Analysis Tools for RNA-seq and Isoform Characterization

Slides: `bit.ly/1DeRjGM`

Gunnar Rätsch

Biomedical Data Science Group
Computational Biology Center
Memorial Sloan Kettering Cancer Center

@gxr #RNA #MMR #SplAdder #riboDiff #Cancer

Memorial Sloan-Kettering
Cancer Center

cBio@MSKCC

Biomedical Data Sciences Group

Facts

- Cost of collecting data drops, amounts increase exponentially.
- We have *more data than accurate algorithms*.

Group's research

- **Data Science** *Algorithms, Models & Tools*
  - ⇝ *Machine Learning,*
  - ⇝ *Bioinformatics.*
- **Biology & Medicine** *Problem Setting & Goals*
  - ⇝ *RNA processing regulation,*
  - ⇝ *Clinical data analysis.*

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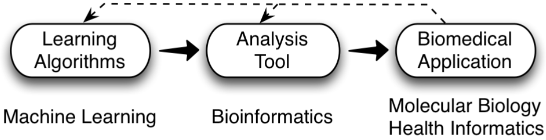
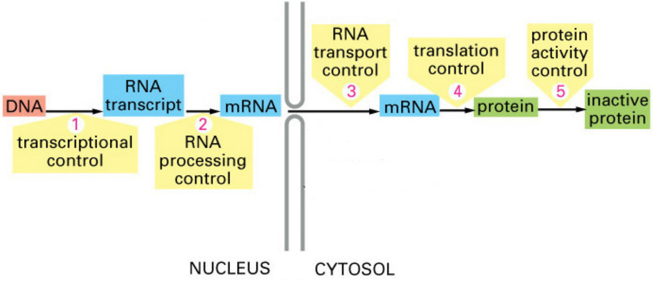


Learning About the Central Dogma



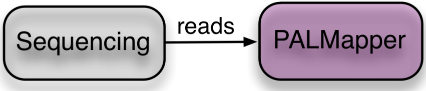
Goal: Learn to predict what these processes accomplish:

- Given the DNA, . . . , predict all gene products

$$f(\text{DNA}, 1\ 2\ 3) = \text{RNA} \qquad g(\text{RNA}, 4\ 5) = \text{protein}$$

- Estimating f, g amounts to cracking the codes of transcription, epigenetics, splicing, . . .

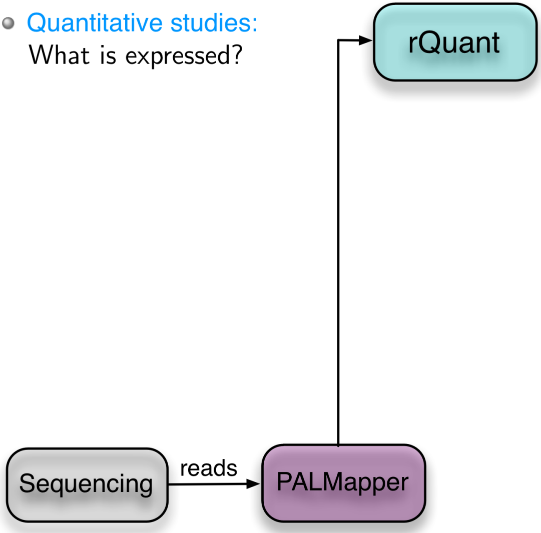
RNA-seq based Transcriptome Characterization



Accurate spliced alignments
[Bona et al., 2008, Jean et al., 2010]

RNA-seq based Transcriptome Characterization

- Quantitative studies:
  What is expressed?



Isoform quantitation and bias modeling
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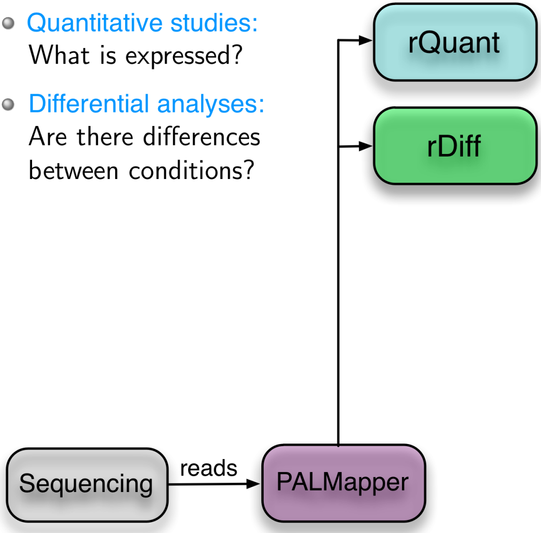
  Are there differences between conditions?



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Tests for differential isoform expression
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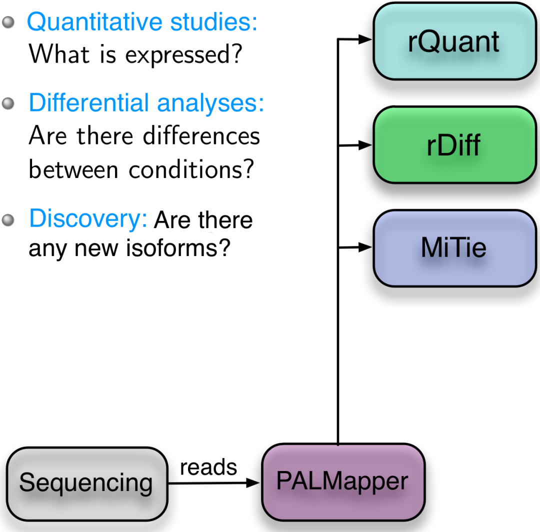

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Simultaneous transcript identification & quantitation
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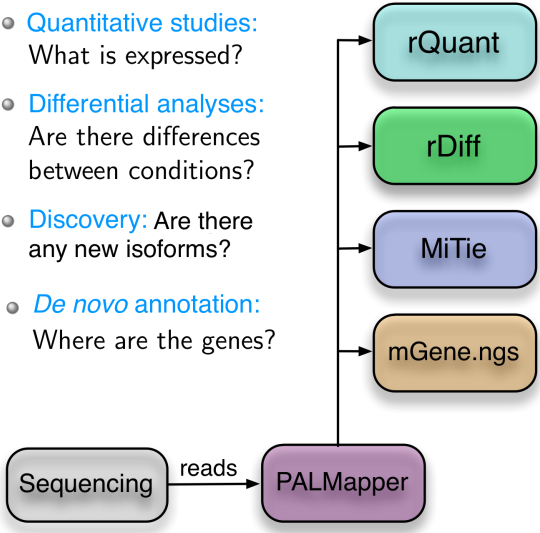
- *De novo* annotation:
  Where are the genes?



Sequencing

reads

PALMapper

rQuant

rDiff

MiTie

mGene.ngs

Isoform quantitation and bias modeling
[Bohnert et al., 2009, 2010]

Tests for differential isoform expression
[Drewe et al., 2013]

Simultaneous transcript identification & quantitation
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Gene finding with RNA-seq evidence
[Behr et al., 2010, 2013, Gan et al., 2011]

Accurate spliced alignments
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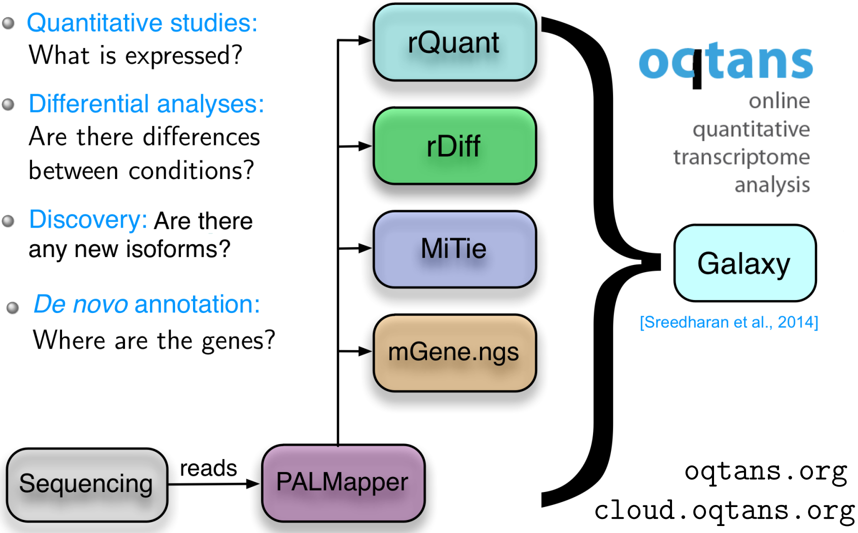

Sequencing

reads

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MiTie

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oqtans

online quantitative transcriptome analysis

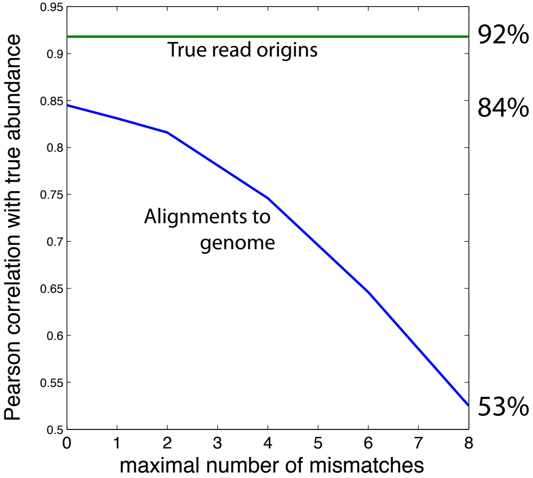
Galaxy

[Sreedharan et al., 2014]

oqtans.org

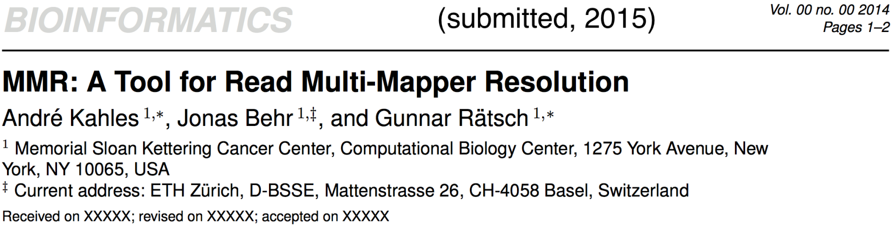
cloud.oqtans.org

Transcript Quantitation and Dependence on Alignments



False alignments, multi-mappers etc. lead to weaker results

Simulated human reads from transcripts of known abundance (Fluxsimulator, [Sammeth, 2009]), 3% error rate, alignment w/ PALMapper [Jean et al., 2010], quantification w/ rQuant [Bohnert et al., 2009], Person correlation over considered transcripts.



BIOINFORMATICS (submitted, 2015) *Vol. 00 no. 00 2014*
Pages 1–2

MMR: A Tool for Read Multi-Mapper Resolution

André Kahles [1,*], Jonas Behr [1,‡], and Gunnar Rätsch [1,*]

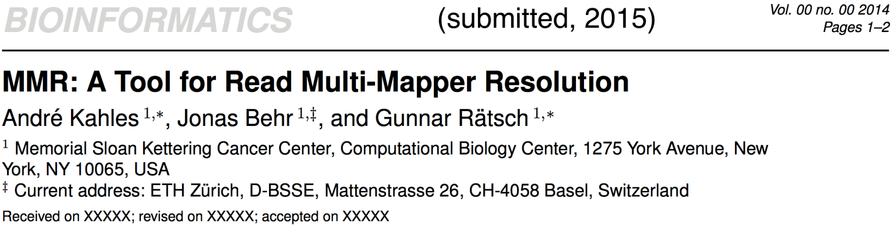
[1] Memorial Sloan Kettering Cancer Center, Computational Biology Center, 1275 York Avenue, New York, NY 10065, USA

[‡] Current address: ETH Zürich, D-BSSE, Mattenstrasse 26, CH-4058 Basel, Switzerland

Received on XXXXX; revised on XXXXX; accepted on XXXXX

bioRxiv `dx.doi.org/10.1101/017103`

- *Efficient* BAM file postprocessor for RNA- & DNA-seq
  - 100M alignments in 20 minutes (10 threads)
- Suitable for **large-scale projects**
- **Improved accuracy** for transcript quantification and prediction
- Open Source `bioweb.me/mmr` (C++)



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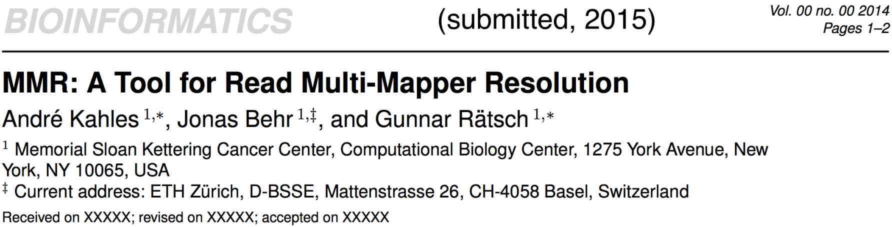

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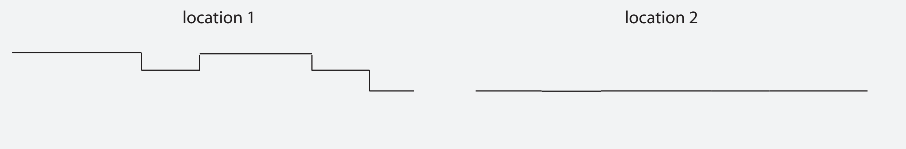
Multiple Mapper Resolution

Principle (Iterated over all reads, N times)

- Use the change of local coverage around read mapping ...
- ... and use its smoothness to identify "better" mapping location



▭ Coverage

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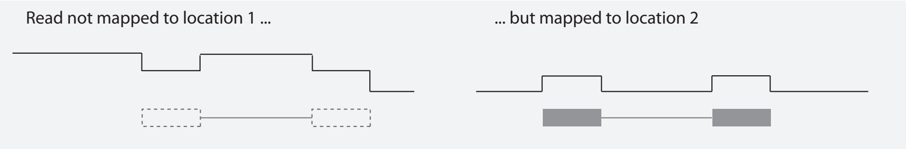

Read not mapped to location 1 ...

... but mapped to location 2

▭ Coverage

▬—▬ Read pair

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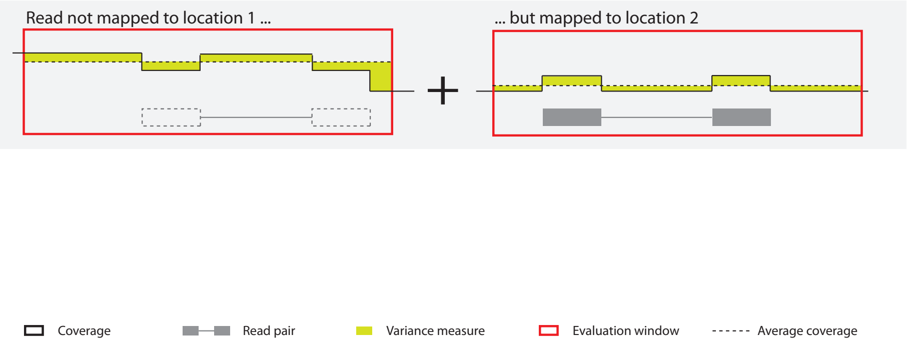

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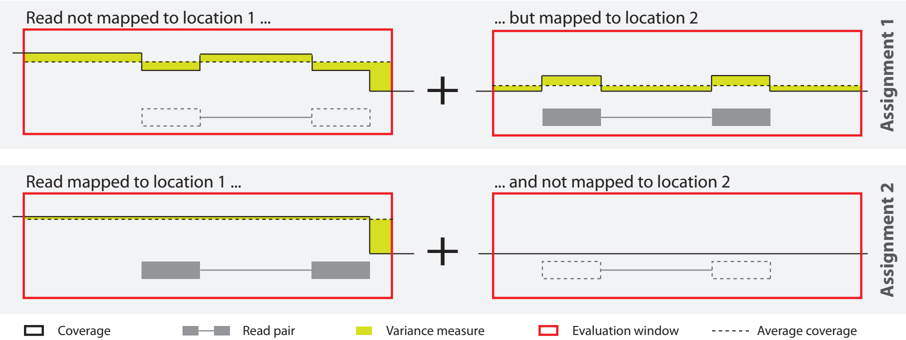
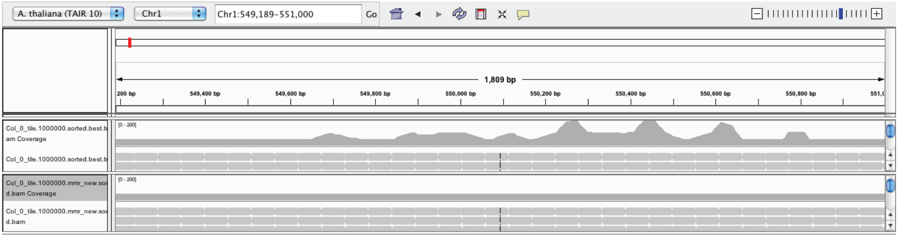

Read not mapped to location 1 ...
... but mapped to location 2
Assignment 1
+
Read mapped to location 1 ...
... and not mapped to location 2
Assignment 2
+
Coverage
Read pair
Variance measure
Evaluation window
Average coverage

Multiple Mapper Resolution

Results for simulated DNA-seq

- Smooths coverage as expected on an artificial dataset



A. thaliana (TAIR 10)

Chr1

Chr1:549,189-551,000

Go

1,809 bp
200 bp
549,400 bp
549,600 bp
549,800 bp
550,000 bp
550,200 bp
550,400 bp
550,600 bp
550,800 bp
551,(
Col_0_tile.1000000.sorted.best.bam Coverage
Col_0_tile.1000000.sorted.best.b
[0 - 200]
Col_0_tile.1000000.mmr_new.sord.bam Coverage
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[0 - 200]

Simulated reads from tiling a part of *A. thaliana* genome, alignment w/ PALMapper [Jean et al., 2010] (with -a option), visualization with IGV [Robinson et al., 2011].

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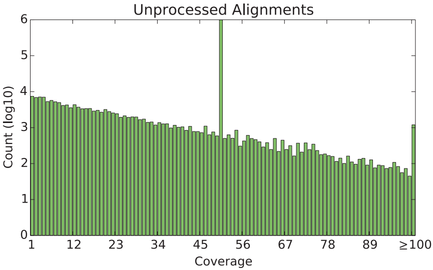

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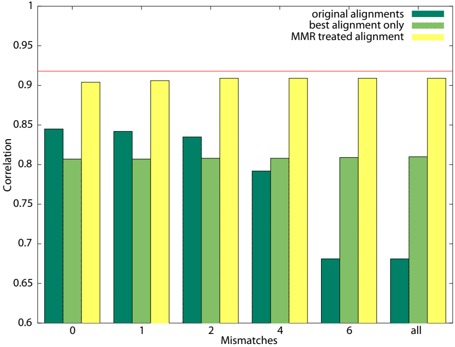
Results for simulated RNA-seq

- Improves performance of transcript quantification



Simulated reads (75nt) from subset of human annotated transcripts with Fluxsimulator [Sammeth, 2009], PALMapper alignments [Jean et al., 2010], rQuant quantitation Bohnert et al. [2009], Pearson correlation over all considered transcripts.

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(Accepted, 2015)

DNA methylation variation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation

Manu Dubin (Vienna Biocenter), Pei Zhang (Vienna Biocenter), Dazhe Meng (Vienna Biocenter), Marie-Stanislas Remigereau (University of Southern California), Edward Osborne (University of Utah), Francesco Paolo Casale (Wellcome Trust Genome Campus), Philip Drewe (Max Planck Society), André Kahles (Max Planck Society), Geraldine Jean (Max Planck Society), Bjarni Vilhjálmsson (Vienna Biocenter), Joanna Jagoda (Vienna Biocenter), Selen Irez (Vienna Biocenter), Viktor Voronin (Vienna Biocenter), Qiang Song (University of Southern California), Quan Long (Vienna Biocenter), Gunnar Rätsch (Max Planck Society), Oliver Stegle (Wellcome Trust Genome Campus), Richard Clark (University of Utah), and Magnus Nordborg (Vienna Biocenter)

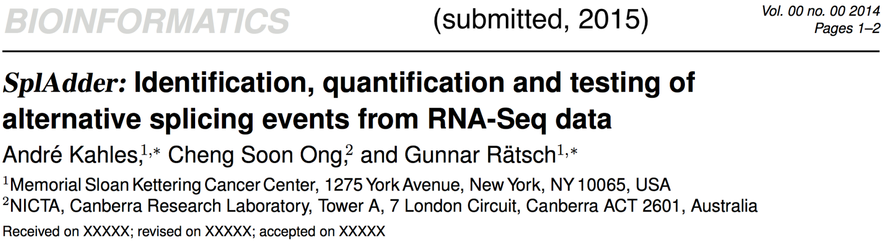
LARGE-SCALE BIOLOGY ARTICLE

Nonsense-Mediated Decay of Alternative Precursor mRNA Splicing Variants Is a Major Determinant of the *Arabidopsis* Steady State Transcriptome[C][W]

Gabriele Drechsel,[a,1] André Kahles,[b,1] Anil K. Kesarwani,[a] Eva Stauffer,[a,2] Jonas Behr,[b] Philipp Drewe,[b] Gunnar Rätsch,[b] and Andreas Wachter[a,3]

[a] Center for Plant Molecular Biology, University of Tübingen, 72076 Tuebingen, Germany

[b] Computational Biology Center, Sloan-Kettering Institute, New York, New York 10065



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SplAdder: Identification, quantification and testing of alternative splicing events from RNA-Seq data

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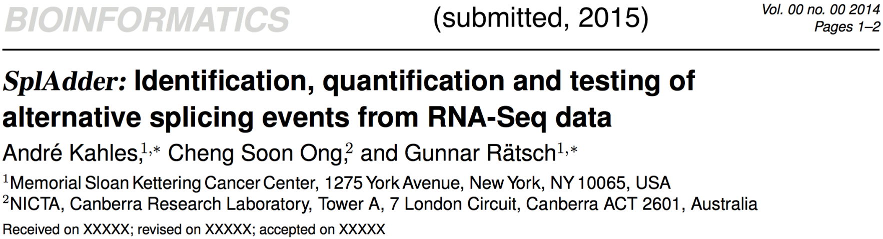
bioRxiv `dx.doi.org/10.1101/017095`

- Analysis of alternative isoforms with RNA-seq data
  - Analyses **known** and identifies **novel** splicing events
  - Quantifies & visualizes splicing-related data
- Suitable for **large-scale projects** (1000's of samples)
- **Improved accuracy** for transcript quantification and prediction
- Open Source `bioweb.me/spladder` (python)



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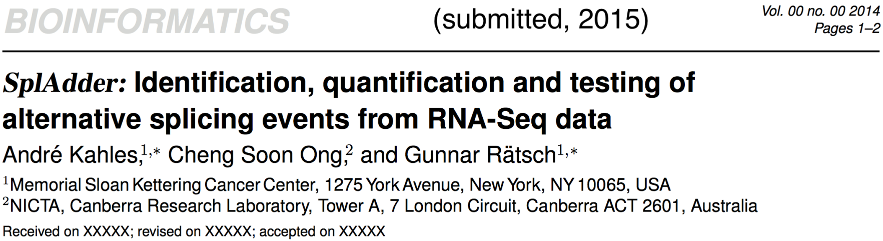

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SplAdder Ideas

Major Problems in Transcriptome Analysis

1. Gene annotations are incomplete and often inaccurate
2. Whole transcript isoforms are difficult to predict/quantify

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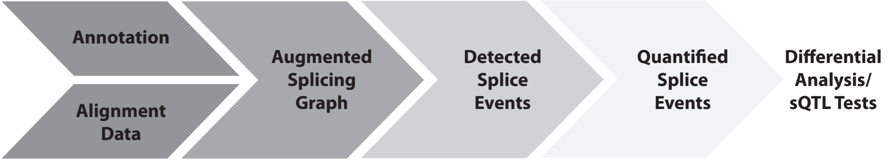

Kahles et al., bioRxiv, 2015

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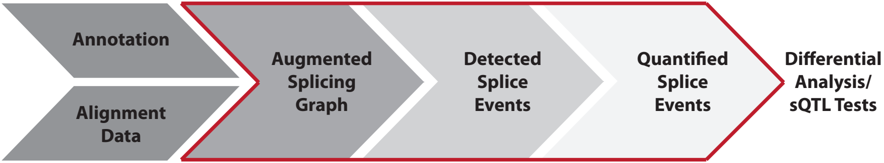

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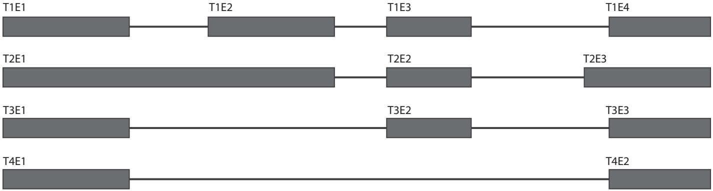
SplAdder Graph Augmentation

Principle

- Collapse annotated transcripts into graph representation
- Use RNA-Seq evidence to add new nodes and edges



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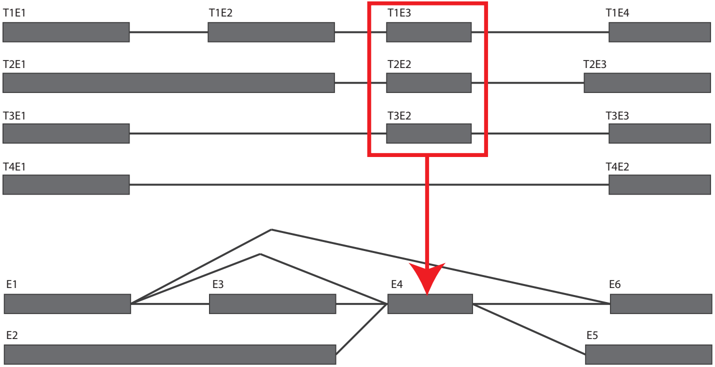

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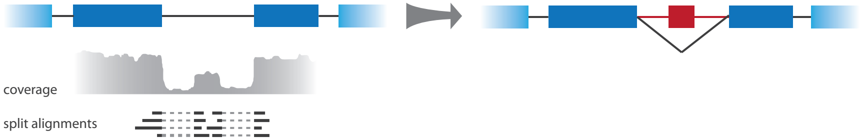
New cassette exon



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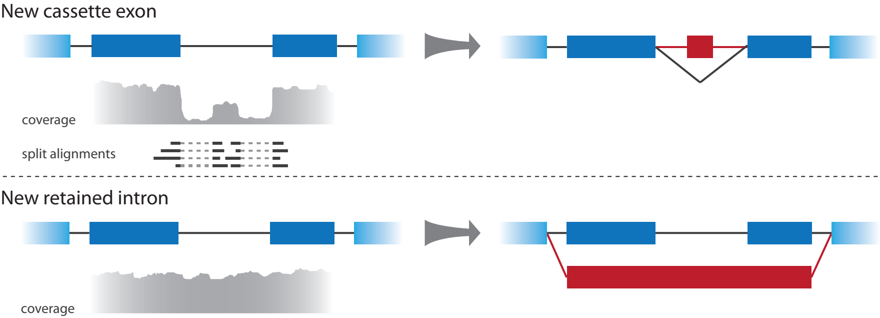

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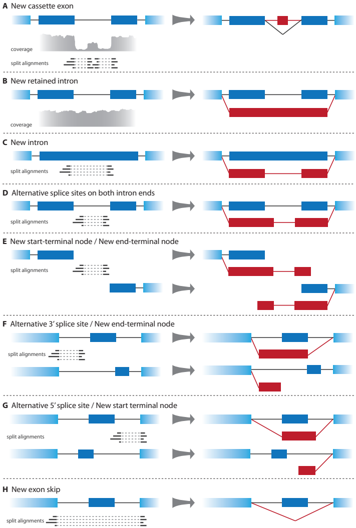
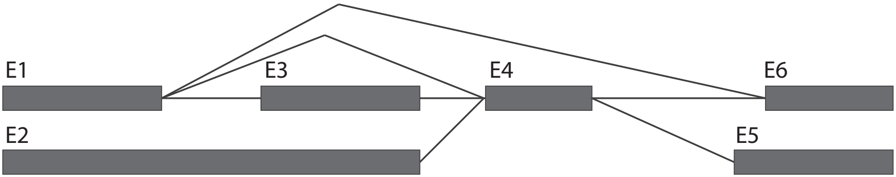

A New cassette exon
coverage
split alignments
B New retained intron
coverage
C New intron
split alignments
D Alternative splice sites on both intron ends
split alignments
E New start-terminal node / New end-terminal node
split alignments
F Alternative 3' splice site / New end-terminal node
split alignments
G Alternative 5' splice site / New start terminal node
split alignments
H New exon skip
split alignments

SplAdder Event Extraction



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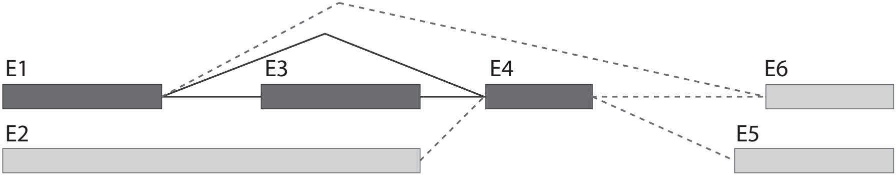
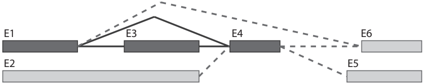
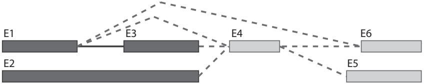


SplAdder Event Extraction

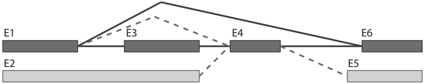
Exon Skip



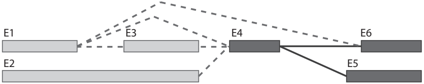
Intron Retention



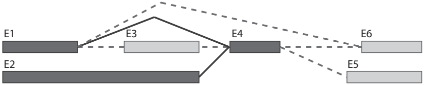
Multiple Exon Skip



Alternative 3' Splice Site

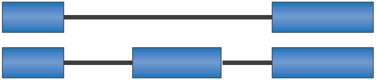


Alternative 5' Splice Site



SplAdder Event Quantification and Visualization

Exon Skip



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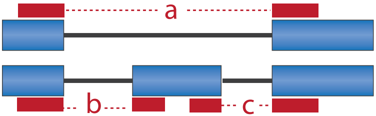

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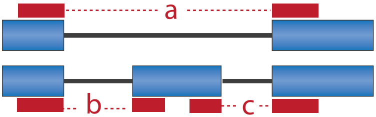
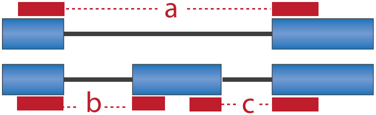


$$\mathrm{PSI} = \frac{b + c}{2 \cdot a + b + c}$$

SplAdder Event Quantification and Visualization

Exon Skip



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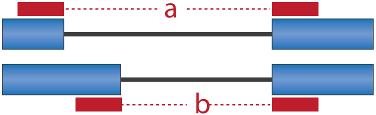

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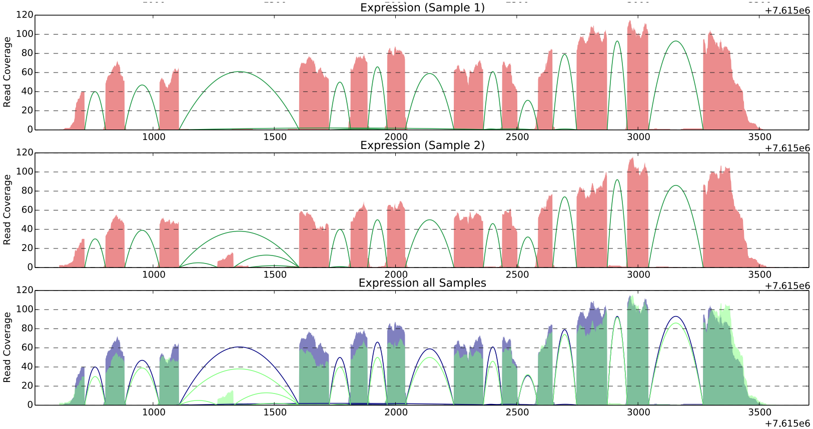

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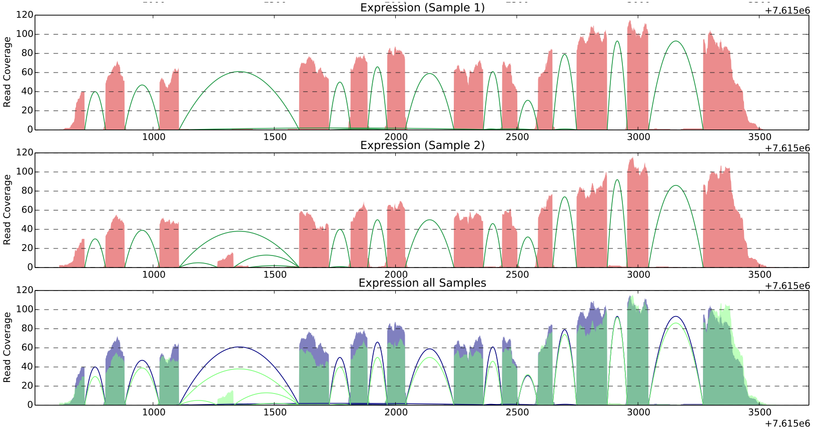

Summary

- SplAdder effectively augments the annotation
- Enables quantitative analysis of events instead of transcripts

Splicing Analysis Across Multiple Cancer Types

Goals

1. Identify cancer-specific splicing patterns
2. Identify variants regulating splicing in same gene (cis)
3. Identify variants regulating splicing in other cancer genes (trans)

TCGA provides RNA-seq and matching exome data

- RNA-seq ⇝ Find & quantify splicing events
- Exome ⇝ Identify variants in exons & flanking intronic regions

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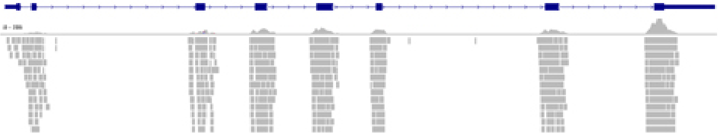
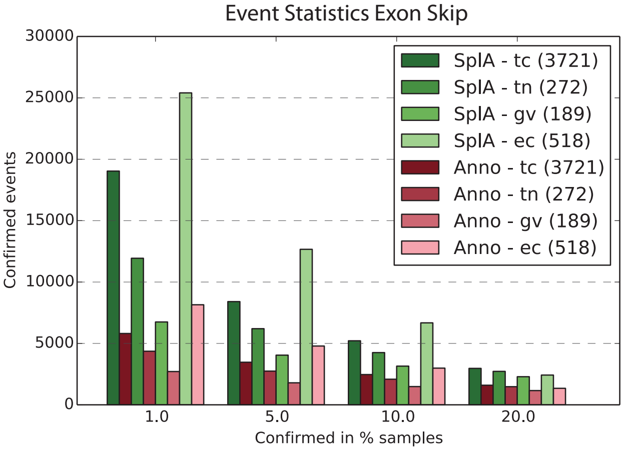
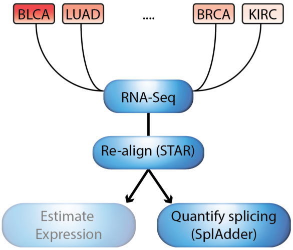


Splicing Variation Across 4,700 Samples



Analysis of a total of 4,700 RNA-seq samples from TCGA normal (tn), TCGA tumors (tc), Encode (ec) and Geuvadis (gv). Alignment w/ STAR [Dobin et al., 2013], analysis w/ SplAdder (SplA) and Gencode annotation (Anno). Figure from [Kahles, 2014].

Uniform analysis of Large-Scale RNA-seq Data



Large-scale Compute

4,700 RNA-seq libraries (≈100 TB)

- ⇒ STAR ≈ 6 CPU years
- ⇒ SplAdder ≈ 0.5 CPU years

[Kahles et al.]

Unified community resources

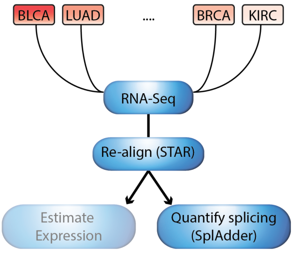
Docker with ICGC RNA-seq alignment SOP

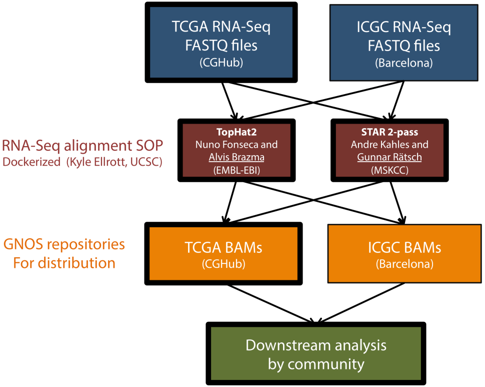
`bioweb.me/ICGC-RNA-SOP`

Syncronize with Encode, gTex, TCGA, . . .

[ICGC PCAWG-3 WG]

Uniform analysis of Large-Scale RNA-seq Data




TCGA RNA-Seq FASTQ files (CGHub)
ICGC RNA-Seq FASTQ files (Barcelona)
RNA-Seq alignment SOP
Dockerized (Kyle Ellrott, UCSC)
TopHat2
Nuno Fonseca and Alvis Brazma (EMBL-EBI)
STAR 2-pass
Andre Kahles and Gunnar Rätsch (MSKCC)
GNOS repositories
For distribution
TCGA BAMs (CGHub)
ICGC BAMs (Barcelona)
Downstream analysis by community

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[Kahles et al.]

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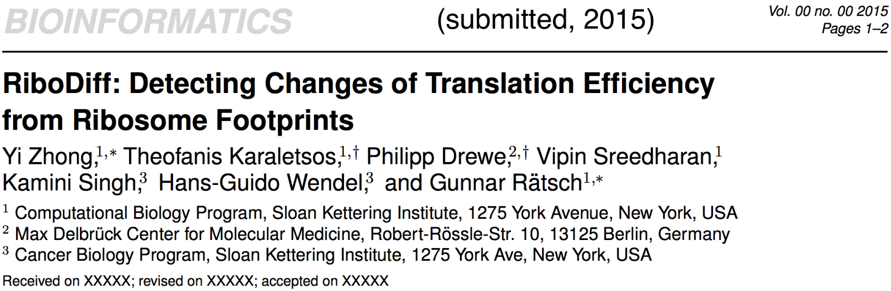

BIOINFORMATICS (submitted, 2015)

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RiboDiff: Detecting Changes of Translation Efficiency from Ribosome Footprints

Yi Zhong,[1,*] Theofanis Karaletsos,[1,†] Philipp Drewe,[2,†] Vipin Sreedharan,[1]
Kamini Singh,[3] Hans-Guido Wendel,[3] and Gunnar Rätsch[1,*]

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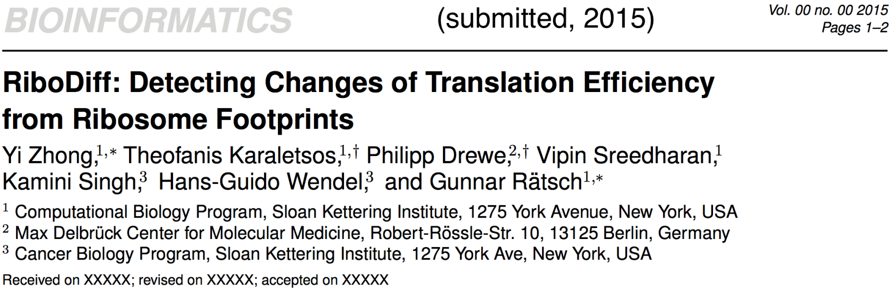
Received on XXXXX; revised on XXXXX; accepted on XXXXX

bioRxiv `dx.doi.org/10.1101/017095`

- Analysis of Ribosome profiling and RNA-seq data
  - Study translation efficiency
  - Adjusts for expression differences
- Accurate method based on dispersion estimates and GLMs
- Open Source `bioweb.me/ribodiff` (python)



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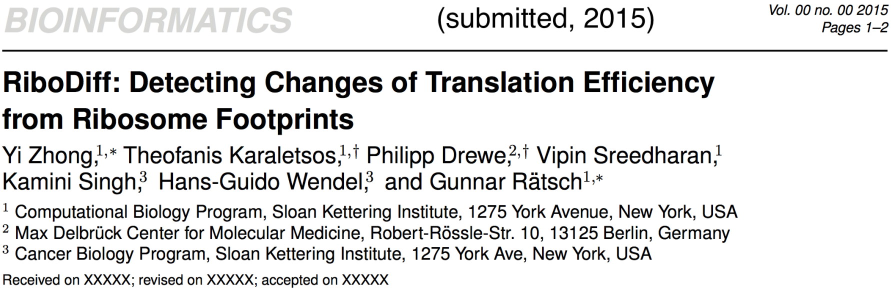

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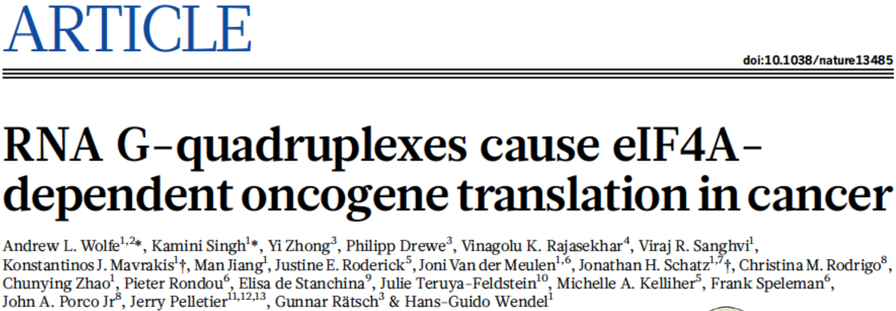
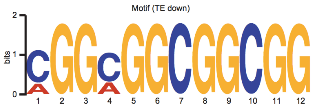


ARTICLE

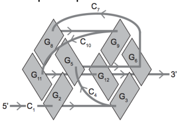
doi:10.1038/nature13485

RNA G-quadruplexes cause eIF4A-dependent oncogene translation in cancer

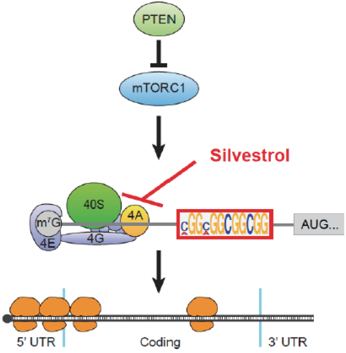
Andrew L. Wolfe[1,2]*, Kamini Singh[1]*, Yi Zhong[3], Philipp Drewe[3], Vinagolu K. Rajasekhar[4], Viraj R. Sanghvi[1], Konstantinos J. Mavrakis[1]†, Man Jiang[1], Justine E. Roderick[5], Joni Van der Meulen[1,6], Jonathan H. Schatz[1,7]†, Christina M. Rodrigo[8], Chunying Zhao[1], Pieter Rondou[6], Elisa de Stanchina[9], Julie Teruya-Feldstein[10], Michelle A. Kelliher[5], Frank Speleman[6], John A. Porco Jr[8], Jerry Pelletier[11,12,13], Gunnar Rätsch[3] & Hans-Guido Wendel[1]



G-quadruplex structures



(Analysis based on related but different strategy [Wolfe et al., 2014].)



5'UTR accumulation and reduction in RF

Summary

- MMR improves alignment choice for multi-mappers
  - ⇒ Helps improving accuracy of tools like Cufflinks
- SplAdder identifies, quantifies & visualizes alternative splicing
  - ⇒ Finds unannotated alternative splicing, tumor/normal splicing differences; splicing reprogramming; sQTLs
- riboDiff accurately detects differential translation efficiency
  - ⇒ Ribosome footprinting revealed RNA G-Quadruplex elements in 5' UTR that interacts with compound via eIF4a
- Tools (+ six other ones) are open source and available
  - ⇒ `ratschlab.org/tools`
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- Jonas Behr @ ETH Basel
- Regina Bohnert @ Molecular Health
- Geraldine Jean @ University of Nantes

Cancer Biology

- Guido Wendel
- Kamini Singh, . . .

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Thank you!

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