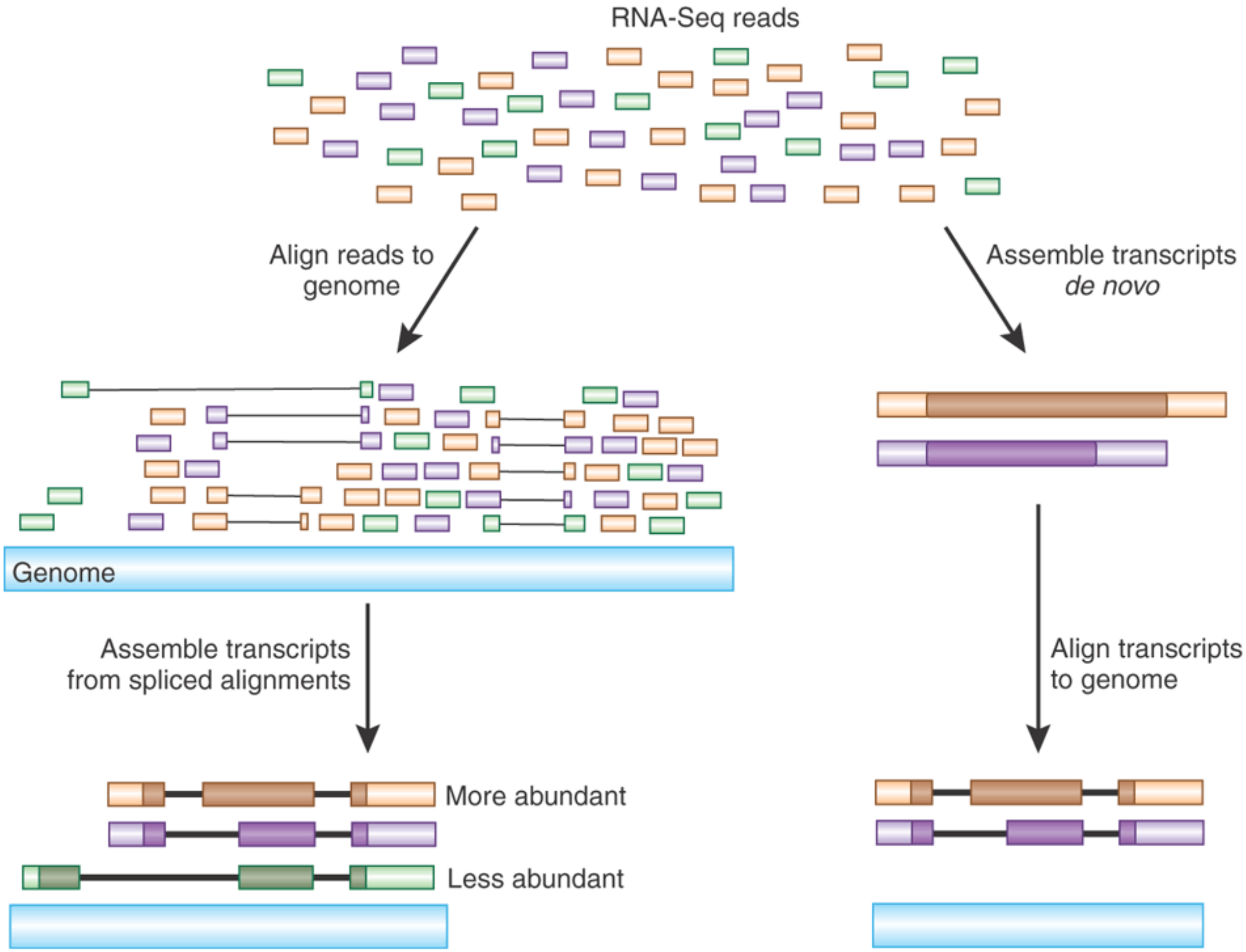
SciLifeLab

Karolinska Institutet KTH Stockholms universitet UPPSALA UNIVERSITET

Transcriptome and isoform reconstruction with short reads

Estelle Proux-Wéra
SciLifeLab RNAseq workshop
October 2016

Transcriptome assembly


RNA-Seq reads
Align reads to genome
Assemble transcripts *de novo*
Genome
Assemble transcripts from spliced alignments
Align transcripts to genome
More abundant
Less abundant

Haas and Zody, Nature Biotechnology 28, 421–423 (2010)

Reference-based assembly

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Case study:
The transcriptome of the domestic dog

An improved canine genome and a comprehensive catalogue of coding genes and non-coding transcripts. Hoeppner MP et al. PLoS One 2014 Mar 13;9(3):e91172

Reference-based assembly

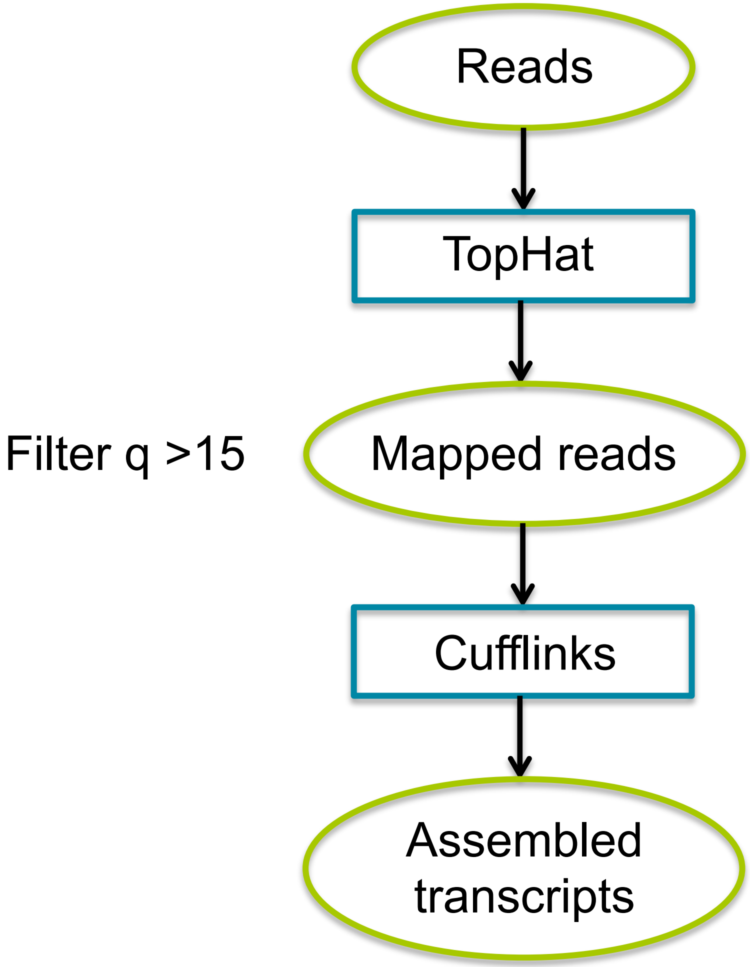
- Why dogs?
  - Shared environment with humans for > 10.000 years
  - Affected by cancer or heart disease
  - Breed-specific disease
- New genome release in 2011 (canFam3.1)
  - 85 Mb of additional sequences integrated
  - 99.8% of euchromatic portion of genome covered, high quality
- Annotation: not so good
  - Mostly homology-based
  - Almost no isoform information

Reference-based assembly

- 10 tissues at great depth (30-100 million paired-end reads)
  - blood, brain, heart, kidney, liver, lung, ovary, skeletal muscle, skin, and testis
- 2 sets of libraries
  - strand-specific dUTP with poly-A selection: captures protein coding genes and other transcripts transcribed by polymerase II
  - duplex-specific nuclease (DSN): targets all RNAs, reduces the levels of the highly abundant ribosomal transcripts through normalization

Reference-based assembly

For each sample (tissue/library)



Reads

TopHat

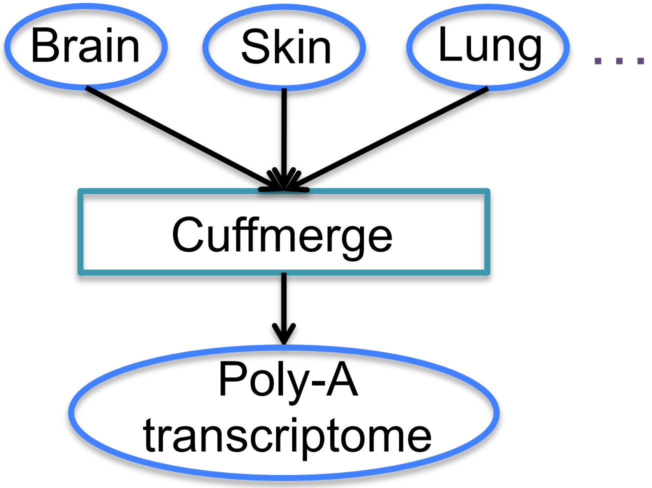
Filter q >15

Mapped reads

Cufflinks

Assembled transcripts

For each library (Poly-A, DSN)



Brain

Skin

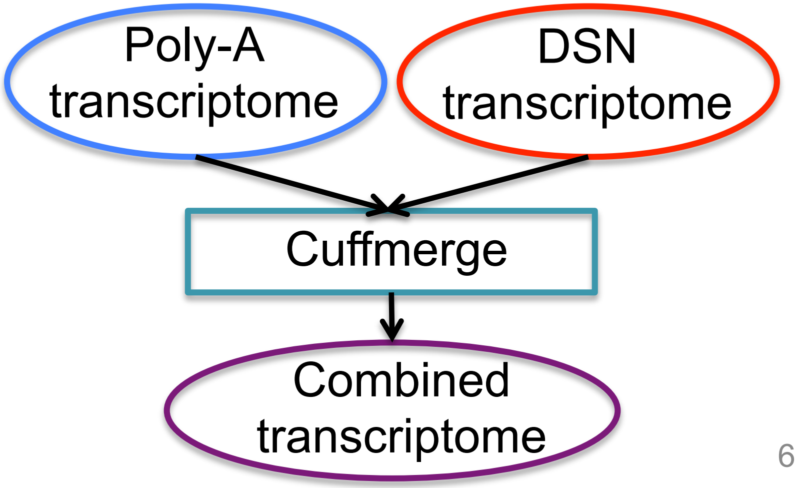
Lung

…

Cuffmerge

Poly-A transcriptome

Final transcriptome



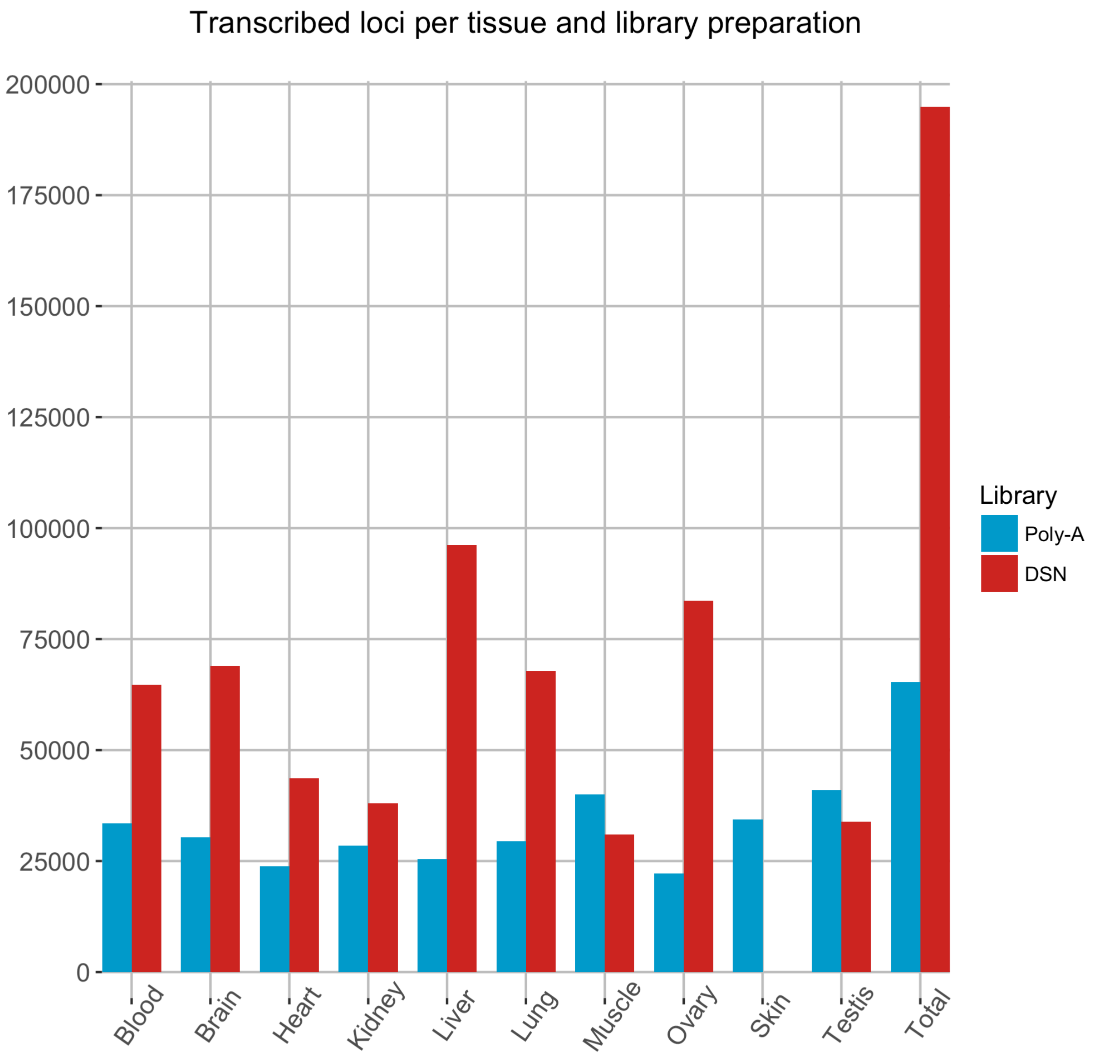
Poly-A transcriptome

DSN transcriptome

Cuffmerge

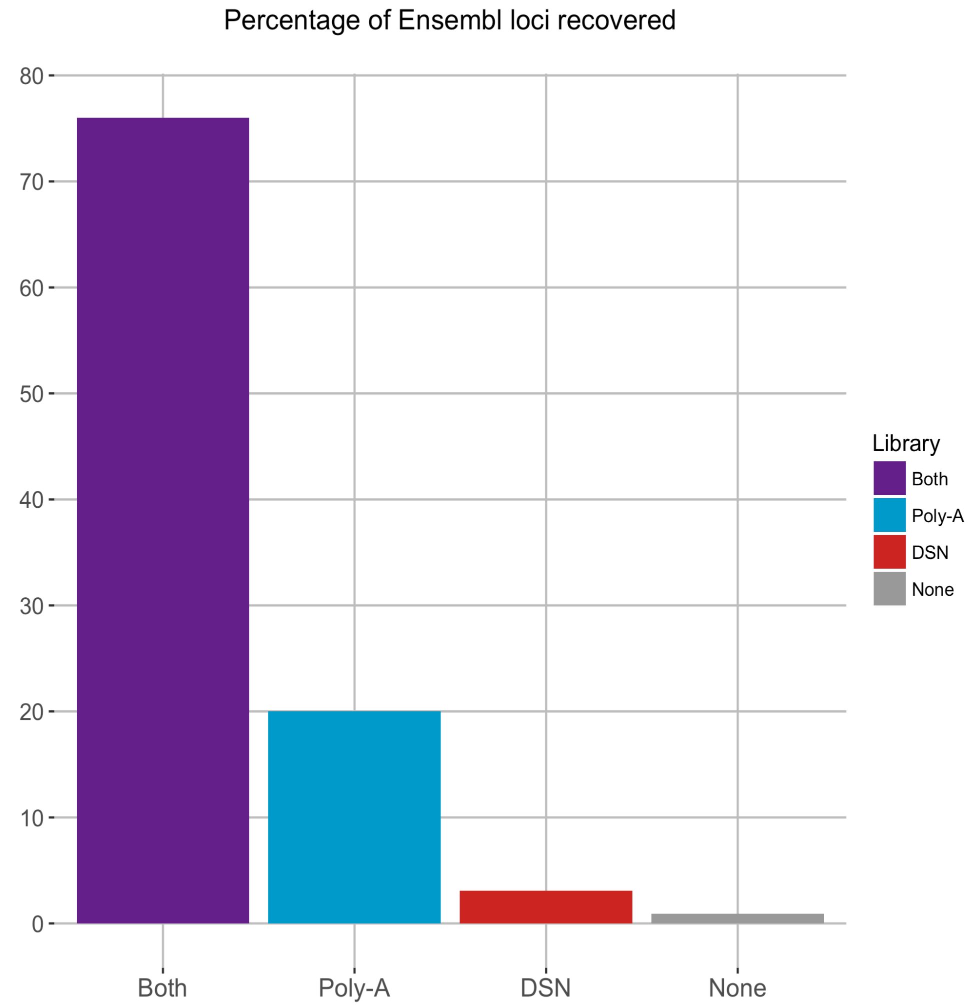
Combined transcriptome

Reference-based assembly



- DSN recovers more transcripts
- Poly-A: highest number in testis, then muscle
- Poly-A: heart and muscle share 88% of loci
- Mean transcript length:
  - Poly-A: 3169 bp
  - DSN: 1485 bp

Reference-based assembly

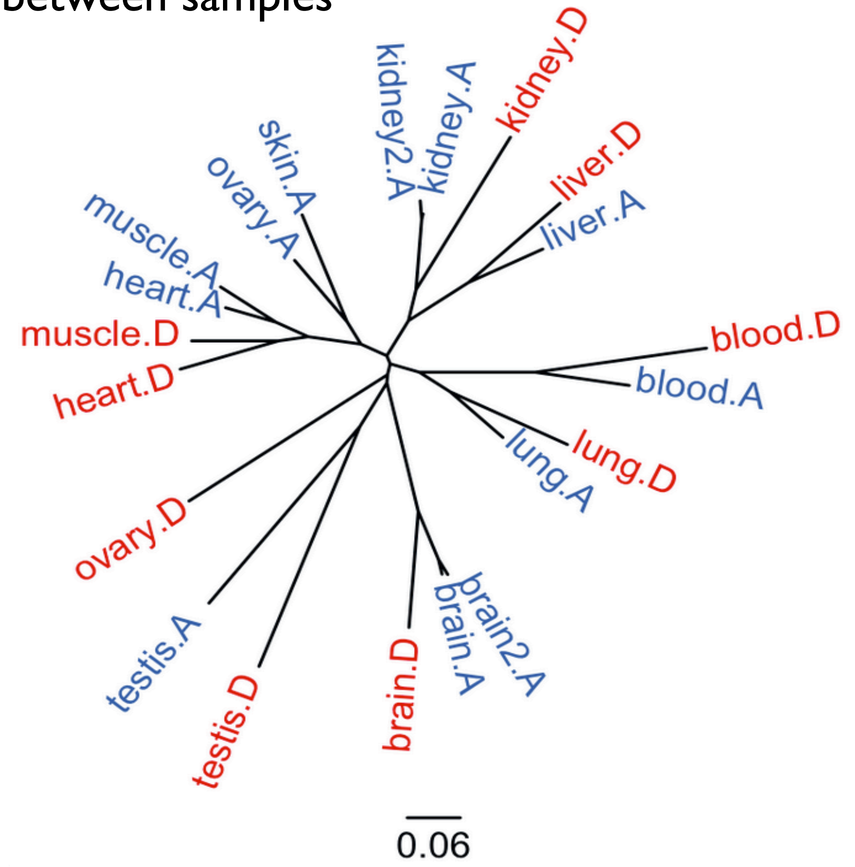


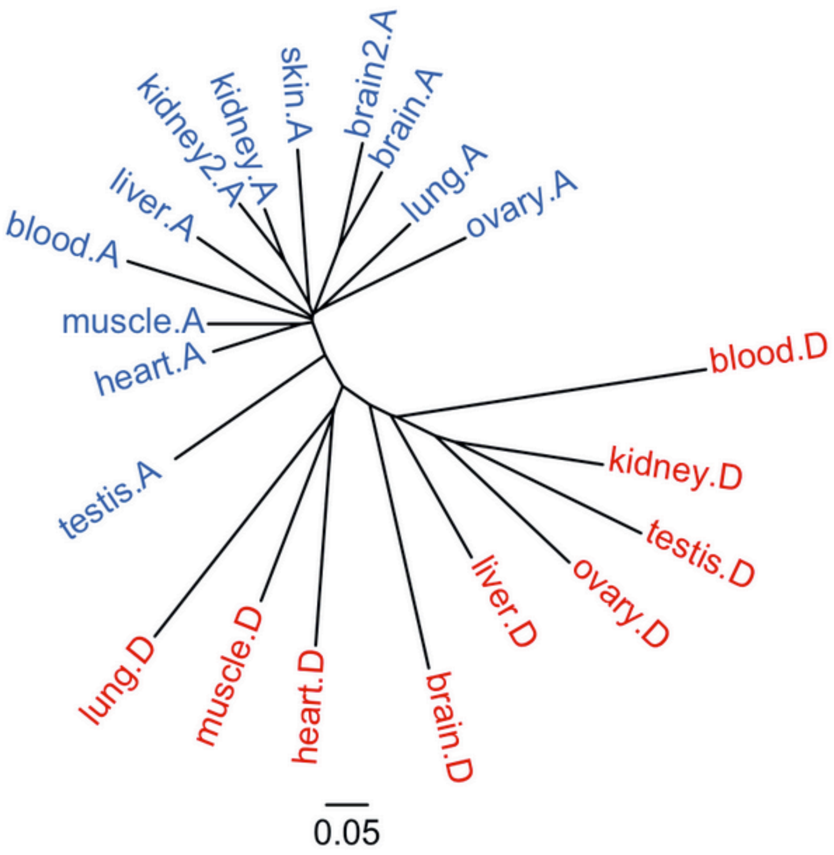
- Ensembl build 64: 19,856 annotated loci
- Combined Poly-A + DSN: 174,336 loci
- Majority located in introns of known genes and transcribed in the same sense
  - potential byproducts of incomplete splicing
- Many located outside of known features, seem independently transcribed

Reference-based assembly

Distance trees of expression profile

Neighbor-joining trees based on the correlation between expression values (FPKM>1.0) between samples





Protein-coding genes with RNA-Seq support

Intergenic and uncharacterized single exons

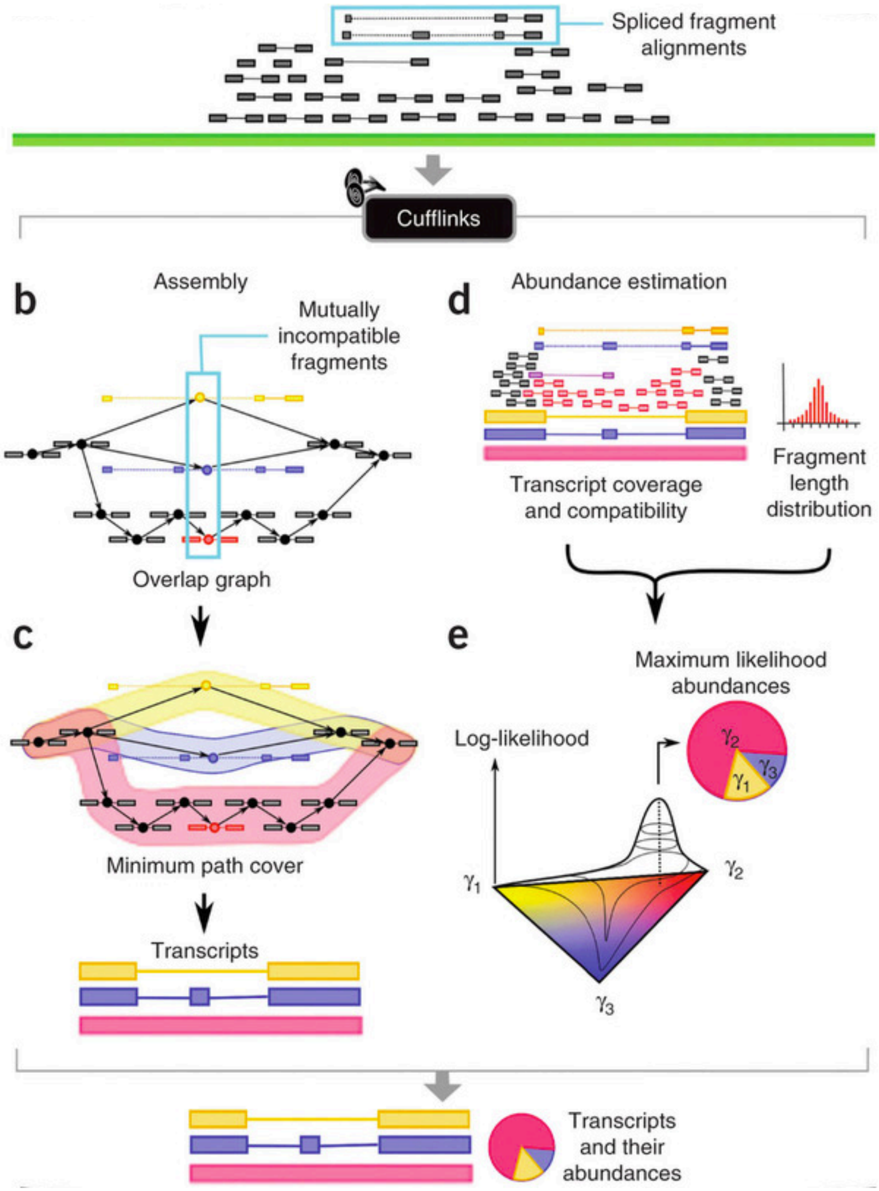
An improved canine genome and a comprehensive catalogue of coding genes and non-coding transcripts. Hoeppner MP et al. PLoS One 2014 Mar 13;9(3):e91172

Reference-based assembly

Cufflinks

From the "Tuxedo" protocol

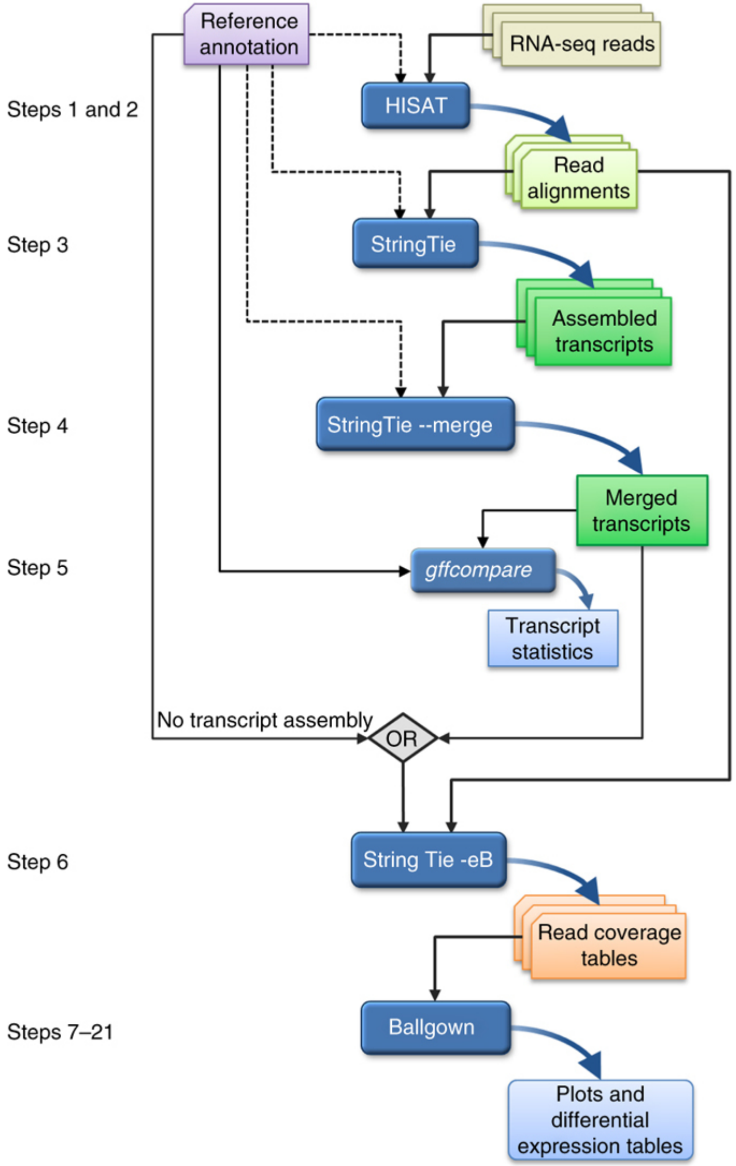
Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Trapnell C. et al. Nature Biotechnology 28, 511–515 (2010)


Spliced fragment alignments
Cufflinks
b
Assembly
Mutually incompatible fragments
Overlap graph
c
Minimum path cover
Transcripts
d
Abundance estimation
Transcript coverage and compatibility
Fragment length distribution
e
Maximum likelihood abundances
Log-likelihood
γ_1
γ_2
γ_3
Transcripts and their abundances

Reference-based assembly

The “new Tuxedo” protocol

Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Pertea M. et al. Nature protocol 11, 1650–1667 (2016)

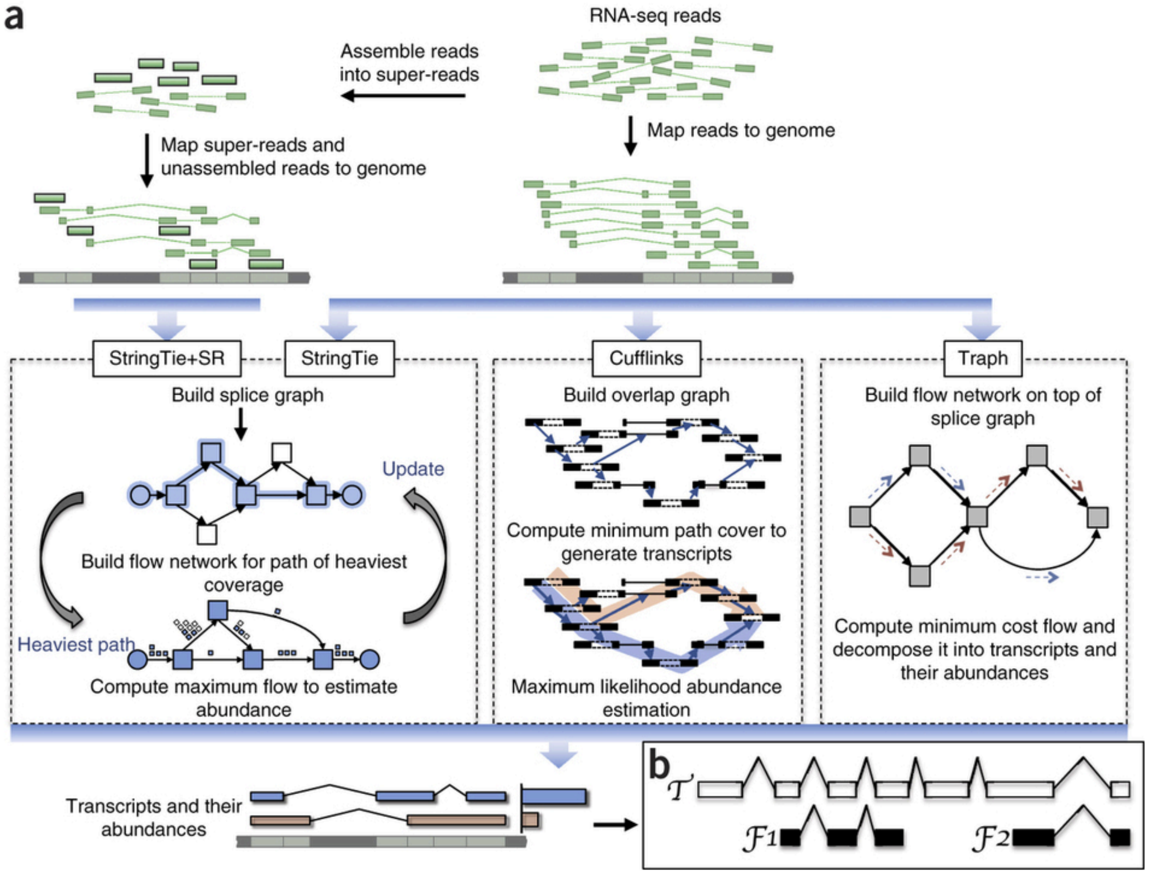


Reference-based assembly

StringTie

From the "new Tuxedo" protocol

StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Pertea M.. et al. Nature Biotechnology 33, 290–295 (2015)



a
RNA-seq reads
Assemble reads into super-reads
Map reads to genome
Map super-reads and unassembled reads to genome
StringTie+SR
StringTie
Cufflinks
Traph
Build splice graph
Update
Build flow network for path of heaviest coverage
Heaviest path
Compute maximum flow to estimate abundance
Build overlap graph
Compute minimum path cover to generate transcripts
Maximum likelihood abundance estimation
Build flow network on top of splice graph
Compute minimum cost flow and decompose it into transcripts and their abundances
Transcripts and their abundances
b
T
F1
F2

Reference-based assembly

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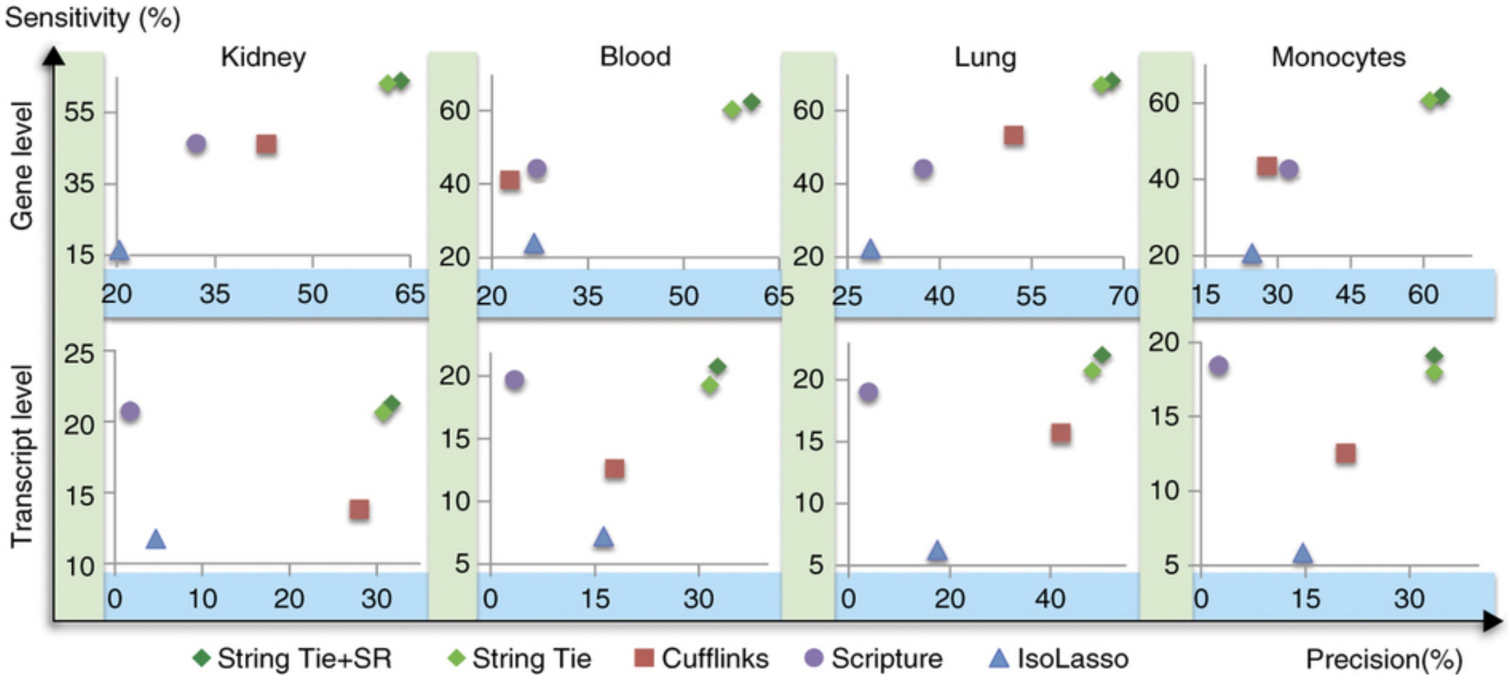
StringTie

Fig.3: Accuracy of transcript assemblers at assembling known genes, measured on real data sets from four different tissues (RefSeq, UCSC or Ensembl human gene databases)



Sensitivity (genes): % of genes for which a program got at least one isoform correct
Sensitivity (transcripts): % of known transcripts that were correctly assembled
Precision: % of all predicted genes/transcripts that match an annotated gene/transcript

Reference-based assembly

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Take-home message

- Need a very good reference (genome most of the time)
- Can use existing annotation (GTF/GFF file)
- Can detect novel transcripts

De novo assembly

De novo transcriptome assembly databases for the butterfly orchid *Phalaenopsis equestris*

Data in Brief

De novo transcriptome assembly of mangosteen (*Garcinia mangostana* L.) fruit

De novo Transcriptome Analysis Reveals Distinct Defense Mechanisms by Young and Mature Leaves of *Hevea brasiliensis* (Para Rubber Tree)

De novo transcriptome assembly and analysis of differentially expressed genes of two barley genotypes reveal root-zone-specific responses to salt exposure

***De Novo* Sequencing and Analysis of Lemongrass Transcriptome Provide First Insights into the Essential Oil Biosynthesis of Aromatic Grasses**

De novo transcriptome assembly of two contrasting pumpkin cultivars

Identification of novel and useful EST-SSR markers from *de novo* transcriptome sequence of wheat (*Triticum aestivum* L.)

De Novo Transcriptome Assembly and Characterization for the Widespread and Stress-Tolerant Conifer *Platycladus orientalis*

De novo* Assembly of Leaf Transcriptome in the Medicinal Plant *Andrographis paniculata

Transcriptome sequencing and de novo characterization of Korean endemic land snail, *Koreanohadra kurodana* for functional transcripts and SSR markers

***De Novo* Assembly of the Transcriptome of *Turritopsis*, a Jellyfish that Repeatedly Rejuvenates**

Transcriptome of the Caribbean stony coral *Porites astreoides* from three developmental stages

De novo transcriptome assembly of the marine gastropod *Reishia clavigera* for supporting toxic mechanism studies

The *De Novo* Transcriptome and Its Functional Annotation in the Seed Beetle *Callosobruchus maculatus*

De Novo Transcriptome Analysis of the Common New Zealand Stick Insect *Clitarchus hookeri* (Phasmatodea) Reveals Genes Involved in Olfaction, Digestion and Sexual Reproduction

Characterization and analysis of a *de novo* transcriptome from the pygmy grasshopper *Tetrix japonica*

Optimizing Hybrid *de Novo* Transcriptome Assembly and Extending Genomic Resources for Giant Freshwater Prawns (*Macrobrachium rosenbergii*): The Identification of Genes and Markers Associated with Reproduction

De Novo Transcriptome Analysis of Two Seahorse Species (*Hippocampus erectus* and *H. mohnikei*) and the Development of Molecular Markers for Population Genetics

De Novo assembly and annotation of the freshwater crayfish *Astacus astacus* transcriptome

De novo assembly

- Most used programs (latest release date):
  - SOAPdenovo-Trans (July 2013)
  - Trans-ABySS (August 2016)
  - Velvet+Oases (March 2015)
  - Trinity (March 2016)
- Originally SOAPdenovo, ABySS and Velvet for de novo genome assembly
- "SOAPdenovo-Trans incorporates the error-removal model from Trinity and the robust heuristic graph traversal method from Oases."
- All based on de Bruijn graph

De novo assembly

The de Bruijn graph

CTTGGAACAATATGAATTGGCAAT
ATTGGCAATTGACTTTTGCCGTAAT
CCGTAATCCGGCATATCTGGATA

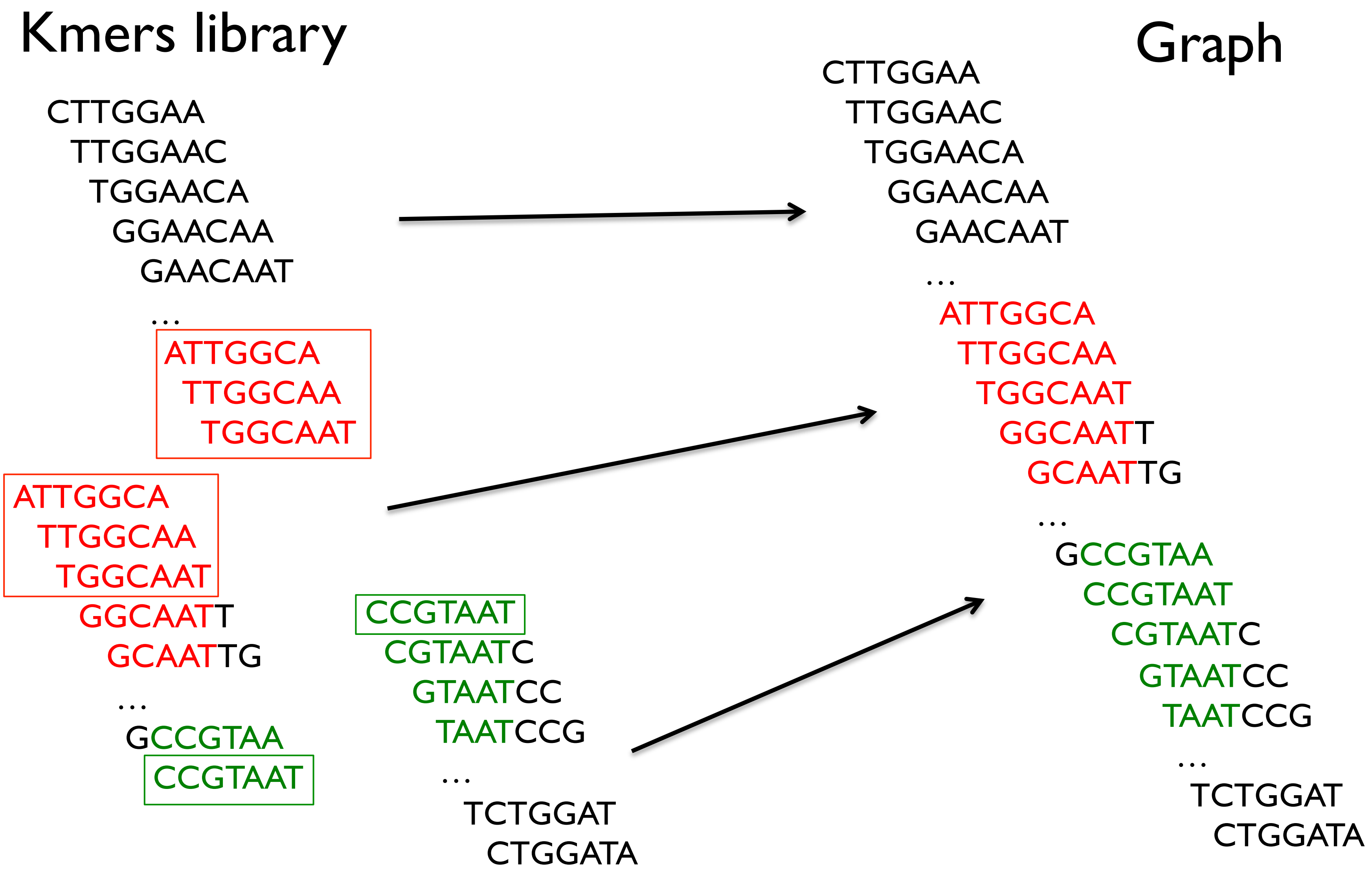
Kmers (k = 7)

CTTGGAA
TTGGAAC
TGGAACA
GGAACAA
GAACAAT
...
ATTGGCA
TTGGCAA
TGGCAAT

ATTGGCA
TTGGCAA
TGGCAAT
GGCAATT
GCAATTG
...
GCCGTAA
CCGTAAT

CCGTAAT
CGTAATC
GTAATCC
TAATCCG
...
TCTGGAT
CTGGATA

De novo assembly



Kmers library

CTTGGAA
TTGGAAC
TGGAACA
GGAACAA
GAACAAT
…
ATTGGCA
TTGGCAA
TGGCAAT

ATTGGCA
TTGGCAA
TGGCAAT
GGCAATT
GCAATTG
…
GCCGTAA
CCGTAAT

CCGTAAT
CGTAATC
GTAATCC
TAATCCG
…
TCTGGAT
CTGGATA

Graph

CTTGGAA
TTGGAAC
TGGAACA
GGAACAA
GAACAAT
…
ATTGGCA
TTGGCAA
TGGCAAT
GGCAATT
GCAATTG
…
GCCGTAA
CCGTAAT
CGTAATC
GTAATCC
TAATCCG
…
TCTGGAT
CTGGATA

De novo assembly

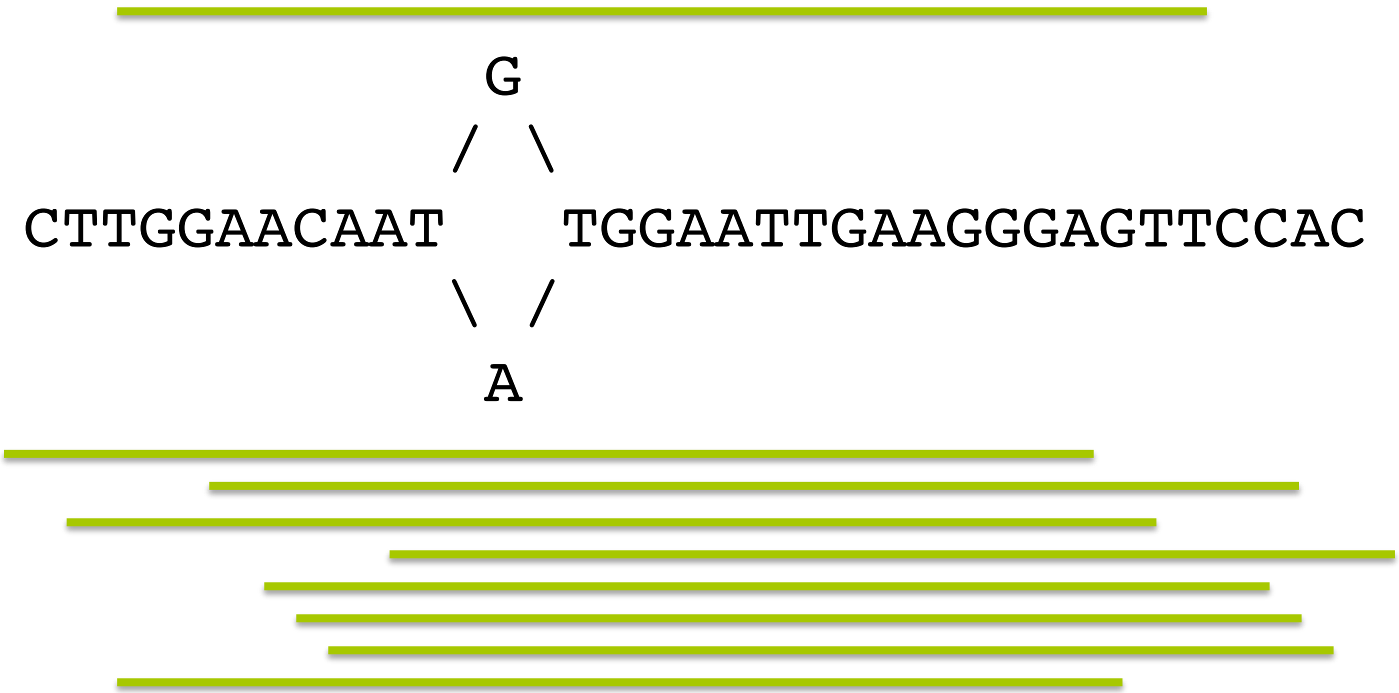
Graphs can have nodes and edges

```
            G         GGCAATTGACTTTT
           / \       /
CTTGGAACAAT   TGGAATT
           \ /       \
            A         GAAGGGAGTTCCAC
```

De novo assembly

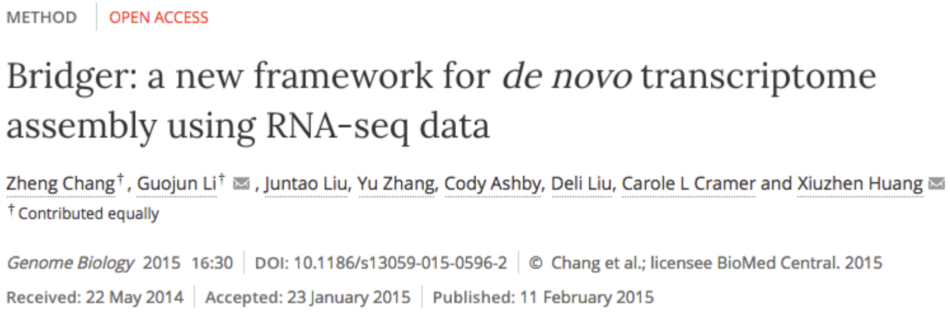
Differences between programs:

- Kmer length
- Removing edges



```
               G
              / \
CTTGGAACAAT       TGGAATTGAAGGGAGTTCCAC
              \ /
               A
```

De novo assembly



METHOD | OPEN ACCESS

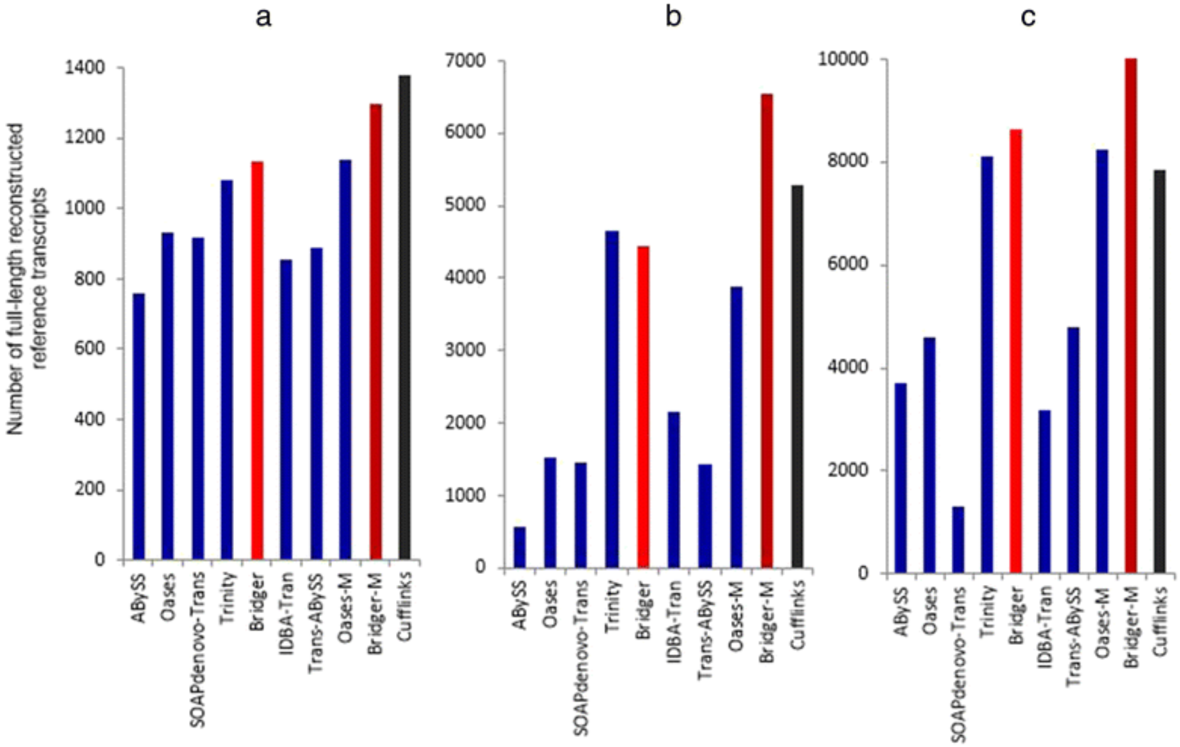
Bridger: a new framework for *de novo* transcriptome assembly using RNA-seq data

Zheng Chang[†], Guojun Li[†], Juntao Liu, Yu Zhang, Cody Ashby, Deli Liu, Carole L Cramer and Xiuzhen Huang

[†] Contributed equally

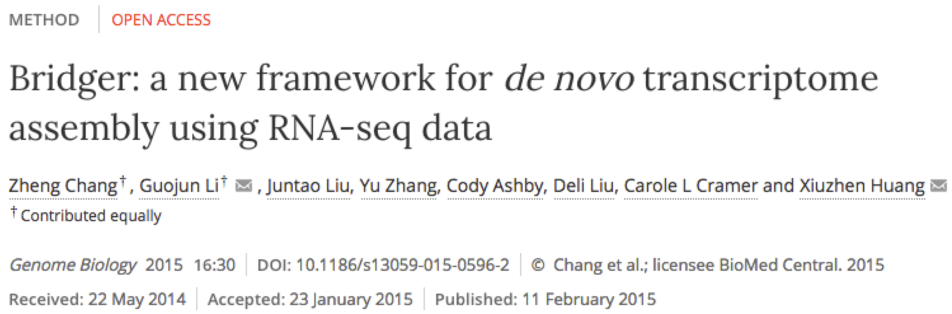
Genome Biology 2015 16:30 | DOI: 10.1186/s13059-015-0596-2 | © Chang et al.; licensee BioMed Central. 2015

Received: 22 May 2014 | Accepted: 23 January 2015 | Published: 11 February 2015



Number of full-length reconstructed reference transcripts for (a) dog, (b) human, and (c) mouse

De novo assembly



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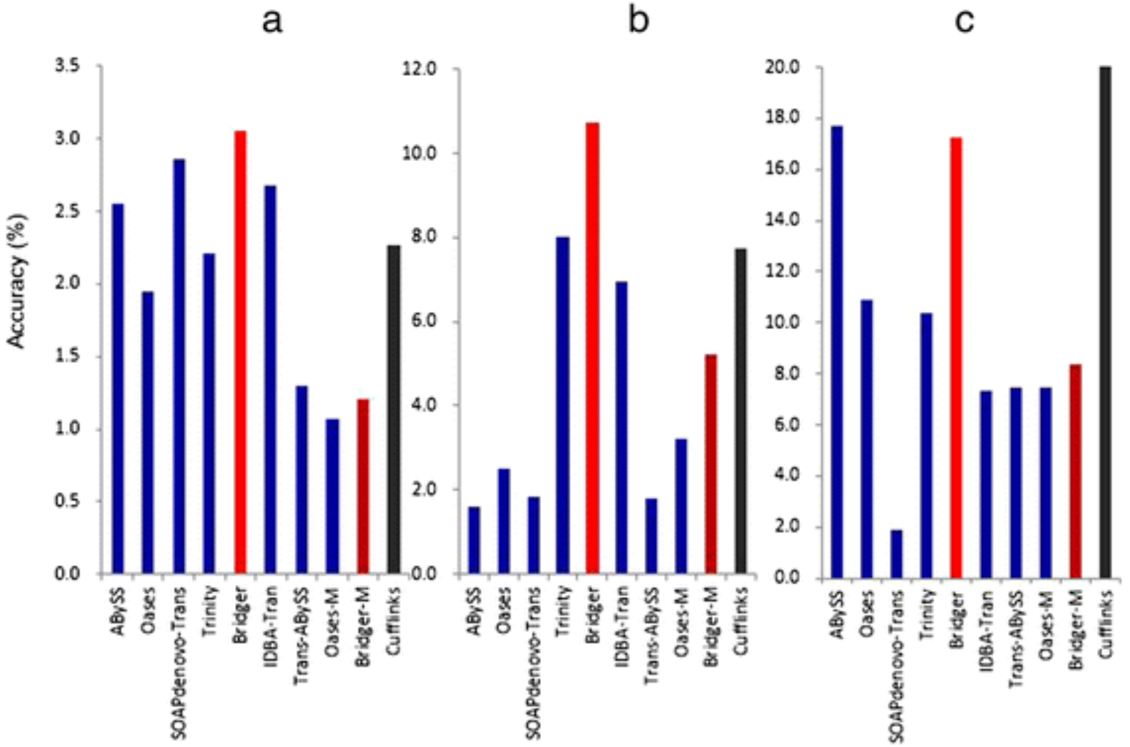
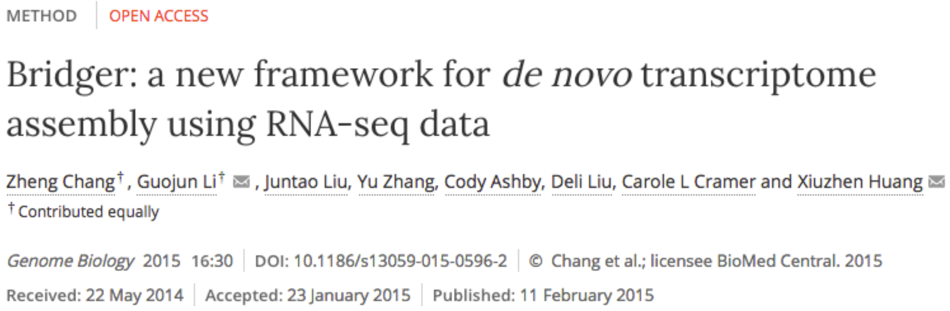


Accuracy for (a) dog, (b) human, and (c) mouse [the most reference transcripts by the least candidate transcripts]

De novo assembly



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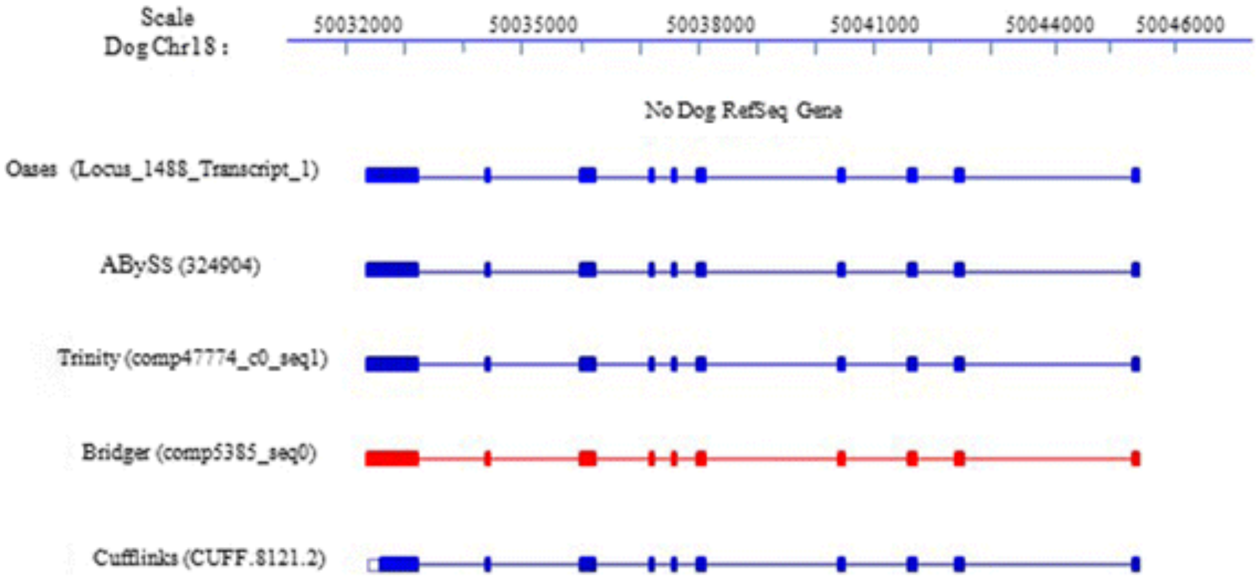
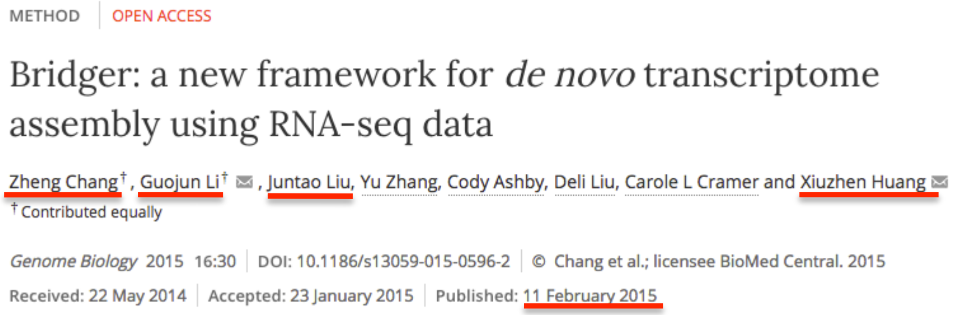


A novel gene containing 10 exons was assembled by all assemblers. Interestingly, all *de novo* assemblers captured longer UTR than the reference-based assembler Cufflinks

De novo assembly



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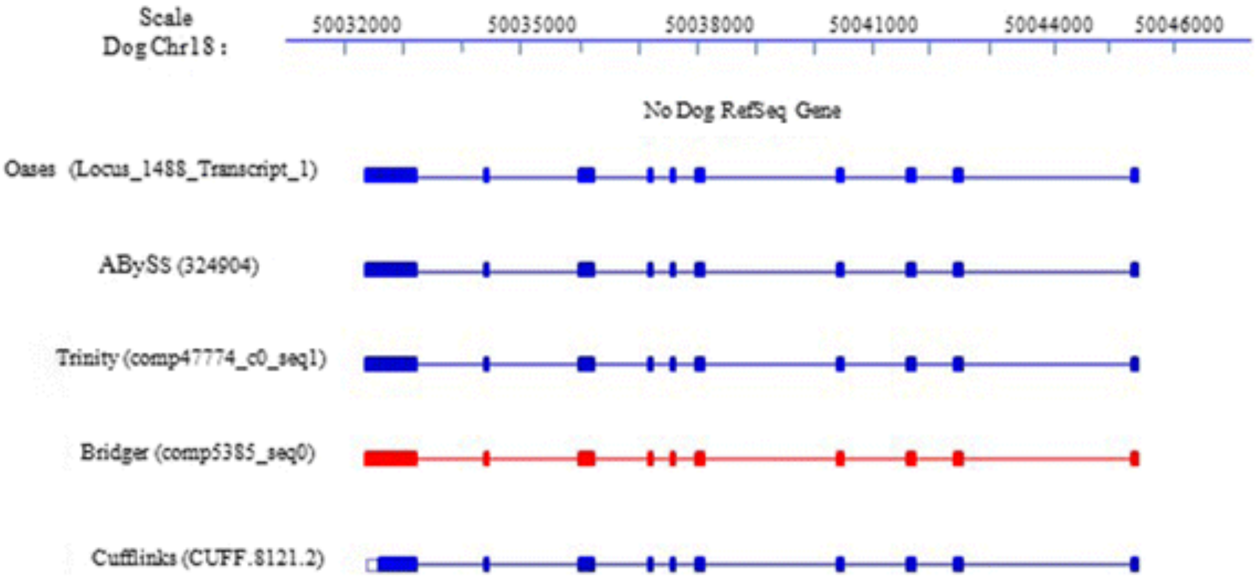

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De novo assembly

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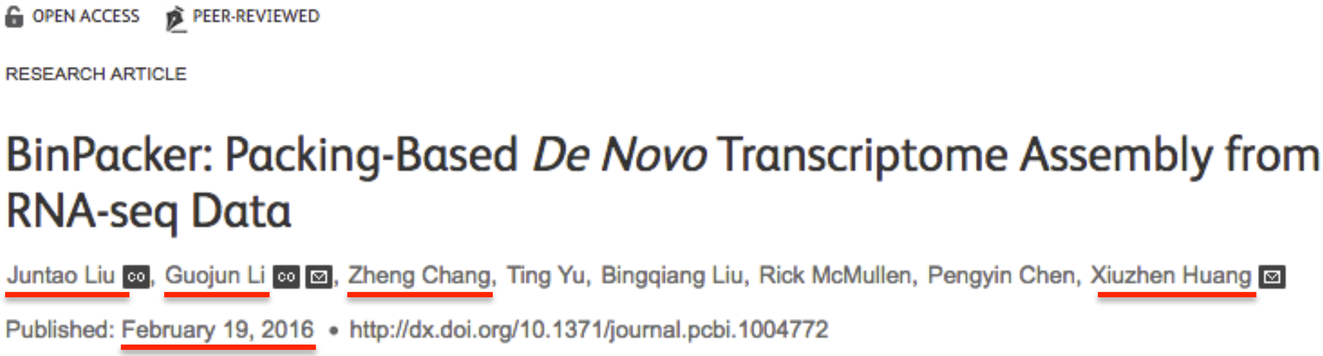


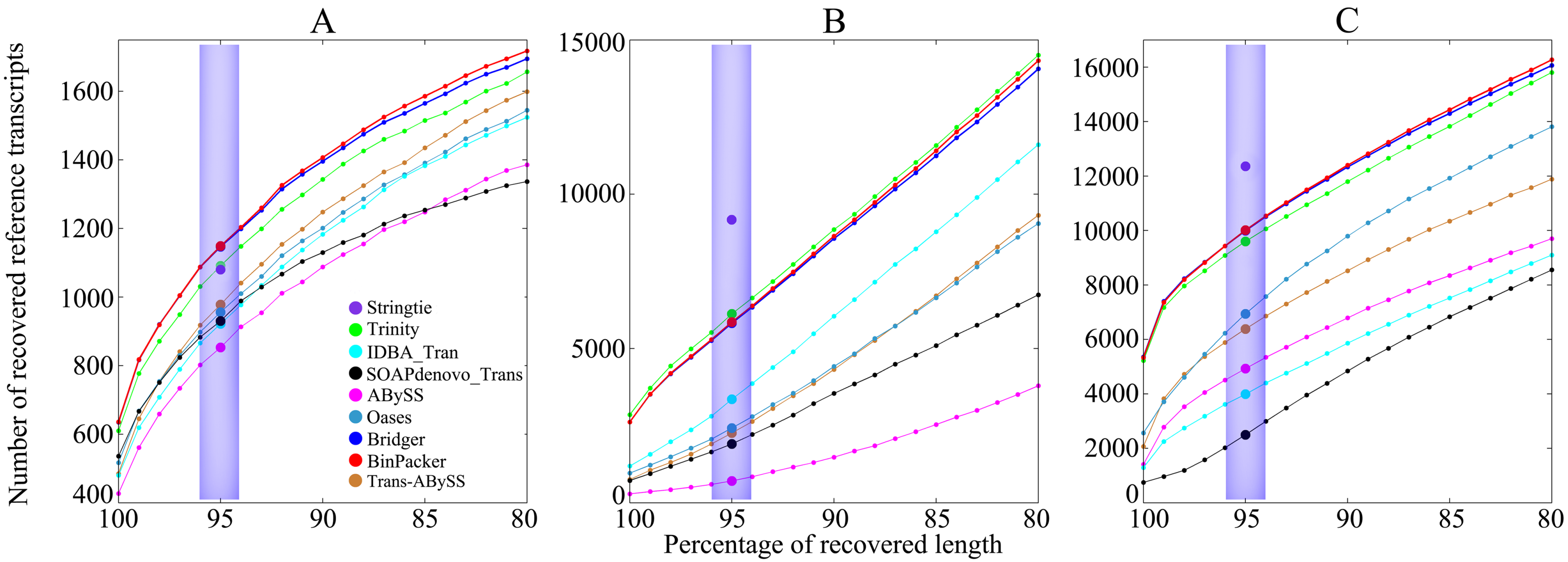
OPEN ACCESS   PEER-REVIEWED

RESEARCH ARTICLE

BinPacker: Packing-Based *De Novo* Transcriptome Assembly from RNA-seq Data

Juntao Liu, Guojun Li, Zheng Chang, Ting Yu, Bingqiang Liu, Rick McMullen, Pengyin Chen, Xiuzhen Huang

Published: February 19, 2016 • http://dx.doi.org/10.1371/journal.pcbi.1004772



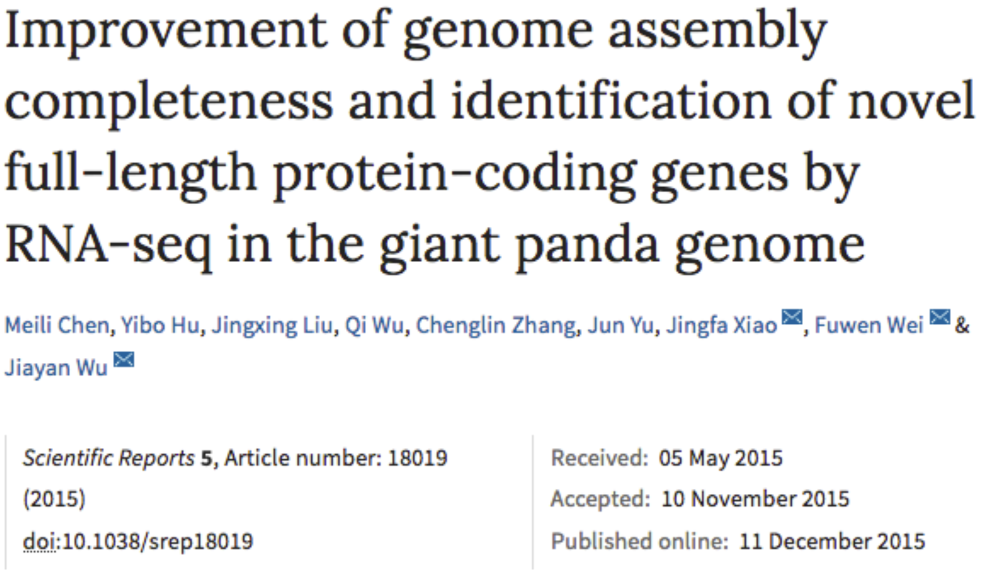
Comparison of recovered reference sensitivity and its distribution against recovered sequence length rates (sequence identity) ranging from 80% to 100% on (A) dog, (B) human and (C) mouse datasets.

De novo assembly

Take-home message

- No reference needed
- Many programs available
- Lots of potential transcripts. Filter!

Combining both methods



Improvement of genome assembly completeness and identification of novel full-length protein-coding genes by RNA-seq in the giant panda genome

Meili Chen, Yibo Hu, Jingxing Liu, Qi Wu, Chenglin Zhang, Jun Yu, Jingfa Xiao ✉, Fuwen Wei ✉ & Jiayan Wu ✉

Scientific Reports **5**, Article number: 18019 (2015)
doi:10.1038/srep18019

Received: 05 May 2015
Accepted: 10 November 2015
Published online: 11 December 2015

Combining both methods

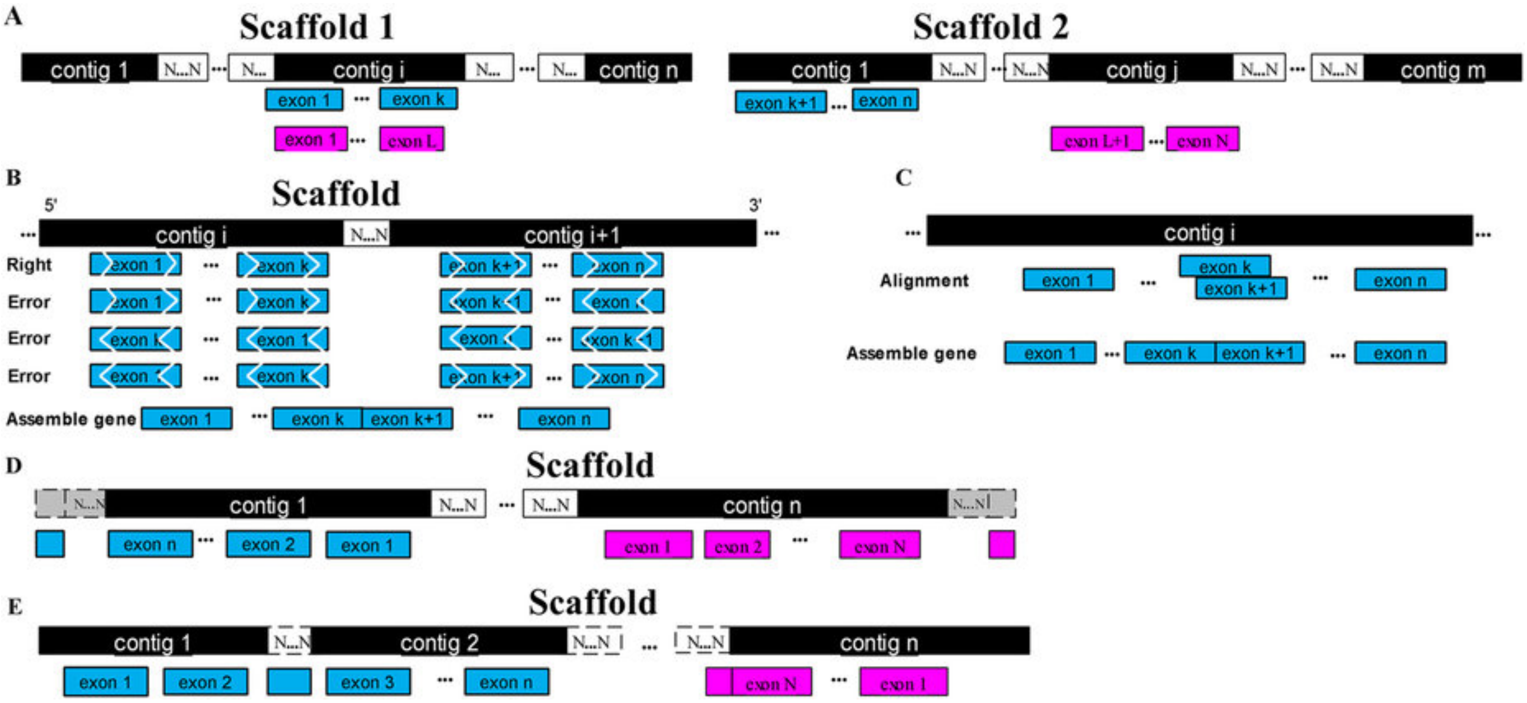
- Background
  - 1st *de novo* assembled genome based solely on short reads (Li et al., Nature 463, 2010)
  - 23,408 genes annotated on the basis of a homology search with human and dog genes and *ab initio* methods
- RNA-seq: 12 tissues
  - liver, stomach, small intestine, colon, pallium and testis from 1 male adult
  - pituitary gland, skeletal muscle, tongue, ovary and skin from 1 female adult

Combining both methods

- Reference-based:
  - Transcripts reconstruction: Cufflinks (alignment: TopHat)
- De novo:
  - Transcripts reconstruction: Trinity
- 24 assemblies (12 tissues * 2 methods)
  - Merge the 12 transcriptomes for each method
  - Merge the 2 method transcriptomes

Combining both methods

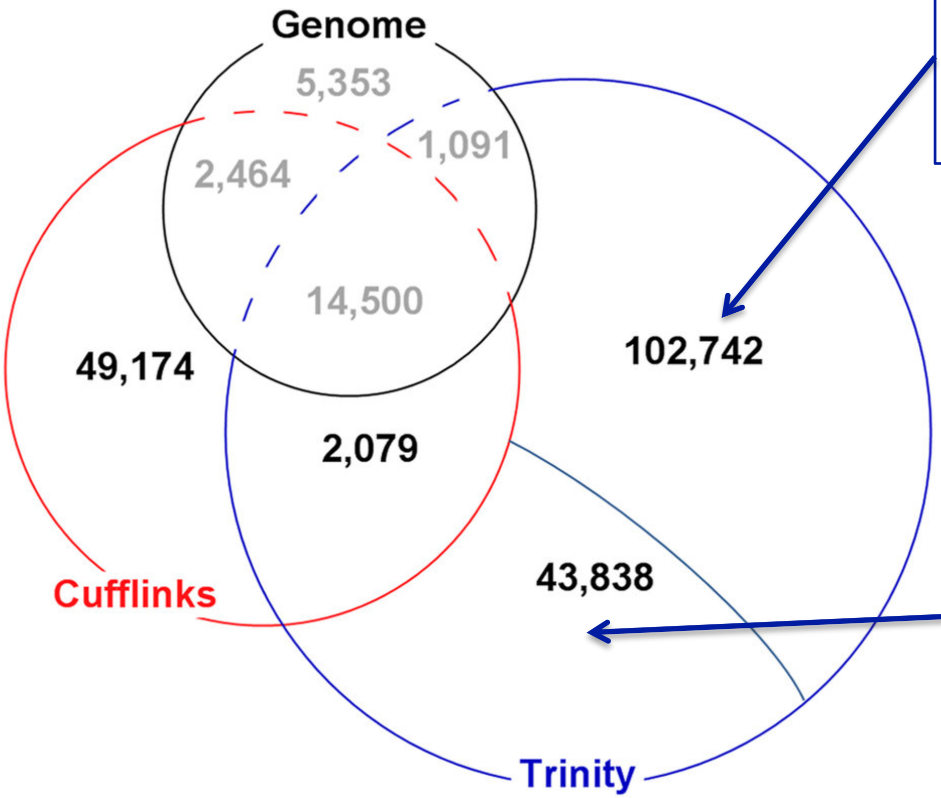
Improvement of genome assembly



(A) Scaffolding improvement; (B) Scaffolding inconsistencies; (C) Nest assembly errors; (D) Boundary extensions; (E) Gap closure

Combining both methods

Transcriptome reconstruction



Transcripts located to scaffolds that did not cover any known gene models

Transcripts unaligned back to the giant panda draft genome

49,174 + 2,079 + 43,838 + 102,742 = 197,833 potential novel transcripts!

Combining both methods

Validation of candidate novel protein-coding genes

- ORF detection (Augustus)
  - 197,833 novel transcripts => 28,522 potential novel protein-coding genes
- Homology search (blast) – 3 categories
  - 551 (1.93%) ***homology-based genes*** that were similar to known proteins in the nr database and known cDNA sequences in the nt database;
  - 6,290 (22.03%) ***unknown genes*** that were similar to EST sequences in dbEST but had no protein or cDNA homology information;
  - 12,575 (44.09%) ***hypothetical genes*** that had a complete ORF but no known homologs.
  - 9,106 ORFs were filtered out (no start or stop codon, too short CDS…)

Combining both methods

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Validation of candidate novel protein-coding genes

- Protein domain search on 19,416 ORFs (InterProScan)
  - 409 out of 551 *homology-based genes*
  - 5,112 out of 6,290 *unknown genes*
  - 7,981 out of 12,575 *hypothetical genes*
- Proteomic analysis in 5 tissues
  - 12,043 peptide hits
  - 1,691 novel protein-coding genes characterized with at least 1 peptide

Combining both methods

Take-home message

- Useful if the reference is incomplete
- Can help improving the reference
- Can help annotating the reference
- Need to filter the results!

Thank you for listening!

Questions?