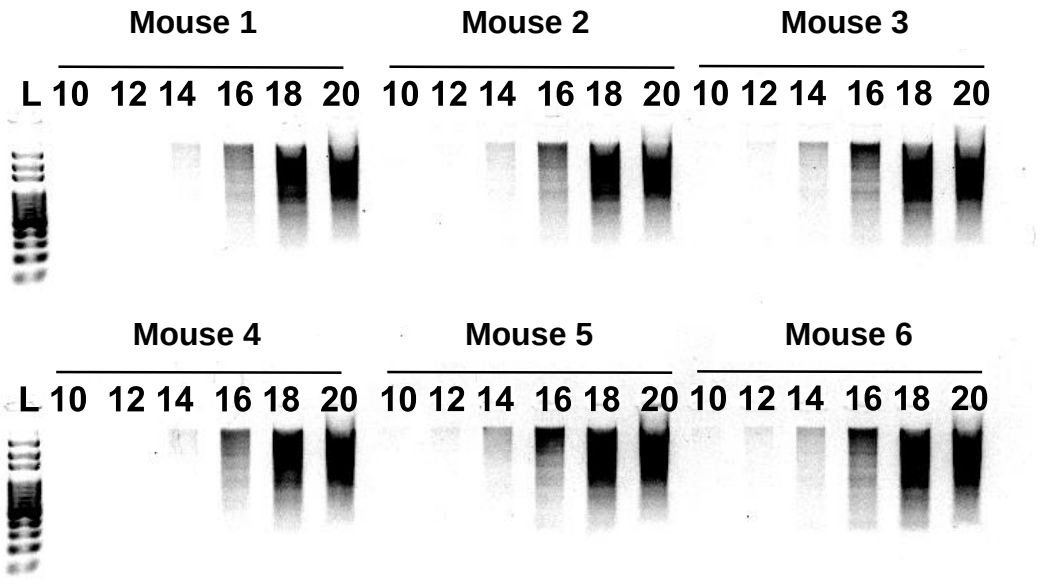
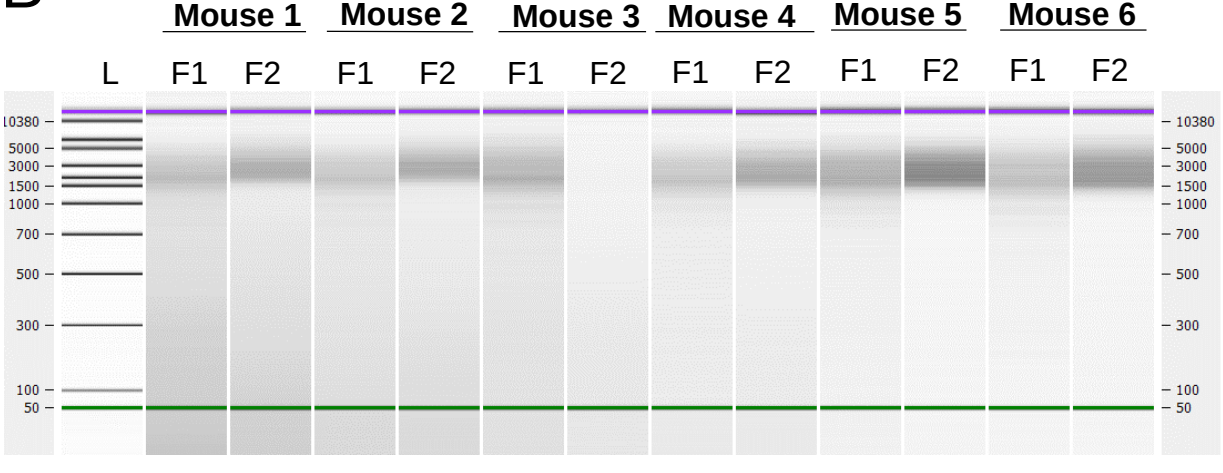
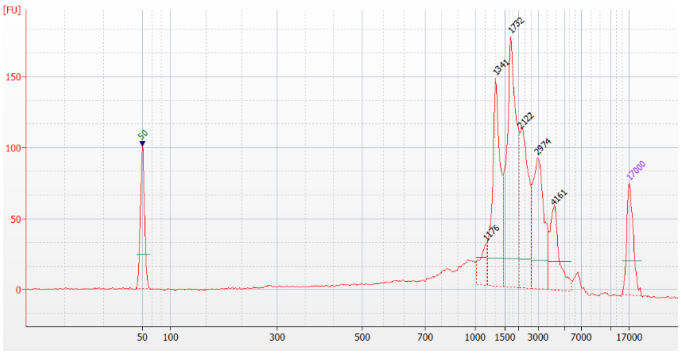
A


Mouse 1
Mouse 2
Mouse 3
L 10 12 14 16 18 20 10 12 14 16 18 20 10 12 14 16 18 20
Mouse 4
Mouse 5
Mouse 6
L 10 12 14 16 18 20 10 12 14 16 18 20 10 12 14 16 18 20

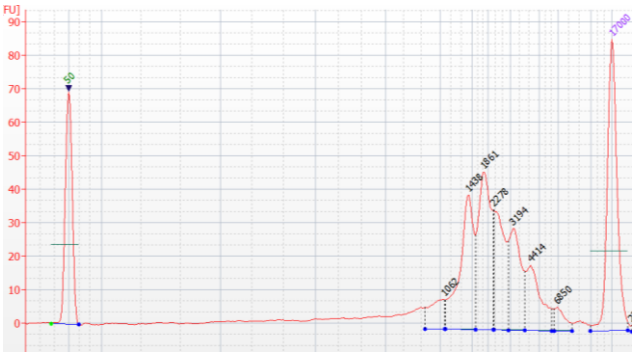
B


Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
L F1 F2 F1 F2 F1 F2 F1 F2 F1 F2 F1 F2
10380
5000
3000
1500
1000
700
500
300
100
50

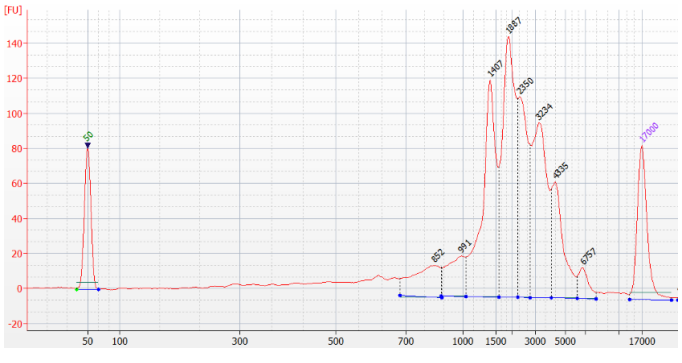
A Batch 1 (Target capture)



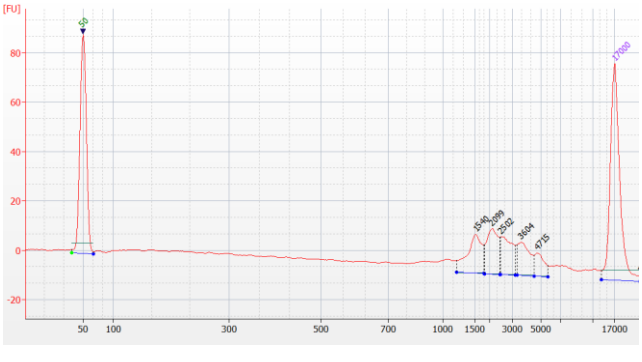
B Batch 1 (Final)



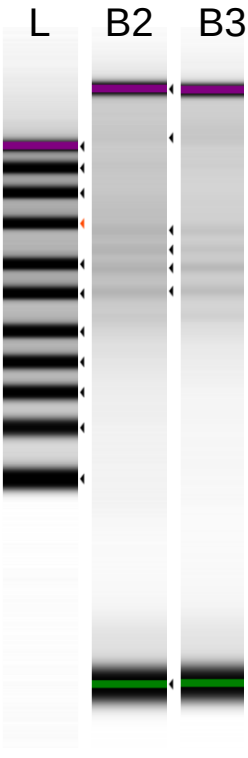
C Batch 2 (Target capture)



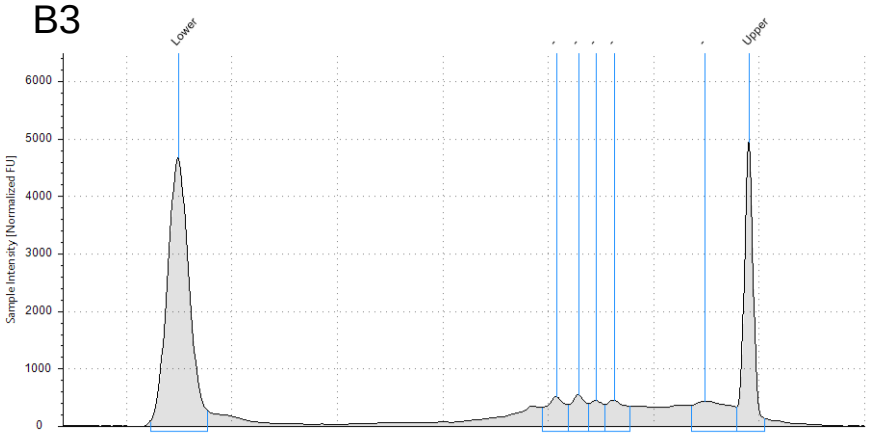
D Batch 2 (Final)

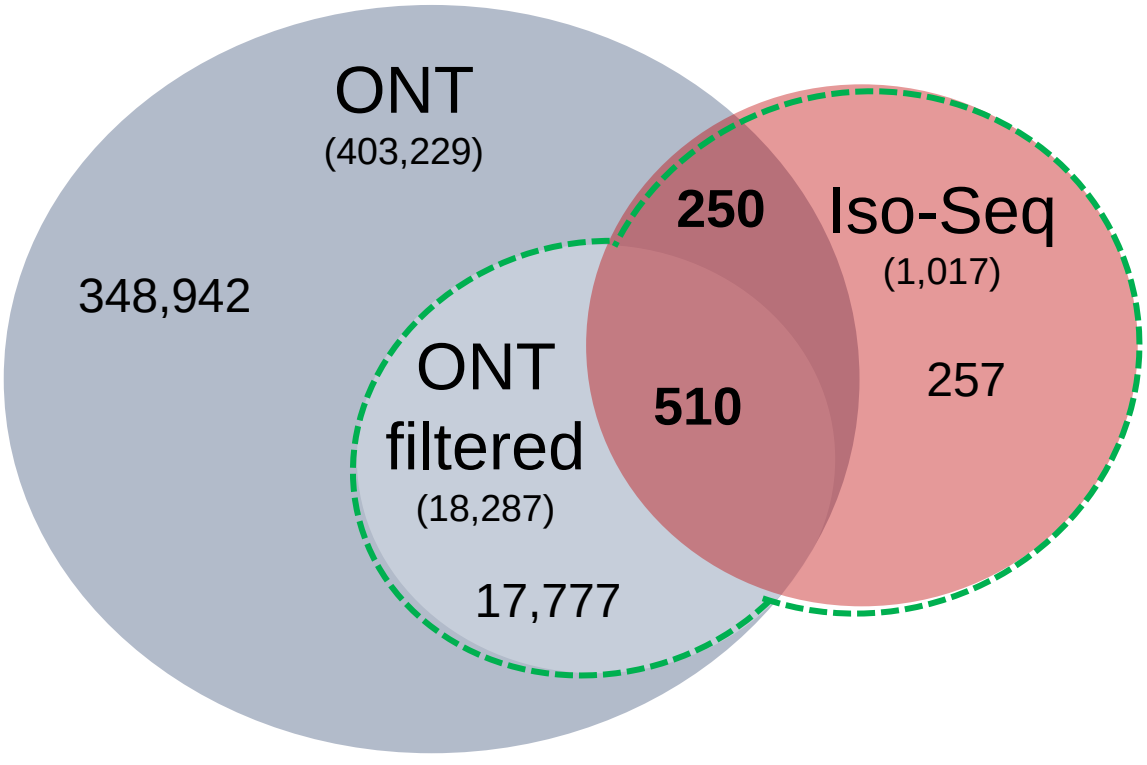


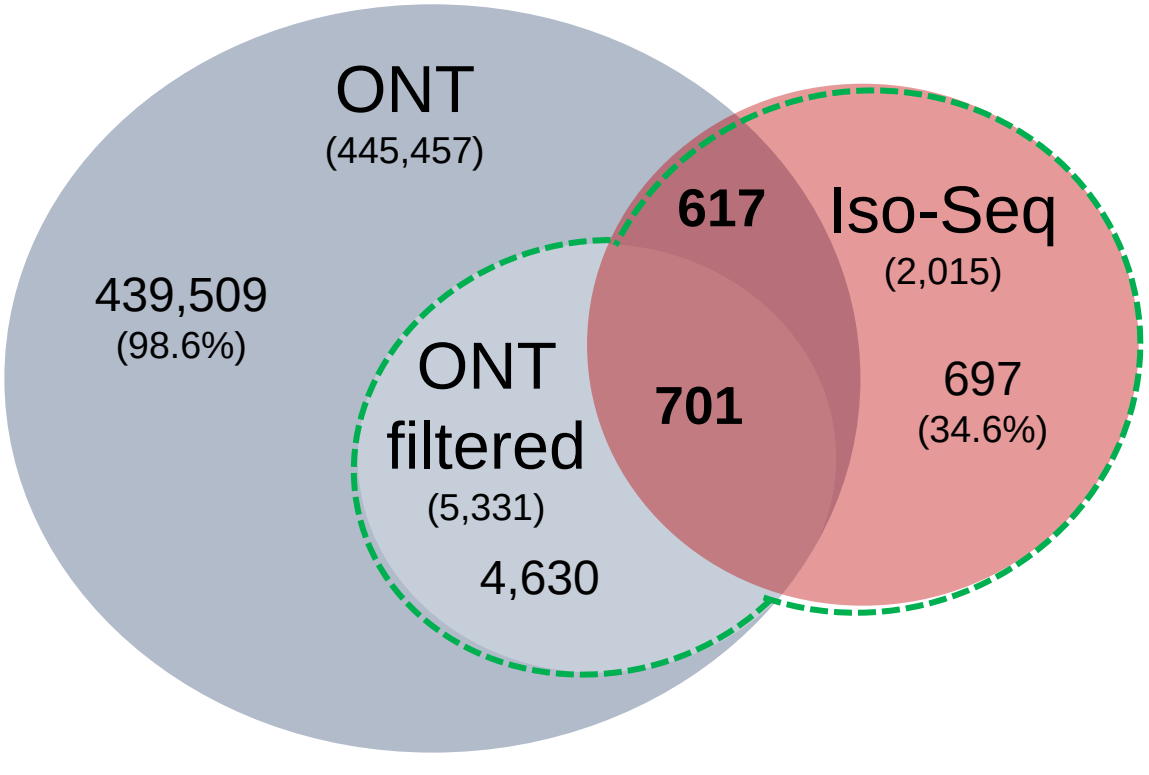
A

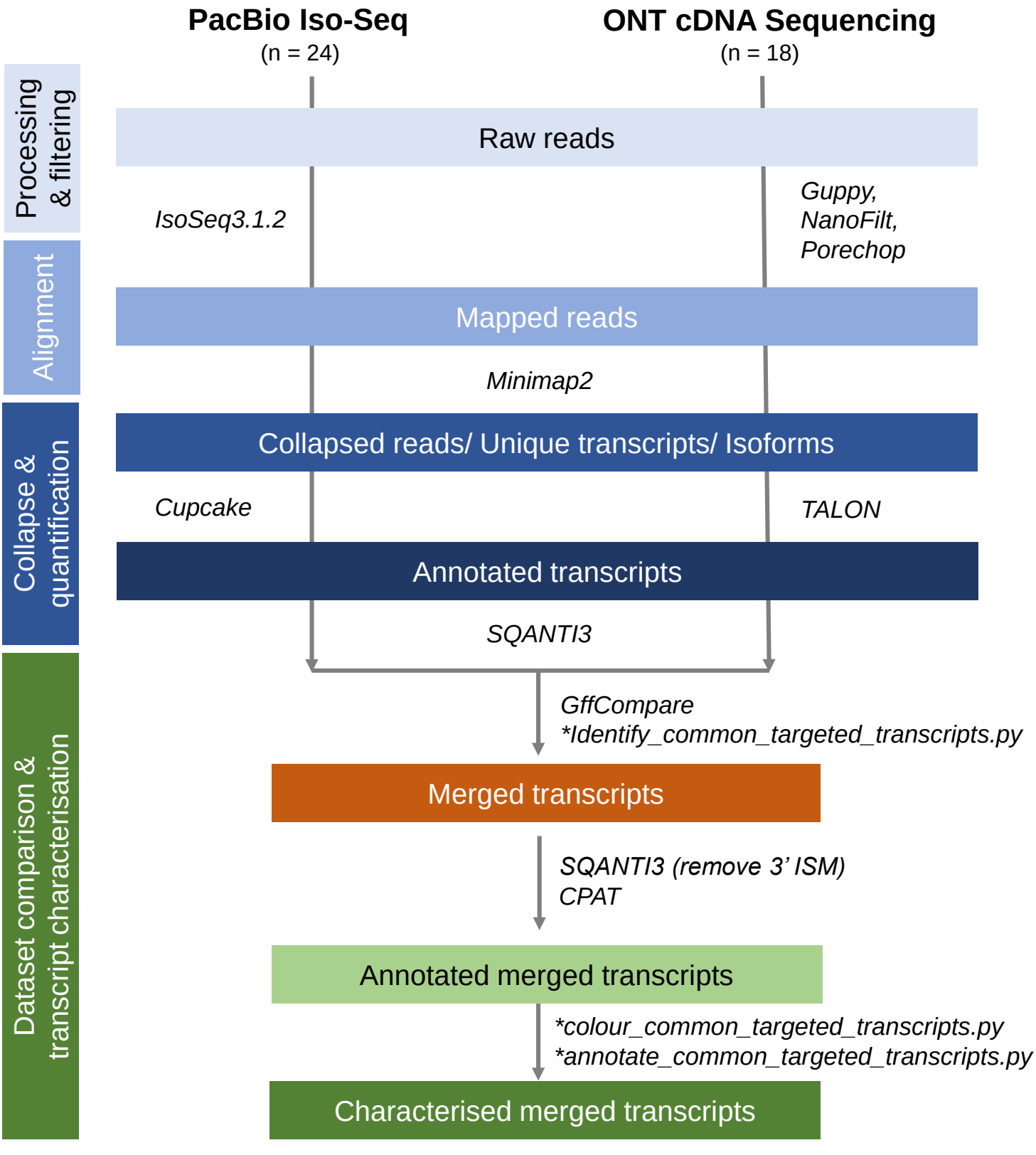

L
B2
B3

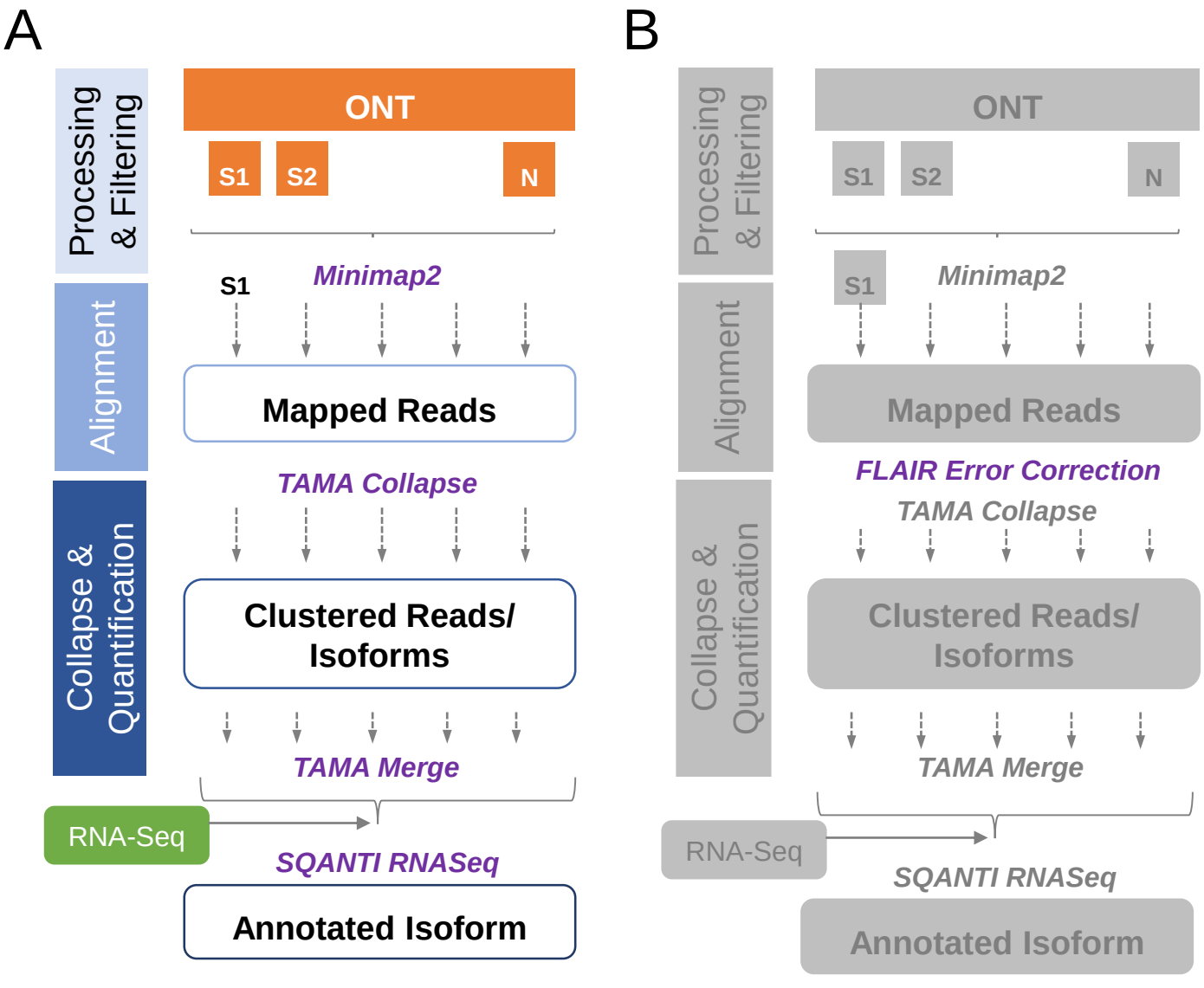
B

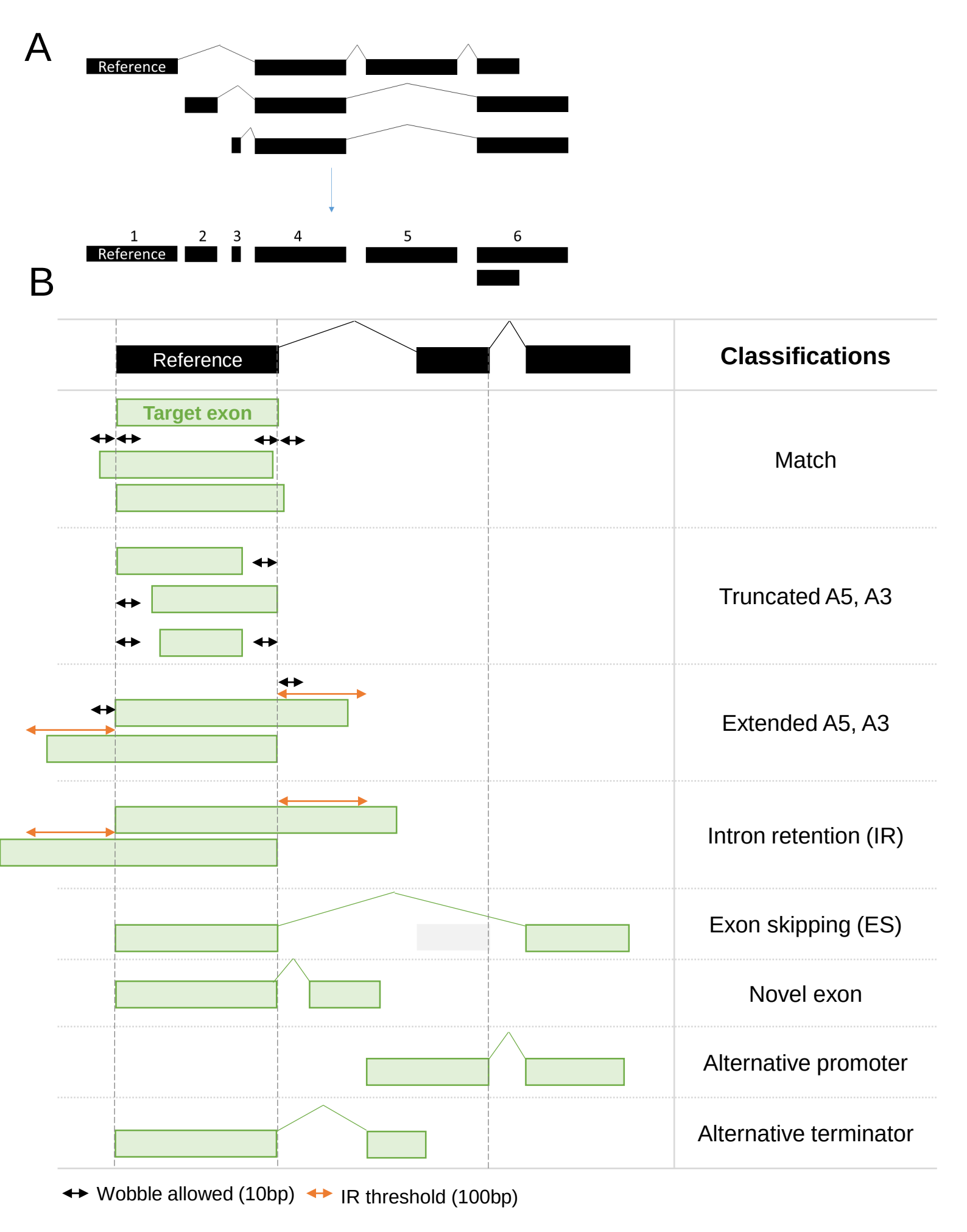

B3
Lower
Upper
Sample Intensity [Normalized FU]
6000
5000
4000
3000
2000
1000
0


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

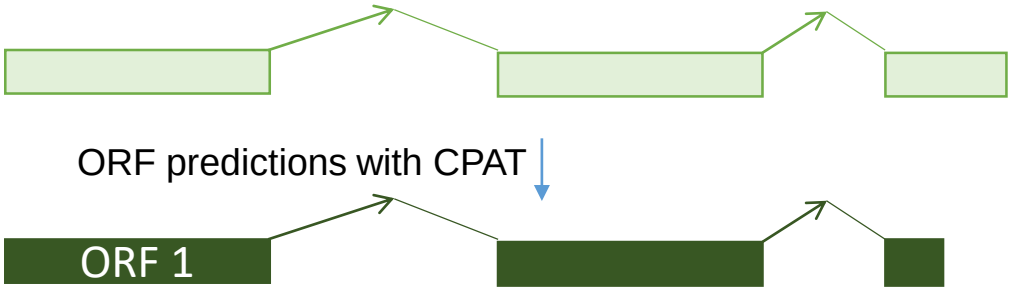

ONT
(445,457)
439,509
(98.6%)
617
Iso-Seq
(2,015)
697
(34.6%)
ONT
filtered
(5,331)
701
4,630
Transcripts retained for annotation (6,645)


PacBio Iso-Seq
(n = 24)
ONT cDNA Sequencing
(n = 18)
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


A
Reference
1
2
3
4
5
6
Reference
B
Reference
Classifications
Target exon
Match
Truncated A5, A3
Extended A5, A3
Intron retention (IR)
Exon skipping (ES)
Novel exon
Alternative promoter
Alternative terminator
Wobble allowed (10bp)
IR threshold (100bp)

A



ORF predictions with CPAT

Best ORF selected with the highest coding probability
If coding probability ≥ 0.44: transcript considered coding

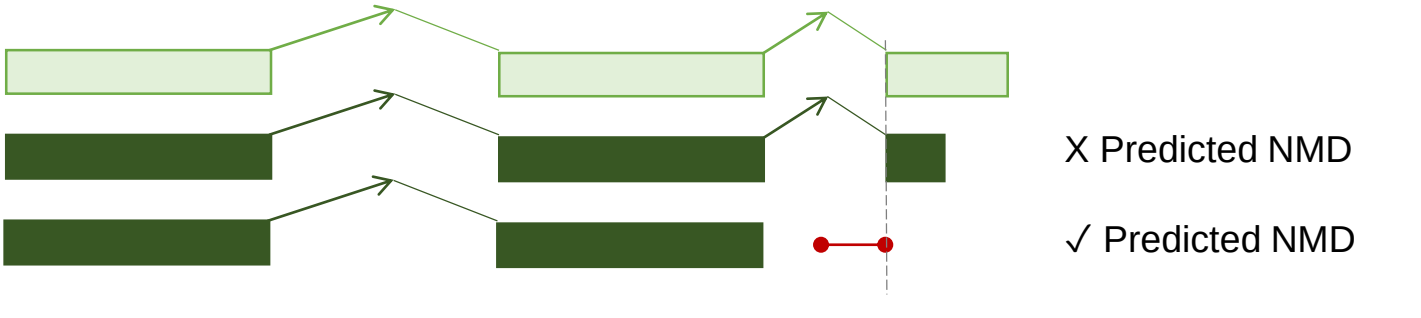
Transcript colour by coding status and shade by abundance

B

Abundance (FL Reads)	Coding Status: Coding	Coding Status: Non-coding	Coding Status: No ORF
≤ 25			
25 < FL ≤ 50			
50 < FL ≤ 100			
100 < FL ≤ 250			
≥ 500			

C

NMD prediction for coding transcripts



Transcript is predicted for NMD if distance between ORF end and last exon-exon junction is more than 50bp