

# RNA-seq: analysis and de novo assembly

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We want to know everything about RNA high throughput sequencing.

- How RNA is sequenced?
- What are the features and differences from DNA sequencing data?
- How to assembly this data?
- How to validate assembled data?

Assemble and analyze RNA is very cool, because of:

- we get only what we interested in genes (mRNA) and ncRNA,
- we can get useful information about organism without full-genome sequencing,
- **Everybody can do it, but we cannot yet!**
- everybody do it awfully.

Oases Scriptre Trinity  
IDBA-Tran Cufflinks  
transABySS KisSplice  
T-IDBA Velvet

In RNA-seq:

- cDNA is sequenced, so we can get paired reads
- eukaryotic mRNA has cap and poly-A tail, which help to identify start and end
- prokaryotic mRNA do not have alternative splicing, so it is easy to assemble them (**FALSE**)
- all the headache starts with alternative splicing...
  - different isoforms have different expression
  - different isoforms have same exons
  - and more, than you can imagine

# What have we done?

- ✓ Read dozens of papers.
- ✓ Understood the process of RNA sequencing.
- ✓ Created the table of (dis)advantages of all popular assemblers.
- ✓ Understood most of RNA De Bruijn graph reducing algorithms.
- ✗ SPAdes 2.4.0 fails on prokaryotic data ☹️.
- ➡ Alternative splicing reducing algorithms are in development.

# What's the problem with SPAdes?

To assemble 25 Gb paired reads of E. Coli's mRNA SPAdes took:

- 26 hours of work on ACE server
- 144 Gb RAM peak load
- Average contig length is  $(k + 1)$

After 26 hours of work SPAdes failed with `std::bad_alloc` exception!



