Direct RNA vs cDNA sequencing of *C. elegans* transcriptome
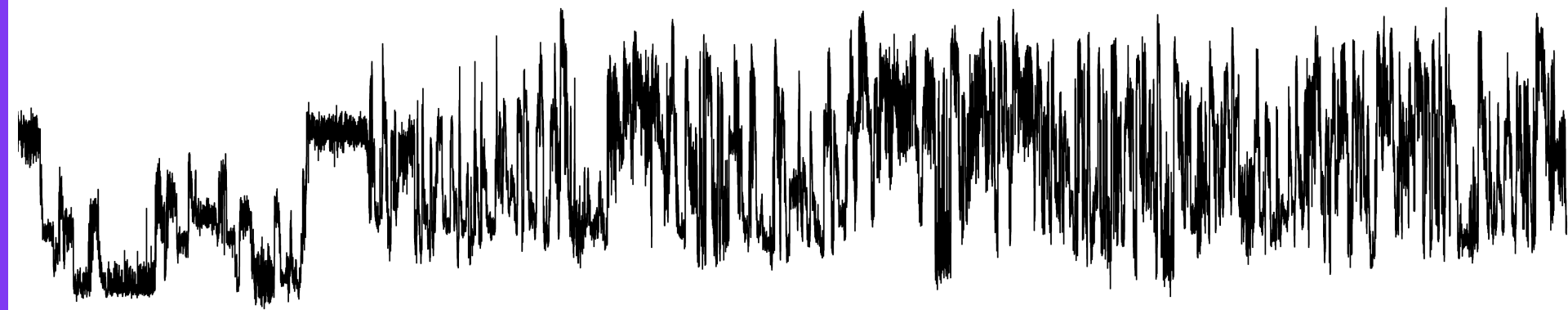
Rachael Workman

Timp Lab

Johns Hopkins University

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Why compare direct RNA vs cDNA sequencing?

Many advantages to direct RNA:
- Poly-A profiling
- Modification detection
- More accurate expression quantification?

But necessary to first understand differences between two data types

What to compare: direct RNA vs cDNA sequencing

Library preparation

Quality

Transcript detection
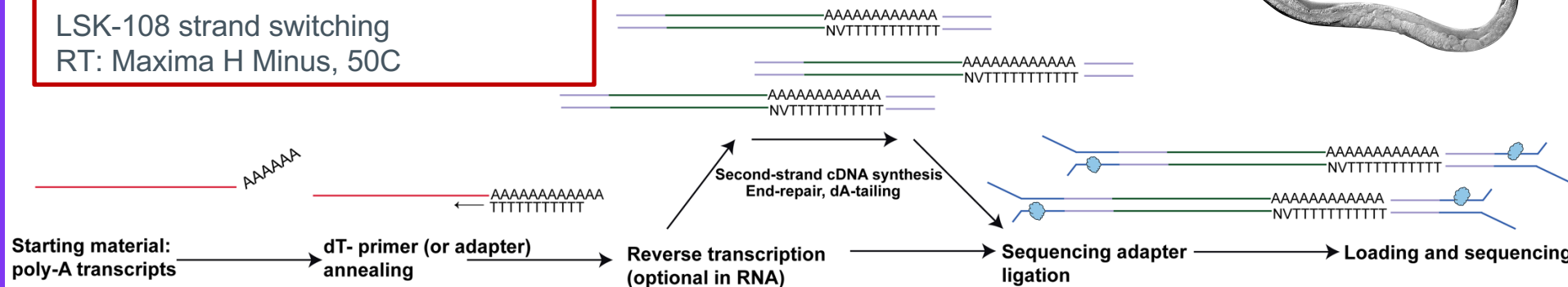
Abundance

Homopolymer calling

Direct RNA and cDNA comparison library preparations

cDNA
LSK-108 strand switching
RT: Maxima H Minus, 50C

Universiteit Utrecht,
Developmental Biology

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**Second-strand cDNA synthesis
End-repair, dA-tailing**

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NVTTTTTTTTTTT

AAAAAAAAAAAA
NVTTTTTTTTTTT

**Starting material:
poly-A transcripts**

**dT- primer (or adapter)
annealing**

**Reverse transcription
(optional in RNA)**

**Sequencing adapter
ligation**

Loading and sequencing

Direct RNA and cDNA comparison library preparations

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Loading and sequencing

AAAAAAAAAAA
TTTTTTTTTT

CCC

AAAAAAAAAAA
NVTTTTTTTTTTTT

CCC

AAAAAAAAAAA
NVTTTTTTTTTTTT

RNA
RNA-001
RT: SuperScript IV, 55C

What to compare: direct RNA vs cDNA sequencing

Library preparation

Quality

Transcript detection
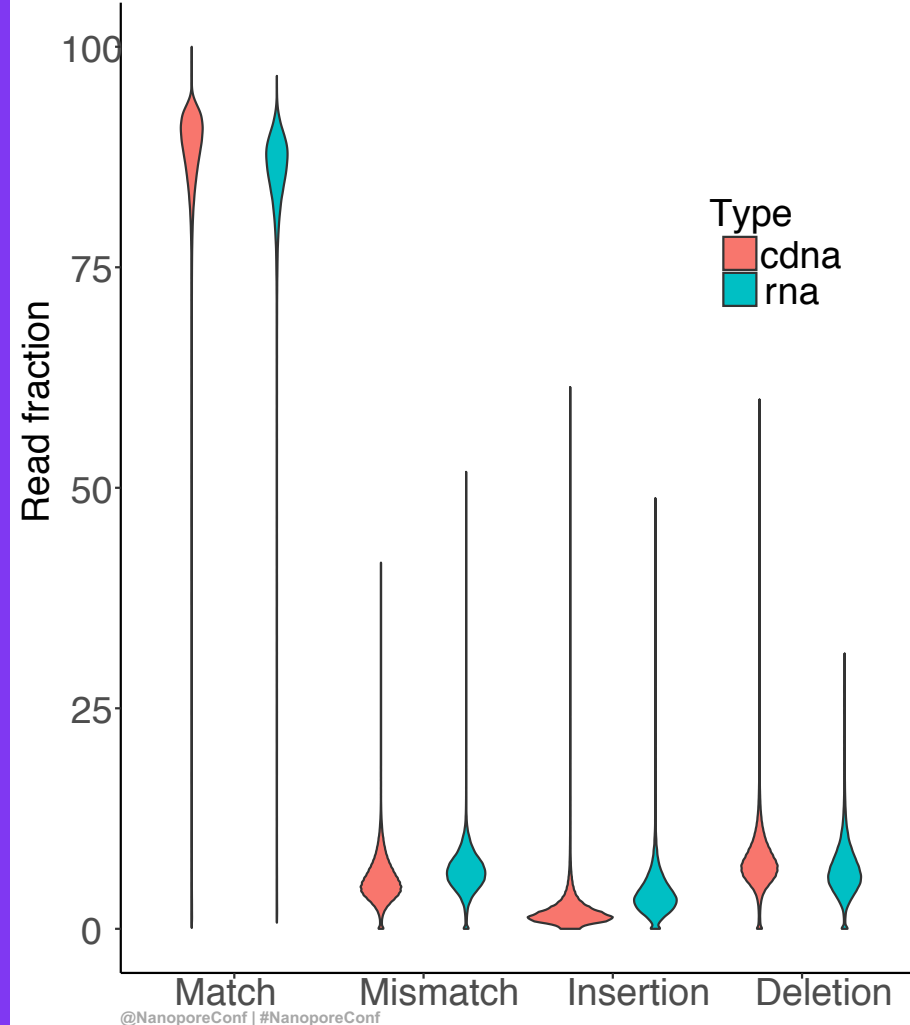
Abundance

Homopolymer calling

Basic run statistics
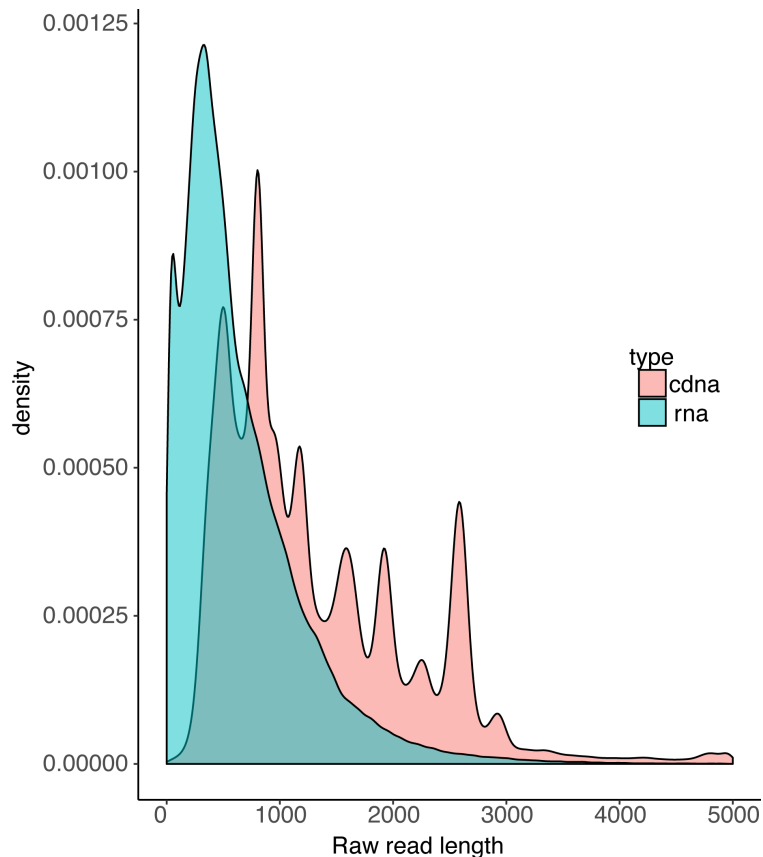
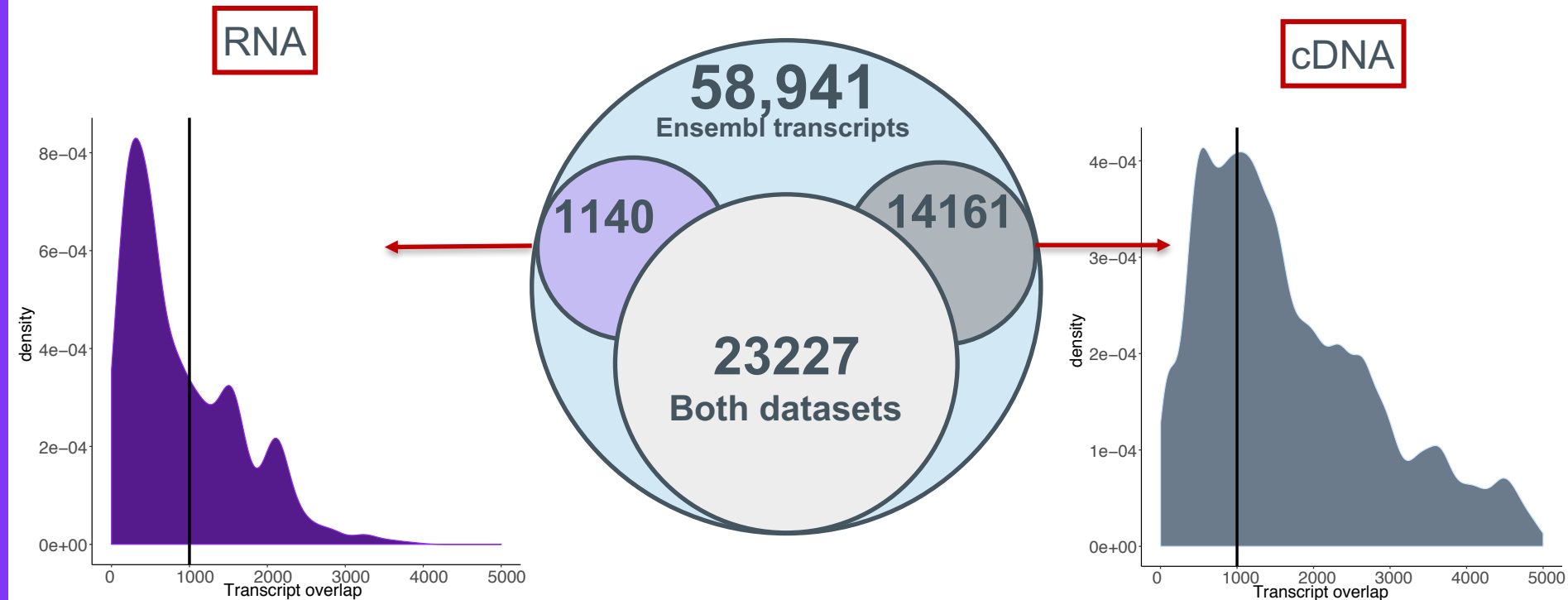|	RNA	cDNA
Reads	240K	2400K
Yield	0.2Gb	3.23Gb
Mean read length	652bp	1340bp

Alignment quality similar between runs

	RNA	cDNA
Alignment	65%	85%
Mapq >10	77K	545K
Mean match len	752bp	1130bp
Median match fraq	82%	87%
% Accuracy	83%	85%

@NanoporeConf | #NanoporeConf

What to compare: direct RNA vs cDNA sequencing

Library preparation

Quality

Transcript detection

Abundance

Homopolymer calling

Large portion curated gene transcripts detected

58,941
Ensembl transcripts, WBcel235
All life cycle stages

RNA

cDNA

1140

14161

23227
Both datasets
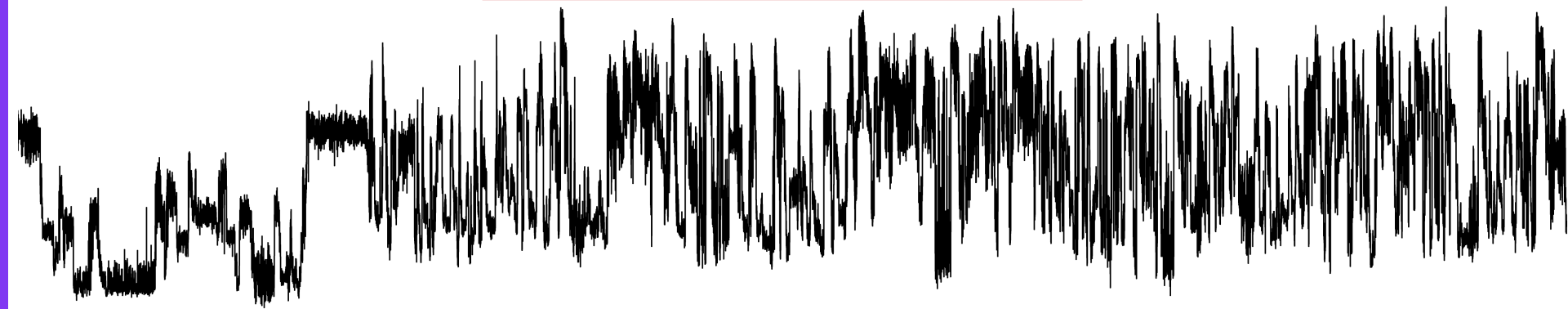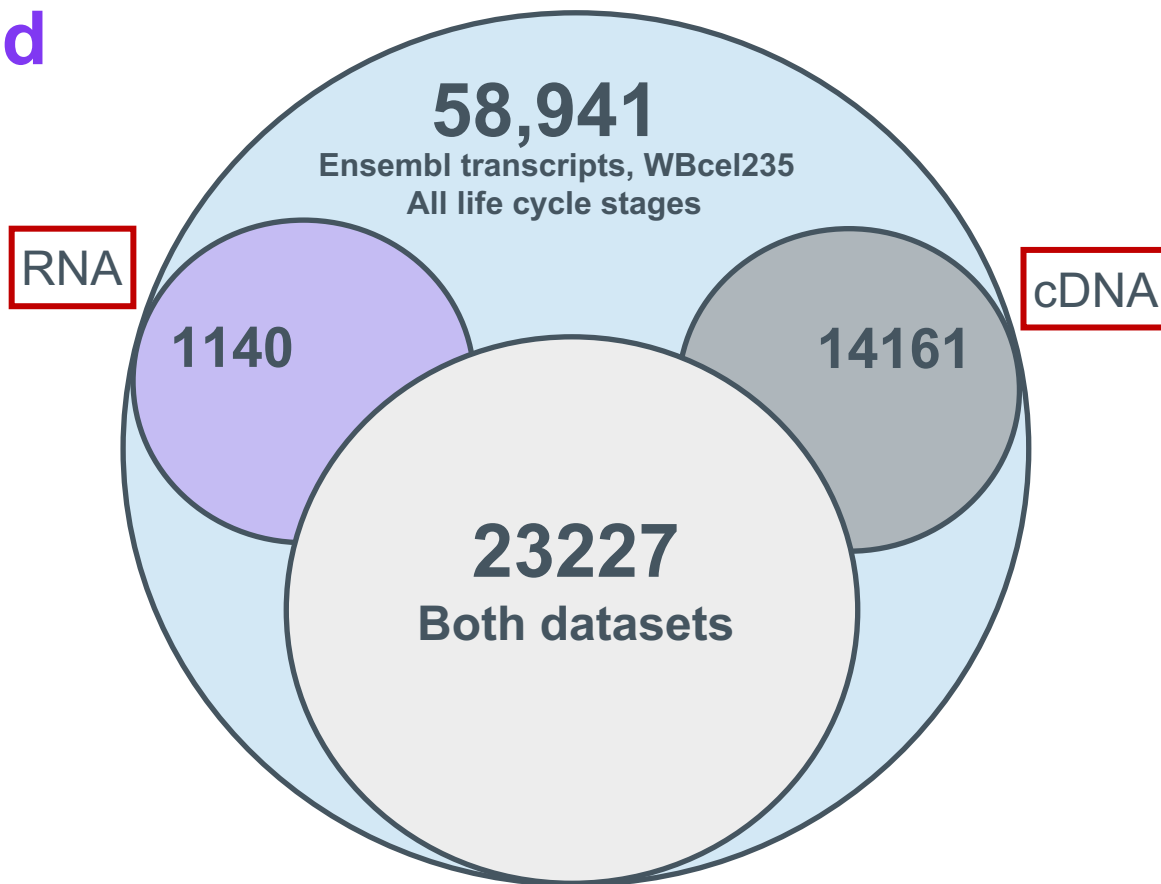
Large portion curated gene transcripts detected

RNA

cDNA

58,941
Ensembl transcripts

1140

14161

23227
Both datasets

density

Transcript overlap

density

Transcript overlap

More full length transcripts in cDNA sequencing
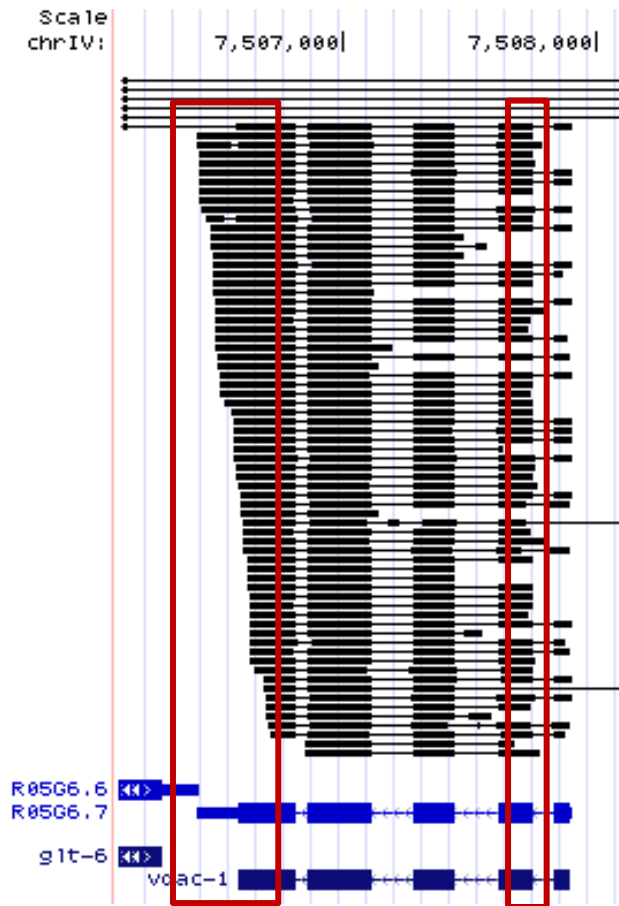
Pileup of percent transcript covered by each read

More degradation in RNA run, respectable lengths in both
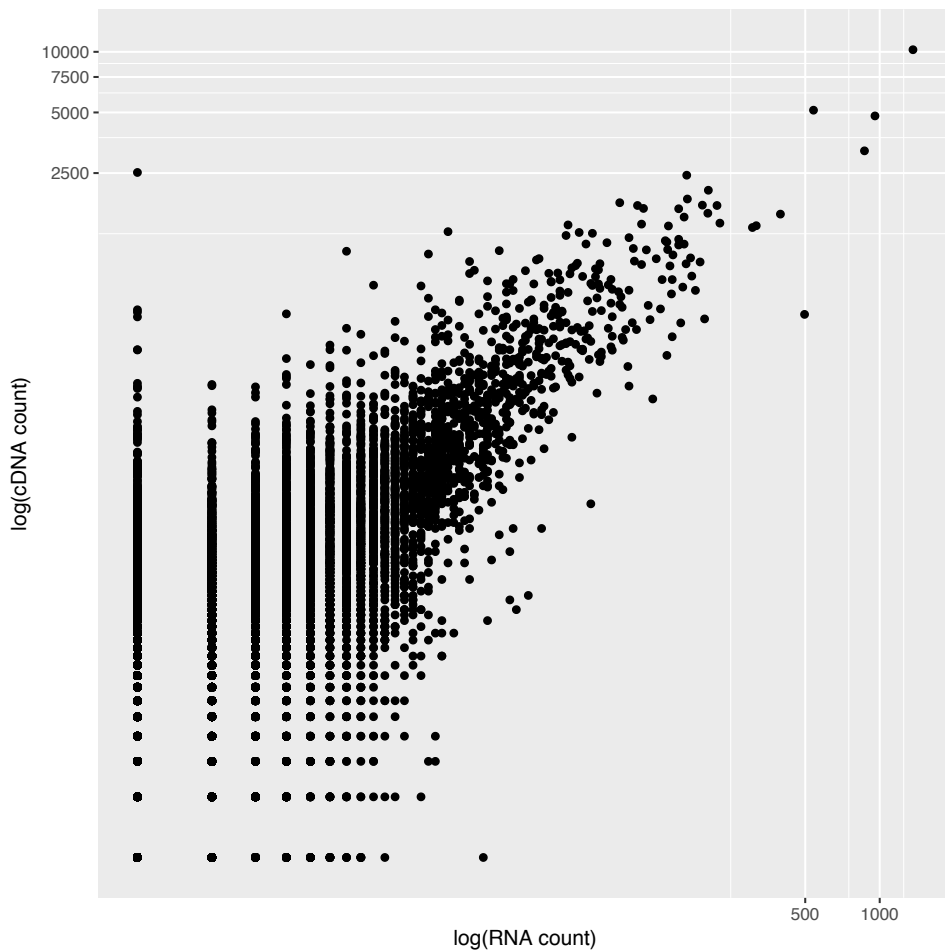
Removing RT step may reduce degradation
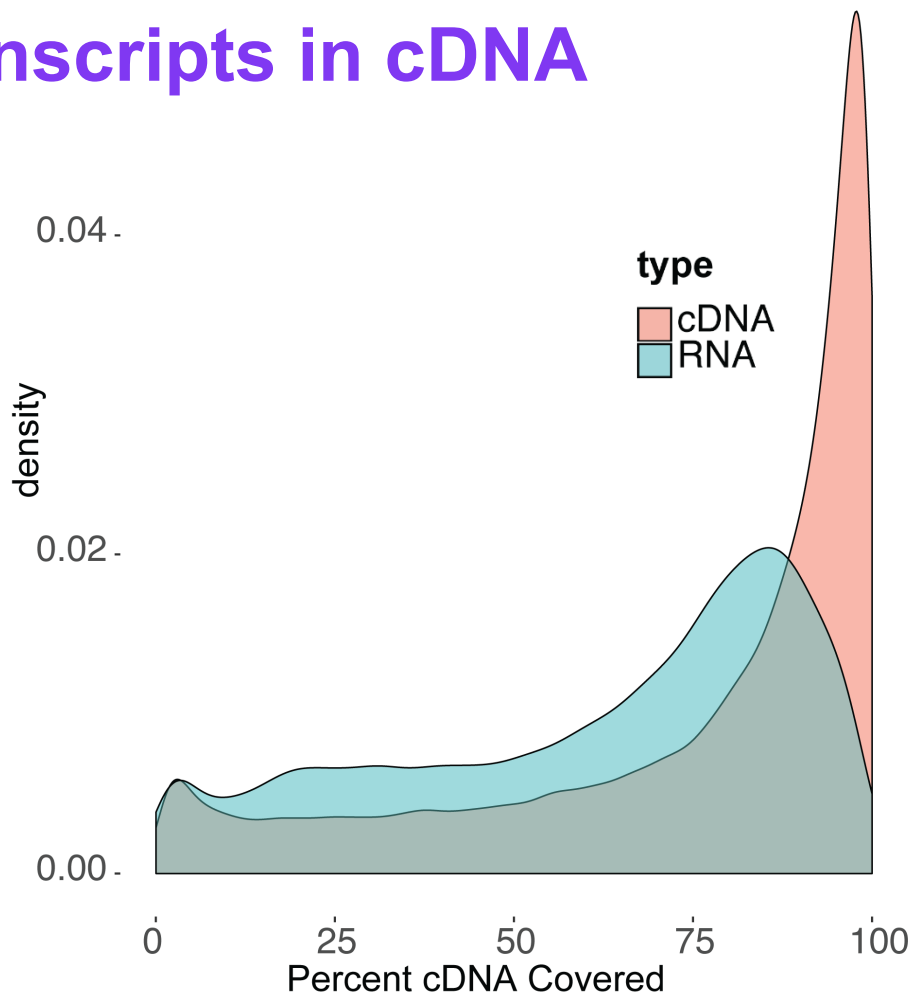
More full length transcripts in cDNA sequencing

Non-full length reads- preparatory degradation, aligner clipping

What to compare: direct RNA vs cDNA sequencing

Library preparation

Quality

Transcript detection

Abundance

Homopolymer calling

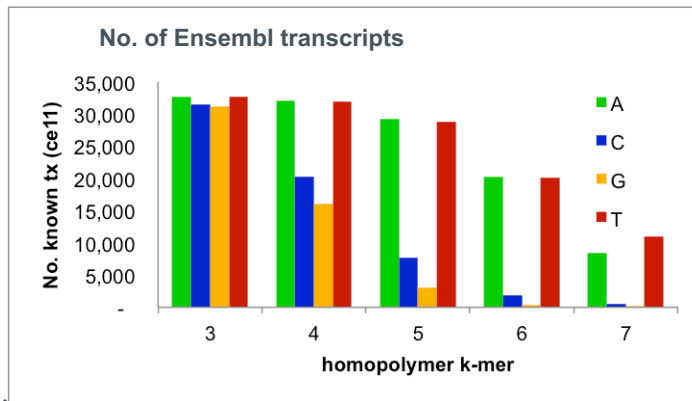**Transcript abundance consistent between cDNA and RNA runs**
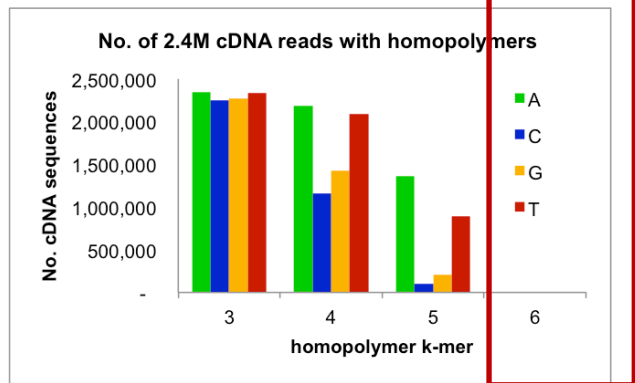
Pearson R = 0.76

What to compare: direct RNA vs cDNA sequencing

Library preparation

Quality
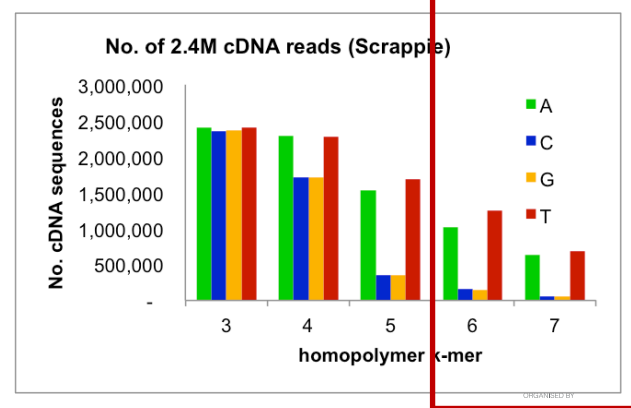
Transcript detection

Abundance

Homopolymer calling

Scrappie greatly improves homopolymer calls

No. of Ensembl transcripts

Albacore (-Scrappie)

No. of 2.4M cDNA reads with homopolymers

Albacore (+Scrappie)

No. of 2.4M cDNA reads (Scrappie)

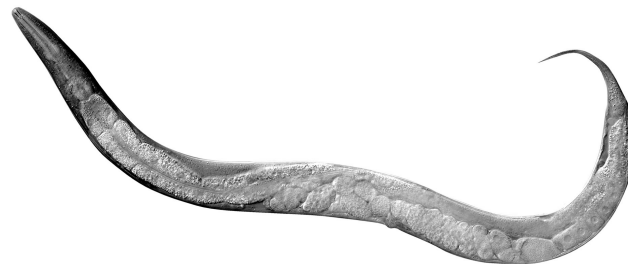# **Conclusions**

Library preparation

- Robust in both, simpler in RNA, mRNA lengths better preserved in cDNA
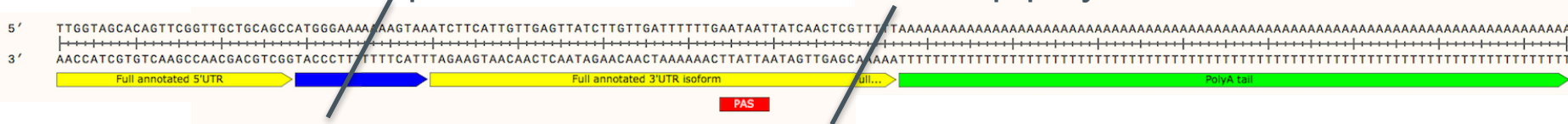
Quality, transcript detection and abundance

- Comparable when taking into account yield differences

Homopolymer calling
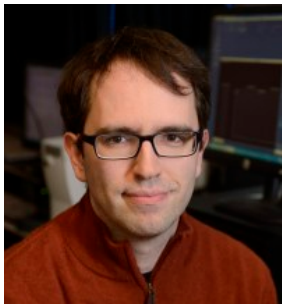
- Next application pA tail detection

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Technologies

Conclusions

Eef-1A.1
Full 5' UTR   1290bp CDS   Full 3' UTR   79bp poly-A tail

Homopolymer calling

- Long poly-A tails aligned, likely requires further training/adapter trimming to refine

Acknowledgements

Timp Lab

Winston Timp

Taylor Lab

James Taylor

Mallory Freeberg

Kim Lab

John Kim

Amelia Alessi

Funding/Reagent Support

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