



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

PEGS, Boston  
May 3<sup>rd</sup> 2012

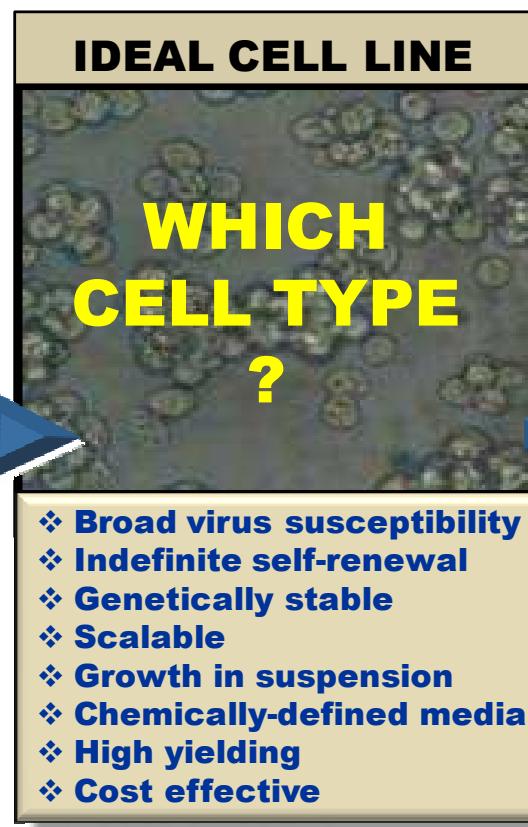
# Cell substrates for vaccines production

*A stem cell alternative to the embryonated chicken eggs*



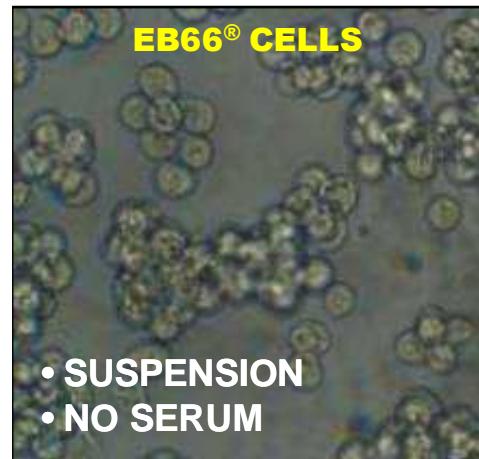
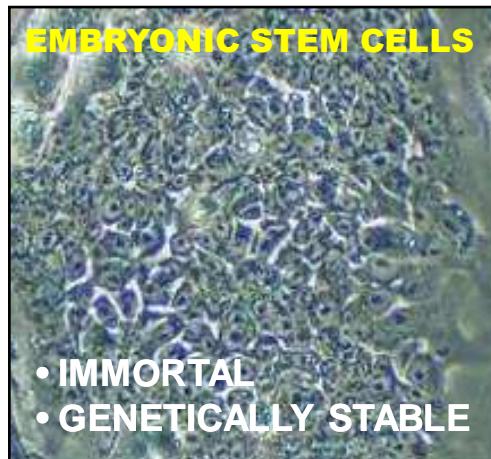
## BUT BESET BY ISSUES

- 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*



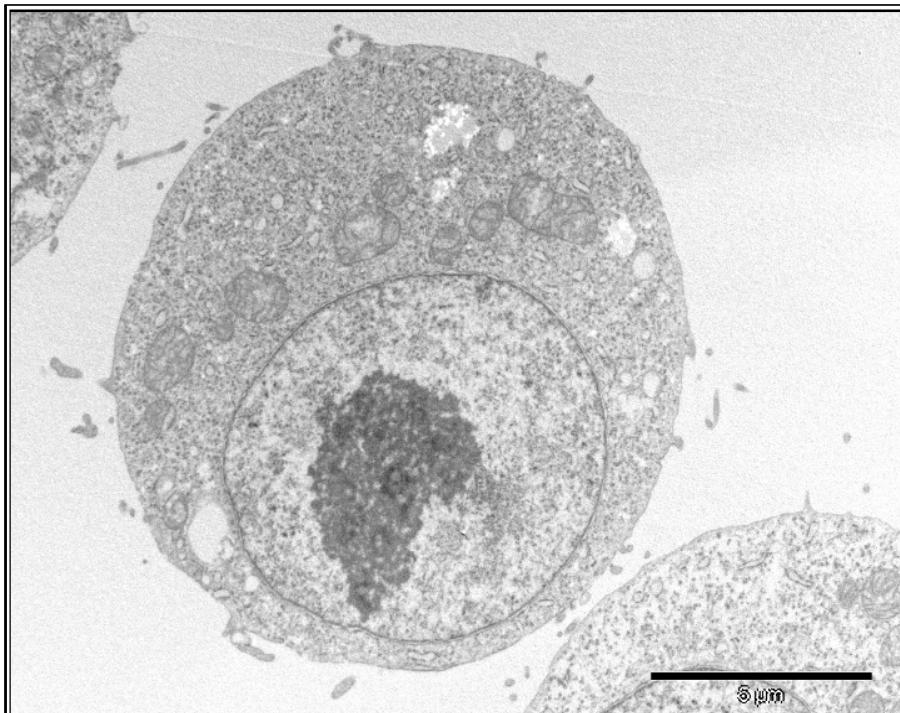
No genetic modifications and no viral nor chemical modifications

Fully documented from animal substrate to isolated EB66® Cells

# The duck EB66® cell line

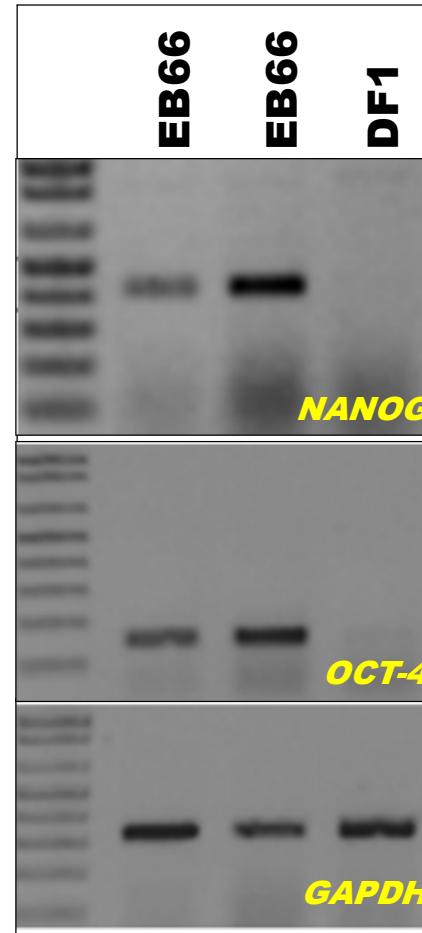
*Maintenance of ES cells unique properties*

## ULTRASTRUCTURE SIMILAR TO EMBRYONIC STEM CELLS

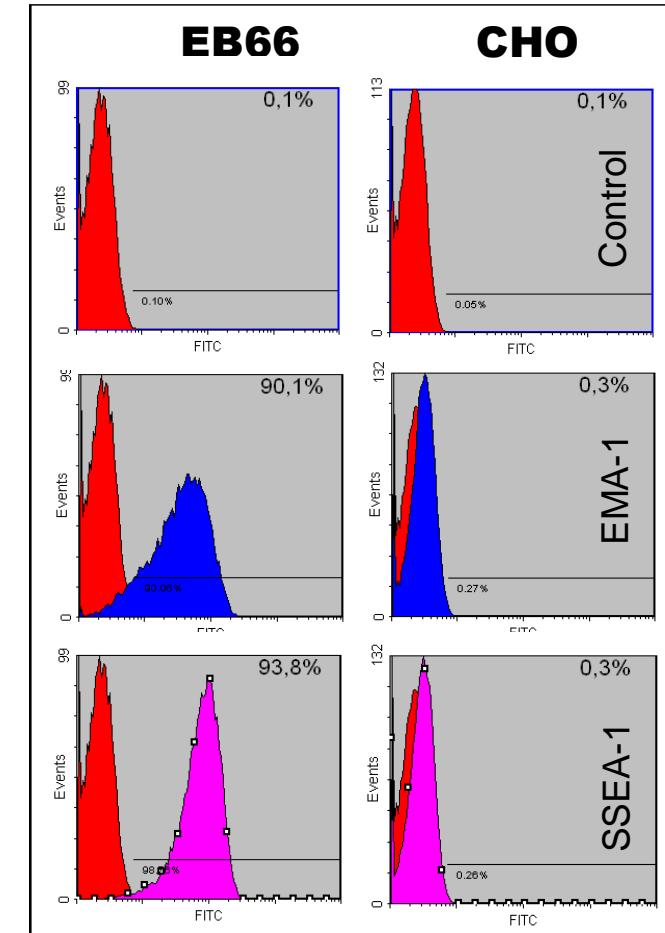


- Abundant mitochondria & ribosomes
- Large nucleus & nuclear bodies
- Small size (~8-10 μm)

## EXPRESSION OF « STEMNESS » GENES



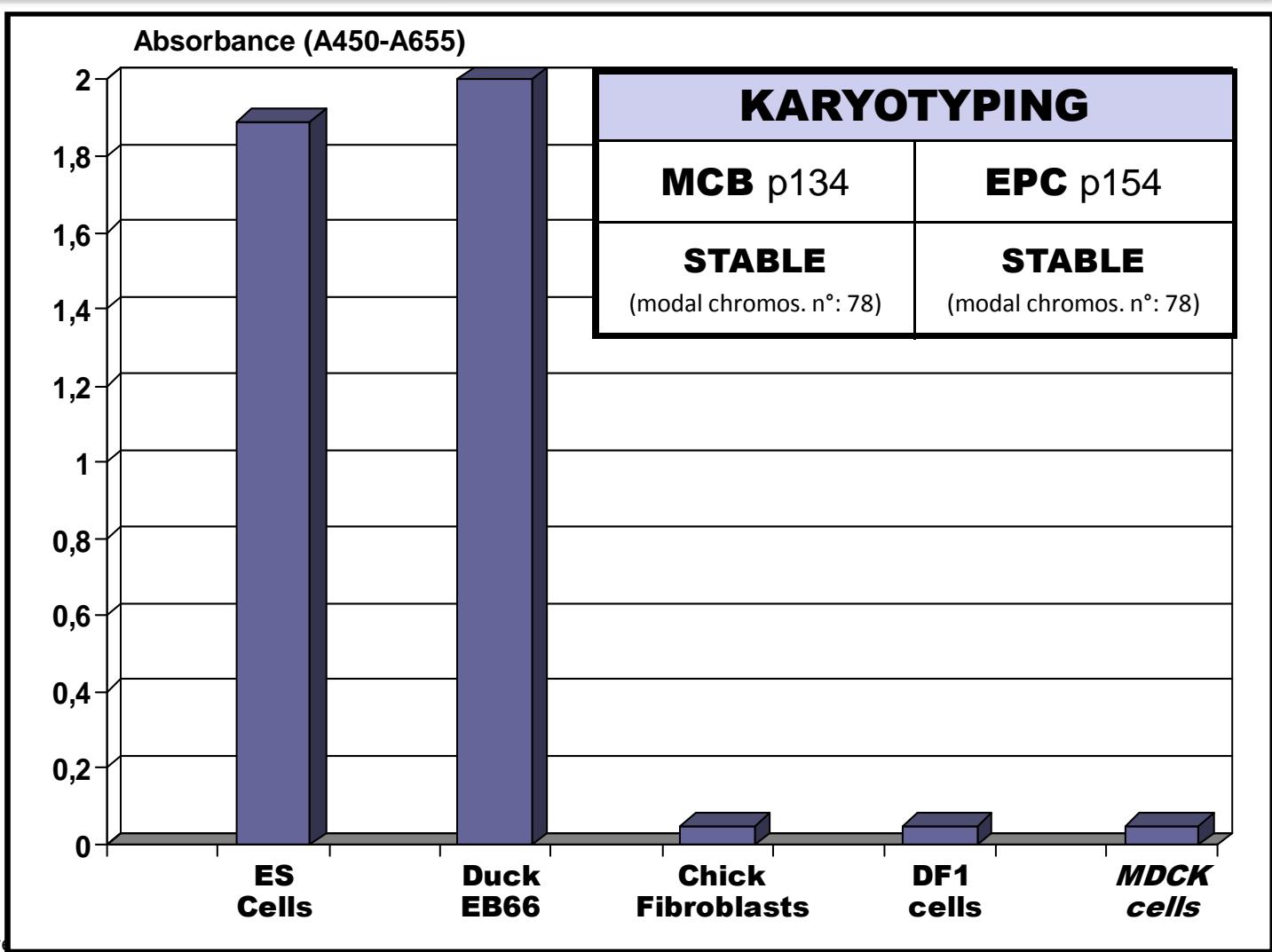
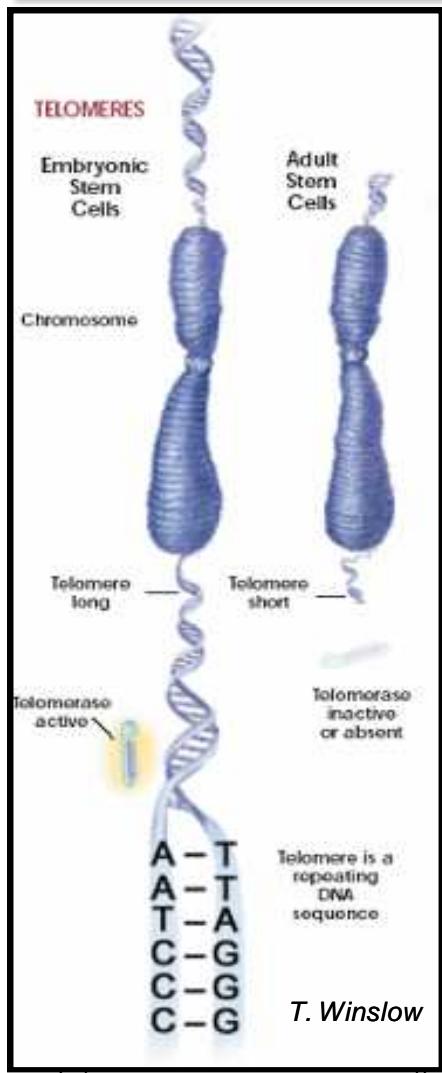
## EXPRESSION OF ES CELL SURFACE MARKERS



# 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

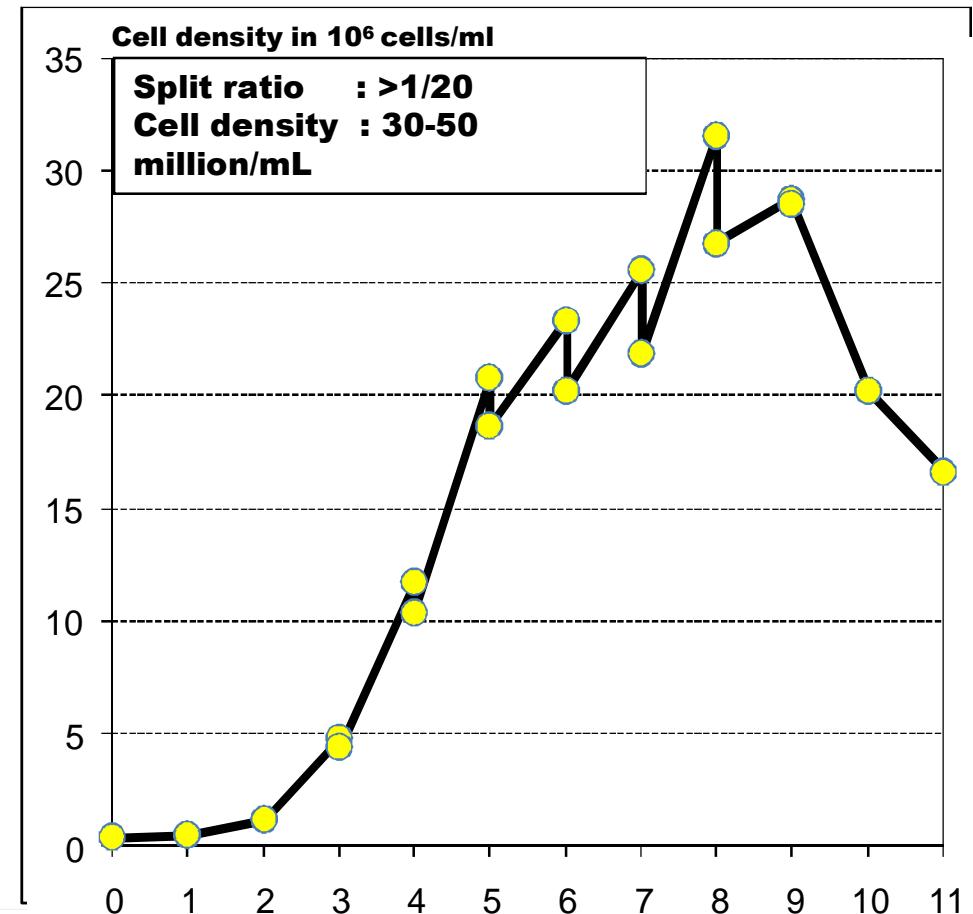
*Cell culture characteristics; currently up to 1000L*



**Single-Use  
Bioreactor**



**Stainless Steel  
Bioreactor**

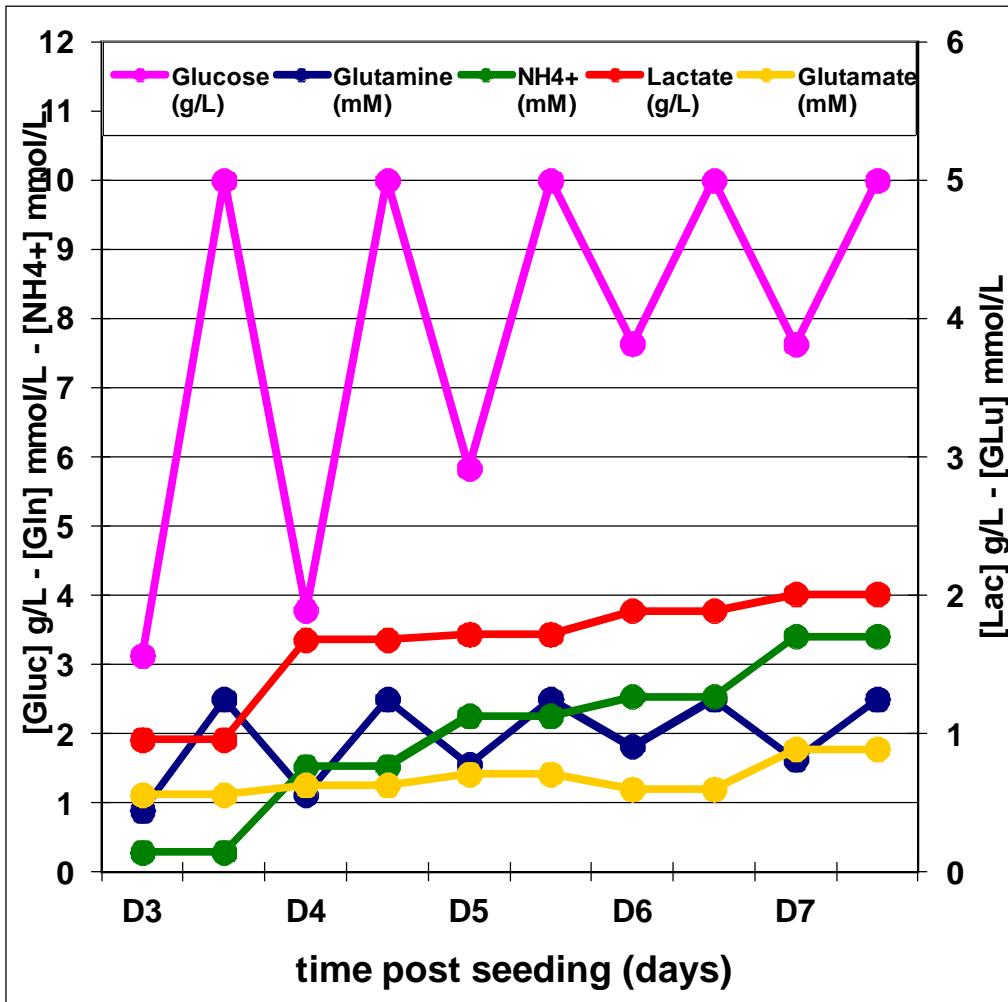


## Custom serum-free medium:

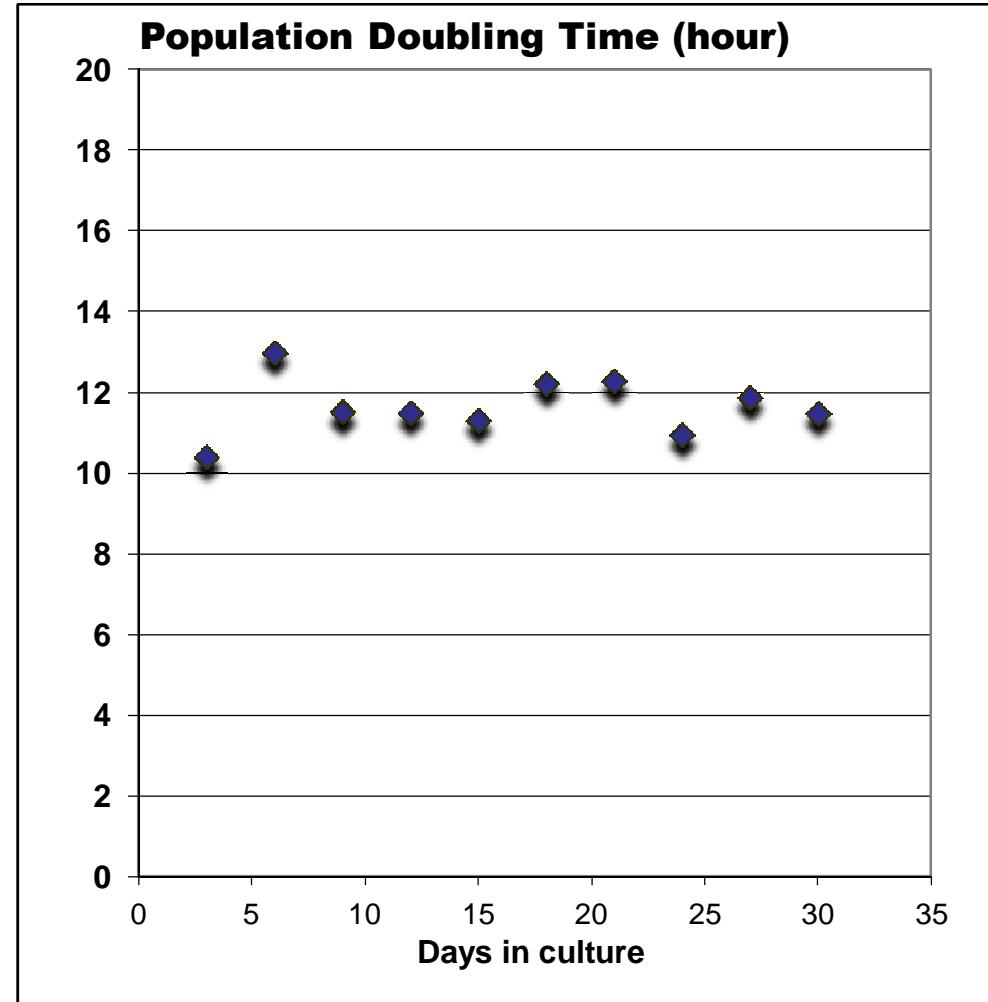
- ✓ Liquid & powder
- ✓ R&D grade & GMP grade
- ✓ Devoid of components of primary & secondary animal origin
- ✓ Cost-effective

# 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

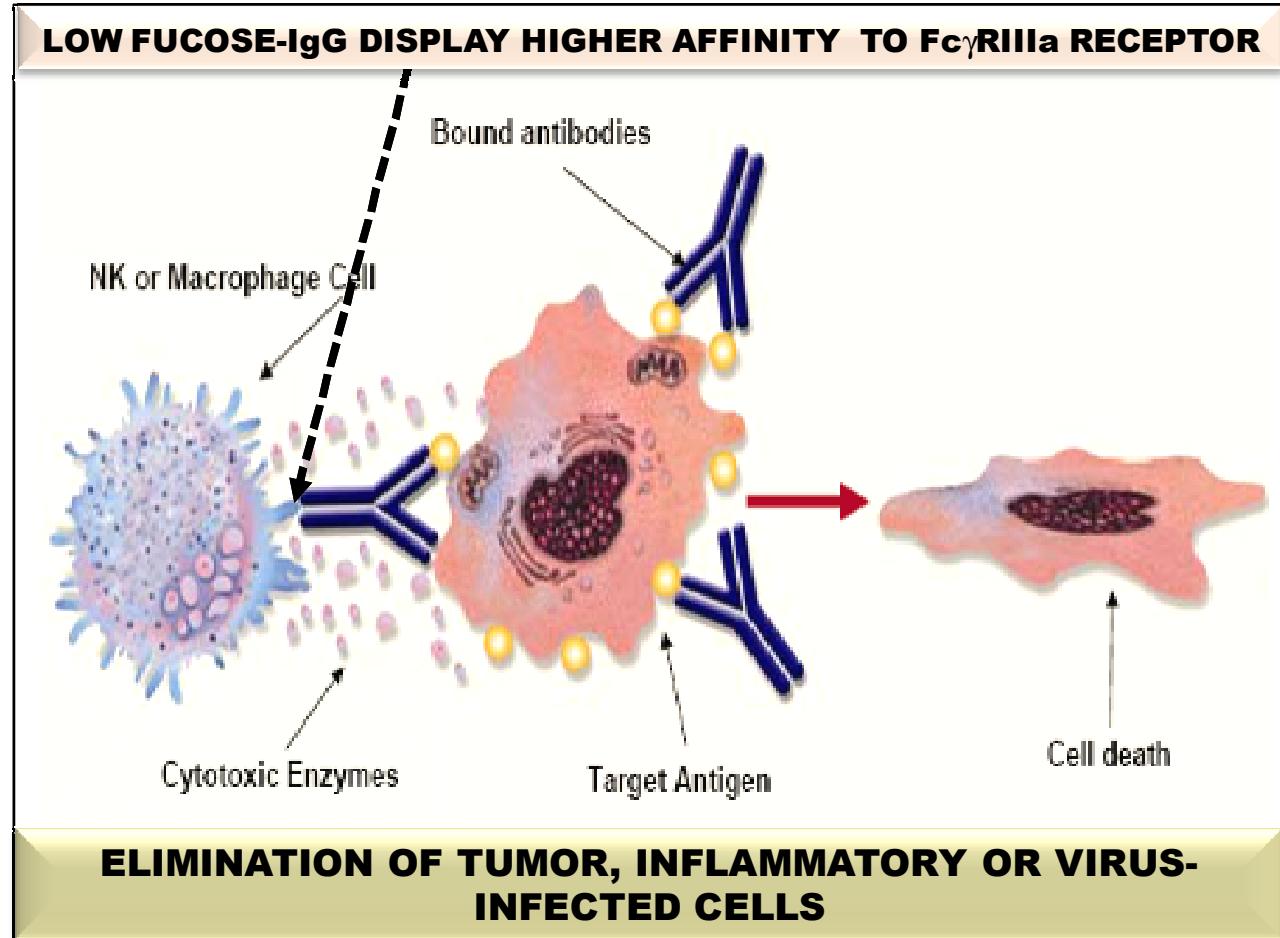
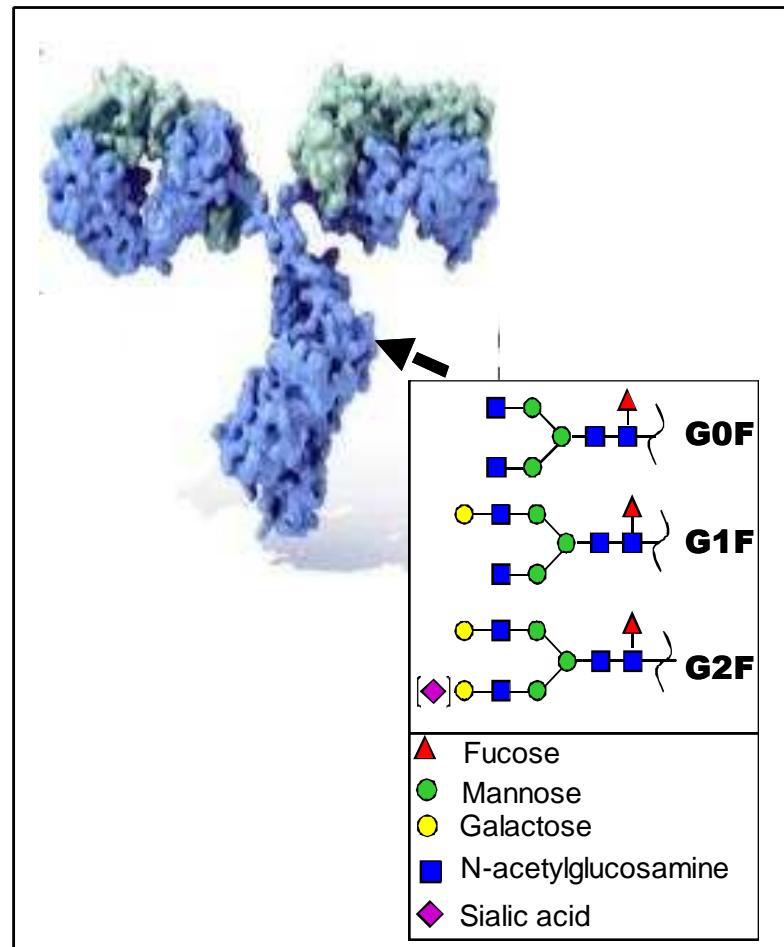
*A new standard for the production of vaccines*



- **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 $\gamma$ RIIIa receptors*



# ■ Biomanufacturing of Antibodies with enhanced ADCC

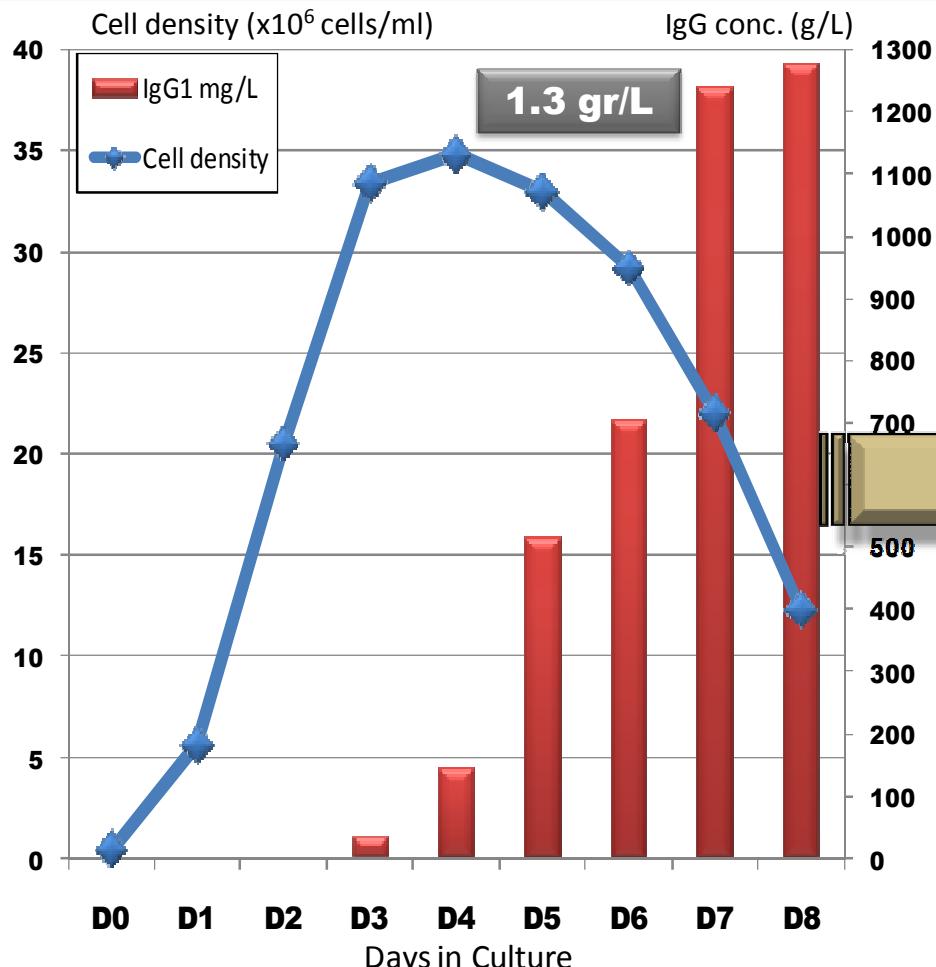
*Development of a novel cell substrate for antibody production*



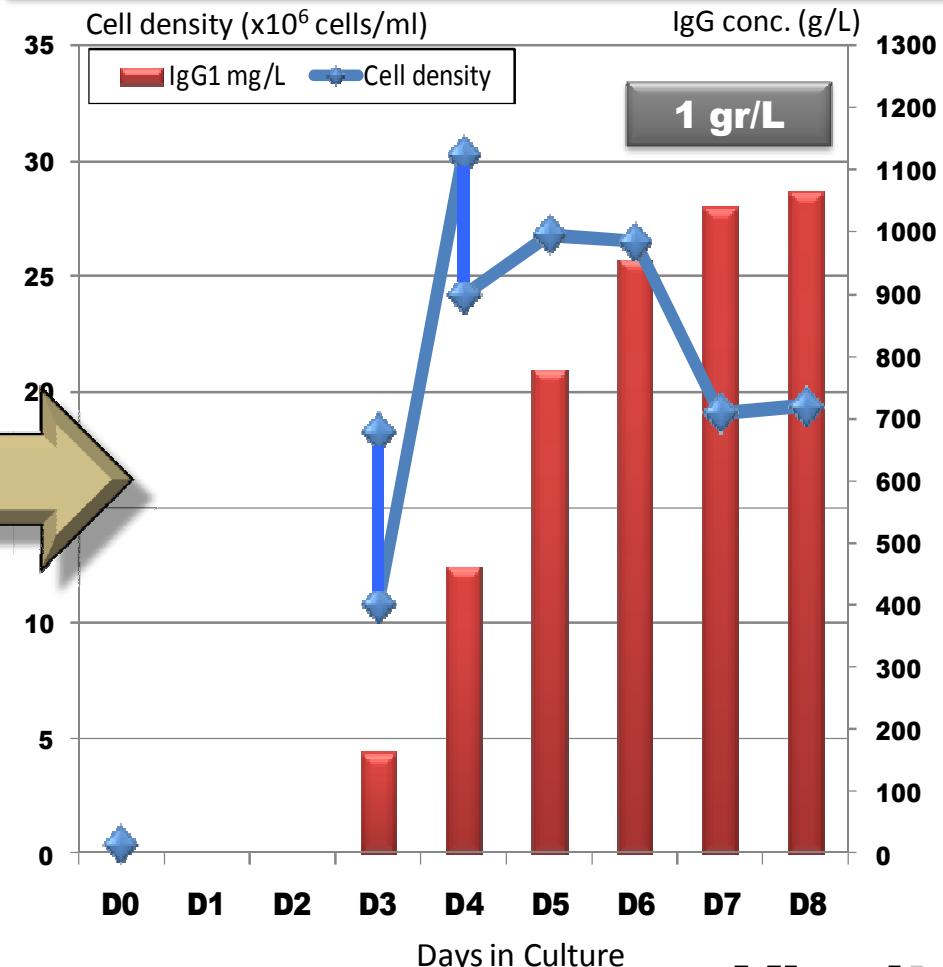
# Cell Culture Media in fed-batch Culture

mAb production and upscaling

## FEDBATCH PRODUCTION PROCESS IN SHAKE FLASK

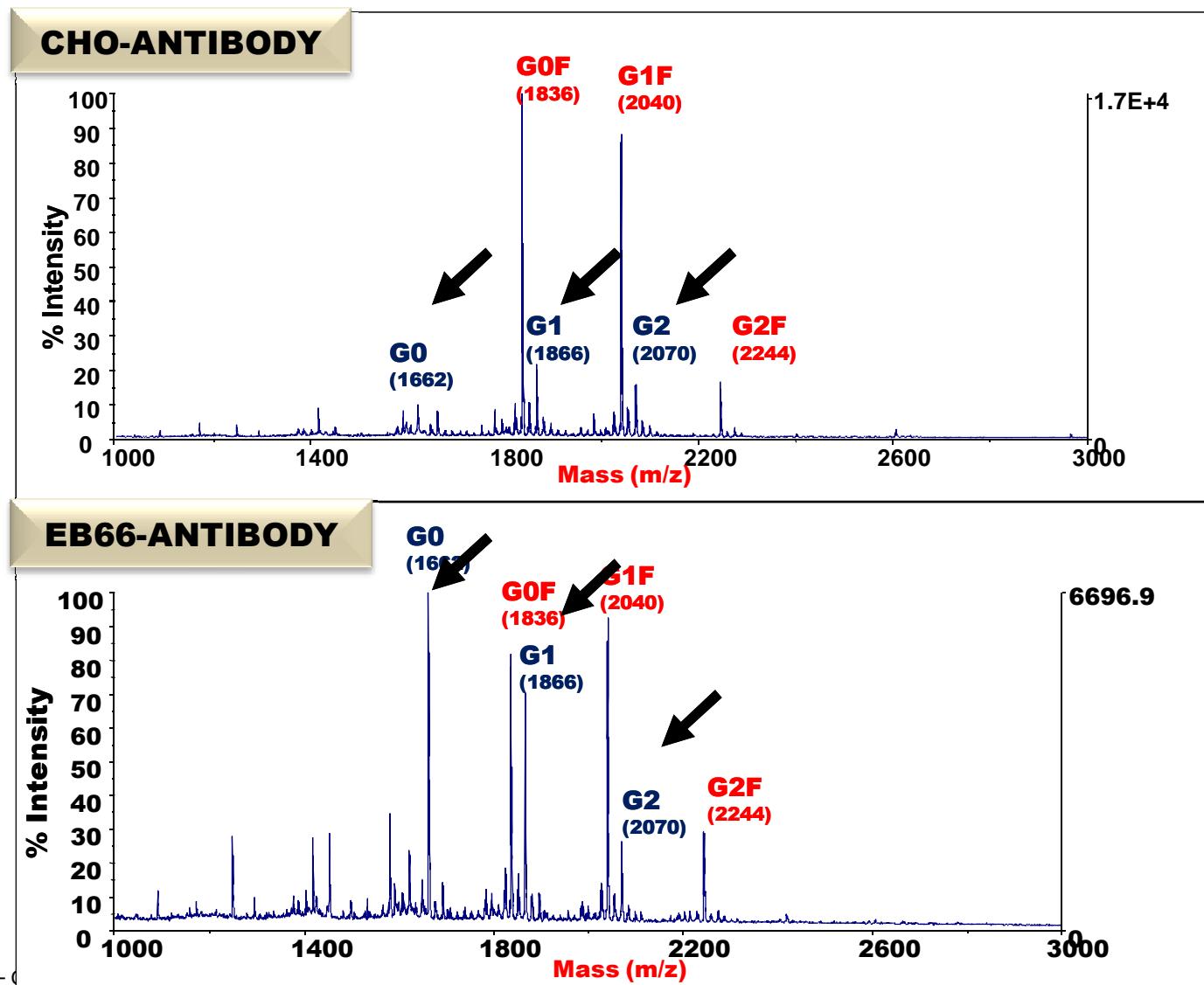
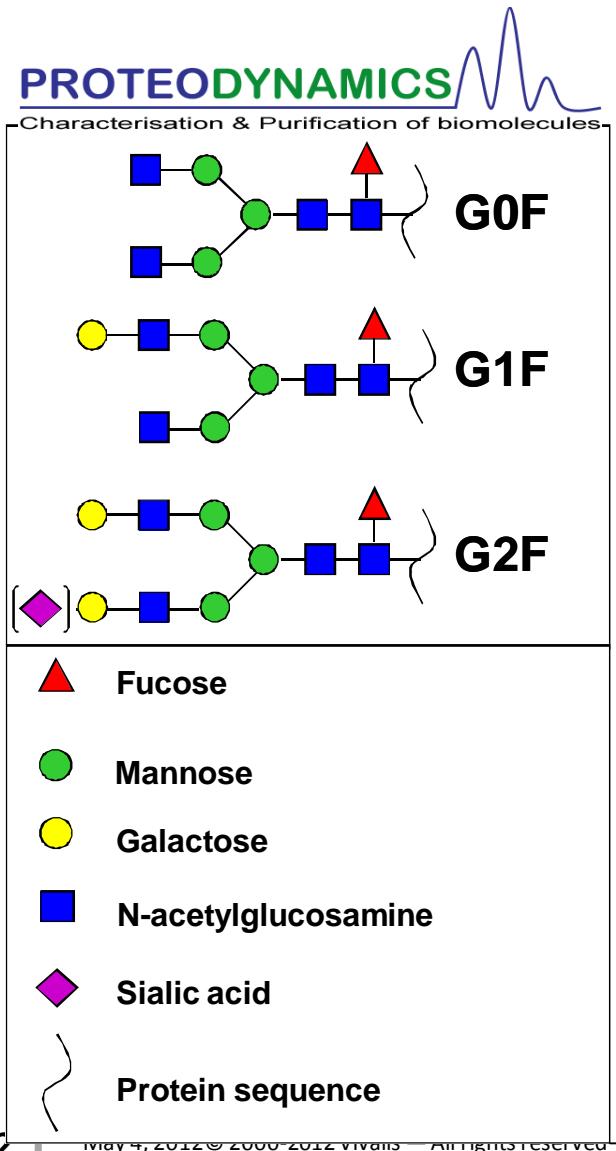


## FEDBATCH PRODUCTION PROCESS IN 20L DISPOSABLE BIOREACTOR



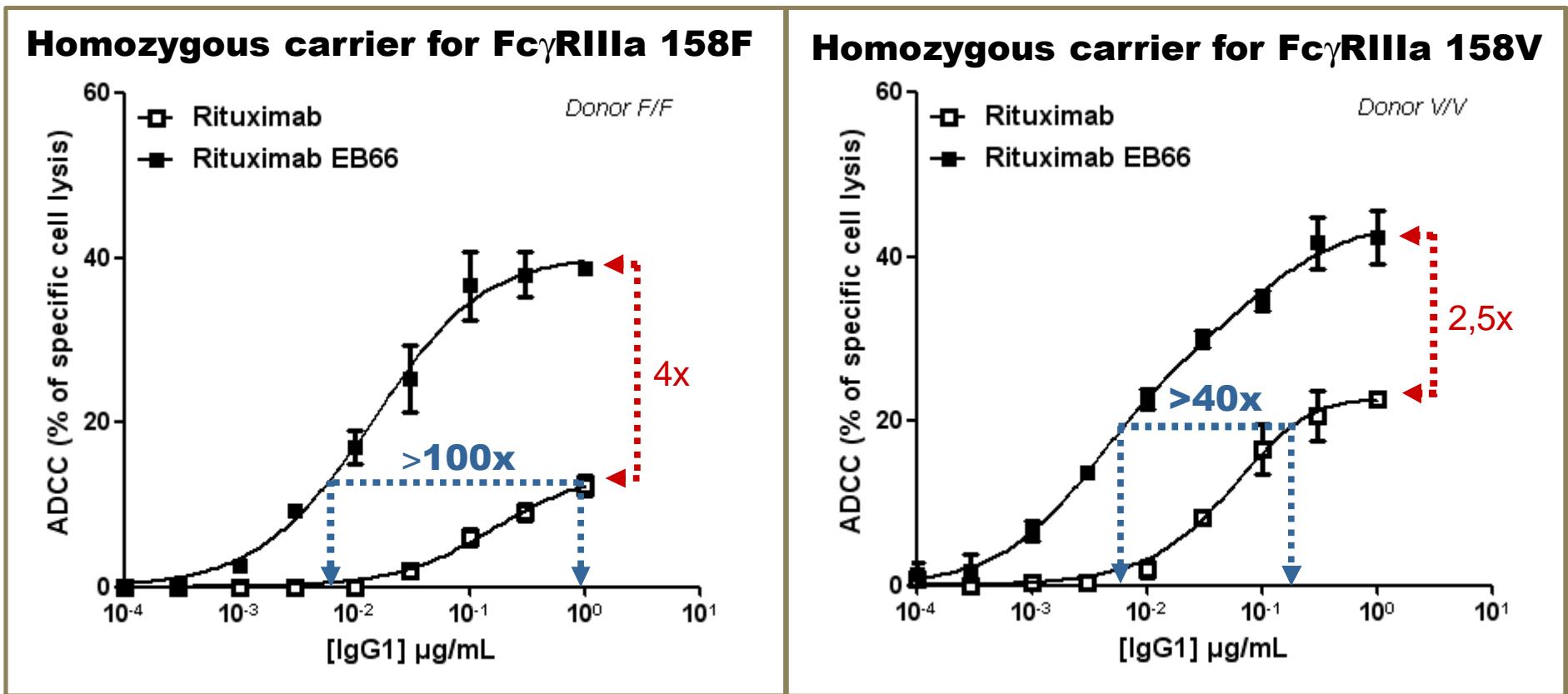
# Glycosylation profile of EB66-IgGs

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



# ■ ADCC activity of EB66-antibodies

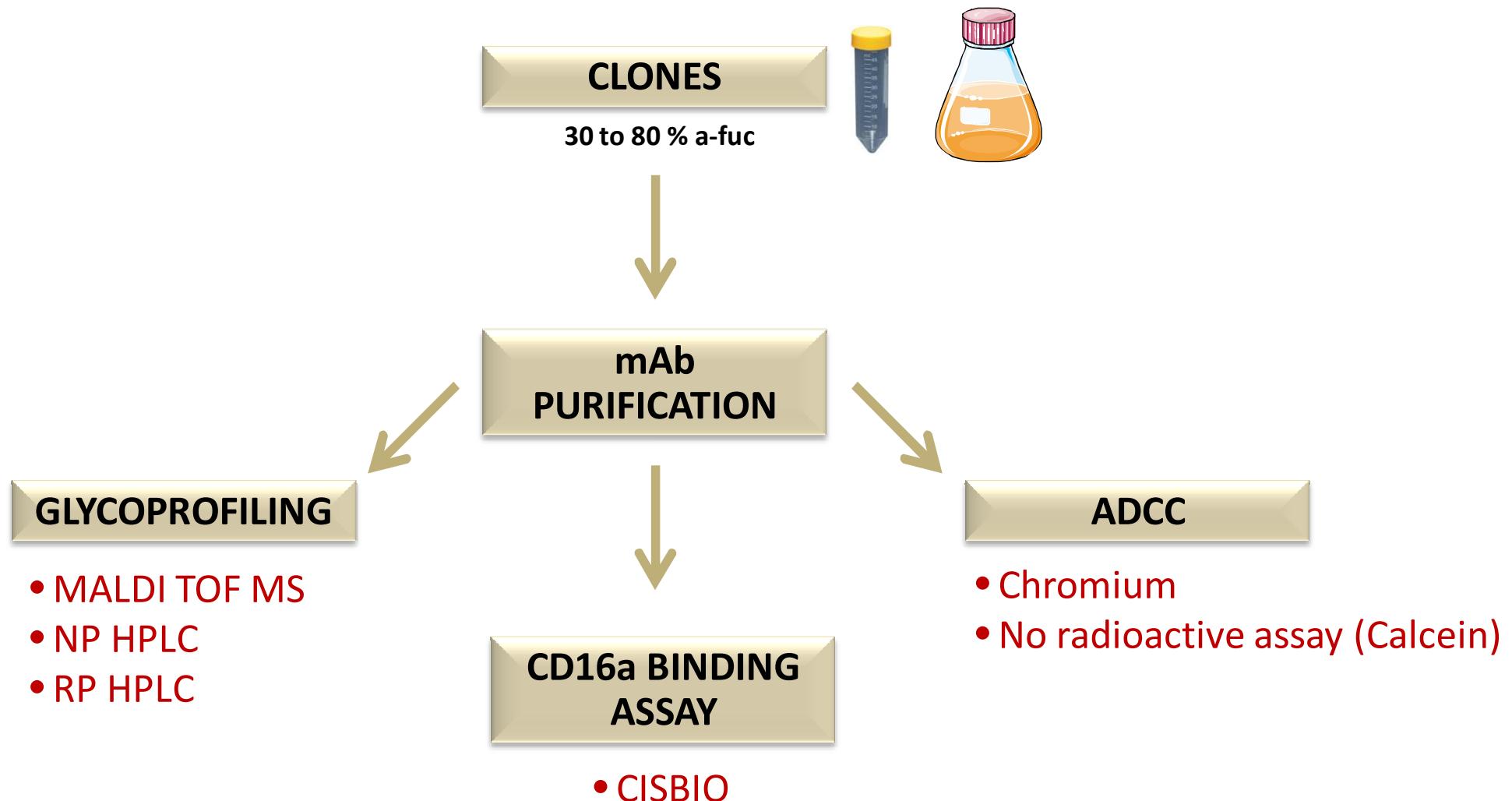
*EB66 antibodies display low fucose and enhanced ADCC*



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

# ■ Selection of low fucosylated mAb producers

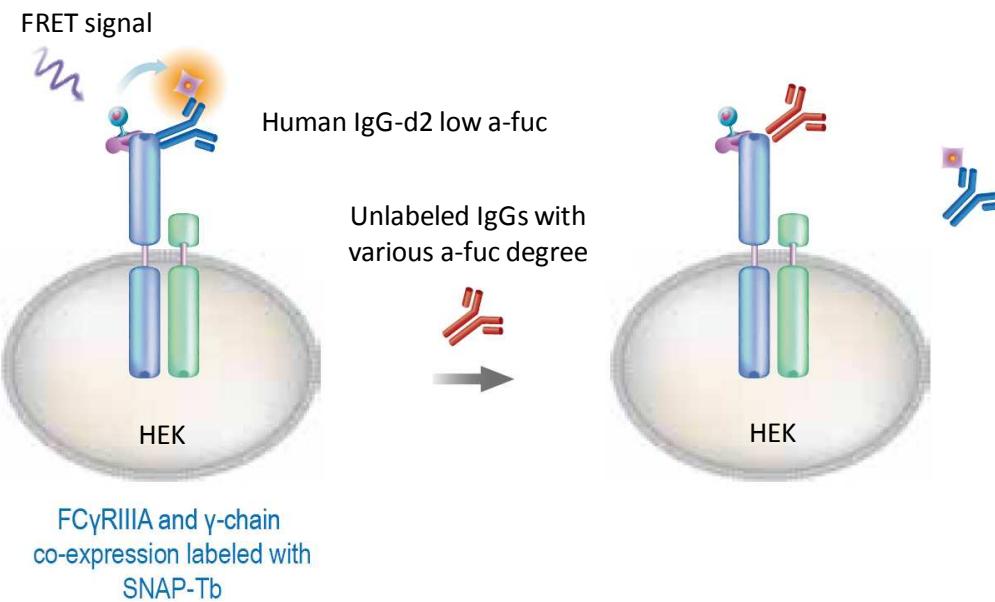
## *Analytical methods*



# ■ TagLite technology

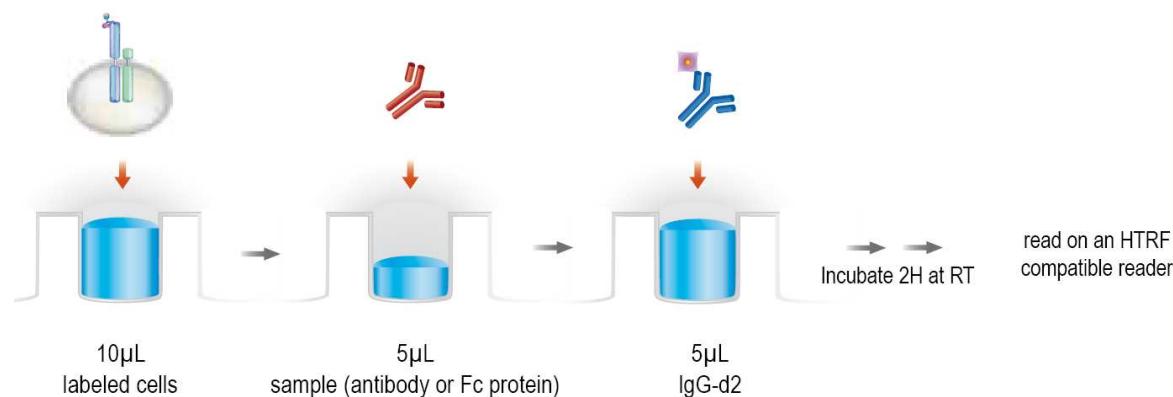
## *Fc $\gamma$ RIIIaV158 competition assay*

### PRINCIPLE



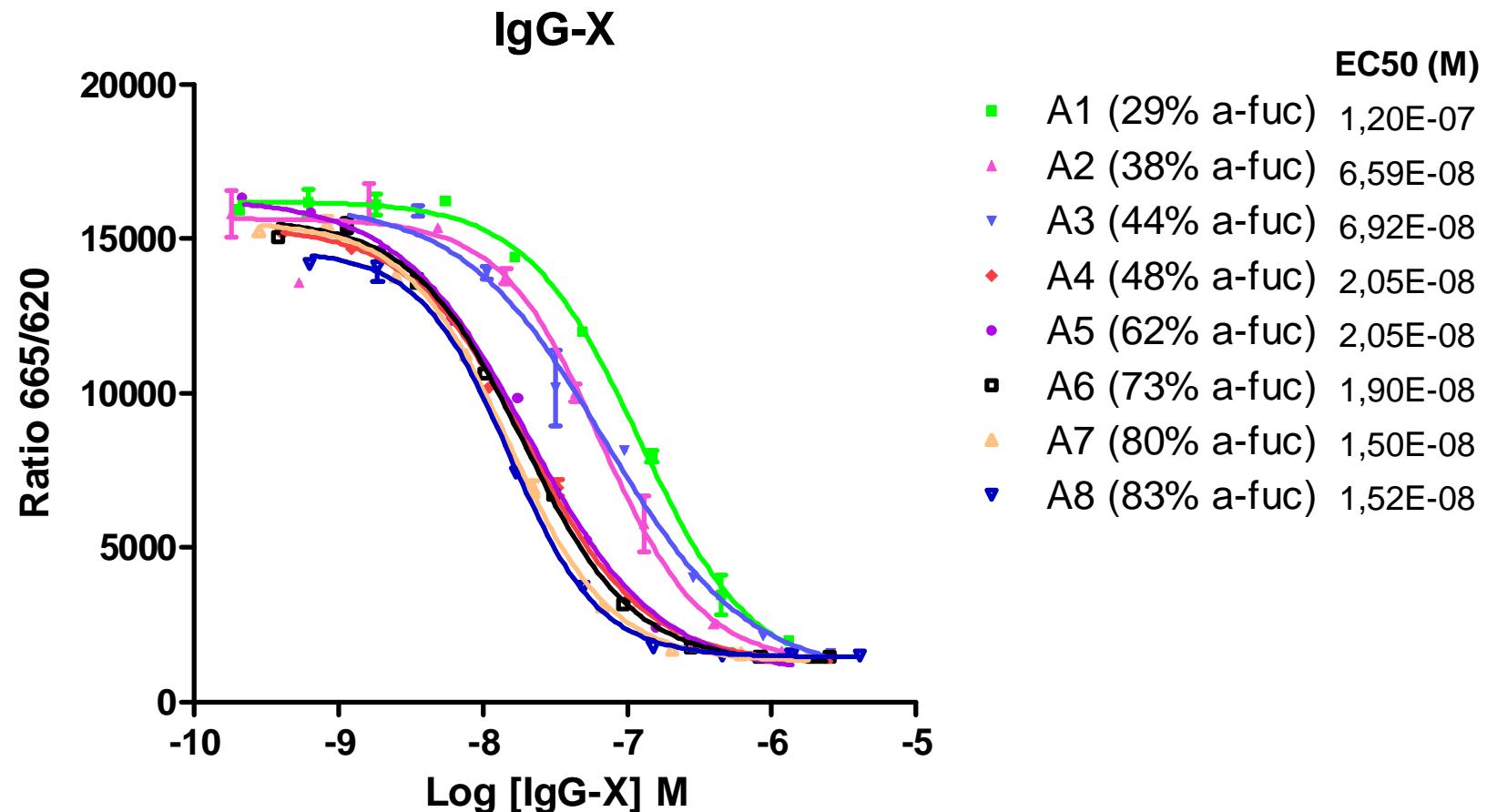
**cisbio**  
Bioassays  
Member of IBA group

### PROTOCOL



# TagLite technology

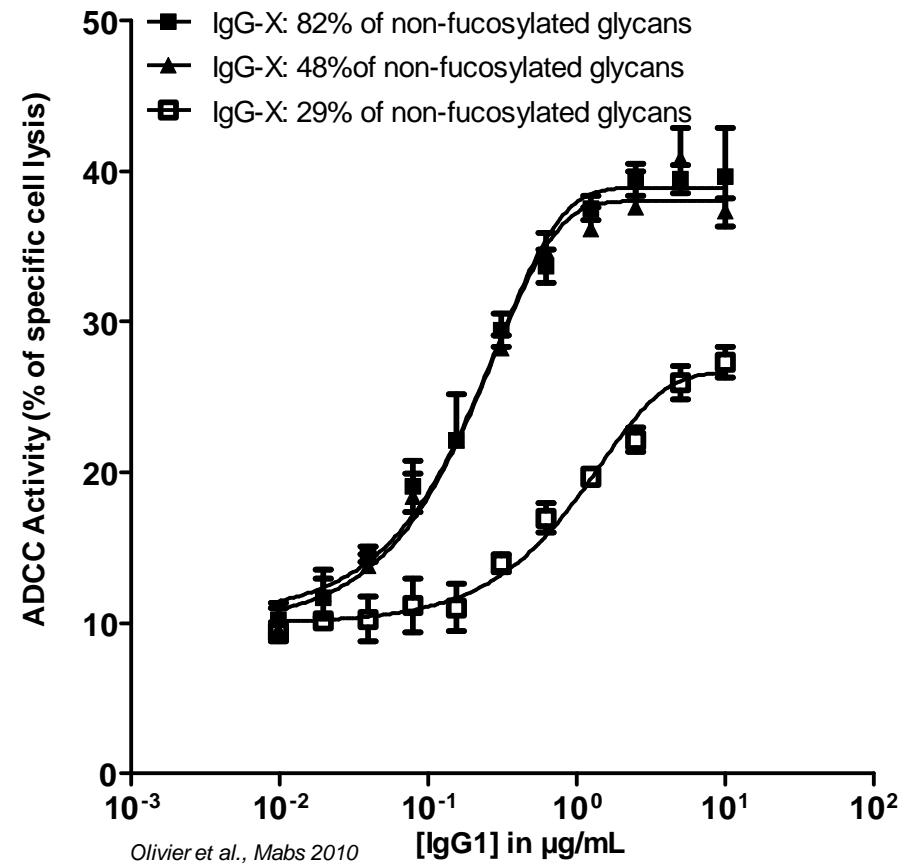
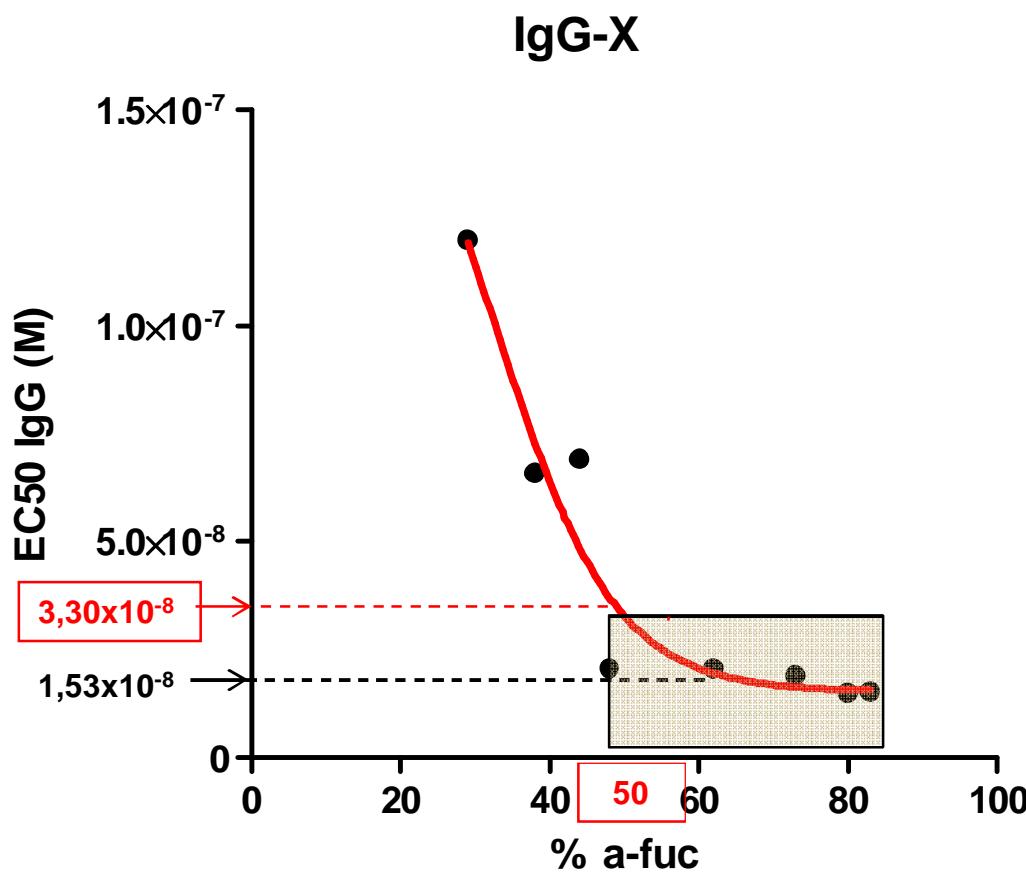
*Fc $\gamma$ RIIIaV158 competition assay - Results*



BINDING OF ANTIBODY Fc PORTIONS TO Fc $\gamma$ RIIIA ON CELLS CORRELATE  
WITH GLYCOPROFILING

# TagLite technology

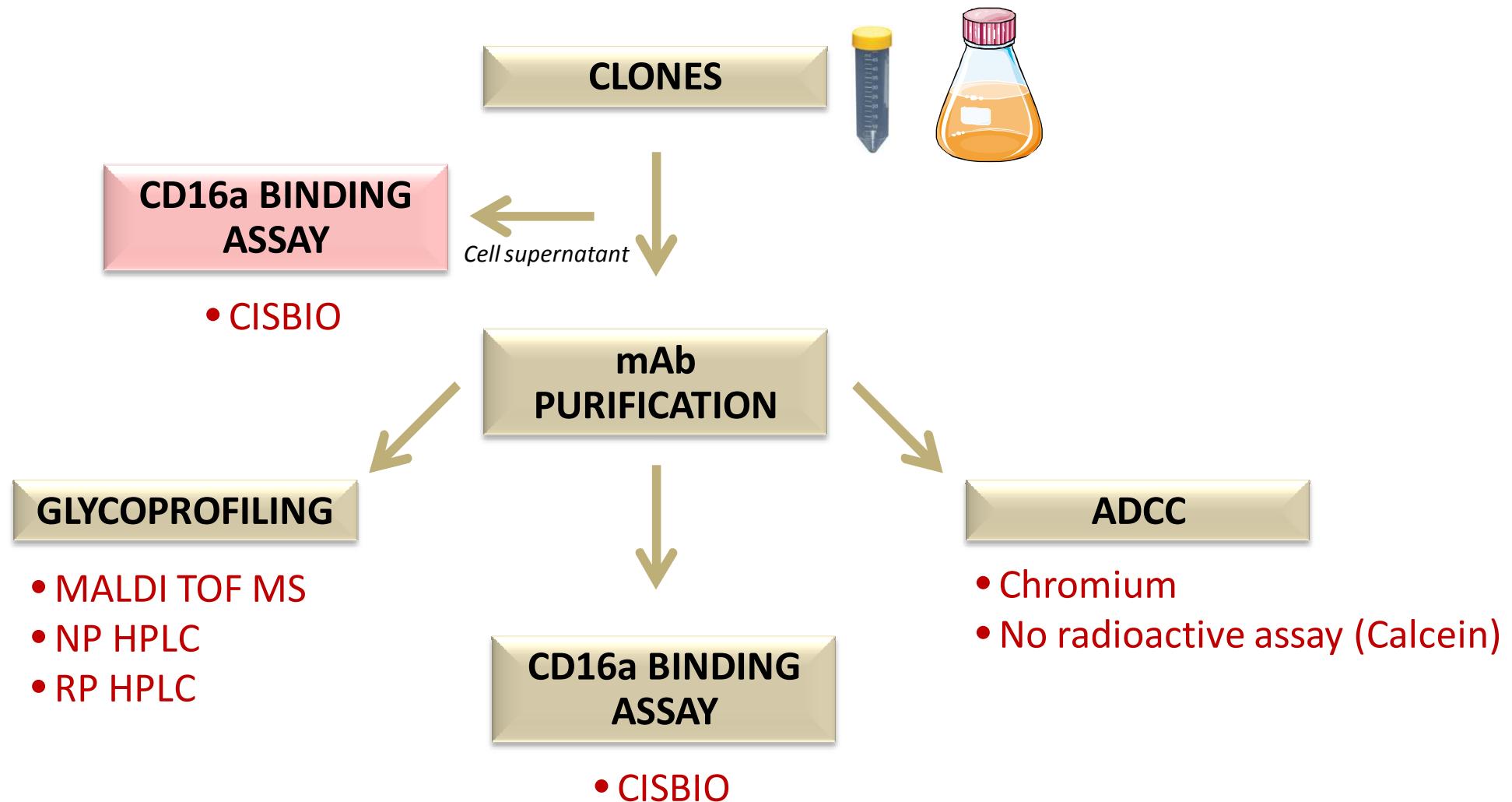
## *Fc $\gamma$ RIIIaV158 competition assay - Results*



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

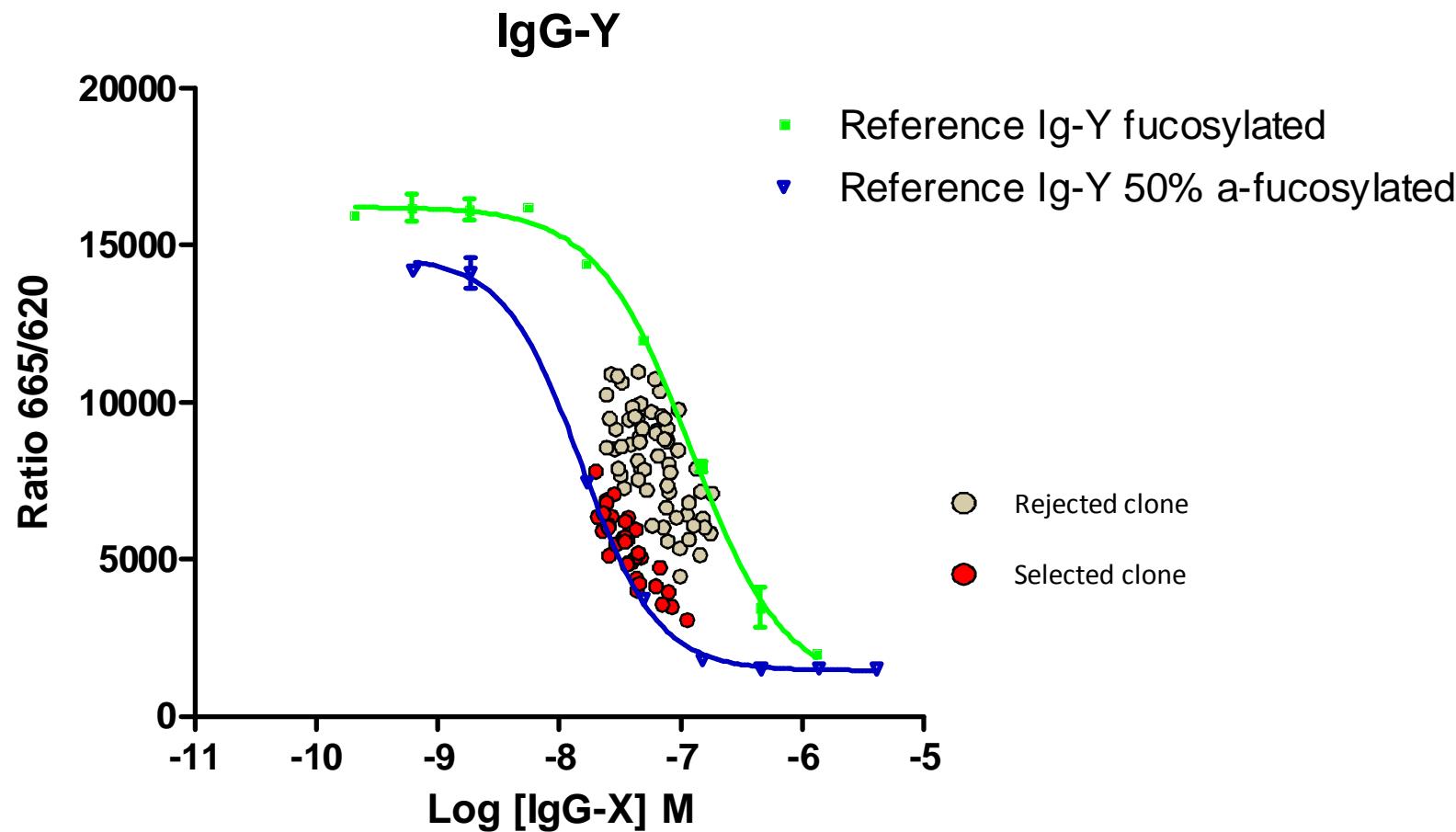
# ■ Selection of low fucosylated mAb producers

## *Analytical methods*



# TagLite technology

*Fc $\gamma$ 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

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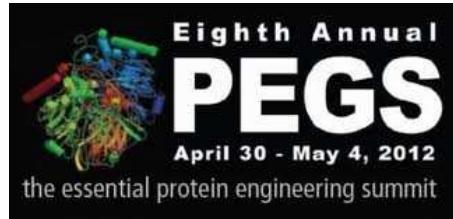
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We invite you to visit our booth, #18 and CISBIO's booth, #64



From cells to therapeutics **Vivalis**®

**Merci**  
**Thank You**  
ありがとうございました