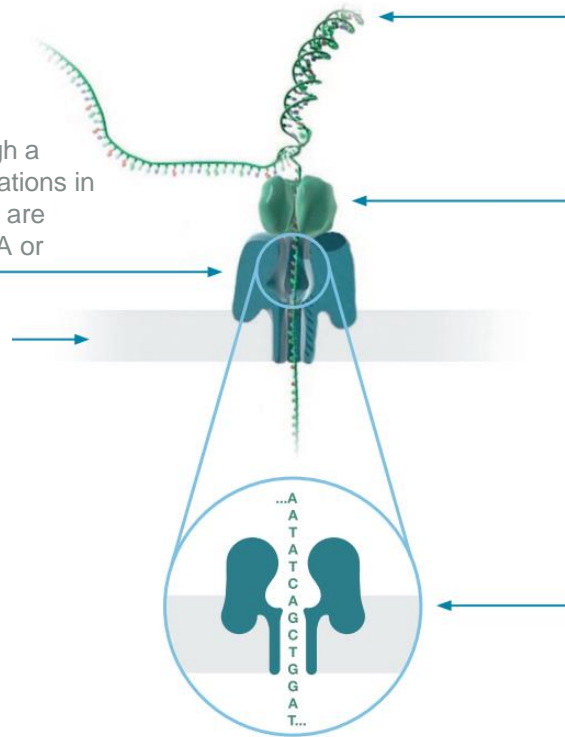


A

Nanopore reader

DNA or RNA passes through a nano-scale hole. The fluctuations in current during translocation are used to determine the DNA or RNA sequence.

An electrically resistant **membrane** means all current must pass through the nanopore, ensuring a clean signal.



The nanopore processes the length of **DNA or RNA** presented to it. The user can control this through the library preparation protocol utilised. (e.g. >2 MB DNAs has been recorded.)

An **enzyme motor** controls the translocation of the DNA or RNA strand through the nanopore. Once the DNA or RNA has passed through, the motor protein detaches and the nanopore is ready to accept the next fragment.

The **nanopore signal**, captured by the ASIC in the device, is characteristic of the sequence of the DNA or RNA fragment. Algorithms are used to convert the signal into basecalls.

B



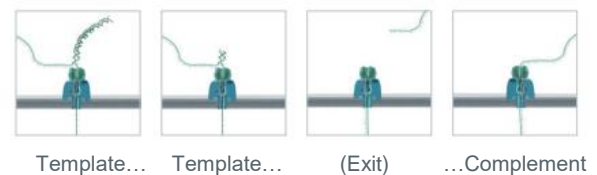
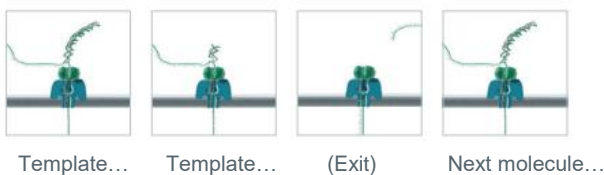
C

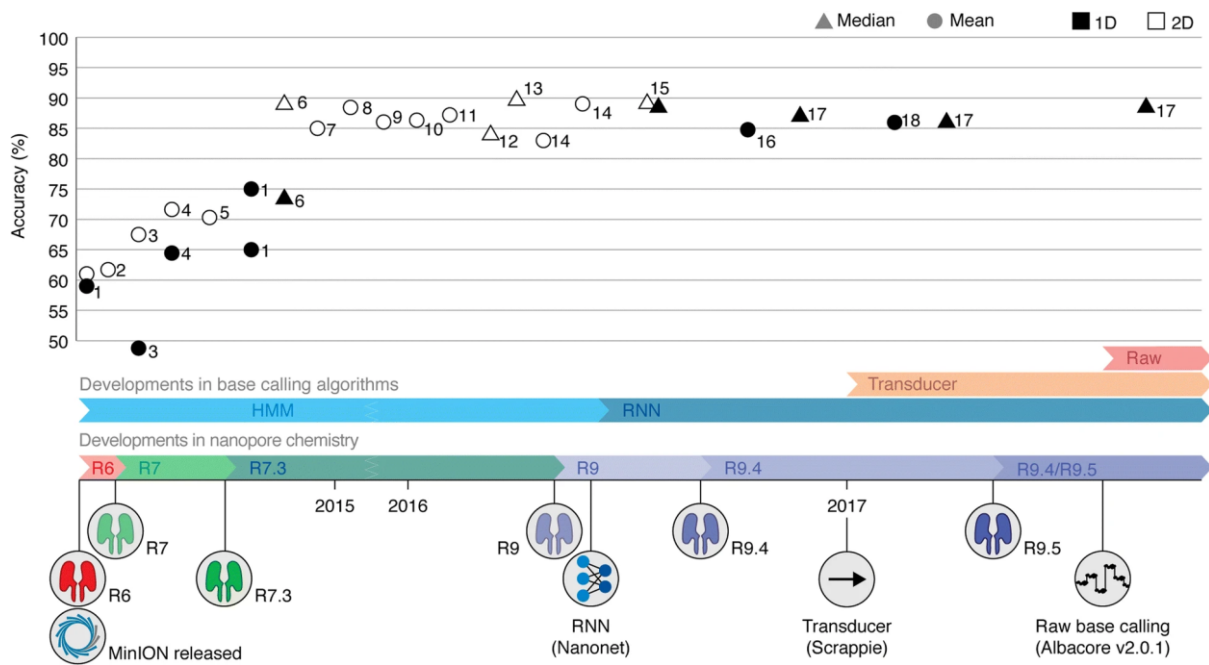
Translocation – 1D

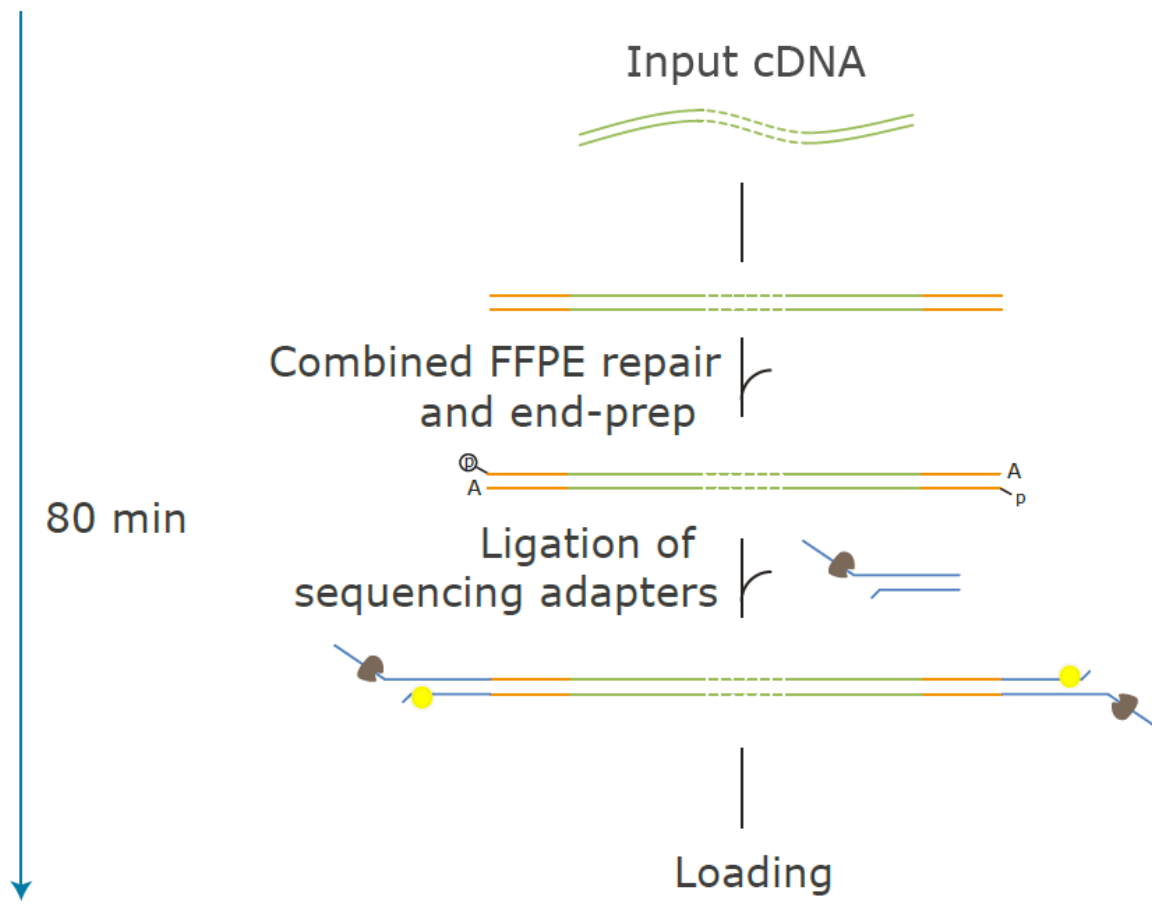
The template and the complement strands are sequenced as individual strands

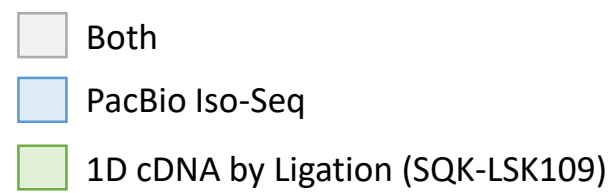
Translocation – 1D²

The 1D² library preparation deploys special adapters that increase the probability that the complement strand will immediately follow the template strand. This method of sequencing when used with 1D² analysis produces a higher accuracy read.









1) cDNA synthesis

Total RNA

cDNA

cDNA

2) Large Scale Amplification

Amplified cDNA

PacBio Isoseq

ONT 1D

3) AMPure Bead
(1x & 0.4x)

1X

0.4X

3) AMPure Bead (0.9x)

0.9X

Qubit, Bioanalyzer

4) Pooled F1 and F2

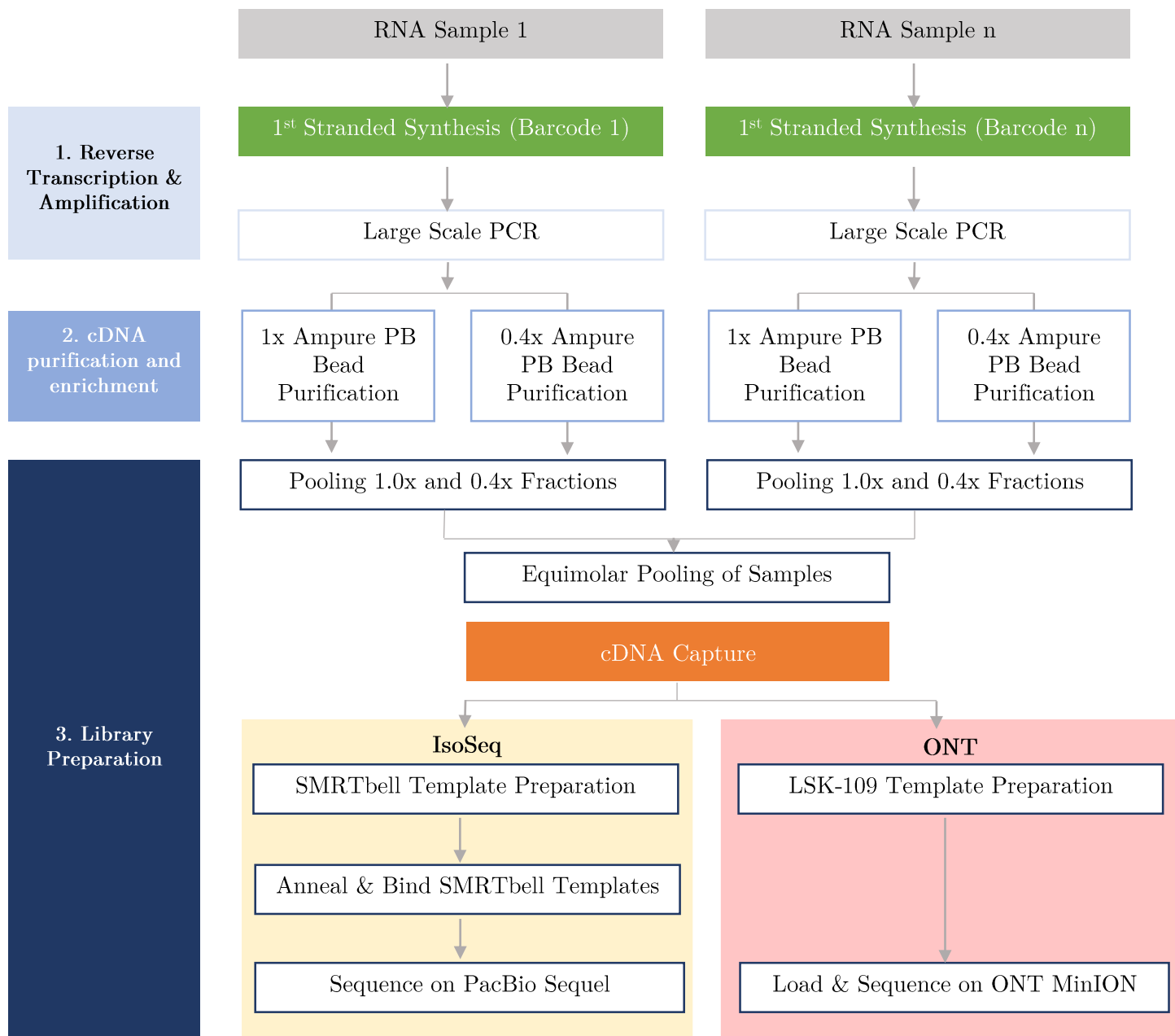
Library Preparation

- 5) Repair DNA Damage
- 6) Repair Ends
- 7) AMPure Bead (1x)
- 8) Blunt Ligation
- 9) Exonuclease treated
- 10) AMPure Bead (1x)
- 11) AMPure Bead (1x)

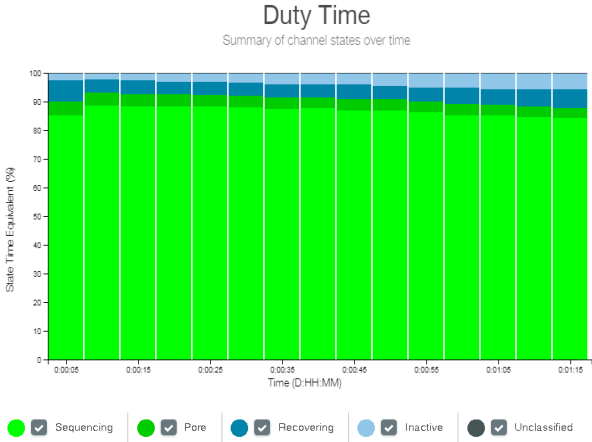
Library Preparation

- 4) Repair DNA Damage
- 5) Repair Ends
- 6) AMPure Bead (1x)
- 7) Adapter Ligation
- 18) AMPure Bead (0.4x)

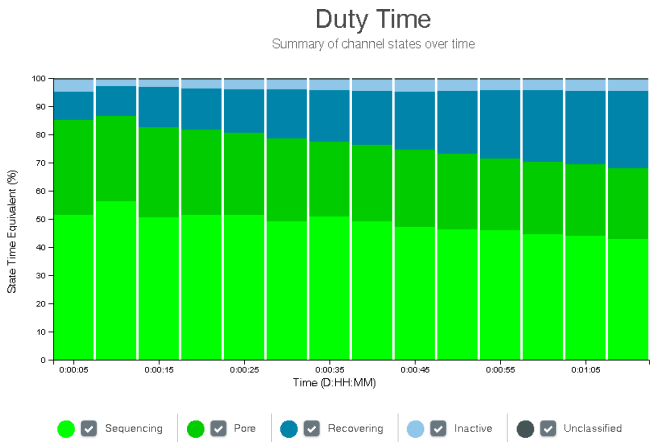
Sequence



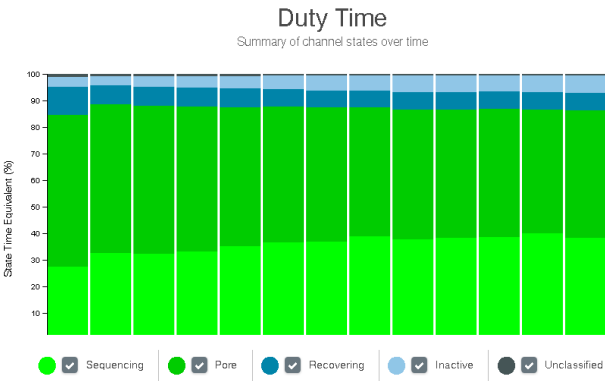
A Good Quality Library



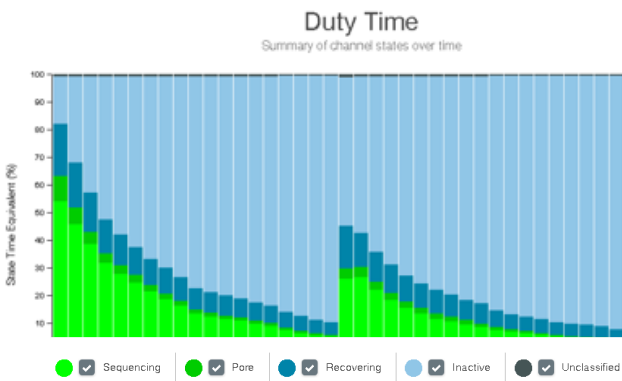
B Channel Blocking



C Low Pore Occupancy



D Flow Cell Failure



1) cDNA synthesis -
with barcode

**2) Large Scale
Amplification**

**3) Pooling - AMPure
Bead (1x & 0.4x)**

4) Target Capture

**SMRTbell Template
Library Preparation**

- 5) Repair DNA Damage
- 6) Repair Ends
- 7) AMPure Bead (1x)
- 8) Blunt Ligation
- 9) Exonuclease treated
- 10) AMPure Bead (1x)
- 11) AMPure Bead (1x)
- 12) Anneal and Bind
SMRTbell templates

RNA Sample 1

RNA Sample n

