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NGS-Driven Quality

Redefining Biosafety and Release Strategies in Advanced Therapy Manufacturing

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CDMO Solutions – Gene Therapy

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Overview

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Our mission is to create healthier lives

We currently operate

110+ Facilities **IN** **20+** Countries

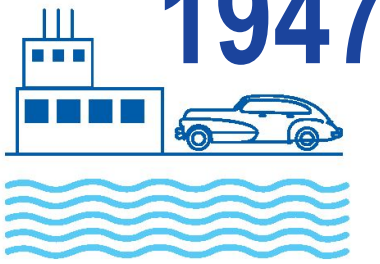
We supported the development of

86%

of the novel FDA-approved drugs

Founded

1947



Conducted

>900

cell & gene therapy studies

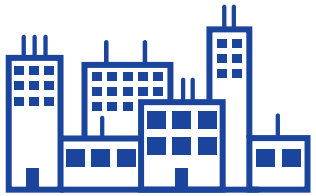
Supported the development of

27

FDA-approved cell & gene therapies

Acquired

6



companies since 2020 to strengthen our end-to-end cell & gene therapy portfolio

3 CDMO Locations | **800+** CDMO Employees | **~400K** CDMO Square Feet | **720+** Testing Employees

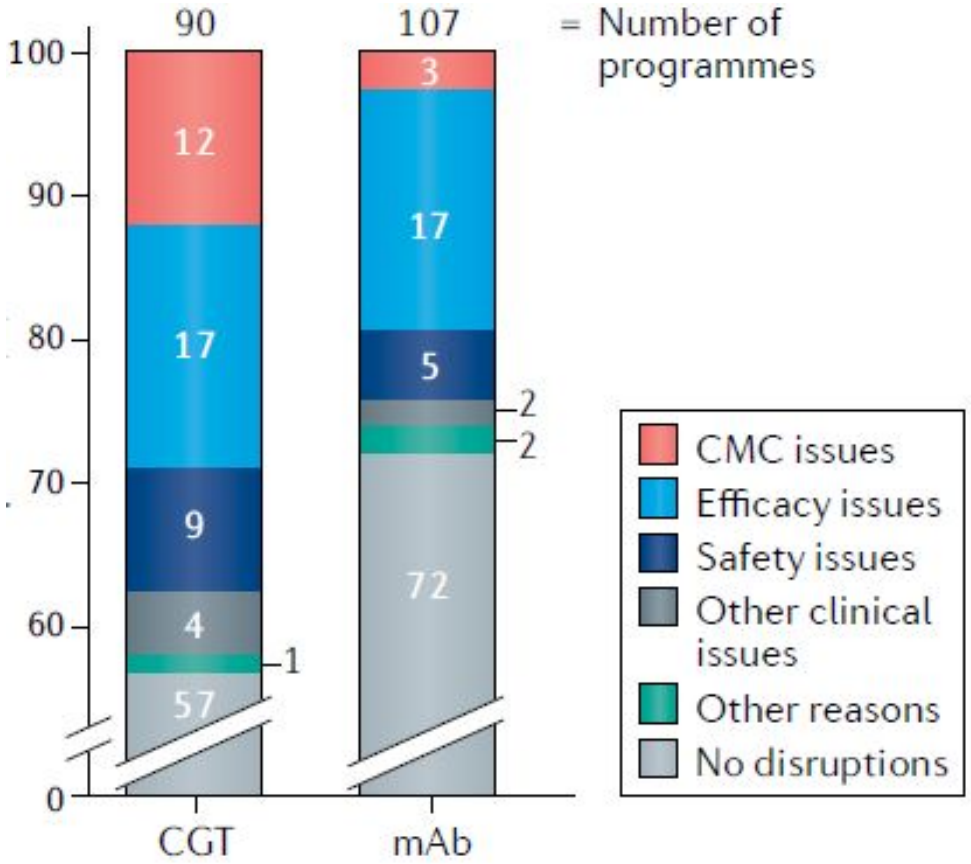
Delays and Disruptions in Development of Cell and Gene Therapies

All CGTs and mAbs entering phase III Trials over 5 yrs (2016-2021)

+ Similar number of mAb vs CGTx Products
+ Similar challenges around efficacy
+ About 2-fold higher Safety issues with CGTx
+ About 4-fold higher CMC issues with CGTx

Conclusions:

These findings indicate a need to proactively address the CMC issues related to CGT products, in order to advance CGT beyond the current period of “growing pains” and towards the consistency observed across mAb development programs.



Guang Yang, et al., Nature Reviews | Drug Discovery Volume 21 | March 2022

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Incorporating NGS into Gene Therapy Development

Integration focuses on efficient translation

Adventitious agent testing
+ Cell lines, viral vectors, cell therapy products, etc.

Vector identity testing
+ AAV, LVV, Ad5, etc.

Manufacturing support
+ Raw material screening
+ Environmental monitoring
+ Contamination identification

Plasmid Manufacturing
+ Plasmid-based products
+ Critical starting material

News Release

View printer-friendly version

Charles River and PathoQuest Announce Publication of a Head-to-Head Study Validating Proprietary Next Generation Sequencing Viral Safety Assay as a Reliable In Vitro Alternative to Animal Testing Methods

Download PDF

NGS assay demonstrated a broader detection range than standard in vivo tests with comparable or higher analytical sensitivity

WILMINGTON, Mass. & PARIS--(BUSINESS WIRE)--Sep. 6, 2023--

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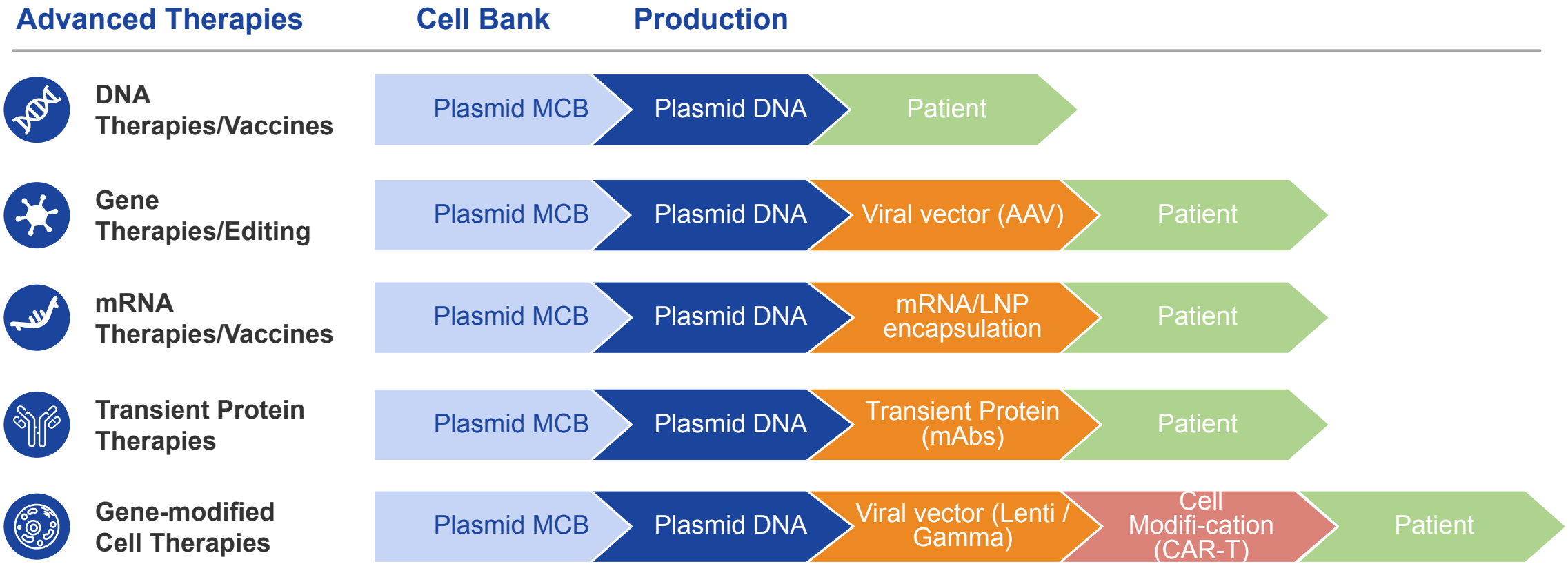
Plasmid DNA: Role within Advanced Therapies

Many advanced therapy approaches are dependent on Plasmid DNA as a Critical Starting Material



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The eXpDNA™ Plasmid Platform

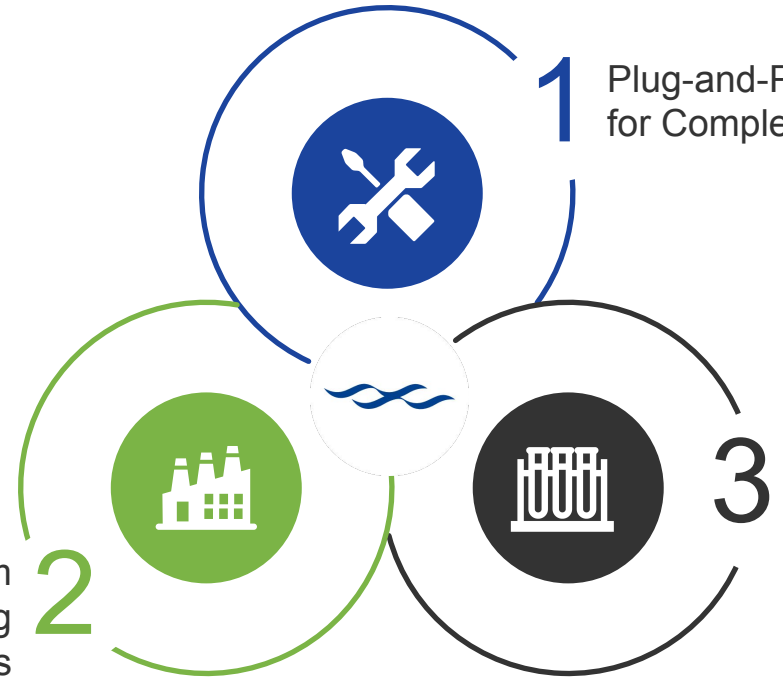
Trust the team to reliably expedite your plasmid production

Reliable and trusted partner of choice

Rated 4.9 out of 5
for “Overall Reliability”
per 2021 client survey

20+ years of experience of gene therapy CDMO services
>95% successfully released pDNA batches
220+ GMP/HQ batches

eXpDNA™ Plasmid Platform



Experience with various complex plasmids

30+ years of experience of biologics testing

5+ weeks HQ I 10+ weeks GMP

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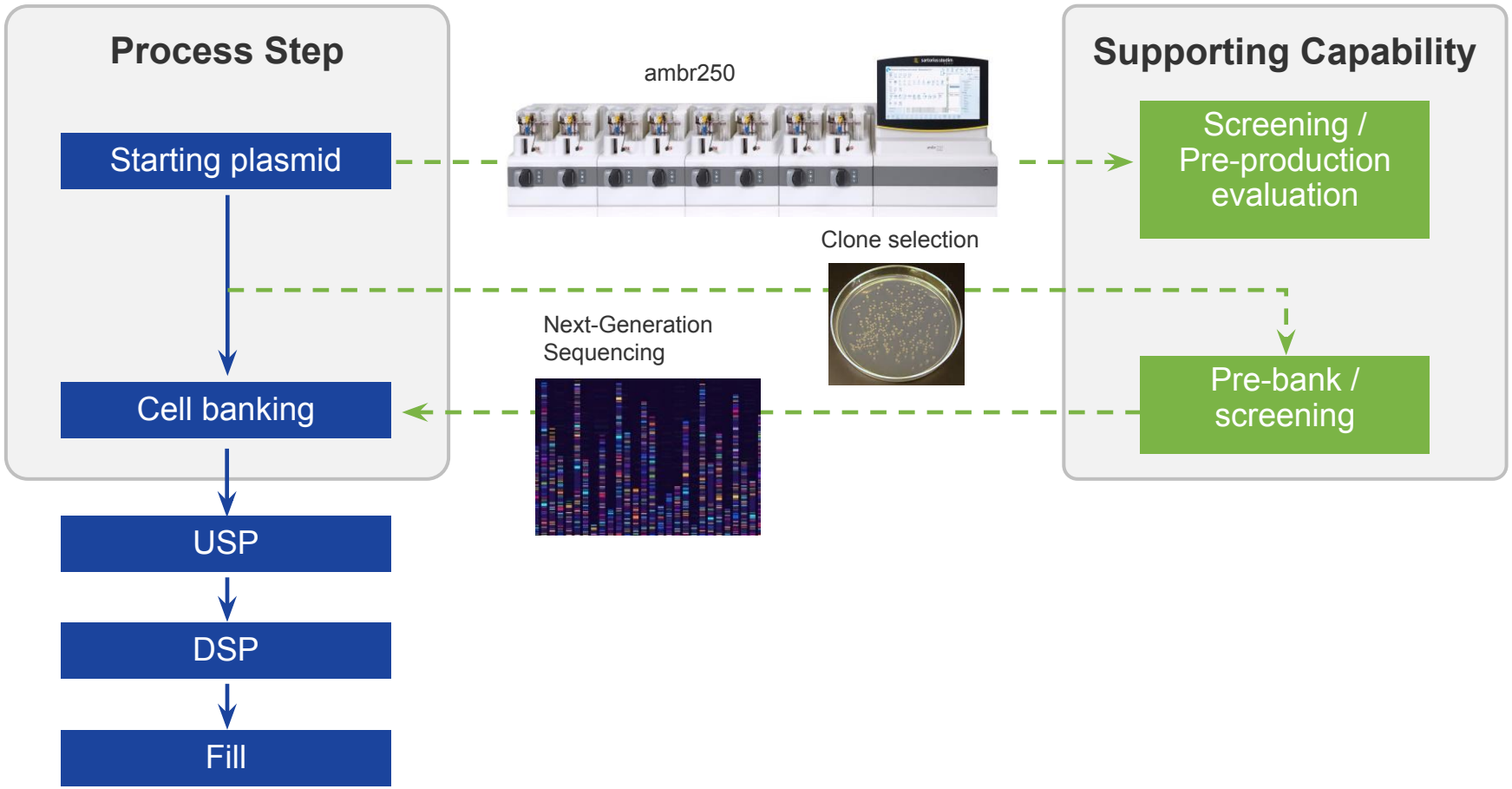
Plasmid Screening Prior to Manufacturing



Outputs / benefits

+ Process performance
+ Productivity assessment
+ Yield prediction/uplift

Outputs / benefits

+ Plasmid integrity / sequence check
+ Robust clone selection

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NGS Overview

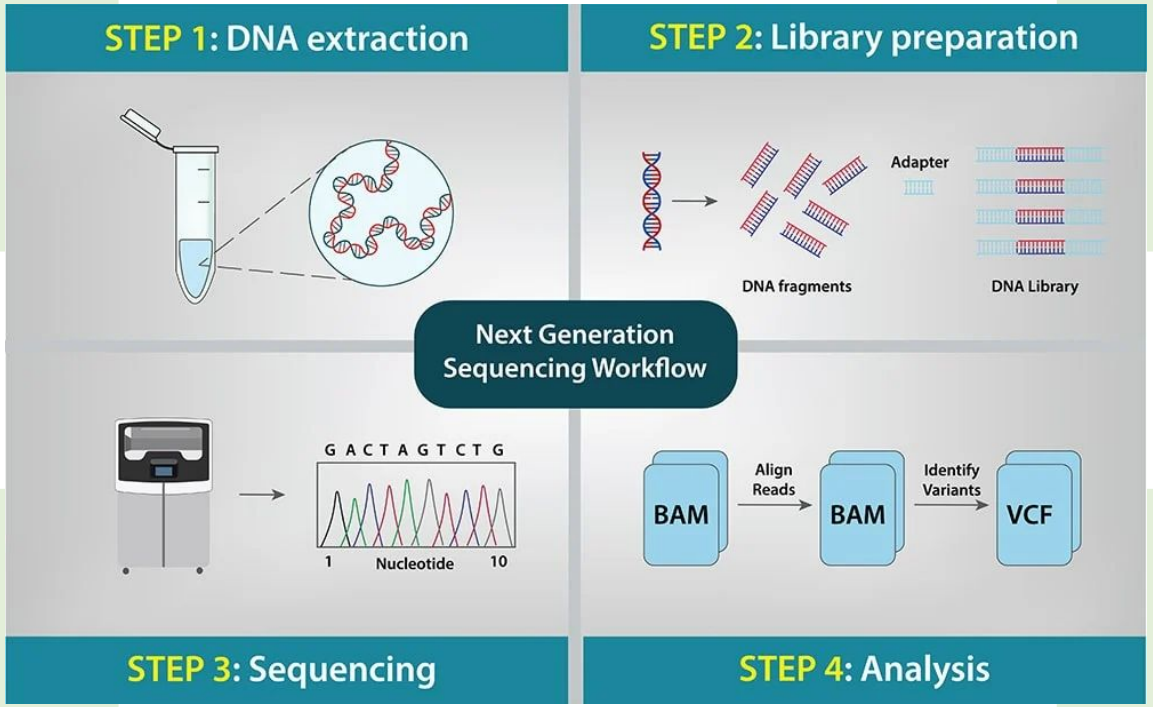
Workflow stages, key considerations, & risks

Sample preparation (nucleic acid extraction)

+ Reagents, personnel training, sample contamination

Sequencing

+ Different chemistry types
+ Potential bias



To ensure reliability, accuracy, and consistency of results, each stage must be assessed for the specific sample type and required variant detection

Library preparation

+ Reagents, PCR, personnel training

Bioinformatics and reporting

+ Variety of tools available
+ Potential alignment errors

https://www.beckman.com/resources/technologies/next-generation-sequencing/importance-of-optimizing-your-ngs-workflow#

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NGS vs. Sanger

Sanger Sequencing

“First generation”

+ Developed in the late 1970’s
+ Low throughput
+ Targeted PCR with fluorochrome-labeled ddNTPs (chain termination method)
+ Consensus sequence taken from electropherogram

Next Generation Sequencing (NGS)

“Next generation”

+ Introduced in the early 2000’s
+ AKA “high-throughput sequencing” or “deep sequencing”
+ Broad range, non-hypothesis-driven
+ Massively parallel sequencing (millions of reads)

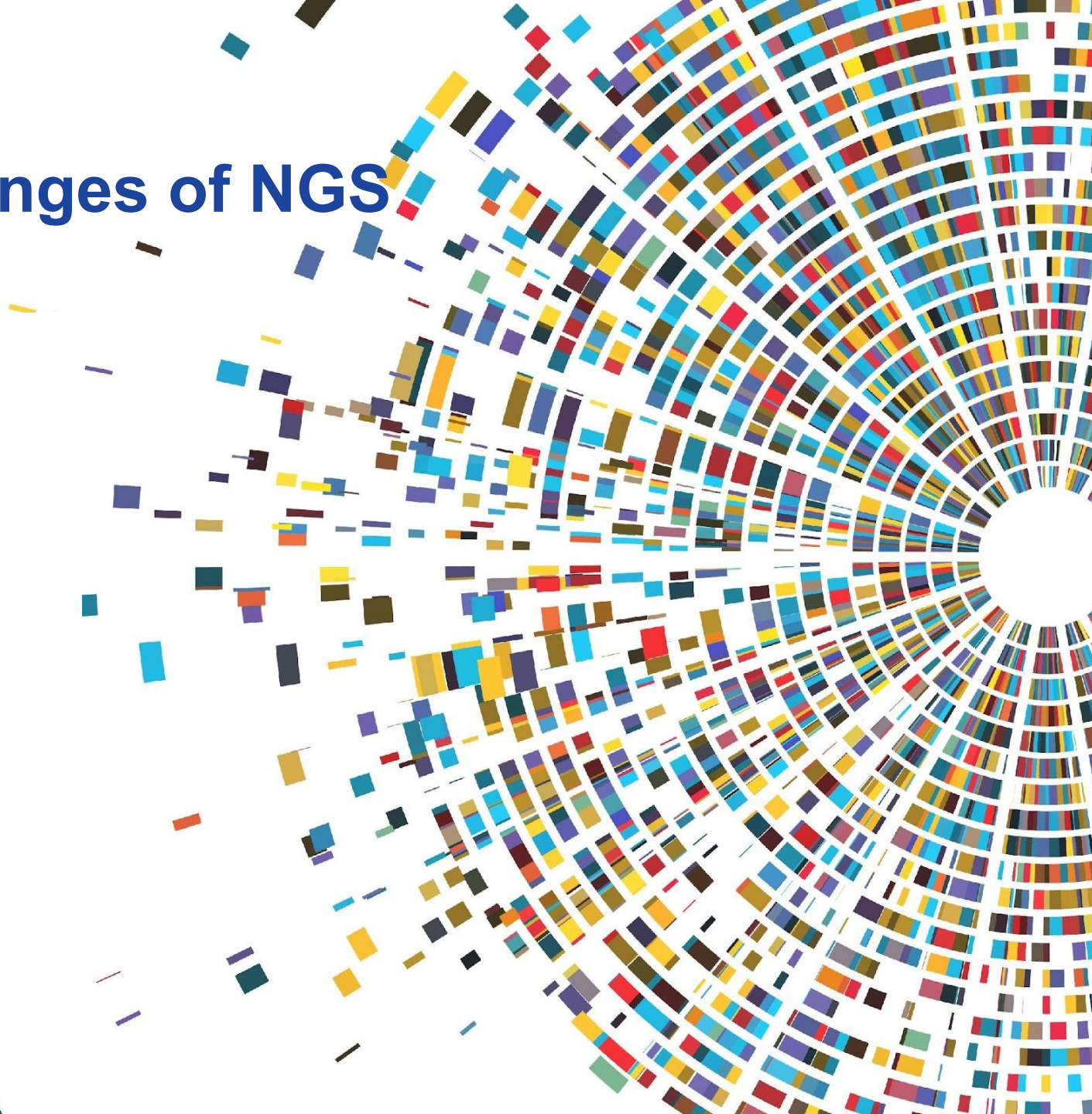
Advantages and Challenges of NGS

Advantages of NGS

+ Higher throughput & faster turnaround time
+ Greater depth
+ Ability to sequence entire plasmid or genome at once
+ Identification of unknown sequences
+ Detection and quantification of low-level variants
+ Ability to sequence through ITRs

Challenges

+ Large amount of data
  + Potential false positives or negatives
  + Interpretation of results



Case Study 1 – AAV Gene Therapy

Experienced gene therapy developer – new AAV pipeline product

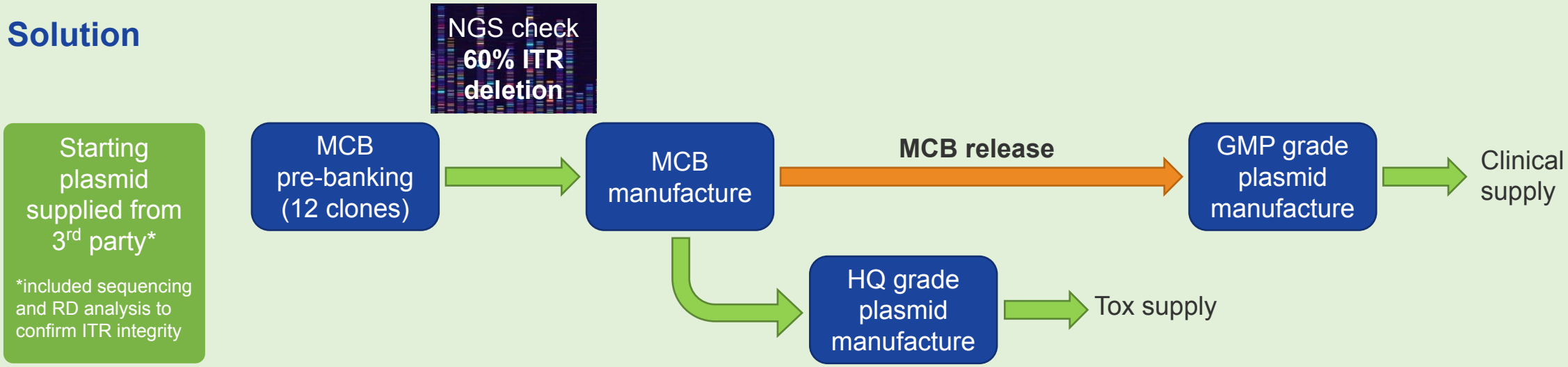
Background

+ ITR containing GOI plasmid for AAV production
+ Previously using SmaI restriction digest as per Wilmott *et al* 2019

Requirement

+ Supply of plasmid DNA for AAV manufacturing to support both toxicology and Phase I/II clinical trial
+ Aggressive timelines

Solution



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Case Study 2 – Impact of Sequencing Coverage

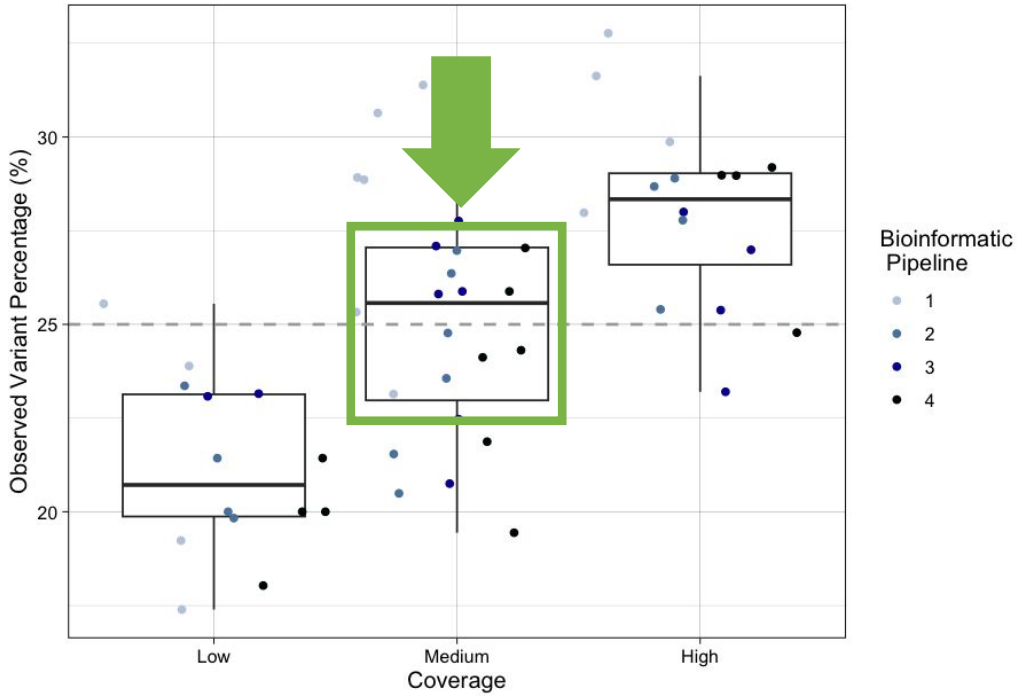
Optimization data from Accugenix®

Experiment

+ Two AAV pGOI plasmids were mixed at a known percentage (25%) of variant (11 bp deletion in one ITR) to wild-type plasmid
+ Variant detection analyzed by four bioinformatic pipelines at low, medium, and high coverage.

Result

Medium coverage provided the most accurate variant detection (high coverage overestimated while low coverage underestimated)



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Summary

NGS-driven quality in advanced therapy manufacturing

NGS has multiple applications for advanced therapies

MCB characterization, plasmid screening, vector product release, safety testing, etc.

Incorporating NGS derisks CGT product manufacturing

Can verify troublesome plasmid sequences (ITRs) before investing in subsequent manufacturing activities

NGS is not a “one size fits all” platform

Chemistry and depth of coverage can impact results

Acknowledgements

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Thank you for your attention

Any Questions?
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