Introduction to Trinity RNA-Seq

Berlin, June 2018

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Broad Institute

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Umeå universitet

Physalia
Courses

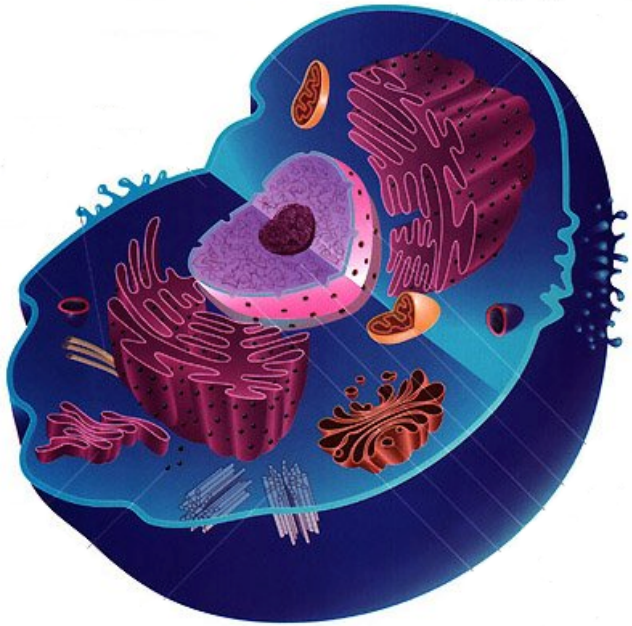
Welcome to the Berlin 2018 Trinity Workshop Wiki!

Day	Time	Activities
Monday, June 11	morning	Workshop Introduction -slides-
		Exploring the Computational Infrastructure -slides-
	afternoon	Unix command-line review
		Data overview and setup
		Using FASTQC and Trimmomatic -slides-
Tuesday, June 12	morning	Trinity de novo transcriptome assembly -slides-
	afternoon	Uploading own data or identifying and downloading SRA studies of interest -slides-
Wednesday, June 13	morning	Expression quantification -slides-
		Quality assessment for assembly -slides-
	afternoon	QC samples and replicates
Thursday, June 14	morning	Statistical methods for differential expression analysis -slides-
	afternoon	Transcript clustering and expression profiling
		Methods for functional annotation -slides-
		Trinotate and TrinotateWeb
Friday, June 15	morning	Functional enrichment analysis
		Review and custom data analyses
		Comments on software installations for later use on different resources

https://github.com/trinityrnaseq/BerlinTrinityWorkshop2018/wiki

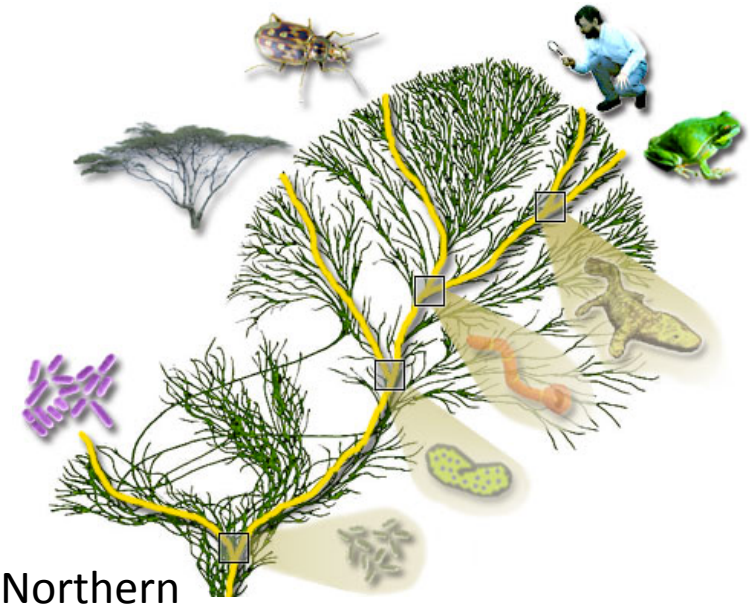
Transcriptomics

A powerful tool for studying molecular biology

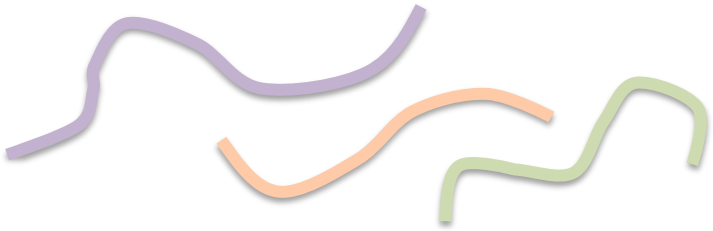


- Yields insights into:
  - Identity of expressed transcripts.
  - Levels of expression.
  - Differences in expression across samples or conditions.

Biological Investigations Empowered by Transcriptomics

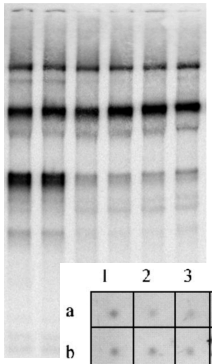


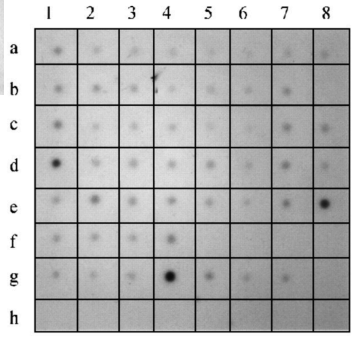
Extract RNA,
… some protocol for processing, …



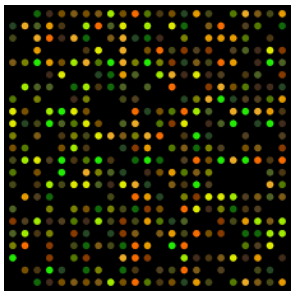
Analysis Method
(pick your favorite)

Northern

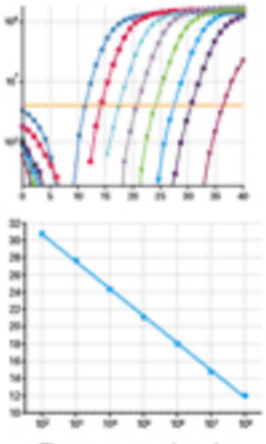




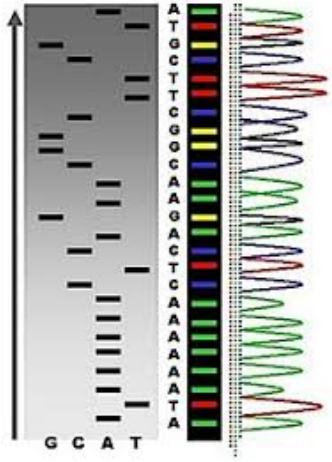
Dot Blot



Microarray



qRT-PCR



Sanger Sequencing

Other…

Minion

MinION

Historical Timeline of Transcriptomics (from 1970)

Reverse Transcription (1970)

Northern Blot
Sanger Sequencing
(1977)

Expressed Sequence Tags (1992)

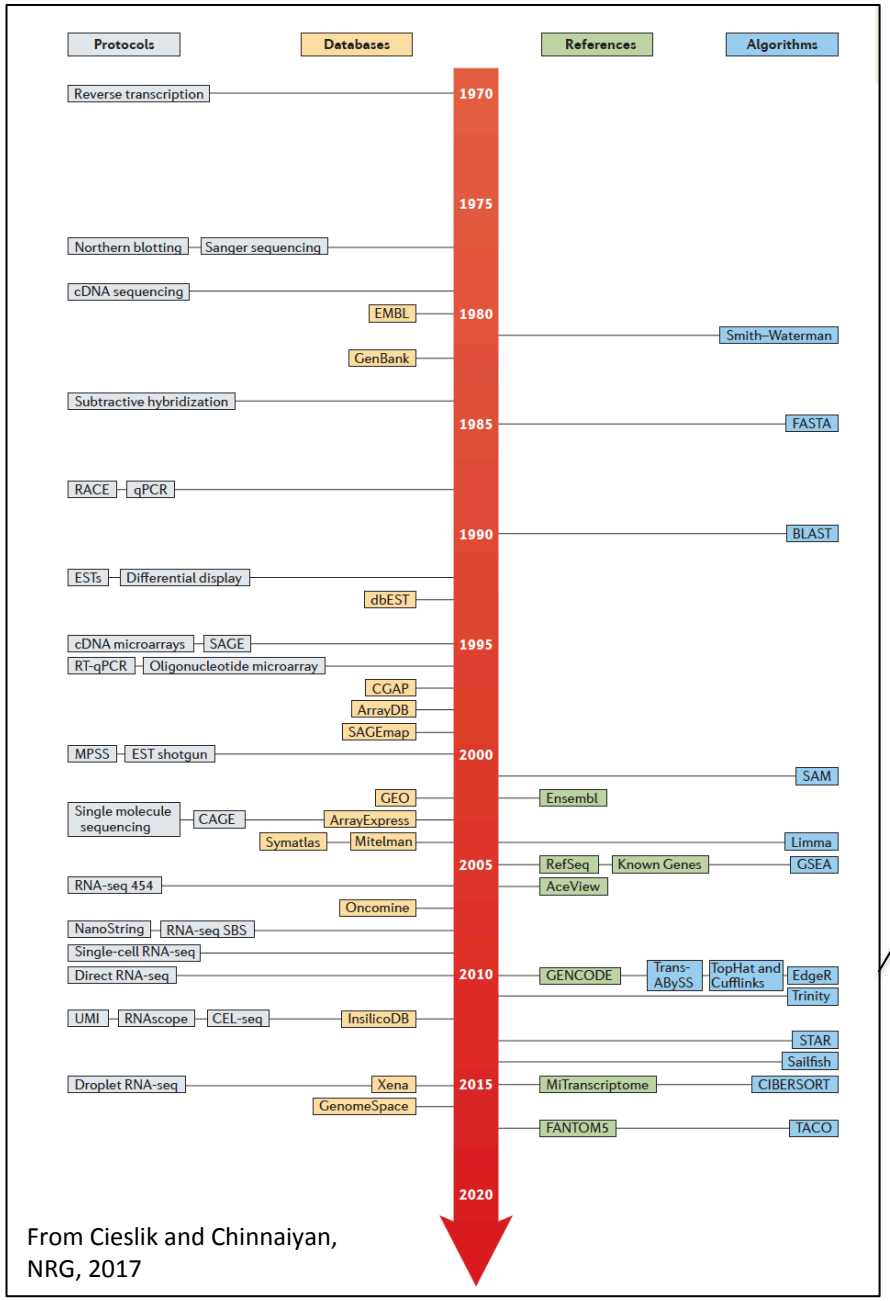
cDNA microarrays (1995)

RNA-Seq (2006-2008)

PacBio IsoSeq (2014)

Droplet single cell RNA-Seq (2015)

Direct RNA Seq Nanopore (2018)



From Cieslik and Chinnaiyan, NRG, 2017

Note: Just a small sampling of what's available.

Smith Waterman (1981)

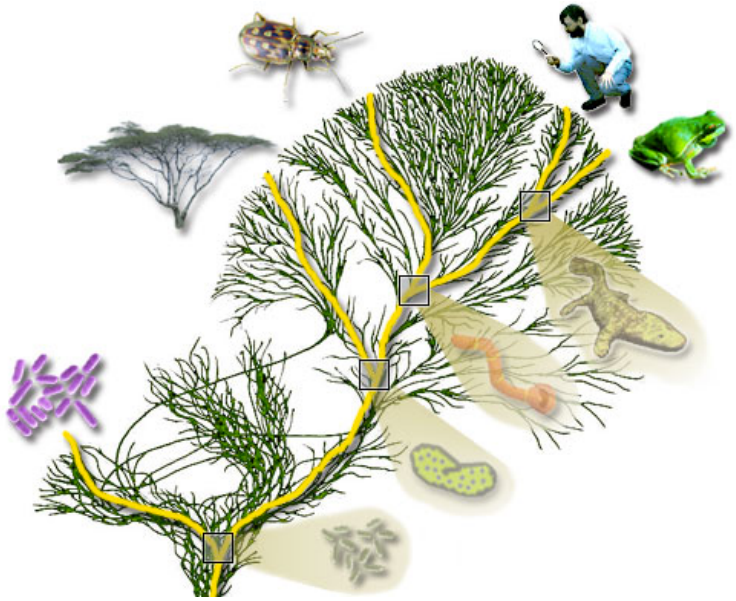
BLAST (1990)

Tophat/Cufflinks (2010)

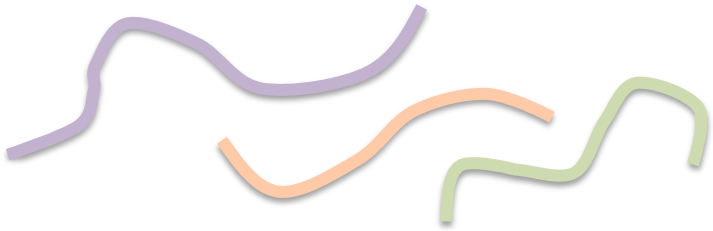
RSEM (2011)

Kallisto (2016)
Salmon (2017)

Modern Transcriptome Studies Empowered by RNA-seq



Extract RNA, convert to cDNA



Next-gen Sequencer
(pick your favorite)

Millions to Billions of Reads

Generating RNA-Seq: *How to Choose?*

Platform	Project Firefly 2018	MiniSeq	MiSeq	Next Seq 550	HiSeq 2500 RR	Hiseq 2500 V3	HiSeq 2500 V4	HiSeq 4000	HiSeq X	Nova Seq S1 2018	Nova Seq S2	Nova Seq S4	5500 XL	318 HiQ 520	Ion 530	Ion Proton P1	PGM HiQ 540	RS P6-C4	Sequel	R&D end 2018	Smidg ION RnD	Mini ION R9.5	Grid ION X5	Prome thION RnD	Prome thION theor etical	QiaGen Gene Reader	BGI SEQ 500	BGI SEQ 50	#
Reads: (M)	4	25	25	400	600	3000	4000	5000	6000	3300	6600	20000	1400	3-5	15-20	165	60-80	5.5	38.5	--	--	--	--	--	--	400	1600	1600	--
Read length: (paired-end*)	150*	150*	300*	150*	100*	100*	125*	150*	150*	150*	150*	150*	60	200 400	200 400	200	200	15K	12K	32K	--	--	--	--	--	--	100*	50	--
Run time: (d)	0.54	1	2	1.2	1.125	11	6	3.5	3	1.66	1.66	1.66	7	0.37	0.16	--	0.16	4.3	--	--	--	2	2	2	--	--	1	0.4	--
Yield: (Gb)	1	7.5	15	120	120	600	1000	1500	1800	1000	2000	6000	180	1.5	7	10	12	12	5	150	4	8	40	2400	11000	80	200	8	--
Rate: (Gb/d)	1.85	7.5	7.5	100	106.6	55	166	400	600	600	1200	3600	30	5.5	50	--	93.75	2.8	--	--	--	4	20	1200	5500	--	200	20	--
Reagents: ($K)	0.1	1.75	1	5	6.145	23.47	29.9	--	--	--	--	--	10.5	0.6	--	1	1.2	2.4	--	1	--	0.5	1.5	--	--	0.5	--	--	--
per-Gb: ($)	100	233	66	50	51.2	39.1	31.7	20.5	7.08	18	15	5.8	58.33	--	--	100	--	200	80	6.6	--	62.5	37.5	20	4.3	--	--	--	--
hg-30x: ($)	12000	28000	8000	5000	6144	4692	3804	2460	849.6	1800	1564	700	7000	--	--	12000	--	24000	9600	1000	--	7500	4500	2400	500	--	600	--	
Machine: ($)	30K	49.5K	99K	250K	740K	690K	690K	900K	1M	999K	999K	999K	595K	50K	65K	243K	242K	695K	350K	350K	--	--	125K	75K	75K	--	200K	--	--
#Page maintained by http://twitter.com/albertvilella http://tinyurl.com/ngslytics #Editable version: http://tinyurl.com/ngsspecsshared																													
#curl "https://docs.google.com/spreadsheets/d/1GMMfhyLK0-q8XkIo3YxlWaZA5vVMuhU1kg41g4xLkXc/export?gid=4&format=csv" | grep -v '^#' | grep -v '^"' | column -t -s\, | less -S																													

*Not all shown at scale

Generating RNA-Seq: *How to Choose?*

Platform	Project Firefly 2018	MiniSeq	MiSeq	Next Seq 550	HiSeq 2500 RR	Hiseq 2500 V
Reads: (M)	4	25	25	400	600	300
Read length: (paired-end*)	150*	150*	300*	150*	100*	100
Run time: (d)	0.54	1	2	1.2	1.125	1
Yield: (Gb)	1	7.5	15	120	120	60
Rate: (Gb/d)	1.85	7.5	7.5	100	106.6	5
Reagents: ($K)	0.1	1.75	1	5	6.145	23.4
per-Gb: ($)	100	233	66	50	51.2	39.
hg-30x: ($)	12000	28000	8000	5000	6144	469
Machine: ($)	30K	49.5K	99K	250K	740K	690

#Page maintained by http://twitter.com/albertvilella http://t

#curl "https://docs.google.com/spreadsheets/d/1GMMfhyLK0-q

g	Mini ION R9.5	Grid ION X5	Prome thION RnD	Prome thION theor etical	QiaGen Gene Reader	BGI SEQ 500	BGI SEQ 50	#
--	--	--	--	--	400	1600	1600	--
--	--	--	--	--	--	100*	50	--
--	2	2	2	--	--	1	0.4	--
4	8	40	2400	11000	80	200	8	--
--	4	20	1200	5500	--	200	20	--
--	0.5	1.5	--	--	0.5	--	--	--
--	62.5	37.5	20	4.3	--	--	--	--
--	7500	4500	2400	500	--	600	--	
--	--	125K	75K	75K	--	200K	--	--

"What I especially like about this baby is this little drawer where I can keep my lunch."

Thx Joshua Levin, for the cartoon. ☺

Small to Large

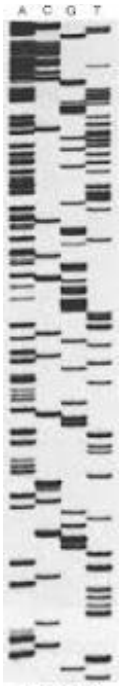
Each has pros/cons

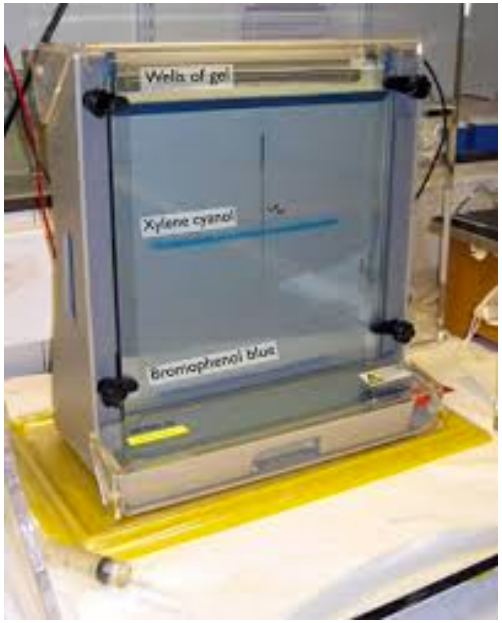
Small to Less Large

Each technology continues to advance

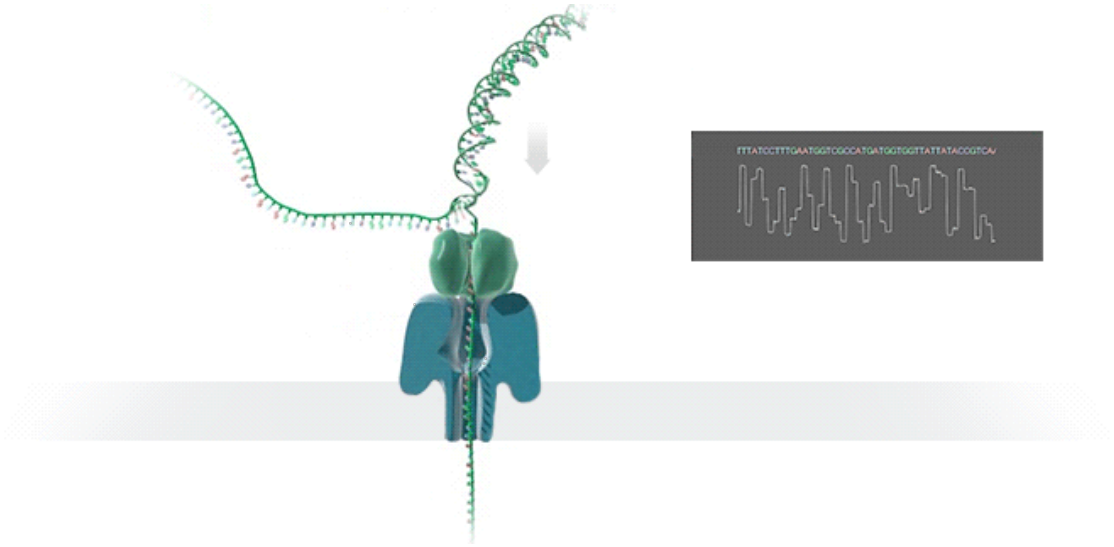
Personal Reflections… (and haunting memories)

Circa 1995

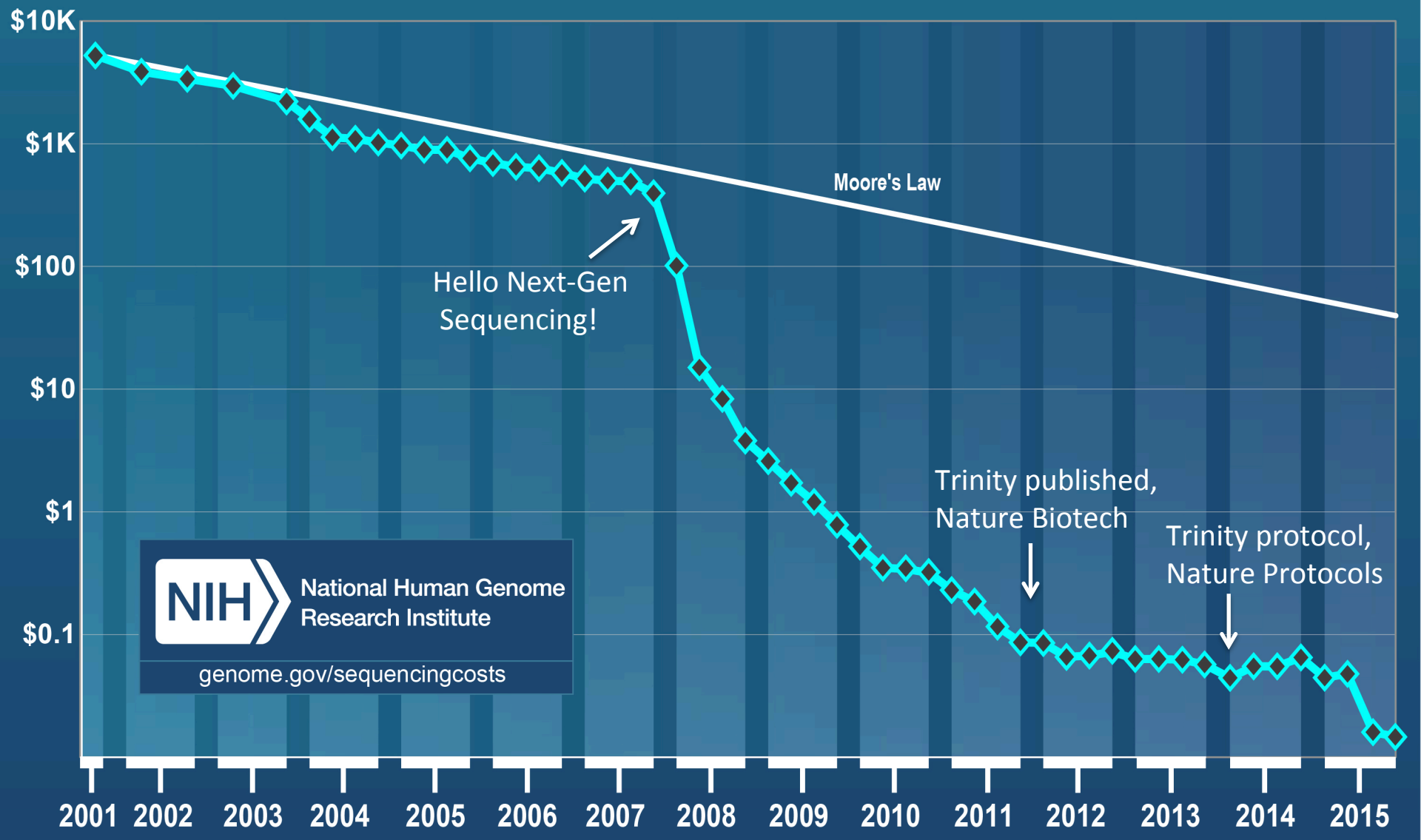




Now (2018)



Cost per Raw Megabase of DNA Sequence



From https://www.genome.gov/sequencingcostsdata/

A Plethora of Biological Sequence Analyses Enabled by RNA-Seq

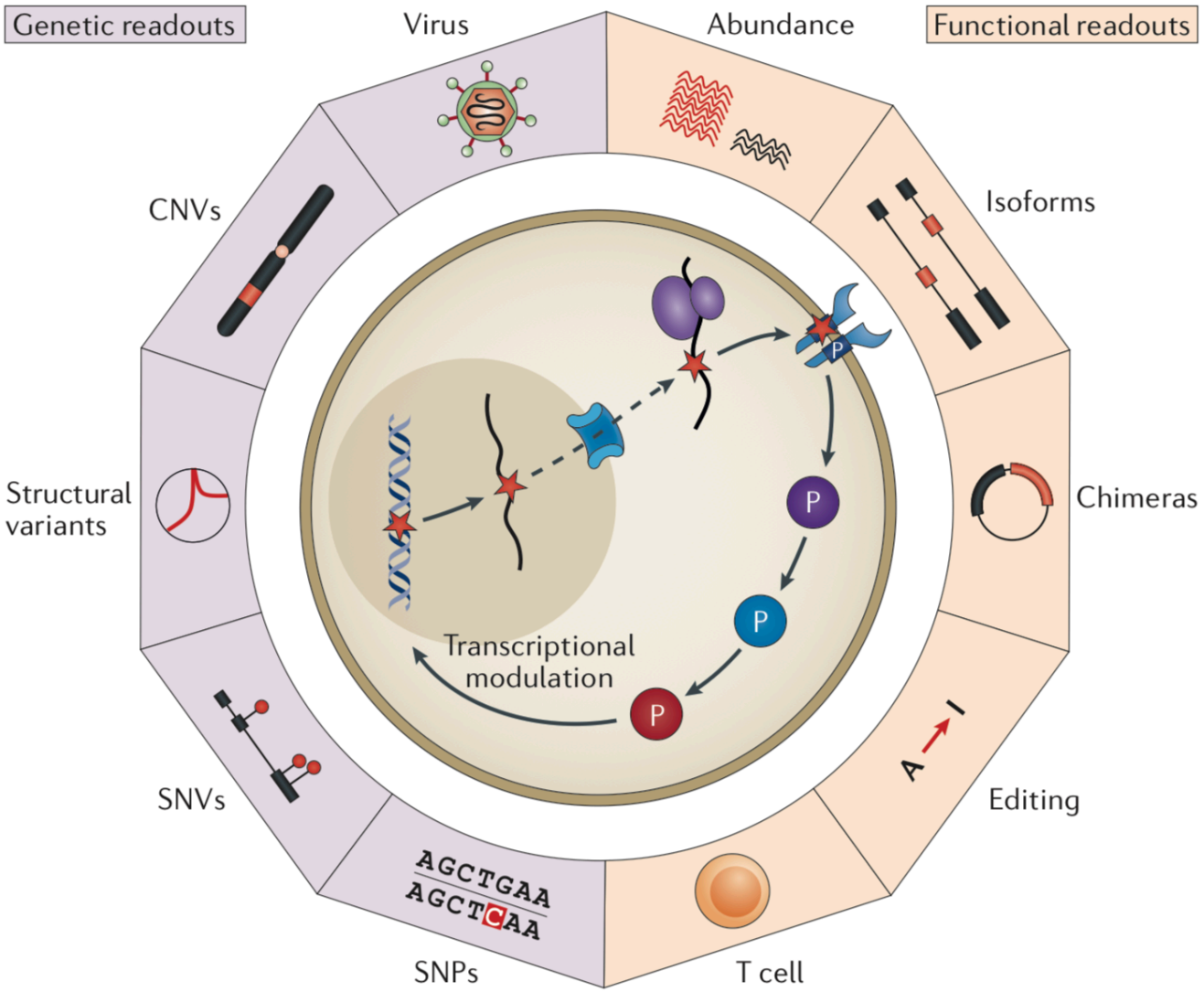
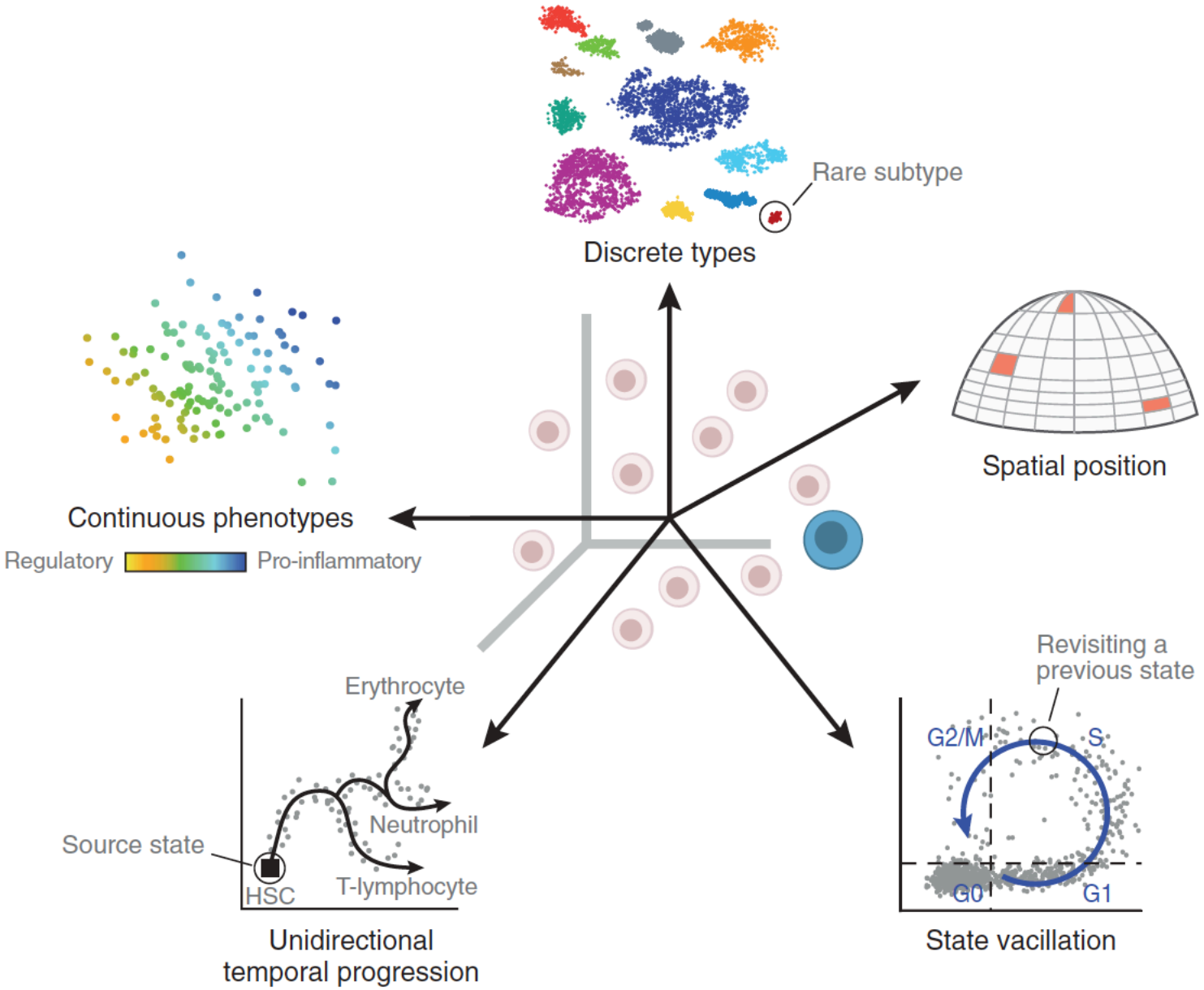


Figure 2 | **Transcriptome profiling for genetic causes and functional phenotypic readouts.**

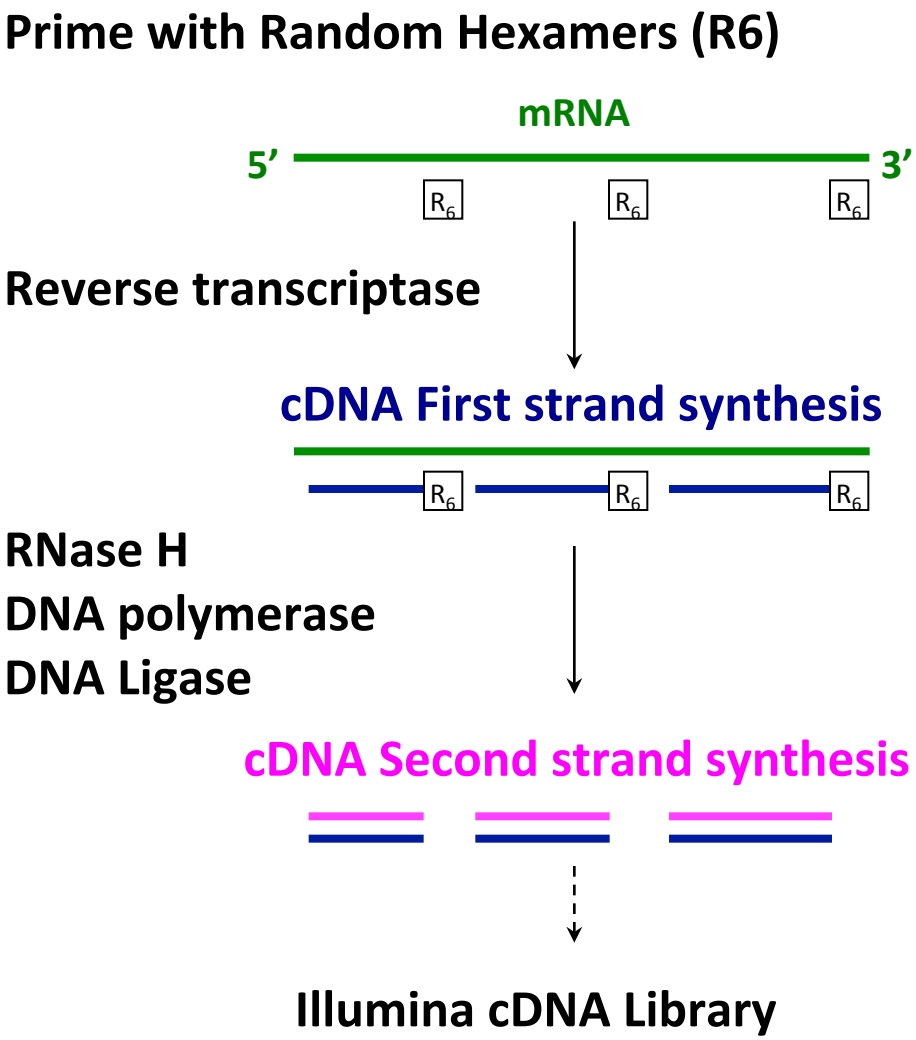
From Cieslik and Chinnaiyan, NRG, 2017

RNA-Seq is Empowering Discovery at Single Cell Resolution



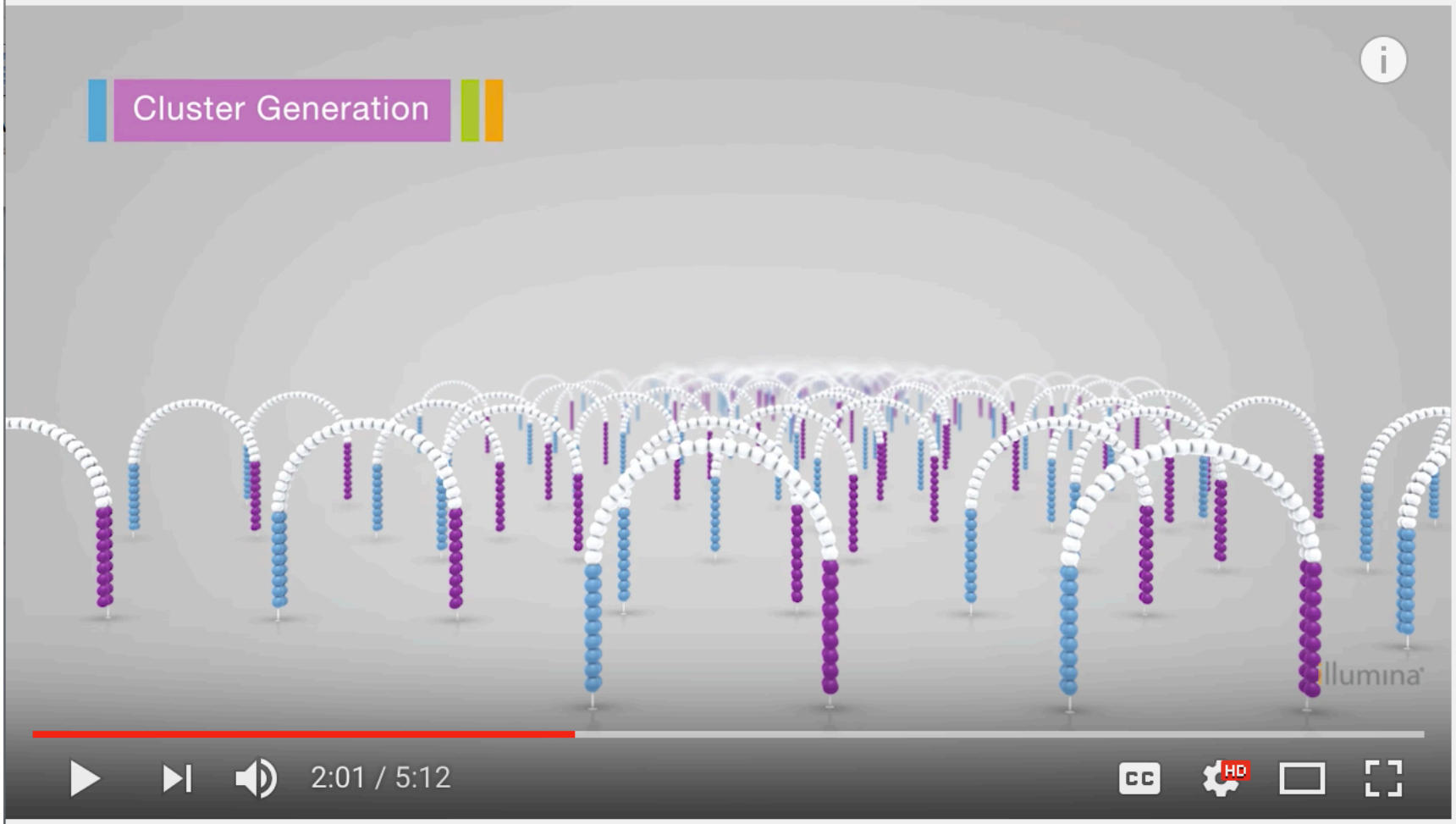
Wagner, Regev, and Yosef. NBT 2016

RNA-Seq: How do we make cDNA?



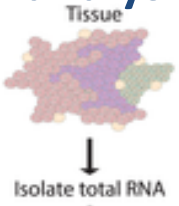
Slide courtesy of Joshua Levin, Broad Institute.

Illumina Sequencing by Synthesis



Illumina Inc https://www.youtube.com/watch?v=fCd6B5HRaZ8

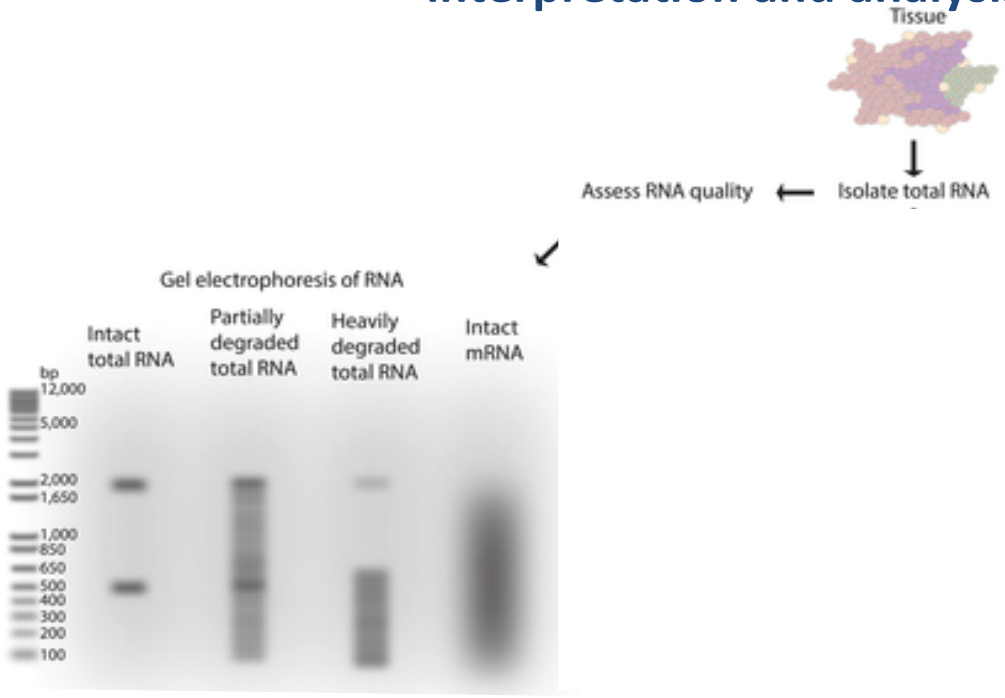
RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

PLOS | COMPUTATIONAL BIOLOGY

RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



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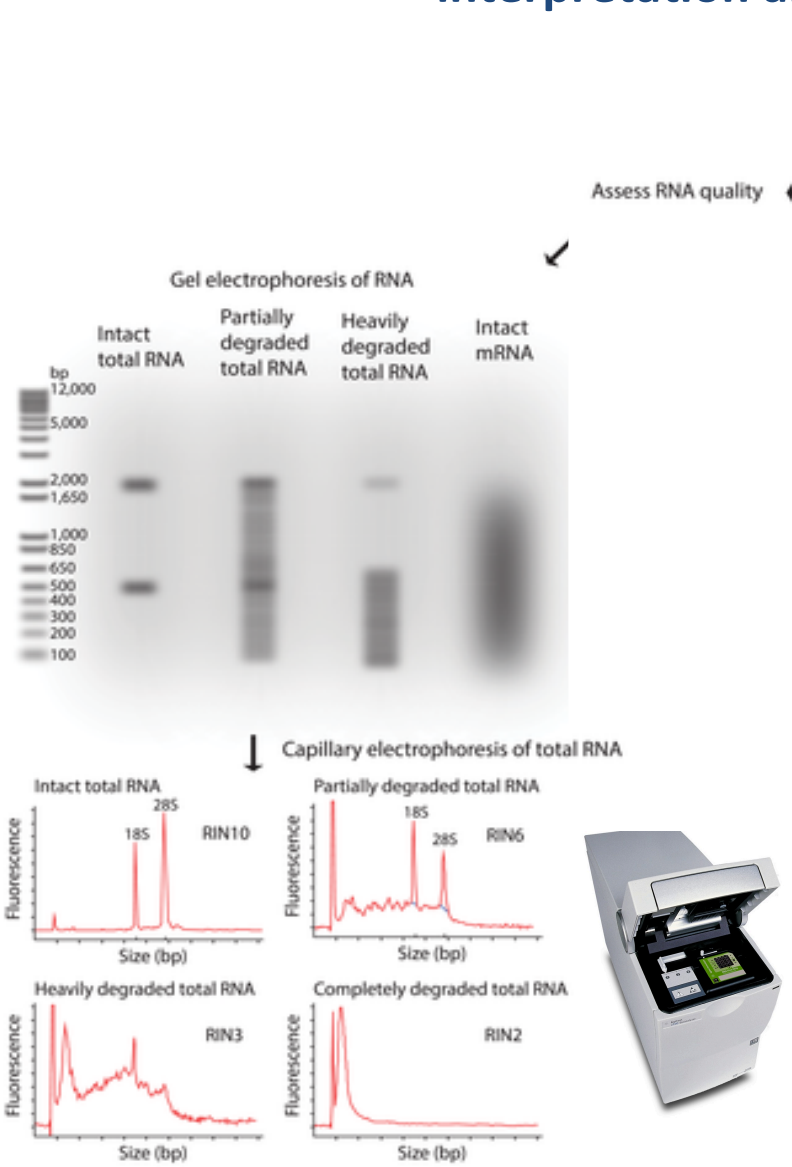

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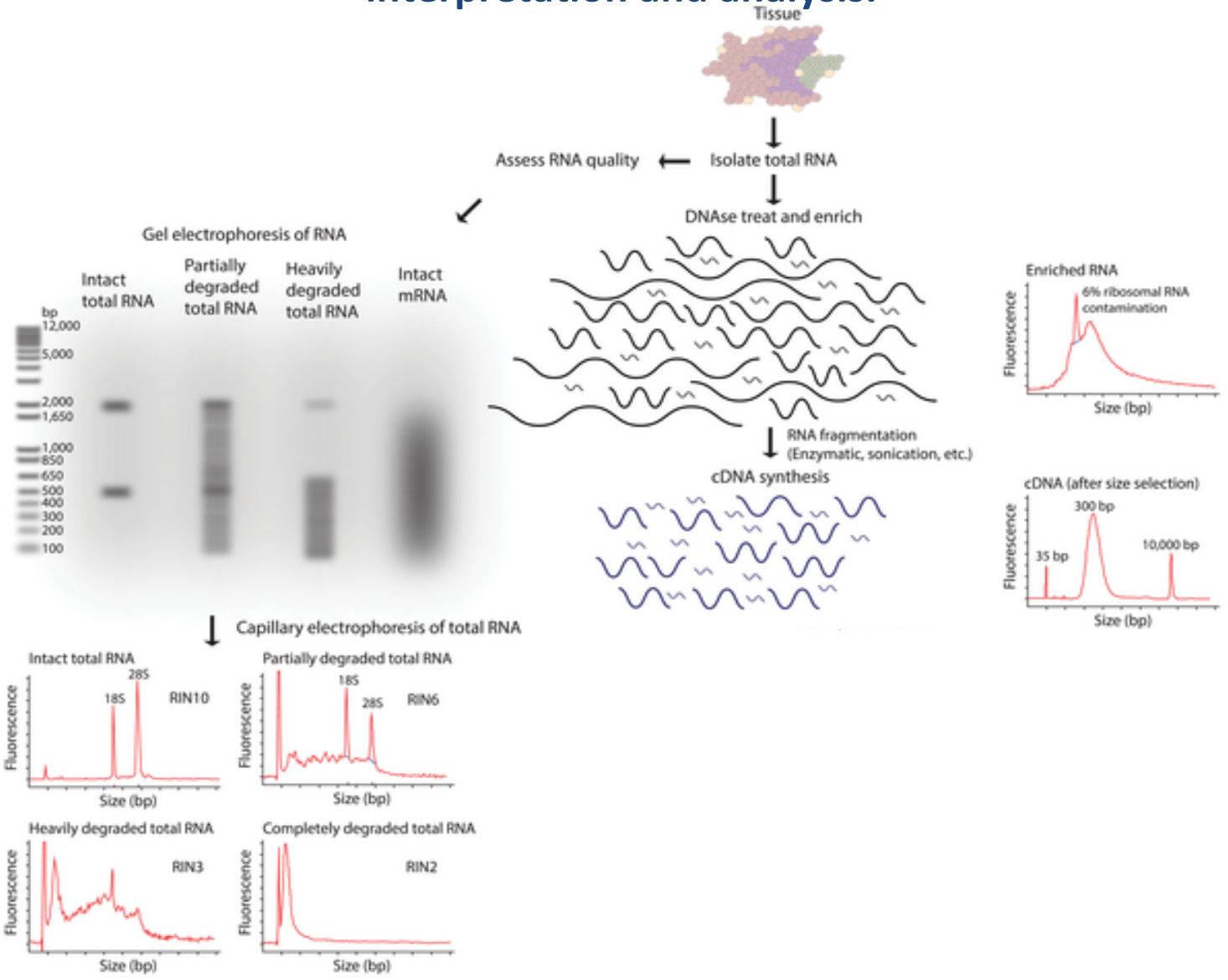

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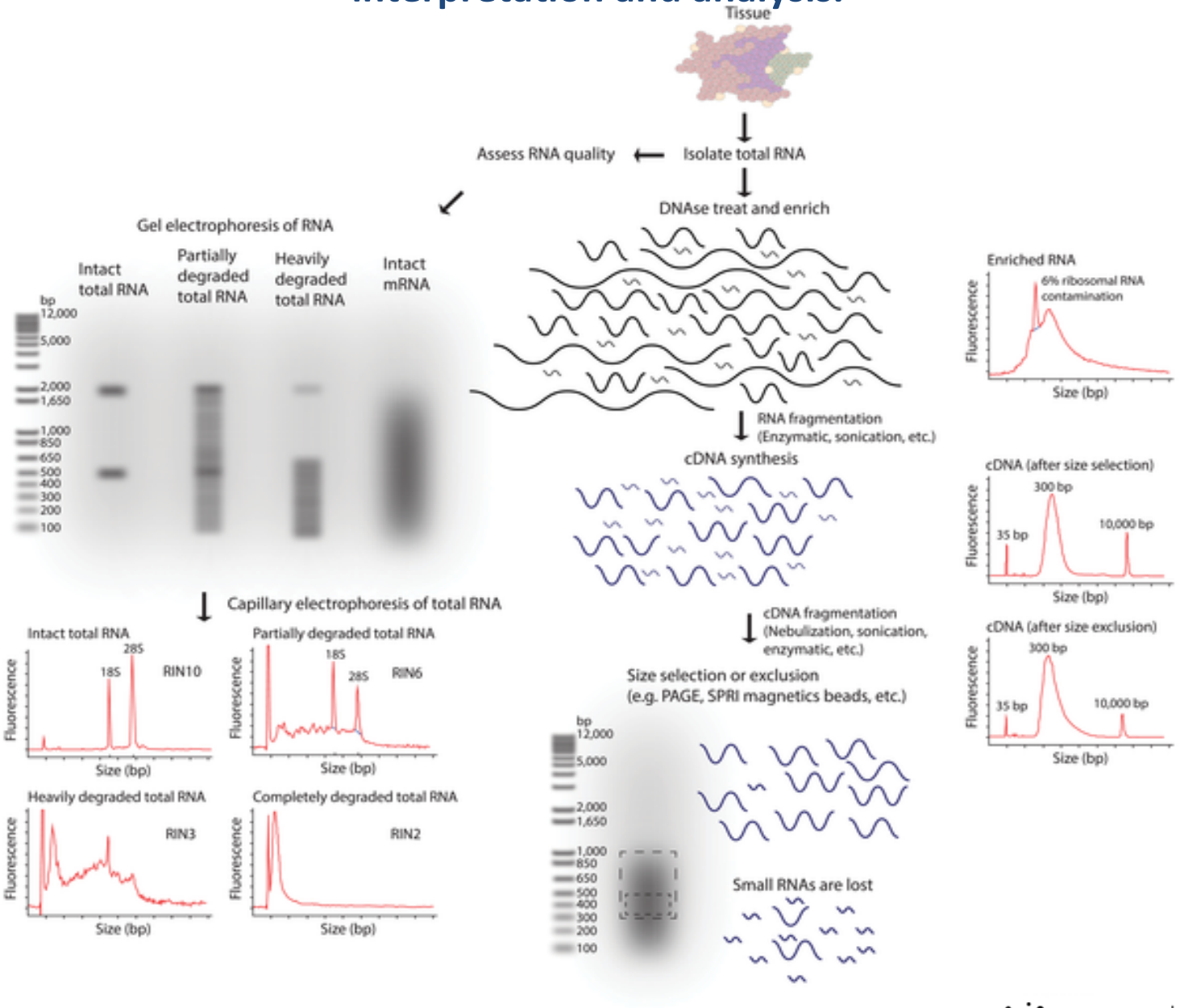

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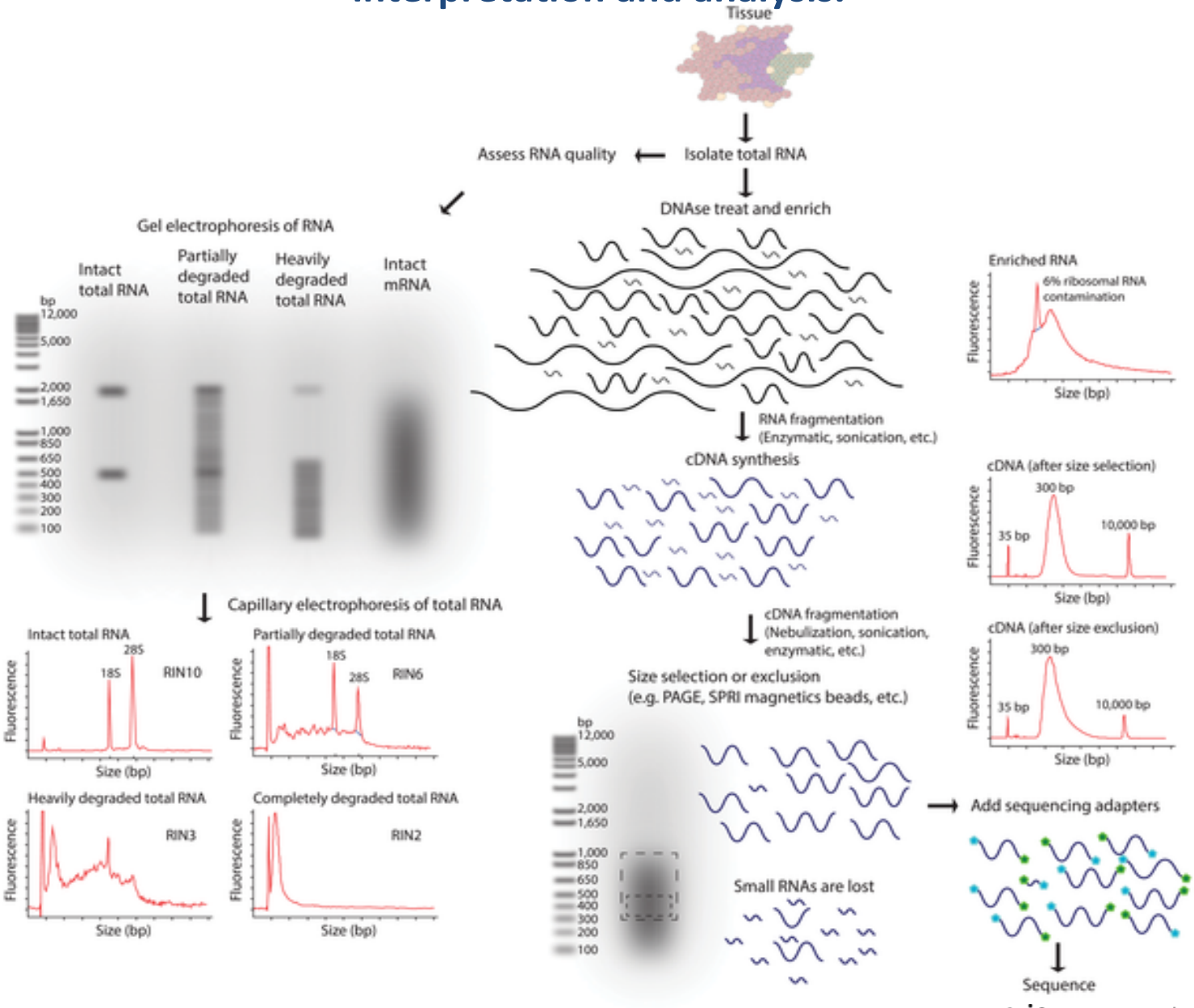

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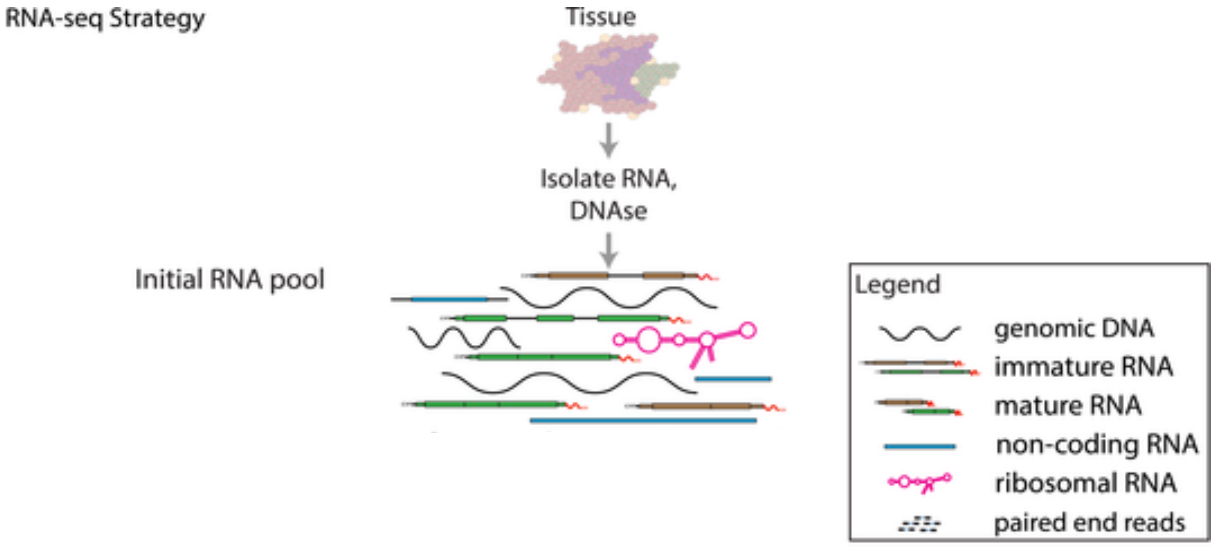
RNA-seq library enrichment strategies that influence interpretation and analysis.



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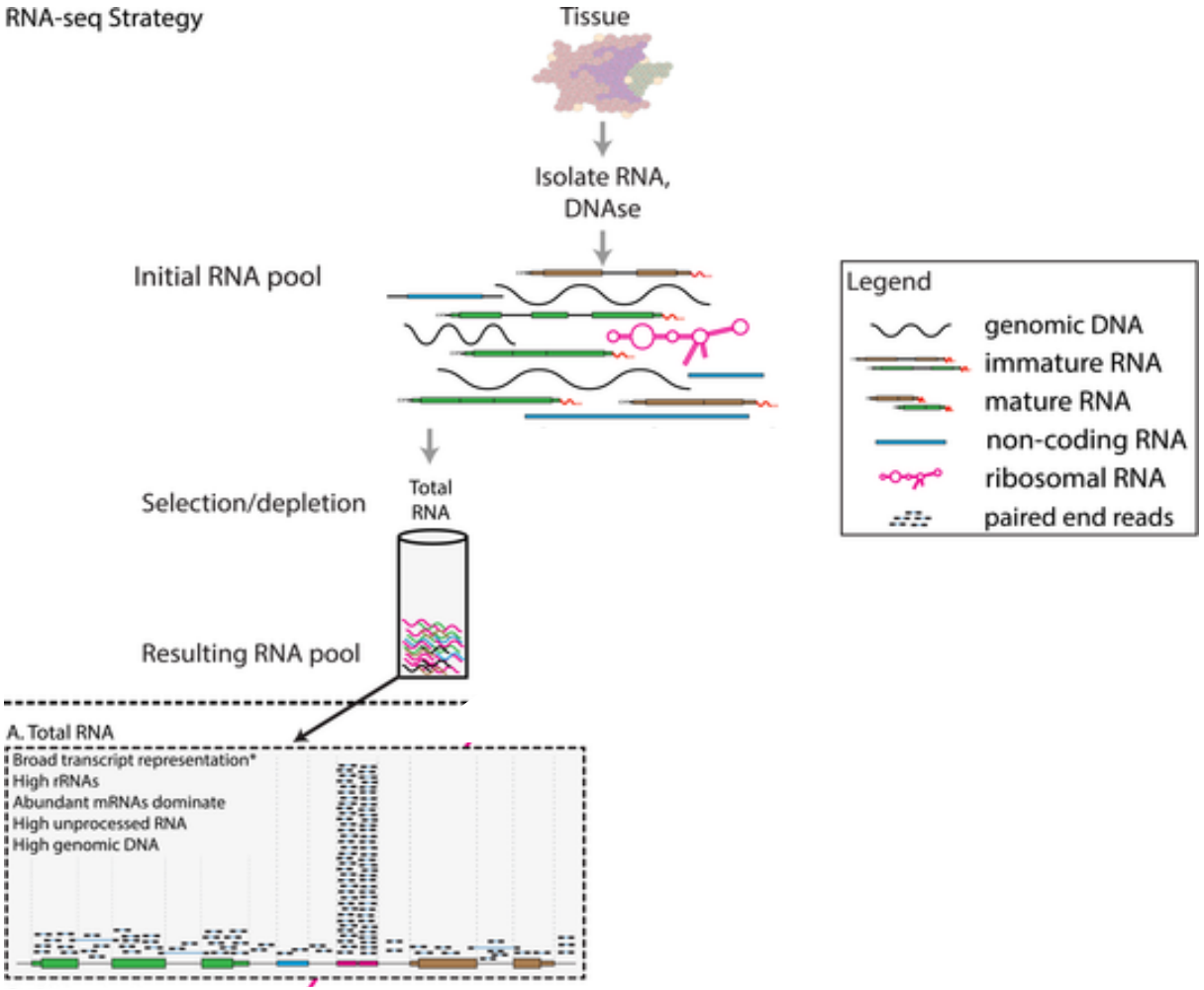

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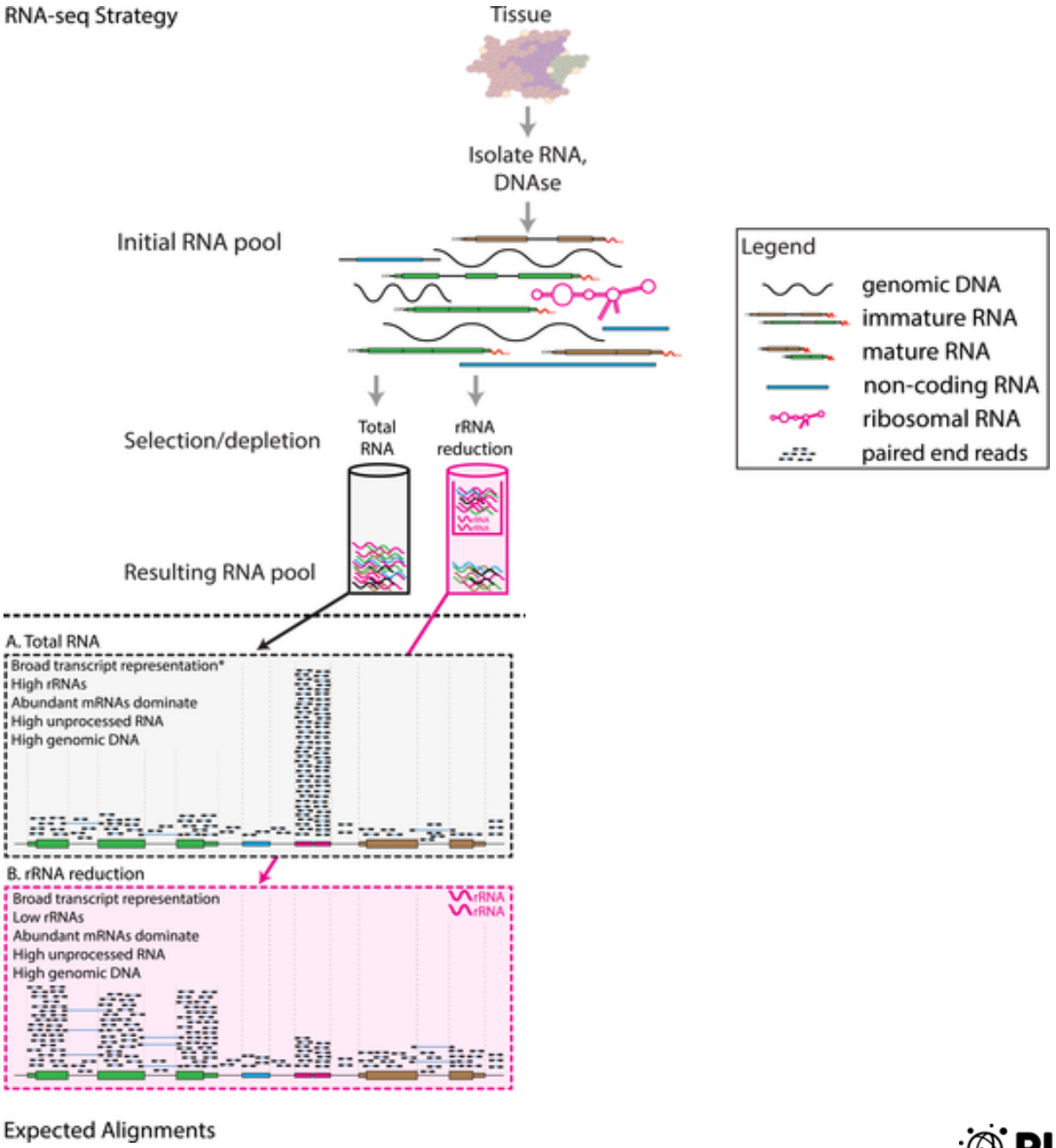

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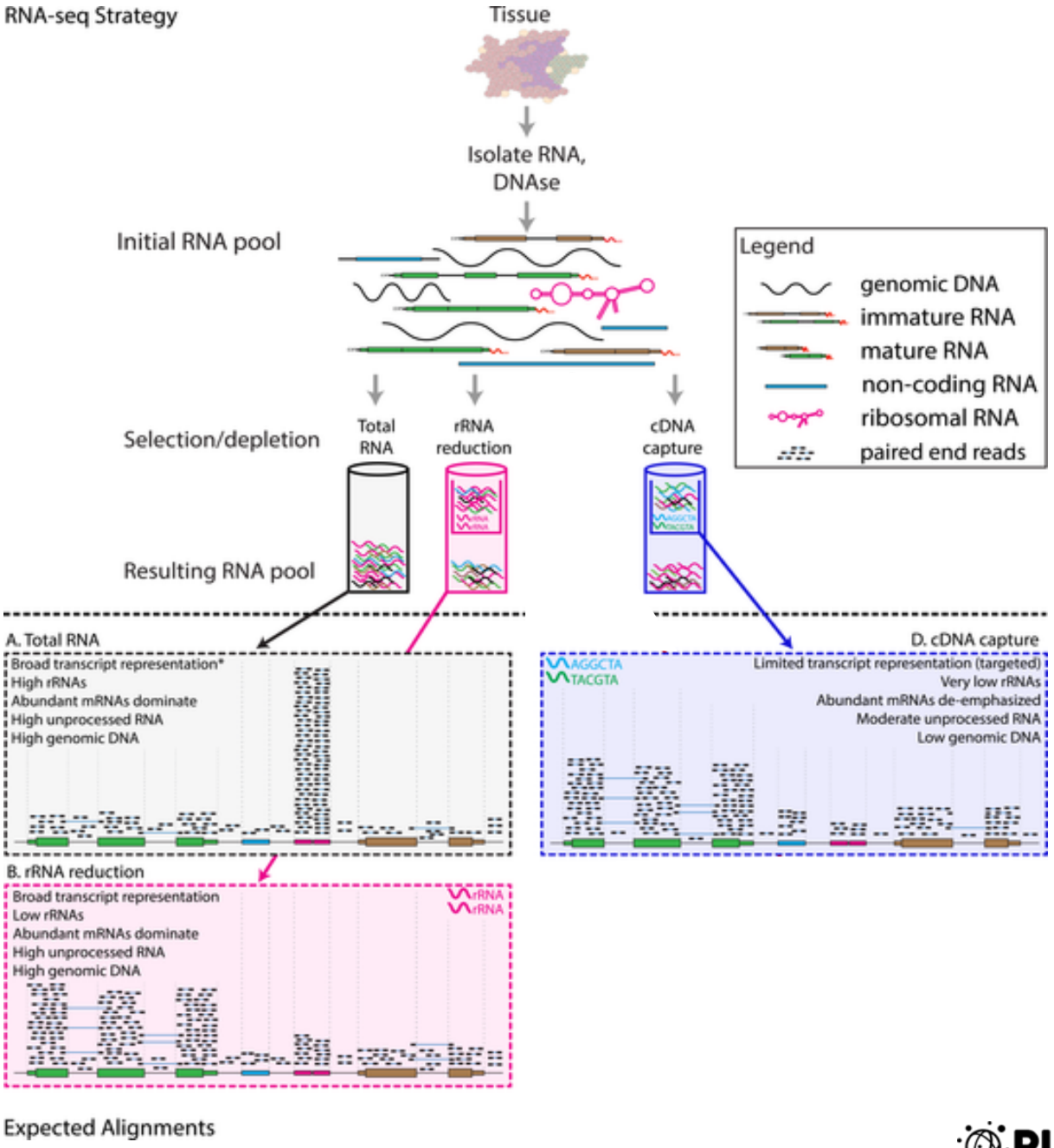

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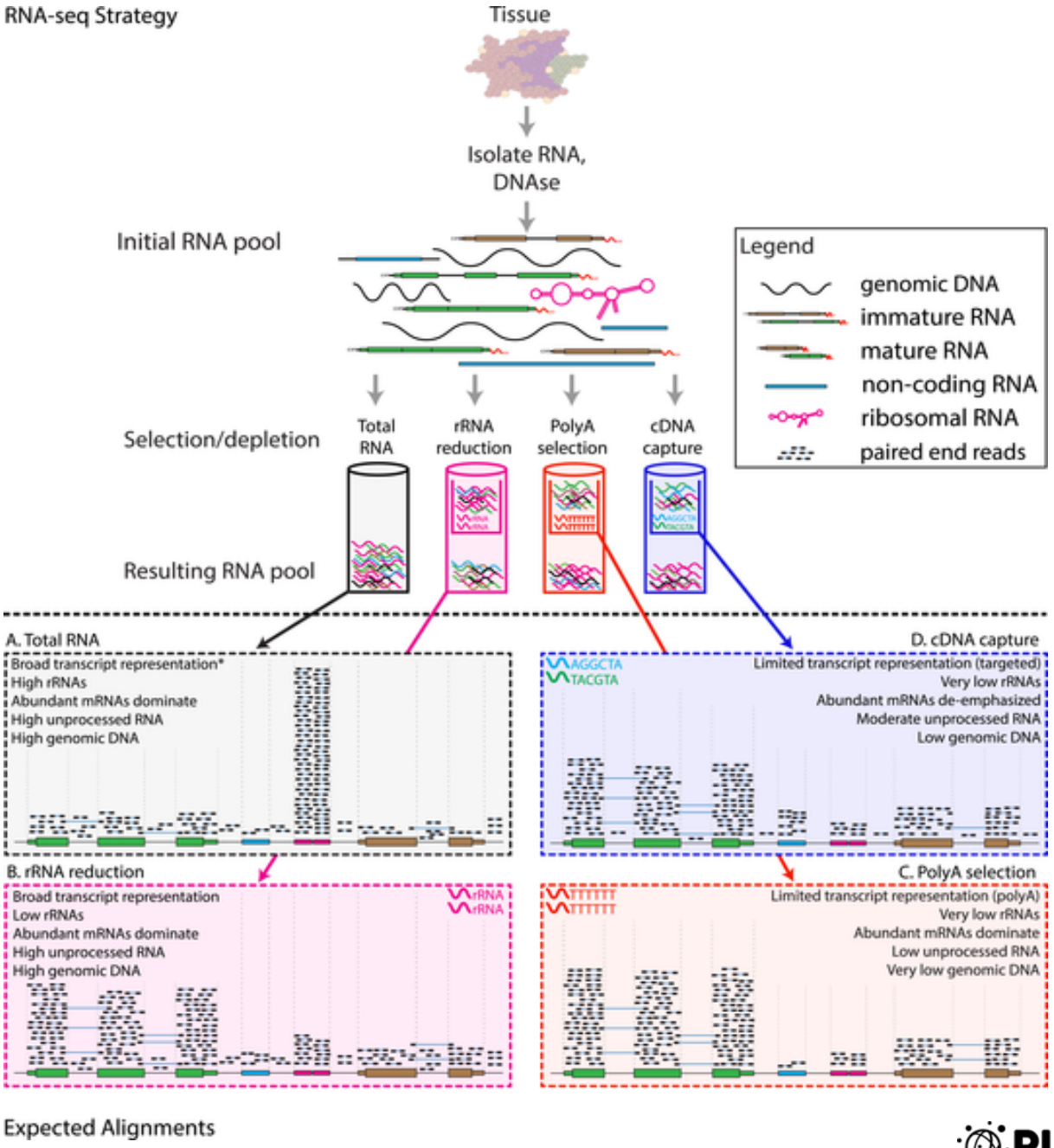

http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

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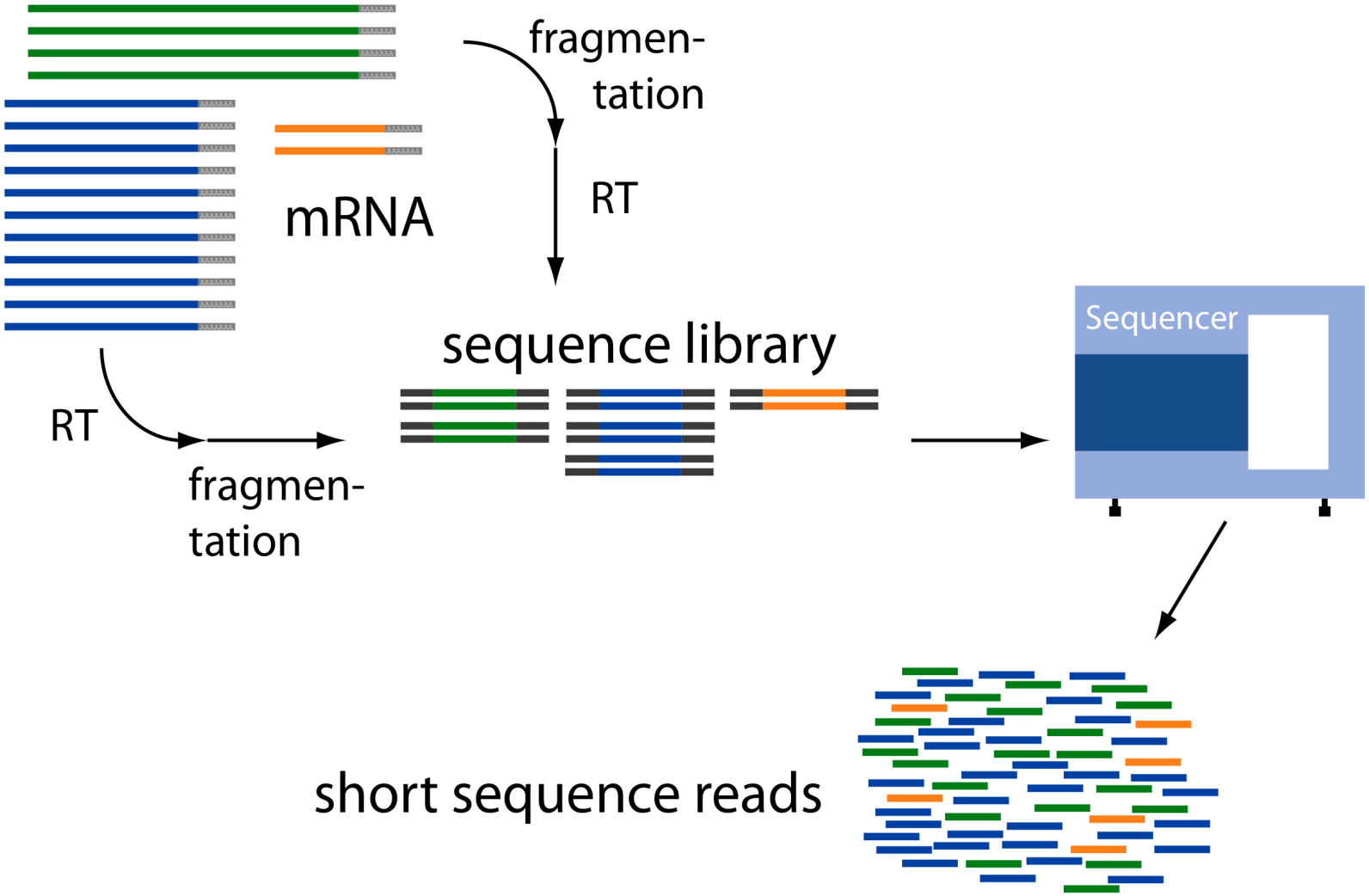
Overview of RNA-Seq



From: http://www2.fml.tuebingen.mpg.de/raetsch/members/research/transcriptomics.html

Common Data Formats for RNA-Seq

FASTA format:

```
>61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
```

FASTQ format:

```
@61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
+
ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBC?CCCCCCCCC@@CACCCCCA
```

Read

Quality values

Interpreting Base Quality Values

Phred_Quality_Value = AsciiEncodedQual('B') – 33 = 30

Phred_Quality_Value = -10 * $\log_{10}$(Pwrong('T'))

Pwrong('T') = 10^(30/-10) = 10^-3 = 0.001

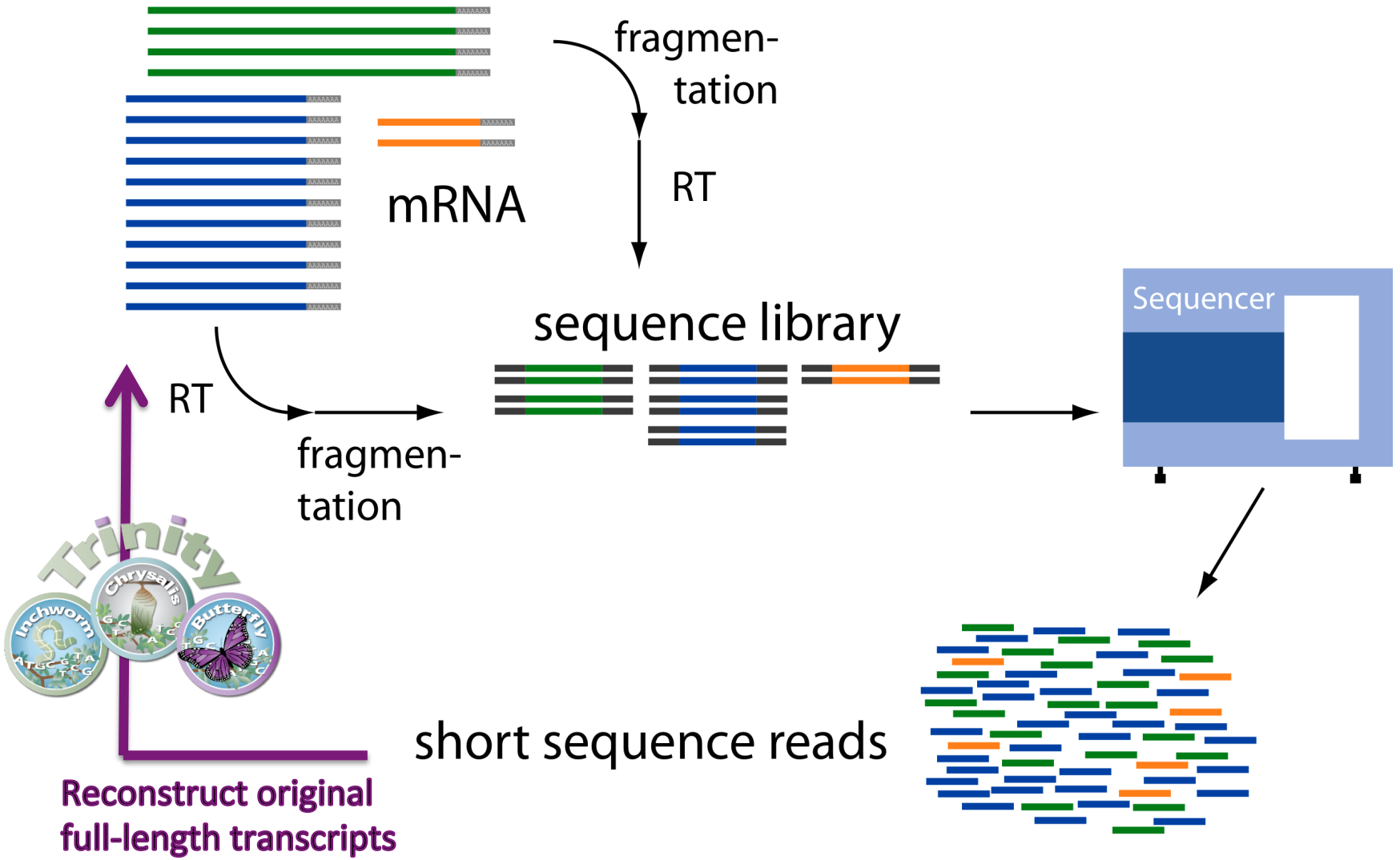
Paired-end Sequences

Two FastQ files, read name indicates left (/1) or right (/2) read of paired-end

```
@61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
+
ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBC?CCCCCCCCC@@CACCCCCA
```

```
@61DFRAAXX100204:1:100:10494:3070/2
CTCAAATGGTTAATTCTCAGGCTGCAAATATTCGTTCAGGATGGAAGAACA
+
C<CCCCCCCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBCCCC
```

Overview of RNA-Seq



From: http://www2.fml.tuebingen.mpg.de/raetsch/members/research/transcriptomics.html

The Ever-Growing Trinity User Community

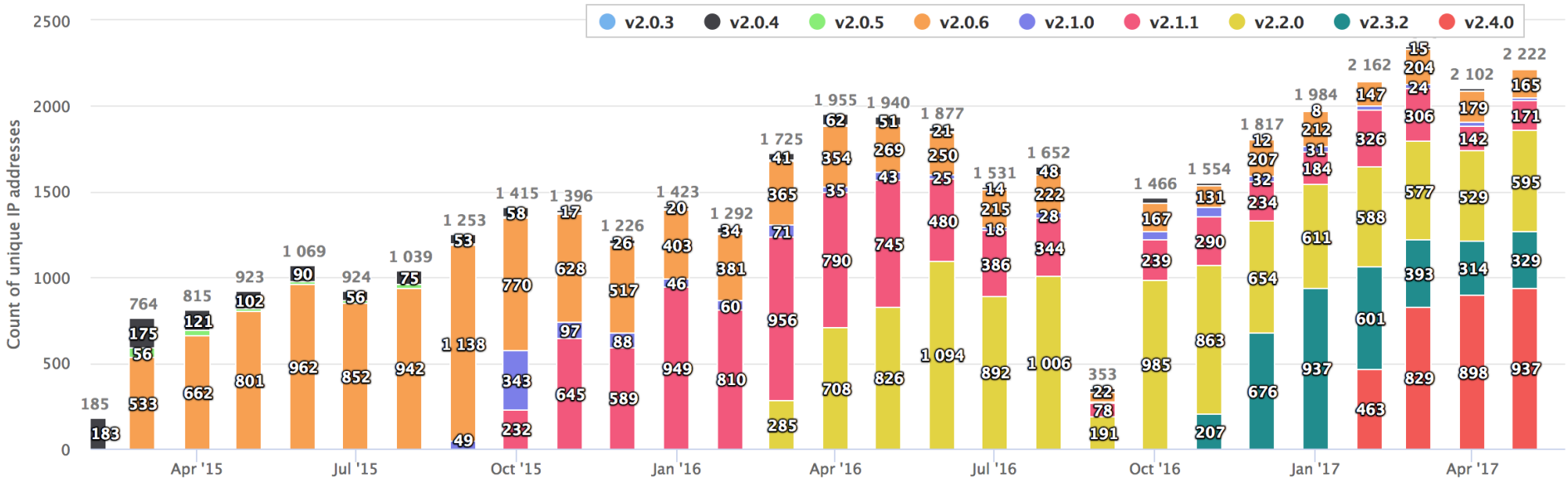
Nature Biotechnology, 2011
Nature Protocols, 2013

- ~3k unique users per month
- ~8k literature citations
- Open Source software development contributions from the Trinity community.

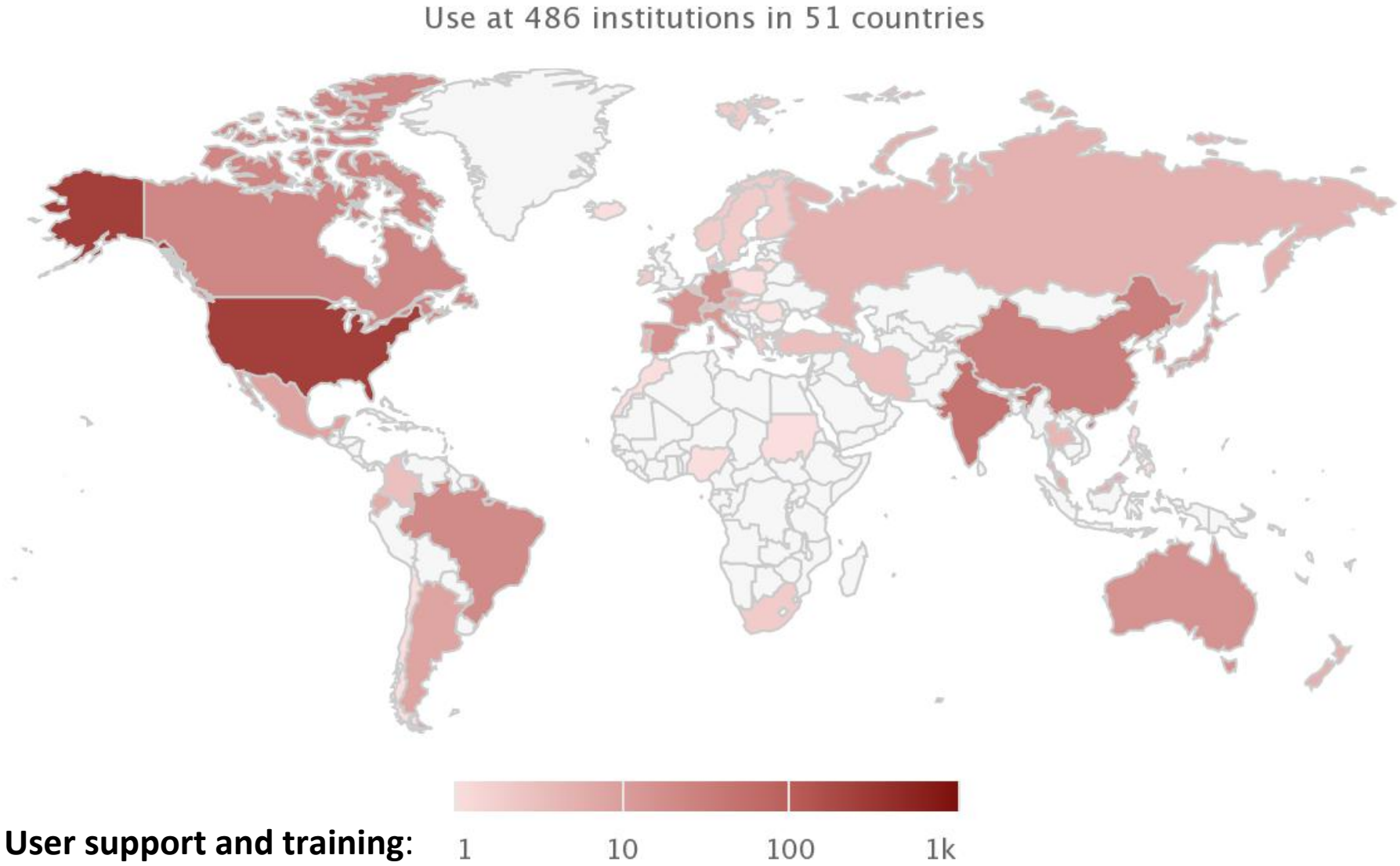
GitHub

http://trinityrnaseq.github.io

Trinity Usage Tracked by Unique IP Address



Trinity Usage is Global



User support and training:

- Google group and Twitter feed for community interaction and support.
- Extensive documentation, user guides, tutorials and protocols
- Demo and training videos
- On-site training workshops

nature protocols

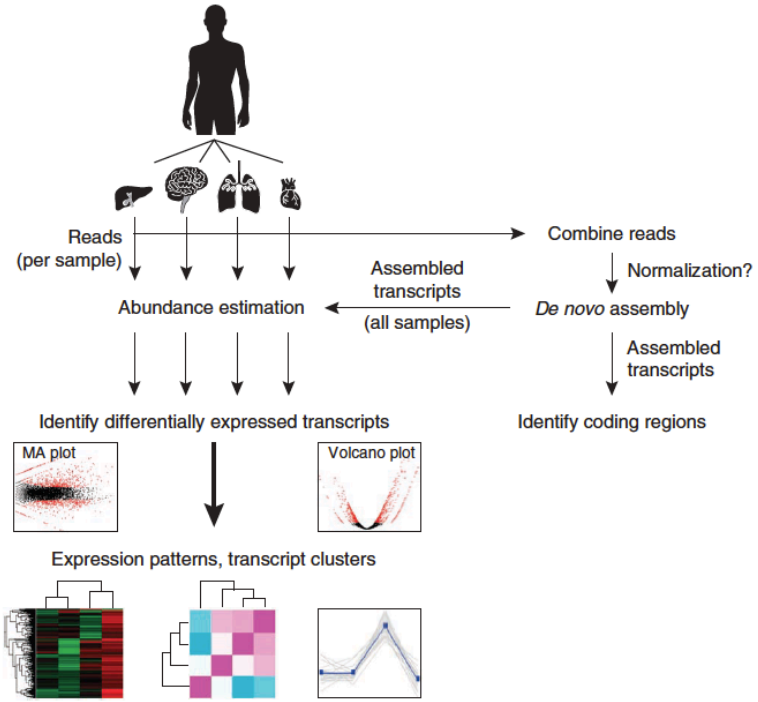
NATURE PROTOCOLS | PROTOCOL

De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis

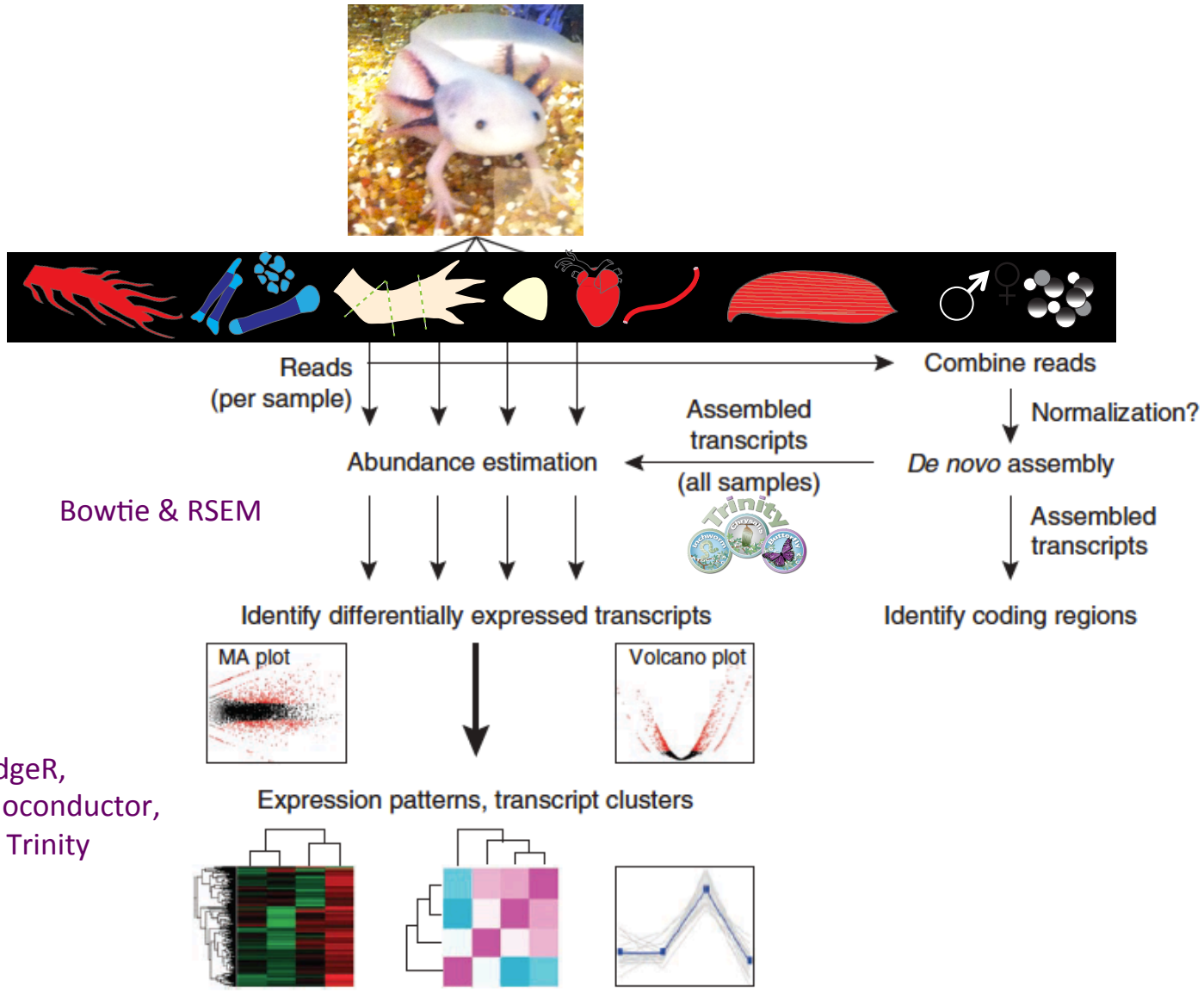
Brian J Haas, Alexie Papanicolaou, Moran Yassour, Manfred Grabherr, Philip D Blood, Joshua Bowden, Matthew Brian Couger, David Eccles, Bo Li, Matthias Lieber, Matthew D MacManes, Michael Ott, Joshua Orvis, Nathalie Pochet, Francesco Strozzi, Nathan Weeks, Rick Westerman, Thomas William, Colin N Dewey, Robert Henschel, Richard D LeDuc, Nir Friedman & Aviv Regev

Affiliations | Contributions | Corresponding authors

Nature Protocols **8**, 1494–1512 (2013) | doi:10.1038/nprot.2013.084
Published online 11 July 2013



Framework for De novo Transcriptome Assembly and Analysis



1.3 Billion
Total Reads

86 Million
Normalized Reads

Bowtie & RSEM

EdgeR,
Bioconductor,
& Trinity

RNA-Seq De novo Assembly Using Trinity

Quick Guide for the Impatient

Trinity assembles transcript sequences from Illumina RNA-Seq data.

Download Trinity here.

Build Trinity by typing 'make' in the base installation directory.

Assemble RNA-Seq data like so:

```
Trinity --seqType fq --left reads_1.fq --right reads_2.fq --CPU 6 --max_memory 20G
```

Find assembled transcripts as: 'trinity_out_dir/Trinity.fasta'

Use the documentation links in the right-sidebar to navigate this documentation, and contact our Google group for technical support.

Example Applications of the Trinity RNA-Seq Protocol

Resource

A Tissue-Mapped Axolotl De Novo Transcriptome Enables Identification of Limb Regeneration Factors

Donald M. Bryant[1,6], Kimberly Johnson[1,6], Tia DiTommaso[1], Timothy Tickle[2], Matthew Brian Couger[3], Duygu Payzin-Dogru[1], Tae J. Lee[1], Nicholas D. Leigh[1], Tzu-Hsing Kuo[1], Francis G. Davis[1], Joel Bateman[1], Sevara Bryant[1], Anna R. Guzikowski[1], Stephanie L. Tsai[4], Steven Coyne[1], William W. Ye[1], Robert M. Freeman Jr.[5], Leonid Peshkin[5], Clifford J. Tabin[4], Aviv Regev[2], Brian J. Haas[2], , , Jessica L. Whited[1,7],

Original Article

Loggerhead sea turtle embryos (*Caretta caretta*) regulate expression of stress response and developmental genes when exposed to a biologically realistic heat stress

Blair P. Bentley, Brian J. Haas, Jamie N. Tedeschi, Oliver Berry

Got RNA-Seq?

Run Trinity