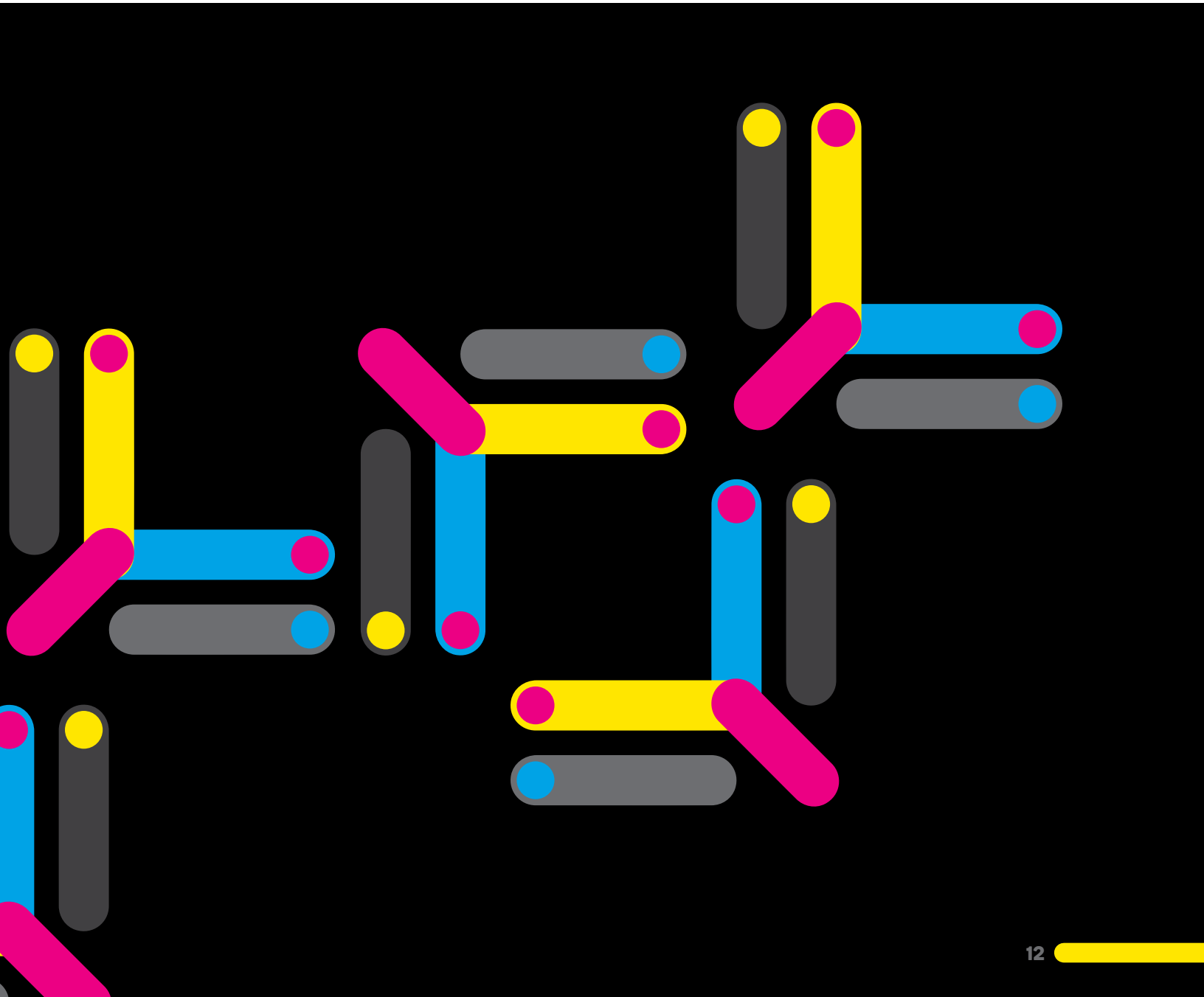
ATUM

antibody services

From virtual to physical
Antibody screening
Antibody production and purification
Engineering antibodies for developability
Antibody humanization
Generating stable cell lines



antibody services:

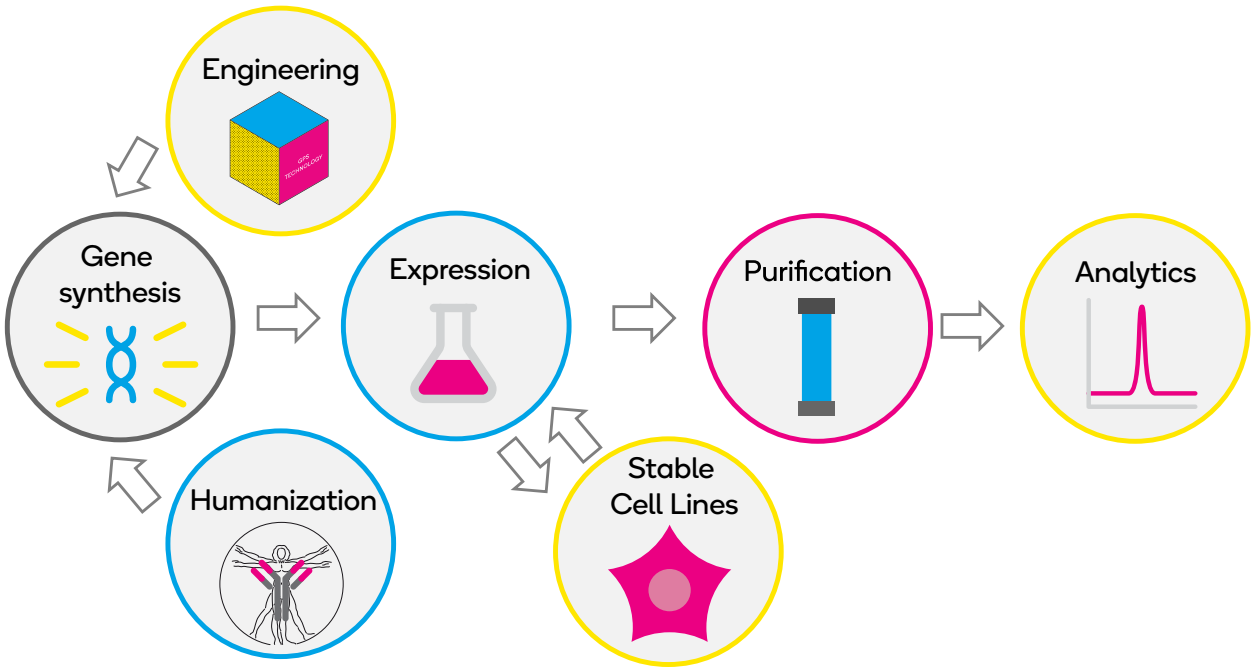
overview

Development of therapeutic monoclonal antibodies (mAbs) began in the early 1980s and the first mAb drug was approved by 1986. Since then, a multitude of different antibody-based formats have reached the market. Today antibody-based drugs dominate the biopharmaceutical market, and ATUM's integrated antibody service platform was built to accommodate the flexibility and efficiency required to make a drug from a binder.

Engineering of better antibodies, improved cell lines and higher production yields require efficient tools and experience. ATUM's integrated pipeline from oligonucleotide synthesis via gene design/gene synthesis/vector optimization all the way to transient and stable mammalian protein production from a GMP Master Cell Bank enable the direct link from lead to therapeutic.

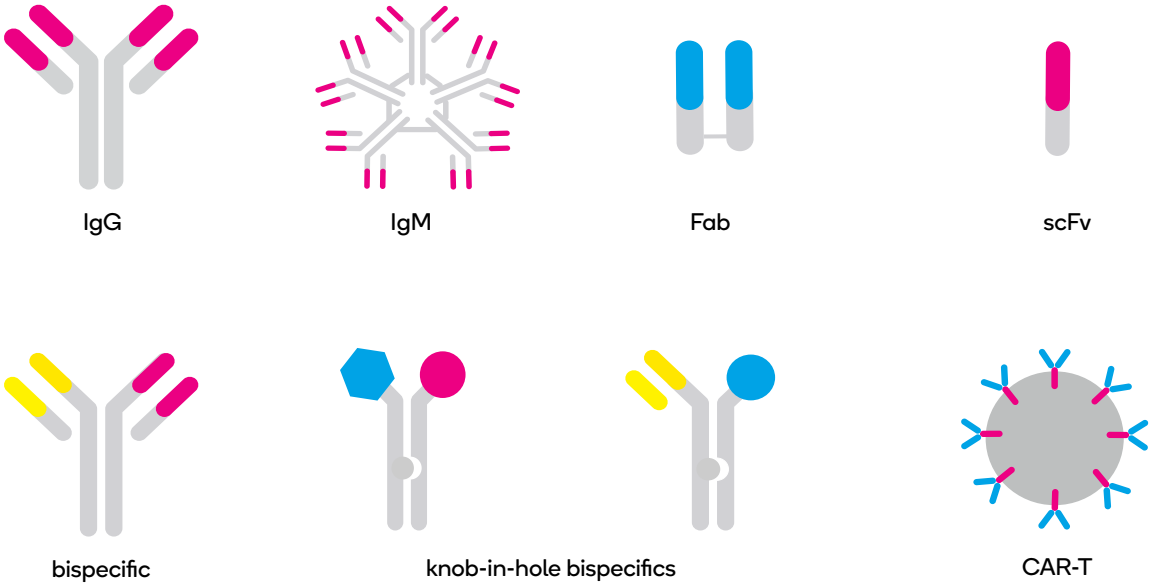
Your antibody is safe in our hands. We express and purify thousands of recombinant antibodies and antibody-based molecules every year, apply our GPS design platform to humanize, affinity maturate, increase their potency and build highly productive cell lines for manufacturing.

A Fully Integrated Ecosystem



Whether you need expression optimization, recombinant protein production, antibody humanization and engineering, or cell line development, ATUM delivers solutions to advance your research and speed your path to market.

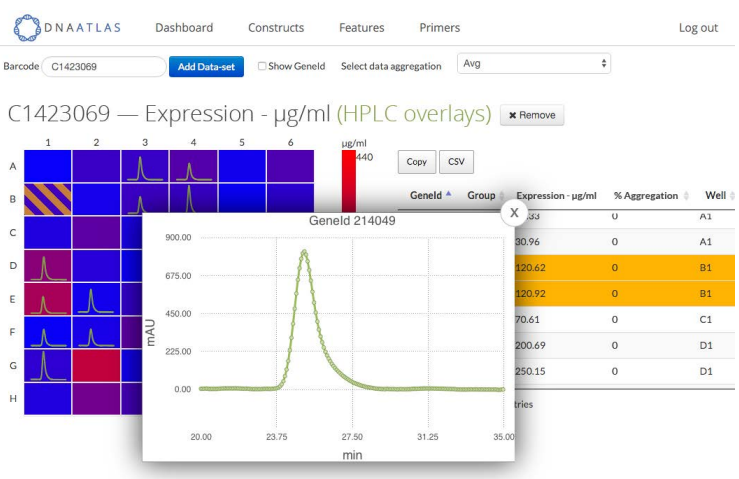
Antibody types

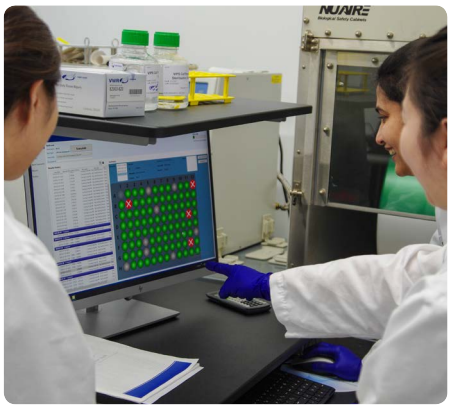


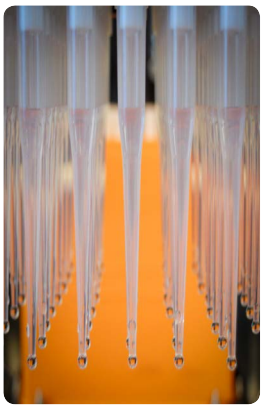
Whatever your discovery platform, we can take the sequence of your variable regions and turn them into real molecules. Variable regions can be combined with the original constant regions, or with new constant regions to generate humanized or isotype-switched antibodies. We apply our GeneGPS® and VectorGPS® design platforms to optimize expression and balance chain ratios, thereby maximizing yield of your functional antibody.

"We are excited that joining forces with ATUM will provide customers with continued access to the highly integrated synthesis-through-purification process that rapidly delivers highly characterized antibody and antibody-like molecules efficiently at high titers. The protein pharmaceutical market is finding this integrated offering an exceptional platform to accelerate their research."

Dr. Michael Feldhaus, Founder of MIGS. ATUM acquired MIGS in April 2016.







"ATUM provides an unparalleled antibody engineering and production platform under one roof. With the acquisition of MIGS, we now have the ability to go from virtual sequence to gram-scale of proteins derived from mammalian cells. Adding this to our machine learning and DNA synthesis platforms, helps our customers to reimagine their research."

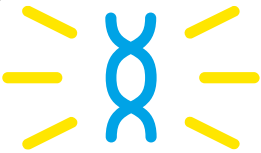
Dr. Claes Gustafsson, Co-founder of ATUM

antigen production

ATUM's protein production services combine proprietary *in silico* codon and vector optimization algorithms to design and synthesize genetic constructs that maximize expression, whether the proteins are expressed in bacterial, yeast or mammalian hosts. ATUM's purification expertise and comprehensive analytical capabilities ensure that the proteins are made according to precise specifications and at high quality. Are you struggling with non-expressing proteins, low solubility or proteins that aggregate? Give us a call, we can help.

hybridoma sequencing

ATUM offers sequencing of your hybridoma cell line to determine sequence of cDNA encoding the antibody variable light (VL) and heavy (VH) domains. Antibody sequence information is essential for making monoclonal antibodies (mAbs) engineering and humanization, function optimization, database banking and patent applications.



Send us two tubes, each with a frozen pellet of ~500,000 hybridoma cells. We will prepare mRNA and make cDNA corresponding to the heavy and light chain by RACE. We will clone and sequence independent clones to get you the sequence of the variable region, native secretion signal and the antibody isotype subclass.

ATUM provides:

- Determination of variable heavy and light chain sequences
- Species and IgG sub-type confirmation
- Follow on services: Combine antibody sequencing with our antibody engineering, humanization and antibody expression services

antibody production

Our efficient transient 96-well antibody production platform is second to none when it comes to speed and efficiency. Send us your in silico CDR sequences from your discovery campaign (phage display, FACS sort, transgenic animals, deep sequencing or similar). ATUM will codon optimize the sequences using our proprietary algorithms, graft the CDR into your preferred scaffold (IgG1, IgG4, IgM, Fab, scFv and other formats) and synthesize the corresponding genes directly into our optimized expression vectors in 96-well format. The synthetic genes will be transfected in HEK 293 cells, expressed, harvested and purified in one integrated process, resulting in unprecedented speed from virtual to mg antibody.

expression & purification

Whether you need micrograms of many different proteins for your discovery programs, or grams of highly purified protein for animal studies, we have the production scale and purification strategy to match. Our cell cultures range from 96-well plates of 1ml cultures through shake flasks to bioreactors delivering multi grams of protein. Expression testing/piloting, desalting, custom buffer formulations, and aliquoting are all available on request.

high throughput

high volume

bioreactor

Cell cultures range from 96-well 1 ml cultures to shake flasks to bioreactors, delivering grams of protein.

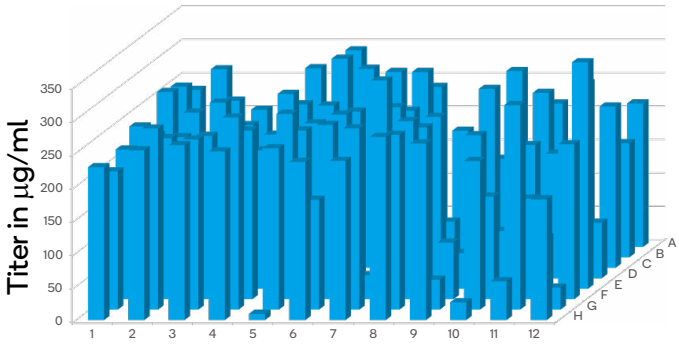
- Expression vector constructs are made with customer's variable heavy and light chain regions grafted into one of several available and validated scaffold sequences. The construct identity is confirmed by sequencing and transfection grade DNA is prepared.
- HEK 293 or CHO cells are transiently transfected in 1 ml 96-well (HTP) format, or up to 10l. Larger volumes available upon request.
- Cells are grown, harvested and proteins are purified using appropriate purification resin.
- Proteins are eluted, neutralized and desalted (if required).
- Second column purification (SEC, IEX, MMC) and buffer formulations are available per client request.

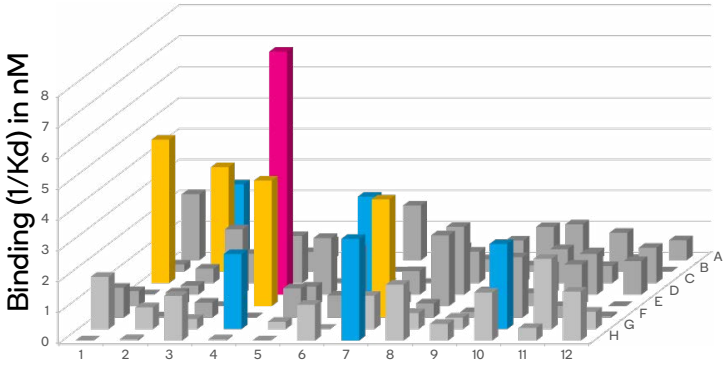
case study: high throughput purification

Customer needed purification and screening of several antibody variants to identify the best variants with desired characteristics to move along their pipeline. ATUM's integrated pipeline of tools helped identify the winners and to quickly move desired pools into stable cell line development programs.

96-well screening, robotics & the Leap-in advantage:

Efficient and robust integration, predictable selection, and high titers with Leap-in technology (see pages 10-11)enable high throughput screening of stable pools that are representative of final stable clonal lines. Initiate analytical development, process development, and GLP toxicology studies early, shaving months off the CMC timelines.





Antibody Fab variants were transiently expressed and purified from 96-well 1 ml cultures. Expression titer and binding was measured for all 96 variants, shown in graphs above. Expression titer was reasonably even across the 96 variants, with only 8 wells expressing below 50µg/ml. We identified antibody variants with high (pink bar), medium (yellow bars) or low (blue bars) binding affinities determined using ForteBio Octet®.

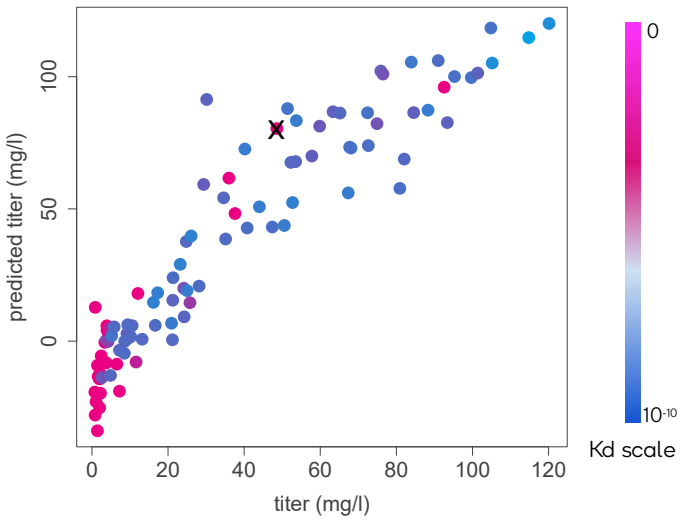
Leap-in enables fast parallel processing and multiple shots at goal.

engineering antibodies for developability

test learn

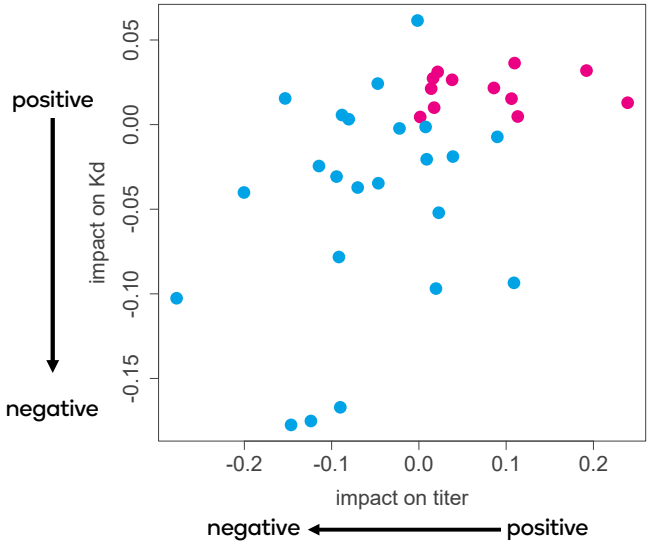
Phylogenetic and structural modeling identifies amino acid residues that may affect affinity, stability, expression yield, aggregation, humanization and other properties that affect the developability of the antibody. Functional data derived from physical testing is modeled against the systematically varied infolog variants and used to generate predictions of new variants with enhanced developability properties. These predictions are then tested in subsequent rounds of engineering.

A. Modeling titer



A total of 96 systematically designed variants of antibody Z. The X-axis denotes the observed (i.e. wet lab) expression yield. The Y-axis denotes the predicted expression yield which is an iterative process using 10% of omitted data per iteration against the remaining 90% from the model, thus providing a predicted expression value. The diagonal distribution represents the accuracy of the model. We have here also color coded the binding affinity of the antibody variants. Parent (mouse) variant is denoted as 'X'.

B. Impact of sequence variables



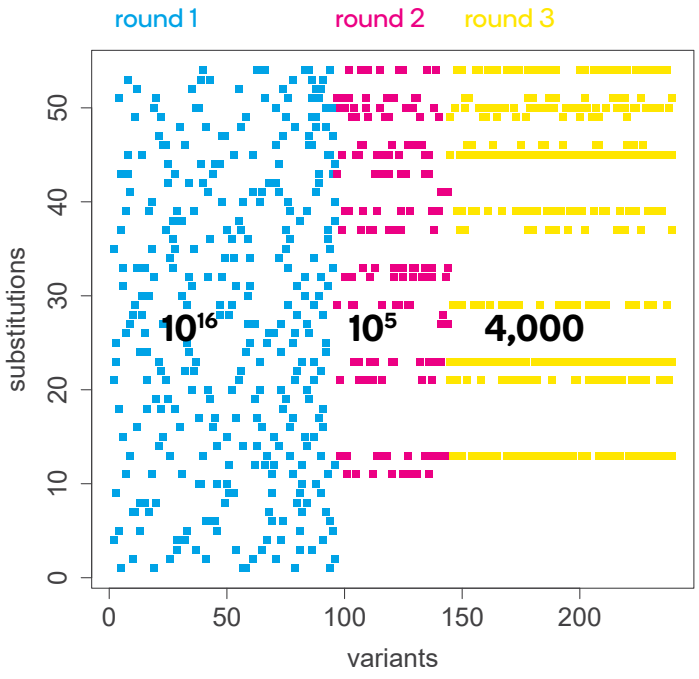
Amino acid substitutions representing the variants from A are distributed by their relative contribution towards titer (X axis) and binding (Y axis). Substitutions contributing positively in both dimensions are denoted in pink.

ATUM's proprietary Design of Experiment (DoE) technology enables systematic exploration of sequence-function relationships, identifying and quantifying amino acid substitutions and their relative contribution in multiple different functional dimensions. Assessing the sequence-function relationship and the amino acid substitutions relative independence provide guidance for generating predictive and testable models of target protein performance, metrics of its humanness, and developability. We typically test a total of 48-400 antibody variants over 1-4 iterations.

learn improve

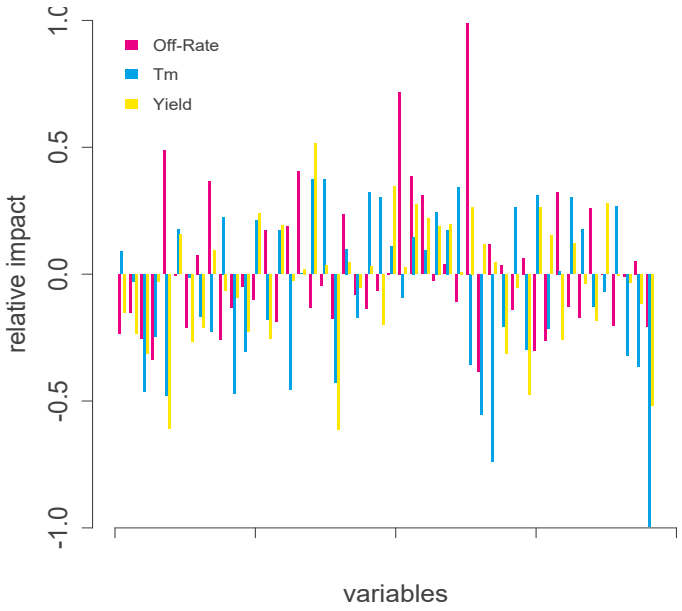
The results from multiple independent antibody humanization experiments impart predictive design strategies for future antibody humanizations.

Efficient search of space



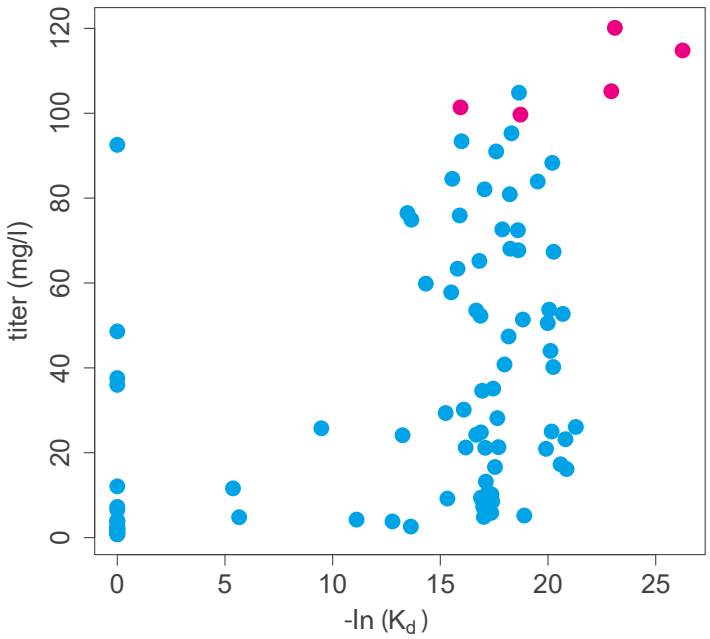
Reduction of tested amino acid substitutions over three iterations of antibody engineering for undisclosed biological activity. The X-axis denotes each tested mAb variant, Y-axis denotes presence/absence of substitutions. First round (R1) in blue tests 54 amino acid substitutions present in 96 mAb variants for a total space of $2^{54} = 10^{16}$ possible variants. Second round (pink) constitutes 48 mAb variants narrowing the search to 18 substitutions ($2^{18} = 10^{5}$). Third round (yellow) constitutes 96 mAb variants further narrowing the search to 12 substitutions ($2^{12} = 4{,}000$ variants).

Weights distribution plot



Relative contribution of 50 amino acid substitutions in the same mAb scaffold; each substitution is represented by 3 bars, binding off-rate (red bar), Tm (blue bar) and expression productivity (yellow bar).

Predicting and validating new variants



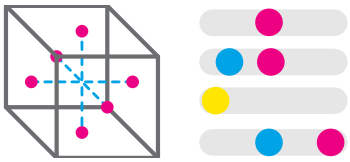
improve validate

Sequence function information for binding (X-axis) and expression yield (Y-axis) derived from antibody engineering round 1 (blue) was used to build models and to predict 5 new improved variants (pink) which were engineered and validated.

case study: purification & scale up

Customer needed gram quantities of a poorly expressing lead candidate antibody. We used our engineering capabilities to optimize codons, vectors and expression to generate high producing stable pools. Formulation optimization of the purified antibody allowed concentration of antibody to desired levels without aggregation. Scale up of the antibody was carried out to produce greater than 200 grams of well-characterized purified protein.

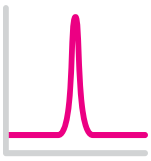
1. design, build



Low expressing antibody optimization

generate high expressing stable pool

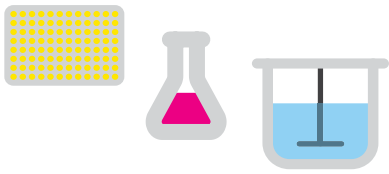
2. analytics



Concentrating protein using tangential flow filtration (TFF)

Custom formulation - no aggregation even at >100 mg/ml

3. scale-up



Scaled up to produce >200 grams of therapeutic antibody

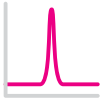
analytics

We offer a range of analytic services to characterize the identity, purity, quantity and quality of your protein. Every protein purified at ATUM includes the following standard analytical package: Appearance description, quantitation by A280, molecular weight and purity analysis by micro-capillary electrophoresis and aggregation analysis by SEC-HPLC.

The standard package can be augmented with other analytical services. We can work with you to build an analytical package appropriate for your molecule and subsequent applications (e.g., IND filing, toxicology studies).

QUANTITY

- A280 reading
- Bradford protein assay
- Productivity (Octet, HPLC, ELISA)



PURITY

- SDS-PAGE
- Reverse phase chromatography (HPLC)
- Aggregation analysis (SEC-HPLC)
- Endotoxin analysis

Standard analytical package: quantitation, molecular weight, purity analysis and aggregations analysis.
Additional analytical services available on request.

STRENGTH & POTENCY

- Octet-based assays
- ELISA

IDENTITY

- Reducing & non-reducing SDS-PAGE
- Size-exclusion chromatography (HPLC)
- Western Blot
- Reverse-phase chromatography (HPLC)
- Intact molecular weight (MS)
- Antibody subunit analysis (MS)
- Glycans profile (micro-CE, HPLC)
- Peptide mapping (HPLC, MS)
- Charge variant analysis (micro-CE, HPLC)

deliverables

PURIFIED PROTEIN

Purified protein shipped frozen or on ice packs as requested.

ANALYTICAL REPORT

Comprehensive report for every analytical service performed.

CHARACTERIZATION

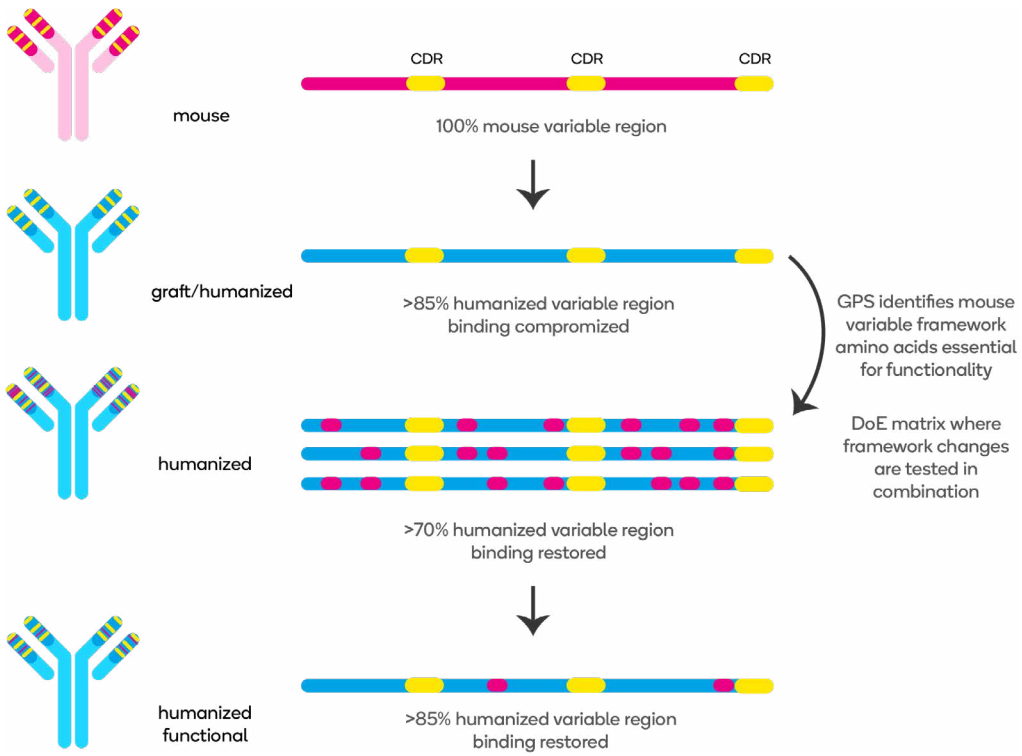
Amino acid sequence information, process and characterization report for each IgG delivered.

antibody humanization

ATUM's antibody humanization platform combines advantages of both rational and empirical antibody humanization approaches. Humanized antibodies are designed using our proprietary humanization and optimization algorithm and made by gene synthesis and transient protein expression in mammalian cells.

Traditional humanization approaches and CDR grafting can be drastically improved by applying DoE and machine learning methodologies to generate a small number of humanized molecules with improved developability profiles including expression titer, aggregation propensity, stability, polyspecificity profile, Tm, in conjunction with retained or enhanced affinity.

- ATUM uses homology modeling between the non-human and human V genes, followed by *in silico* CDR grafting.
- Sequences are analyzed for the presence of known T cell epitopes, N-linked glycosylation sites, unpaired cysteine residues, potential amino acid modification sites and other sequence liabilities.
- Humanized antibodies are designed consistent with the current World Health Organization (WHO) humanization standards (INN). Specifically, the V-gene of the humanized heavy and light chain must align most closely with a human V-gene sequence, and be >85% identical to a human V-gene sequence.
- Antibody genes are cloned and expressed in ATUM's expression vectors that have been optimized for cell line, isotype and signal sequences to obtain maximal yields.



ATUM's HuAbGPS design tool begins by grafting heavy and light chain CDRs into human germline frameworks. We then identify amino acid differences between the original (murine) framework and the new human framework. To find the murine framework amino acids essential for proper presentation of the CDRs within the human framework context, we create a DoE matrix where murine framework amino acids are systematically tested in different combinations. The result is a functional antibody containing the minimal number of murine framework amino acids whose heavy and light chain sequences are closer to human than to any other species in accordance with the INN standards.

cell line development

ATUM's Leap-In transposases and synthetic transposons enable a cost-effective, fast and robust cell line development workflow.

Leap-In transposases integrate single copies of the entire synthetic transposon into multiple transcriptionally active genomic loci. Expression constructs integrated by Leap-In transposases do not exhibit the rearrangements and concatemerization that are common, problematic hallmarks of random non-homologous recombination driven integrations. Instead, they maintain structural integrity of the sequence integrated into the host genome and ensure that regulatory elements remain associated with the appropriate open reading frames. Desired balances between multiple open reading frames are maintained.

The technology delivers high productivity pools and cell lines, resulting in consistent product quality. The Leap-In technology underpins ATUM's cell line development services and is also licensable for your in-house use. ATUM currently uses two host cell lines with lineage traceability and documented provenance and are adapted to commercially-available chemically defined serum-free formulations - HD-BIOP3 GS null CHOK1 cell line from Horizon Discovery and the DG44 (from Dr. Lawrence Chasin, Columbia University lab).

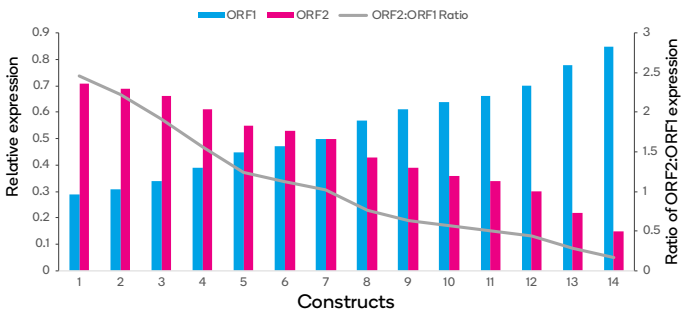
Rapid timelines

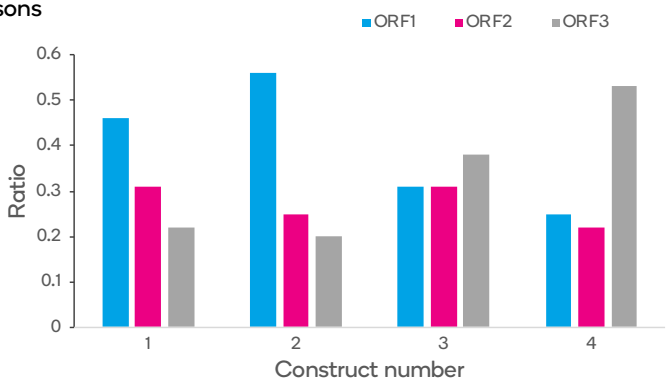
- Efficient and robust integration = Predictable selection
- From transfection to RCB in ~12 weeks
- Predictive high titer stable pools
- Leveraging >decade of ATUM proprietary vector elements and algorithms
- Highly uniform cell pools up to 5g/L and clones up to 14g/L
- Genetic stability - Transposase mechanism provides very high genetic stability
- No loss in productivity or transgene copy number after 60+ populatioh doublings
- Enabling for next generation biologics
- Compatible with very large inserts (eg., >100kb)
- Able to co-express multiple genes and tune ratios
- Multiple transposases enable unique genetic engineering strategies
- Approved for IND filing

Expression balancing and optimization with VectorGPS®

The balance of expression for each subunit in a multi-subunit protein affects productivity and product quality. Using our VectorGPS design platform, we can design, synthesize and transfect several synthetic transposons in parallel, each harboring up to 4 independently-controlled, codon-optimized ORFs with altered expression ratios.

Control of protein subunit expresion ratios in 2-,3-ORF Leap-In transposons

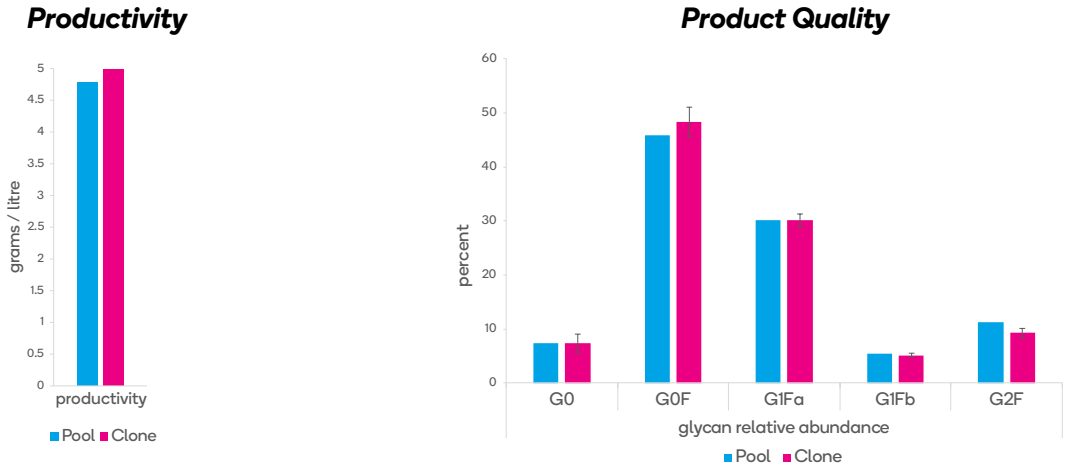




"The Leap-In transposases give us stable, highly productive cell lines in very short timeframes and we can rapidly insert DNA of unlimited size into a target genome. The availability of two independent transposases allows us to make sequential genome modifications. For example, genes to modify glycosylation pathways using one transposase, then integrate 4 new genes to produce a bispecific antibody with an improved glycosylation profile with a second transposase."

Dr. Ferenc Boldog, Director of Cell Line Development

High productivity stable pools



Product qaulity (glycan relative abundance %, charge %) and productivity is comparable between stable pool and derivative clones.

Cells that are transfected with Leap-In tranpsosons will integrate multiple copies of the transposon into their genomes. This results in rapid recovery of stable pools consisting of many similar cells. Productivity and product quality of clones derived from these pools are similar to the pools themselves. Process and analytical development can therefore start as early as a few weeks post-transfection. Material for toxicology studies can be prepared from stable pools to further shorten overall CMC timelines.

Leap-In Transposase technology is available for licensing.

Contact us for information on licensing options & cell line development services.

+1 650 853 8347 ext. 200
+1 877 DNA TOGO ext. 200
info@atum.bio

References

GenEng News 2019. Leap-In Transposases and Transposons Accelerate Cell Line Development. Balasubramanian et. al.

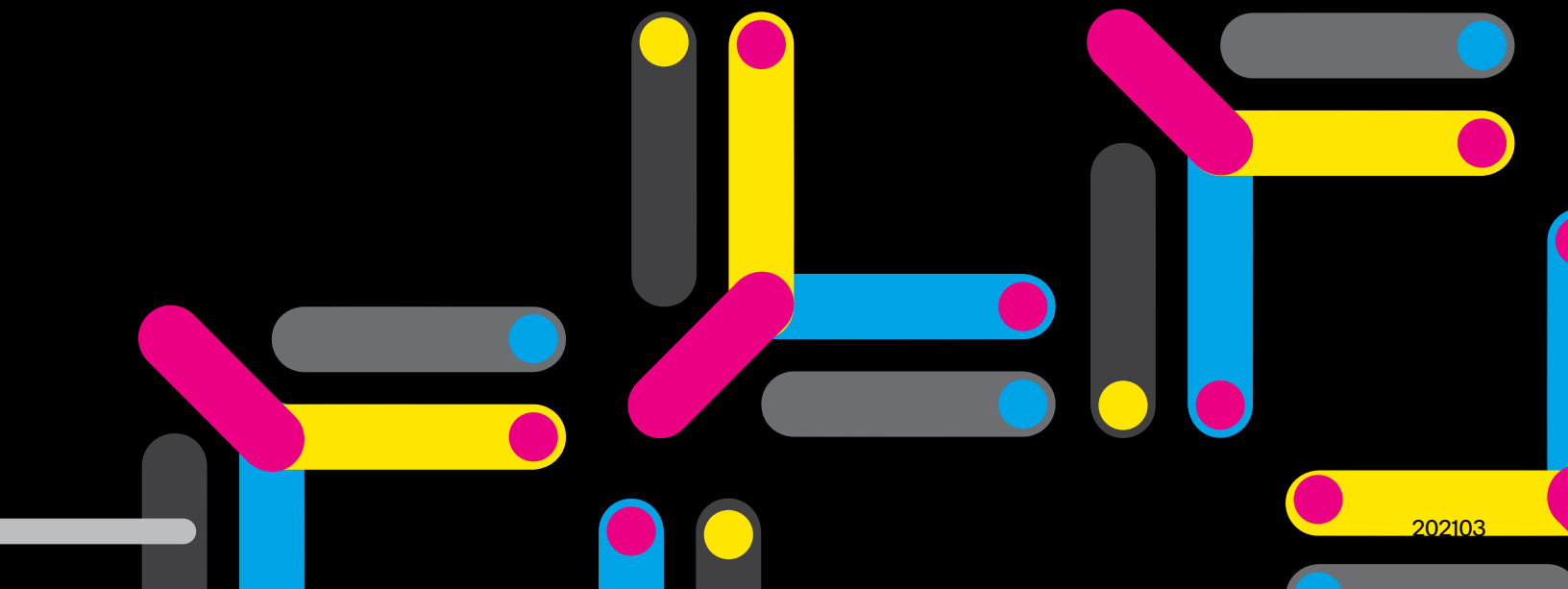
Biotech J 2018. Generation of high expressing Chinese Hamster Ovary cell pools using the Leap-In transposon system. Balasubramanian et. al.

Curr Prot in Prot Sc 2018. Optimization of Protein Expression in Mammalian Cells. Hunter et. al.

ATUM Patents

+1 877 DNA TOGO
+1 650 853 8347
info@atum.bio

research. create. break through.



202103