An integrated platform for the analysis and quality control of rAAV vectors based on long-read sequencing

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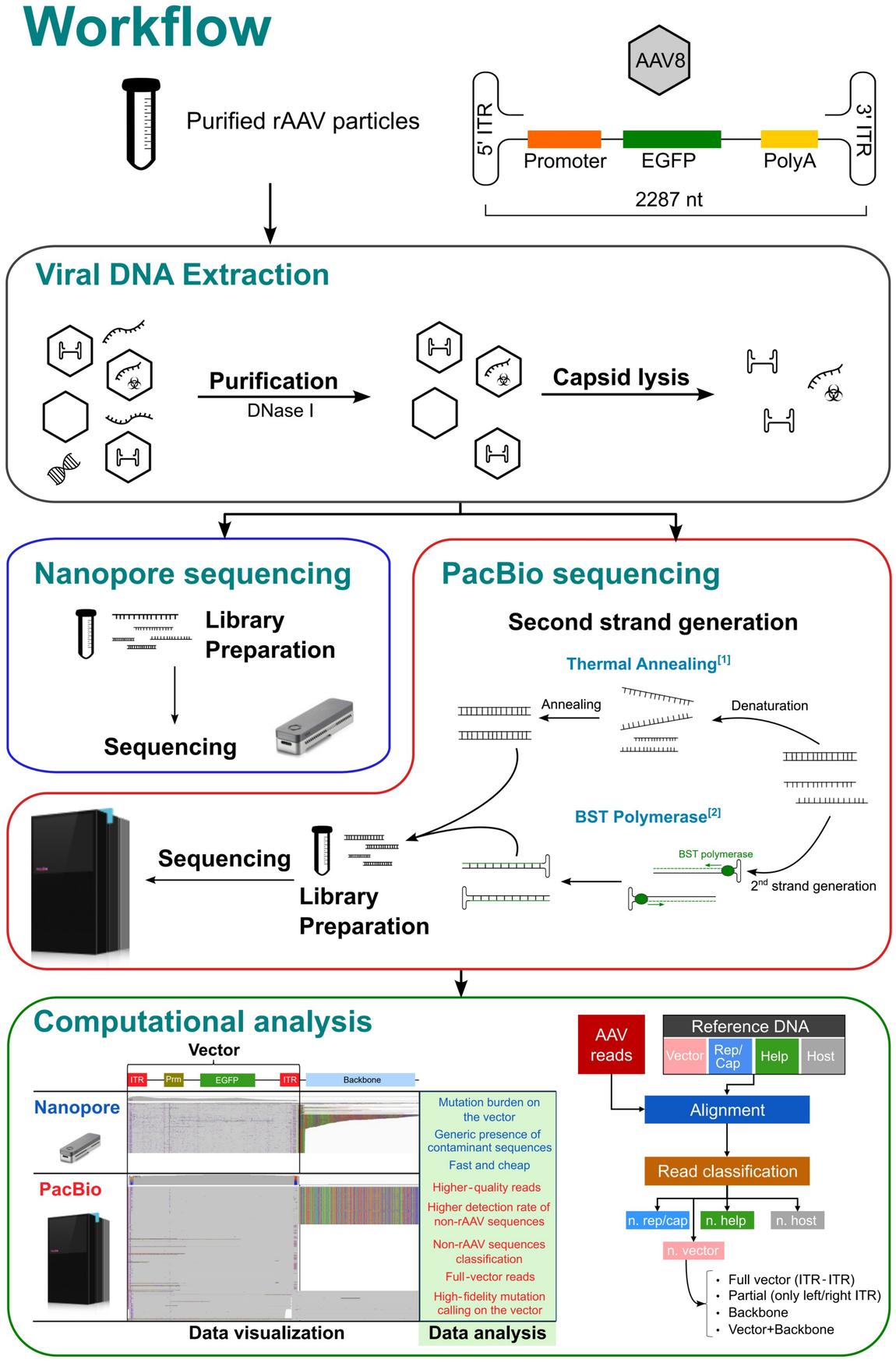
Summary

- Identifying and ensuring the quality of recombinant AAV (rAAV) products is a challenging, but essential effort for the development of gene therapy products.
- **NewBiologix** (**NBX**) developed an analytical method for the genomic sequencing of rAAV vectors based on **long-reads**, using **PacBio** and **Oxford Nanopore** technologies, together with a comprehensive **bioinformatic pipeline**.
- We compared both technologies with a commercially manufactured rAAV product. **PacBio** sequencing provides **better vector genome coverage** (10% of reads covering the full ITR-to-ITR sequence) as compared to Nanopore (~0.5%). **Both technologies** detect **non-rAAV DNA sequences** (~10% of sequences). A significant source of non-rAAV DNA comprises **Rep/Cap** sequences.
- We analyzed encapsidated rAAV DNA produced by our **NBX-HEK293** cell line, revealing **higher genome quality and integrity** when compared to the **leading commercially available cell line**
- NBX aims to provide **quantitative** and **qualitative data** on the **encapsidated** genomic DNA of **rAAVs** as well as enhance the detection and classification of non-rAAV DNA sequences.

Workflow



Results

PacBio sequencing best evaluates vector integrity

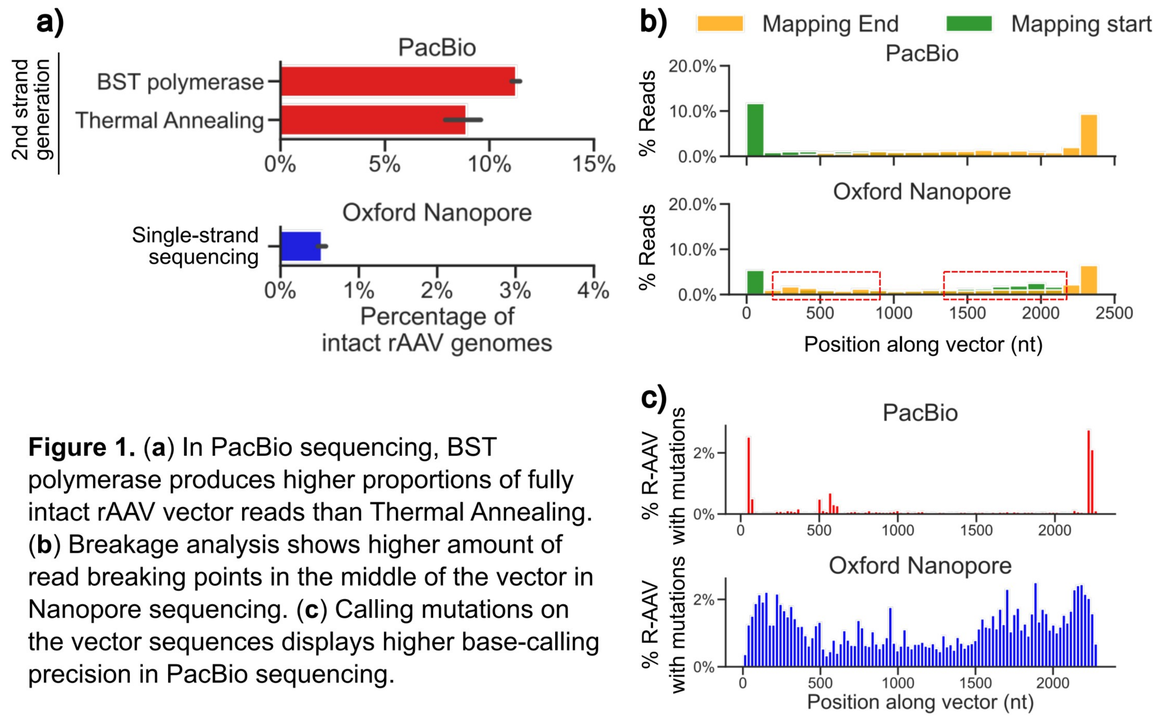


Figure 1. (**a**) In PacBio sequencing, BST polymerase produces higher proportions of fully intact rAAV vector reads than Thermal Annealing. (**b**) Breakage analysis shows higher amount of read breaking points in the middle of the vector in Nanopore sequencing. (**c**) Calling mutations on the vector sequences displays higher base-calling precision in PacBio sequencing.

PacBio and Nanopore can detect non-rAAV sequences

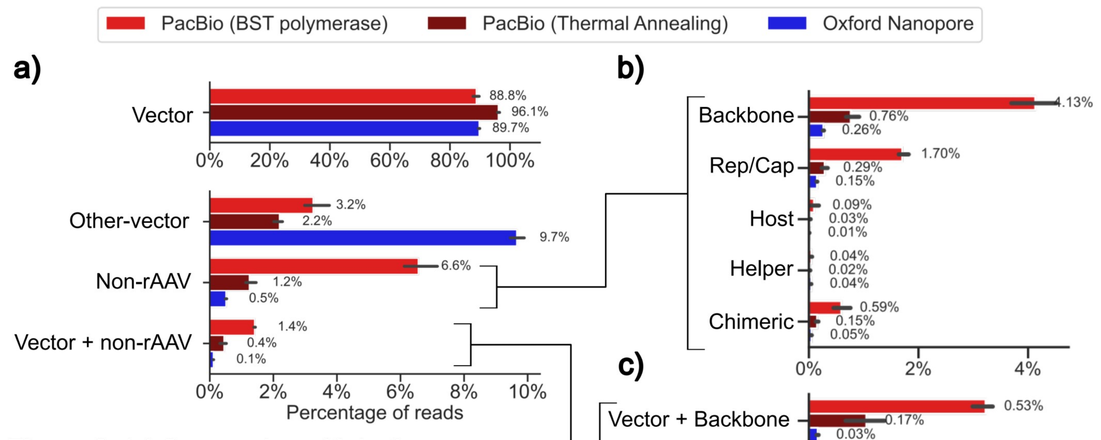


Figure 2. (**a**) Sequencing with both technologies identified different types of non-rAAV sequences. (**b**) The majority are constituted of backbone and Rep/Cap plasmid sequences. (**c**) Deeper analysis revealed the presence of vector + non-rAAV sequences.

NBX engineered HEK293 outperforms leading commercial cell line in rAAV production quality

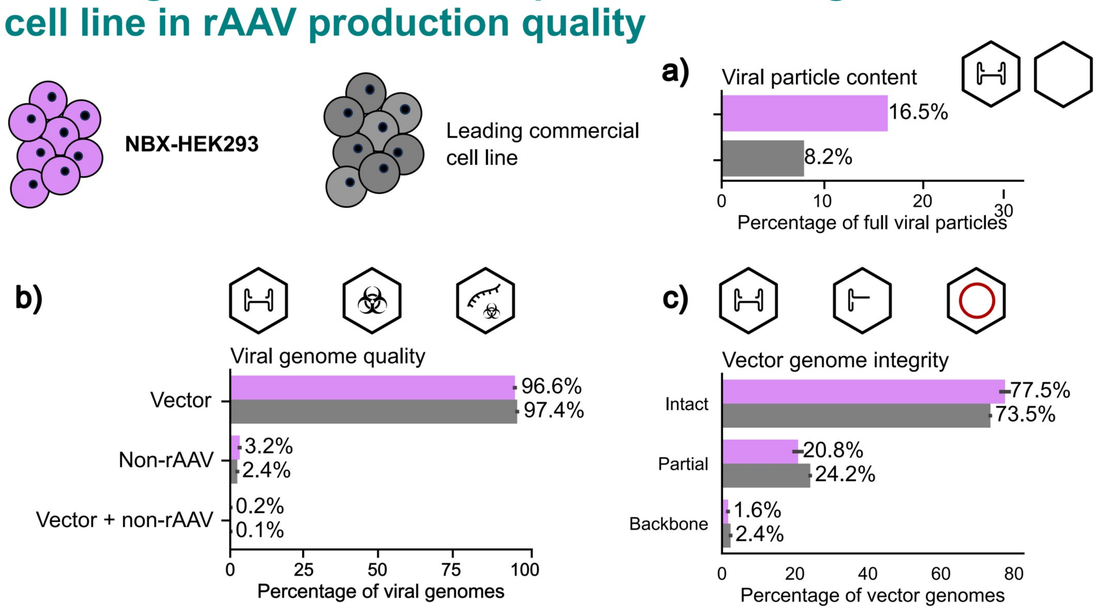


Figure 3. We compared rAAVs produced by our engineered HEK293 cell line with the leading rAAV producing cell line. (**a**) Using mass photometry, NBX-HEK293 displayed higher amount of full capsids. (**b**) Using PacBio sequencing, NBX-HEK293 displayed comparable amount of encapsidated non-rAAV sequences and (**c**) higher percentage of fully intact vector genomes.

Conclusions

NBX offers you **rAAV analytics** solutions using **long-read sequencing**, with the following **services**:

	PacBio	Nanopore
Detection of non-rAAV DNA	✓	✓
Calling alterations along the vector	✓	✓
Assesement of vector integrity	✓	✗

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References

1. Tran, Ngoc Tam, et al. *AAV-genome population sequencing of vectors packaging CRISPR components reveals design-influenced heterogeneity*. Molecular Therapy Methods & Clinical Development 18 (2020).

2. Zhang, Junping, et al. *Thorough molecular configuration analysis of noncanonical AAV genomes in AAV vector preparations*. Molecular Therapy Methods & Clinical Development 32.1 (2024).