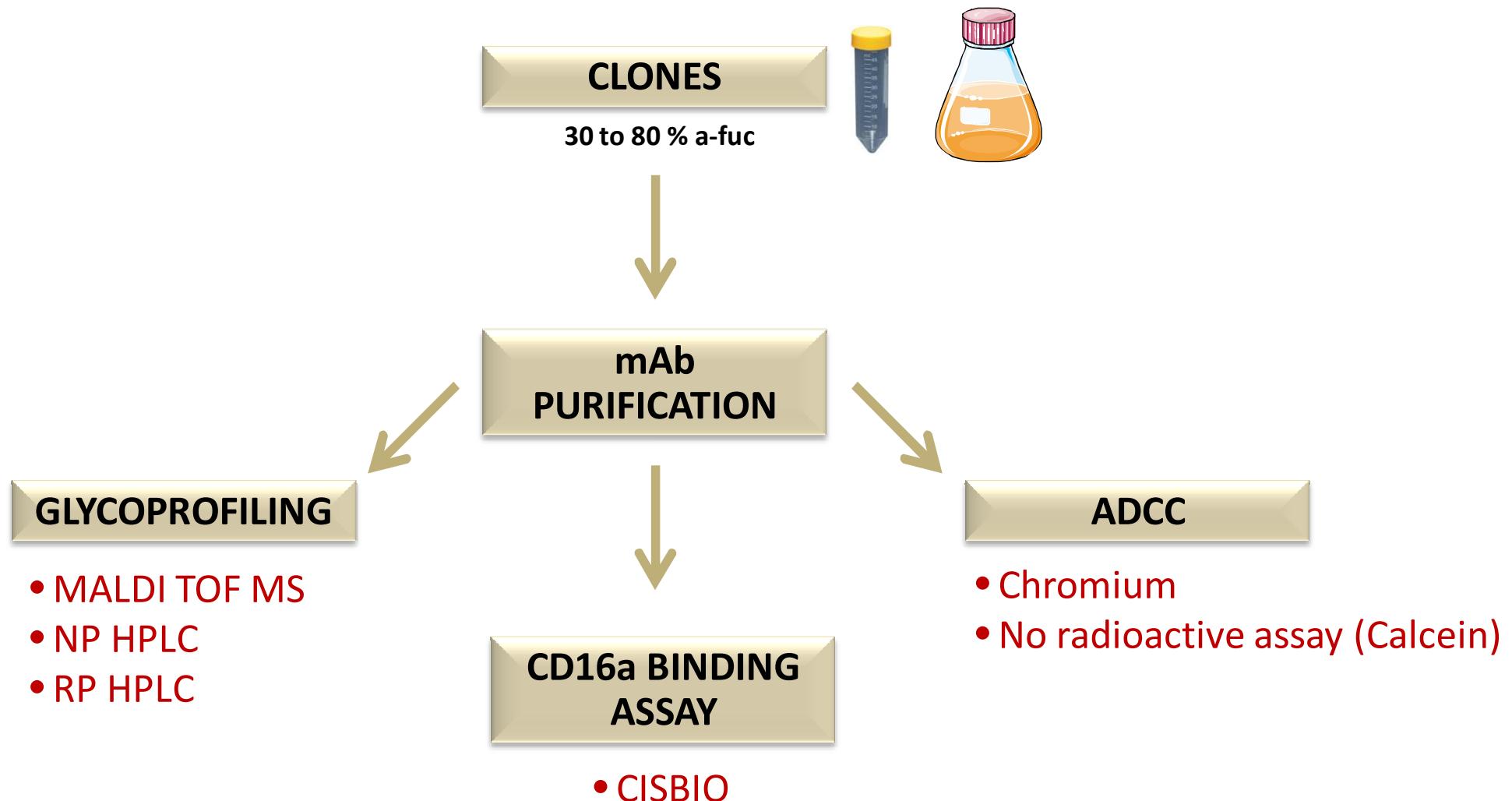
EB66 antibodies display low fucose and enhanced ADCC



LOW FUCOSE ANTIBODIES PRODUCED BY EB66 SHOW IMPROVED ADCC ACTIVITIES (*Similar results with 5 independent antibodies*)

■ Selection of low fucosylated mAb producers

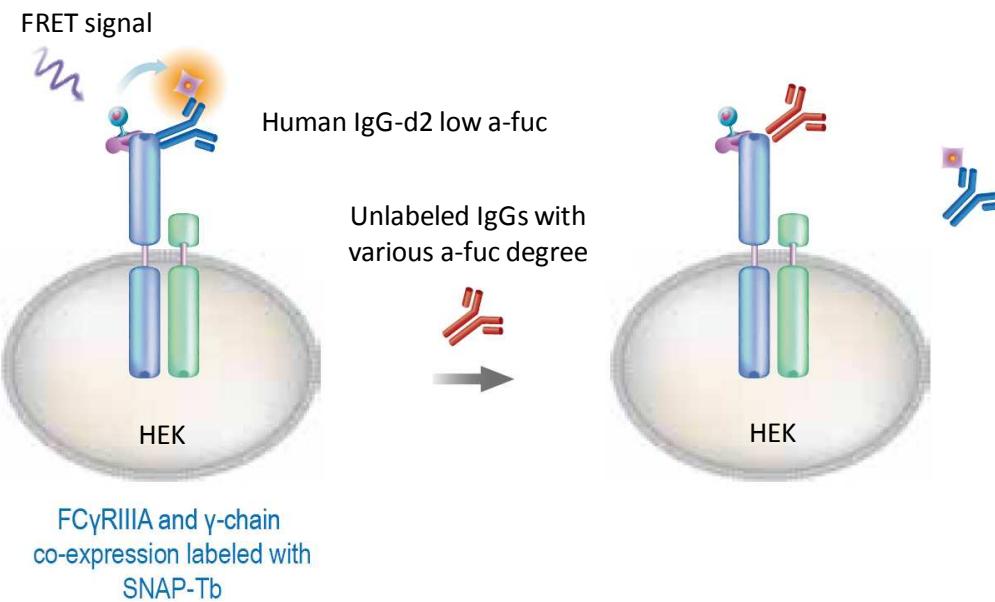
Analytical methods



■ TagLite technology

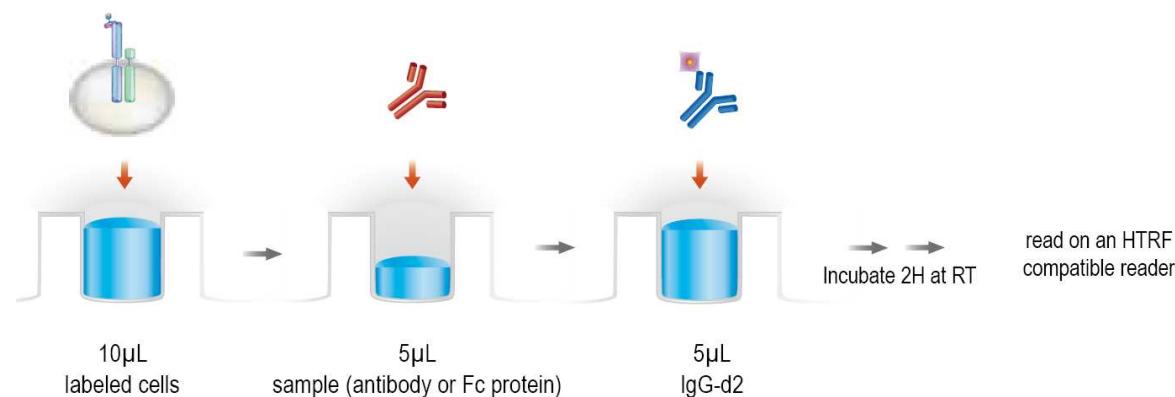
Fc γ RIIIaV158 competition assay

PRINCIPLE



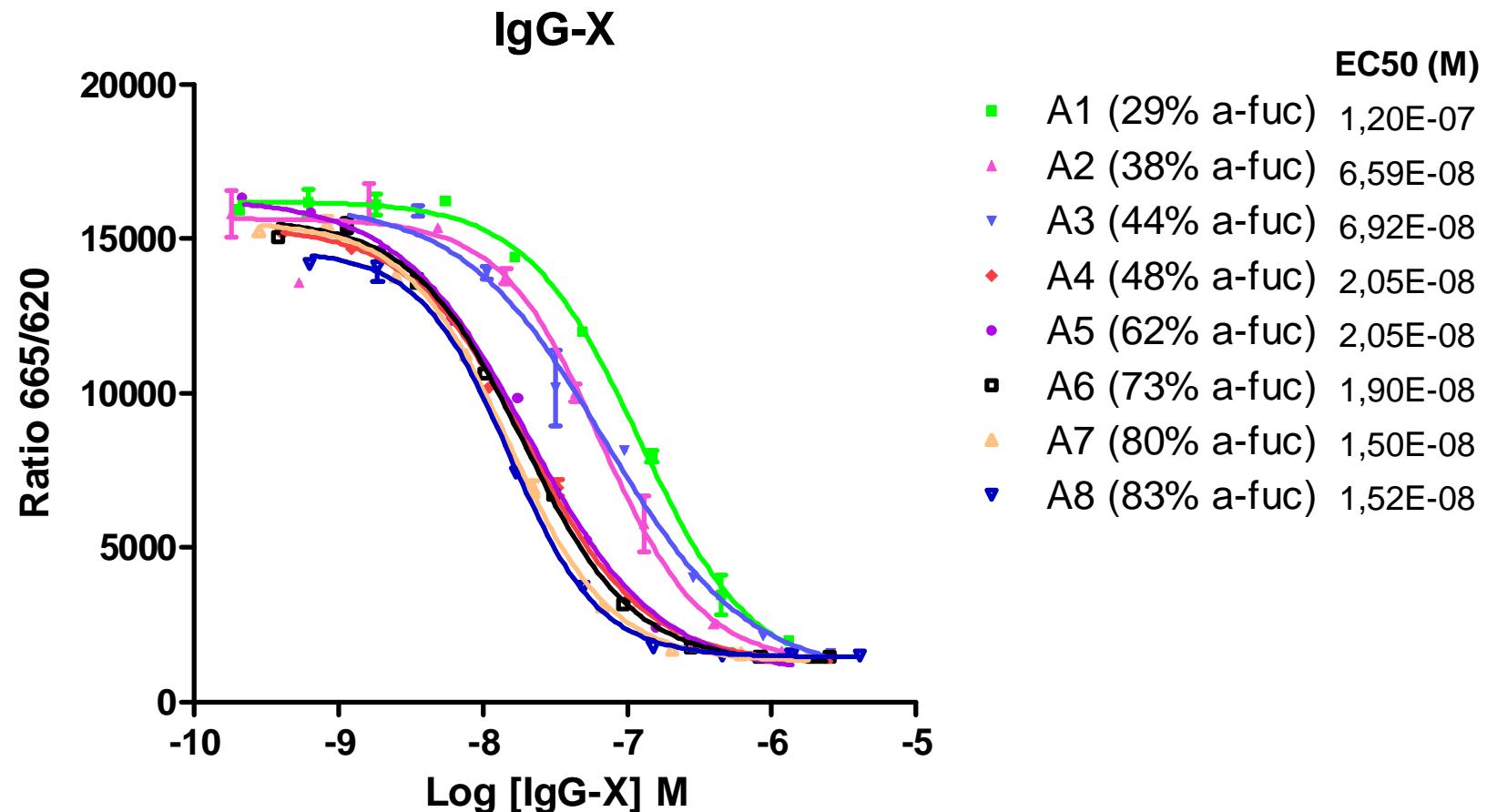
cisbio
Bioassays
Member of IBA group

PROTOCOL



TagLite technology

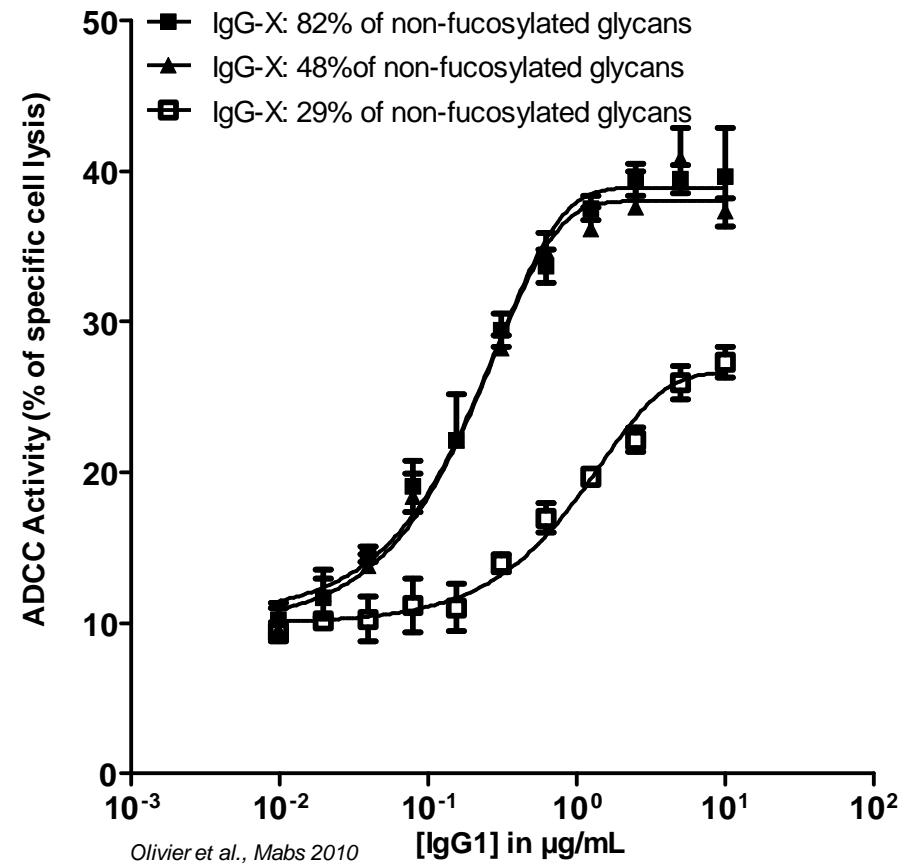
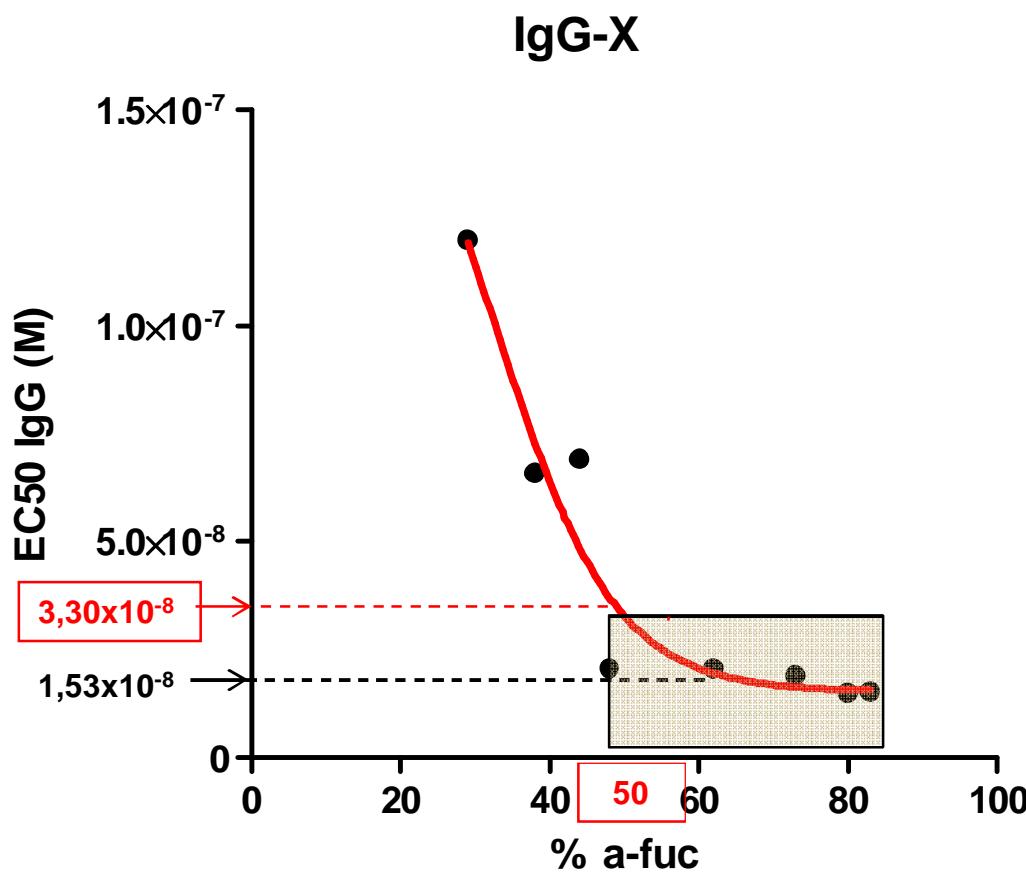
Fc γ RIIIaV158 competition assay - Results



BINDING OF ANTIBODY Fc PORTIONS TO Fc γ RIIIA ON CELLS CORRELATE
WITH GLYCOPROFILING

TagLite technology

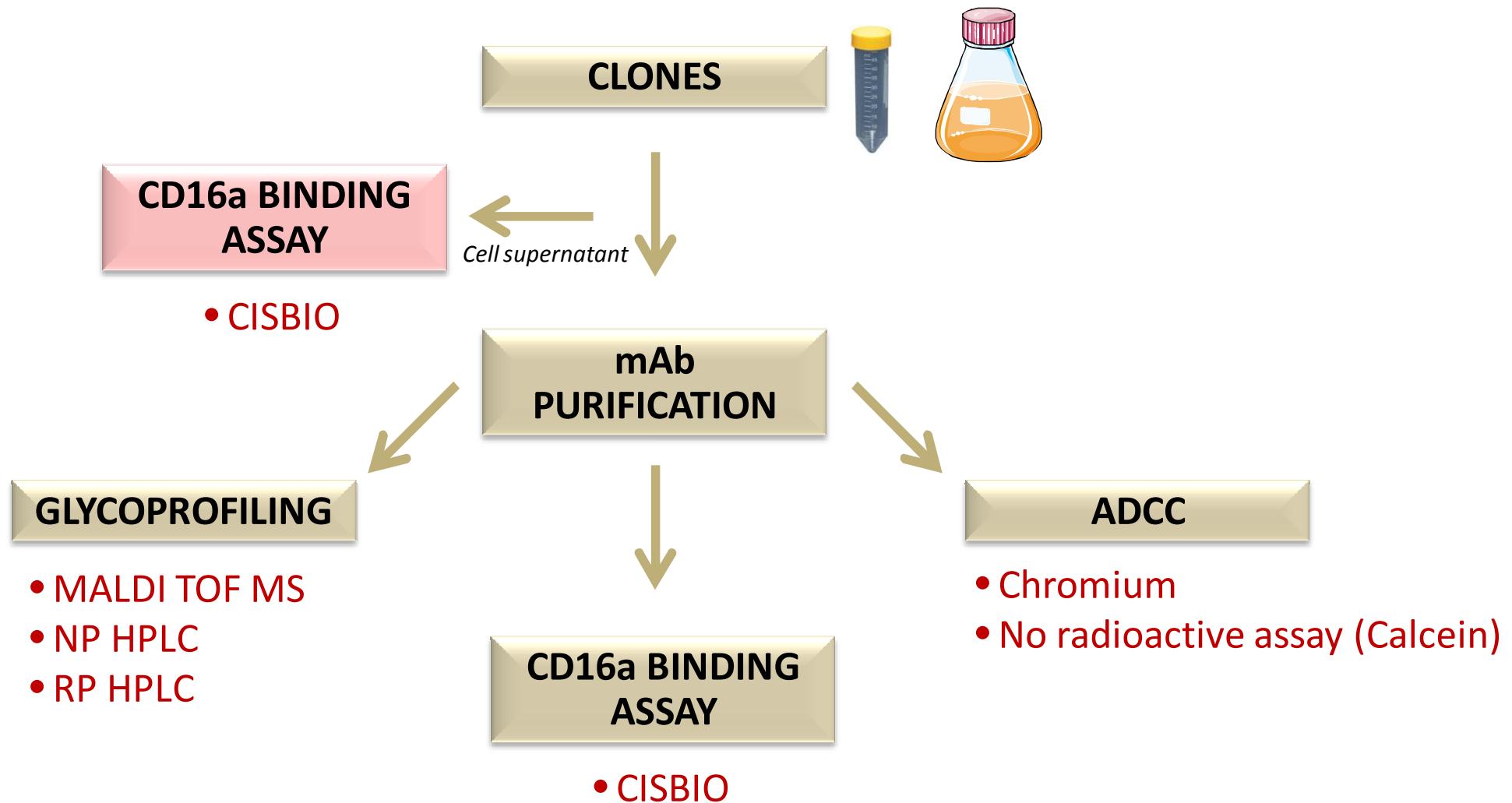
Fc γ RIIIaV158 competition assay - Results



50% of non-fucosylated oligosaccharides is sufficient to have the best CD16a affinity and thereby to confer maximum ADCC activity

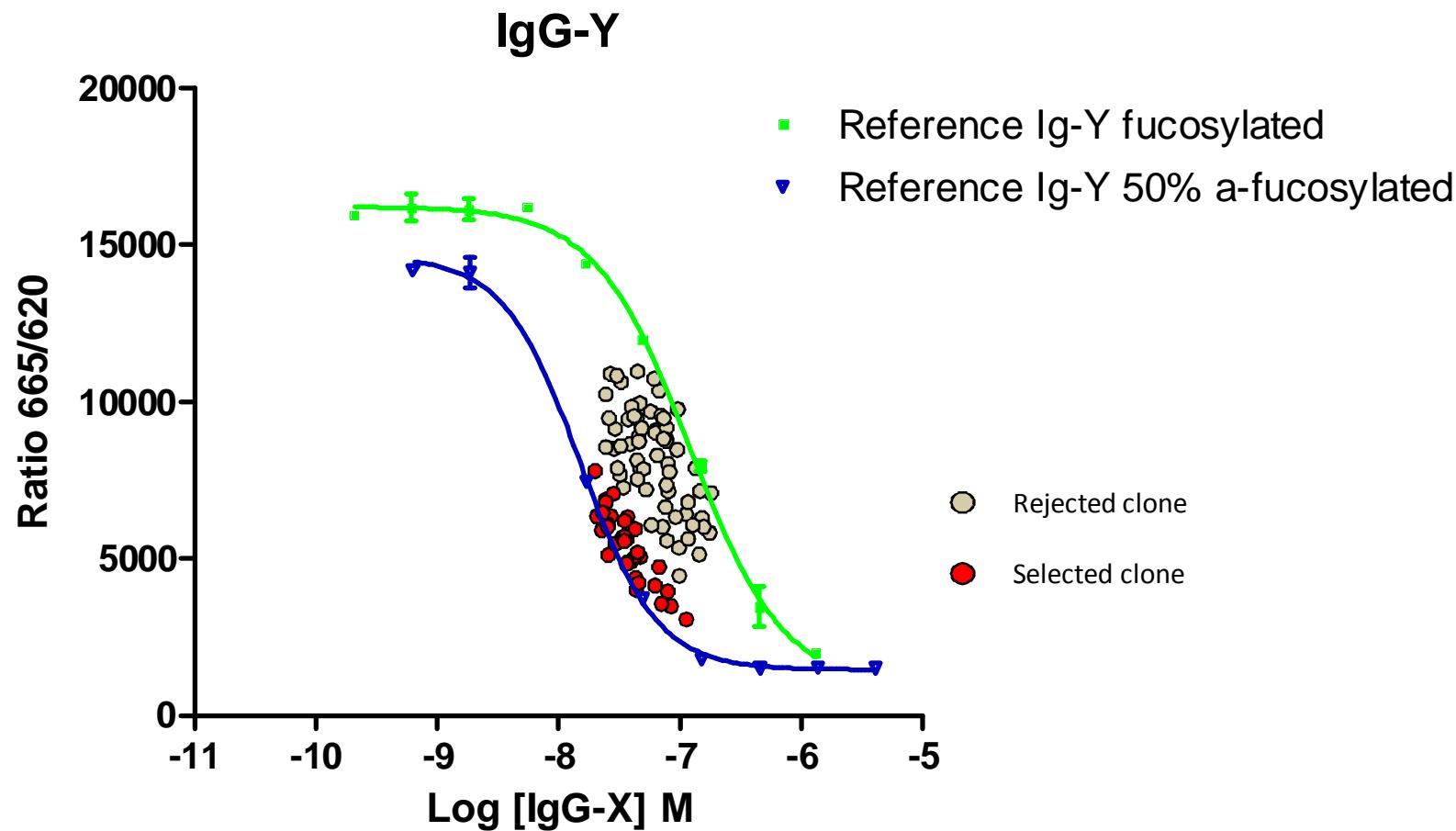
■ Selection of low fucosylated mAb producers

Analytical methods



TagLite technology

Fc γ RIIIaV158 competition assay - perspective



USE THE TagLite ASSAY FOR CLONE SCREENING FROM CELL SUPERNANT ?

The EB66® Platform

A unique technology in Biologics manufacturing

EB66® cells display unique technical, industrial & regulatory features

□ A safe substrate.

- Derivation from duck ES cells with no genetic, viral or chemical modifications
- Absence of endogenous retroviral particles
- cGMP Master Cell Bank available & Full process documentation & traceability
- Biological Master File (BMF) filed with the U.S. FDA.

□ Unique industrial properties.

- Long term genetic stability, short PDT, high cell densities (>40 million cells/mL) in suspension
- Broad susceptibility to human and veterinary vaccines
- mAbs produced in EB66 cells display low fucose content & enhanced ADCC activity
- Potential platform for difficult to express therapeutic proteins

□ Substrate for the production mAbs

- Promising production yields: >1.2gr/L in basic fedbatch process
- Low fucose content: 30 to 80% α-fucosylated IgG
- Optimization of screening process to selected best candidates based on glycosylation profile and mAb productivity
- Implementation of several analytical methods including TagLite CD16a binding assay which is a robust and easy to use tool to study binding of antibody Fc portions to FcγRIIIa

Acknowledgements

Fabrice LeGall

Fabienne Guehenneux

Goeffrey Blanc

Marine Jacoby

Majid Mehtali

From Cisbio

Delphine Jaga

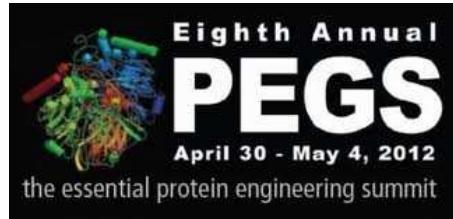
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We invite you to visit our booth, #18 and CISBIO's booth, #64



From cells to therapeutics **Vivalis**®

Merci
Thank You
ありがとうございました