two anacura companies

Single-assay mRNA vaccine cQA testing based on nanopore sequencing

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Strict European guidelines (ICH-Q5 – EMA) regulate QC of biologics as mRNA vaccines. Our extensive service portfolio for mRNA-based therapeutics and vaccines, adeno-associated and other viral vectors, plasmids, microbiomes and/or cell lines, includes analytical solutions based on long-read Nanopore sequencing (Oxford Nanopore Technology, ONT).

We offer a set of assays to assess the combined read-out for several different critical quality attributes (cQAs) of an mRNA drug substance (DS) and drug product (DP).

mRNA cQAs

IDENTITY

- Sequence confirmation
- Sequence modifications (m1Ψ and Ψ)

INTEGRITY

- Intact transcripts
- 5′ cap detection
- Poly(A) tail length
- off-target contaminants (as a proxy to dsRNA)

Sequencing assay overview

Oxford NANOPORE Technologies

Sample prep
Library prep
QC

Direct RNA protocol | Direct cDNA protocol

5′ cap – 5′ UTR – gene of interest coding sequence – 3′ UTR – poly(A)

Read lengths of >20kb

Bioinformatics analysis[1]

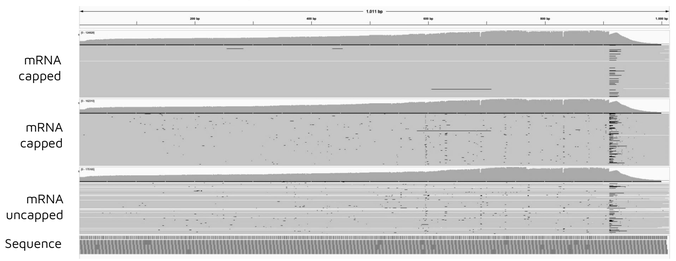
Full report
sequencing quality & metrics
calculated mRNA cQAs

[1] Gunter H.M. *et al.* 2023. Nat Comms.

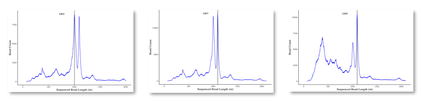
Full sequence confirmation

- Using Direct cDNA sequencing protocol

Full mRNA sequence confirmation

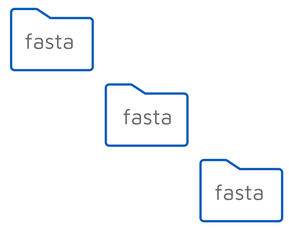


Sequence length distribution



Capping	Reference length	Identified read lengths	
Yes	1010	1005	±1100
Yes	1010	1097	±1010
No	1010	1095	±1010

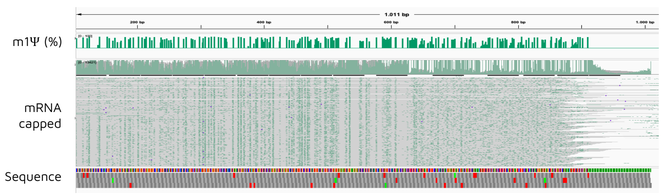
Full mRNA sequence



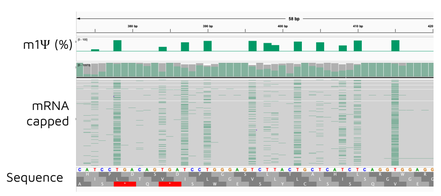
Sequence modification

- Using Direct RNA sequencing protocol
- Based on deep learning basecalling models specific for m1Ψ and Ψ

Full m1-pseudouridine (m1Ψ) modification profile

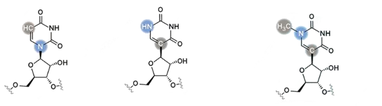


Detailed zoom in



Modification at base level

Uridine nucleosides in mRNA-vaccines are modified to reduce immunoreactivity and enhance protein production

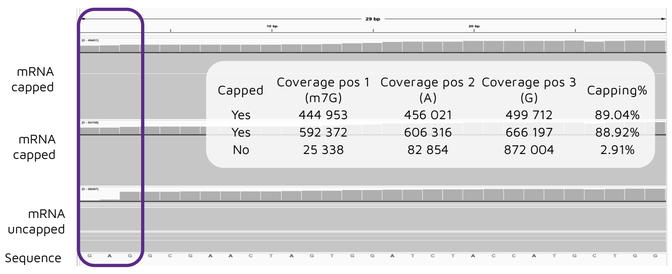


Uridine (standard) | Pseudouridine (Ψ) | 1-methylpseudouridine (m1Ψ)

3′ poly(A) tail / 5′ capping

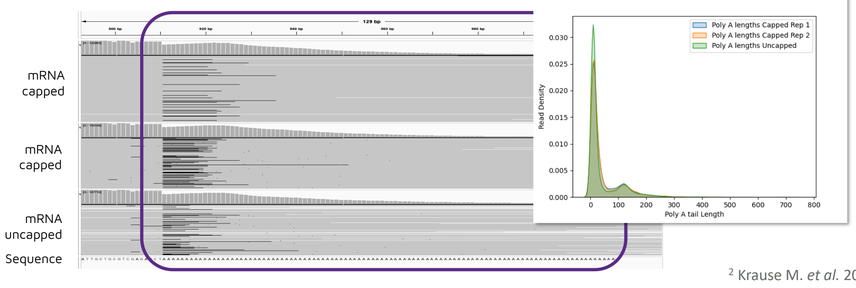
- Using Direct cDNA sequencing protocol
- Based on custom bioinformatics pipelines[2]

5′ cap detection



Capped	Coverage pos 1 (m7G)	Coverage pos 2 (A)	Coverage pos 3 (G)	Capping%
Yes	444 953	456 021	499 712	89.04%
Yes	592 372	606 316	666 197	88.92%
No	25 338	82 854	872 004	2.91%

3′ poly(A) tail

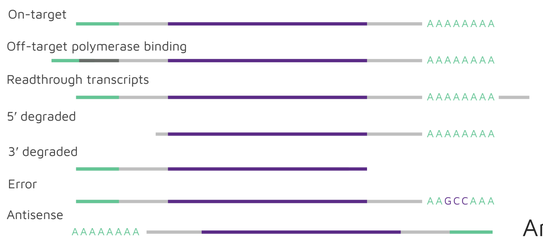


[2] Krause M. *et al.* 2019. RNA

Off-target contaminants

- Using Direct RNA sequencing protocol
- Additional poly(A)-tailing
- Based on custom bioinformatics pipelines

mRNA species identifications[1]



On-target and E. coli contaminant mapping

Capped	On-target mapping (%)	*E. coli* mapping (%)
Yes	99.13%	0.43%
Yes	99.18%	0.27%
No	95.74%	2.94%

Antisense as proxy to dsRNA %

[1] Gunter H.M. *et al.* 2023. Nat Comms

Towards GMP

Oxford Nanopore Q-line GridION system

- Proven, locked-down technology
- Compliance with 21 CFR Part 11 and EU GMP Annex 11 requirements
- Full operating software and consumable version support and supply for three years post-release
- ISO 9001:2015 certified product manufacturing process

- RUO only
- TAT 1-2 weeks
- Non-standardized reporting

- GMP
- TAT <1 week
- Standardized reporting