BioReliance®
Contract Testing Services

Superior Quality Control for Next Generation mRNA Therapeutics

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MilliporeSigma

Critical Quality Attributes

Identity

Potency

Safety

Purity

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Regulatory guidance for mRNA requires Identity testing

Table 2: Characterization and release testing for mRNA Drug Substance

Quality	Attribute	Method
Identity	mRNA sequence identity confirmation	High throughput sequencing (HTS)
		Sanger sequencing
		Reverse Transcriptase – PCR (RT-PCR)

Aka: Next Generation Sequencing (NGS)

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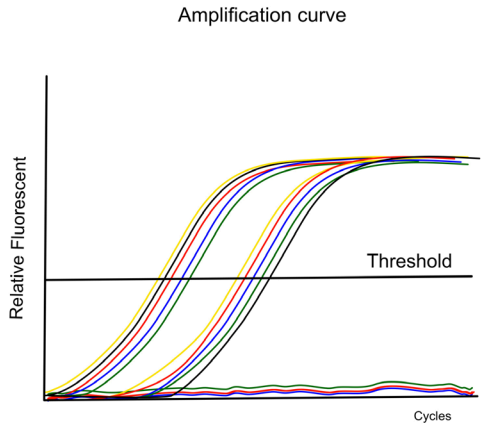
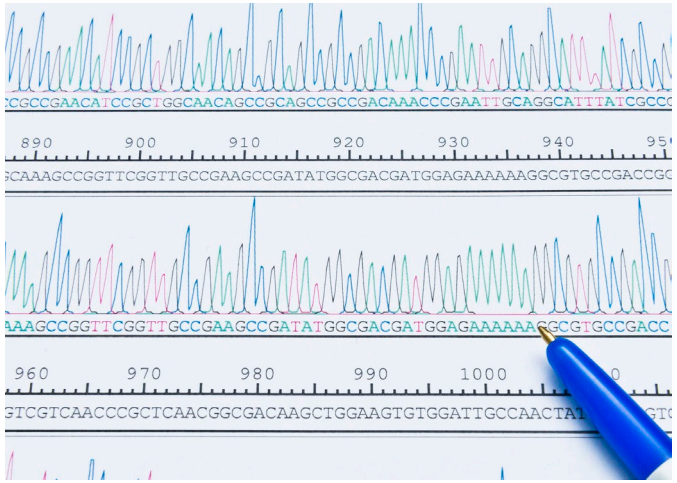
What are your options when it comes to performing ID?

RT-PCR/RT-qPCR



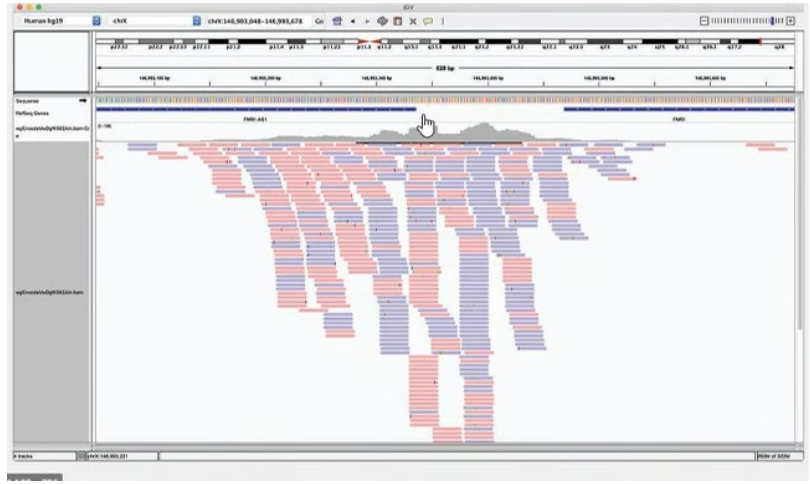
Confirms presence of amplification of a small specific sequence

Sanger Sequencing



Confirms a consensus sequence against a reference sequence

NGS/HTS (short read)



Sequencing which can differentiate sub-populations in sequence variation

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General Overview- What is NGS?
Next Generation Sequencing (HTS)

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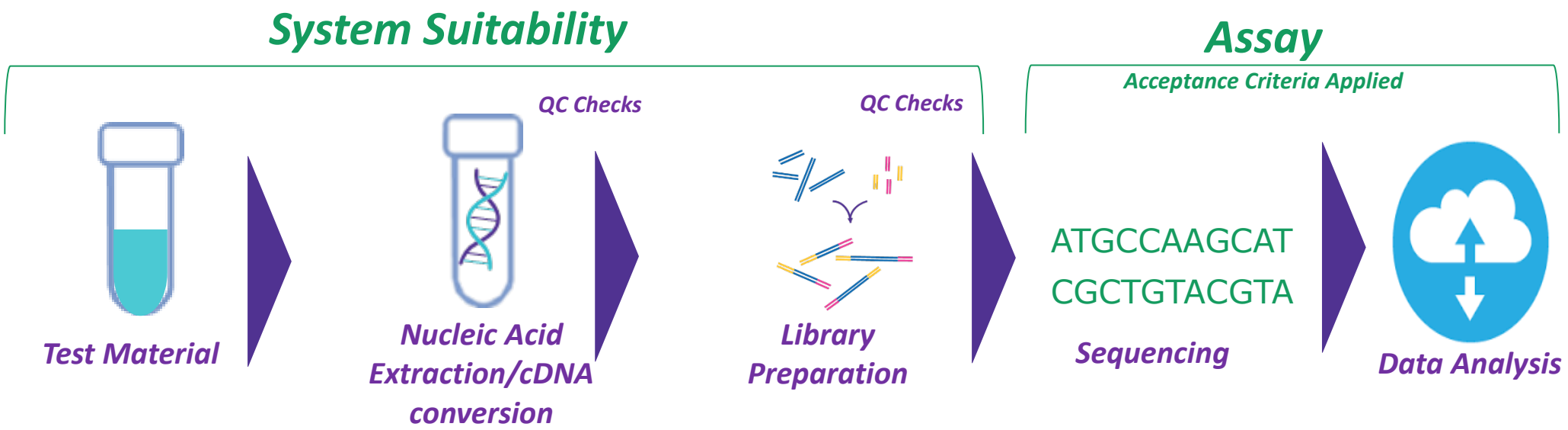
- Individual Molecule Sequencing with Millions of data points (small sequence fragments) generated
- Sequences represent the full genomic profile of all Nucleic Acids present in a test sample
- A technology with application across all modalities
- Simple methodology, complex equipment, mind-blowing bioinformatics!



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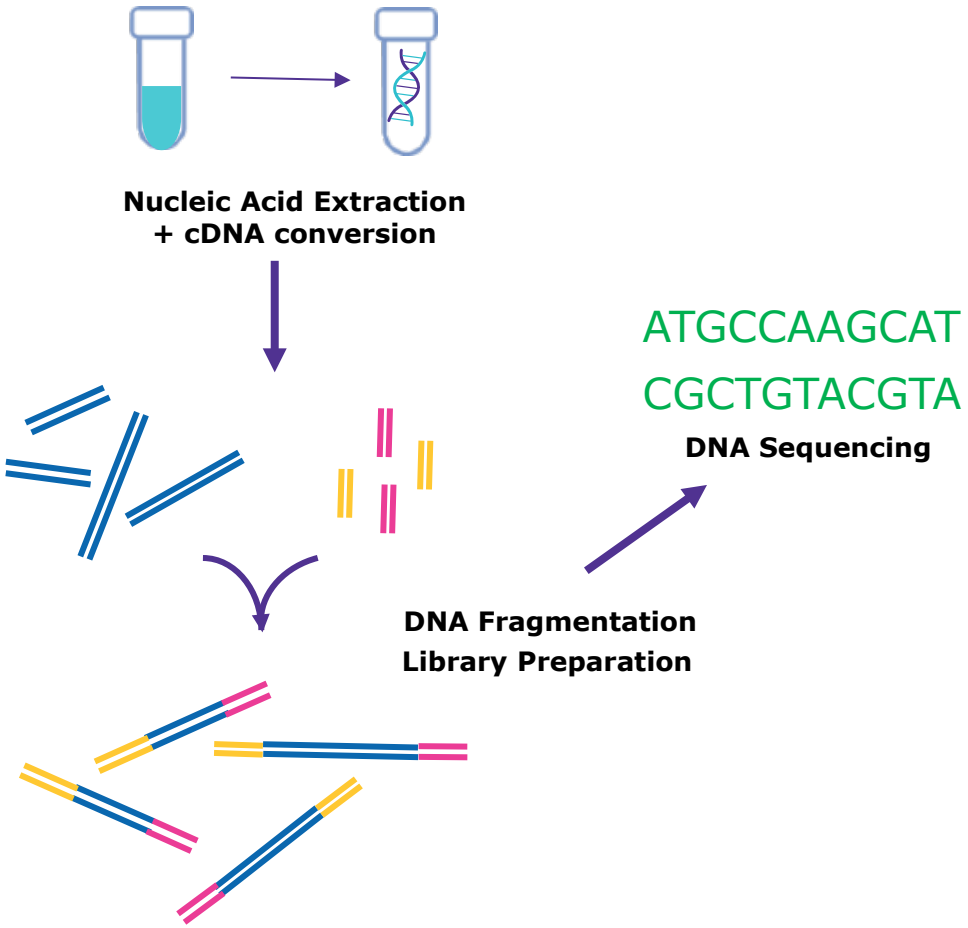
ID Testing Workflow



Bioinformatics

- Read mapping against a client-provided reference sequence (typically theoretical or derived from Sanger consensus)
- Algorithm utilizes an aligner and a variant caller(s):
- Detected variants:
  - Insertions
  - Deletions
  - Substitutions
  - Multi-nucleotide variants (MNV's)
- Variant frequency of detection down to 1% or less in the population.
- Short-read sequencing offers the highest fidelity
- Fully validated algorithm

Why would we use NGS for ID and can it do more?

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1. A platform-based test for Molecular Identity
2. Ease of implementation with no additional validation requirements or up front primer design
3. Ability of NGS to sequence the full molecule, fulfilling regulatory requirements for vector characterization
4. Low level variant detection and tracking from lot to lot
5. **Superior risk mitigation profile** when compared to other ID methods
6. Can be used as a surrogate for potency via sequence purity analysis

CRO visibility across industry

Regulatory agencies have been recommending NGS with supplemental qualification of sponsor specific sequences and variant detection capabilities

Industry precedent

Position paper

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What can we do to mitigate risk
Why should we be concerned about variants

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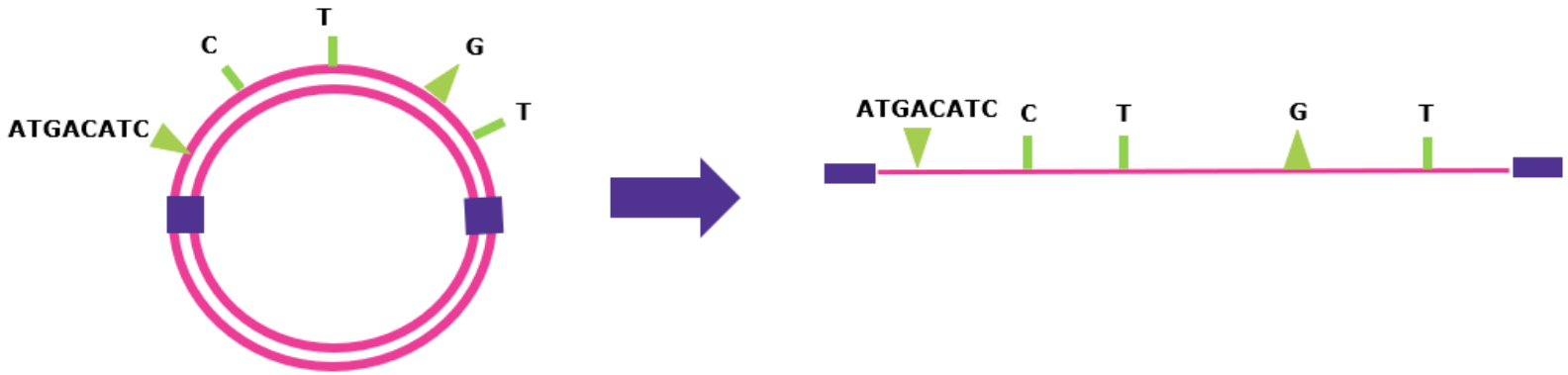
- Variation in sequence composition as the ability to impact your product
- Can be performed at multiple points in the process (e.g. plasmid)
  - Allows for early identification of sequence variation



- Allows for detection of low level variation in sequence
- Allows for lot to lot monitoring for the presence of sequence variation
- Time, money and re-work!!

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Provides results that offer both analysis & interpretation

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Identity match to reference sequence

ASSAY NUMBER	SPECIFICATION	RESULT*
706510GMP.BSV	Report Result	Reference Sequence Coverage= 99.99%. Similarity to Covered Reference = 99.78%.

Quality metrics to demonstrate appropriate molecule coverage for sequence and variant evaluation

IDENTITY TESTING (ID) RESULTS

Total # of Reads Used for Mapping	Reference Sequence Used for Mapping	Accession Length (Bases)	Total # of Mapped Reads	% of Population Mapped	Reads in Aligned Pairs	Average Depth of Coverage[1]	Consensus Length Generated By Mapping[2]	% Reference Coverage	% Similarity to Reference	Total Number of Unmapped or Low-Quality Positions[3]
22,463,940	EXM-2345	7,557	413,176	1.84	322,734	5,396.69	7,556	99.99	99.78	1

Sequence variant characterization: insertions, deletions and single nucleotide changes with associated frequencies

CHARACTERIZATION OF VARIANTS

Position	Reference Base	Variant Base	Number of High-Quality Ref (fwd)	Number of High-Quality Ref (rev)	Number of High-Quality Var (fwd)	Number of High-Quality Var (rev)	Max Number of Reads Supporting INDEL (IDV)	Raw Read Depth (DP)	Variant Frequency (%)	Variant Type
5,702	G	GTACGGCC	N/A	N/A	N/A	N/A	1,047	1,298	80.66	Insertion
5,811	C	T	5,401	286	2,267	714	N/A	9,349	34.39	Substitution
5,856	T	C	7,306	517	3,798	1,997	N/A	18,848	42.55	Substitution
5,885	T	A	4,672	905	1,917	995	N/A	13,726	34.3	Substitution
5,885	ATGACATC	T	N/A	N/A	N/A	N/A	3,845	13,726	28.01	Deletion

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Identity and variant detection
Validation of NGS Methodology and implementation

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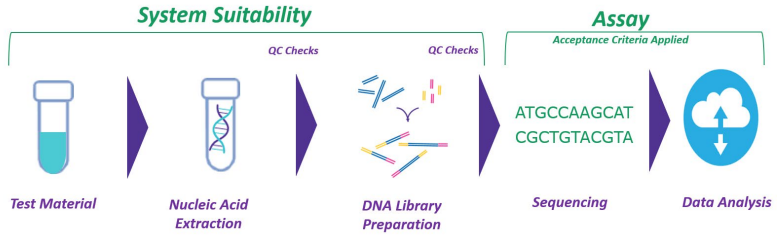
NGS Assay is validated in a modular fashion

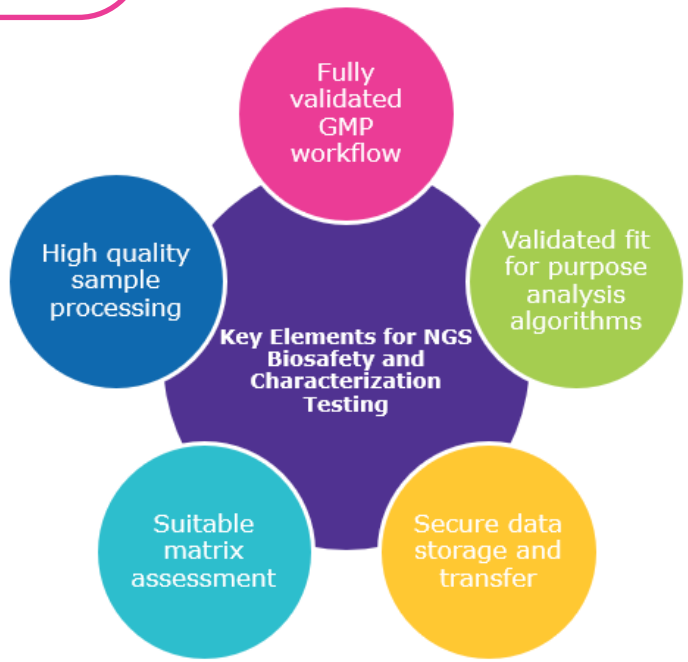
- **Extraction**
- **Library**
- **Sequencing**
- **Bioinformatics**

This approach confirms that each portion of the assay behaves as expected and offers flexibility for updating rapidly changing technology



Suitability criteria are built in between modules as "QC checks"

GMP assay combines different modules which are put together and qualified within the context of an application or product



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Additional capabilities of NGS- "value-add"

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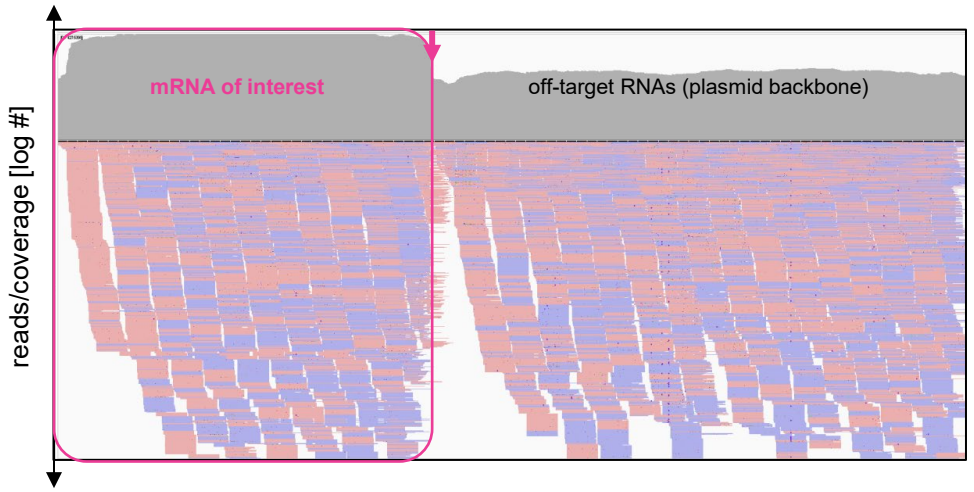
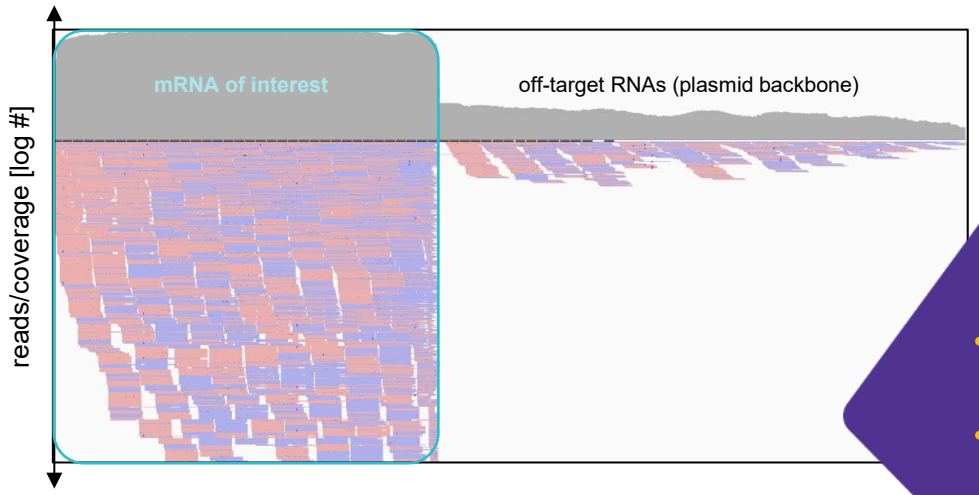
PCR-based manufacturing results reduced off-target species (*FLuc*)

plasmid-based mRNA



region	mapped reads	fraction
FLuc gene	114475225	0.97556
plasmid backbone	678076	**0.00578**

PCR-based mRNA



region	mapped reads	fraction
FLuc gene	128332305	0.98178
plasmid backbone	2109	**0.00002**

Sequence Identity Testing (NGS)

- Samples provided by mRNA manufacturing
- Analyzed by Rockville Contract Testing Services site.

NGS-based analysis of off-target mRNA synthesis in plasmid-based and PCR-based mRNA manufacturing. Arrow indicates restriction site for plasmid-based manufacturing. Coverage plots of mRNAs mapped to full length plasmid sequence. Plot generated IGV, coverage is log-scaled. Process optimization and samples provided by Eike Joest, Head of Innovation mRNA manufacturing (Merck KGaA Darmstadt Germany). Sequencing performed by GMP NGS laboratory (MilliporeSigma) and analysis by Mo Heydarian & Melisa Wilson (R&D, MilliporeSigma).

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Takeaways

Next Generation QC of mRNA therapeutics

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1

NGS offers superior risk mitigation profile throughout the manufacturing process

2

CRO insight:

Highly sensitive methodology is being more commonly requested by regulators

3

Value-add capabilities of NGS can help during process development

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