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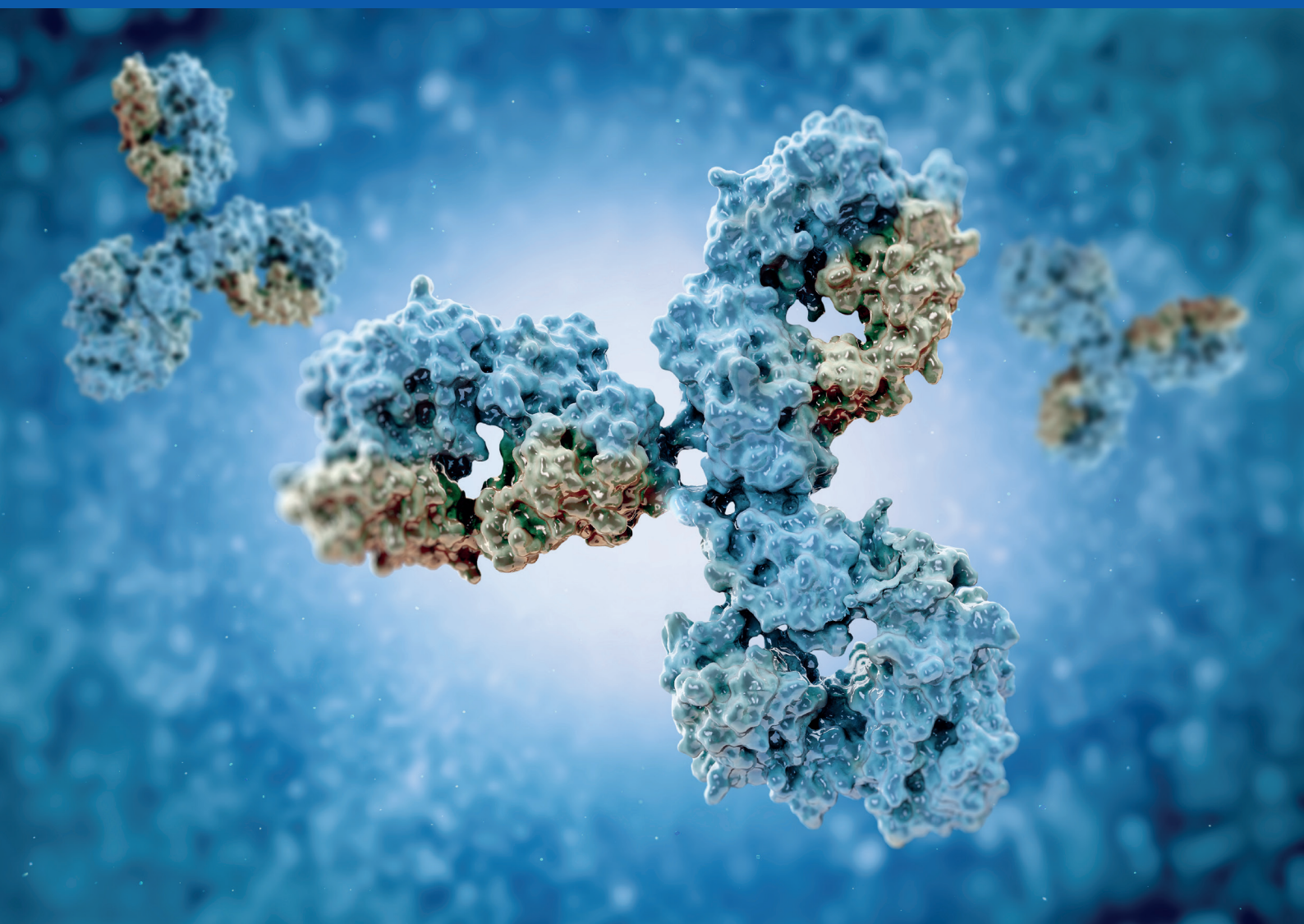
How to rescue a poorly performing hybridoma cell line

Case Study



How to rescue a poorly performing hybridoma cell line: Case examples

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Introduction

Antibodies accounted of the largest share of the *in-vitro* diagnostic (IVD) market in 2020, driven by their widespread application in medicine for the purpose of research, diagnosis, drug & IVD development, and disease treatment[1]. Antibody-based biologics are also one of the most popular classes of biomolecules in the modern biopharmaceutical market[2]. Their growth can be attributed to advancing monoclonal antibody (mAb) generation technologies in recent years, which facilitate the identification of new target antigens to be explored in diagnostic and therapeutic approaches[3,4].These methods of mAb production – including recombinant strategies (such as phage, yeast, and ribosomal display), antigen-specific single B cell technologies, and hybridoma technology – each have their own advantages and limitations.

Hybridoma technology, developed in 1975, is a traditional isolation method that is able to produce highly specific, naturally matured *in vivo* mAbs. Antibody-producing B cells from an animal source are fused with cultivated myeloma cells to generate an immortalized hybrid cell line that is selected for its ability to produce high-yield mAbs. In recent years, hybridoma technology has been expanded to develop mAbs against a range of antigens from different animal species, beyond the commonly used murine hosts, and even transgenic humanized animals.

Despite its ability to produce high affinity mAbs, hybridoma technology still suffers from some common challenges. Very low yield through weak antibody expression rates, loss of antibody production capability, or production of additional functional variable regions can impact hybridoma cell line performance. In addition, hybridoma cell line development timelines are relatively long due to the time needed to cultivate and harvest B lymphocytes, and the expression and purification of antibodies can be time-consuming and cost prohibitive.

There are several ways in which a poorly performing hybridoma cell line can be rescued, depending on the requirements of the researcher or manufacturer. InVivo BioTech Services provides customized hybridoma development and scale-up services to help optimize mAb production and increase yield. With its extensive service portfolio, InVivo uniquely offers three solutions that can be adapted to meet customers' specific yield requirements, product validation needs, and timelines:

1. Recloning of hybridoma cell line and selection for high-producer clones

2. Transition from mAb to recombinant antibody (recAb) production via transient gene expression (TGE) in human embryonic kidney (HEK) cells

3. Transition from mAb to recombinant antibody (recAb) production via stable gene expression (SGE) in Chinese hamster ovary (CHO) cells

This case book outlines three examples of how InVivo identified the most suitable antibody production method according to the customer's needs and project requirements. In each case, the InVivo team evaluated the current hybridoma clone's performance and selected a solution that would provide the best results for the customer, enabling them to speed up development timelines, deliver assays to market sooner, and explore new markets that are only available with high antibody yields or recombinantly modified antibodies.

Case example 1: Single cell cloning

Background

A mAb distribution company that resells its product to various end customers, who use the antibody for their specific assays, was experiencing low producing hybridoma clones with considerable variation between production batches. As the product is already available on the market, transitioning the mAb to a recAb was not a viable option due to the cost and time involved in all end customers revalidating their assays. InVivo therefore identified single cell cloning as the most suitable method to rescue the hybridoma cell line and improve productivity.

To achieve successful hybridoma production through single cell cloning, high-producing clones must first be selected, and it has to be ensured that the resulting hybridoma cells producing the antibody are truly monoclonal.

Materials & methods

Hybridoma cells producing the antibody of interest were automatically seeded in 96-well (100 µl/well) multi-titer plates and analyzed three times over a period of approximately 14 days with NyONE FL10 cell imager on clonality (Figure 1). Only wells containing one colony, traced back to exactly one cell on seeding day, were further considered.

If the viability and confluence were sufficient, the resulting clones were transferred to an additional 96-well multi-titer plate for an in-plate productivity assay. Resulting supernatant from this assay was used for titer screening via enzyme-linked immunosorbent assay (ELISA) method. High-producing clones were selected for further cultivation and batch production under stirring or shaking conditions in InVivo's proprietary and customized ISF-1 medium, sera and antibiotic free, over a period of 14 days. The supernatant was harvested and purified using standard procedures established at InVivo.

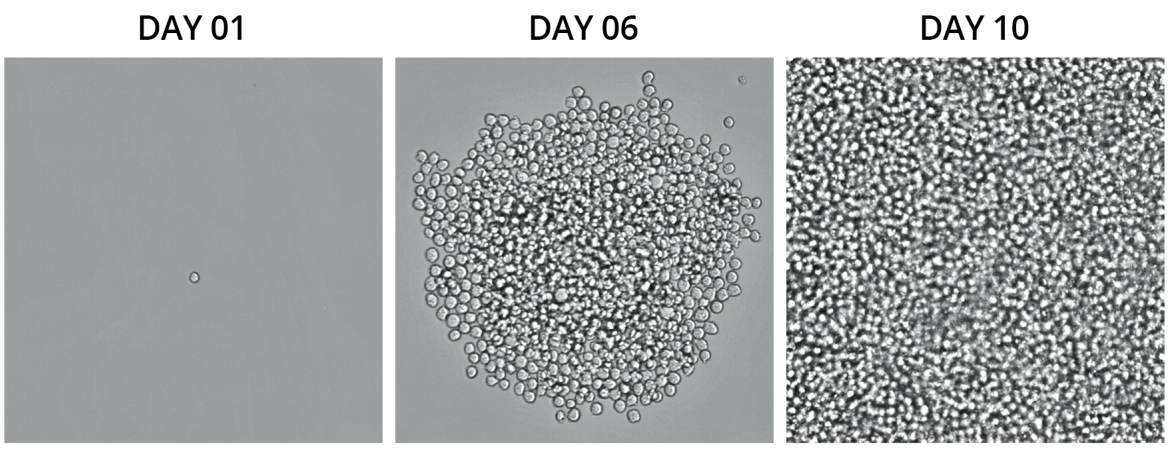

Figure 1: Monoclonality screening using NyONE FL10 cell imager on day 1, day 6 and day 10 of hybridoma cell seeding.

Results & discussion

The original hybridoma clone produced consistently poor antibody yields, with a mean antibody yield of 22.3 mg/L. The subclone produced through single cell cloning, provided a mean yield of 83.4 mg/L, increasing production by ~four-fold (Figure 2). The higher yield from the optimized clone not only allows the distributor to supply to more end users, but higher expression rates lead to less production costs as less media is required. Faster bioreactor runs also reduce timelines and free up trained personnel time for other value-added tasks. In addition, a larger batch size requires fewer testing procedures and more consolidated validation efforts for use in IVDs.

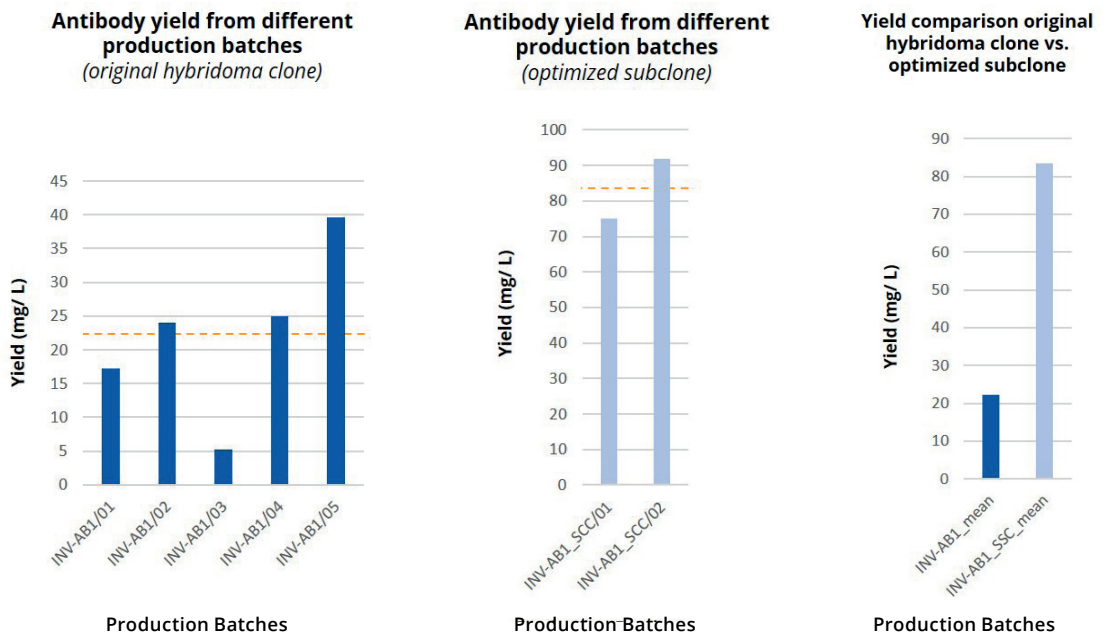

Figure 2: Antibody yield (mg/L) from original hybridoma clone (left) and optimized clone (middle). Mean value = orange line. Comparison between original and optimized clones (right) shows ~4-fold increase in antibody yield.

Case example 2: recAb via transient gene expression

Background

A manufacturer of a new IVD kit was experiencing very low hybridoma clone productivity. Given that the assay was still under development, the manufacturer was open to switching to a recAb. TGE was selected as the most appropriate solution as the recAb could be generated with a fast turnaround time, allowing the manufacturer to validate in a short timeframe and evaluate the performance against the original clones. A short time-to-market was a key deciding factor in proceeding with recAb via TGE.

The process of producing a recAb from existing hybridoma cells begins (Figure 4) with Ab sequencing and complementary DNA (cDNA) synthesis. The cDNA is cloned into a suitable expression vector, such as pINV, which delivers it into mammalian cells – most commonly HEK cells. These transiently transfected cells express the foreign gene and produce recAbs.

The InVivo Expression System for Transient Transfection: InVEST system is a flexible HEK TGE system that includes an advanced cell line and vector system, a novel transfection reagent as well as a unique and customized media, all synergistically optimized for highly efficient production of recAbs.

Materials & methods

Transient gene expression of recombinant antibodies

Transient antibody production in HEK cells was performed using InVivo's InVEST. This includes the proprietary suspension-adapted human cell line HEK-INV and the novel expression vector pINV. HEK-INV cells are routinely cultivated in suspension and split in a 2-2-3 day scheme per week in XellVivo serum free medium. The cells were transiently transfected in fresh media with the gene harboring pINV expression vectors using a polycationic polymer in shake flask scale. 1 L productions were performed using established InVivo standard protocols including a fed-batch procedure (Figure 3). Cell culture supernatant was harvested by centrifugation and antibodies purified by Protein-A affinity chromatography using established InVivo standard protocols.
For more detailed information about InVEST, please visit www.transient-transfection.com.

Figure 3: Transient gene expression (TGE) of recombinant antibodies.

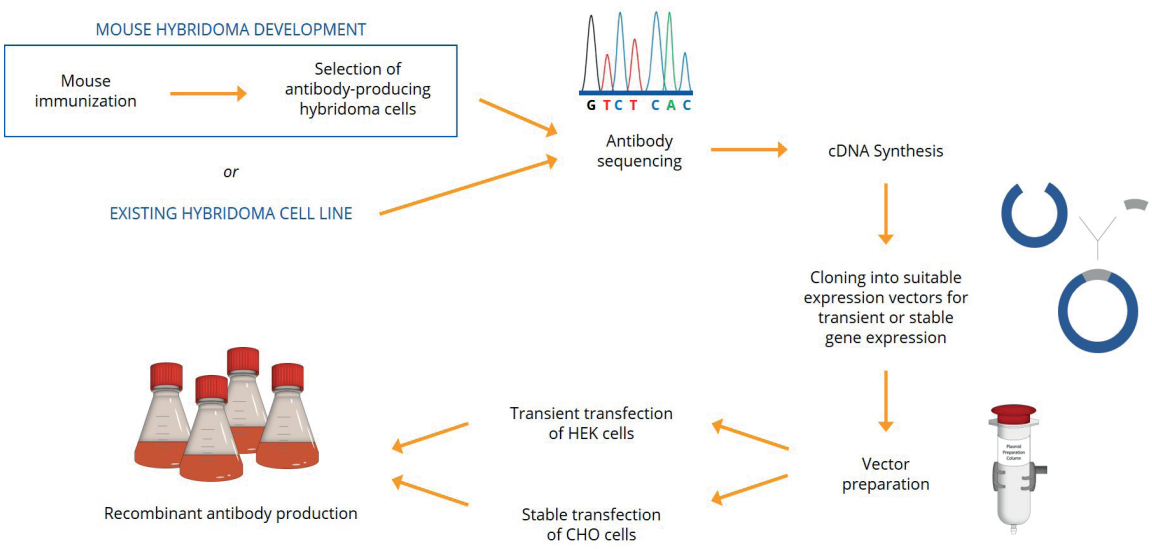

Figure 4: Workflow for recombinant antibody (recAb) production via transient gene expression or stable gene expression following the transition of a monoclonal hybridoma produced antibody into a recombinant version.

Results & discussion

The antibody yield from several production batches using the original hybridoma clone was poor, with an average antibody yield of 6.2 mg/L. This was increased by ~30-fold to 191.5 mg/L after recAb production via transient gene expression in HEK cells (Figure 5). The recAb was tested and found to perform well in the assay. The significant increase in productivity within a three-month timeframe and being able to rely on an industry-leading TGE system with an optimized workflow, allowed the manufacturer to speed up time-to-market for its assay.

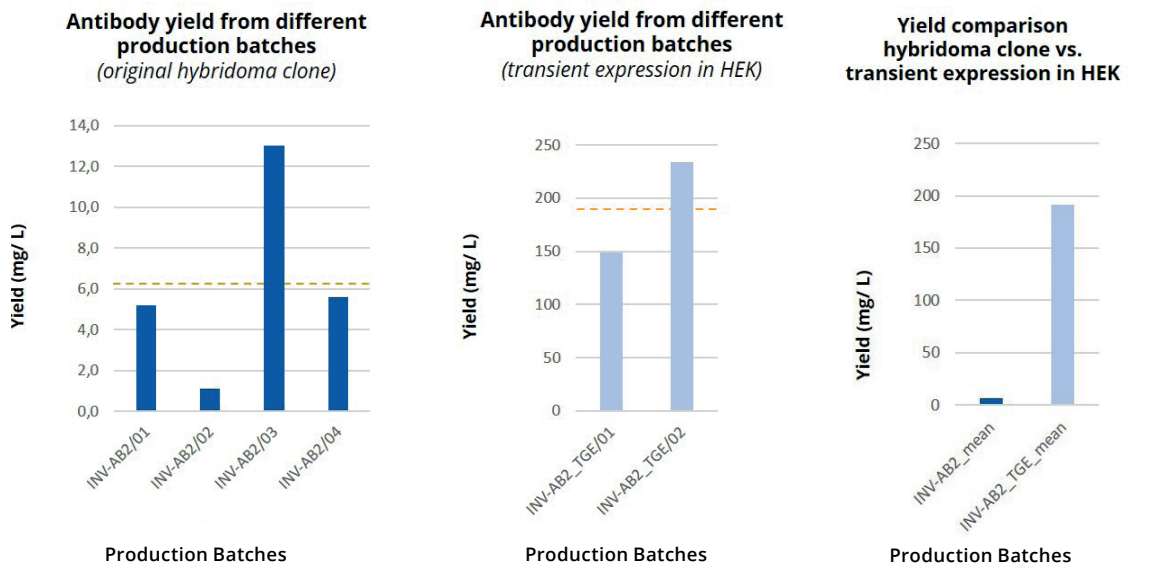

Figure 5: Antibody yield (mg/L) from original hybridoma clone (left) and transient gene expression (TGE) in HEK cells (middle). Mean value = orange line. Comparison between original hybridoma clone and TGE in HEK (right) shows approx. 30-fold increase in recombinant antibody yield.

"Our transient gene expression platform, InVEST, has been established for more than ten years. Our experts have optimized the HEK cell line, expression vector, the media itself, and the plasmid preparation to offer a highly reproducible recombinant antibody solution in a short time frame."

Tim Welsink, Head of Research & Development Services,
InVivo BioTech Services.

Case example 3: recAb via stable gene expression

Background

An IVD manufacturer required large quantities of antibody for kit manufacturing and was experiencing extremely low productivity with its original hybridoma clone. The manufacturer was open to transferring to recAb to compare antibody features in the final assay and establish whether the new recAb showed the same activity, affinity, and sensitivity as the original mAb. SGE in CHO cells was chosen as the most appropriate option given the customer's need for high expression rates and more flexible timeline.

In recAb production from stable CHO cell pools, ABs are sequenced from the existing hybridoma cells and cDNA is synthesized (Figure 4). The cDNA is cloned into a suitable expression vector, which delivers it into CHO cells, which are favored for their

ease of upscaling and larger batch sizes. In stably transfected cells, the foreign gene is integrated into the genome and is replicated. Descendants of these cells will therefore also express the new gene, resulting in a stably transfected cell line.

InVivo's stable CHO cell pool platform has been developed that combines the benefits of large batch sizes and easy upscaling with a shortened timeline, while maintaining good antigen or antibody titer. This is made possible by eliminating the time-consuming screening step required in stable monoclonal cell line production. The pool contains a mixture of cells with different numbers of insertion(s) at different locations of the genome, which produce the same antibody in sufficient quantities for longer-term production and batch-to-batch consistency, ensuring product quality.

Materials & methods

Stable gene expression of recombinant antibodies

Using the piggyBac transposon-mediated active gene transfer, suspension adapted CHO cells were co-transfected with the recombinant antibody chains-encoding expression vector (pPB-double; piggyBac donor plasmid) and the piggy-Bac helper plasmid (pPLE wtPB_Transposase) coding the transposase (Figure 6).

Through a two-step selection procedure, stable cell pools were generated and titers were measured from the cell culture supernatant analytical affinity chromatography via Protein A (Figure 6). 1 L fed-batch productions in shake flasks, which are easily convertible to large-scale productions in a bioreactor system, were performed using established InVivo standard protocols.

Comparison of original and recombinant antibody affinities

Affinity evaluation was performed in duplo on a fiber optic surface plasmon resonance (SPR)-based molecular interaction analyzer (White FOx instrument, Fox Biosystems, Diepenbeek, Belgium). Briefly, carboxylated optic sensors were activated with EDC/NHS (N-ethyl-N-(3-(dimethylamino)propyl)carbodiimide/ N-hydroxy-succinimide) and purified antibody was subsequently immobilized. After quenching and washing, sensors were dipped into the analyte (peptide Ag) and the wavelength shifts (nm) were measured after 900 seconds of incubation.
In addition, a chemiluminescence (CL)-based sandwich assay was used to determine the antibody-antigen affinity. For this, the INV-AB3 Ab was coupled to magnetic beads and incubated with peptide Ag. A second specific biotinylated "detector Ab" was added and the response upon binding was measured (counts).

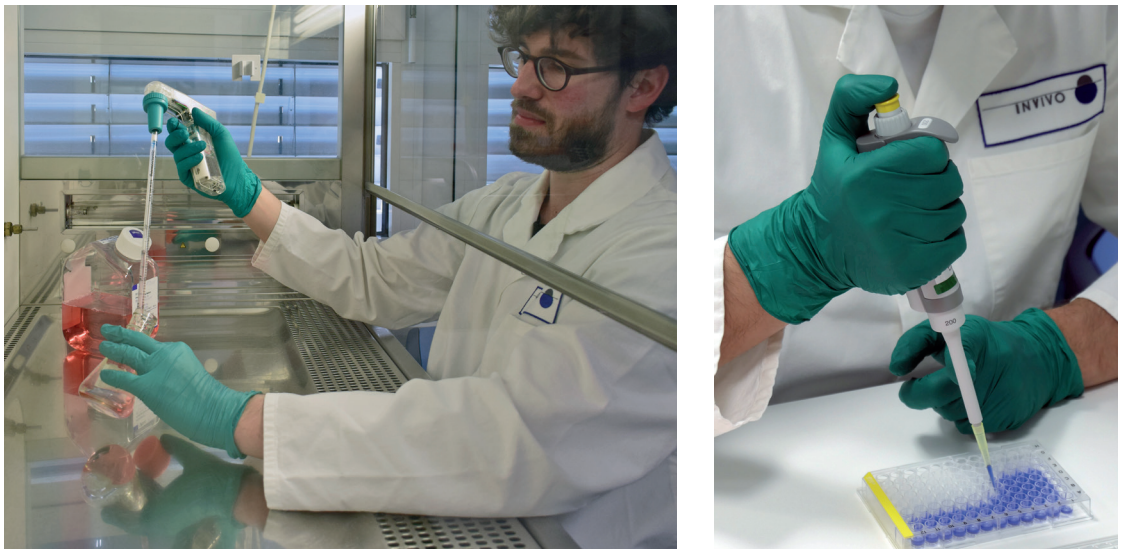

Figure 6: Handling of CHO suspension cells (left) and tryptan blue staining to determine cell count and viability (right).

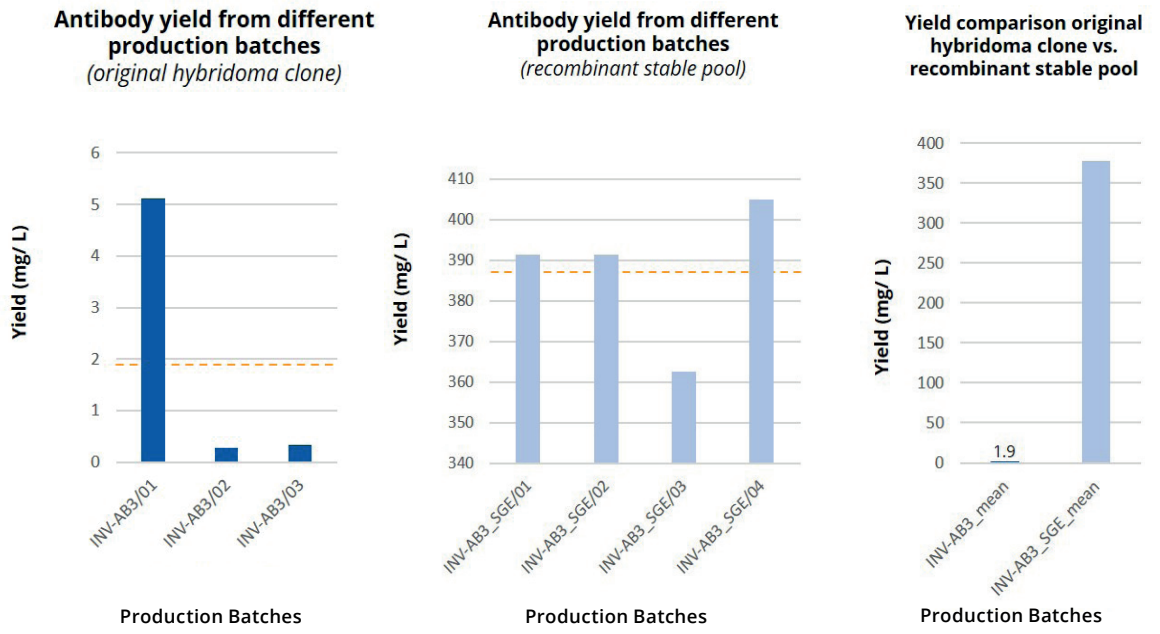

Figure 7: Antibody yield (mg/L) from original hybridoma clone (left) and stable gene expression (SGE) from CHO cell pools (middle). Mean value = orange line. Comparison between original hybridoma clone and SGE in CHO pools (right) shows approx. 200-fold increase in recombinant antibody yield.

"CHO cells are common production systems for pharmaceutical companies making therapeutic antibodies, but the economic support in this market is more substantial. By continuing to invest in our stable gene expression system using the stable cell pool approach, we are trying to open up this technique to more cost-sensitive markets such as the IVD market. The CHO pool development is a unique offering for the IVD market."

Caterina Farnleitner, Head of Antibody and Protein Production,
InVivo BioTech Services.

Results & discussion

The antibody yield of the original hybridoma clone was highly variable across different production batches and with a mean yield of 1.9 mg/L also extremely low. This was increased ~200-fold after recAb production via stable gene expression in CHO cells, to provide a yield of 387.5 mg/L (Figure 7). This substantial increase in antibody production provides the scale-up volume needed by the manufacturer, within a relatively short timeframe. The faster turnaround was made possible by achieving sufficient antibody yield from stable cell pools, rather than developing a stable cell line

from scratch. In bypassing the time-consuming screening step in stable cell line development, biotech service providers such as InVivo can offer a high producing recAb in a fraction of the time.

In addition, experiments to determine the performance of the recAb compared with the original mAb showed an almost perfect correlation in binding activities (Figure 8), demonstrating the utility of the recAb in a commercial assay.

mAb (Source)	Wavelength shift (nm)
INV-AB3 (hybridoma)	3.48
INV-AB3_SGE (CHO) #1	3.74
INV-AB3_SGE (CHO) #2	4.19
INV-AB3_SGE (CHO) #3	3.41
INV-AB3_SGE (CHO) #4	3.06

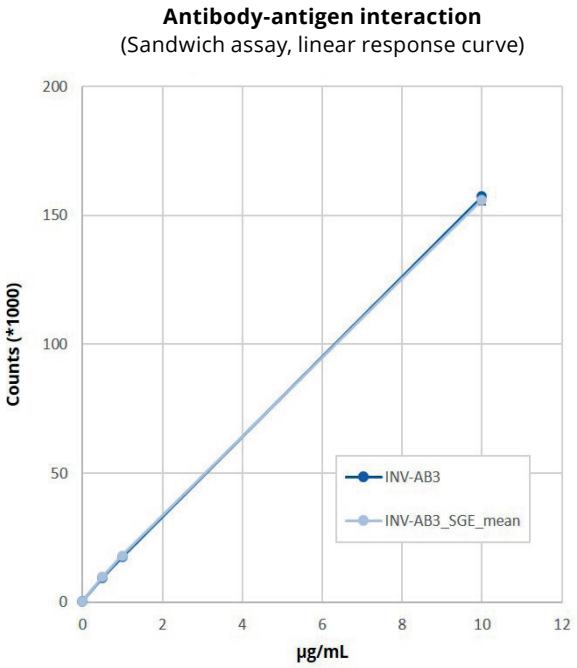

Figure 8: Affinity evaluation of the antibody-antigen interaction. *Left*: Wavelength shifts (nm) measured by surface plasmon resonance (SPR) show comparable values for the hybridoma-derived antibodies and for the recombinant antibodies (stable pools). *Right*: Chemoluminescence-based sandwich assay: Measurements at different antigen concentrations show identical linear response curves. Values for INV-AB3_SGE represent means ± SD (n=4).

Conclusion

Scaling up hybridoma cell lines presents both opportunities and risks for antibody producers. A well-established hybridoma clone can provide high affinity mAbs but if the clone doesn't succeed or production becomes compromised, for example due to incomplete selection, decline in monoclonal antibody productivity with passaging, weak immunogen, or errors in the screening process, establishing a new line is costly and time intensive, and the chance of success is uncertain. In addition, several optimizations and process developments do not require operation under Good Manufacturing Practice (GMP) regulations so, where bioreactor capacity on the market is limited, these can be outsourced to non-GMP facilities.

Not only do InVivo's mAb and recAb production solutions minimize these supply chain risks, but switching to a recAb offers the additional benefit of an available antibody sequence, providing an important level of security for many antibody producers. By offering three distinct solutions to rescue poorly performing hybridoma cell lines, InVivo ensures increased antibody productivity regardless of the customer's specific challenges. This enables IVD manufacturers to speed up development timelines and time-to-market, and even enter new markets that were not previously possible due to low productivity or high production costs.

InVivo is committed to developing innovative antibody production solutions that meet the needs of all IVD manufacturers and researchers. As well as continuously optimizing its stable gene expression system in CHO cells to lower the barrier to market entry and cost for customers, by improving timelines and enhancing efficiency, InVivo experts are now developing different antibody isotypes in the recombinant format. This aims to mimic the natural isotypic variation in antibodies produced by B-cells or hybridoma cell lines and conserve them in recAb form, providing the opportunity to engineer antibodies after obtaining the sequence and enabling flexibility for already approved assays and products.

For more information on how InVivo BioTech Services can help rescue your hybridoma cell line, please visit https://www.invivo.de/cell-line-development/hybridoma-development/.

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"Hybridoma cell lines produce different antibody isotypes, the most common of which is IgG. We wanted to be able to offer production of different isotypes, such as IgM and IgA, in recombinant form as well as mAbs. The ability to conserve the isotype in recAbs will benefit a number of our customers in terms of assay design and regulatory compliance."

Tim Welsink, Head of Research and Development Services,
InVivo BioTech Services.

About InVivo BioTech Services

InVivo BioTech Services GmbH is the leading European contract manufacturing organization in mammalian cell culture for production of monoclonal antibodies and recombinant proteins for research & development, in vitro diagnostics and pre-clinical studies. With more than 20 years of experience, the company has consistently grown over time and exhibits today approximately 2,850 square meters (~ 30,700 square feet) of laboratory and operational space in the Berlin greater area. In 2017, Bruker Daltonics integrated InVivo to gain a reliable partner for the production of in-vitro diagnostic kits, biological standards and chemical matrices. For more information, please visit www.invivo.de.

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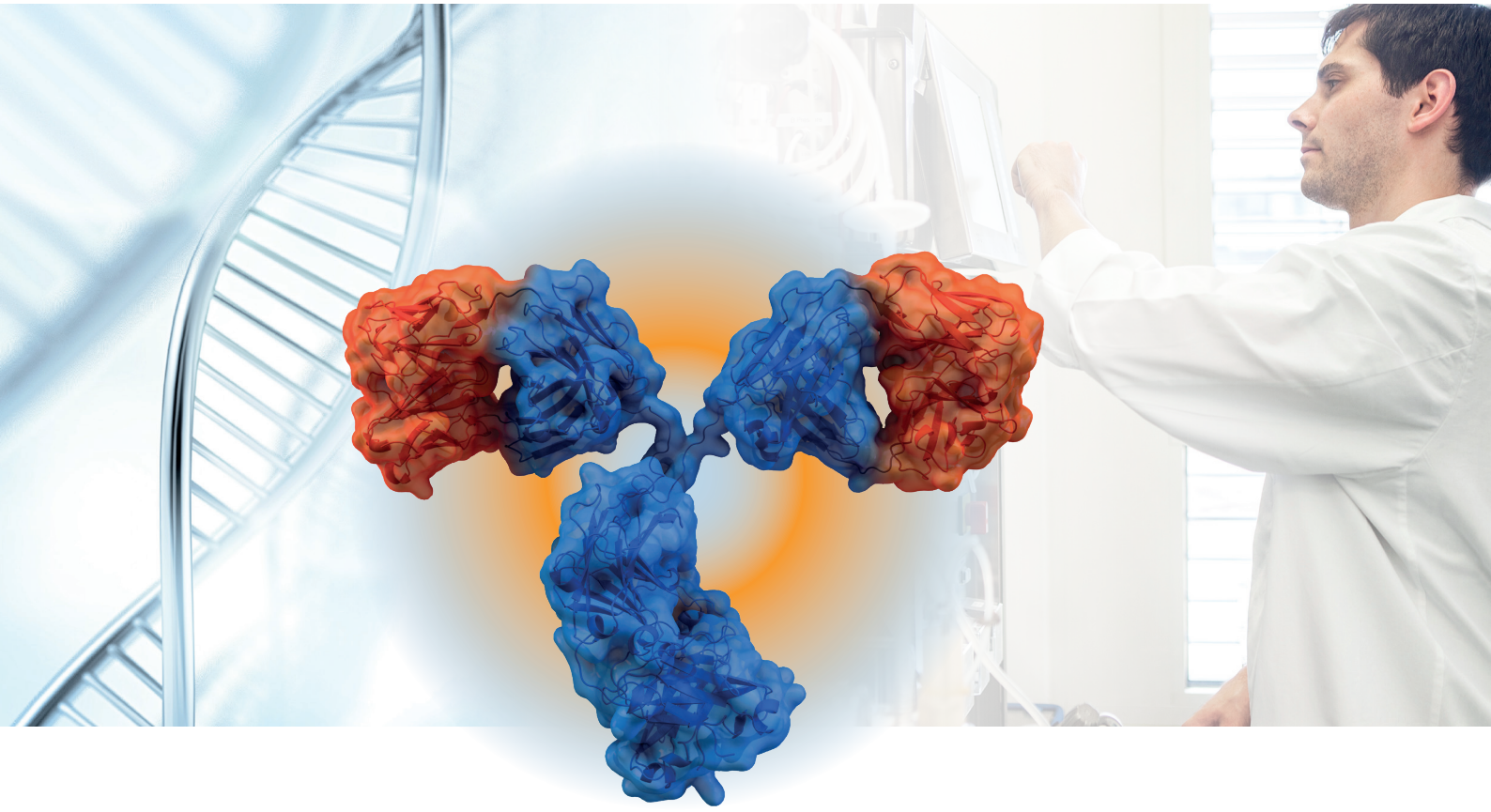

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