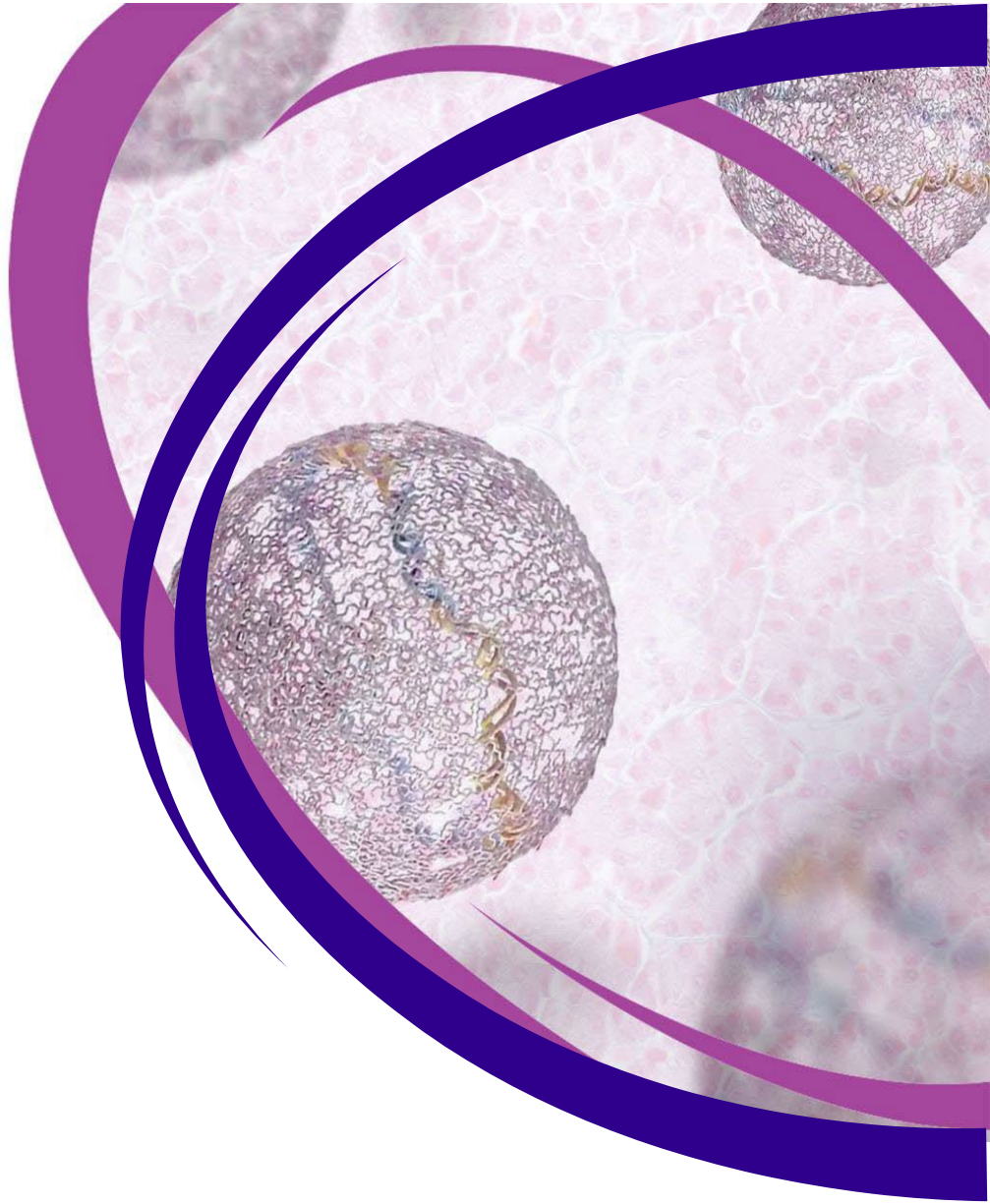
Bridging Small & Medium-Scale DNA Production:

Practical Strategies for Scalable Manufacturing with High Quality



DNA Process Development & Manufacturing Summit
Boston, MA
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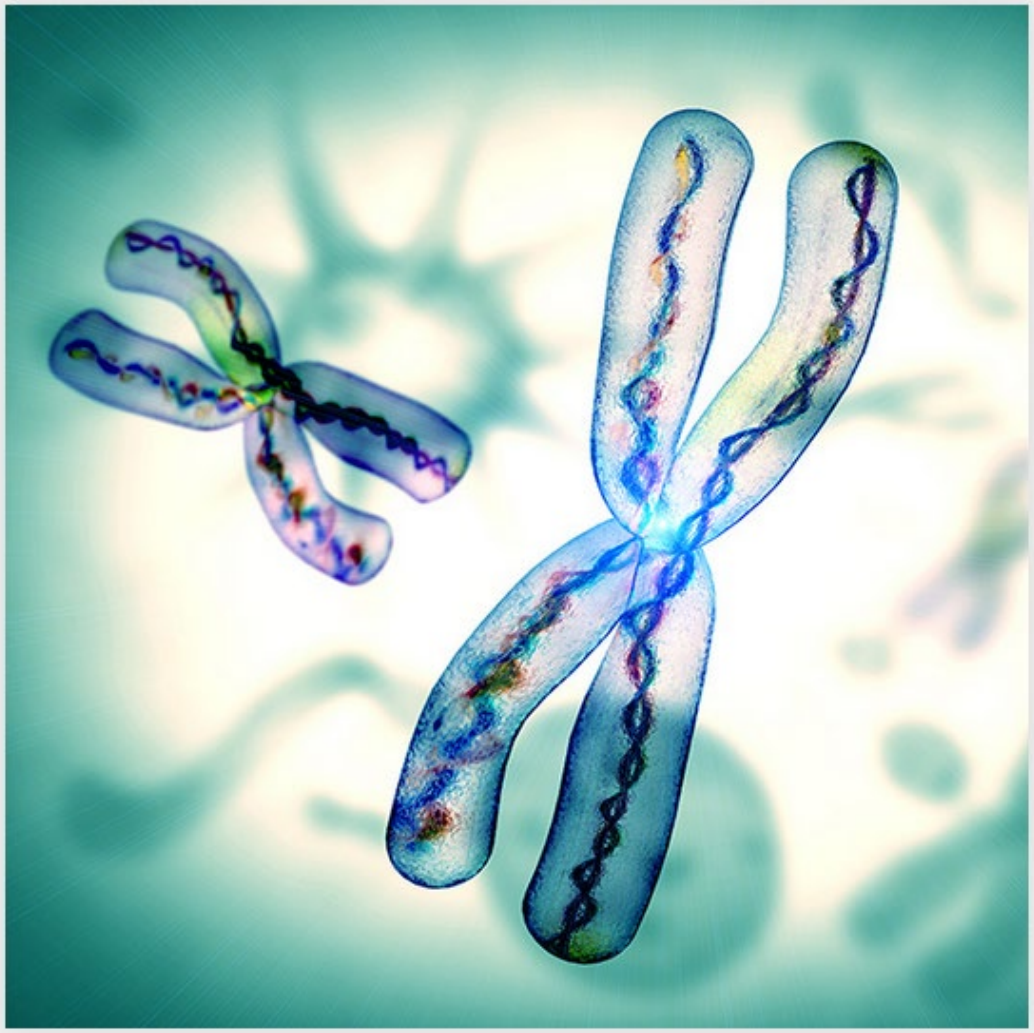
Topics

- Plasmid Manufacturing scale-up case study: strategy and lessons learned from a well-controlled process resulting in high quality and competitive yield
- Value of in-process testing within development plan to build a more robust, scalable process
- Practical considerations to enable rapid development of a well-controlled process with high quality within budget



Imunon's Manufacturing Story for High Quality Plasmid DNA-based Therapy

- To support the company's pDNA vaccine program, a small internal team produced >100 pDNA constructs at gigaprep scale
- To enable multiple in vivo studies from single pDNA batch, fed-batch fermentation and small-scale, highly inefficient DSP was established
- To enable safety/tox studies and later Phase 1 trial, larger scale, efficient DSP was successfully developed (multi-gram batches)
- Today, we are producing batches of commercial-quality pDNA sufficient to supply Phase 3 gene therapy trial

Lead Candidate – phIL-12 Non-viral Gene Therapy

Status: Enrolling Phase 3 *(OVATION 3; randomized, 500-patient trial)*

IMNN-001

IMNN-001 offers a novel way to harness the powerful immunological properties of IL-12: the "Master Switch" to the body's immune system.

- ✓ Six completed ovarian cancer trials demonstrate **biologic and clinical activity**
- ✓ Safety and activity signals in Phase 1; Mechanism of action confirmed; Clinical proof of concept achieved in OVATION 2
- ✓ **OVATION 2 offers new hope for ovarian cancer patients.** Topline data are promising, with data suggesting overall survival benefits of 11 months over standard of care
- ✓ Now enrolling patients in Phase 3 trial studying 1st line indication for newly diagnosed ovarian cancer patients

Motivation for Internal Development of Manufacturing Capabilities

- After several years of reliance on external CDMO partners for clinical API supply, where prices were consistently increasing, we projected that the COGS would prove to be unaffordable if we did not take action
- Manufacturing capability was established internally to produce API for IMNN-001
- This internally manufactured API (plus internally-manufactured synthetic delivery system) is then formulated into final sterile Drug Product at external CDMO
- Today, Imunon has corporate headquarters in Lawrenceville, NJ and GMP manufacturing/testing facility in Huntsville, AL

pDNA Manufacturing Process Requirements

- **In evaluating potential of internal manufacturing capabilities to meet the challenge, the process must be:**
  - ✓ Efficient, balancing cost and productivity
  - ✓ Easily scaled to sufficient batch sizes, maintaining high quality and control
  - ✓ Ideally fully enclosed, utilize SUAs, and generally reduce environmental risks
  - ✓ Sufficiently robust to allow reliable supply chain for Drug Product

pDNA Manufacturing Process Summary

Key Aspect	Description
Nucleic Acid	pDNA
End Use	API
Biomass Production	Fed-Batch Microbial Fermentation
Strain	*E. coli* Stbl2 (Small Scale-vaccine) *E. coli* DH10B (Medium Scale-gene therapy)
DSP Method	Alkaline Lysis

Primary Considerations

At the outset, what were the key steps and associated questions?

1) How can the lysis system be scaled?

- What single-use assembly configurations are available?
- If none are available, could similar assemblies be modified to specifications?

2) What depth filter train surface area is required?

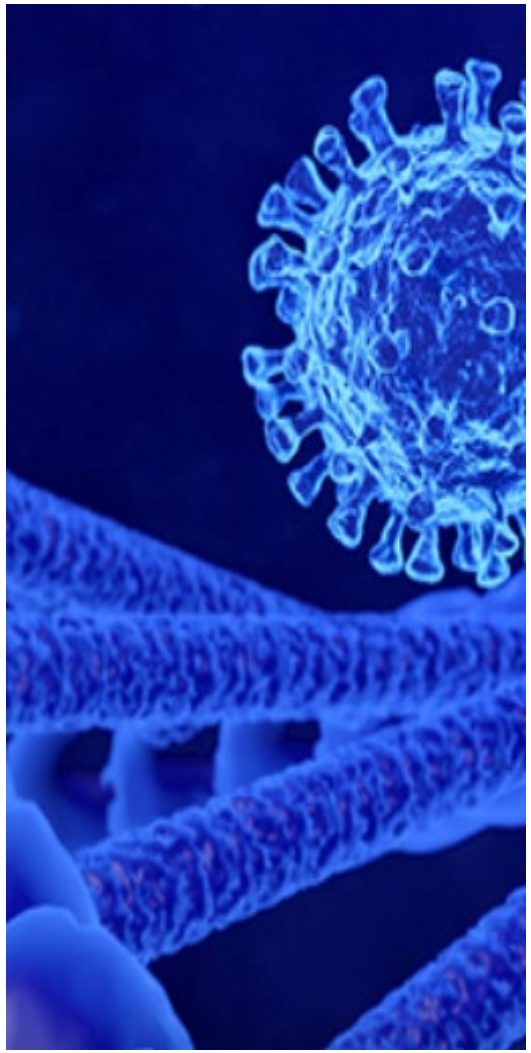
- Can we change the format to decrease process time?
- Could this be scaled down to test different options?

3) What hollow fiber filter surface area is required?

- Are multiple parallel filters required and what should the flow path look like?
- How should our TFF system be configured?

4) What chromatography resin volume is required?

- Can we change the format used from self-packed to pre-packed?
- Can our chromatography system handle the larger column size?



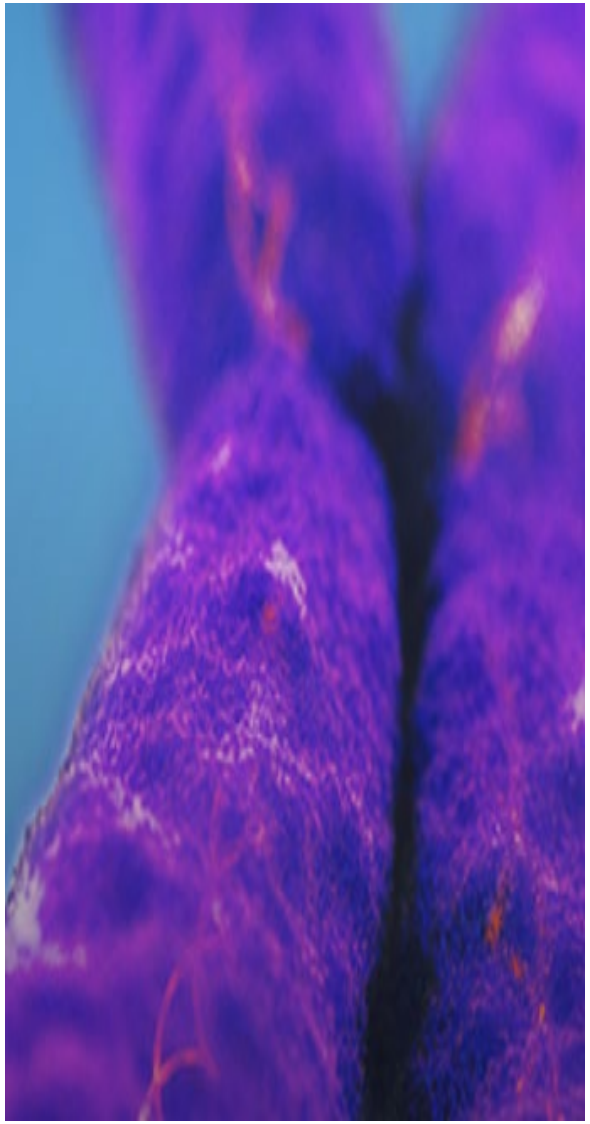
USP/Fermentation Scale-Up

10L to 100L

- **Staying with same fermenter manufacturer reduces reactor design & control variability**

- **Remaining development work is simpler**
  - Determine actual max fermentation volume
  - Feed pump calibration & feed rate scaling
  - Sterile feed medium production, including SUA configuration

- **Once configuration is determined, consider custom pre-packaged media from trusted suppliers**
  - Switch to non animal-derived media as soon as possible

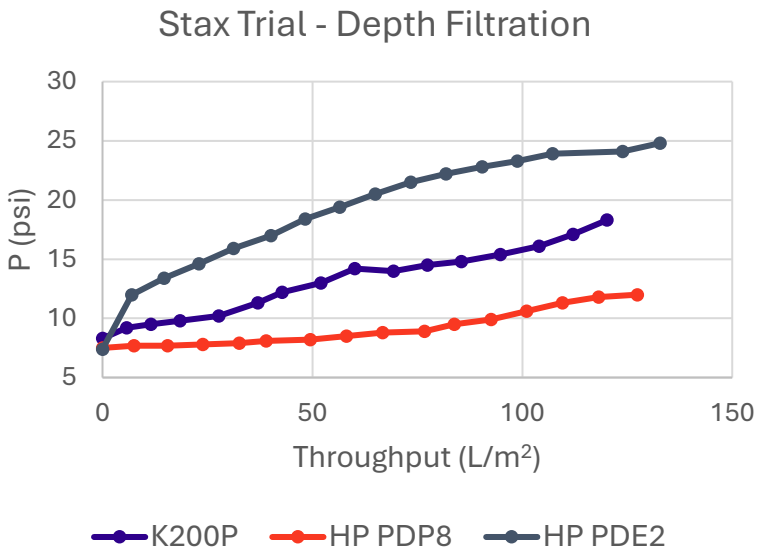


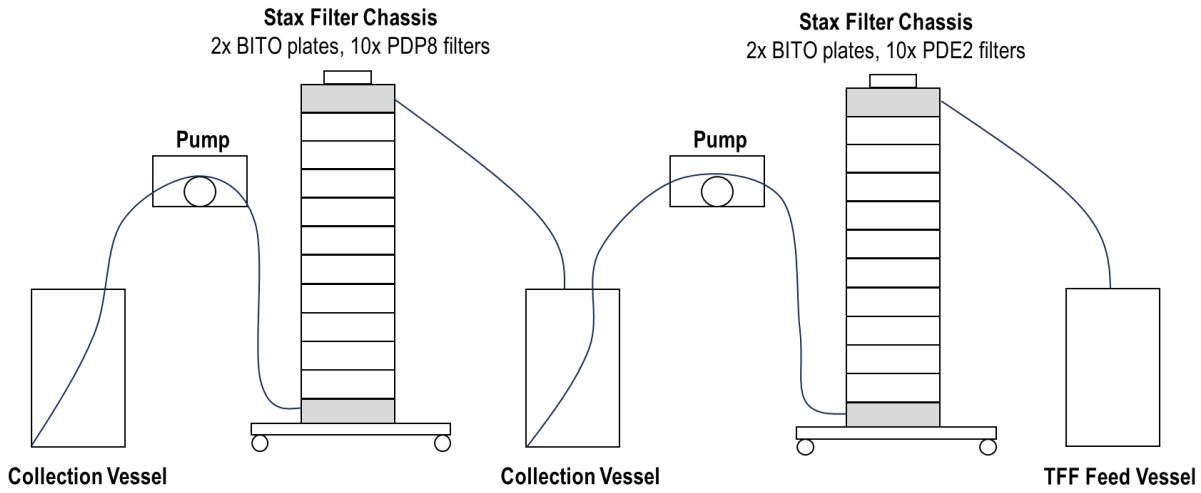
General Approaches to Scaling Lysis

- **Design process that is modular or utilizes components that are readily available in different sizes**
  - Similar to chromatography, fix one or two dimensions, vary third to meet requirements
- **Measure as many system parameters as possible**
- **Calibrate equipment prior to use (even if just a "check")**
  - Long term, qualification is the goal
- **Fix the critical parameters known in the field (Birnboim & Doly published in 1979!)**
  - Keep contact time 3-7 min
  - Low-shear neutralization
  - Buffer concentrations

General Approaches to Scaling Clarification

- **Disc-type centrifuges can be cost-effective if available**
- **Depth filter trains are effective but can be the most expensive DSP component per batch**
  - Vendors often offer on-site development support to aid in filter selection
  - Number of filters in stack are easily determined





General Approaches to Scaling UFDF

- **General principles of TFF processing of pDNA steams should be followed**
  - Membrane surface area chosen to enable reasonable processing time
  - Crossflow rate must be high enough to prevent membrane fouling
  - Membrane flux should be optimized

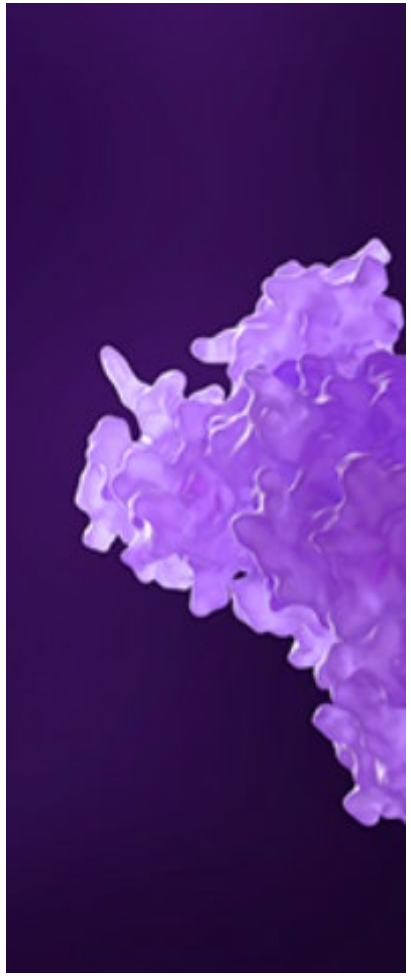
- **Optimal membrane flux <u>will likely not</u> result from highest crossflow rate that enables P_F to stay below a specific value**

$$TMP = \frac{P_F + P_R}{2} - P_P$$

  - At low [pDNA], TMP = P_F = P_R
  - If flow rate is too high, as [pDNA] rises, TMP drops due to P_R drop (gel layer)
  - Flux is suboptimal, process extended

- **Scale up should instead focus on moving to <u>TMP control</u> to minimize processing time**

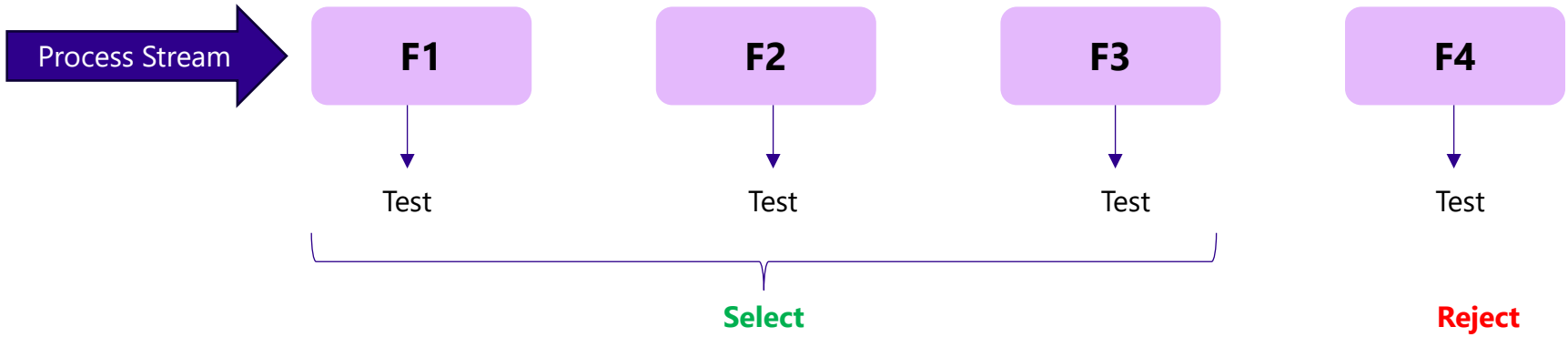
General Approaches to Scaling Chromatography



- **Generally, most straight-forward DSP unit operation to scale up**
- **When scaling, keep column height constant and increase column diameter**
- **Adjust flow rate to stay within pressure limits of packed column**
- **Approach is typically bind-and-elute**
  - Mobile phase compositions/concentrations are unchanged
  - Step lengths/volumes are a function of column volumes
- **If budget allows, pre-packed columns eliminate packing development**

In-Process Testing/PAT Strategy to Build Robust, Scalable Process

- **Early on, sample and retain everything** (buffers, process steps, pre-/post-filtration, waste, etc.)
- **Establish in-process acceptance criteria when data support them** (flexibility is critical early on)
- **In continuous processes, use segmented sampling in combination with in-process testing to lower risk**

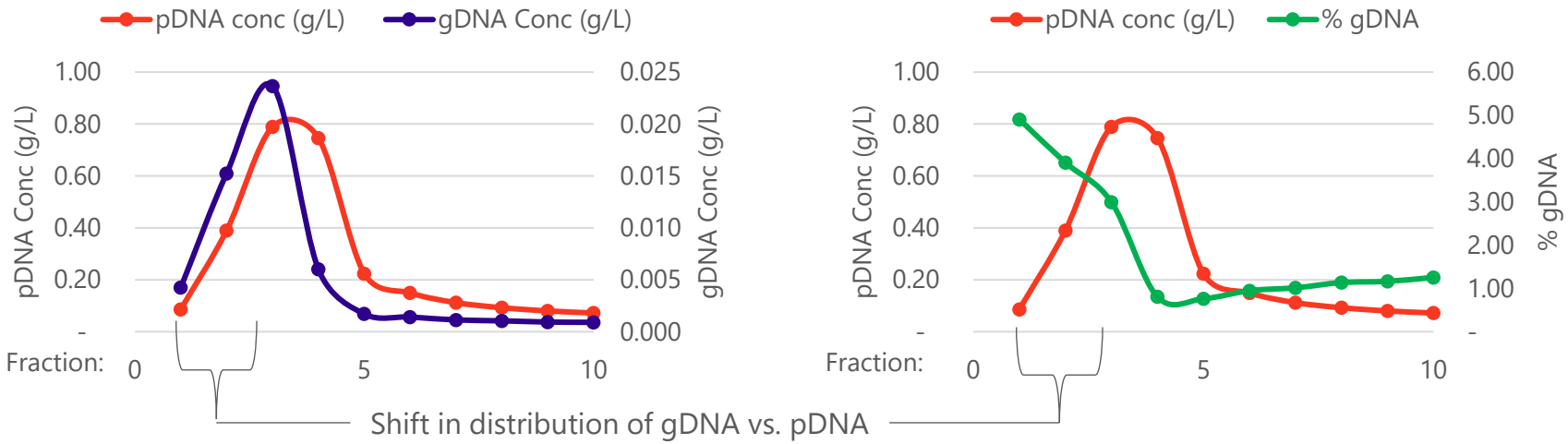


- **Consider building impurity-dilution calculations into selection process**

In-Process Testing/PAT Strategy to Build Robust, Scalable Process

- **Consider fraction volumes and reality of dilution when setting in-process specs/strategy**
  - For example, gDNA is a troublesome impurity in pDNA process streams
  - We may be tempted to:
    - Set in-process spec the same as final product (<1%)
    - Minimize fraction volume to minimize impurity in selected fractions

AEC Fractions:



In-Process Testing/PAT Strategy to Build Robust, Scalable Process

- **However, being too conservative may cause unnecessary pDNA loss**
- **See the single batch below, purified by AEC over two cycles (identical loads)**
  - In Cycle 1, early fraction volume is low (0.3 L) to select out fractions high in gDNA
  - In Cycle 2, early fraction volume is high (1.2 L), but since [pDNA] is higher slightly later, the gDNA in the fraction is only very moderately increased
  - Difference in final gDNA content in batch could be 0.35% vs. 0.27%. Is this worth a potentially 5-10% pDNA yield loss and added complexity of collection timing, volume determination, etc.?

F	Cycle 1			Cycle 2		
	Vol (L)	[DNA]	gDNA	Vol (L)	[DNA]	gDNA
1	0.3	0.19	1.4%	1.2	0.80	0.4%
2	0.3	0.52	1.1%	10.0	0.76	0.2%
3	0.3	0.78	1.0%	10.0	0.12	0.2%
4	10.0	1.00	0.2%			
5	10.0	0.30	0.2%			
6	7.0	0.10	0.2%			

Rejected fractions, spec < 1%

Practical Considerations to Enable Rapid Development of a Well-Controlled Process with High Quality Within Budget

- **Project annual material/supply needs, communicate to vendors**
  - Develop key relationships into partnerships
- **Determine if early-DSP steps require highest purity reagents, sterile SUAs, etc.**
  - Perform risk assessment
  - Further characterization of process may show ultra-pure materials needed only in later steps

DSP Unit Operation	Level of Material Purity	Justification
Lysis/Clarification	Reagent grade	Mixed with bacterial cells → lysate
UFDF1	Reagent grade	Mixed with lysate
Chromatography	High/sterile	Polishing step (BET removal)
UFDF2	High/sterile	= Final Fill buffer

Thank you!