NICKING LOOP™: REDEFINING LIBRARY PREP FOR THE NEXT SEQUENCING ERA

Simona Adamusová[1,2], Nea Laine[1], Anttoni Korkiakoski[1,2], Tatu Hirvonen[1], Anna Musku[1], Tuula Rantasalo[1], Jorma Kim[1], Juuso Blomster[1,2], Jukka Laine[1,2,3], Ian McLaughlin[4], Manu Tamminen[1,2], Juha-Pekka Pursiheimo[1]

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SCAN ME

PCR-FREE PREP FOR SIMPLICITY AND PRECISION

Library prep methods produce PCR-amplified linear libraries that must be converted into circular form for new sequencers, creating complexity and bias

Nicking Loop™ converts linear DNA into circular ssDNA, creating a simple, PCR-free workflow with unbiased amplification for precise sequencing.

UNIVERSAL SEQUENCING READ-OUT

Sequencing platforms require specialized library prep, limiting flexibility and slowing adoption of new sequencing technologies.

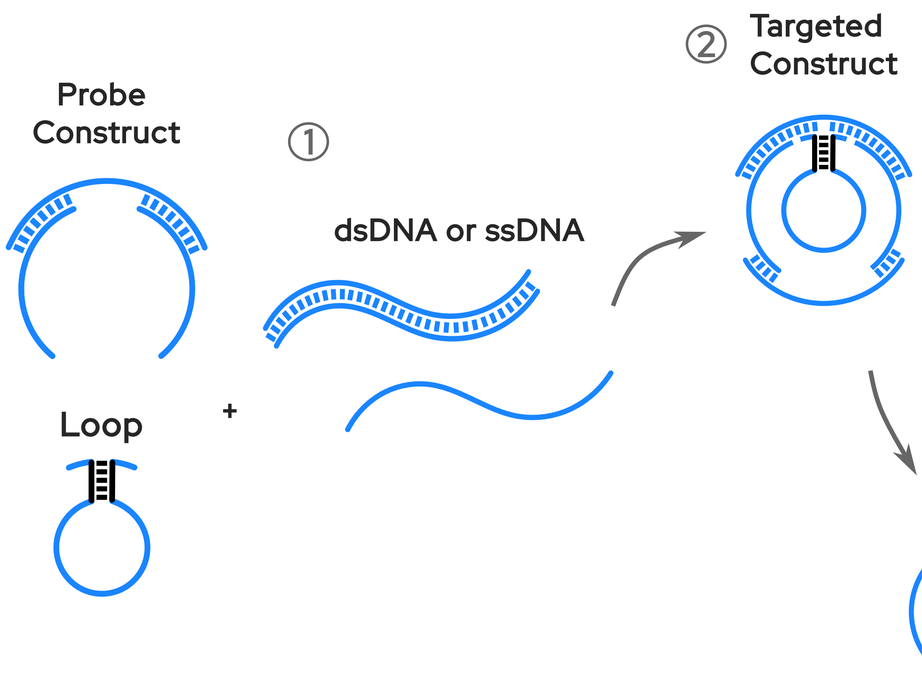
Nicking Loop™ generates circular, concatemeric, and linear ssDNA templates that serve directly as libraries for NGS platforms.

EARLY INDEXING FOR EXTREME SCALABILITY

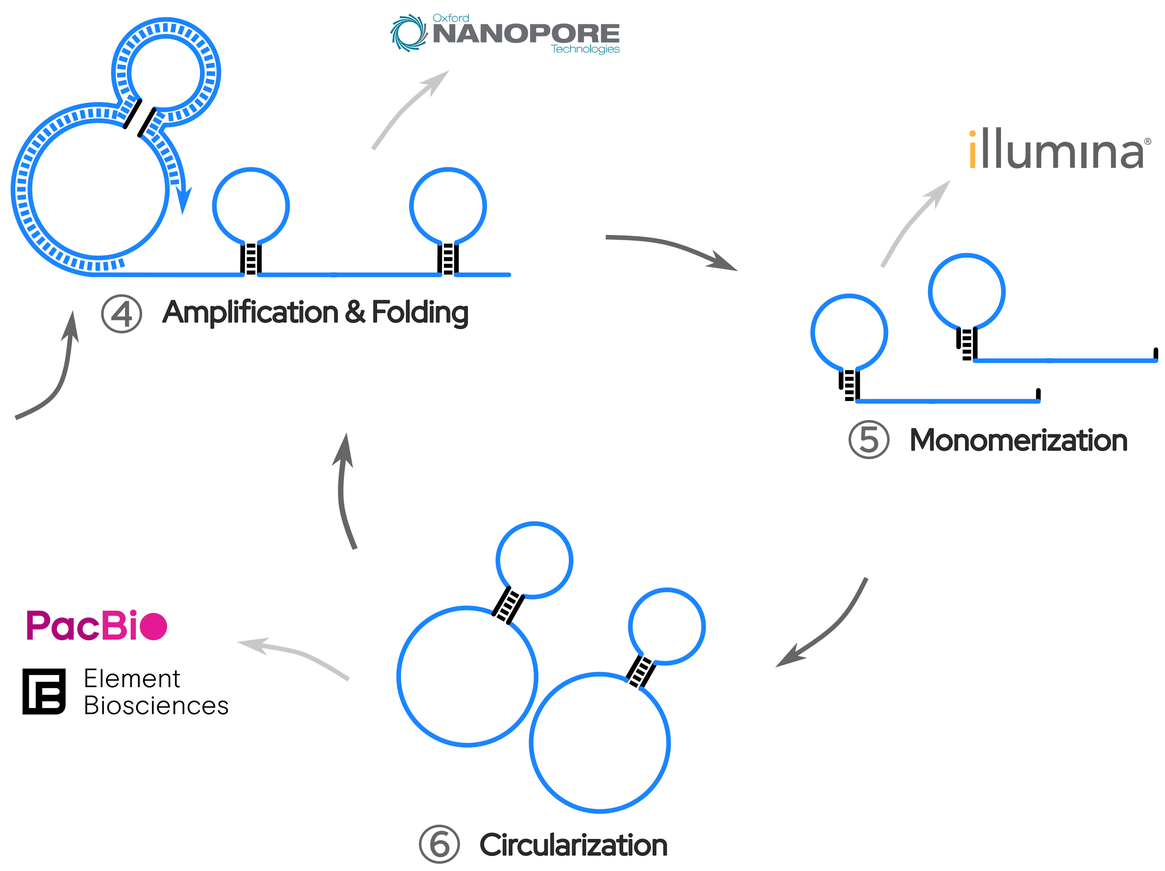
Current workflows require separate processing of each sample, creating bottlenecks and limiting throughput.

Nicking Loop™ enables early sample indexing, allowing first-step sample tagging and pooling, reducing a multi-sample workflow into a single tube.

HOW IS LINEAR DNA CONVERTED TO CIRCULAR?



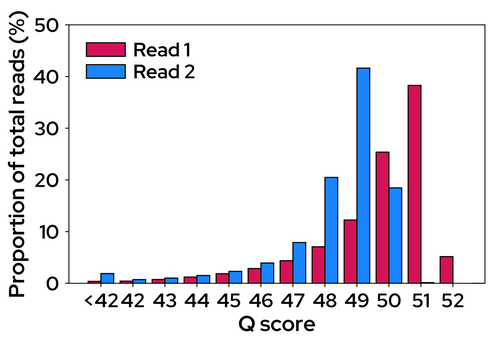
HOW ARE DIFFERENT LIBRARIES PRODUCED?



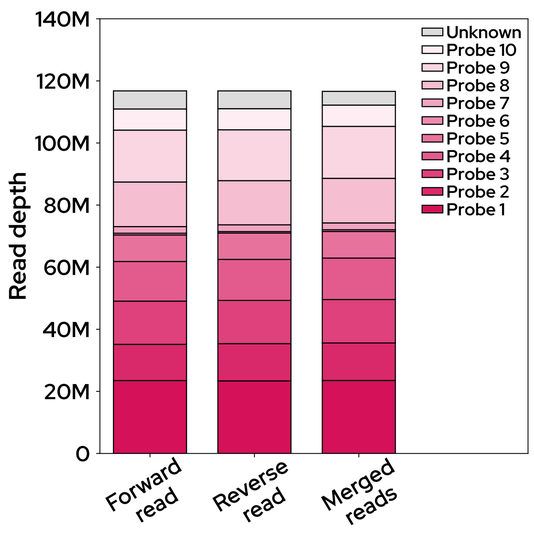
(1) The Probe Construct combines a bridge and probes targeting dsDNA or ssDNA. Loop enables amplification and carries sample indices or functional motifs. (2) The Targeted Construct is formed when all components are bound together. (3) Nicking Loop Converted DNA is produced after gaps are sealed and excess oligomers or DNA are degraded.

(4) Amplification is initiated by a strand-displacing enzyme from a DNA primer, producing concatemers that fold into Loop structures. (5) These are cleaved into monomers by a nicking endonuclease, which then (6) fold on themselves and are ligated, completing circularization. For cyclical amplificiation, steps 4-6 can be repeated. Intermediate products serve as libraries for various NGS platforms.

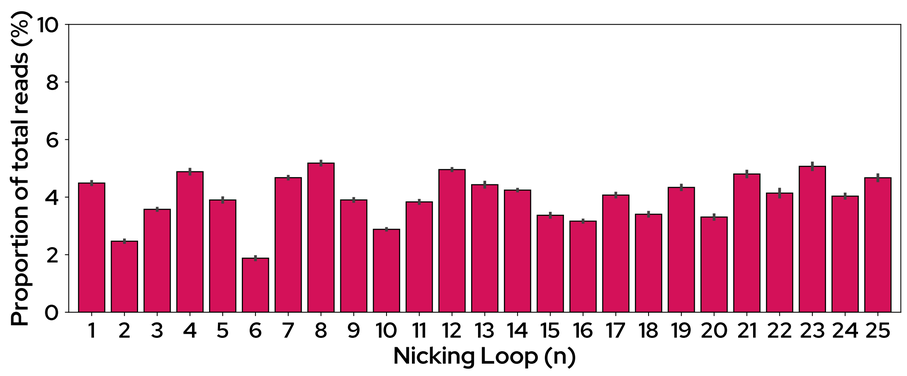
DIRECT SEQUENCING OF CIRCULAR LIBRARIES



Direct circular sequencing on PacBio Onso™ yielded high Q scores and uniform read composition across ten probes for both forward and reverse reads.

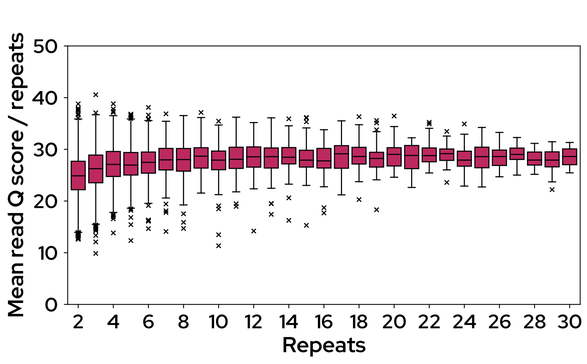


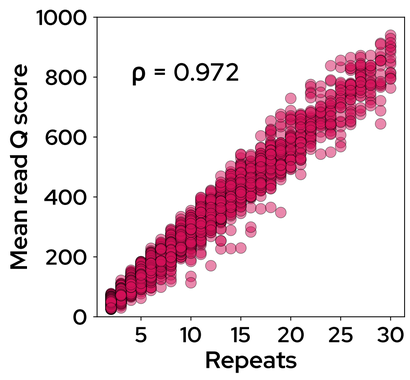
EARLY SAMPLE INDEXING WITH LOOPS



Twenty-five different Loops competed for their incorporation into Nicking Loop™-converted DNA. The Loops performed comparably with no preferential enrichment, supporting feasibility of early sample indexing.

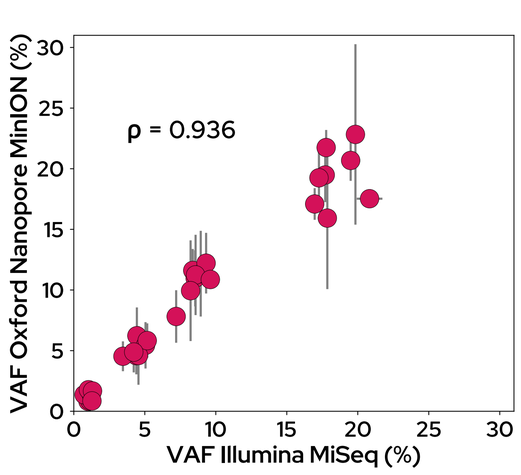
SHORT-READ NANOPORE SEQUENCING





Nicking Loop™ concatemers, built from short-read repeats, form Nanopore-compatible libraries. Aligning repeats improves accuracy with repeat count, reaching unprecedented Q scores (up to 1000).

SEQUENCING OF LINEAR DNA LIBRARIES



Nicking Loop™ concatemers sequenced using Oxford Nanopore and matching linear templates sequenced on Illumina MiSeq showed strong agreement between the variant allele frequencies (VAF) (ρ = 0.936).

[1]Genomill Health Inc., Turku, Finland; [2]University of Turku, Turku, Finland; [3]Department of Pathology, TYKS Laboratories, Turku University Hospital, Turku, Finland; [4]Pacific Biosciences, Menlo Park, CA, USA

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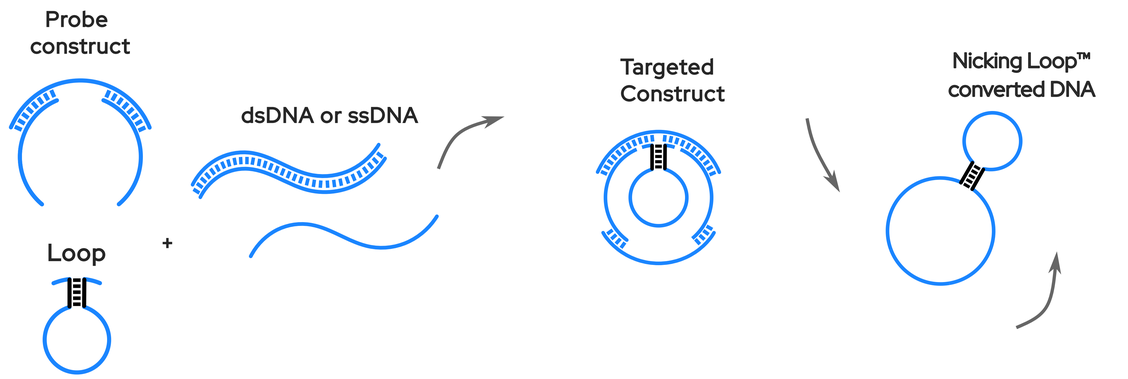
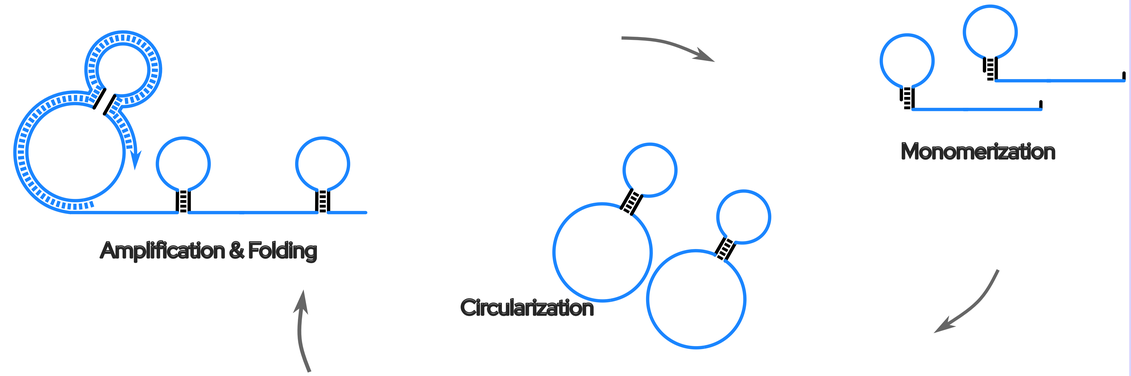


Bridge holds probes and Loop together.
Probes target DNA of interest.
Loop enables circularization, cyclical amplification, carries samples indices and functional motives.

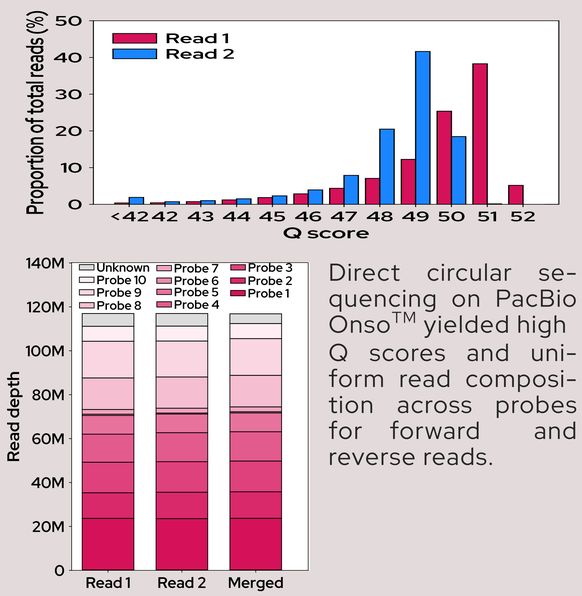
All components and targeted DNA are bound together Polymerase and ligase close the gaps, bridge and leftover DNA are degraded.

HOW ARE DIFFERENT LIBRARIES PRODUCED?



Amplification is initiated by strand displacing enzyme from DNA primer. Nascent concatemer folds on its own, creating a Loop structure. Folded concatemer is cleaved into the monomers by nicking endonuclease. Monomers folds on intself and nick in the loop is ligated

DIRECT CIRCULAR SEQUENCING



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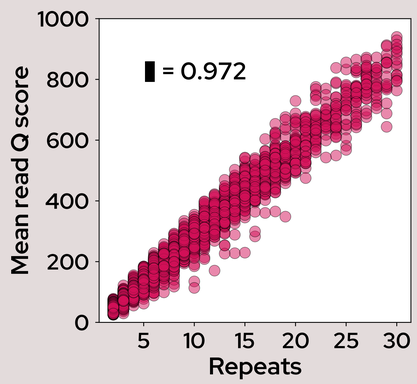
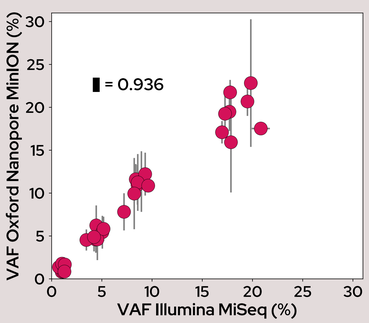
SHORT READ NANOPORE SEQUENCING



Nicking Loop™ concatemers enables sequenced on Oxford Nanopore enables unprecedented short-read quality sequencing.

SEQUENCING OF LINEAR DNA LIBRARIES



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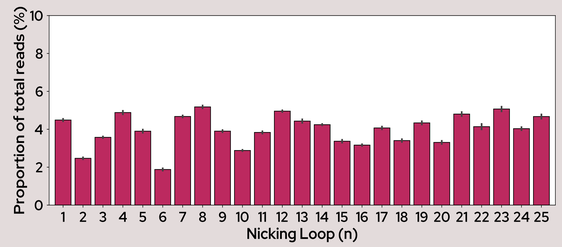

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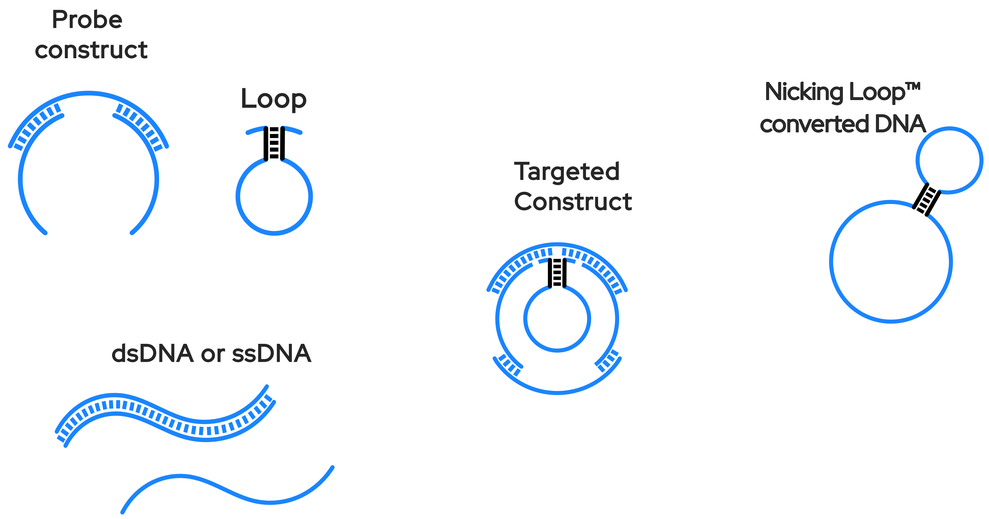

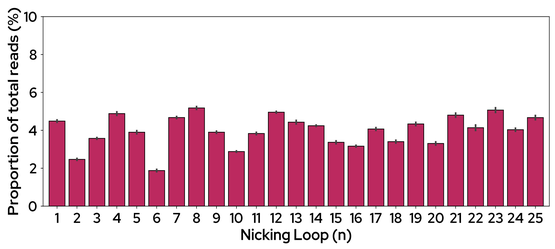

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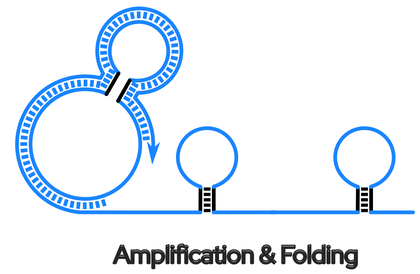

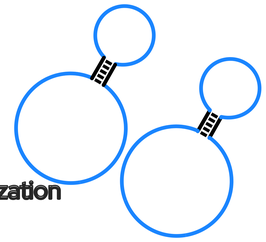

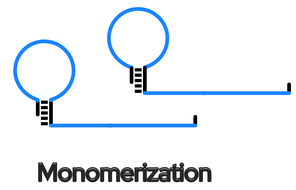

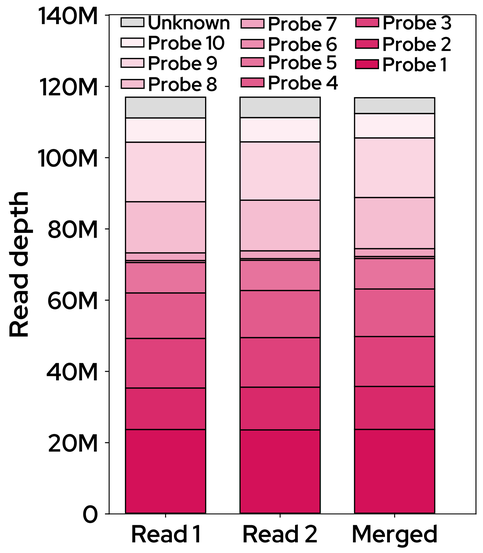

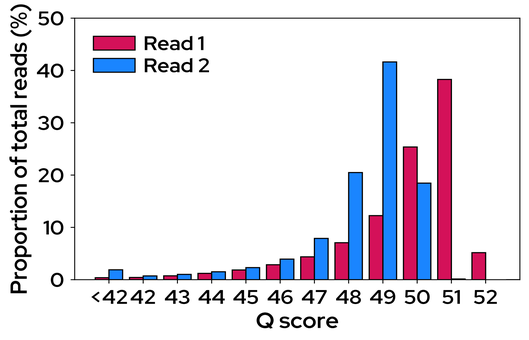

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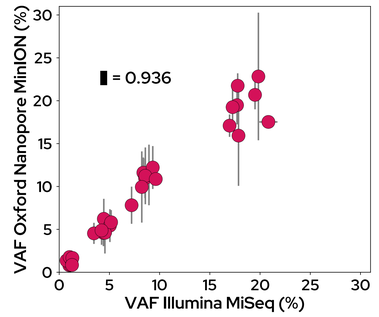

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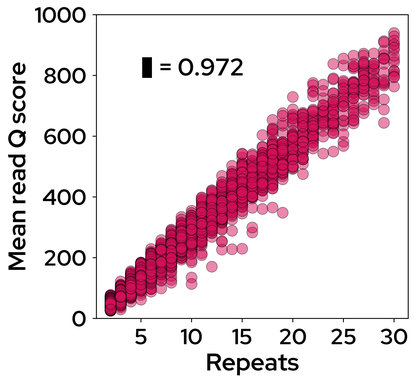

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