Pharma&Biotech

Lonza

XS™ Microbial Expression Technologies

Optimize Productivity, Speed and Process Robustness

Embracing Complexity

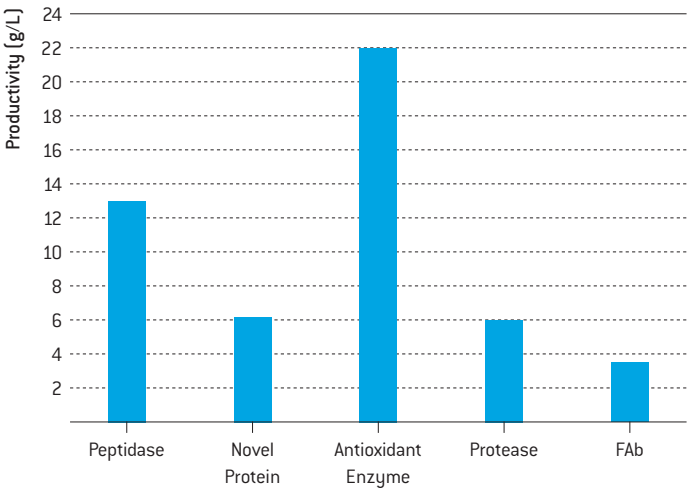
Based on 30 years of innovation in microbial biotechnology, Lonza offers the XS™ Microbial Expression Technologies Portfolio, an advanced and versatile production platform for biological candidates.

No single host type or vector is capable of being the best expression option for all types of biotherapeutics and vaccines. Therefore, Lonza has assembled a collection of proprietary expression systems that are screened in a high throughput manner to identify the best production clone for your product. Various *E. coli* options form the foundation of the XS™ Technologies Toolbox and are complemented by several host options, including *P. pastoris* (Pichia) and *B. subtilis* (Bacillus).

In combination with our well-established upstream and downstream production platforms, our XS™ Technologies Toolbox quickly delivers cGMP processes that are productive, robust, scalable, and efficient.

Find the Right Tool for Your Microbial Expression

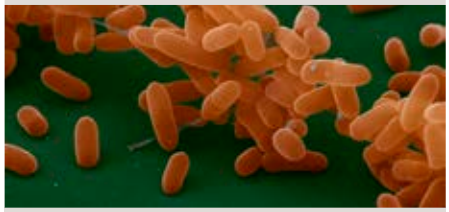
The various options in the XS™ Technologies Toolbox were developed to increase the probability of expression success. Platform fed-batch fermentation and recovery protocols have been tailored to each XS™ promoter system in order to form robust and scalable processes. Our goal is to provide the right tool for producing the highest titers as well as making recovery and downstream processing easier and more efficient.



Expression levels achieved for various therapeutic proteins using XS™ Systems

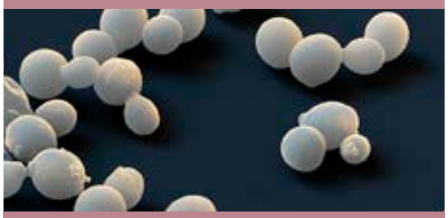
XS™ Technologies Toolbox

E. coli



Sugar Inducible
Depletion Inducible
IPTG Inducible

P. pastoris



Methanol Inducible
Glucose Regulated
Strong Constitutive
GAP Constitutive

B. subtilis

Sugar Inducible
Auto Inducible

E. coli is the Toolbox Foundation

Due to the historical success of *Escherichia coli* for the expression of recombinant proteins, this host type forms the foundation of Lonza's XS™ Technology Toolbox. Our *E. coli* systems are based on the commonly accepted K-12 and B microbial strains. Lonza's XS™ *E. coli* offering includes 3 different means of induction: sugar, IPTG and nutrient depletion, leveraging different regulatory mechanisms: positive and negative exponential, and positive stationary phase. This diverse set of options increases the probability of expression success for a variety of recombinant proteins.

Plasmid stability is a common concern when expressing complex proteins in microbial systems. Lonza's auxotrophic selection system achieves 100% plasmid stability throughout the fermentation process via the deletion of an essential gene in our proprietary *E. coli* strains. This gene is complemented in Lonza's upgraded XS™ plasmid backbone to ensure that only cells that have retained the plasmid will survive and produce the target protein.

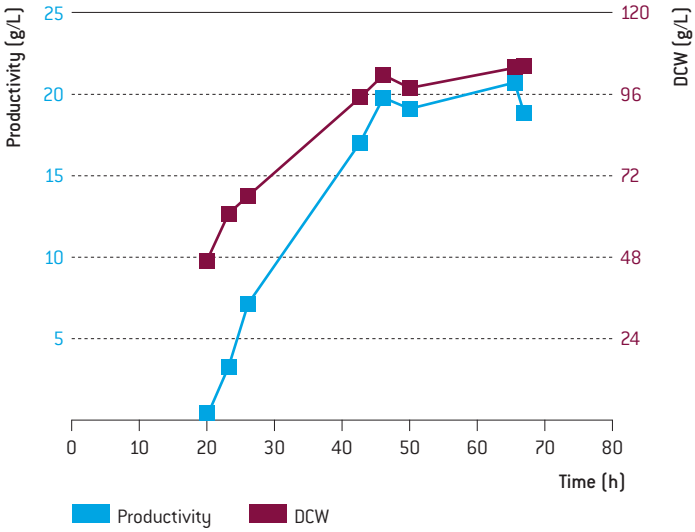
All Lonza *E. coli* systems share the same plasmid backbone, which enables the efficient use of our high throughput cloning platform for rapid strain development.

***E. coli* System Components Include:**

- Multiple *E. coli* strains
- Generic plasmid backbone with stabilizing elements: pXSE
- Same multi-cloning site for all plasmids
- Options for intracellular or periplasmic expression
- Numerous signal peptide options
- Options for monocistronic or polycistronic expression
- Fed-batch fermentation platform protocols

E. coli Sugar Inducible System

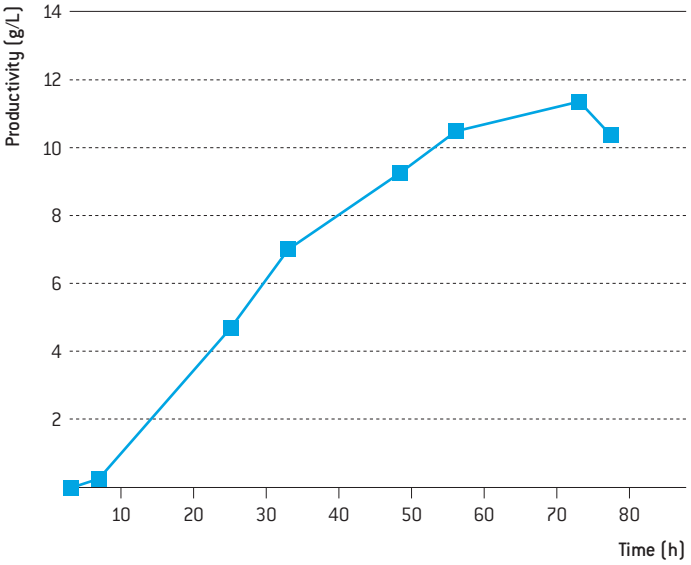
One of our most popular XS™ Technologies is Lonza's *E. coli* Sugar Inducible System. This tightly-regulated expression system can be induced by either rhamnose or melibiose. High cell density is achieved without premature induction, resulting in higher titers. In addition, the system features tunable expression, where very slow induction kinetics favor the production of soluble, functional target protein, especially for periplasmic production. Lonza's patented *E. coli* Sugar Inducible System has been successfully used in over 30 customer projects and is capable of achieving expression levels in excess of 20 g/L, a 10–20 fold improvement compared to a commercially available *E. coli* system. This proven technology has been shown to express many types of products, most notably, various difficult-to-express proteins.



Fed-batch fermentation of a sugar-induced XS™ *E. coli* strain producing an intracellular target protein reaching a maximum titer in excess of 20 g/L. (DCW, dry cell weight)

E. coli Depletion Inducible System

Lonza's *E. coli* Depletion Inducible System controls induction by limiting a key metabolite. This system contains an array of different strength promoters which enables the fine tuning of the expression rate, maximizing soluble expression. This is in contrast to Lonza's Sugar Inducible System, where expression tuning is performed using different sugar/inducer concentrations. In addition, the Depletion Inducible System expresses protein during the stationary phase of cell growth which expands the design space for experimentation in *E. coli*.

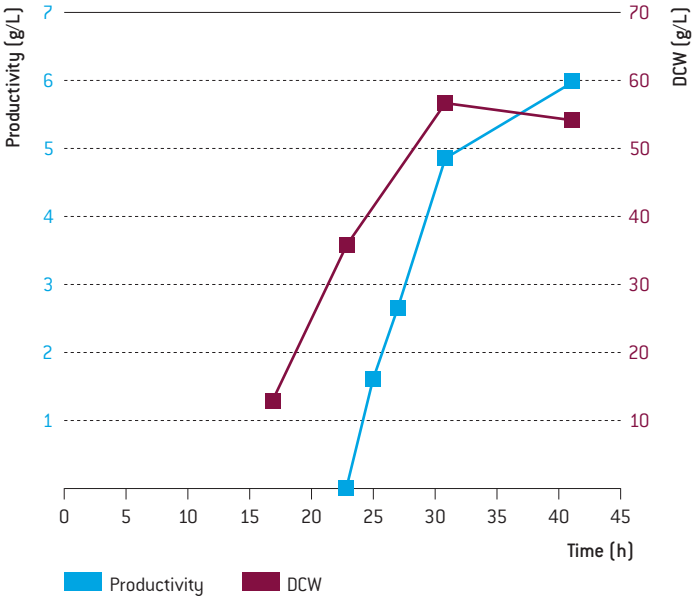


A high cell density fermentation run using the XS™ *E. coli* Depletion Inducible System is shown where the productivity is peaking after about 70 hours with 11.6 g/L of the target protein.

E. coli IPTG Inducible System

Lonza's *E. coli* Toolbox includes a set of well-accepted and well-known expression options, all based on the use of IPTG as inducer. We offer the T5 phage promoter which drives very high titers with very short induction times and, optionally, the lac and tac promoters, which continue to be used in commercial production processes. When coupled with our proprietary XS™ strains and vectors, this system generates highly stable commercially-viable cGMP strains. As shown below, our XS™ strains can produce high cell densities (57 g/L DCW) while maintaining plasmid stability and, therefore, improved protein expression.

The IPTG Inducible System complements our other *E. coli* Systems and is typically tested as part of an overall expression feasibility study. Screening all three XS™ *E. coli* Systems in parallel increases the probability of establishing the best production clone for your product.



The productivity and DCW over time for an IPTG-induced XS™ *E. coli* strain producing an intracellular target protein.

Pichia Supports More Complex Protein Candidates

Although *E. coli* host systems remain the industry workhorse, there are times when an ideal expression outcome for your product is not achieved. In these situations, we offer an alternative approach to microbial expression with a range of *Pichia pastoris* expression systems.

Lonza's proprietary Pichia LP1 host was developed for excellent growth performance. Our XS™ Pichia Toolbox includes four different promoter systems: Methanol Inducible, Glucose Regulated, Strong Constitutive, and GAP Constitutive. This range of promoter options provides complementary mechanisms to allow for the optimal expression of your product.

Pichia is a natural secretion host, which helps it to express soluble secreted product as opposed to undesirable intracellular inclusion bodies. In addition, Pichia lacks endotoxin, therefore eliminating this typical purification challenge.

As a eukaryotic host organism, Pichia is able to express small polypeptides effectively, as well as large, complex proteins that require higher order post translational modifications.

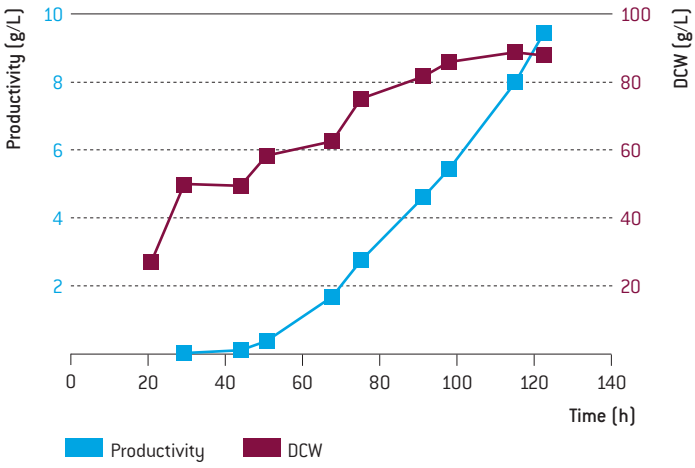
Pichia System Components Include:

- Proprietary plasmid backbone: pXSP
- Same multi-cloning site for all plasmids
- Selection marker: zeocin
- *E. coli* type origin of replication
- Single or multi-copy insertion possible at 2 different chromosomal loci
- Proprietary signal peptides
- Fed-batch fermentation platform protocols

Pichia Methanol Inducible System

Pichia is a methylotrophic yeast, capable of metabolizing methanol as its sole carbon source. Growth on methanol induces the expression of genes whose products are required for its metabolism. The AOX1 promoter regulating the first gene of the methanol utilization pathway has been the most widely-used Pichia system in the industry. Induction by methanol requires volatile solvent handling and, if not carefully controlled, can affect protein production. In some cases however, the advantages of higher titers may outweigh the issues related to methanol.

Lonza has combined its proprietary XS™ Pichia host and signal sequences with this proven promoter system to offer customers this reliable Pichia option. Multiple gram per liter titers have been achieved with this system.

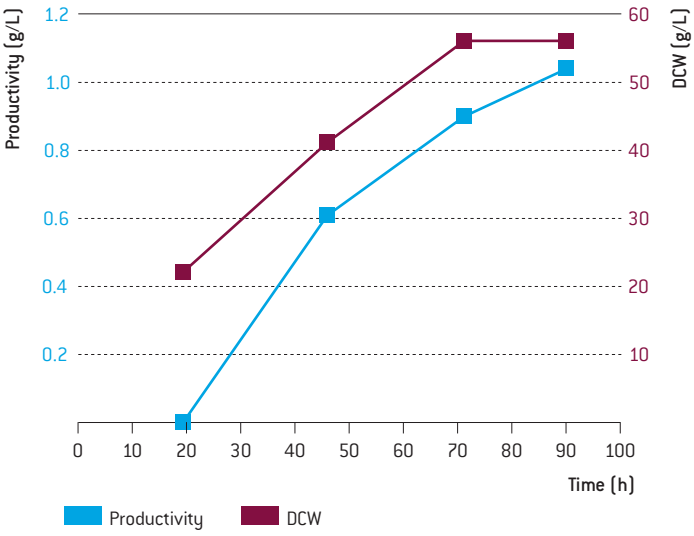


10 L fed-batch fermentation of a protein using the XS™ Pichia Methanol Inducible System showing titers in excess of 9 g/L.

Pichia Glucose Regulated System

Inducible promoters are beneficial for the production of heterologous proteins that interfere with cell metabolism and the viability of the cells. By using inducible promoters, the growth phase can be decoupled from the production phase. This enables high cell densities to be achieved prior to product expression leading to high titers.

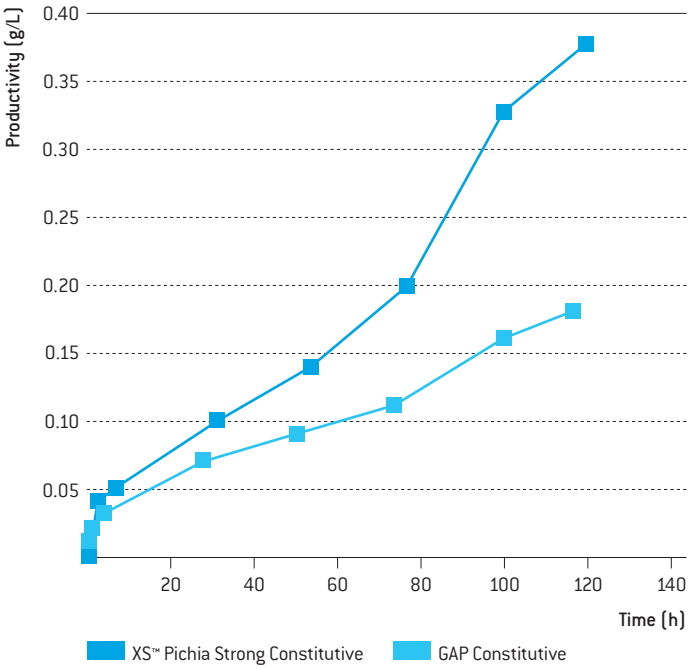
The AOX1 promoter is widely used in the industry and is induced by methanol requiring volatile solvent handling. Methanol feeds need to be carefully controlled since methanol accumulation during fermentation is toxic to the Pichia cells. In addition, methanol induces a stress response which can ultimately lead to target protein degradation. Lonza's proprietary Pichia Inducible System avoids methanol and uses glucose as a trigger for induction.



10 L fed-batch fermentation of a target protein using the XS™ Pichia Glucose Regulated Promoter System. Expression levels in excess of 1 g/L were achieved.

Pichia Strong Constitutive System

For products that do not interfere with Pichia metabolism and cell viability, non-induced constitutive can be an attractive expression strategy. Lonza's proprietary promoter achieves higher titers and consistently outperforms the commonly used GAP Constitutive promoter. An additional benefit of this system is that it does not require the use of methanol.

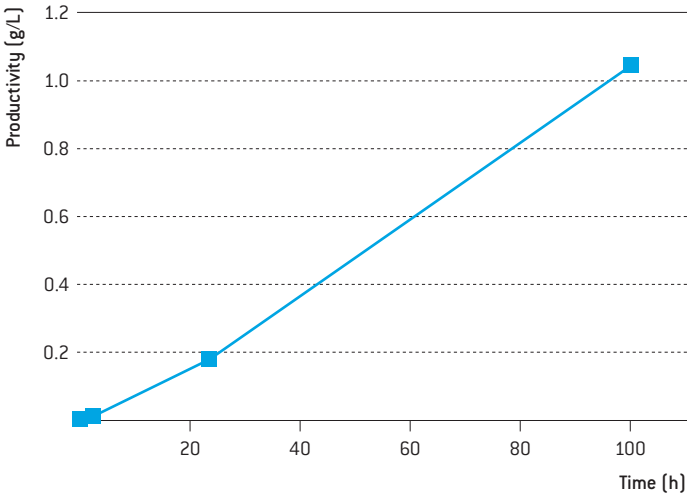


10 L fed-batch fermentation of a therapeutic protein using XS™ Pichia Strong Constitutive System versus a commercially available GAP Constitutive promoter system using strains with the same gene copy. Twice the expression of the target protein was achieved with the Lonza Strong Constitutive System.

Pichia GAP Constitutive System

Vectors containing a constitutive Pichia promoter derived from the glyceraldehyde-3-phosphate dehydrogenase gene (GAP) are widely known and used in the industry. For products that do not interfere with Pichia metabolism and cell viability, non-induced constitutive can be an attractive expression strategy.

Lonza has combined this promoter with our novel XS™ Pichia host and signal sequences to offer customers an additional Pichia option to screen. This system has shown productivity levels in excess of 1 g/L for a novel therapeutic candidate. And similar to the Pichia Strong Constitutive System, it does not require the use of methanol.



10 L fed-batch fermentation of a novel therapeutic candidate using XS™ Pichia GAP Constitutive System showing titers in excess of 1 g/L.

Bacillus for Secretion of Monomeric Proteins

Bacillus subtilis is the latest strain addition to Lonza's comprehensive XS™ Toolbox. This organism has been thoroughly characterized and investigated. Its genome has been sequenced completely and all essential genes and metabolic pathways are known. It also has achieved Generally Recognized as Safe (GRAS) status with the US FDA. Currently this organism is widely used for production of prokaryotic industrial enzymes. Lonza has developed two XS™ Bacillus Expression Systems for the production of recombinant biopharmaceuticals and vaccines: Sugar Inducible and Auto Inducible. The system uses our proprietary *B. subtilis* 168 derivative host and has over 10 signal sequences for screening. XS™ Bacillus is an interesting option to explore for monomeric protein products possessing some intrinsic folding propensity. It is also an attractive host for biopharmaceutical production because it can express soluble properly-folded heterologous protein, lacks endotoxin, and grows easily to high cell densities in minimal media.

Bacillus Sugar Inducible System

Lonza's patented XS™ Bacillus Sugar Inducible System includes a positively regulated promoter and is induced by D-mannose. This XS™ System has been successful at expressing proteins in excess of 5 g/L in high cell density fermentations.

Bacillus Auto Inducible System

Lonza's auto inducible mannose promoter provides an alternative expression approach that eliminates the need for a D-mannose feed. In the XS™ Bacillus Auto Inducible System, induction occurs at the transition from a batch phase into a fed-batch phase. Auto induction takes place as the glucose level falls below a defined concentration. Throughout the fed-batch phase, the desired product is expressed as the cells grow to high cell densities. This auto induction strategy often generates higher titers, up to 15 g/L, and offers the added cost-savings from the lack of D-mannose.

The XS™ Technologies Toolbox Has Complementary Options to Meet All of Your Expression Needs

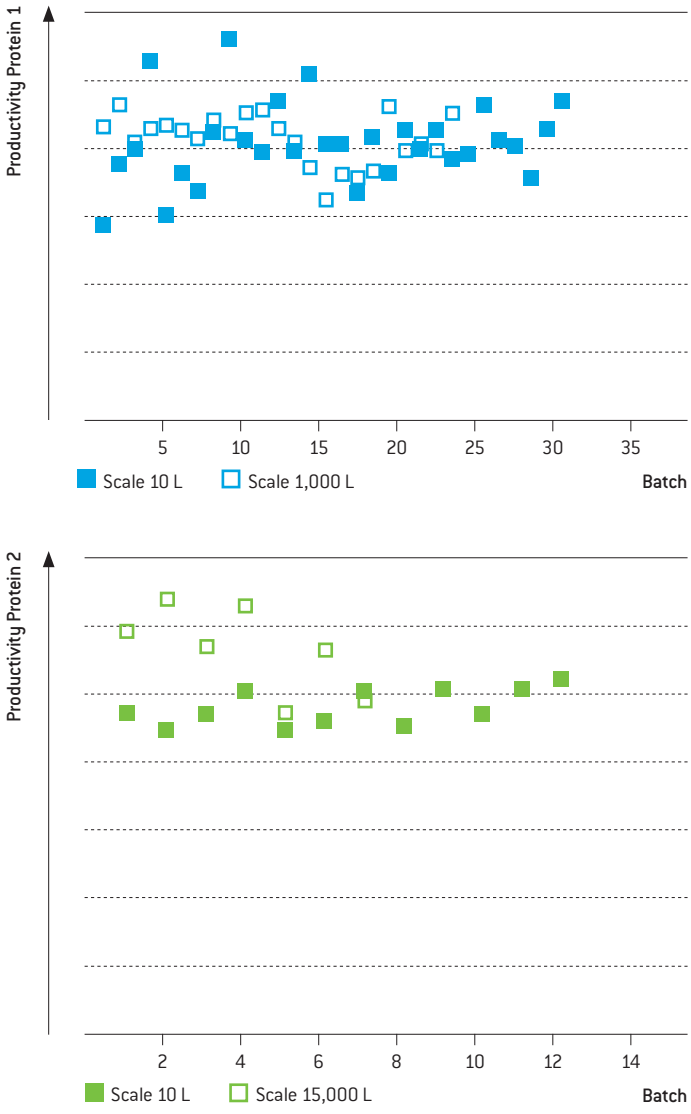
XS™ Expression Systems	*E. coli*	Pichia	Bacillus
High Titers	■	▪	▪
Expression of FAbs	■	■	–
Soluble Expression	■	■	■
Stable	■	■	■
Scalable	■	■	■
Secretes Product	▪	■	▪
Endotoxin Free	–	■	■
Lacks Glycosylation	■	–	■
Approved by US FDA for Biopharma	■	■	–

■ Yes ▪ Sometimes – No

Focus on Productivity and Robustness

With Lonza's multi-host XS™ System, several microbial options exist to allow you to find the right tool for your expression needs. Whether it is *E.coli*, Pichia or Bacillus, these systems have been shown to achieve high expression levels, some in excess of 20 g/L. For difficult-to-express proteins, the XS™ Portfolio has delivered expression levels of up to 1 g/L, where alternatives were only achieving a few milligrams per liter.

These industry-leading results are achieved through Lonza's novel molecular biology machinery combined with our world-class fermentation expertise. Improving your cost of goods begins with high expression levels, but it does not end there. XS™ Microbial Expression Technologies also drive soluble expression which results in higher downstream yields by avoiding inefficient refolding steps.



A comparison between specific productivities for a series of XS™ *E. coli* fermentation runs for 10 L high cell density fermentation and large scale fed-batch fermentation (1,000 L and 15,000 L) for two different proteins, showing the scalability of the XS™ Systems.

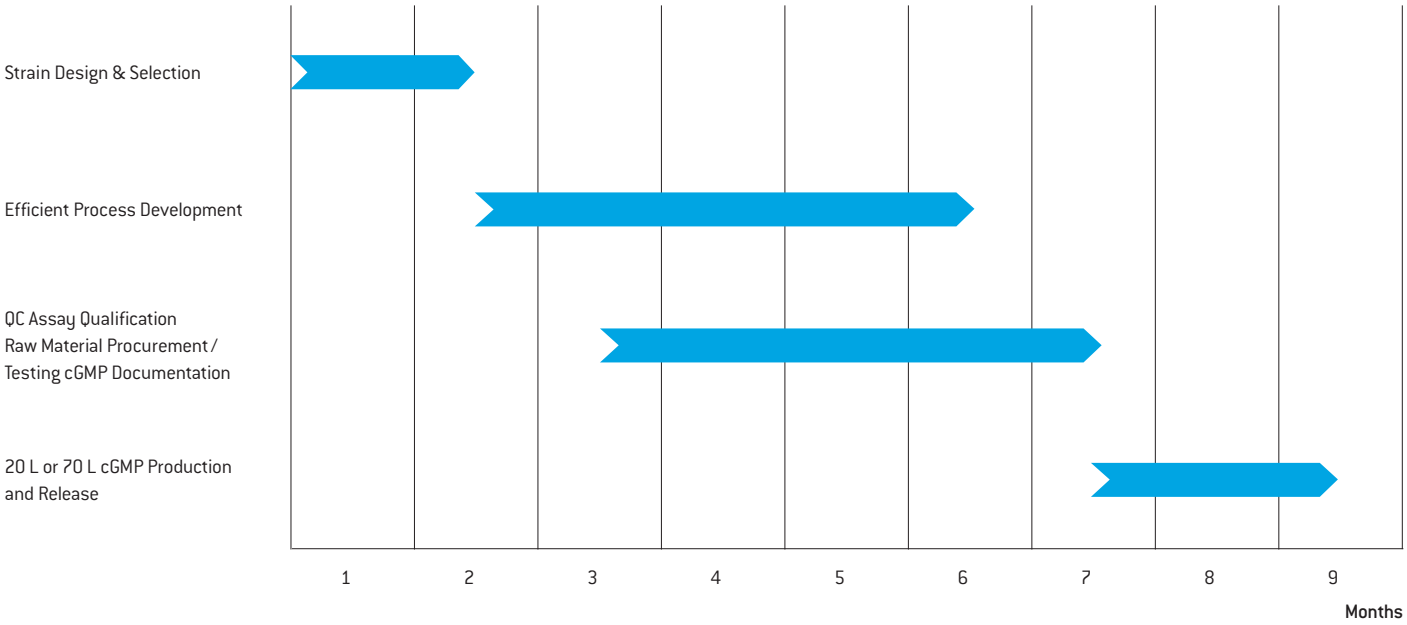
Lonza's XS™ Technologies Toolbox Was Designed to Help Establish a Reliable and Scalable cGMP Process

Productivity	– In excess of 20 g/L for monomeric proteins, if possible – 2 to 5 g/L common for typical therapeutics – up to 3.5 g/L for FAbs
Speed	– Hundreds of host-vector combinations are screened to identify the best production clone within 4–6 weeks – Optimized process development workflows and automated systems can enable delivery of clinical grade product in less than 9 months
Simple, Scalable & Robust Processes	– Complete plasmid retention results in reliable fermentations – Robust platform fed-batch fermentation and recovery protocols ensure scalability – Soluble and secretion expression options result in simpler recovery and downstream unit operations

Faster to the Clinic, Faster to Market

Lonza understands that filing your IND/IMPD and moving your biotherapeutic or vaccine into the clinic is a critical step in the progression of your product towards the marketplace. With the help of new automated equipment, we have incorporated a high throughput element into our XS™ Technologies Platform, making it possible to quickly explore all system options.

Several high throughput tools, including robotic workstations and detection methods, are used for analytics, screening, transformation, and colony isolation. Within 4–6 weeks, this automated approach allows hundreds of host-vector combinations to be screened to identify the best production clone. In addition, optimized platform processes can enable delivery of cGMP clinical grade product in less than 9 months.



Accessing XS™ Technologies is Easy

The benefits of XS™ Technologies can be explored in two ways. Lonza offers a complete range of strain development services in Visp, Switzerland. In addition, a select portion of the XS™ Technologies Toolbox is available for use in your laboratory under a Research Evaluation Agreement (REA).

With Lonza's XS™ REA, you will have access to our microbial expression hosts and vectors to use in your development laboratories*. The research agreement includes licensing options for both *E. coli* and Pichia systems, along with detailed fed-batch fermentation protocols designed to quickly create commercially-viable production strains and cGMP processes. The kit contains all the components necessary to identify the best production clone for your product. In addition, there are numerous options for technical support including notification of system upgrades, visits to customer sites as part of XS™ Technology tours, and access to technical experts by phone or e-mail.

The REA is renewable on an annual fee basis for as long as you have XS™-expressed products in preclinical stages. This fee covers access to 5 *E. coli* and Pichia systems. Once you are ready to file an IND/IMPD for a biotherapeutic or vaccine candidate, an XS™ Commercial License must be obtained. This license covers the clinical development and commercial stages for the biological candidate.

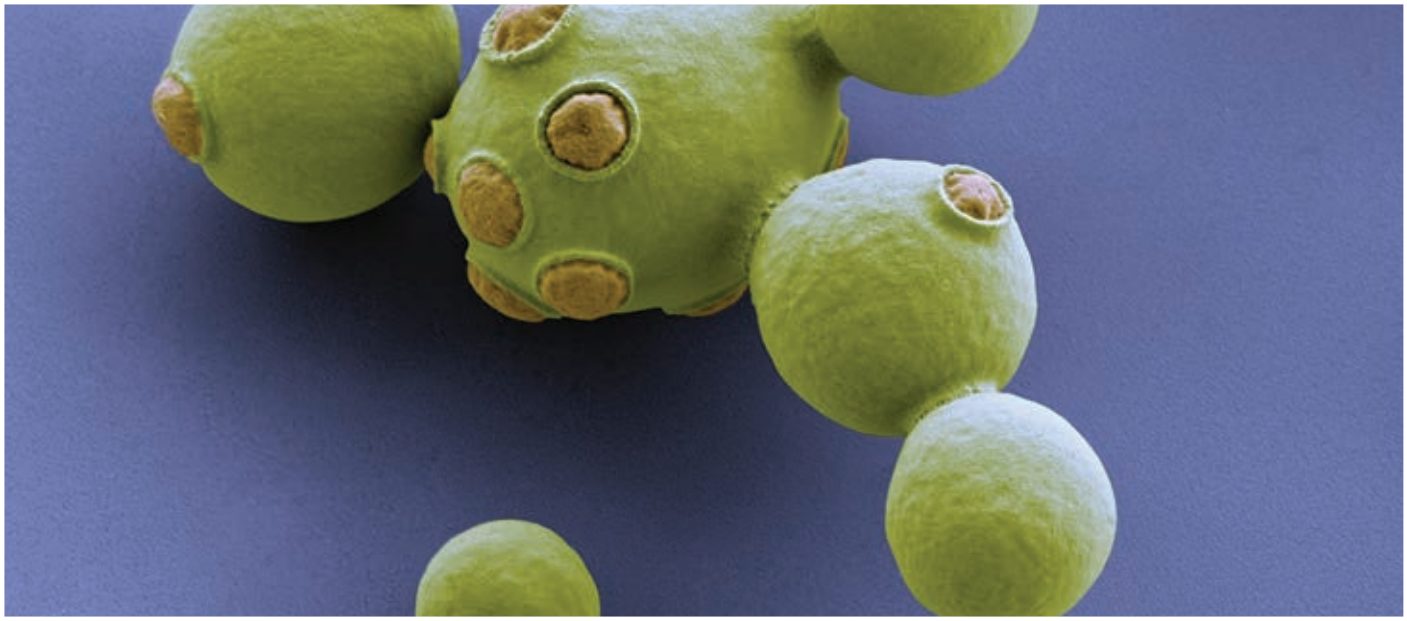
* System availability varies by country

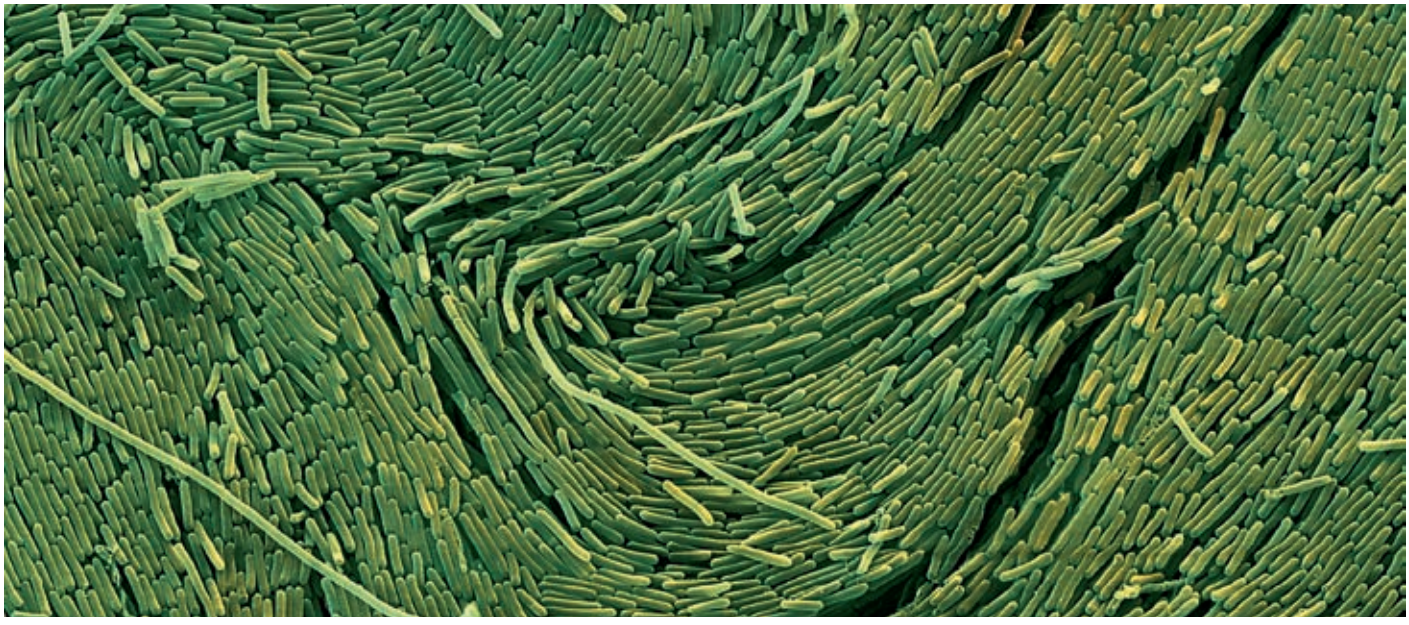
REA Package for XS™ *E. coli* Systems

	Sugar Inducible	IPTG Inducible
Hosts	5 Hosts	2 Hosts
Promoters	Melibiose, Rhamnose (kanamycin resistance)	T5/IPTG (kanamycin resistance)
Strain Development Manual	Manual includes: – Plasmid construction & transformation protocols – Clone screening methods for desirable expression attributes – Plasmid maps and sequences	
Fermentation Protocols	Fed-batch fermentation protocols tailored to each XS™ System	

REA Package for XS™ Pichia Systems

	Methanol Inducible	Glucose Regulated	GAP Constitutive
Hosts	1 Host	1 Host	1 Host
Promoters	AOX1 (zeocin resistance)	G1 (zeocin resistance)	GAP (zeocin resistance)
Strain Development Manual	Manual includes: – Plasmid construction & transformation protocols – Clone screening methods for desirable expression attributes – Plasmid maps and sequences		
Fermentation Protocols	Fed-batch fermentation protocols tailored to each XS™ System		





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