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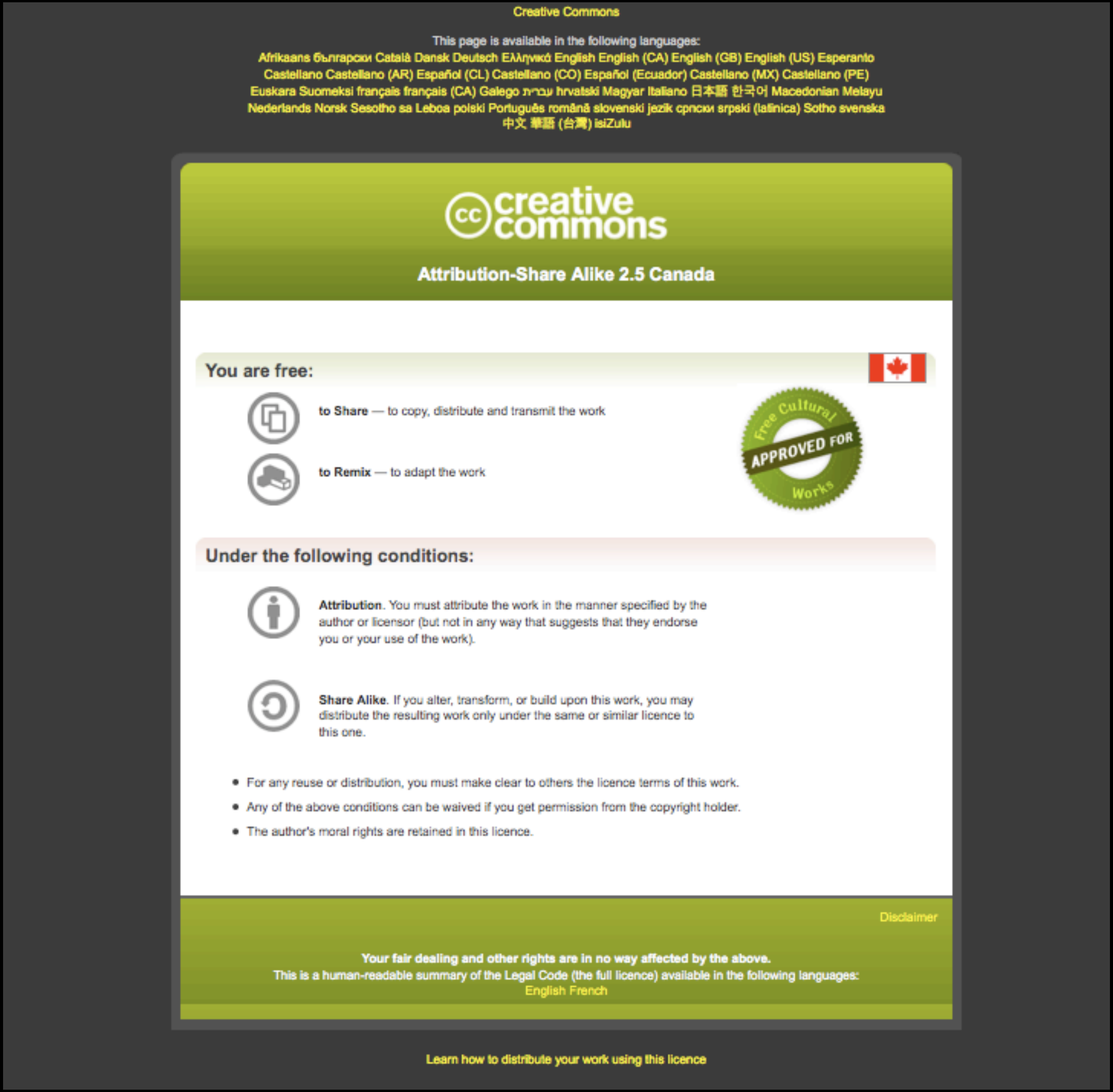

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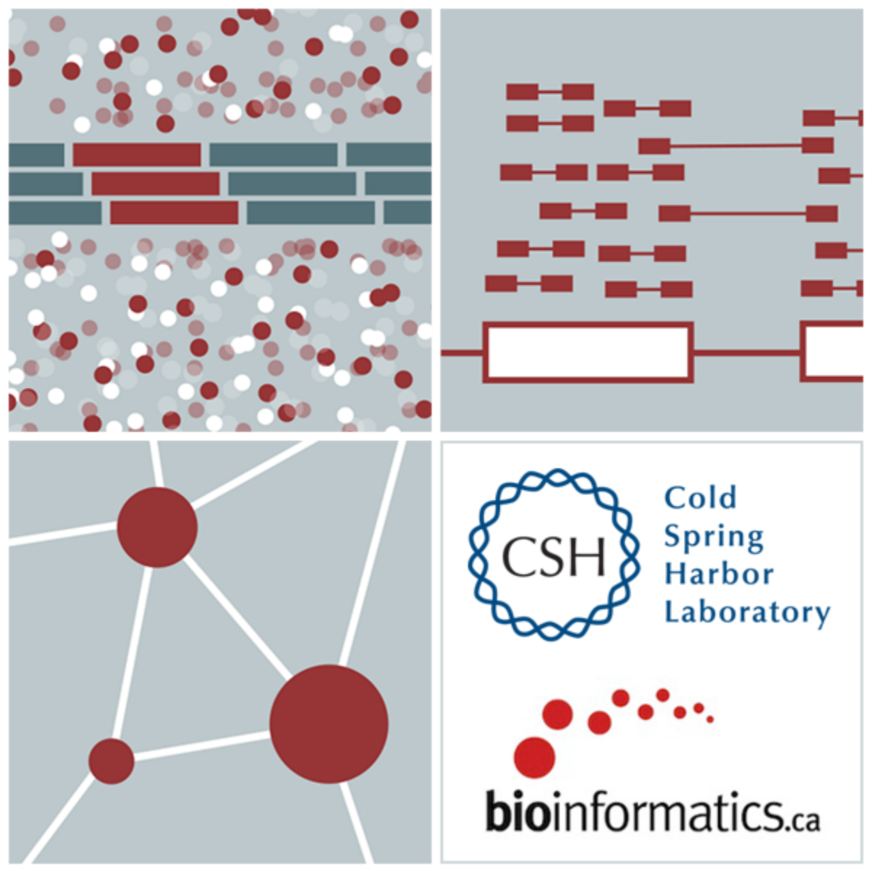
RNA-Seq Module 4
Alignment Free Expression Estimation (lecture)

Malachi Griffith, Obi Griffith

Informatics for RNA-seq Analysis

May 28-30, 2018

bioinformatics.ca



THE GENOME INSTITUTE at Washington University

Learning objectives of the course

- Module 0: Introduction to Cloud Computing
- Module 1: Introduction to RNA Sequencing
- Module 2: Alignment and Visualization
- Module 3: Expression and Differential Expression
- **Module 4: Alignment Free Expression Estimation**
- Module 5: Isoform Discovery and Alternative Expression

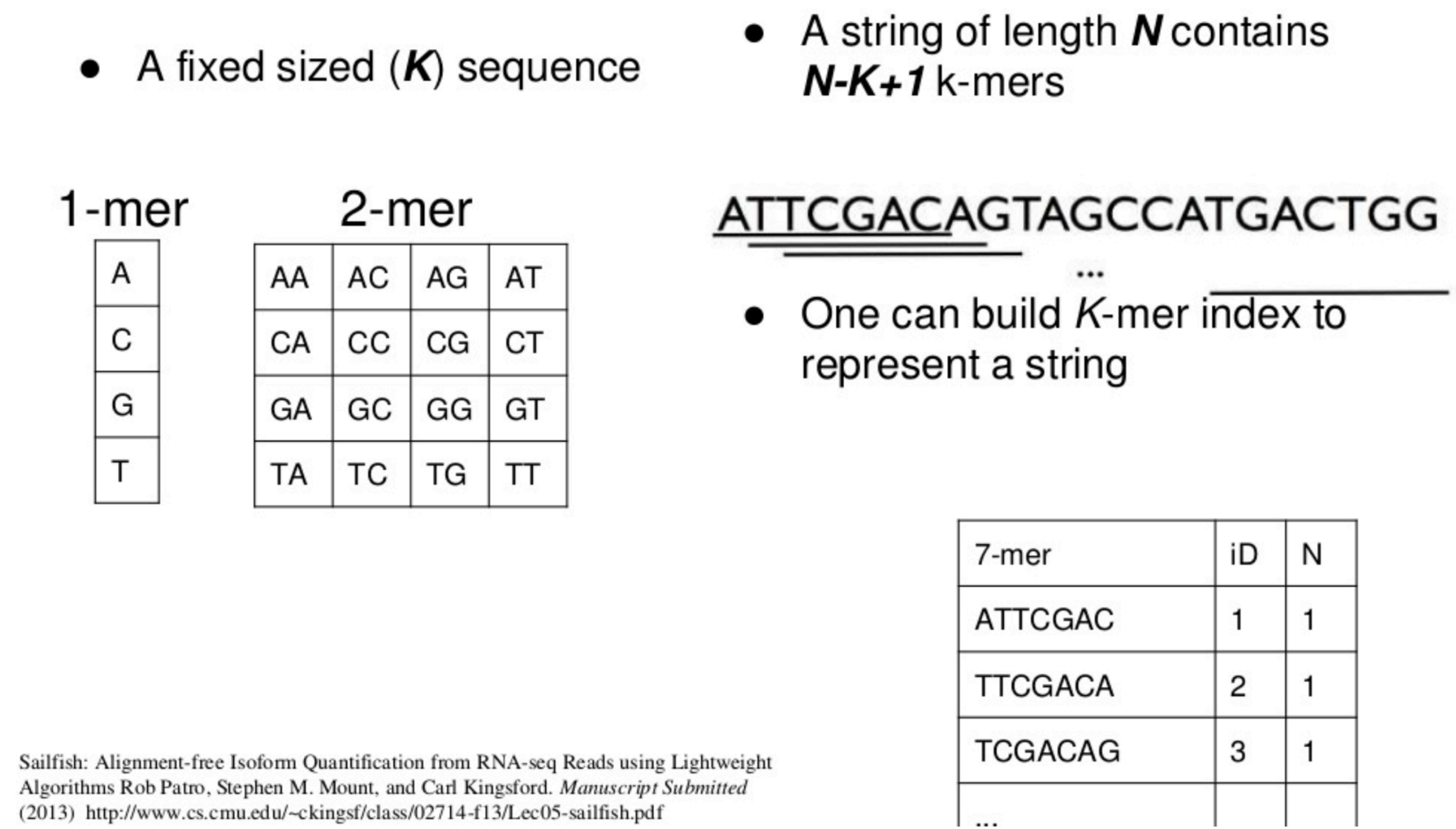
- Tutorials
  - Provide a working example of an RNA-seq analysis pipeline
  - Run in a 'reasonable' amount of time with modest computer resources
  - Self contained, self explanatory, portable

Learning objectives of module 4

- Alignment free estimation of transcript abundance

- Introduction to k-mers
- Alignment free tools
  - Sailfish, RNA-Skim, Kallisto, Salmon
- Abundance estimation and differential expression analysis with Kallisto and Sleuth

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

Basic concept of 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 occurring in each transcript sequence
   - Store each k-mer and its position within the transcript. "hashing"
3. Parse all RNA-seq reads and count how many times each k-mer occurs within each 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.
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
  - 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