FUTURE FIELDS

Transgenic Drosophila: Turning Drug Discovery Dead-ends into Runways for Development

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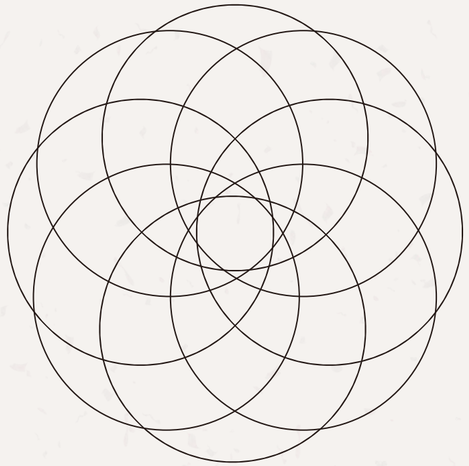
POWERED BY PEOPLE, FLY BY DESIGN

What project did you shelve because you couldn't access the protein you needed?

Protein production bottlenecks for drug discovery programs:

1. Scale-up cliff
2. Expression challenges
3. Poor quality
4. Unstable clones



Reality: traditional expression systems have trade-offs

	Cost	Volumes	Quality	Time
E. coli	✓	✓	✗	✓
Yeast	✓	✓	✗	✓
CHO (transient)	✓	✗	✗	✓
CHO (stable)	✗	✓	✓	✗

The EntoEngine™ checks all the boxes so that you don't have to make trade-offs

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CHO (transient)	✓	✗	✗	✓
CHO (stable)	✗	✓	✓	✗
EntoEngine™	✓	✓	✓	✓

The EntoEngine™ leverages whole fruit flies to make custom proteins.

Drosophila larvae

THE ENTOENGINE™

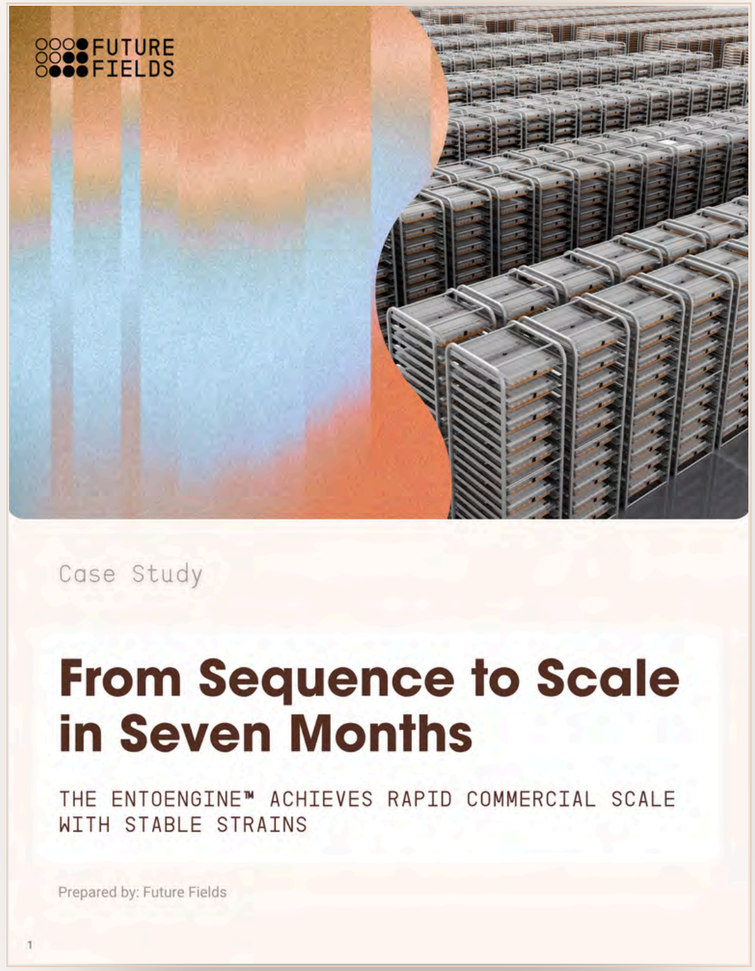
protein production at scale with transgenic *Drosophila melanogaster*

+ VECTOR SELECTION TO MANUFACTURING-READY IN **28 WEEKS**
+ INHERENT STABILITY GUARANTEES QUALITY AT SCALE
+ FLEXIBLE BATCH SIZES, SHORT LEAD TIMES
+ UNLOCKS CHALLENGING PROTEINS AT SCALE

1 Addressing the scale-up cliff

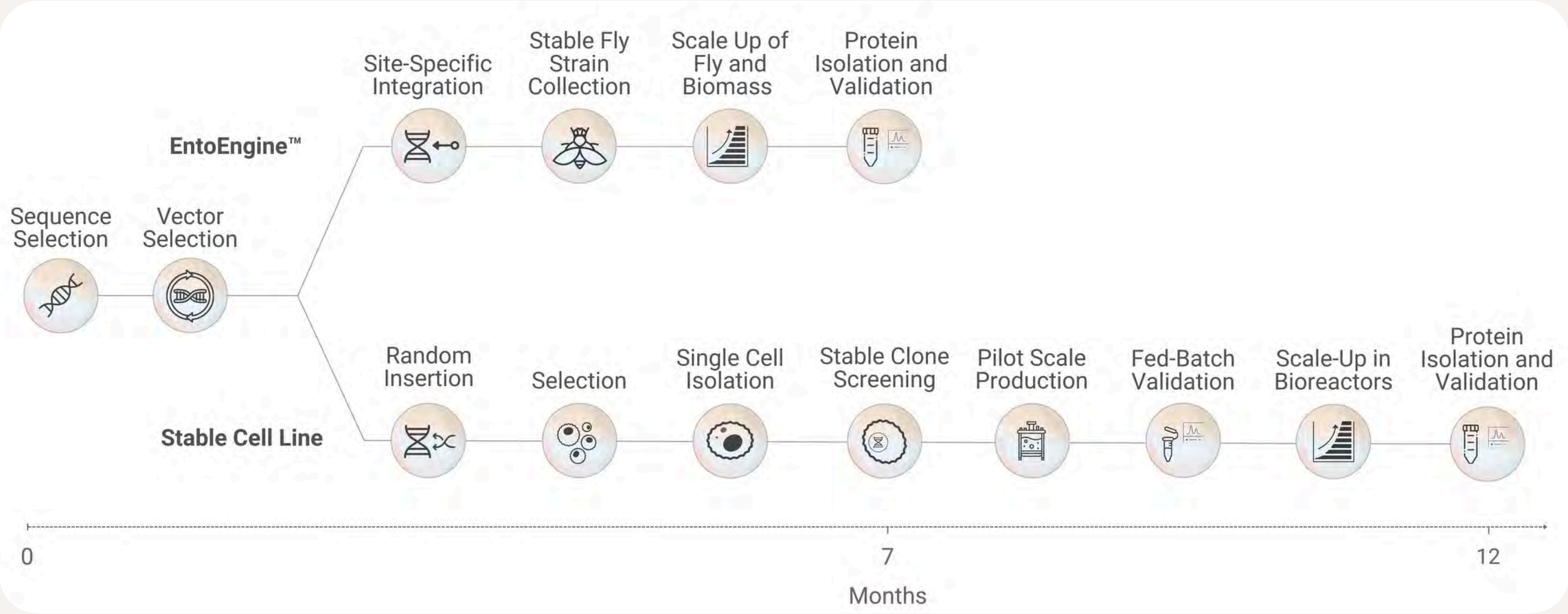
Problem:
Successful screens die in the 'Scale-Up Gap'

Solution: The EntoEngine moves your protein from HTP hits to manufacturing-ready without restarting the clock



PRODUCTION TIMELINES

7 vs 12+ months



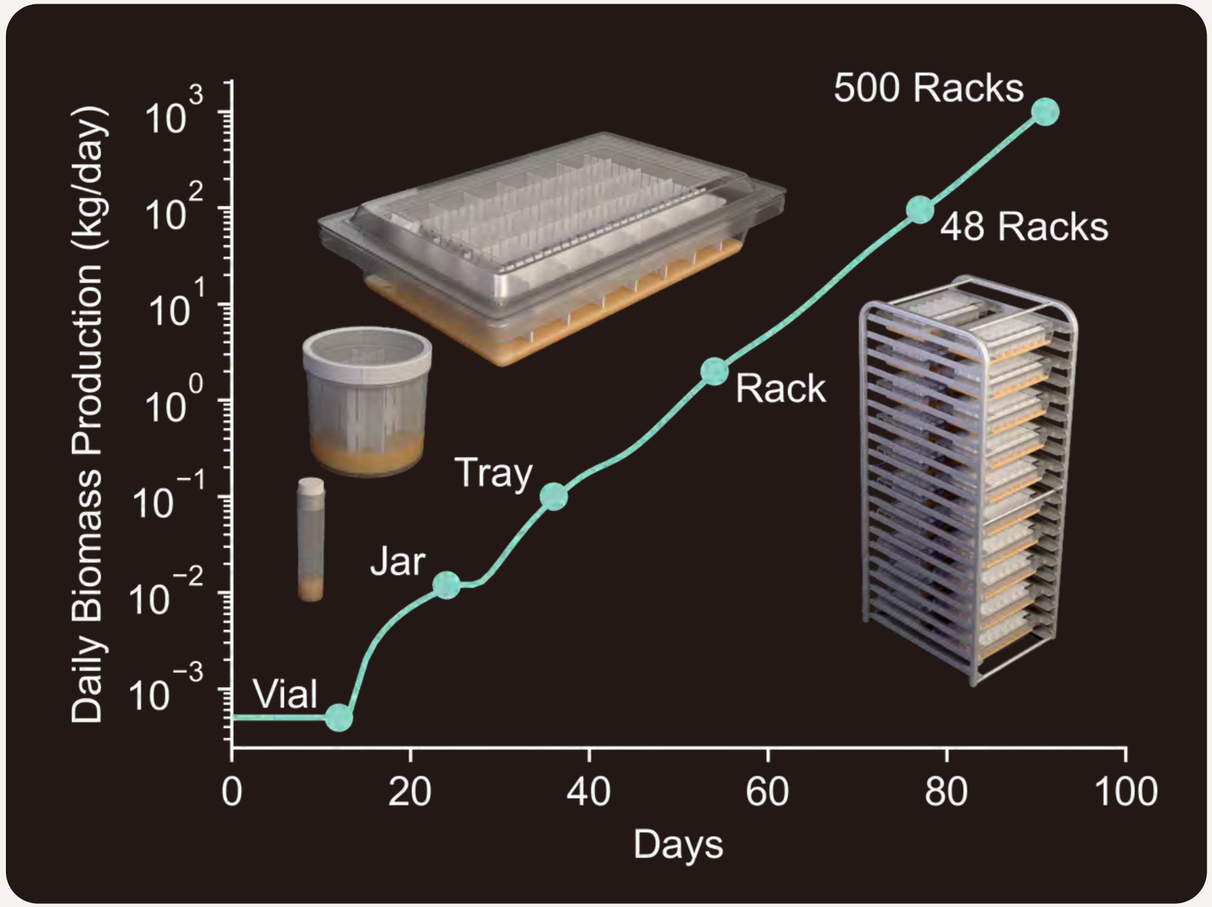
THE APPROACH

modular production

- **Removes scale-up cliff:** Once a stable strain is established, scaling up is simply increasing the number of rearing trays
- **No re-optimization required:** R&D larvae conditions are identical to that in commercial production
- **Eliminates translation loss:** Providing a level of predictive certainty that traditional bioreactors cannot match
- **Low infrastructure cost:** Scaling involves inexpensive infrastructure, leading to cost-savings for the client

THE APPROACH

modular scalability



- Rapid, predictable, and exponential growth of stable strains
- Low cost, low maintenance system
- Protein-agnostic

Figure 1: Continuous Transgenic *D. mel* Expansion for Recombinant Protein Manufacturing with the EntoEngine. Population expansion from vials to jars to trays to racks within one month for rapid and continuous production.

batch-to-batch consistency

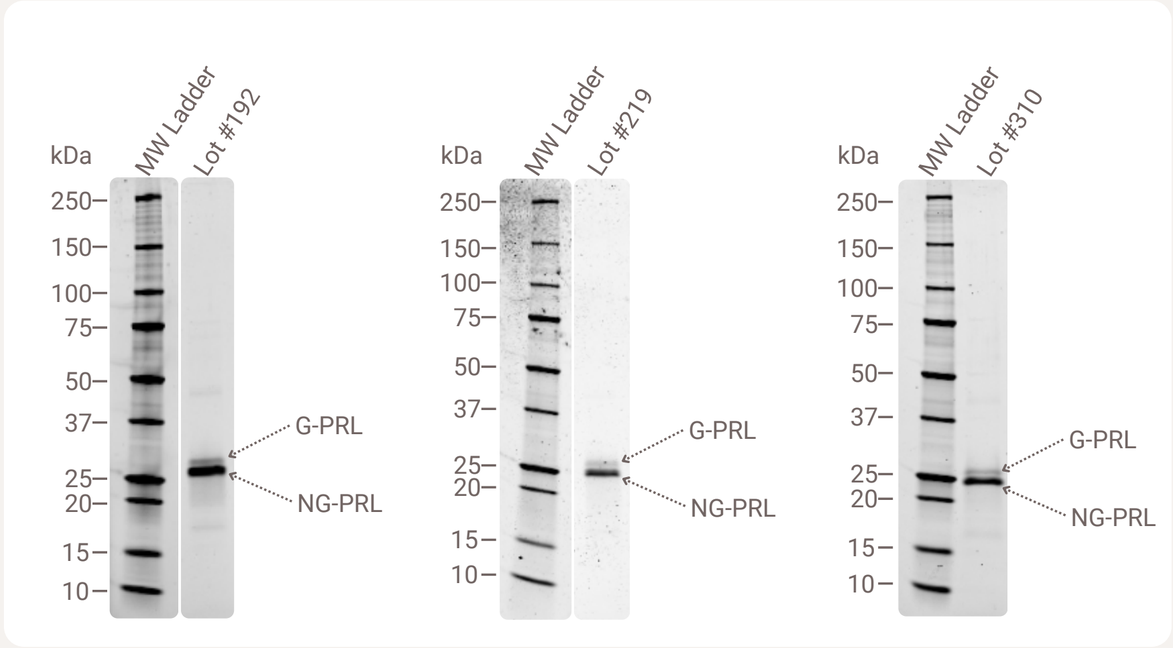
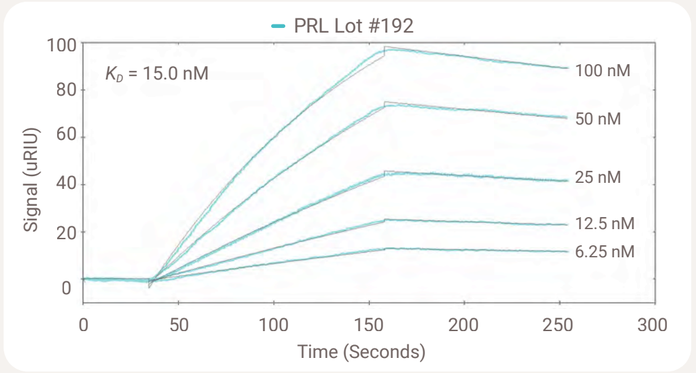
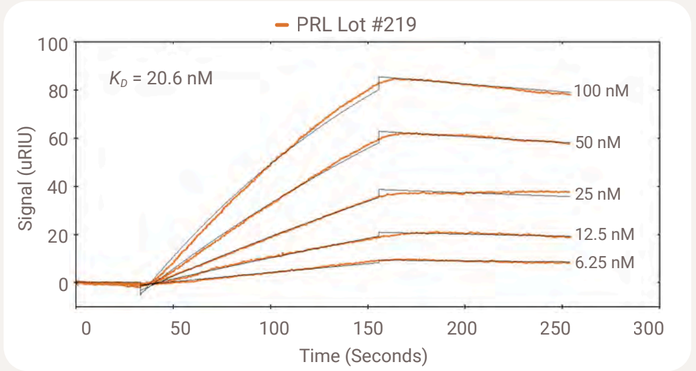


Figure 2: Consistent purity of recombinant Prolactin manufactured by the EntoEngine platform. SDS-PAGE analysis with SYPRO Ruby staining shows uniform band patterns for NG-PRL (~23 kDa) and G-PRL (~25 kDa). Densitometry confirms ≥ 90% purity.





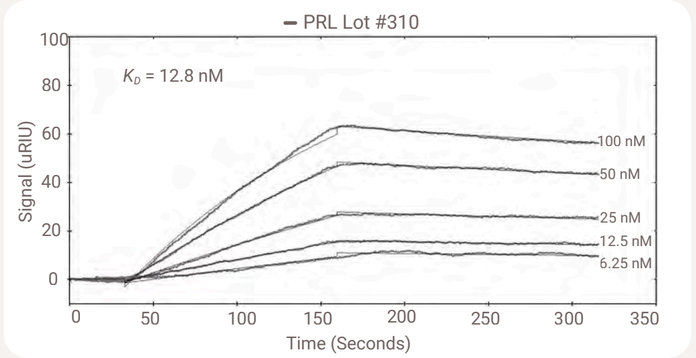
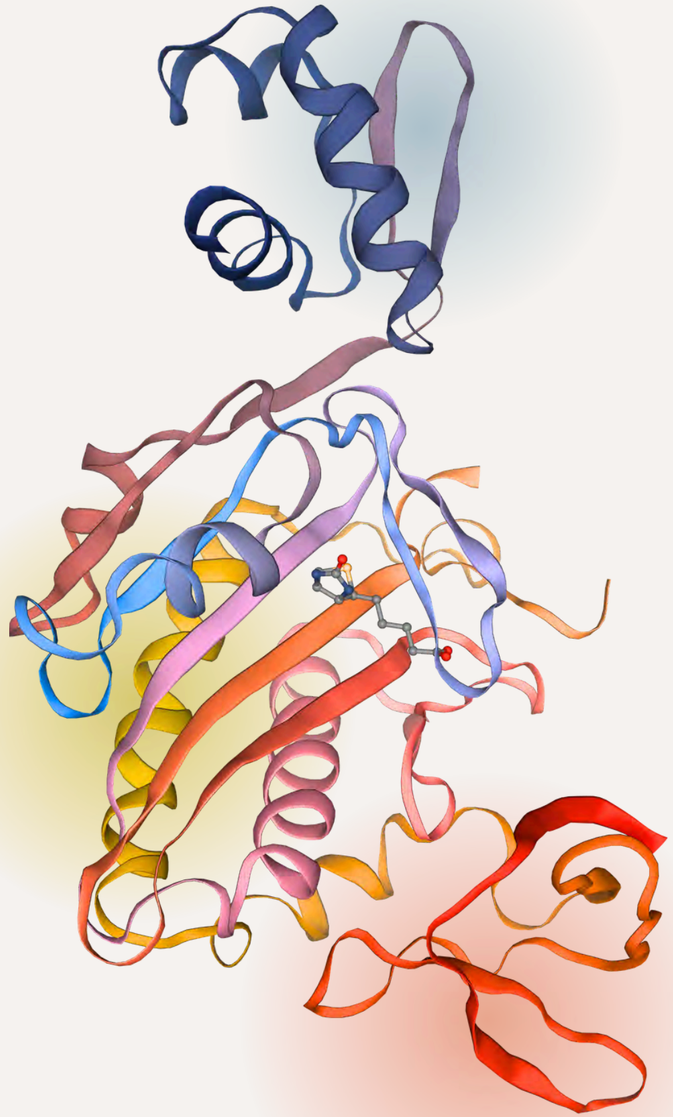


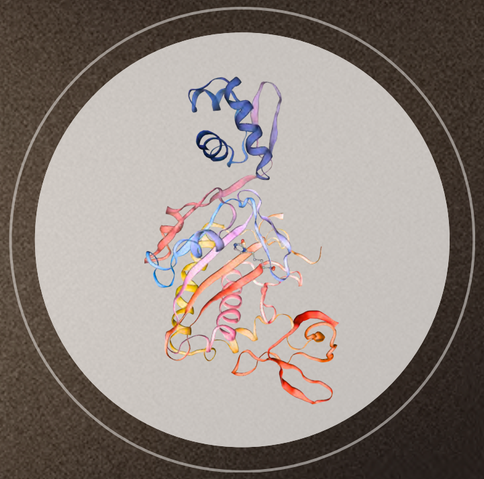
Figure 3: High functional affinity of the EntoEngine-produced PRL to its receptor.
The average binding affinity (K_D) of ~16 nM confirms high functional integrity of different PRL lots.

2 Addressing the expression wall

Problem:
Protein won't express well

Solution:
The EntoEngine is the high-complexity specialist that can

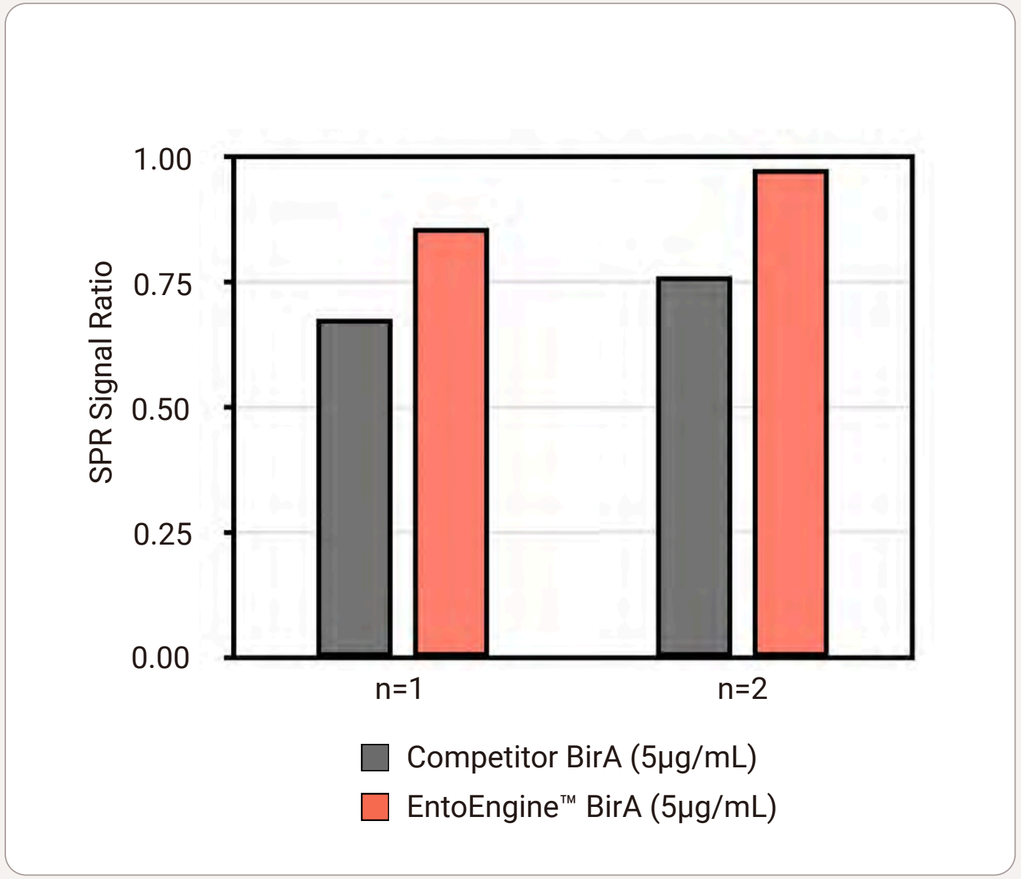


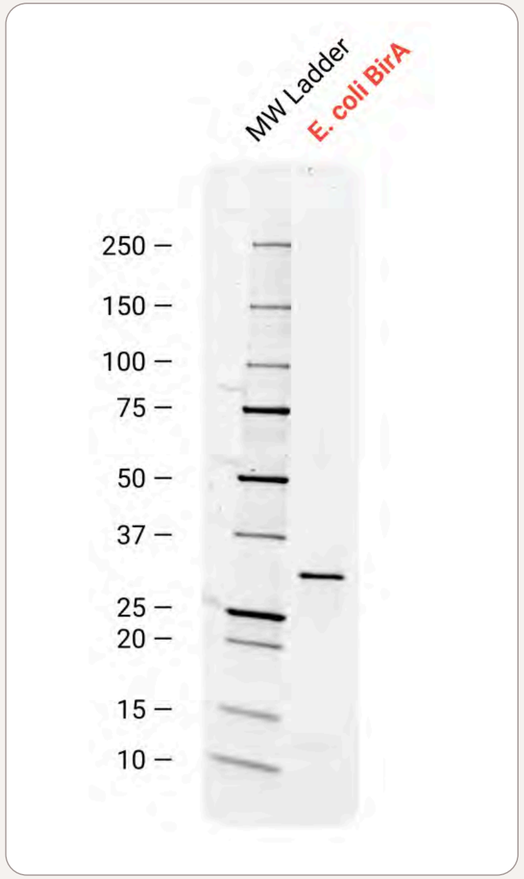


Case Study:

BirA

+ TOXIC WHEN OVEREXPRESSED
+ INSOLUBLE AGGREGATES
+ INCLUSION BODIES





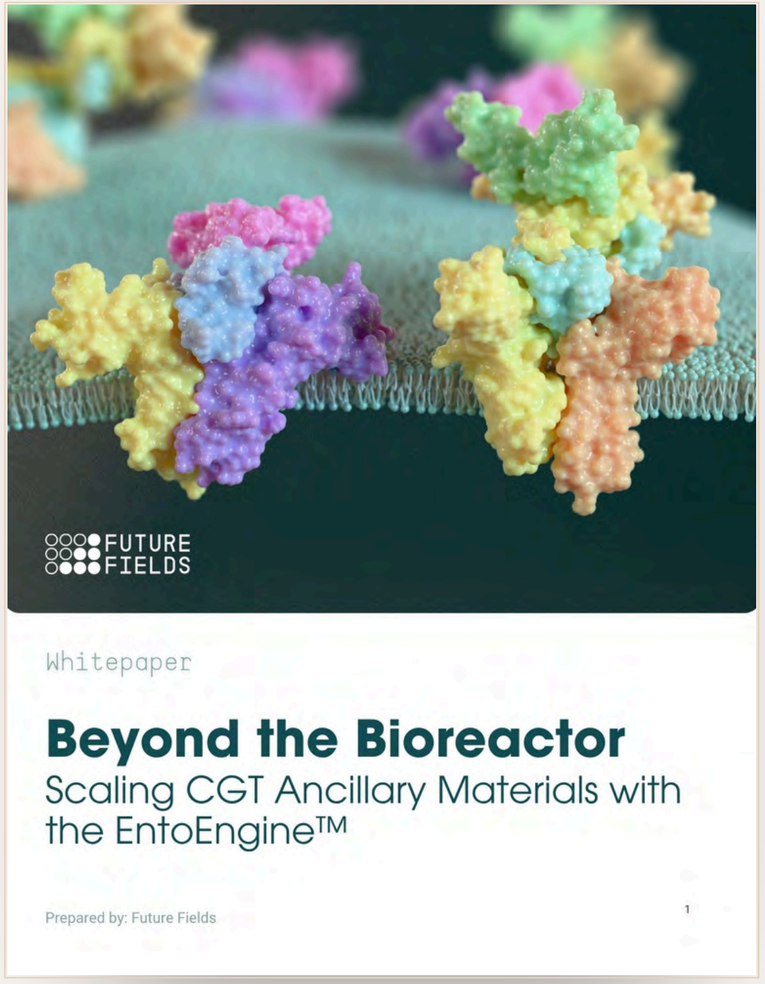
Activity: 500 Units/µg of BirA
Definition of Activity: One Unit (U) of BirA is the amount of enzyme that will biotinylate 1 pmol of Avi Tag substrate in solution at 2.3 µM within 30 minutes at room temperature.

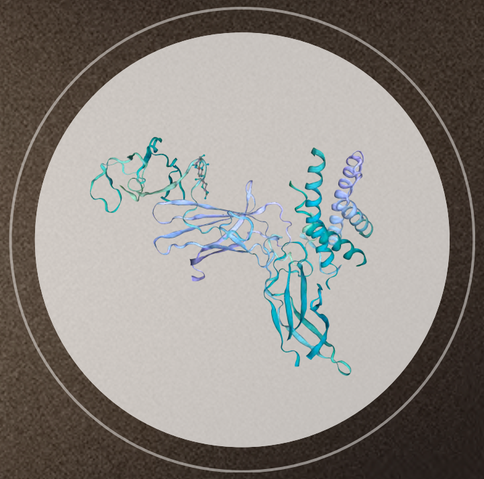
Purity: 91%
BirA purified to > 90% from the EntoEngine™.

3 Addressing quality challenges

Problem:
The protein expresses, but quality is poor

Solution:
The EntoEngine has the complexity of a multicellular organism, which can be leveraged to produce highly functional, high quality active proteins

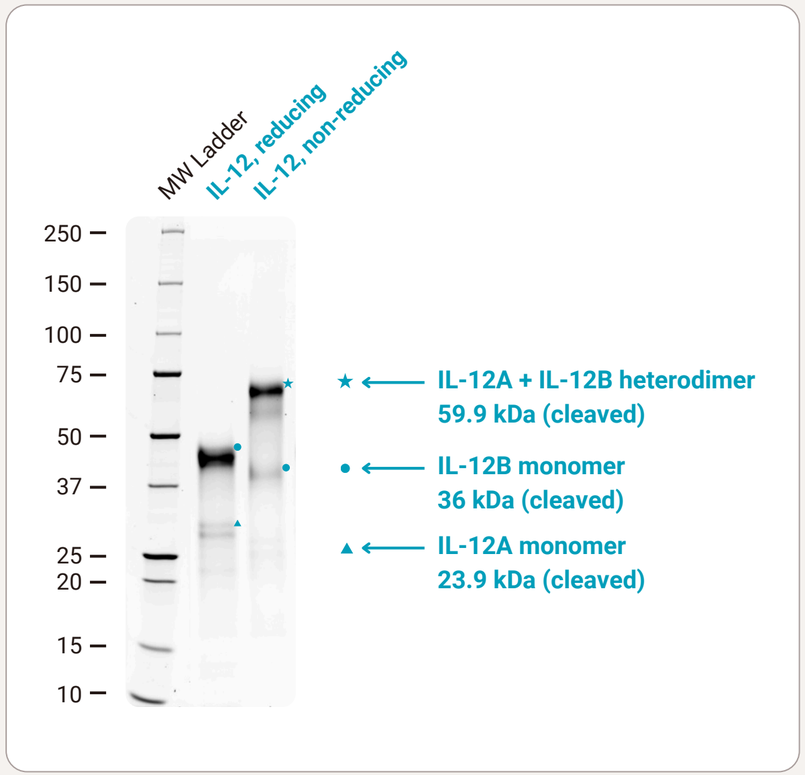


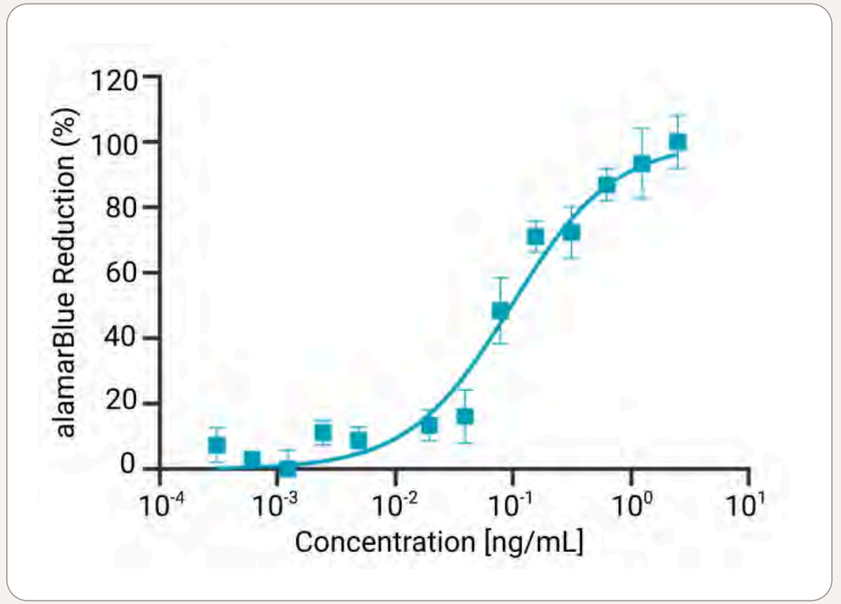


Case Study:

IL-12

+ HETERODIMER
+ DISULFIDE BOND FORMATION
+ STRICT STOICHIOMETRIC BALANCE NEEDED FOR SUBUNIT EXPRESSION





Heterodimer:
IL-12A + IL-12B heterodimer at 59.9 kDa produced in the EntoEngine™.

Activity:
IL-12 heterodimer bioactivity assessed in PHA-stimulated human T-lymphoblast proliferation.

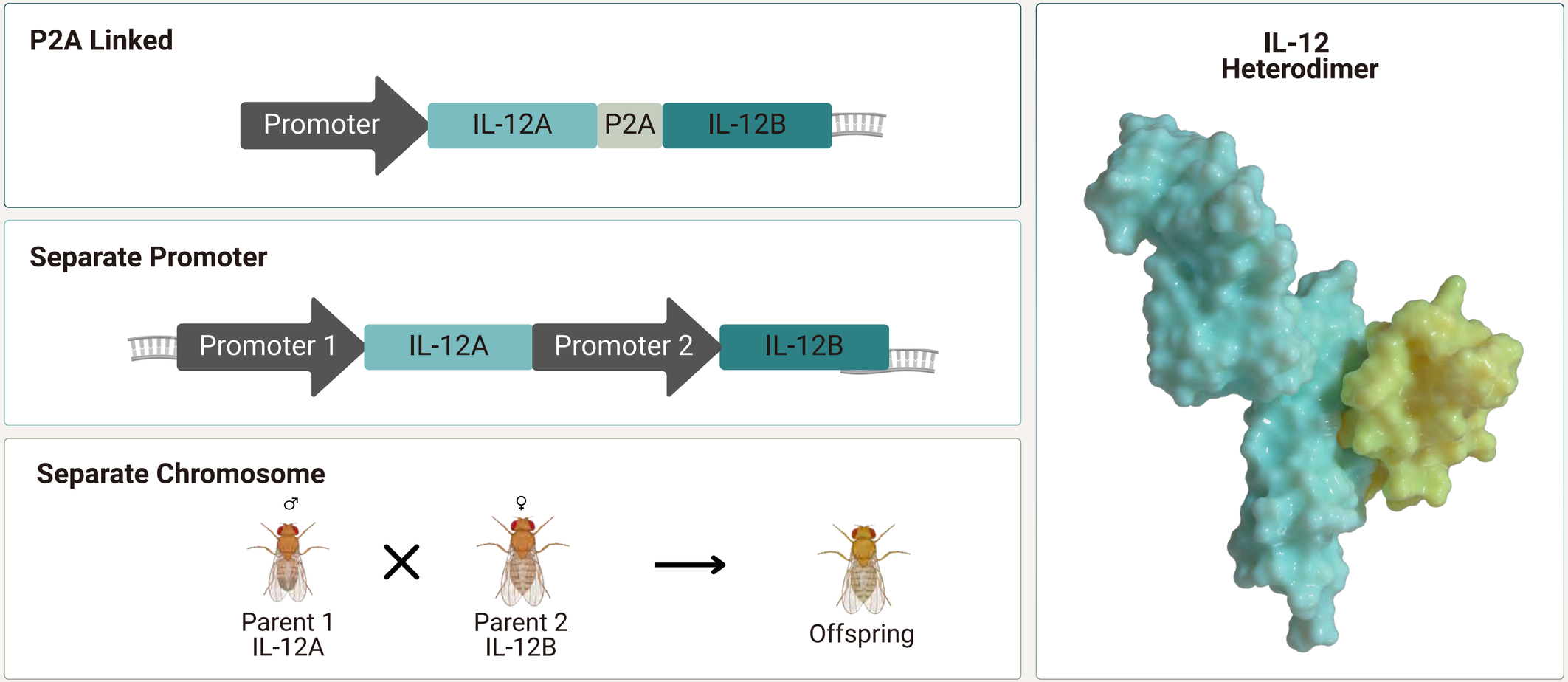


Illustration of three different molecular configurations for IL-12 heterodimer production: P2A linked IL-12A and IL-12B subunit under a single promoter, subunits under separate promoters and introduction of subunits into two separate chromosomes crossed to form IL-12 heterodimer.

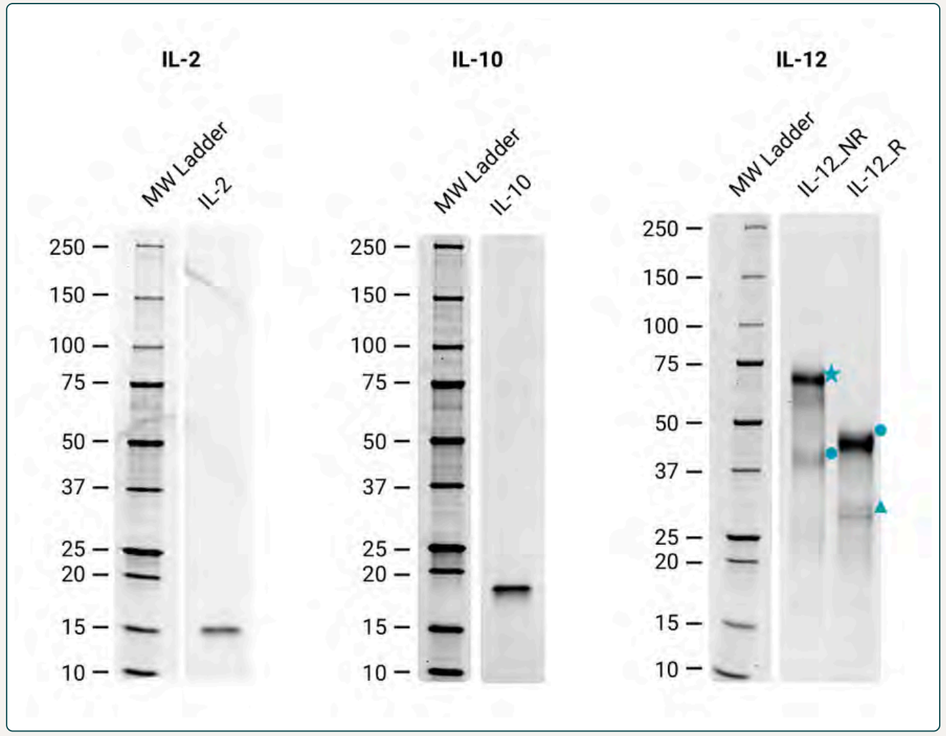


Figure 1: SDS-PAGE analysis of purified interleukins expressed in the EntoEngine platform. IL-2 at ~15 kDa, IL-10 at ~18 kDa, cleaved IL-12 non-reduced (NR) at ~60 kDa (star) and IL-12 reduced (R) at ~24 kDa (IL-12A, triangle) and ~36 kDa (IL-12B, circle).

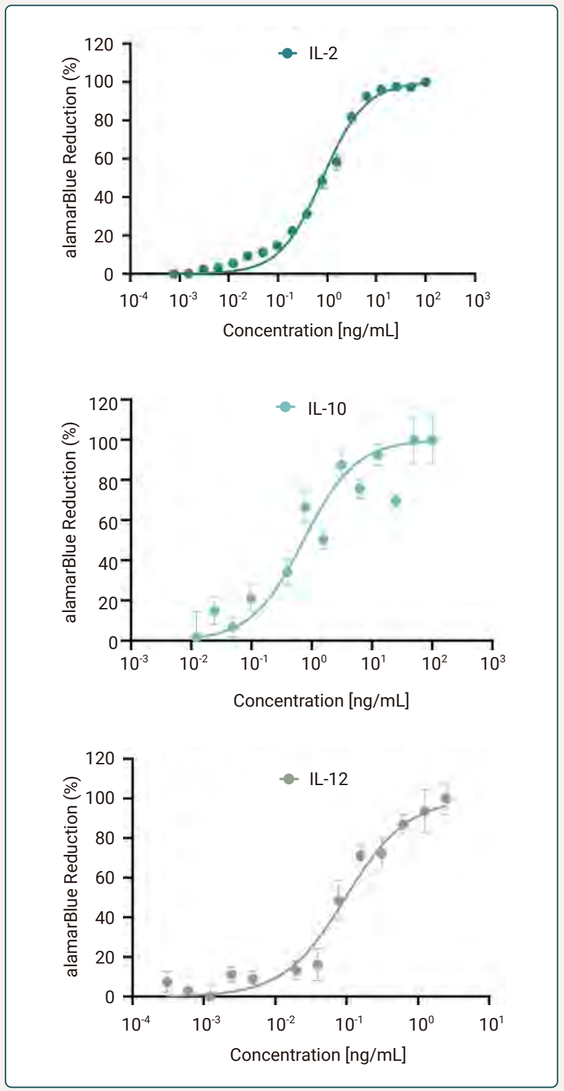


Figure 2: alamarBlue Proliferation Assay to determine the activity of EntoEngine-made interleukins. IL-2: EC_{50} 0.80 ng/mL (specific activity >1x10^6 units/mg) in CTLL-2 mouse cytotoxic T cells. IL-10: EC_{50} 0.77 ng/mL (specific activity >1x10^6 units/mg) in MC/9 mouse lymphoblast cells. IL-12: EC_{50} 0.09 ng/mL (specific activity >1x10^7 units/mg) in PHA stimulated PBMCs.

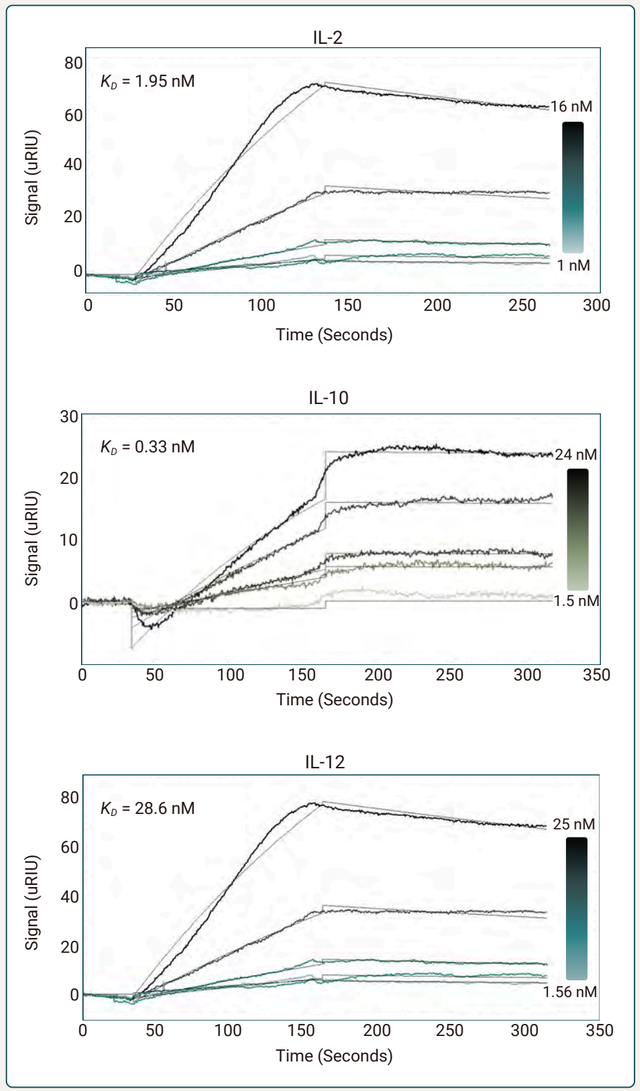


Figure 3: SPR binding affinity analysis of the EntoEngine-made interleukins to their respective receptors. K_D values of IL-2, IL-10 and IL-12 were 1.95 nM, 0.33 nM and 28.6 nM respectively.

	Endotoxin (EU/µg) USP〈85〉	Mycoplasma USP〈63〉
IL-2	< 0.3 EU/µg	Negative
IL-10	< 0.8 EU/µg	Negative

Table 1: Representative endotoxin quantification and mycoplasma detection outcomes for IL-2 and IL-10 purified from the EntoEngine platform.

IL-2	**Lot 1**	**Lot 2**
EC_{50}/ Specific Activity	1.69 ng/mL >5 x 10^5 units/mg	0.80 ng/mL >1 x 10^6 units/mg
SPR Binding Kinetics	K_D = 1.95 nM	K_D = 1.89 nM
Endotoxin PyroSmart LAL	<1 EU/µg	<1 EU/µg
Purity SDS-PAGE	>98%	>98%

Table 2: Consistent activity and safety profiles of two different IL-2 lots expressed and purified from the EntoEngine platform.

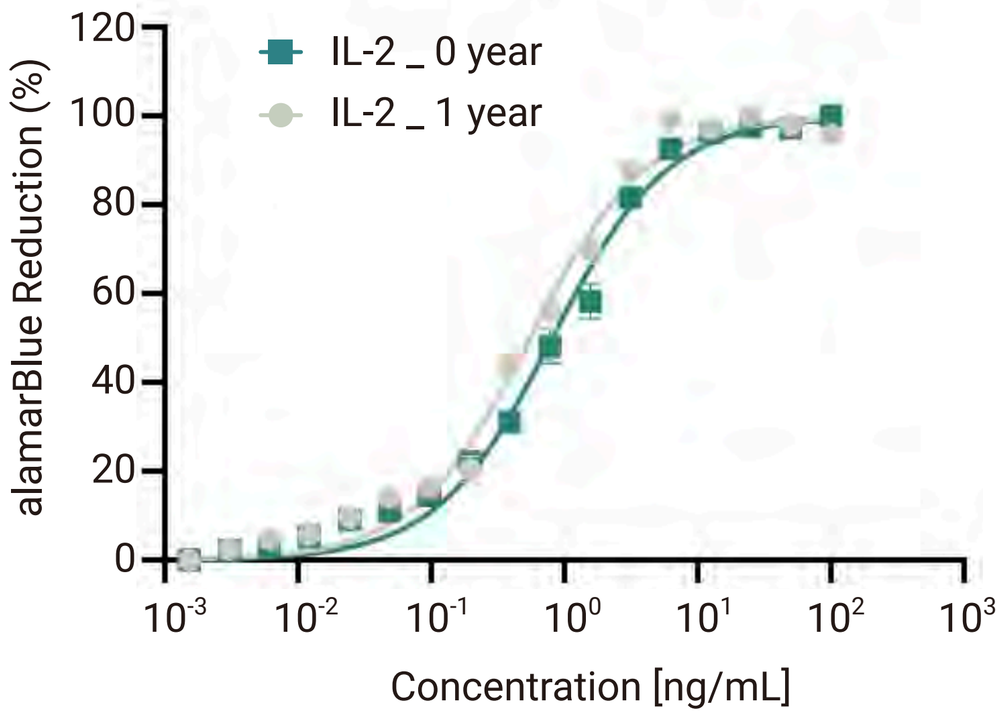


Figure 4: Stability of the EntoEngine-made interleukin assessed by alamarBlue proliferation assay with long-term stored IL-2. A new lot of IL-2 activity compared to one year -20°C stored sample.

4 Addressing stablility

Problem:
Transient systems work, but you can't make a stable line

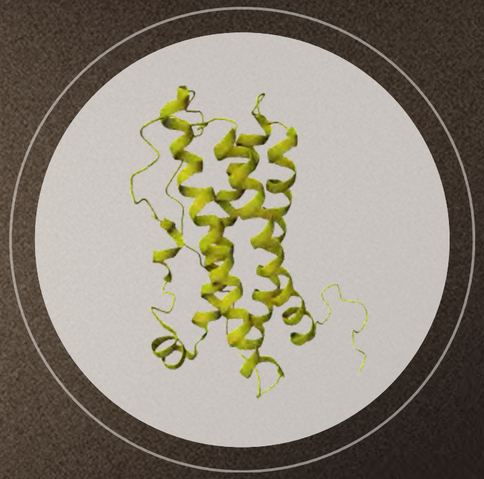
Solution:
The EntoEngine predictably and reliably produces stable strains, which are used from benchtop to scale

THE APPROACH

stability through structural genetics

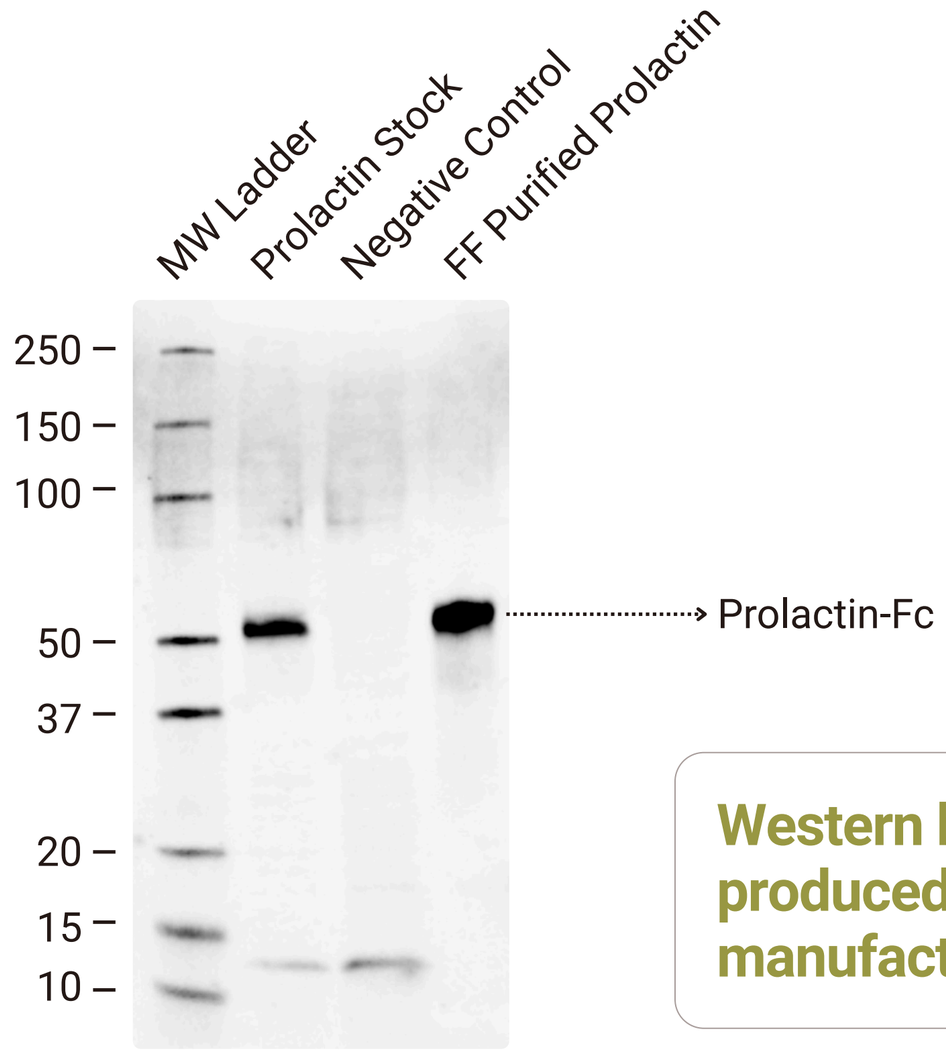
Genetic recombination adds **diversity** to the gene pool.

The risk shifts from **stochastic to deterministic**, which essentially **guarantees** the generation of a **stable production strain** with **predictable timelines**.



THE DATA

prolactin stability



Western blot of Prolactin (PRL) produced from a 3-year-old manufacturing strain

The EntoEngine™ checks all the boxes so that you don't have to make trade-offs.

	Cost	Volumes	Quality	Time
E. coli	✓	✓	✗	✓
Yeast	✓	✓	✗	✓
CHO (transient)	✓	✗	✗	✓
CHO (stable)	✗	✓	✓	✗
EntoEngine™	✓	✓	✓	✓

Stop making trade offs with your protein production workflows.

Don't let your program die in discovery. Innovation is only a breakthrough if it reaches the patient.

Transgenic Drosophila can help you get there.

FUTURE FIELDS

questions?

We're here to help.
Submit an inquiry here.