Optimized Transient Production: Faster Preclinical Studies with Enhanced Quality Control

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ABSTRACT

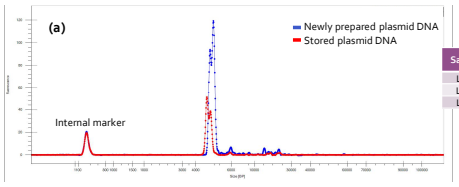
Optimizing transient protein production is vital for accelerating biotherapeutic development. High-quality plasmid DNA enhances transfection efficiency, while optimized expression systems boost titers and maintain product integrity. Additionally, developability assessments evaluate critical biophysical properties to identify high-risk candidates early. These strategies compress preclinical timelines, enabling rapid access to proteins for early decision-making and streamlining the transition from discovery to clinical translation, without compromising product quality or consistency.

Gene Synthesis & Molecular Construction | Plasmid DNA Scale up & Quality Control | Transient Production | Purification & Characterization QC | Developability Assessment

Figure 1. Curia's antibody transient production workflow begins with in-house gene synthesis, followed by seamless molecular cloning to obtain endotoxin-free, transfection-grade DNA. Sequencing QC is performed at every step, and capillary electrophoresis of plasmid DNA can be used to assess supercoiled content. The process then transitions to Curia's proprietary TunaCHO^{SM} platform for transient transfection in a streamlined workflow optimized for consistent productivity and scalability. After purification and quality control using capillary electrophoresis (CE-SDS), size exclusion (SE-UPLC) and intact mass measurement, developability assessments can be integrated early to help identify and address potential issues in later stages of development. These evaluations include assessing the biophysical and chemical properties of the drug candidate.

Supercoil Content QC of Plasmid DNA for Transfection

- Increased stability: Circular supercoiled DNA is less vulnerable to degradation by exonucleases, improving its survival in the cellular environment.
- Enhanced cellular uptake: Supercoiled DNA forms more compact structures, allowing it to enter cells more effectively than linear or relaxed circular DNA.
- Better complexation with transfection reagents: Supercoiled DNA forms more compact complexes, potentially enhancing endocytosis.



(b)

Sample	Supercoil %	Yield (mg)	VCD (e6 cells/mL)	Viability (%)
Lot 1	88.3	7.83	2.29	31
Lot 2	81.7	6.68	3.31	37
Lot 3	85.0	7.22	2.09	30

Figure 2. Supercoil content quality control for plasmid for transfection. (a) The LabChip™ (Revvity) Plasmid DNA assay separates the three primary isoforms of plasmid DNA and provides supercoil content based on corrected area percentages for each conformation. (a) Overlay electropherograms of newly prepared plasmid DNA for transfection and plasmid DNA stored for an extended period. Newly prepared plasmid DNA contains high supercoiled content, while stored plasmid DNA may form dimers or aggregates. **(b)** Small-scale transient transfection for the sample antibody was performed using different lots of plasmid DNA. The supercoiled content of the plasmid DNA correlated with the purified yield from transient production but did not impact cell culture growth or viability.

TunaCHO^{SM} High Yield Transient Production (2 mL – 300 L)

- Start in TunaCHO^{SM} platform and progress into the same parental line of CHO-GSN^{SM} platform suitable for GMP development.
- Available from 96X2 mL, 96X10 mL, to 300 L. Supports consistent scalability to streamline manufacturing in clinic.
- Quick turnaround time 3 to 6 weeks
  - Gene synthesis to purified antibodies
  - Complementary Mass Spec QC and developability option available

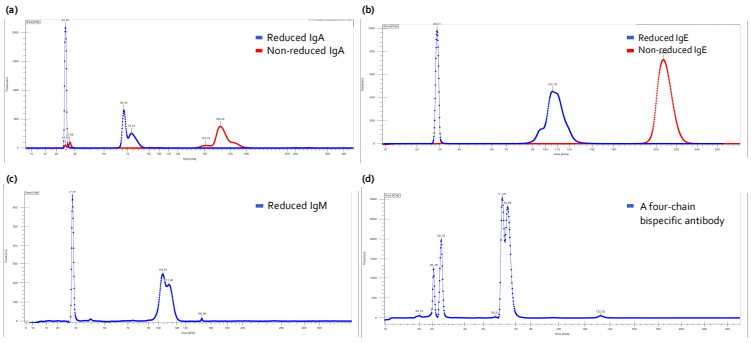


Figure 3. Small scale pilot productions on various biologics molecules, CE-SDS of (a) IgA, (b) IgE, (c) IgM, (d) a four-chain bispecific antibody.

Integrated Developability Assessment

Curia offers comprehensive developability assessments for biologics candidates, complementing our protein production services. Beyond simply generating material, we delve deeper into the critical biochemical and biophysical properties of drug candidates providing valuable insights into its potential for successful development.

- Developability Package I
  - In silico Sequence Liability analysis and Immunogenicity analysis
- Developability Package II
  - Polyspecificity assessment
  - Integrity and Stability assessment
    - Aggregation/Purity/Charge Variant/Thermostability/Post-translational modifications
- Extended Developability Package II
  - Preliminary Formulation study
  - Accelerated stability study

(a)

Formulation	Tm1 (°C)	Tm2 (°C)	Tagg 266 (°C)	Tagg 473 (°C)	Pk1 Diameter(nm)	PDI
Formulation_1	72.7	82.1	83.6	83.8	13.20	1.461
Formulation_2	72.6	84.2	79.1	79.4	12.21	1.604
Formulation_3	66.3	75.3	77.3	77.9	10.42	1.385
Formulation_4	70.5	81.6	N/A	N/A	10.41	0.934
Formulation_5	64.7	75.1	77.7	78.1	12.21	0.610
Formulation_6	72.6	83.8	77.9	78.7	13.21	1.645

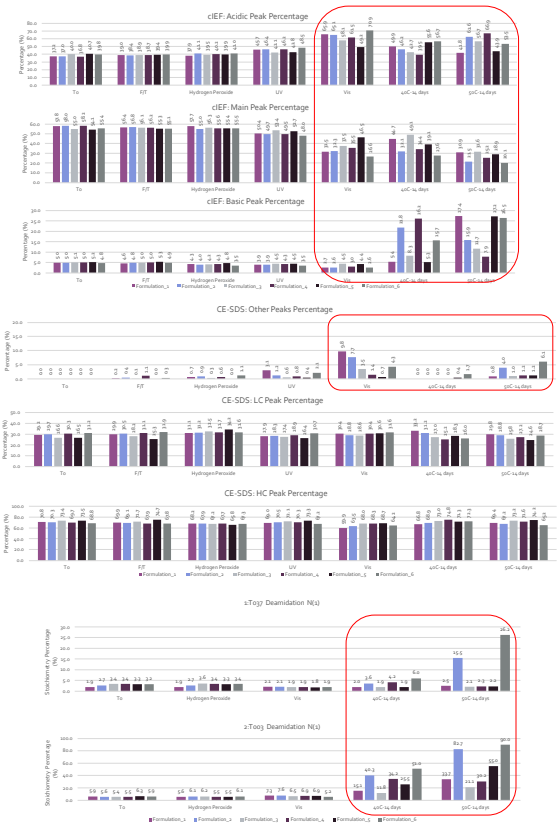


Figure 4. Application Example: Extended Developability Package II. Short-term accelerated stability studies enable rapid assessment of antibody developability by evaluating critical parameters. This example utilized a combination of assay techniques of **(a)** DSF/DLS, **(b)** cIEF, **(c)** CE-SDS, SE-UPLC and **(d)** mass spec., to profile molecule and identify potential formulations.

Large Scale Transient Production To Support Non-GLP Efficacy And Toxicology Study

Transient production systems yield antibodies of sufficient quality and consistency to support toxicology studies. Optimized platforms produce antibodies with comparable critical quality attributes to those from stable cell lines. The resulting antibodies exhibit comparable purity profiles and biological activity, making them suitable for early-stage toxicology studies and accelerating preclinical development without compromising data quality.

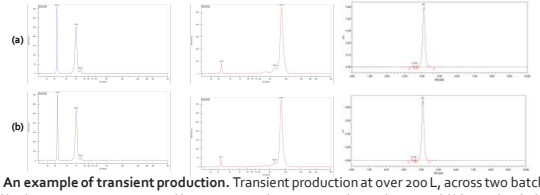


Figure 5. An example of transient production. Transient production at over 200 L, across two batches (a) and (b), exhibited high consistency as assessed by CE-SDS and SE-UPLC. The authors would like to thank the Gates Foundation for providing the data in this section, which was supported by grant (INV-004259).

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