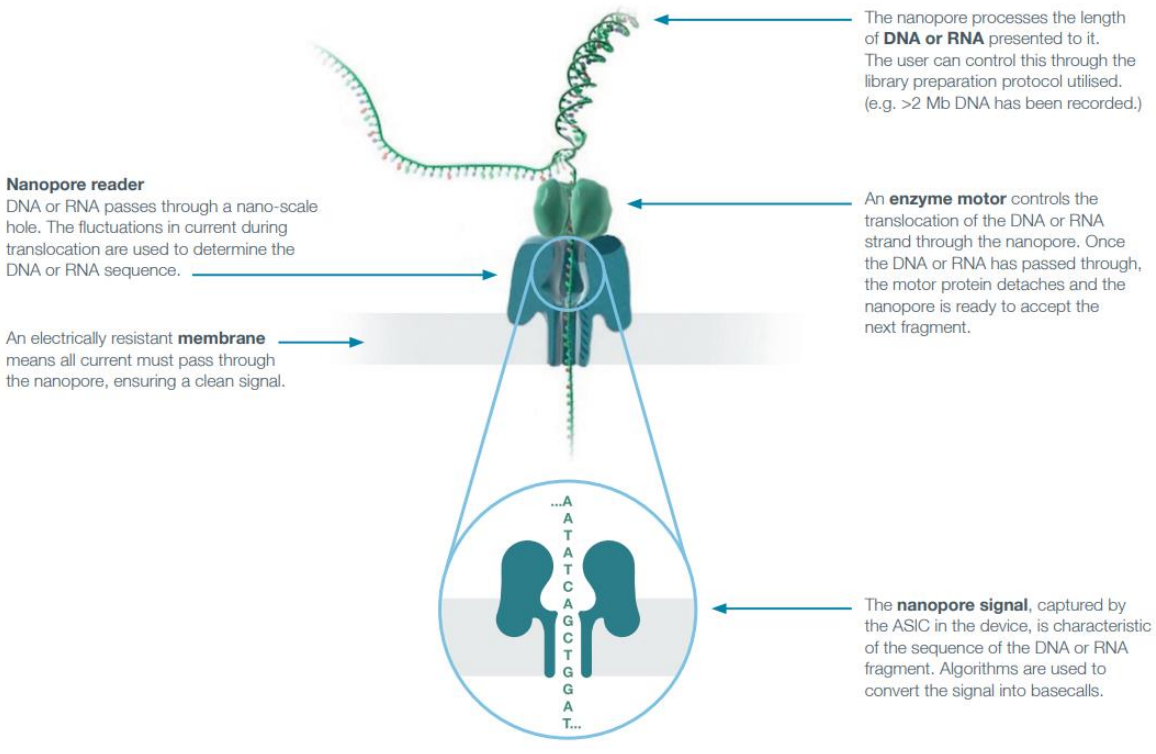
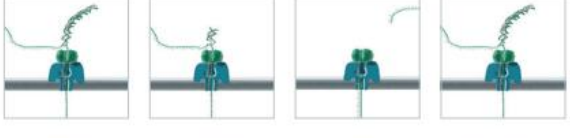
a)



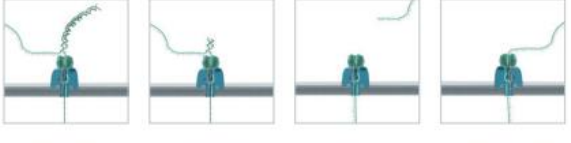
b)

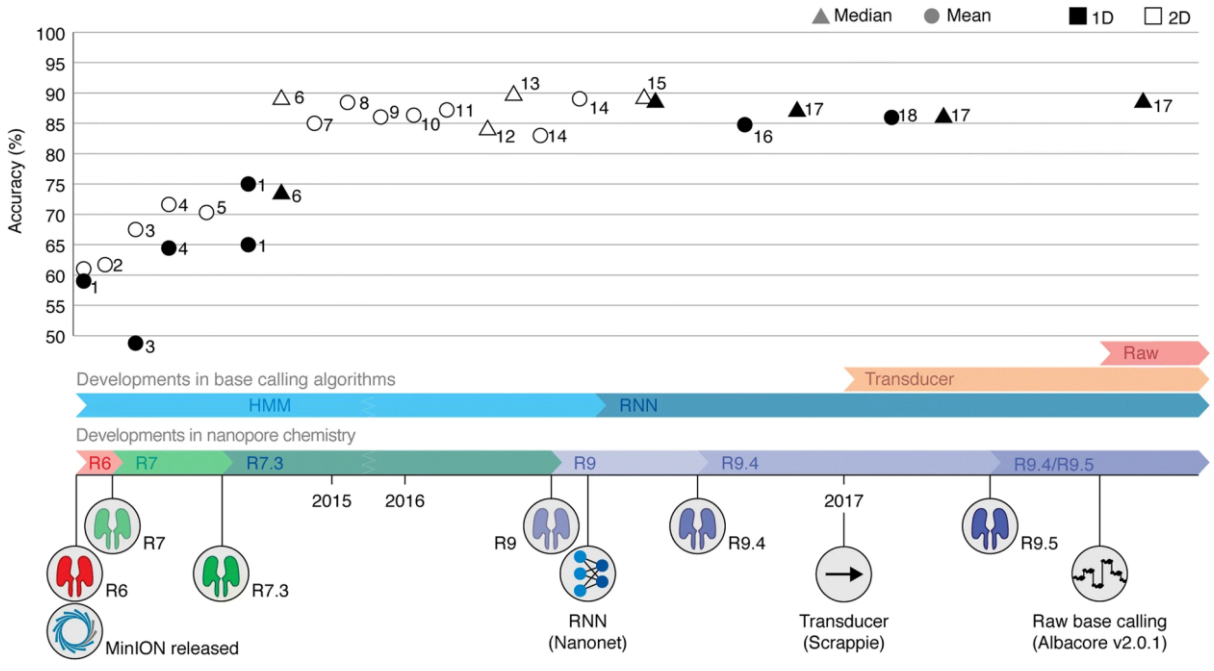
c)

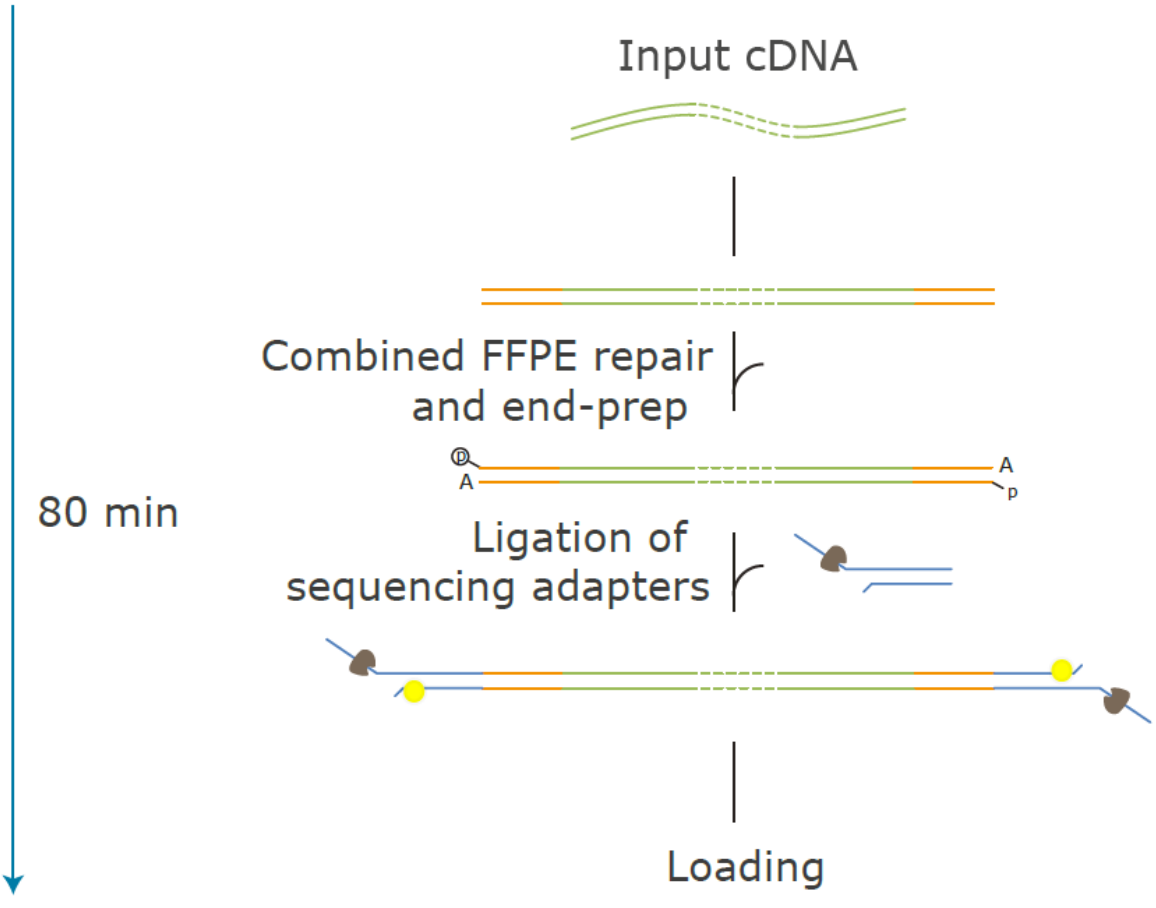
Translocation – 1D
The template and the complement strands are sequenced as individual strands.



Translocation – 1D²
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.




Median
Mean
1D
2D
Accuracy (%)
100
95
90
85
80
75
70
65
60
55
50
Raw
Developments in base calling algorithms
Transducer
HMM
RNN
Developments in nanopore chemistry
R6
R7
R7.3
R9
R9.4
R9.4/R9.5
2015
2016
2017
R7
R6
R7.3
R9
R9.4
R9.5
MinION released
RNN
(Nanonet)
Transducer
(Scrappie)
Raw base calling
(Albacore v2.0.1)

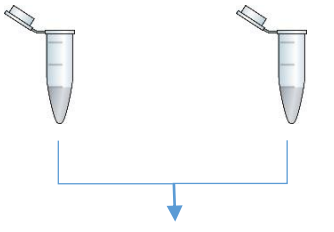

Input cDNA
Combined FFPE repair
and end-prep
80 min
Ligation of
sequencing adapters
Loading

Both
PacBio Iso-Seq
1D cDNA by Ligation (SQK-LSK109)

1) cDNA synthesis

Total RNA

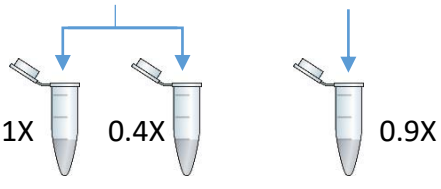
cDNA **cDNA**



2) Large Scale Amplification

Amplified cDNA

PacBio Isoseq **ONT 1D**

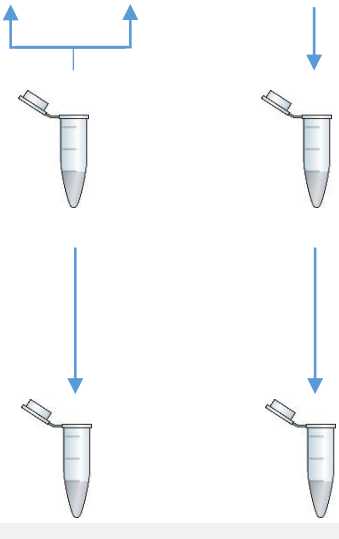


3) AMPure Bead (1x & 0.4x)

3) AMPure Bead (0.9x)

Qubit, Bioanalyzer

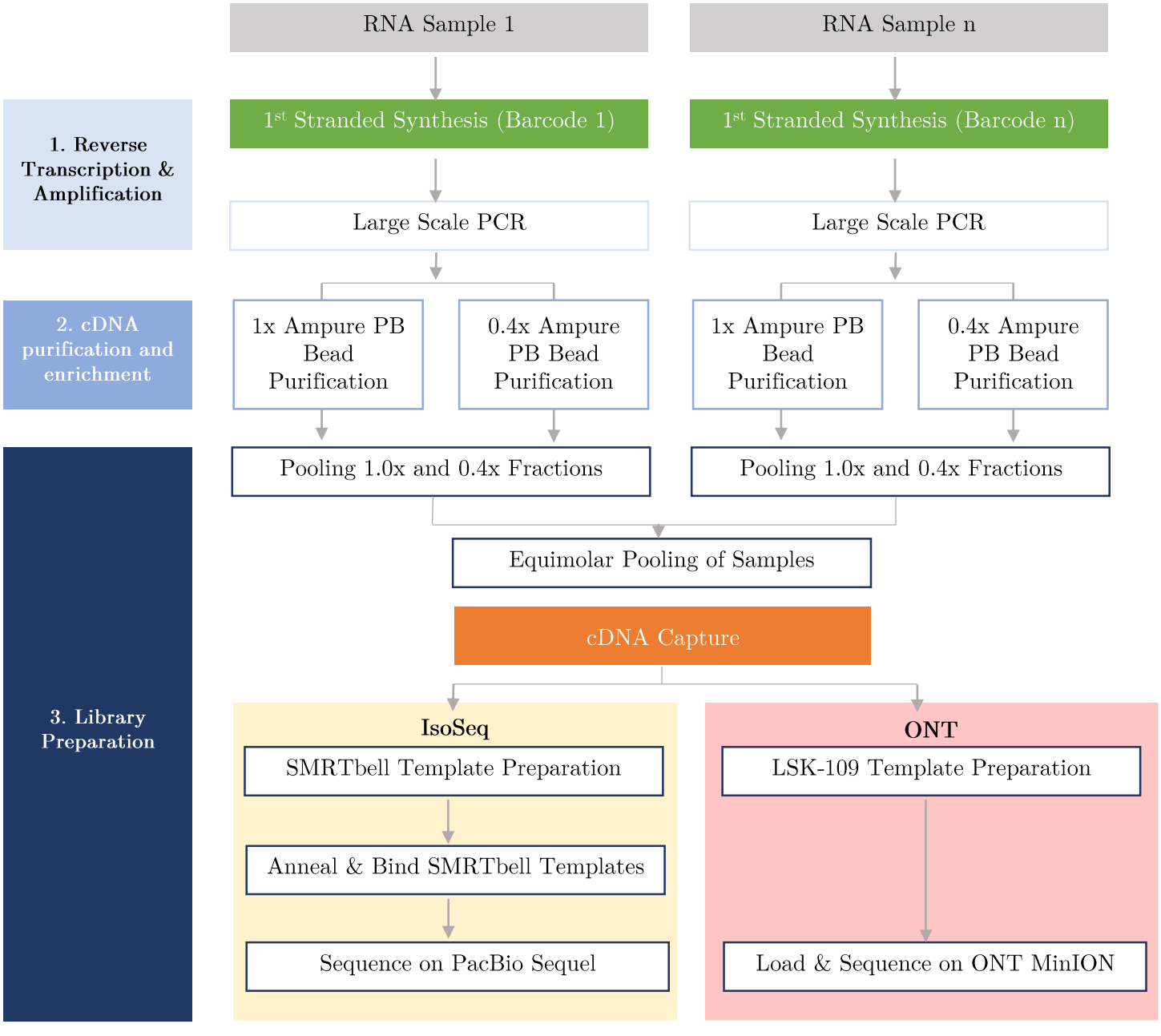
4) Pooled F1 and F2



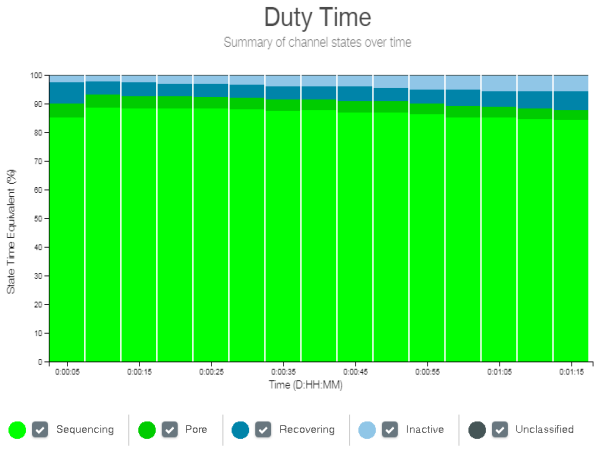
Library Preparation
5) Repair DNA Damage
6) Repair Ends
7) AMPure Bead (1x)
8) Blunt Ligation
9) Exonuclease treated
10) AMPure Bead (1x)
11) AMPure Bead (1x)

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

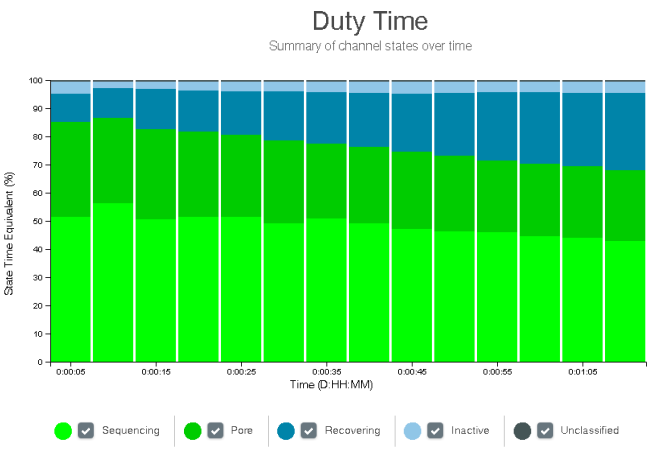
Sequence


1. Reverse Transcription & Amplification
2. cDNA purification and enrichment
3. Library Preparation
RNA Sample 1
RNA Sample n
1st Stranded Synthesis (Barcode 1)
1st Stranded Synthesis (Barcode n)
Large Scale PCR
Large Scale PCR
1x Ampure PB Bead Purification
0.4x Ampure PB Bead Purification
1x Ampure PB Bead Purification
0.4x Ampure PB Bead Purification
Pooling 1.0x and 0.4x Fractions
Pooling 1.0x and 0.4x Fractions
Equimolar Pooling of Samples
cDNA Capture
IsoSeq
SMRTbell Template Preparation
Anneal & Bind SMRTbell Templates
Sequence on PacBio Sequel
ONT
LSK-109 Template Preparation
Load & Sequence on ONT MinION

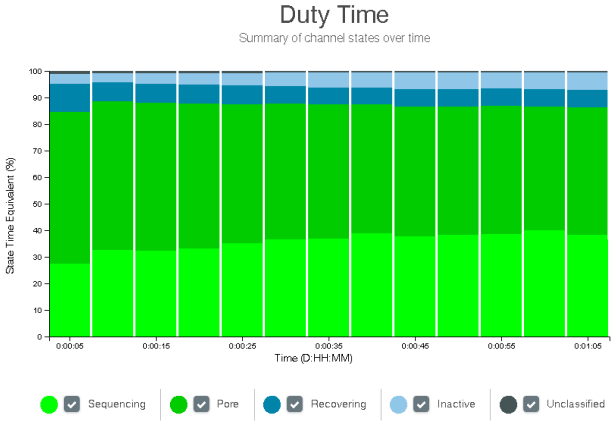
a) Good Quality Library



b) Channel Blocking



c) Low Pore Occupancy



b) Flow Cell Failure