

Canadian Bioinformatics Workshops

www.bioinformatics.ca

bioinformaticsdotca.github.io

Supported by



This page is available in the following languages:

Afrikaans Ελληνικά Català Dansk Deutsch Ελληνικά English English (CA) English (GB) English (US) Esperanto Castellano Castellano (AR) Español (CL) Castellano (CO) Español (Ecuador) Castellano (MX) Castellano (PE) Euskara Suomi français français (CA) Galego עברית hrvatski Magyar Italiano 日本語 한국어 Macedonian Melayu Nederlands Norsk Sesotho sa Leboa polski Português română slovenski jezik срpski srpski (latinica) Sotho svenska 中文 華語 (台灣) isiZulu



Attribution-Share Alike 2.5 Canada

You are free:



to **Share** — to copy, distribute and transmit the work



to **Remix** — to adapt the work



Under the following conditions:



Attribution. You must attribute the work in the manner specified by the author or licensor (but not in any way that suggests that they endorse you or your use of the work).



Share Alike. If you alter, transform, or build upon this work, you may distribute the resulting work only under the same or similar licence to this one.

- For any reuse or distribution, you must make clear to others the licence terms of this work.
- Any of the above conditions can be waived if you get permission from the copyright holder.
- The author's moral rights are retained in this licence.

[Disclaimer](#)

Your fair dealing and other rights are in no way affected by the above.
This is a human-readable summary of the Legal Code (the full licence) available in the following languages:
[English](#) [French](#)

[Learn how to distribute your work using this licence](#)

Alignment Free Expression Estimation (Kallisto)

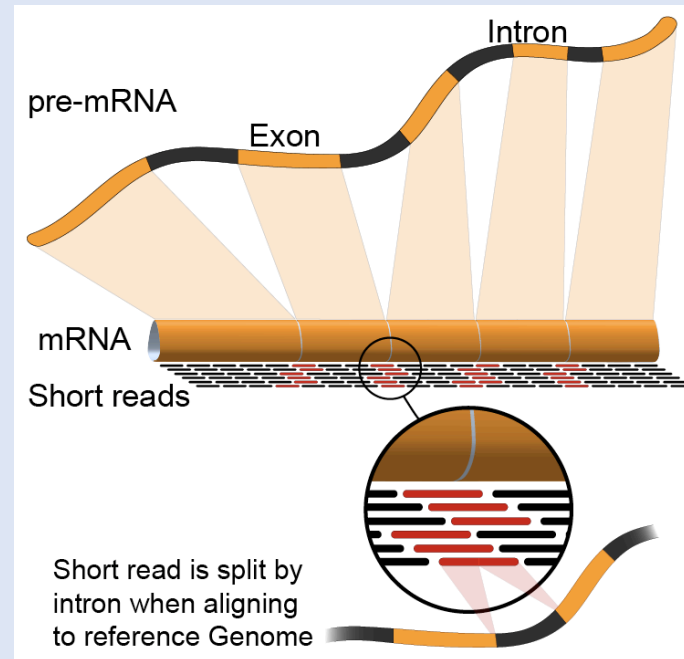
Kelsy Cotto, Obi Griffith, Malachi Griffith, Saad Khan, Allegra Petti, Huiming Xia

Informatics for RNA-seq Analysis

June 17-19, 2020



A collage of four panels. Top-left: A grid of red and white dots. Top-right: A diagram of a gene with exons and introns. Bottom-left: A network diagram with red nodes and white lines. Bottom-right: Logos for CSH Cold Spring Harbor Laboratory and bioinformatics.ca.



What is a k-mer?

- A fixed sized (K) sequence
- A string of length N contains $N-K+1$ k-mers

1-mer

A
C
G
T

2-mer

AA	AC	AG	AT
CA	CC	CG	CT
GA	GC	GG	GT
TA	TC	TG	TT

ATTCGACAGTAGCCATGACTGG

- One can build K -mer index to represent a string

7-mer	iD	N
ATTCGAC	1	1
TTCGACA	2	1
TCGACAG	3	1
...		

Sailfish: Alignment-free Isoform Quantification from RNA-seq Reads using Lightweight Algorithms Rob Patro, Stephen M. Mount, and Carl Kingsford. *Manuscript Submitted* (2013) <http://www.cs.cmu.edu/~ckingsf/class/02714-f13/Lec05-sailfish.pdf>

<https://www.slideshare.net/duruofei/cmsc702-project-final-presentation>

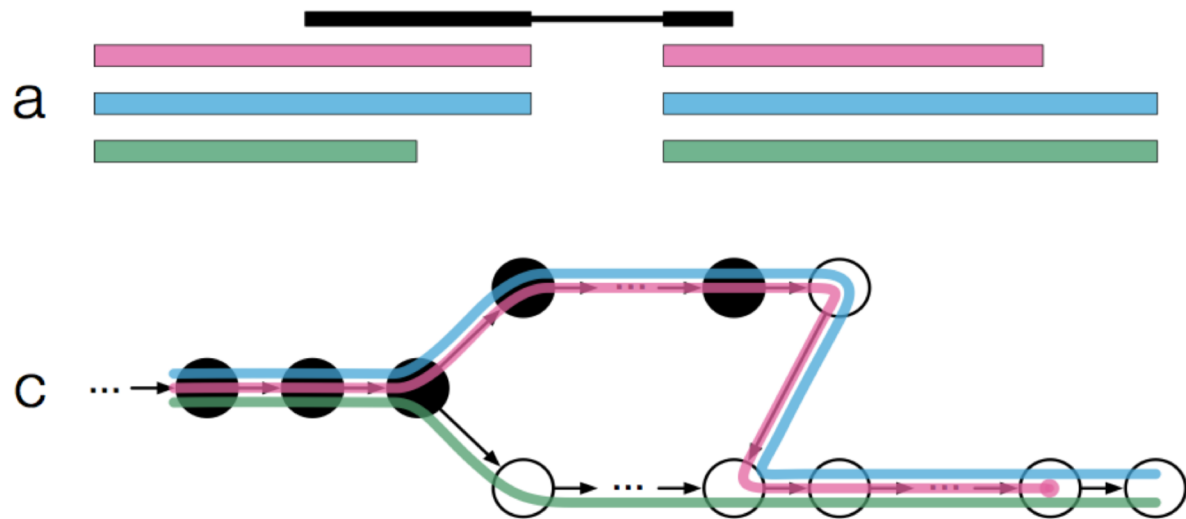
Alignment free approaches for transcript abundance

1. Obtain reference transcript sequences
 - e.g. Ensembl, Refseq, or GENCODE
2. Build a **k-mer index** of all of the k-mers in each transcript sequence
 - Store each k-mer and its position within the transcript. “hashing”

Alignment free approaches for transcript abundance

3. Count number of times each k-mer occurs within each RNAseq read

- Model relationship between RNA-seq read k-mers and the transcript k-mer index.
- What transcript is the most likely source for each read?
- Called “pseudoalignment”, “quasi-mapping”, etc.



Bray, 2016 doi:10.1038/nbt.3519

<https://tinyheero.github.io/2015/09/02/pseud-alignments-kallisto.html>

4. Handle sequencing errors, isoforms, ambiguity, and determine abundance estimates

- Transcriptome de Bruijn graphs, likelihood function, expectation maximization, etc.

Advantages/disadvantages of alignment free approaches

- Advantages
 - Very fast and efficient
 - Similar accuracy to alignment based approach but with much, much shorter run time.
 - Do not need a reference genome, only a reference transcriptome
- Disadvantages
 - You don't get a proper BAM file (though a pseudo-bam can be created)
 - Information in reads with sequence errors may be ignored
 - Limited potential for transcript discovery, variant calling, fusion detection, etc.

Common alignment free tools

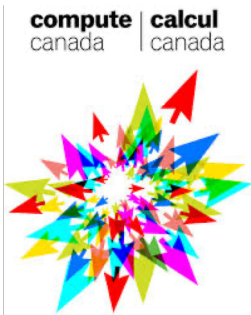
- Sailfish
 - “Sailfish enables alignment-free isoform quantification from RNA-seq reads using lightweight algorithms.” 2014
 - <https://www.ncbi.nlm.nih.gov/pubmed/24752080>
- RNA-Skim
 - “RNA-Skim: a rapid method for RNA-Seq quantification at transcript level.” 2014
 - <https://www.ncbi.nlm.nih.gov/pubmed/24931995>
- Kallisto
 - “Near-optimal probabilistic RNA-seq quantification.” 2016
 - <https://www.ncbi.nlm.nih.gov/pubmed/27043002>
- Salmon
 - “Salmon provides fast and bias-aware quantification of transcript expression.” 2017
 - <https://www.ncbi.nlm.nih.gov/pubmed/28263959>

Which is best?

- Somewhat controversial ...
- <https://liorpachter.wordpress.com/2017/08/02/how-not-to-perform-a-differential-expression-analysis-or-science/>
- Various sources suggest that Salmon, Kallisto, and Sailfish results are quite comparable
- Usability, documentation, and supporting downstream tools could be used to decide

We are on a Coffee Break & Networking Session

Workshop Sponsors:



Canadian Centre for
Computational
Genomics

