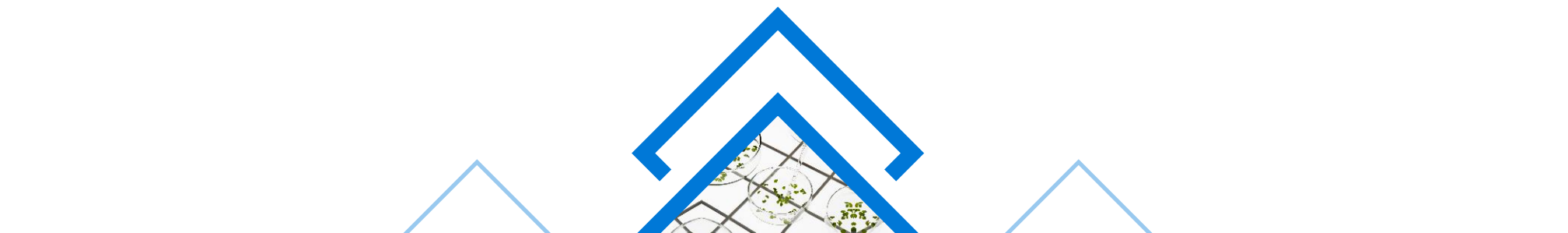
Creative Biolabs®

Recombinant Antibody Expression Overview

01 Introduction

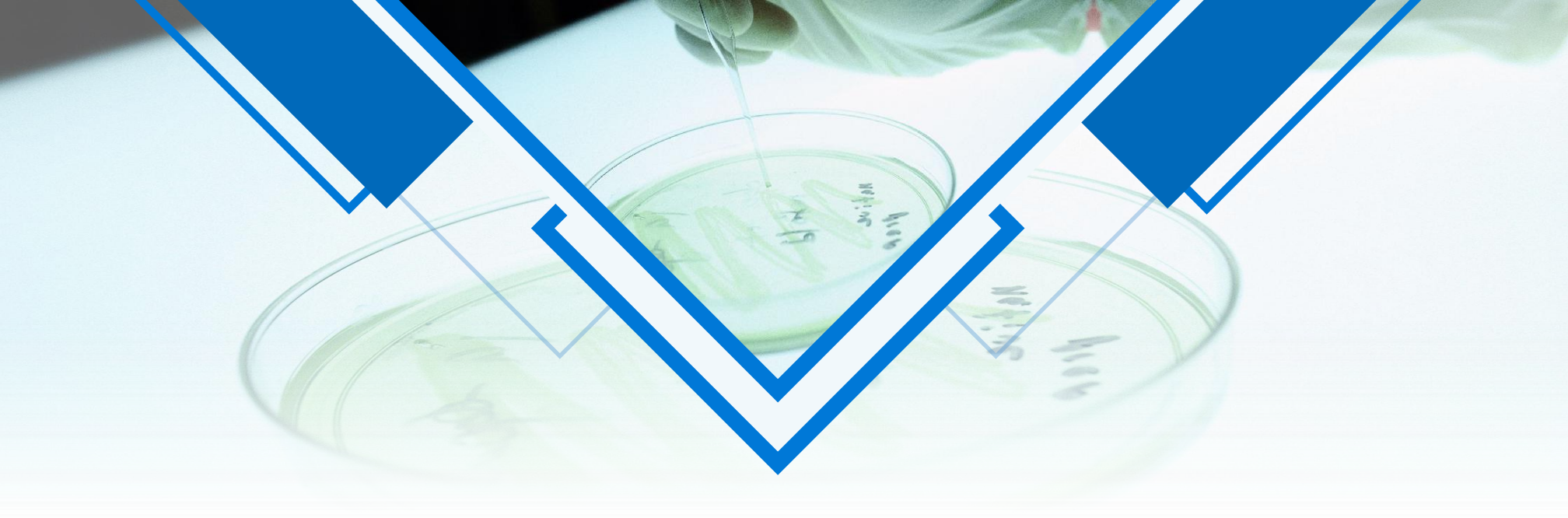
An increasing portion of marketed drugs and drugs under development are biopharmaceuticals, of which antibodies currently are the most common molecule class. Among those, recombinant antibodies are the fastest growing group and a large number of new antibody products are in clinical and preclinical development. Recombinant antibodies take many advantages over traditionally generated monoclonal antibodies that are only beginning to be explored. Several expression systems are available for producing recombinant antibodies and antibody fragments, ranging from prokaryotic expression system including bacteria to eukaryotic expression system including yeasts, insect cell lines, mammalian cells, and transgenic plants and animals. Generalized features of antibody expressed in different biological systems are listed in Table 1. However, it should be kept in mind that there are exceptions to this table for specific product/expression systems. Each has advantages, potential applications, and bottlenecks. For example, bacteria cannot assemble whole glycosylated antibodies but are suitable for the production of antigen-binding antibody fragments. Intact antibodies have been expressed in yeast, but they show defects in effector functions owing to different glycosylation patterns. Plant and insect cells can also produce antibodies, but with unnatural carbohydrate structures. Currently, almost all therapeutic antibodies are still produced in mammalian cell lines in order to reduce the risk of immunogenicity due to altered, non-human glycosylation patterns. To assure sufficient yields, it is necessary to develop customized expression and purification procedures adapted to the construct specificities. The choice of a particular system will be determined to a large extent by the nature and origin of the desired protein, the intended use of the product, the amount needed, and the cost. Such a complex context requires the capacity of comparing advantages and shortcomings of alternative production system. In this overview, we focus on current antibody production systems including their usability for different applications.



This review aims at summarizing the recent trends, tries to evaluate the level of feasibility and reliability reached by the different methodologies, and will describe some recent innovative proposals.

Table. 1 Generalized features of antibody in different biological expression systems.

Antibody Feature	Prokaryotic System	Eukaryotic System
Molecular weight	Low	High
Antibody format	Fragments, scfv, fab	Intact Ig, Fragments
S-S bridges	Limitation	No limitation
Secretion	No	Yes/no
Aggregation state	Inclusion body	Singular, native
Folding	Risk of misfolding	Correct folding
Glycosylation	Limited	Possible
Cost to manufacture	Low	High/low



02 Prokaryotic Expression System - Production of Antibody Fragments

02-1 *Escherichia coli*

Escherichia coli is the most important production system for recombinant proteins reaching volumetric yields in the gram per liter scale for extracellular production. There are objective advantages for this expression host, such as its simplicity, the availability of a large amount of well-tested reagents (vectors, strains), and the enormous experience (protocols, common expertise) that the research community accumulated over the last 30 years using this specific bacterium. The negative side effects implicit in choosing *E. coli*-based expression systems are that this solution is often driven by consuetude rather than by rational considerations. For the production of functional antibody fragments, the key to success is the secretion of both V chains into the periplasmic space of E. coli where the oxidizing environment, the combination of specific chaperone and isomerase activities allow the correct formation of disulfide bonds and the assembly to a functional Fv fragment (Figure. 1). The production of recombinant antibodies in the reducing cytoplasmic compartment results mostly in non-functional aggregates. Recovery of functional antibody fragments from cytoplasmic inclusion bodies by complete denaturation and refolding is often not efficient. Stable cysteine free mutants of some scFvs are successfully produced in the cytoplasm of *E. coli*.

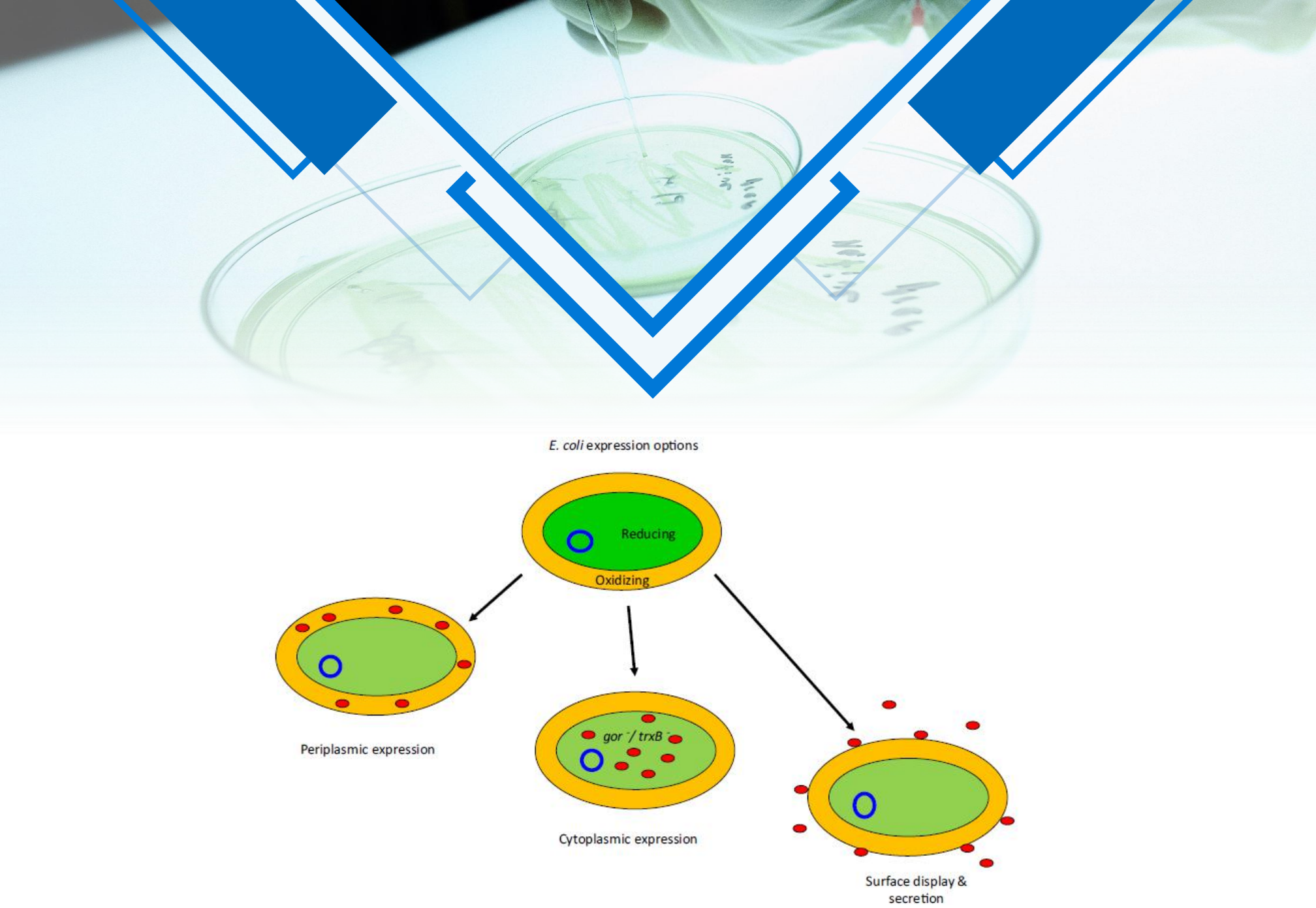


Figure. 1 Conventional antibody expression in E. coli. Most of the recombinant antibodies rely on the formation of disulfide bonds in order to reach their native structure. Periplasm is the only E. coli oxidizing compartment compatible with disulfide bond formation and consequently it has been considered the logic environment for antibody accumulation despite its small volume. Mutant strains (*gor-/trxB-*) with partially oxidizing cytoplasm represent an alternative (cytoplasmic accumulation) as well as expression systems that enable the antibody secretion in the medium or at the cell surface (antibody display). (Ario de Marco. Recombinant antibody production evolves into multiple options aimed at yielding reagents suitable for application-specific needs)

An effort to engineer *E. coli* for the production of difficult-to-express antibodies has been made. For example: 1) *E. coli* strains with mutations in the glutathione and thioredoxin reductase in combination with coexpression of cytoplasmic chaperones GroEL/ES, trigger factor, DnaK/J as well as signal sequence-less variants of periplasmic chaperones DsbC and Skp increased the yield of functional Fab (Figure. 2). 2) Coexpression of Erv1p sulfhydryl oxidase increased the yield of camelid single domain antibodies (VHH) in the cytoplasm. Despite these efforts, most antibody fragments are produced in the periplasm of *E. coli* using N-terminal leader sequences targeting the periplasmic Sec-secretion pathway.

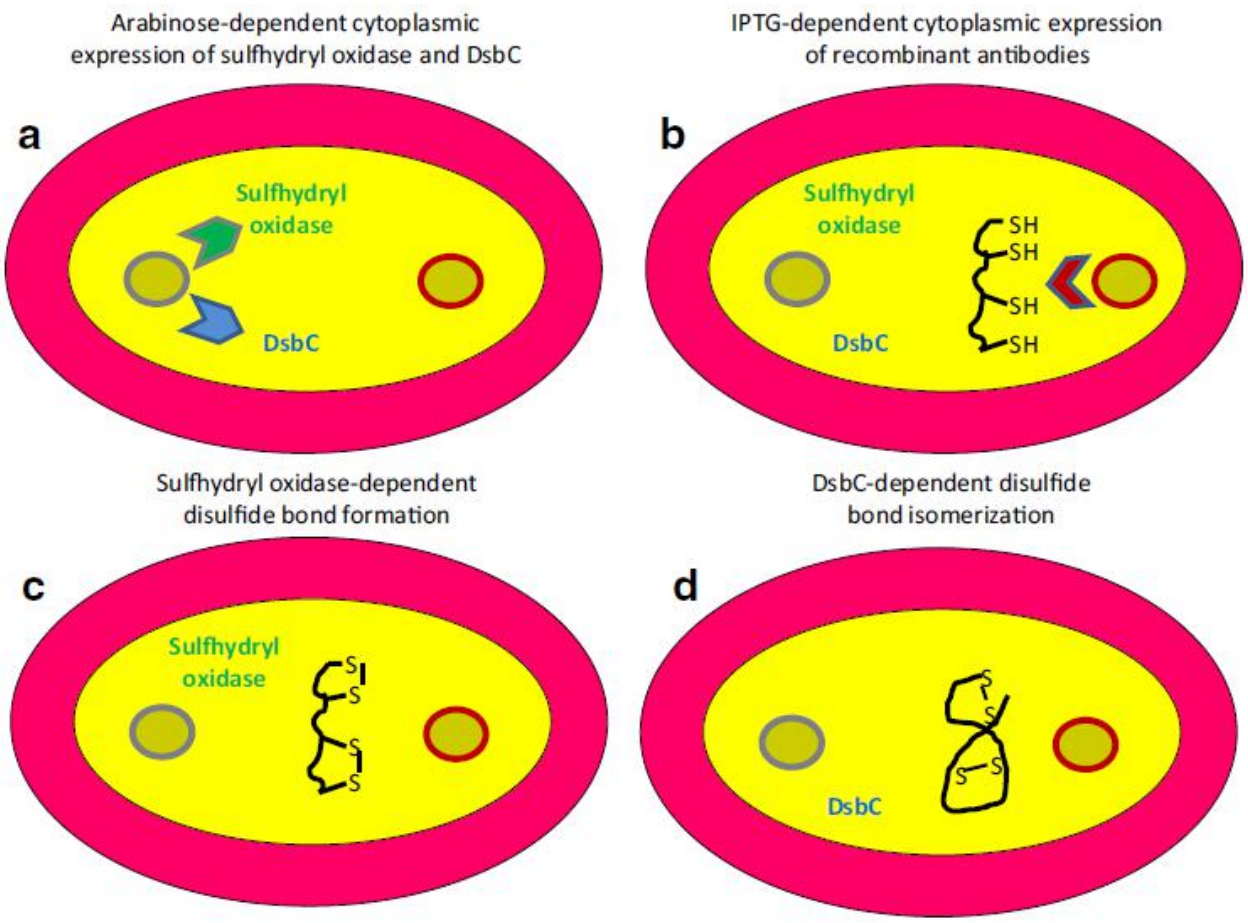
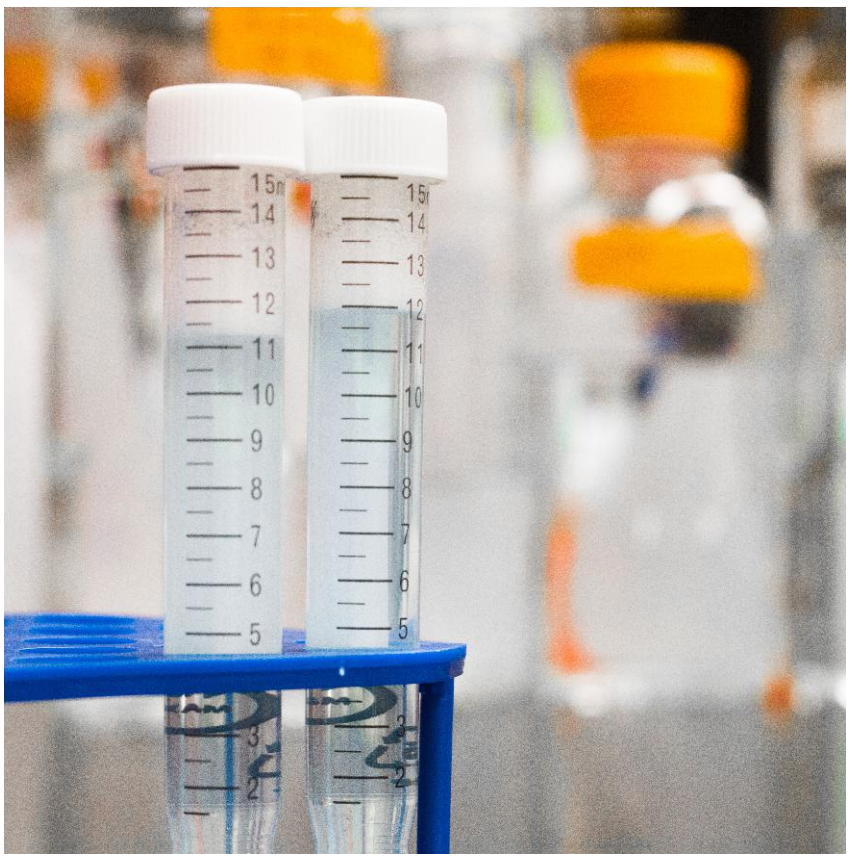


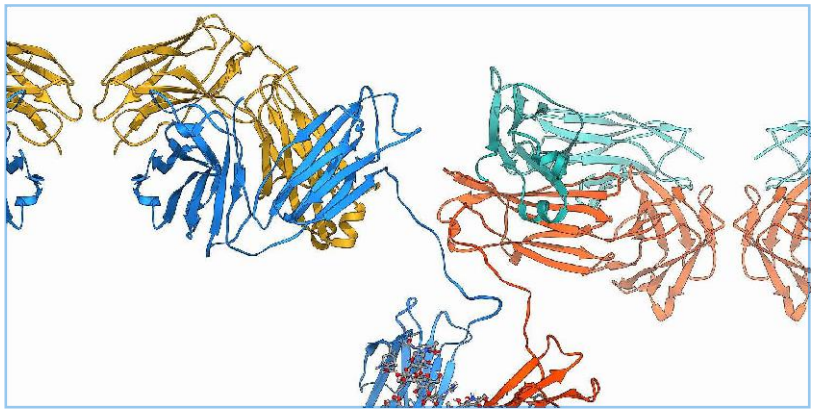
Figure. 2 Alternative antibody expression in *E. coli* cytoplasm. Effective accumulation of functional recombinant antibodies can be obtained by expressing sulfhydryl oxidase and DsbC isomerase (a) in the cytoplasm before inducing antibody expression in the same cell compartment (b). The two foldases have complimentary activities: the cysteine SH groups are converted into disulfide bonds by sulfhydryl oxidase (c) and, if necessary, these are scrambled by DsbC to achieve the native folding (d.(Ario de Marco.Recombinant antibody production evolves into multiple options aimed at yielding reagents suitable for application-specific needs)

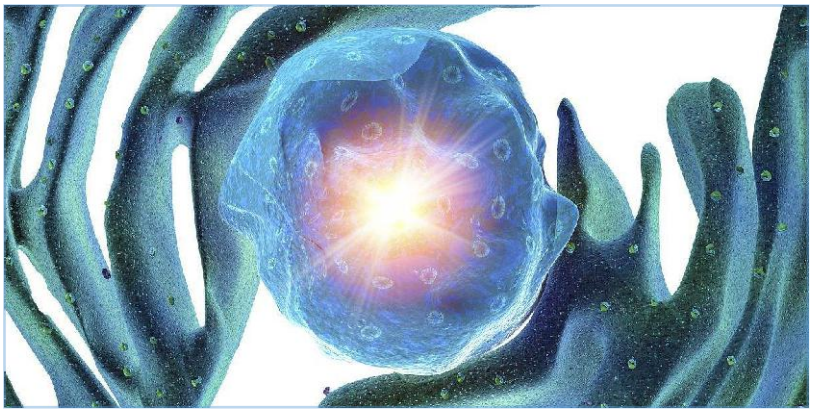
To improve folding efficiency, several strategies for increasing the solubility of recombinant proteins expressed in *E. coli* have been optimized. Proteins such as isomerases and chaperones have been overexpressed, the bacterial growth has been performed at low temperatures and in the presence of osmolytes and alcohols known for stimulating heat-shock response, and even specific strains have been developed. By contrast, the optimization of medium composition and fermentation conditions is often neglected in academia with the exception of labs performing more industrial-oriented R&D. Very high yields of antibody fragments produced in *E. coli* are mainly provided by high-cell density fermentation in bioreactors: the expression of a hapten-specific scFv produced in a bioreactor leads to yields up to 1.2 g/L compared to 16.5 mg/L yield of the same antibody obtained by optimized shake flask production, which can be mostly addressed to the over 100-fold higher cell density in the bioreactor.

The main disadvantages of prokaryotic expression systems, especially for antibody expression, are the limited glycosylation, folding, and secretion capabilities of the host cells. Here, we only focus on the characteristics of antibody expression in *E. coli* which is the almost exclusive prokaryotic production system. Furthermore, Creative Biolabs has established a novel prokaryotic recombinant antibody production service by our magic™ platform.

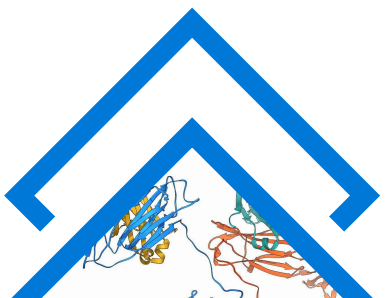


03 Eukaryotic Expression System - Production of Antibody Fragments





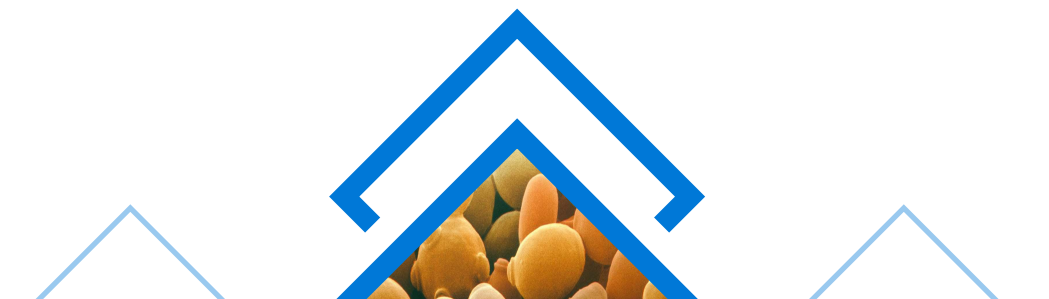
If antibody expression products are to be glycosylated for its biological functions, eukaryotic systems are the optional method. Protein secretion in eukaryotes occurs via the endoplasmic reticulum and Golgi apparatus and is aided by chaperones and cofactors. This is a complex process but the principles are the same for all eukaryotic organisms. However, the pattern of protein glycosylation differs between different species, even if they are very closely related. For recombinant antibodies it is aimed to have a glycosylation pattern as close to the human one as possible to minimize immunogenicity and, for some applications, to maximize induction of immunological effector functions. Besides production levels and cost, the glycosylation pattern of eukaryotic expression hosts, therefore, is one of the most noted rating characteristics of the production system. The set of eukaryotic hosts that in the meantime have been developed for and applied to antibody expression ranges from rather simple organisms such as yeast to transgenic mammals and plants.



03-1 Yeast

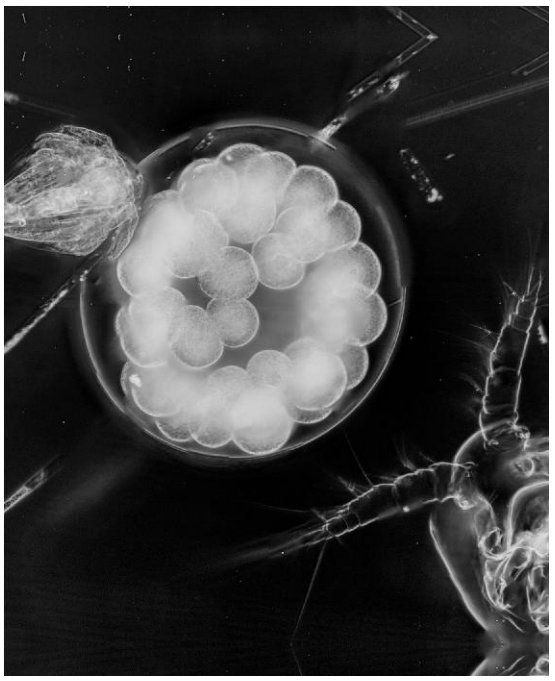
Yeasts combine the properties of eukaryotic cells short generation time and ease of genetic manipulation with the robustness and simple medium requirements of unicellular microbial hosts. Among the yeast species used for recombinant antibody expression are the well-characterized species *Saccharomyces cerevisiae* as well as more unconventional hosts such as *Yarrowia lipolytica* and *Kluyveromyces lactis* and, with very promising secretion yields and emerging popularity, the methylotrophic yeast *Pichia pastoris*. The main issues for antibody expression in yeast are secretion rate, proteolytic activity, and the glycosylation pattern.

P. pastoris shows overall optimal capacity for the production and secretion of heterologous proteins than *S. cerevisiae* and does not secrete large amounts of its own protein which simplifies the downstream processing. Moreover, *P. pastoris* prefers respiratory growth resulting in high-cell densities of more than 100 g/L dry weight. Probably the most prominent feature of *P. pastoris* is the metabolization of methanol as sole carbon source. Since the alcohol oxidase (AOX) enzyme of the methanol assimilating pathway can reach up to 30% of the total cellular protein, the endogenous AOX promoter provides a tightly regulated and powerful expression regulator. The N-glycosylation pattern of *P. pastoris* differs from that of *S. cerevisiae*, although both mainly produce N-linked glycosylation of the high-mannose type. In *P. pastoris*, there is less hyper-glycosylation found and the average length of the added oligosaccharide chains is much shorter. The terminal α-1,3-glycans found on the core oligosaccharides of *S. cerevisiae* are believed to be primarily responsible for the high antigenic nature of glycoproteins produced in *S. cerevisiae*. Moreover, genetically modified glyco-engineered *P. pastoris* strains have been generated which produce humanized glycosylation patterns. Production processes employing glyco-engineered yeasts are currently optimized for commercial antibody production as well as for high throughput screening.



03-2 Insect Cells

Insect cells represent a very versatile eukaryotic expression system. They can be efficiently transfected with insect-specific viruses from the family of *Baculoviridae*, particularly the *Autographa californica* nuclear polyhedrosis virus. There are numerous examples of recombinant antibody expression using baculovirus infected insect cells including scFv, Fab, scFv-Fc, scFv-based immunotoxins, fluorescent scFv fusions, and full-length IgG and IgA antibodies. Thereby, yields of up to 200 mgl−1 for a scFv and 70mgl−1 for entire IgG can be reached. Baculoviral protein expression is normally performed in insect cell lines like Sf-9 and Sf-21 of *Spodoptera frugiperda*, DS2 cells of *Drosophila melanogaster*, or High Five cells (BTI-TN-5B1-4) of *Trichopulsia ni*. Important parameters for optimizing baculoviral protein production are multiplicity of infection (m.o.i.), production length (usually up to 96 h), addition of protease inhibitors due to the release of viral proteases, temperature (usually 25–30°C), and media pH (pH 6.0–6.4).



The N-glycosylation pattern of insect cells differs from that of mammalian cells. Insect cells can assemble N-glycans of the high mannose and paucimannose type but typically fail to produce N-glycans of the complex mammalian type with terminal galactose or sialic acid residues. This limits the suitability of the expression system for therapeutic approaches, which leads to intensive research on the humanization of the glycosylation pattern of insect cells. To solve these problems, transgenic insect cell lines are under development to express mammalian glycosyltransferases, which produce a recombinant protein with highly homogeneous biantennary sialylated N-glycans.

03-3 Mammalian Cells

Today, 60–70% of all recombinant protein pharmaceuticals and 95% of the currently approved therapeutic antibodies are still produced in mammalian cell lines despite relatively high production costs and difficulty in handling. The main reasons for this are the sophisticated folding and secretion machinery as well as the ability to perform post-translational modifications that are not distinguishable from the ones in the human body. However, the risks of contamination by pathogens or bovine spongiform encephalopathy (TSE/BSE) agents have been eliminated by well-documented Good Manufacturing Practice (GMP) compliant designer cell substrates and chemical defined media without the need of supplementing animal serum components.

Production yields of recombinant mammalian cell lines quite drastically increased during the past 2 decades (Figure. 3). Over 100-fold improvement in volumetric productivity is due to increased cell densities in modern cell culture processes, but part of it also to improved cell lines with about tenfold higher cell specific productivities.

Chinese hamster ovary (CHO) cells are the most common cells applied in the commercial production of biopharmaceuticals. This cell line isolated in the 1950s. Today different genetic progenies have been developed, for example, K1, DukX B11, or DG44 that differ in product quality and yield. Besides this, murine myeloma (NS0, SP2/0), BHK (baby hamster kidney), and HEK293 (human embryonic kidney) are also used to a lesser extent. Although glycosylation patterns of mammalian glycoproteins are very similar to that in humans, even small differences can influence pharmacokinetics and effector functions of antibodies. Alternative designer cell lines with improved glycosylation patterns have been generated.

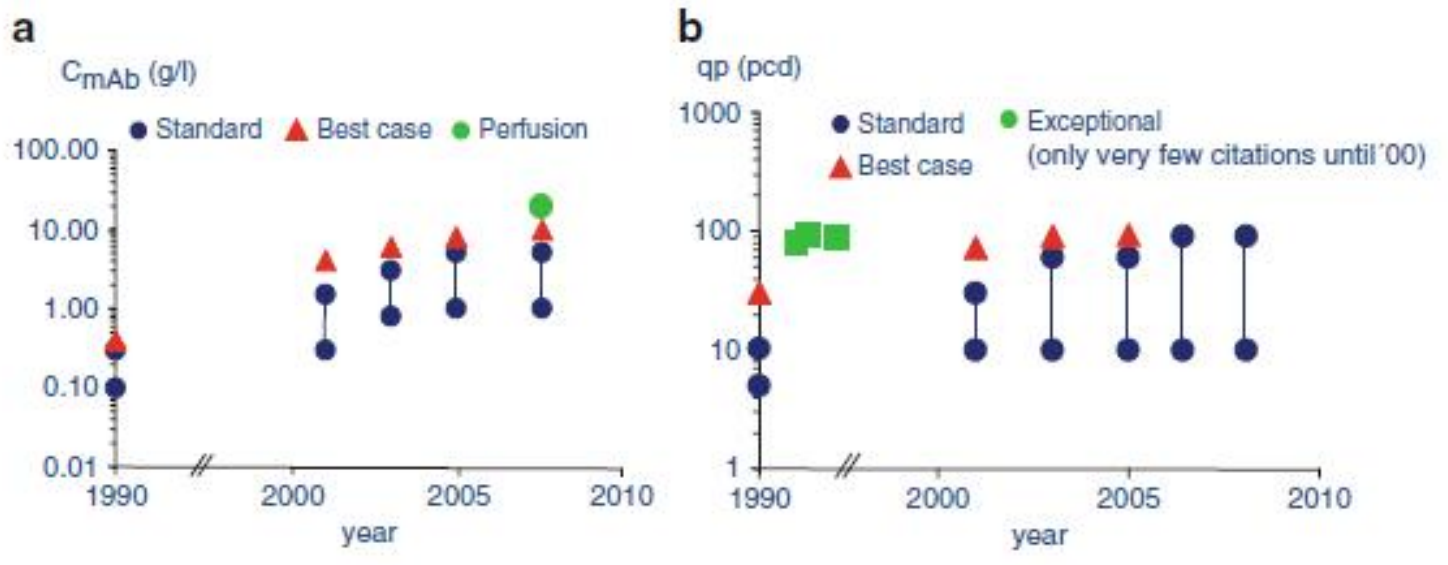
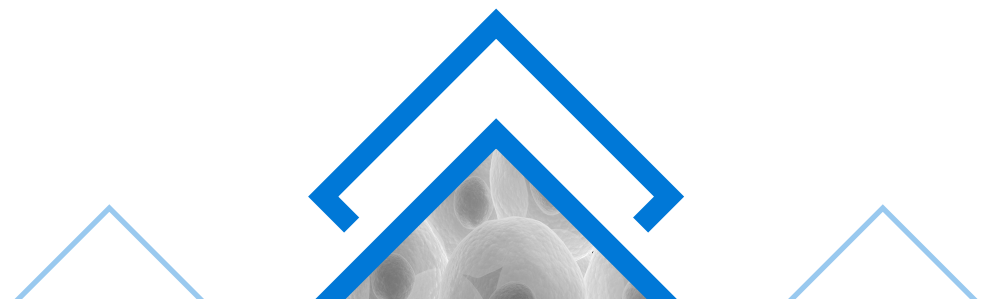


Figure.3 Evolution of antibody production yields. (a) Evolution of volumetric productivity of antibody production processes in mammalian cells. An increase of about 100-fold was achieved in the past 20 years. The values and ranges shown in the graph are based on personal experience, literature and conference presentations without claiming completeness. The Y-axis is drawn in logarithmic scale. (b) Evolution of cell specific productivity (qp) of mammalian antibody producing cell lines. An increase of about tenfold was achieved during the past 20 years. The values and ranges shown in the graph are based on personal experience, literature and conference presentations without claiming completeness. The Y-axis is showing productivities in pg per cell and day (pcd) and drawn in logarithmic scale. (Modified from (Knopf 2008))

The generation of a stable master cell line requires the integration of the expression cassette into the host cell genome. Strong promoters like the immediate early cytomegalovirus (CMV) or the cellular elongation factor (EF) 1-alpha promoter and polyadenylation sites from the simian virus (SV) 40 or the bovine growth hormone (BGH) for improved mRNA stability and translation efficiency are usually implemented into the expression vector. Furthermore, there are different methods to enhance antibody expression by increasing the number of antibody gene copies in the genome through gene amplification. The generation of producer cell lines is still a time-consuming, laborious, and quite expensive process, although the procedure has been dramatically improved and accelerated. Therefore, transient expression is helpful, especially when a large number of different antibodies have to be expressed at lower or medium yields or the product homogeneity is not critical, for example, in research and development. Moreover, transient mammalian antibody production can be scaled up by employing batch or fed-batch bioreactor processes to more than 150 L production volumes. Therefore, transient antibody production is suitable for small-scale production in antibody screening, but also capable to generate grams of antibodies.



03-4 Transgenic Plants

Because of the nearly unlimited upscaling possibility, transgenic plants represent a very promising expression system for recombinant antibodies. In contrast to mammalian cell culture, where the upscaling process leads to strongly increasing production costs, the costs for the expression of an IgA in plants are only 1–10% compared to the expression in hybridoma cells.

Recombinant antibody fragments have been successfully produced in different higher plants. The generation of genetically modified dicotyledonous plants is mainly done by the transfer of the expression cassette of the transgene with the help of *Agrobacterium tumefaciens*. In principle, leaves, leaf disks, or nodes serve as explants for the transfer of the expression cassette including a marker for the selection of successfully transformed plantlets. The transgenic DNA can either be inserted into the genomic DNA of the nucleus or in the genome of the plastid. As this procedure requires several months of transformation and special regeneration protocols, transient expression systems have been developed which allow time-saving production of recombinant proteins: McCormick and colleagues designed a tobacco mosaic virus (TMV) based vector for the secretory expression of different scFvs for the treatment of non-Hodgkin's lymphoma.

Immunogenicity can be induced by proteins expressed in higher-plant due to the presence of some plant-specific sugar residues. The problem has been addressed by using RNA interfering technology to down-regulate glycosylation. A meaningful line of research is aimed at developing transgenic crops able to produce antifungal recombinant antibodies that neutralize pathogens *in vivo*. The approach is not absolutely new, but its efficacy strongly profited from the late technological progress.

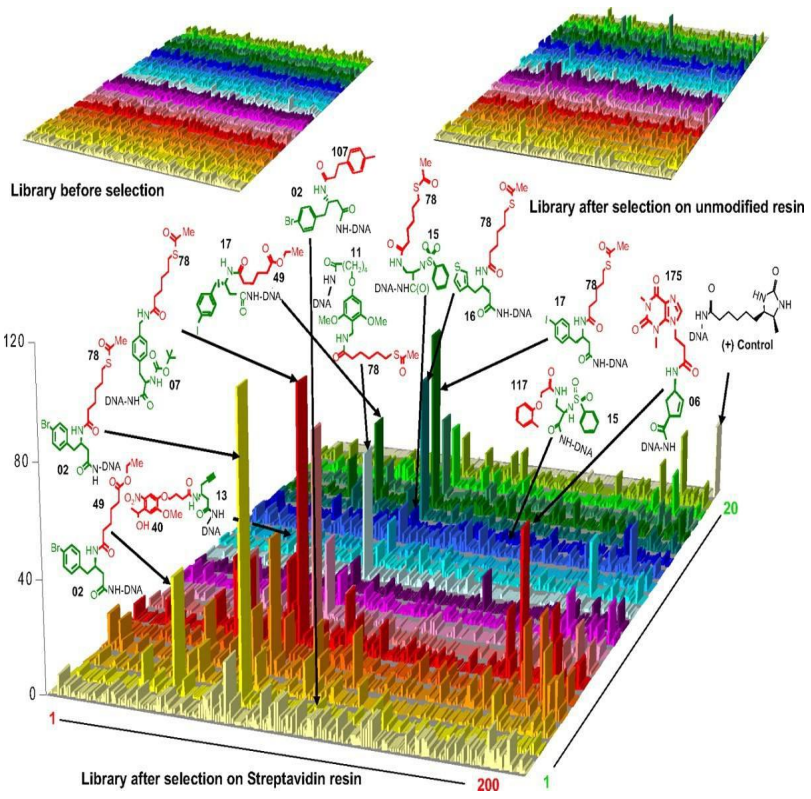
03-5 Transgenic Animals

In recent years the idea of expressing human antibodies in transgenic animals has increased. On the one hand, the humanization of antibodies for therapeutics derived from hybridoma technology is still a laborious and time-consuming procedure which often requires the generation and characterization of a set of different humanized versions of the antibody. On the other hand, the mouse or rat derived antibodies may elicit an immune response in patients. Several transgenic animals have been developed for the production and expression of human monoclonal and polyclonal antibodies. Mostly, transgenic mice have been used for secretion of the recombinant antibodies into the milk.

Much of the energy in transgenic production has been set on the development of humanized mice or rats. The well-established property of generating hybridoma cells from these species facilitates a streamlined approach for the generation of a cell line which stably expresses monoclonal antibodies. The main advantages of transgenic animals for antibody production are the flexible scalability and cost-efficient maintenance of the production facilities. Disadvantages are time- and labor-intensive generation of founder animals as well as safety issues regarding the animal-derived material if intravenous application of the product is planned.

04 Conclusion

Currently, mammalian cell lines are the dominant systems for production processes of full size antibodies and *E. coli* for antibody fragments. Both systems still may be further optimized by genetic engineering of the host cell lines for improved folding, secretion, and growth characteristics. In order to produce antibody with human-like glycosylation patterns, several expression systems have been developed. Bacteria, yeasts, filamentous fungi, and insect cells can be employed in order to lower the production costs of these products. In principle, transgenic plants and animals have the highest potential for up-scaling processes to theoretically unlimited production amounts. An overview of recombinant antibodies produced in different hosts is shown in Table 2.



In summary, substantial effort is still undertaken to develop new alternative production systems for the growing market of recombinant antibody therapeutics. Some of the systems are close to market maturity while others are pretty much in an early phase of development. With biosimilars coming up for therapeutics with expired patents, the pressure to reduce production costs will further rise. Recently, Creative Biolabs has developed a novel gram-scale production system for recombinant antibody to boost your research, especially magic™ high-throughput production service.

Table. 2 Production of recombinant antibodies by host.

Host	Antigen	Antibody format(clone)	Production system	Yield
Eukaryotics Expression System				
Escherichia coli	Digoxin	Fab (26–10)	Shake flask	0.8 mg/L/OD600
Escherichia coli	CD18	F (abf)2	Fermentor	2.5 g/L
Escherichia coli	Lysozyme	scFv (D1.3)	250/400 mL shake flask	0.3–1.0 mg/L
Escherichia coli	CRP	scFv (LA13-IIE3)	300 mL shake flask	0.55 mg/L
Escherichia coli	Lysozyme	scFab (D1.3)	300 mL shake flask	9.5 µg/L
Escherichia coli	MUC1	VHH	100 L shake flask	10 mg/L
Escherichia coli	Clostridium difficiletoxin A	VHH (14 different)	Shake flask?	1.2–72.3 mg/L
Escherichia coli	MUC1	scFv (2 different)	250 mL shake flask	0.46/1.3 mg/L
Escherichia coli	Atrazine	Fab (K411B)	2 L fermenter	13.8 mg/L
Escherichia coli	PPL	VL dAb	1.5 L fermenter	35–65 mg/L
Escherichia coli	phOx	scFv	50 mL shake flask	16.2 mg/L
Escherichia coli	phOx	scFv	3 L fermenter	1.2 g/L
Escherichia coli	Scorpion toxin Cn2	scFv; Fab (BCF2)	n. d.	0.3 mg/L;1.0 mg/L
Escherichia coli	TNF alpha	scFv	Shake flask?	45 mg/L
Escherichia coli	HSP70	Fab (cmHsp70.1)	8 L fermenter	>15 mg/L
Escherichia coli	Tissue factor	IgG	10 L fermenter	130–150 mg/L
Escherichia coli	TAG-72	Fv (B72.3)	Shake flask;fermentor	40 mg/L;450 mg/L
Escherichia coli	VEGF	scFv::SUMO	50 mL shake flask?	50.3 mg/L
Escherichia coli	HIV capsid	Fab, engineered	Shake flask	12 mg/L
Escherichia coli	Ovarian carcinoma/CD3	scFv–scFv	250 mL shake flask	1.2 g/L
Escherichia coli	Fibroblast growth factor receptor FGFR1	VHH	Shake flask	10–15 mg/L
Escherichia coli	Human prion	scFv	Shake flask	35 mg/L
Escherichia coli	Lysozyme	scFv (D1.3)	LEX bioreactor (1.5 L)	~ 2 mg/L
Escherichia coli	MUC1	scFv (HT186-D11)	LEX bioreactor (1.5 L)	~ 40 mg/L
Escherichia coli	CD30	scFv (SH313-B5)	LEX bioreactor (1.5 L)	~ 38 mg/L
Escherichia coli	Crf2	scFv (MS112-IIB1)	LEX bioreactor (1.5 L)	~ 4.5 mg/L
Escherichia coli	Tubulin	scFv (different ones)	Shake flask(intracellular)	up to 50 mg/L
Escherichia coli	n. d.	Fab	20 L; 75 L fed-batch bioreactor	0.7 g/L; 0.5 g/L
Escherichia coli	Digoxin	Fab (26–10)	Shake flask	0.8 mg/L/OD600
Escherichia coli	n. d.	Fab	20 L; 75 L fed-batch bioreactor	0.7 g/L; 0.5 g/L

Table. 2 Production of recombinant antibodies by host. | Continued

Host	Antigen	Antibody format(clone)	Production system	Yield
Proteus mirabilis	FAP	scFv (OS4)	50 mL shake flask	~ 12 mg/L
Proteus mirabilis	Phosphorylcholine	scFv-dHLX	n. d.	10–18 mg/L
Pseudomonas putidas	Lysozyme	scFv (D1.3)	200 mL shake flask	1.5 mg/L
Pseudomonas putidas	MUC1	scFv (HT186-D11)	200 mL shake flask	3.6 mg/L
Pseudomonas	CRP	scFv (TOB5-D4)	200 mL shake flask	2.9 mg/L
Bacillus megaterium	CRP	scFv (LA13-IIE3)	300 mL shake flask	0.39 mg/L
Bacillus megaterium	Lysozyme	scFab (D1.3)	300 mL shake flask	3.5 µg/L
Bacillus subtilis	Digoxin	scFv	n. d.	12 mg/L
Lactobacillus paracasei	Rotavirus	VHH	n. d.	~ 1 mg/L
Streptomyces lividans	Lysozyme	Fv	n. d.	~ 1 mg/L
EUKARYOTES				
Yeast				
Yarrowia lipolytica, Kluyveromyces lactis	Ras	scFv	Shake flasks	10–20 mg/L
Pichia pastoris	Muc1	VHH	Baffled flasks	10–15 mg/L
Pichia pastoris	TNFα	VHH-Fc	Shake flasks	5 mg/L
Pichia pastoris	AaHI	VHH	Shake flasks	17 mg/L
Pichia pastoris	B-type natriuretic peptide	scFv	Shake flasks	150 mg/L
Pichia pastoris	Atrazine	Fab-HRP	Shake flasks	3–10 mg/L
Pichia pastoris	Muc1	Bibody, tribody	Shake flasks	12–36 mg/L
Saccharomyces cerevisiae	71 Different	VHH	Shake flasks	<1 to >100 mg/L
Pichia pastoris	HER2	scFv	Shake flasks	15–20 mg/L
Pichia pastoris	n. d.	scFv	n. d.	300 mg/L
Pichia pastoris	Keratin 8	sc (Fv)2	Baffled shake flasks	4–5 mg/L
Pichia pastoris	n. d.	IgG	0.5 L bioreactor	0.5–1 g/L
Pichia pastoris	Rabies virus	scFv-Fc	80 L fermenter	60 mg/L
Pichia pastoris	HER2	IgG	3 L bioreactor	148–227 mg/L
Insect cell				
Spodoptera frugiperda	Blood coagulation factor VIII	scFv	Shake flasks	3.2–10 mg/L
Drosophila, S2	Glycoprotein H	Fab	Spinner flasks	16 mg/L

Table. 2 Production of recombinant antibodies by host. | Continued

Host	Antigen	Antibody format(clone)	Production system	Yield
Drosophila, S2	Bovine viral diarrhea virus, hepatitis C virus	scFv	n. d.	5–12 mg/L
Spodoptera frugiperda, SF-9	gp41	I gG	T-flasks	3 mg/L
Trichoplusia ni	gp41	IgG	T-flasks	12 mg/L
Sf SWT-1 Mimic	gp41	IgG	T-flasks	3 mg/L
MAMMALIAN CELLS				
HEK293T	CD200, SIRPγ	Fab	Genejuice, roller bottles	4 mg/L
HEK293T	n. d.	IgG	HEKfectin, tissue culture plates	1–14 mg/L
CHO	n. d.	IgG1, IgG4	Lipofectamine	140 mg/L
HEK293F	n. d.	IgG	293fectin	100–400 mg/L
CHO DG44	RhD	IgG	PEI, square-shaped bottles	90 mg/L
HEK293E	n. d.	IgG	PEI, square-shaped bottles	200 mg/L
CHO	n. d.	IgG	PEI, square-shaped bottles	60–80 mg/L
HEK293E Stable	RhD	IgG	PEI, square-shaped bottles	1.1 g/L
CHO-K1	n. d.	IgG	Lipofectamine	0.05–0.45 mg/L
CHO	HIV-1	scFv-Fc	PEI	5.78–45.49 mg/L
Insect cell				
Chlamydomonas reinhardtii	CD22	Immunotoxin, exotoxin A	Particle bombardement	0.2–0.4% chloroplasts
Nicotiana tabacum	BoNT/A	scFv	Agrobacterium tumefaciens	20–40 mg/kg
Nicotiana benthamiana	HIV	IgG	CPMV	105.1 mg/kg
Transgenic animals				
Mouse	CD147	Chimeric IgG	Milk	1.1–7.4 mg/mL
Mouse	Hepatitis A virus	IgG	Milk	32 mg/mL
Mouse	HER2	scFv-Fc	Milk	~ 120 ng/mL
Chicken	CD2, prion peptide	Chimeric IgG	Egg white	<150 μg/mL