Strategies to Accelerate Process Development from Preclinical to Manufacturing for Gene Therapy

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ABSTRACT

Gene therapy is a fast-growing industry. Demand on its products such as pDNA, AAV, retrovirus, lentivirus, etc. has significantly increased during last 10 years while its production challenges remain unresolved. It is a complex process with a number of unoptimized and low yielding steps. A typical process development (PD) cycle from preclinical to late phase manufacturing (Mfg) takes 3 to 5 years. It is desired to rapidly advance to manufacturing for early clinical candidates using well established and high yielding processes. The following strategies were successfully employed by BIB to accelerate the process development activities:

(1) A platform approach for early stage process development,
(2) Efficient DoE application for process optimization and characterization,
(3) Interaction improvement between PD and Mfg to streamline scale-up, tech transfer and manufacture support,
(4) Development of a new PAT tools application to understand process design space and hence develop a control strategy.

Case studies for BIB AAV and pDNA production platforms will be covered in this presentation.

GENE THERAPY IS A COMPLEX PROCESS: PROCESS FLOW CHART FOR AAV PRODUCTION

Plasmid Manufacturing	Viral Manufacturing		Primary Recovery	Downstream Processing	Analytical Support
Vial thaw (E.coli)	Vial thaw		Harvest transfected cells	2-step purification	Identity: SDS-PAGE, MS, PCR
Fermentation	Adherent	Suspension	Cell lysis (mech., chem.)	Buffer exchange by TFF	Titer: qPCR, ddPCR, A260/A280
Plasmid recovery	Cell Expansion T-flask, roller bottle, multilayer tray	Cell Expansion SF, wave or stirred-tank bioreactors	Nuclease treatment	Formulation and characterization	Potency: PFU, FFU
2-step purification					
Formulation	Serial passaging of cells	Serial passaging of cells	Concentration and diafiltration of harvest by TFF		Purity: TEM, MS, HCP,DNA
	Transfection/ Production Multi-layer tray, packed bed	Transfection/ Production Stirred-tank, wave, perfusion			Safety: Sterility, Endotox, Myco,
					Stability: pH, Osmo, Aggregate

Analytical Support					
Identity: Capsid ELISA, MS, PCR	**Titer:** qPCR, ddPCR	**Potency:** TCID50, Gene Function Test	**Purity:** SDS-PAGE, A260/A280, TEM, HCP,DNA	**Safety:** Sterility, Endotox, Myco, RCV, Adven. Agent	**Stability:** pH, Osmo, Aggregate, titer, potency

STRATEGIES TO ACCELERATE PROCESS DEVELOPMENT FROM PRECLINICAL TO MANUFACTURING

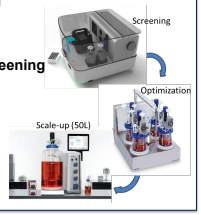
1. **Development of platform for early-stage Process Development (PD)**
2. **Use of efficient DoE for late-stage PD**
3. **Improvement of interactions between PD and Mfg**
4. **Application of new Process Analytical Technology (PAT)**

Note: Only BIB's AAV and pDNA production platforms are covered in this presentation.

DEVELOPMENT OF PLATFORM TO ACCELERATE PROCESS DEVELOPMENT

Strategies

1. **Advanced expression toolbox with high-throughput screening**
2. **Standard media & feed solutions**
3. **Standard seed expansion & production conditions**
4. **Single use bioreactors and high-throughput analytics**



BIB's AAV MEDIA TOOLBOX PLATFORM FOR QUICK AND EFFICIENT PRODUCTION

BIB's platform is a collection of high performing media/reagents/cell lines for screening high AAV titer producing combos

Media Transfection Reagent, Cell Line Screening	Optimize VG Production in Top Performing Combos	Production In Bioreactor
• Identify top media/reagent/Cell combos based on Viral Genome (VG) production, growth, metabolism, and product quality	• Optimize AAV production in top performing combos using DoE • Vector ratio, reagent: vector ratio, cocktail volume, Infection VCD, Infection Duration	• Bioreactor test of top 1-3 media/cell combos • Choose final media/cell combo based on growth, metabolism, GC and product quality

BIB's pDNA PLATFORM FOR QUICK AND EFFICIENT PRODUCTION

BIB's pDNA platform includes strain development, fermentation, recovery, purification, and analytical methods. It is scalable to a thousand liter.

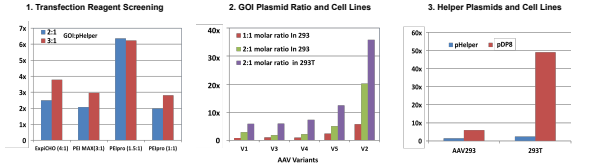
Strain	Fermentation	Recovery	Purification
BIB's platform is compatible with common industry strains such as DH5α & DH1	Unique fed-batch high-cell-density fermentation reliably produces ≥200 mg/L pDNA (for typical ~6kb pDNA vaccines).	Novel efficient primary recovery step that improves yield A single novel filtration step replaces the conventional 3-step primary recovery (flocculation⇨centrifugation ⇨filtration) which reduces processing time & improves yield	High-yielding purification process for isolating high purity super-coiled pDNA Overall downstream yield is >50% (overall yield 100 mg/L pDNA, >98% purity)

CASE STUDY 1: EFFECTS OF MEDIA TOOLBOX ON AAV PRODUCTION

Background:

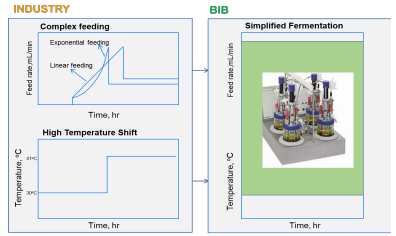
1. **Needed large quantity of 5 AAV variants for preclinical studies**
2. **Client had an old and low titer producing process**
3. **CaCl2 is used for their transfection process**
4. **BIB convinced sponsor to test different reagents and DNA ratio using the BIB "AAV Toolbox" to improve titer**

CASE STUDY 1: EFFECTS OF MEDIA TOOLBOX ON AAV PRODUCTION

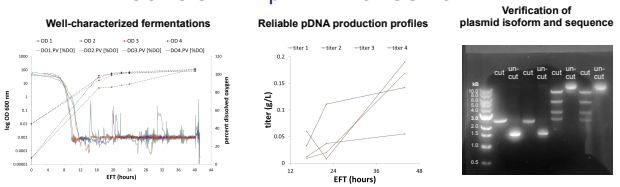


BIB platform resulted in 50-fold titer increase compared to the client's initial process

CASE STUDY 2. BIB'S pDNA FERMENTATION IS SIMPLE AND SCALABLE



CASE STUDY 2. BIB'S pDNA FERMENTATION PROVIDES CONSISTENT pDNA PRODUCTION



SUMMARY FOR BIB PLATFORM APPROACH FOR GENE THERAPHY

BIB successfully employed platform approach to accelerate gene therapy process development. It provides

- **Timeline reduction**
- **Cost saving**
- **Robust process**
- **High yielding titer**
- **Reproducible product titer and quality**