基礎生物学研究所　ゲノムインフォマティクス・トレーニングコース　2018年 7月

RNA-seq解析パイプライン： *de novo*

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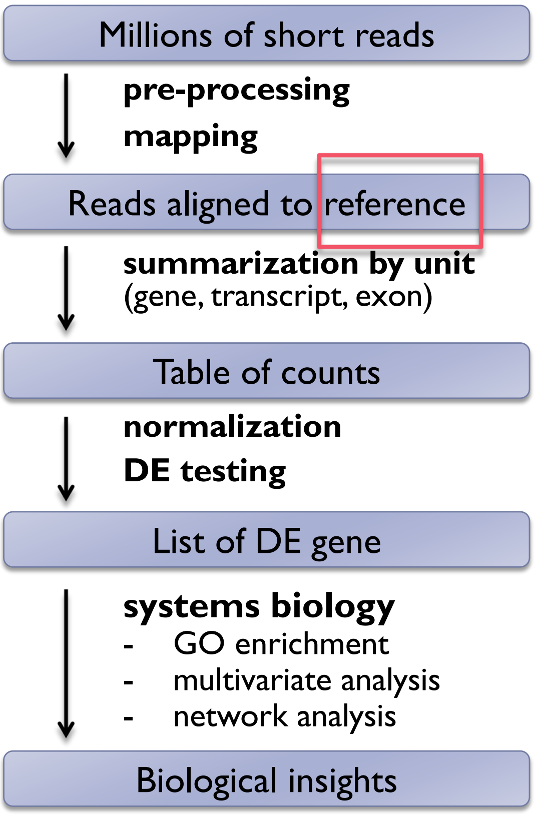
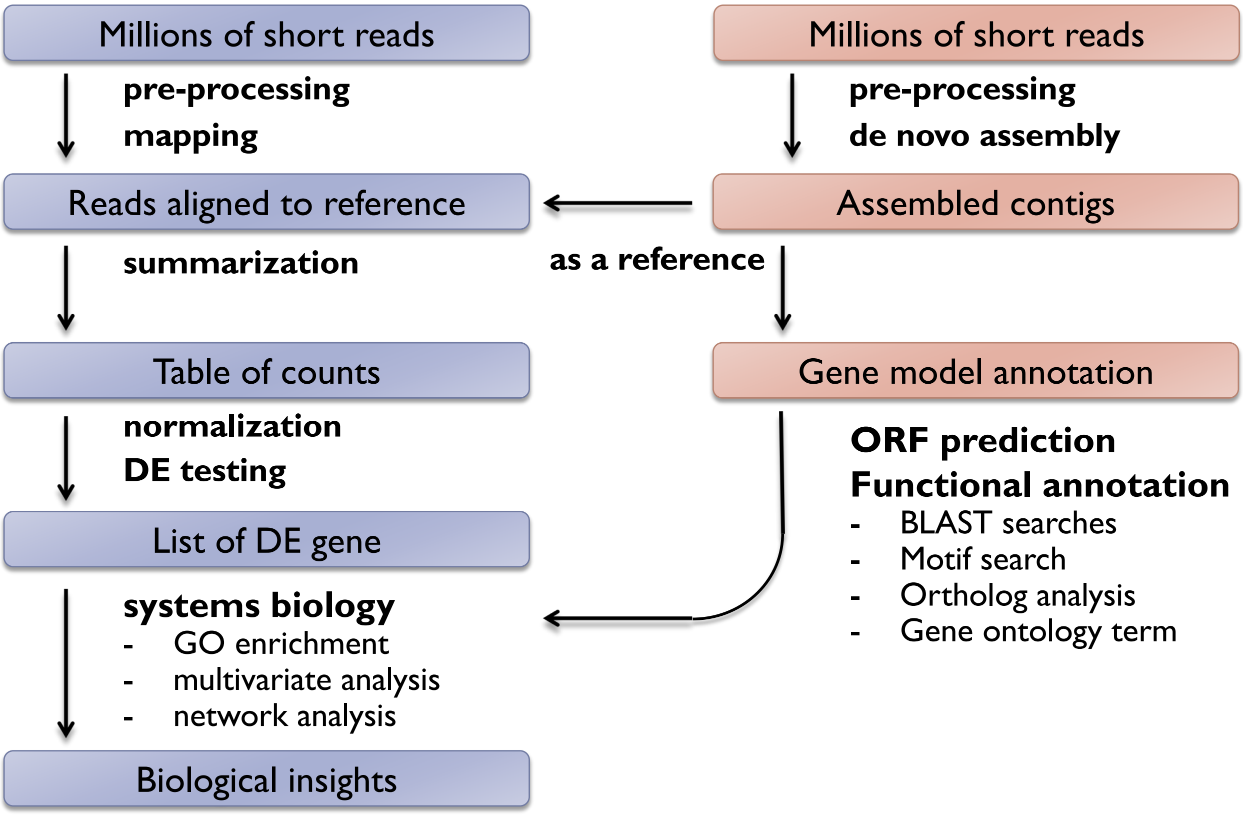
NIBB

de novo RNA-seq



1. **Build** reference
2. **Characterize** reference

RNA-seq analysis pipeline (***de novo*** strategy)



Millions of short reads

pre-processing

mapping

Reads aligned to reference

summarization

Table of counts

normalization

DE testing

List of DE gene

systems biology

- GO enrichment
- multivariate analysis
- network analysis

Biological insights

Millions of short reads

pre-processing

de novo assembly

Assembled contigs

as a reference

Gene model annotation

ORF prediction

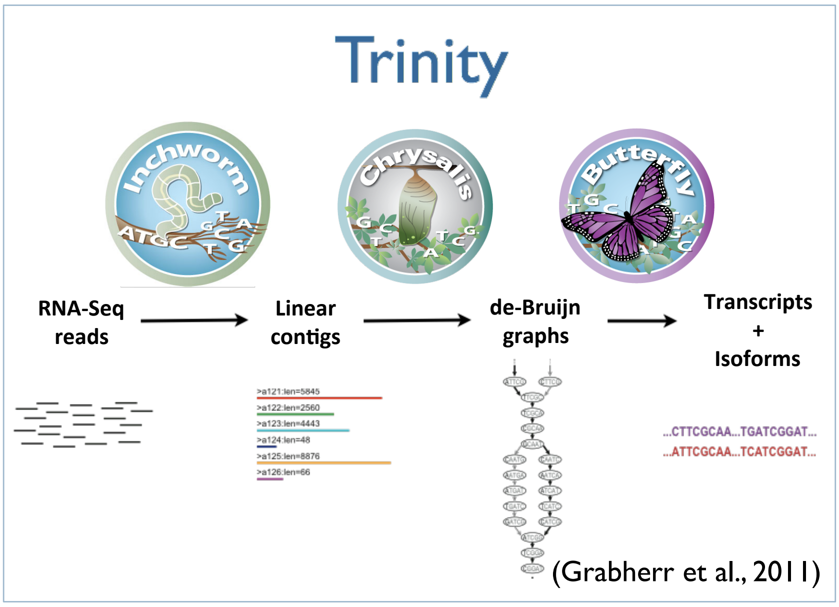
Functional annotation

- BLAST searches
- Motif search
- Ortholog analysis
- Gene ontology term

de novo assemblers of RNA-seq

De novo assemblers use reads to assemble transcripts directly, which does not depend on a reference gnome.

- Trinity
- Oases
- TransAbyss
- EBARDenovo
- …



(Grabherr et al., 2011)

https://github.com/trinityrnaseq/trinityrnaseq/wiki

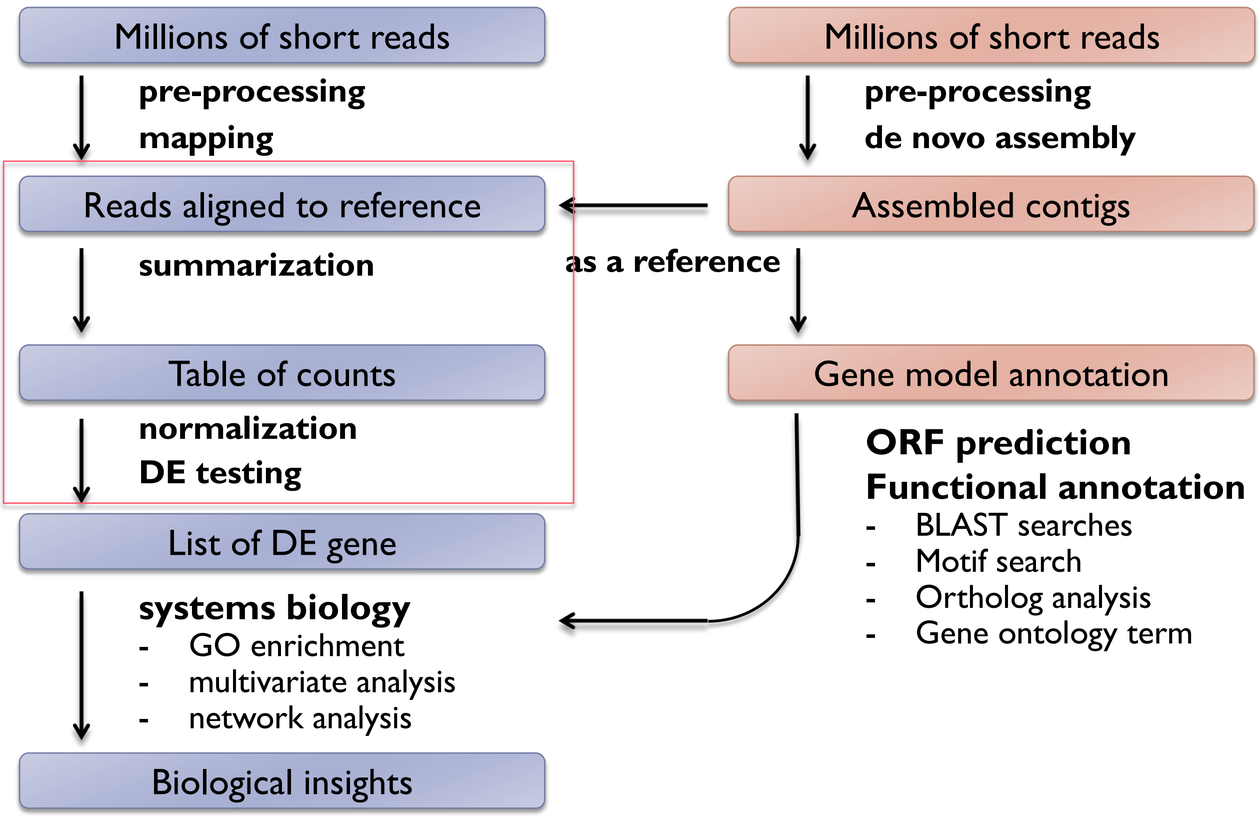
Trinity example

- Input: Illumina short reads in FASTQ | FASTA format
- Output: assembled contigs in FASTA format

```
# Run Trinity
$ Trinity --seqType fq --left left_all.fq --right right_all.fq \
          --CPU 8 --max_memory 20G
```

(Trinity is supported on only Linux)

RNA-seq analysis pipeline (*de novo* strategy)



DEG analysis

- Follow transcript-based RNA-seq pipeline

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Advanced

Clean up reference sequences

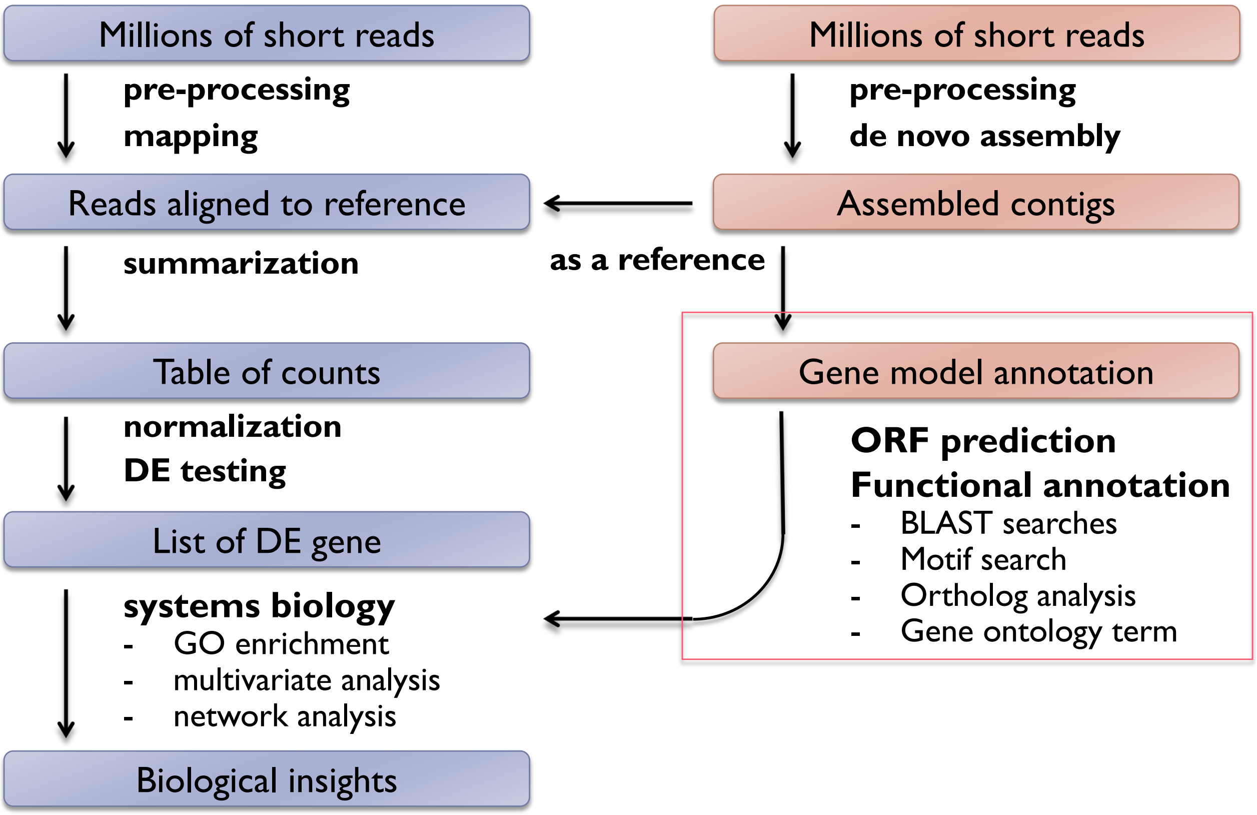
- Issues: Inflation of the number of Trinity contigs is often observed.
  - Trinity outputs splicing variants separately
  - Contaminations
  - Artifacts (bad contigs)
  - Incomplete contigs with very low expression.
- Solution
  - Filter out unwanted contigs.
  - Filter out very lowly expressed transcripts.
  - Clustering similar sequences.

Advanced

Remove redundancy in reference sequences

- Strategy and Tools
  - Choose one representative transcript from each cluster based on Trinity component information. (longest or highest expression)
  - Clustering
    - CDHIT-EST (http://weizhongli-lab.org/cd-hit/)
    - Corset (Davidson et al., 2014).
    - RapClust (https://github.com/COMBINE-lab/RapClust)

RNA-seq analysis pipeline (*de novo* strategy)



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ORF prediction

- Special consideration in ORF prediction after de novo RNA-seq assembly
  - Sometimes partial: Start Met or terminal codon may be missing.
  - Ideally one ORF is present per contig, but erroneously joined contigs may include multiple ORFs.
  - Possible frame shifts.
    - Frame shifts do not occur so often in Illumina, while it happens very frequently in 454 and IonProton.
- Recommended software: TransDecoder

Functional Annotation of Predicted ORFs

- BLAST
  - NCBI NR (or UniProt)
  - species of interest (model organisms, close relatives etc)
  - specific DB (SwissProt, rRNA DB, CEGMA etc)
  - self (assembly v.s. assembly)
- Motif search
  - Pfam, SignalP etc.
- Ortholog analysis
  - vs model organism
  - ortholog database (OrthoDB, eggNOG, OrthoMCL etc)
  - close relatives
- Gene Ontology term assignment

Quick annotation by BLASTX

- Query: assembled contigs

  (nucleotide sequences in multi-fasta format)
- DB: Protein sequences of a model organism

Format DB

```
$ makeblastdb –in protein.fa -dbtype prot
```

Search

```
$ blastx -query trinity_contigs –db protein.fa \
  -num_threads 8 -evalue 1.0e-8 –outfmt 0 > blastxout.txt
```

Protein motif search using InterProScan

- Query: Translated ORF sequences
- Software: InterProScan
  - https://github.com/ebi-pf-team/interproscan/wiki

Search

```
$ interproscan.sh  -I proteins.fasta -f XML,TSV --goterms
--pathways
```

Assign Gene Ontology terms

- Tools
  - InterProScan
  - BLAST2GO
  - Transfer model organisms GO terms based on orthology.

Let's try Trinity assembly

- ex9: de novo RNA-seq assembly using Trinity