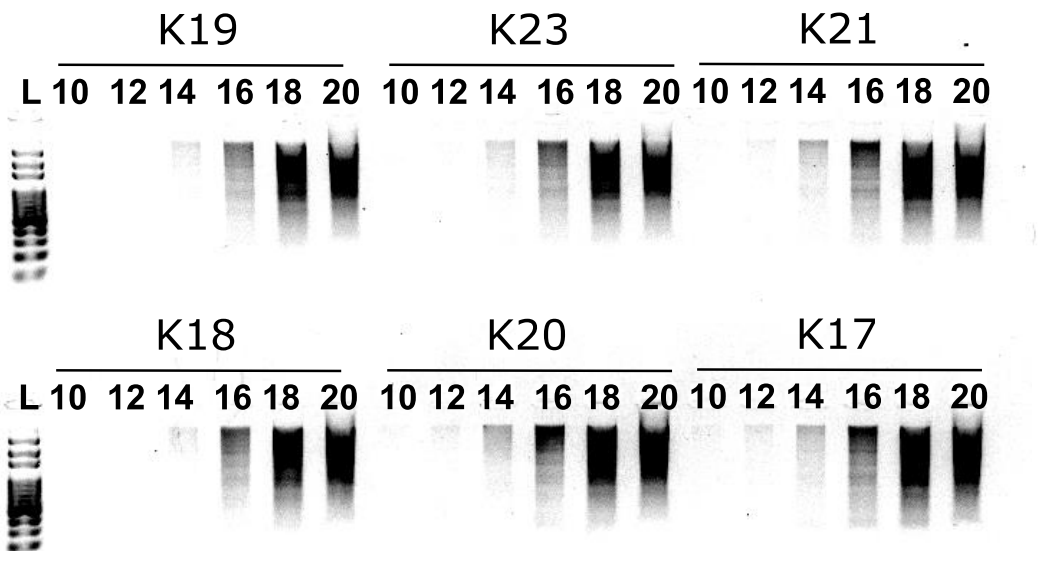
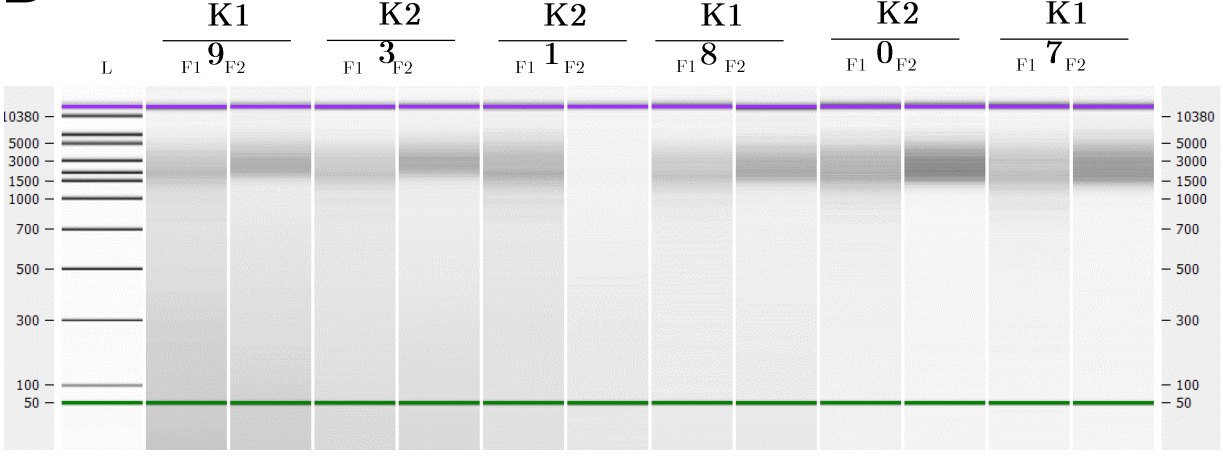
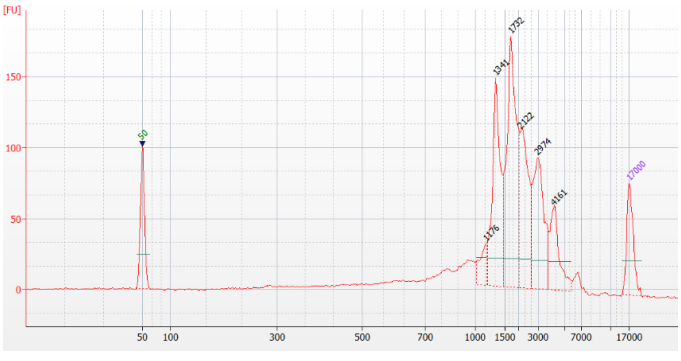
A


K19
K23
K21
L 10 12 14 16 18 20 10 12 14 16 18 20 10 12 14 16 18 20
K18
K20
K17
L 10 12 14 16 18 20 10 12 14 16 18 20 10 12 14 16 18 20

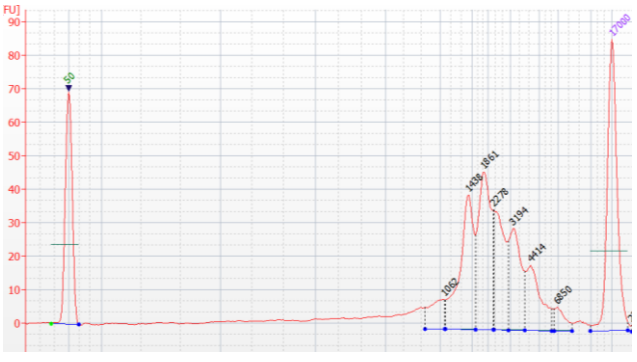
B


L
K1 F1 9 F2
K2 F1 3 F2
K2 F1 1 F2
K1 F1 8 F2
K2 F1 0 F2
K1 F1 7 F2
10380
5000
3000
1500
1000
700
500
300
100
50

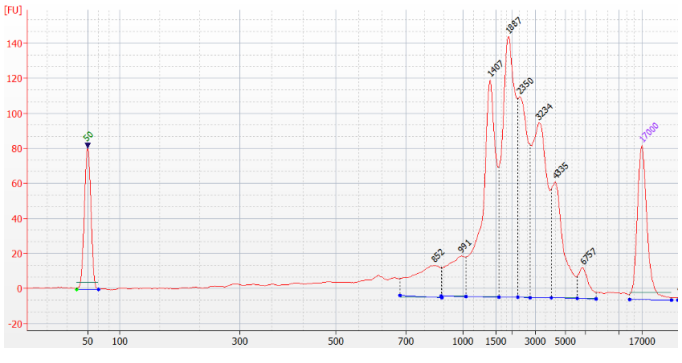
A Batch 1 (Target Capture)



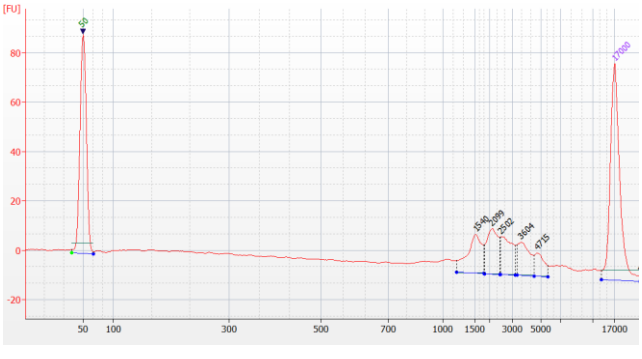
B Batch 1(Final)



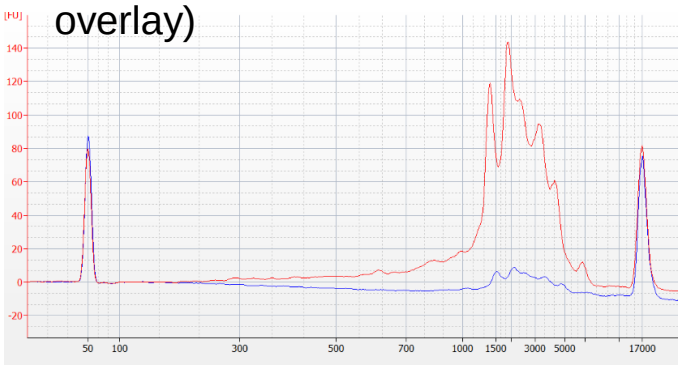
C Batch 2 (Target Capture)



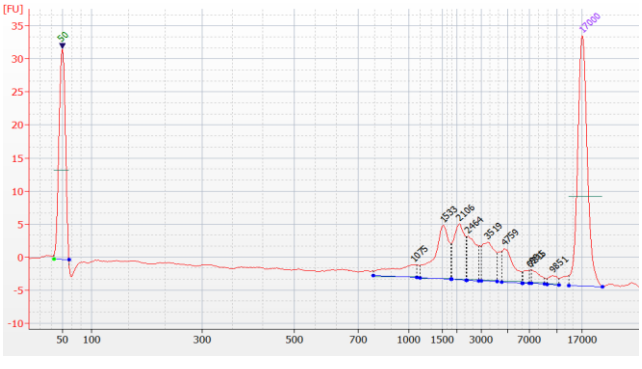
D Batch 2 (Final)



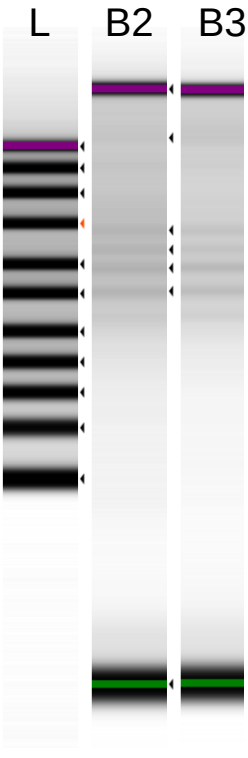
E Batch 2 (Target Capture & Final overlay)



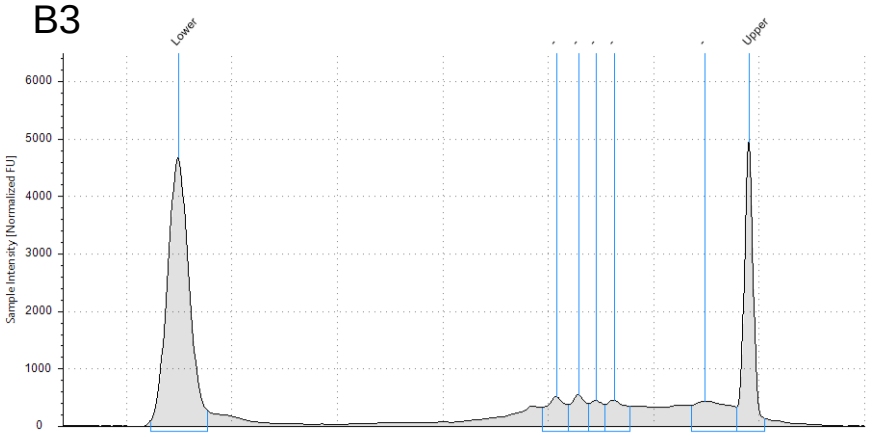
F Batch 3 (Final)

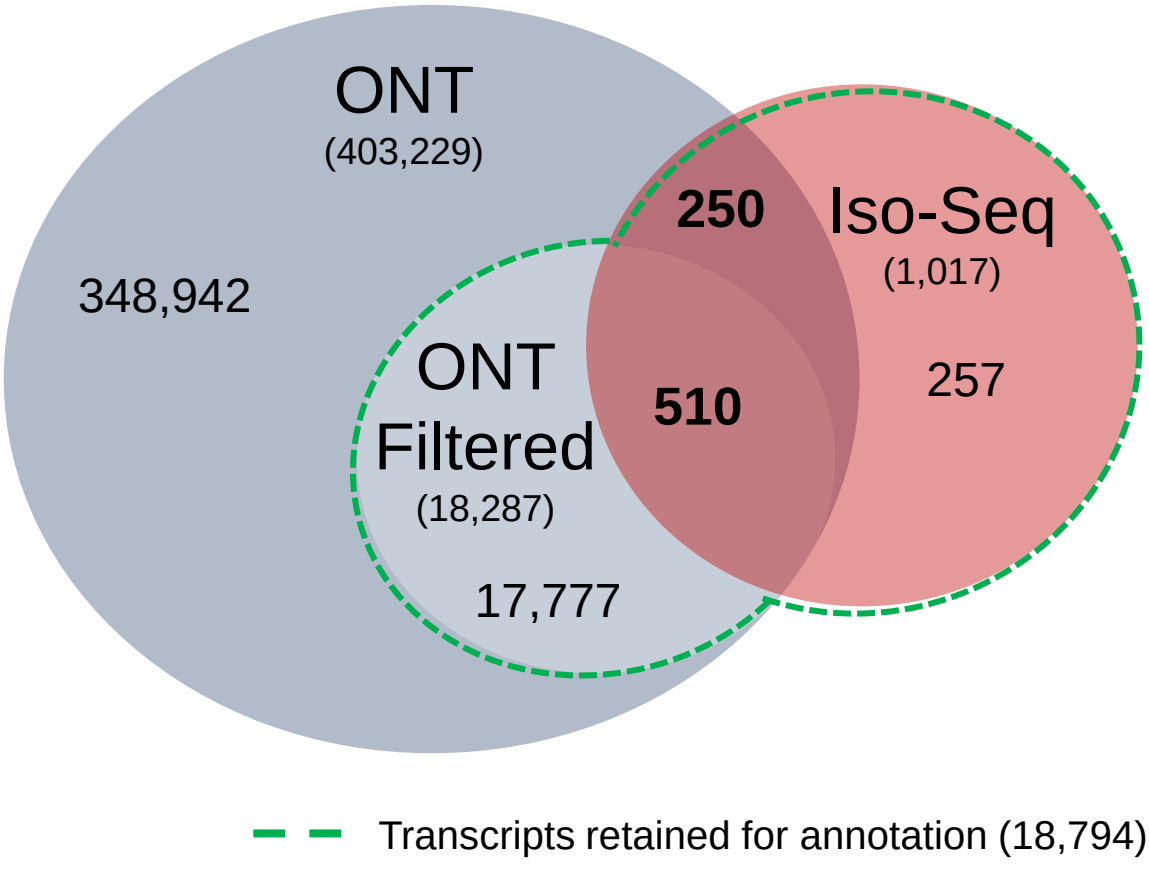


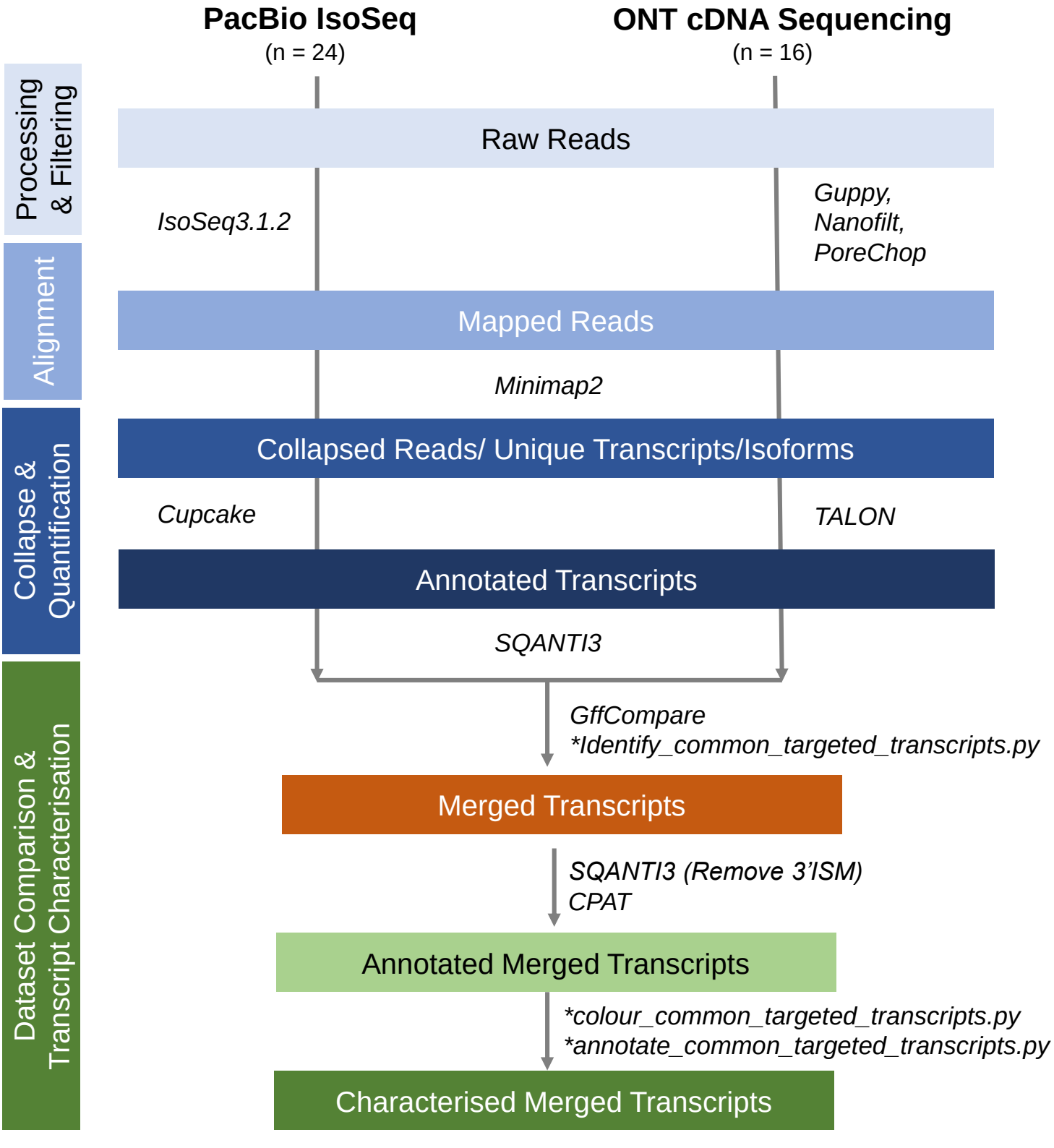
A

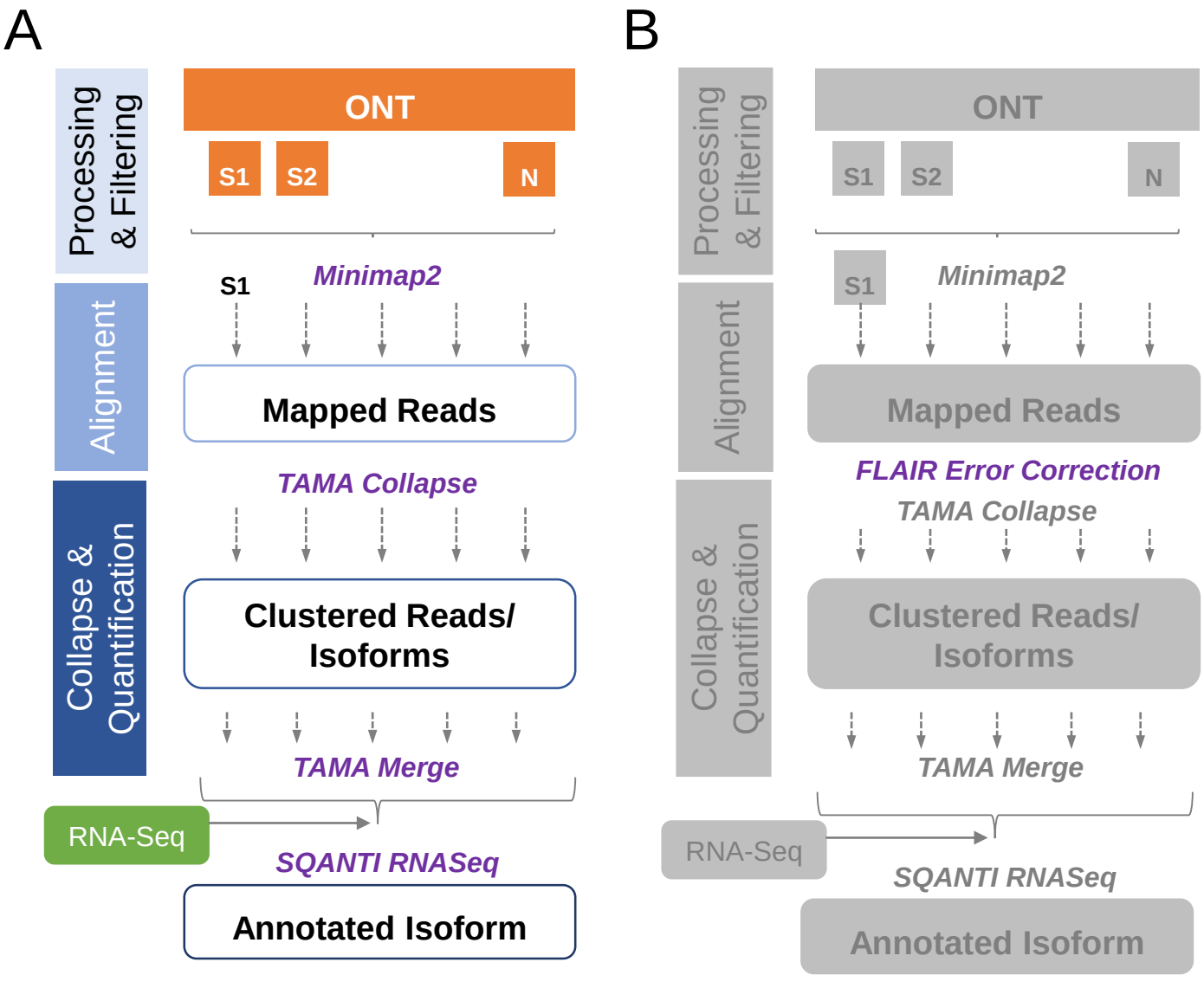

L
B2
B3

B


B3
Lower
Upper
Sample Intensity [Normalized FU]
6000
5000
4000
3000
2000
1000
0


ONT
(403,229)
348,942
250
Iso-Seq
(1,017)
257
ONT
Filtered
(18,287)
510
17,777
Transcripts retained for annotation (18,794)


PacBio IsoSeq
(n = 24)
ONT cDNA Sequencing
(n = 16)
Processing & Filtering
Raw Reads
IsoSeq3.1.2
Guppy, Nanofilt, PoreChop
Alignment
Mapped Reads
Minimap2
Collapse & Quantification
Collapsed Reads/ Unique Transcripts/Isoforms
Cupcake
TALON
Annotated Transcripts
SQANTI3
GffCompare
*Identify_common_targeted_transcripts.py
Dataset Comparison & Transcript Characterisation
Merged Transcripts
SQANTI3 (Remove 3'ISM)
CPAT
Annotated Merged Transcripts
*colour_common_targeted_transcripts.py
*annotate_common_targeted_transcripts.py
Characterised Merged Transcripts


A
Processing & Filtering
Alignment
Collapse & Quantification
ONT
S1
S2
N
S1
Minimap2
Mapped Reads
TAMA Collapse
Clustered Reads/ Isoforms
TAMA Merge
RNA-Seq
SQANTI RNASeq
Annotated Isoform
B
Processing & Filtering
Alignment
Collapse & Quantification
ONT
S1
S2
N
S1
Minimap2
Mapped Reads
FLAIR Error Correction
TAMA Collapse
Clustered Reads/ Isoforms
TAMA Merge
RNA-Seq
SQANTI RNASeq
Annotated Isoform