Gene expression analysis with an R intro

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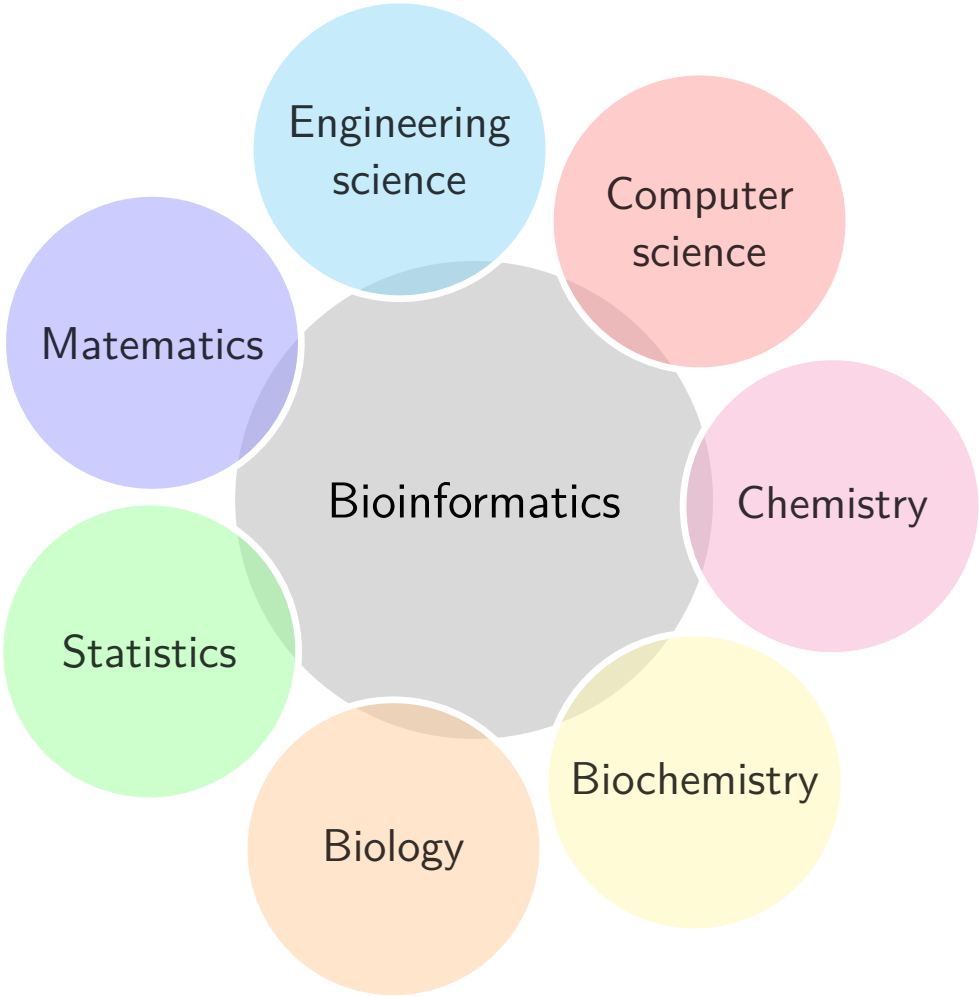
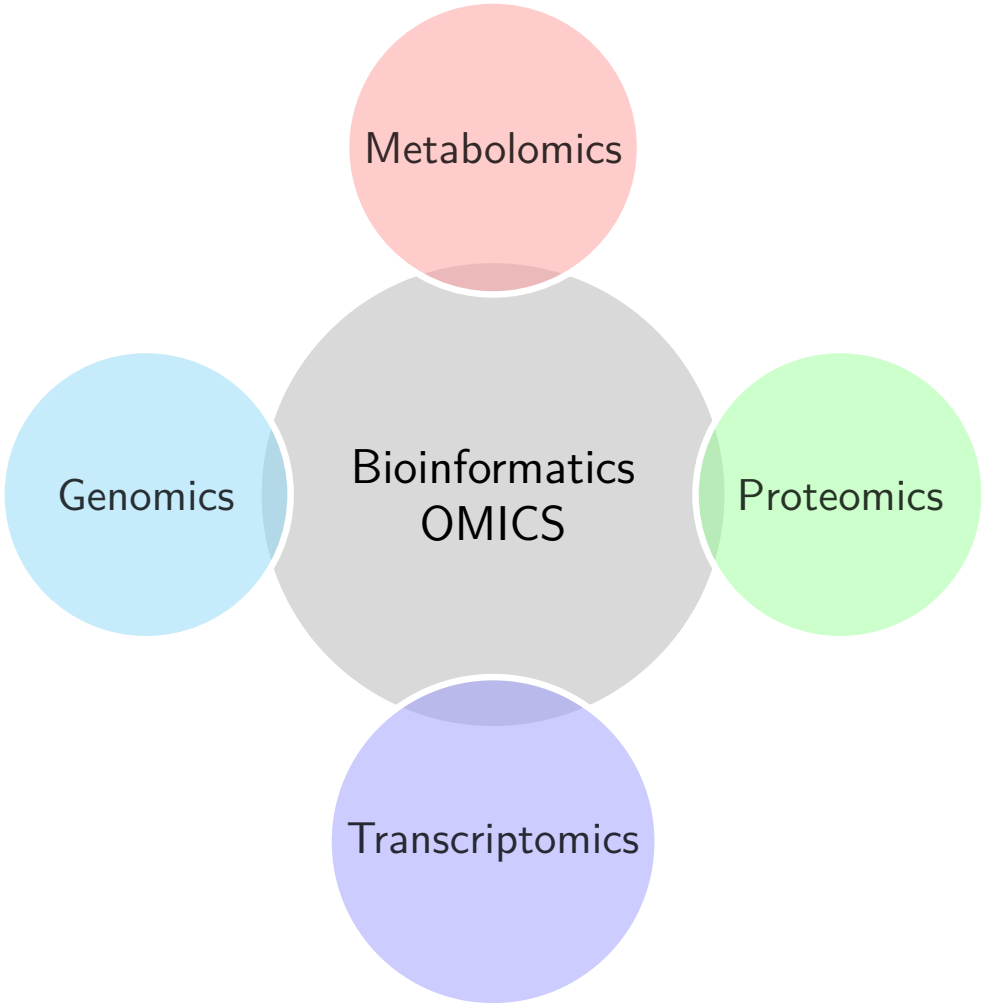
Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data, in particular when the data sets are large and complex.



Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data, in particular when the data sets are large and complex.



1. Sanger
2. Next (New) Generation Sequencing
   - short reads
   - Illumina , Ion Torrent
   - Applied Biosystems (Solid), Roche 454
3. Third Generation
   - long reads
   - PacBio
   - Oxford Nanopore

- shotgun
- targeted (pl. 16S rRNA, RNA-seq)
- DNA, RNA

Nucleic acid extracted from sample: thousands – millions

...CTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGAC
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Nucleic acid fragmentation (physical, enzimatic): 200 – 1000

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...

...

Sequence detection → short read

C

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G

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T

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Sequence detection → short read

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Sequence detection → short read

CTG

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TAC

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Sequence detection → short read

CTGT

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TACT

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Sequence detection → short read

CTGTT

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GTACA

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TACTT

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CATCAGAGGCTGCTCGTGTTGTACGATCAATTTTCTCCCGCAC

Sequence detection → short read

CTGTTC

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GAATTTGACTAAAGAAGGTGCCACTACTTGTGGT

TACTTA

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Sequence detection → short read

CTGTTCT

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TACTTAC

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Sequence detection → short read

CTGTTCTC

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TACTTACC

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Sequence detection → short read

CTGTTCTCT

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Sequence detection → short read

CTGTTCTCTA

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Sequence detection → short read

CTGTTCTCTAA

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Sequence detection → short read

CTGTTCTCTAAA

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Sequence detection → short read

CTGTTCTCTAAAC

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Sequence detection → short read

CTGTTCTCTAAACG

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Sequence detection → short read

CTGTTCTCTAAACGA

CTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACG
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Sequence detection → short read

CTGTTCTCTAAACGAA

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Sequence detection → short read

CTGTTCTCTAAACGAAC

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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Sequence detection → short read

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TTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTTCATCAAACGTTC
GGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTC
ATGTGGGCGAAATACCAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTA
GGCGACGAGCTTGGCACTGATCCTTATGAAGATTTTCAAGAAAACTGGAACACTAAACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGC
ATACACTCGCTATGTCGATAACAACTTCTGTGGCCCTGATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAAGCTTCATGCACTTTGTCCG
AACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAACATGAGCATGAAATTGCTTG

GTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAA

GTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAATTAAATTGGCAAAGAAATTTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTC
CCTTAAATTCCATAATCAAGACTATTCAACCAAGGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAATGAA
TGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTGGTGAAACTTCATGGCAGACGGGCGATTTTGTTAAAGCCACTTGCGAATTTTGTGGCACTGA
GAATTTGACTAAAGAAGGTGCCACTACTTGTGGT

TACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAA

TACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAA
AACCATTCTTCGTAAGGGTGGTCGCACTATTGCCTTTGGAGGCTGTGTGTTCTCTTATGTTGGTTGCCATAACAAGTGTGCCTATTGGGTTCCACGTGCTAGCGCTA
ACATAGGTTGTAACCATACAGGTGTTGTTGGAGAAGGTTCCGAAGGTCTTAATGACAACCTTCTTGAAATACTCCAAAAAGAGAAAGTCAACATCAATATTGTTGGT
GACTTTAAACTTAATGAAGAGATCGCCATTATTTTGGCATCTTTTTCTGCTTCCACAAGTGCTTTTGTGGAAACTGTGAAAGGTTTGGATTATAAAGCATTCAAACA
AATTGTTGAATCCTGTGGTAATTTTAAAGTTACAAAAGGAAAAGCTAAAAAAGGTGCCTGGAATATTGGTGAACAGAAATCAATACTGAGTCCTCTTTATGCATTTG
CATCAGAGGCTGCTCGTGTTGTACGATCAATTTTCTCCCGCAC

Sequence detection → short read

CTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATG

CTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACG
AGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGA
GCCTTGTCCCTGGTTTCAACGAGAAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTC
TTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAACTTGAACAGCCCTATGTGTTCATCAAACGTTC
GGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTC
ATGTGGGCGAAATACCAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTA
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GTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAAT

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CCTTAAATTCCATAATCAAGACTATTCAACCAAGGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAATGAA
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TACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAAT

TACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAA
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ACATAGGTTGTAACCATACAGGTGTTGTTGGAGAAGGTTCCGAAGGTCTTAATGACAACCTTCTTGAAATACTCCAAAAAGAGAAAGTCAACATCAATATTGTTGGT
GACTTTAAACTTAATGAAGAGATCGCCATTATTTTGGCATCTTTTTCTGCTTCCACAAGTGCTTTTGTGGAAACTGTGAAAGGTTTGGATTATAAAGCATTCAAACA
AATTGTTGAATCCTGTGGTAATTTTAAAGTTACAAAAGGAAAAGCTAAAAAAGGTGCCTGGAATATTGGTGAACAGAAATCAATACTGAGTCCTCTTTATGCATTTG
CATCAGAGGCTGCTCGTGTTGTACGATCAATTTTCTCCCGCAC

Sequence detection → short read – SINGLE END

CTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGC

CTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTAATAACTAATTACTGTCGTTGACAGGACACG
AGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCAGCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGA
GCCTTGTCCCTGGTTTCAACGAGAAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGAGGTC
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GGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCAGAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTC
ATGTGGGCGAAATACCAGTGGCTTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTGACTTA
GGCGACGAGCTTGGCACTGATCCTTATGAAGATTTTCAAGAAAACTGGAACACTAAACATAGCAGTGGTGTTACCCGTGAACTCATGCGTGAGCTTAACGGAGGGGC
ATACACTCGCTATGTCGATAACAACTTCTGTGGCCCTGATGGCTACCCTCTTGAGTGCATTAAAGACCTTCTAGCACGTGCTGGTAAAGCTTCATGCACTTTGTCCG
AACAACTGGACTTTATTGACACTAAGAGGGGTGTATACTGCTGCCGTGAACATGAGCATGAAATTGCTTG

GTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAATT

GTACACGGAACGTTCTGAAAAGAGCTATGAATTGCAGACACCTTTTGAAATTAAATTGGCAAAGAAATTTGACACCTTCAATGGGGAATGTCCAAATTTTGTATTTC
CCTTAAATTCCATAATCAAGACTATTCAACCAAGGGTTGAAAAGAAAAAGCTTGATGGCTTTATGGGTAGAATTCGATCTGTCTATCCAGTTGCGTCACCAAATGAA
TGCAACCAAATGTGCCTTTCAACTCTCATGAAGTGTGATCATTGTGGTGAAACTTCATGGCAGACGGGCGATTTTGTTAAAGCCACTTGCGAATTTTGTGGCACTGA
GAATTTGACTAAAGAAGGTGCCACTACTTGTGGT

TACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAATT

TACTTACCCCAAAATGCTGTTGTTAAAATTTATTGTCCAGCATGTCACAATTCAGAAGTAGGACCTGAGCATAGTCTTGCCGAATACCATAATGAATCTGGCTTGAA
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GACTTTAAACTTAATGAAGAGATCGCCATTATTTTGGCATCTTTTTCTGCTTCCACAAGTGCTTTTGTGGAAACTGTGAAAGGTTTGGATTATAAAGCATTCAAACA
AATTGTTGAATCCTGTGGTAATTTTAAAGTTACAAAAGGAAAAGCTAAAAAAGGTGCCTGGAATATTGGTGAACAGAAATCAATACTGAGTCCTCTTTATGCATTTG
CATCAGAGGCTGCTCGTGTTGTACGATCAATTTTCTCCCGCAC

Sequence detection → short read – PAIRED END

```
@SRR11177792.1 1
TTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATATTTTAAGAA
+
AC-B--CCEEFG-C--CCF---CF,,,;CE,CEC,,,,,,,,,;C,,,C,,,<,CEEF9,,,,
@SRR11177792.2 2
CTAAAGCATACAATGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATT
+
8B----C<F-C--C-C9-C-C-,,CEEFE,+@++,;+C,,:CC,,,,;,,,;@@,,,,,,,CC
@SRR11177792.3 3
AACACCAGCTTCTGATCTTTCACAAGTTCCGTGTCTACAGTACTCAGAATCAAAAGTTGTTAC
+
--8-A@--CEECF--CEFFGE9C,,,C,<C,C,CCE,;,,<,;CC,,,,<C,,,,,;E,CE9C
@SRR11177792.4 4
TCATTGCAAAAGCAGACATAGCAATAATACCCATAGCAAAAGGTAAAAAGGCATTTTCATACA
+
C@9CC9E9----C<--;-E9<C99F9,E9CCE9E,,6,,,,,C,,,,6,,C,CFFGF9F9C9
@SRR11177792.5 5
GTATTATCTTTCTGTGCTTTTGCTGTAGATGCTGCTAAAGCTTACAAAGATTATCTAGCTAGT
+
-A<CC9EEFGGFF9F9CFFGG<CE9F9,,C,6E,@E9,,,CEE9@,,,,,CC9CCE9,;C,,C
```

```
@SRR11177792.1 1
TTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATATTTTAAGAA
+
AC-B--CCEEFG-C--CCF---CF,,,;CE,CEC,,,,,,,,,;C,,,C,,,<,CEEF9,,,,
```

Phred Quality Score

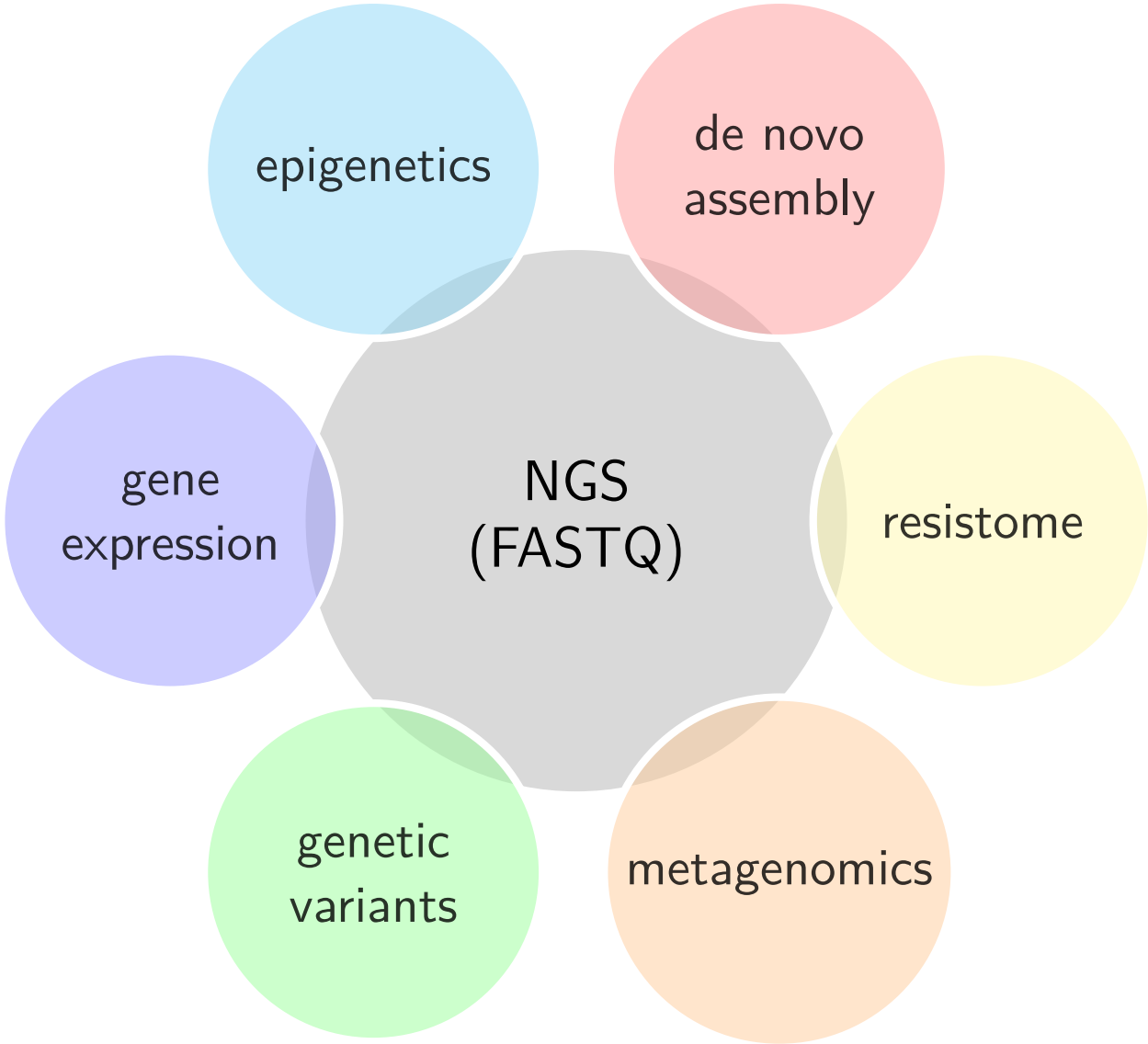
$$Q = -10 \log_{10} P$$

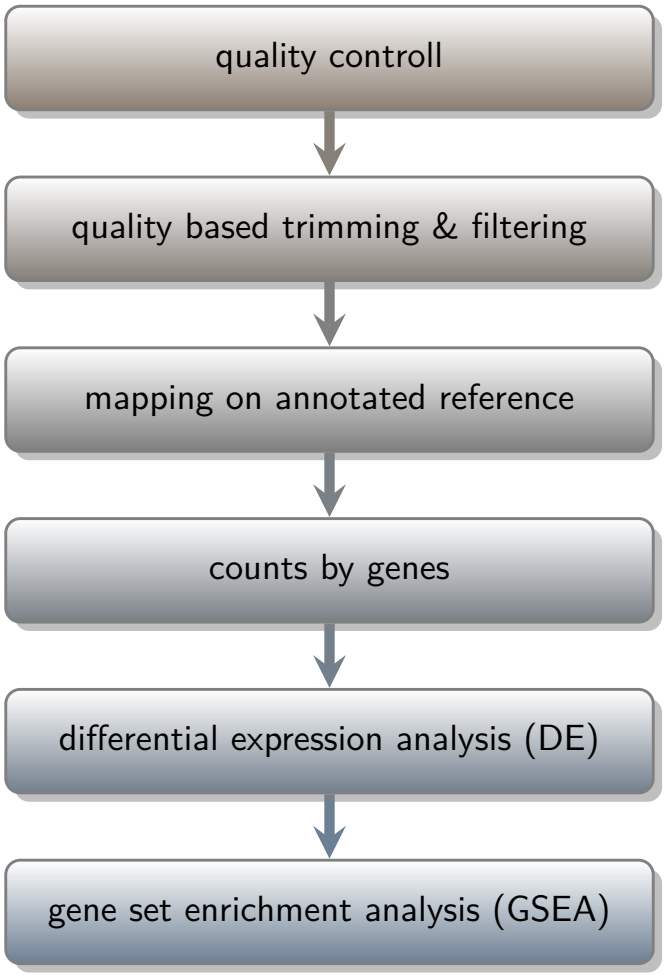
$$P = 10^{\frac{-Q}{10}}$$

Q	Probability of incorrect base call	Base call accuracy	Chr
10	1/10	90%	+
20	1/100	99%	5
30	1/1000	99.9%	?
40	1/10 000	99.99%	I
50	1/100 000	99.999%	S
60	1/1 000 000	99.9999%	]

```
@SRR11177792.1 1
TTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATATTTTAAGAA
+
AC-B--CCEEFG-C--CCF---CF,,,;CE,CEC,,,,,,,,,;C,,,C,,,<,CEEF9,,,,
```

Chr	Q	P
-	12	0.0631
,	11	0.07943
;	26	0.00251
<	27	0.002
9	24	0.00398
A	32	0.00063
B	33	0.0005
C	34	0.0004
E	36	0.00025
F	37	0.0002
G	38	0.00016


epigenetics
de novo assembly
gene expression
NGS (FASTQ)
resistome
genetic variants
metagenomics



PNAS RESEARCH ARTICLE | NEUROSCIENCE

Transcriptome profiling of kisspeptin neurons from the mouse arcuate nucleus reveals new mechanisms in estrogenic control of fertility

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Edited by Donald Pfaff, The Rockefeller University, New York, NY; received July 30, 2021; accepted May 5, 2022

Kisspeptin neurons in the mediobasal hypothalamus (MBH) are critical targets of ovarian estrogen feedback regulating mammalian fertility. To reveal molecular mechanisms underlying this signaling, we thoroughly characterized the estrogen-regulated transcriptome of kisspeptin cells from ovariectomized transgenic mice substituted with 17β-estradiol or vehicle. MBH kisspeptin neurons were harvested using laser-capture microdissection, pooled, and subjected to RNA sequencing. Estrogen treatment significantly (*p.adj.* < 0.05) up-regulated 1,190 and down-regulated 1,139 transcripts, including transcription factors, neuropeptides, ribosomal and mitochondrial proteins, ion channels, transporters, receptors, and regulatory RNAs. Reduced expression of the excitatory serotonin receptor-4 transcript (*Htr4*) diminished kisspeptin neuron responsiveness to serotonergic stimulation. Many estrogen-regulated transcripts have been implicated in puberty/fertility disorders. Patients ($n = 337$) with congenital hypogonadotropic hypogonadism (CHH) showed enrichment of rare variants in putative CHH-candidate genes (e.g., *LRP1B*, *CACNA1G*, *FNDC3A*). Comprehensive characterization of the estrogen-dependent kisspeptin neuron transcriptome sheds light on the molecular mechanisms of ovary–brain communication and informs genetic research on human fertility disorders.

fertility | gene expression | neuropeptides | reproduction | RNA sequencing

Endocrine homeostasis depends on the complex interplay between the hypothalamus and the pituitary and peripheral endocrine organs. Gonadotropin-releasing hormone (GnRH)-synthesizing neurons constitute the final output conduit from the hypothalamus for the control of reproduction (1, 2). The neurosecretory axons of these neurons terminate in the external zone of the median eminence. Episodic release of GnRH at this site into the hypothalamic-hypophysial portal circulation system evokes pulsatile luteinizing hormone (LH) and follicle-stimulating hormone secretion from the anterior pituitary, which, in turn, stimulates gametogenesis and sex steroid synthesis in the male and female gonads. In both sexes, gonadal steroids inhibit the hypothalamic-pituitary-gonadal axis via homeostatic negative feedback to the hypothalamus and the anterior pituitary. In females, rising blood levels of ovarian estrogen hormones at the late follicular phase of the reproductive cycle cause a switch from negative to positive feedback. This rise is a key signal for the midcycle GnRH/LH surge, which triggers ovulation (1, 2).

Hypothalamic peptidergic neurons synthesizing kisspeptin (KP) express estrogen receptor-α (ERα) and play crucial roles in mediating the positive and negative estrogen feedback to GnRH neurons via KP/KP receptor signaling. In rodents, KP neurons located in the rostral periventricular area of the third ventricle (RP3V; also referred to as the KP neuron population of the anteroventral periventricular nucleus) are critically involved in the induction of preovulatory GnRH/LH surges during positive feedback. The arcuate nucleus (ARC) in the mediobasal hypothalamus (MBH) contains an additional large KP neuron population. This anatomical region has long been known as a critical feedback site in the communication between the ovary and the hypothalamus. In postmenopausal women, absence of estrogen feedback causes profound morphofunctional changes within this region, characterized by neuronal hypertrophy (3) and increased neurokinin B (NKB) (4, 5), KP (5, 6), and substance P (4, 7) biosynthesis. In various mammals, KP neurons of the ARC (aka KNDy neurons) coexpress KP, NKB, and dynorphin. Growing evidence suggests that ARC KNDy neurons are key players in negative estrogen feedback (2, 8), and their KP output also regulates the pattern of pulsatile GnRH/LH secretion (9).

Significance

The arcuate nucleus (ARC) of the mediobasal hypothalamus is critically involved in hormonal communication from ovary to brain. Negative estrogen feedback to kisspeptin synthesizing neurons of the ARC is a crucial determinant of hypothalamic gonadotropin-releasing hormone secretion regulating fertility. We performed deep transcriptome profiling of ARC kisspeptin neurons with RNA sequencing and identified over 2,000 estrogen-sensitive transcripts. Several genes responding to estrogen treatment in ovariectomized mice exhibited rare variants in a patient database with pubertal defects and emerge as candidate genes for a role in puberty/fertility disorders. Comprehensive characterization of the estrogen-dependent kisspeptin neuron transcriptome in mice has important clinical implications for the hypothalamic regulation of human menstrual cycle and for the putative molecular consequences of postmenopausal estrogen deficiency.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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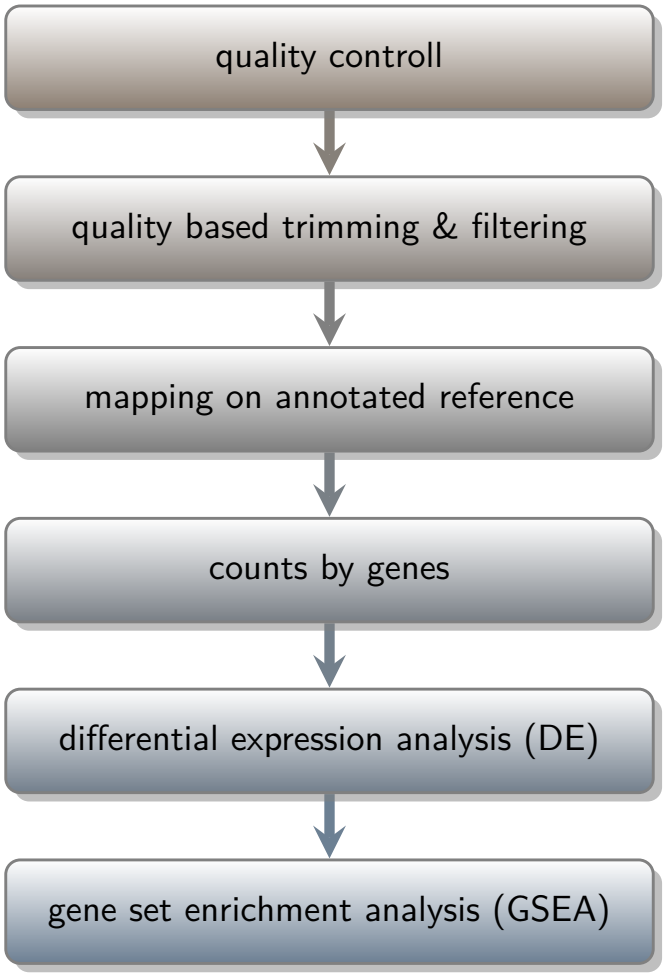
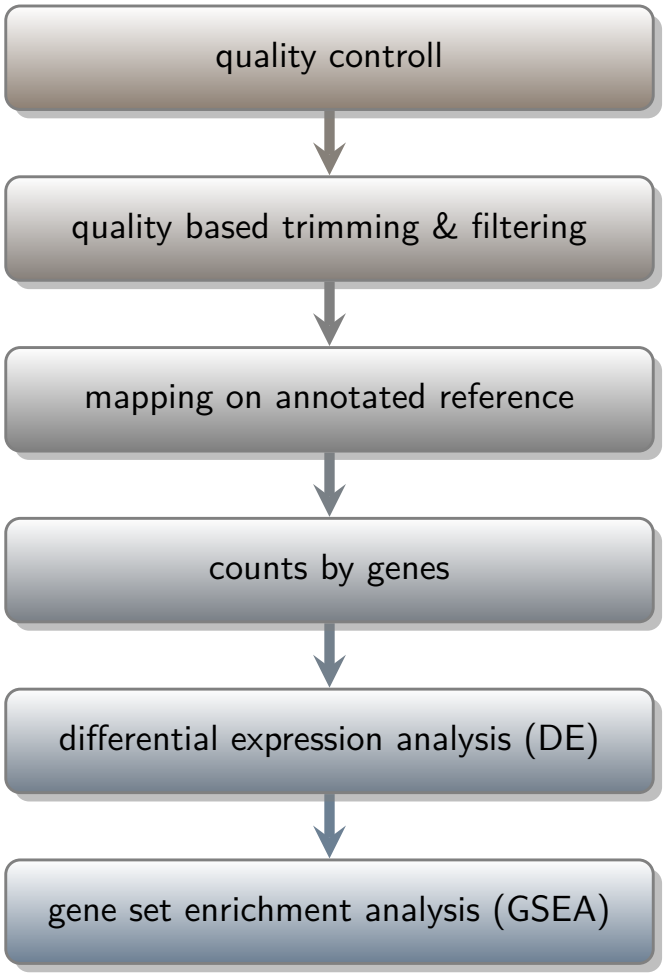
This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2113749119/-/DCSupplemental.

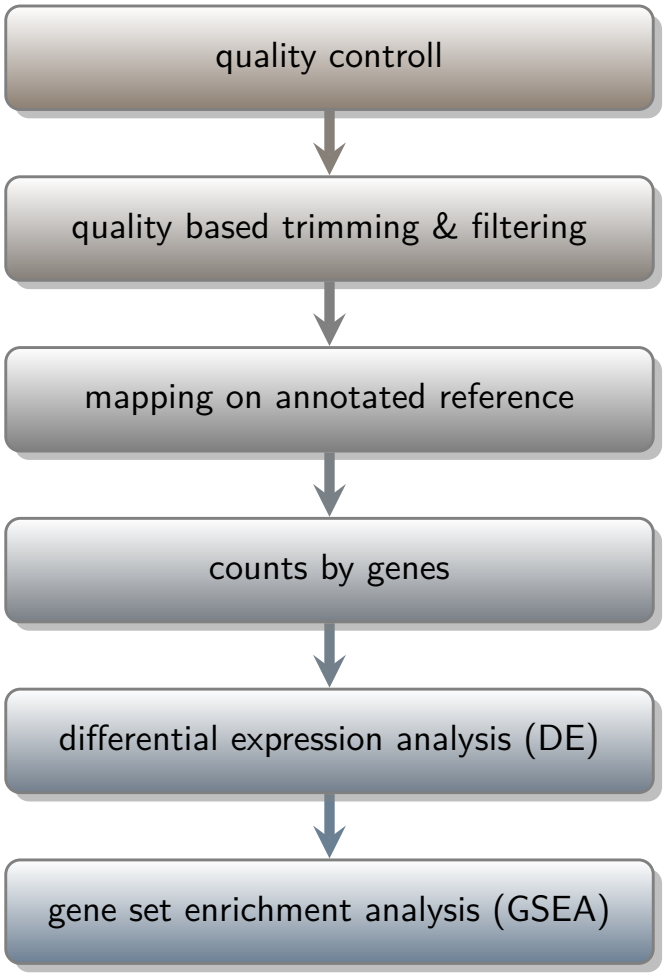
Published June 28, 2022.



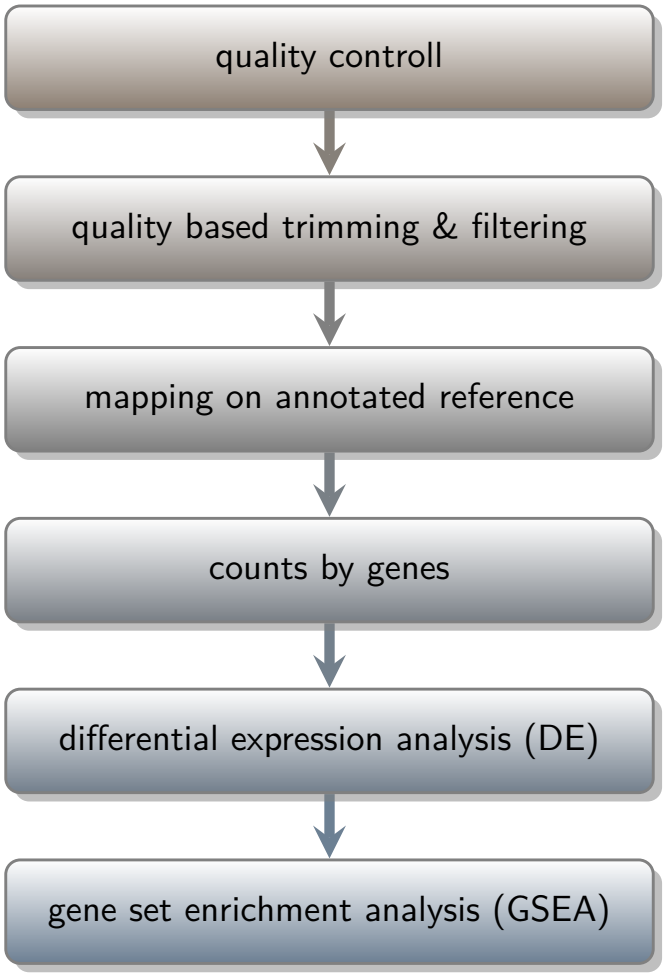

```
export PATH=/data/tools/FastQC:$PATH

for f in *fastq.gz
do
  fastqc -t 38 $f -o qc
done

multiqc qc
```



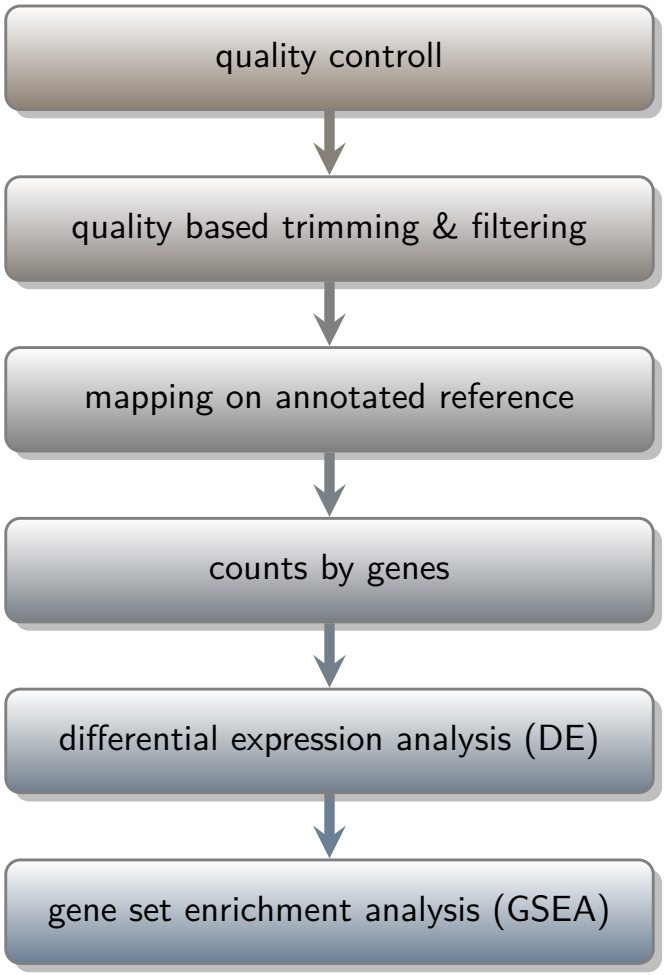

```
TRIM='Trimmomatic-0.38/trimmomatic-0.38.jar'

for f in *.fastq.gz
do
  o=${f/'.fastq.gz'/'_trimmed.fastq'}
  java -jar $TRIM SE -threads 38 \
    $f $o \
    LEADING:3 \
    TRAILING:3 \
    SLIDINGWINDOW:4:30 \
    MINLEN:50
done
```



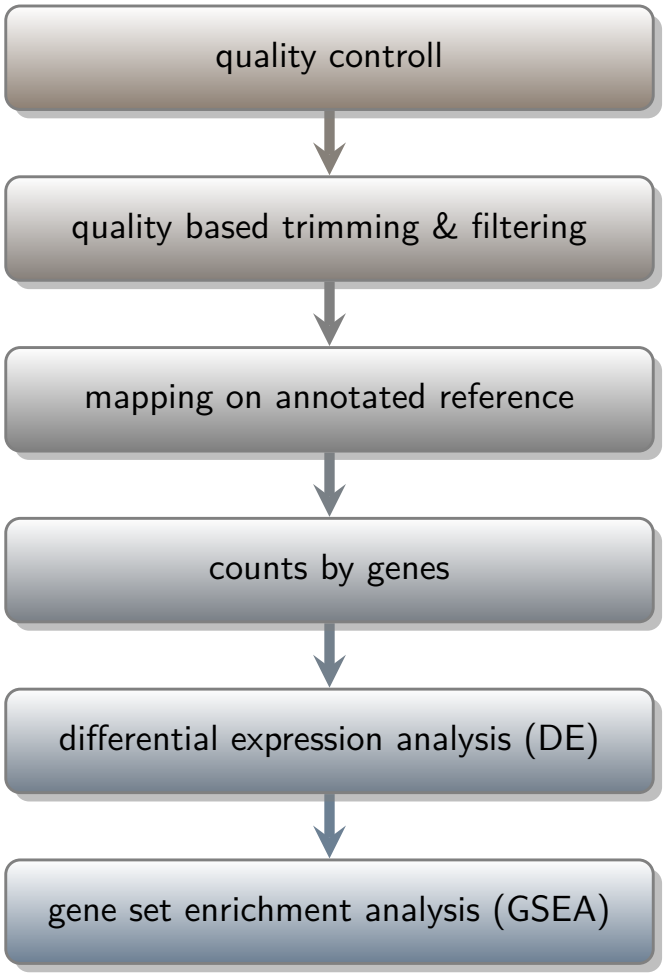

```
export PATH=STAR-2.7.3a/bin/Linux_x86_64:$PATH
export PATH=subread-2.0.0-source/bin:$PATH

cd STAR/GRCm38_100

STAR -- runMode genomeGenerate \
     -- genomeDir . \
     -- genomeFastaFiles \
     Mus_musculus.GRCm38.dna.primary_assembly.fa \
     -- sjdbGTFfile Mus_musculus.GRCm38.100.gtf \
     -- runThreadN 14
```




```
idx='STAR/GRCm38_100'

for f in *_trimmed.fastq
do
  root=${f/'.fastq'/''}
  STAR -- genomeDir $idx \
       -- readFilesIn $f \
       -- outFileNamePrefix 'GRCm38_100_'$root \
       -- outFilterMultimapNmax 1 \
       -- outReadsUnmapped Fastx \
       -- outSAMtype BAM SortedByCoordinate \
       -- twopassMode Basic \
       -- runThreadN 14
done
```



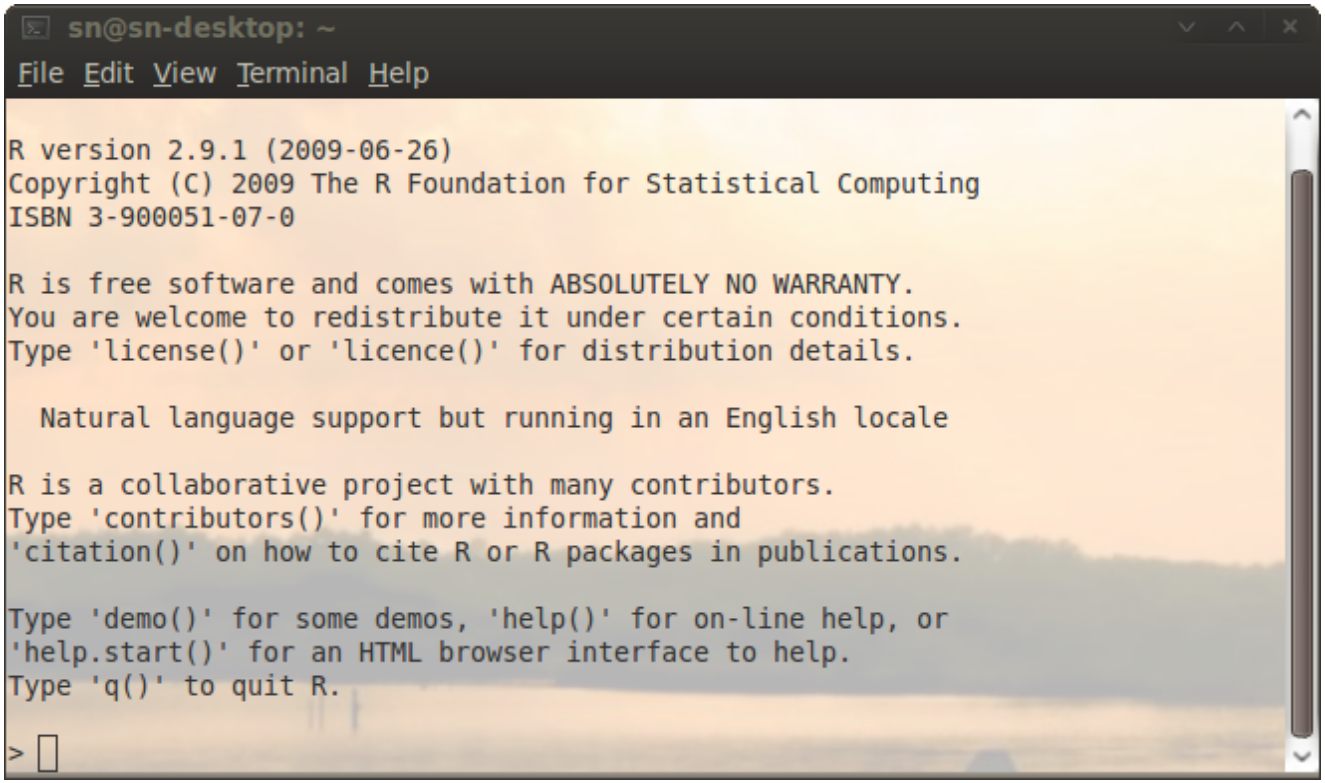

```
idx='STAR/GRCm38_100'

for f in *_trimmed.fastq
do
  root=${f/'.fastq'/''}
  STAR -- genomeDir $idx \
       -- readFilesIn $f \
       -- outFileNamePrefix 'GRCm38_100_'$root \
       -- outFilterMultimapNmax 1 \
       -- outReadsUnmapped Fastx \
       -- outSAMtype BAM SortedByCoordinate \
       -- twopassMode Basic \
       -- runThreadN 14
done
```




```
featureCounts -O \
  -a $idx/Mus_musculus.GRCm38.100.gtf \
  -o featureCounts_GRCm38_100_O.txt \
  GRCm38_100*bam
```

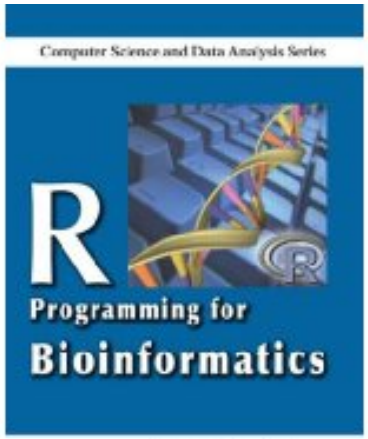
http://www.r-project.org/

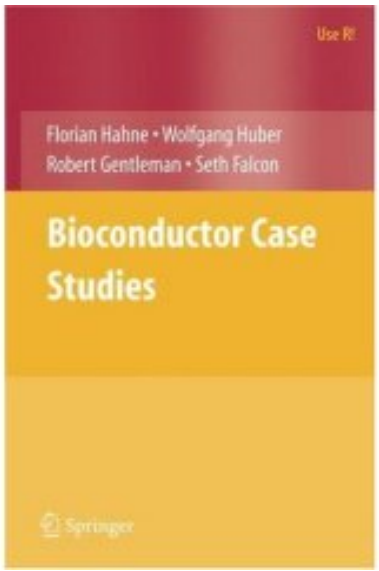
- S, S-Plus
- Robert Gentleman, Ross Ihaka
- Script language
- Functions (packages, libraries)

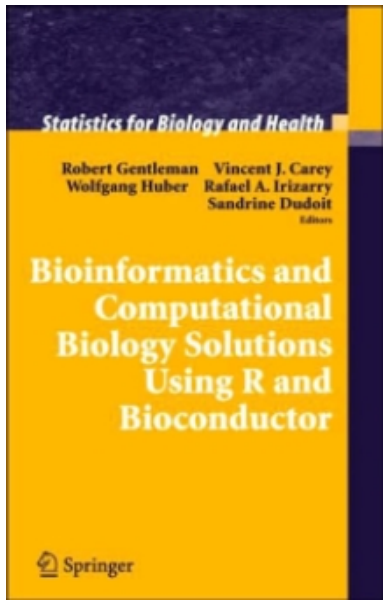


http://www.bioconductor.org/

- Robert Gentleman
- Packages
  - Software
  - Metadata (Annotation, CDF and Probe)
  - Custom CDF
  - Experiment Data
  - Complete Taxonomy







R

- http://cran.r-project.org/
- Binary – source
- Base installation packages
- Package installation

```
> install.packages('vcd')
```

Bioconductor

- Package installation

```
> setRepositories()
> install.packages('BiocManager')
```

- Package groups installation

```
> BiocManager::install('DESeq2')
```

```
>
> 1 + 2
[1] 3
object <- expression
> a <- 1 + 2
> a
[1] 3
> (a <- 1 + 2)
[1] 3
> (a <- 5)
[1] 5
> function.name(arg1,arg2,...)
> length(a)
[1] 1
```

- The functions are stored in libraries

```
> library(DESeq2)
Loading required package: S4Vectors
Loading required package: stats4
Loading required package: BiocGenerics

Attaching package: 'BiocGenerics'

The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

    anyDuplicated, append, as.data.frame, basename, cbind, colnames,
    dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
    grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
    order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
    rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
    union, unique, unsplit, which.max, which.min


Attaching package: 'S4Vectors'

The following objects are masked from 'package:base':

    expand.grid, I, unname

Loading required package: IRanges
Loading required package: GenomicRanges
Loading required package: GenomeInfoDb
Loading required package: SummarizedExperiment
Loading required package: MatrixGenerics
Loading required package: matrixStats
```

```
> help(t.test)
> ?t.test
t.test                  package:stats                  R Documentation

Student's t-Test

Description:
     Performs one and two sample t-tests on vectors of data.

Usage:
     t.test(x, ...)

     ## Default S3 method:
     t.test(x, y = NULL,
            alternative = c("two.sided", "less", "greater"),
            mu = 0, paired = FALSE, var.equal = FALSE,
            conf.level = 0.95, ...)

     ## S3 method for class 'formula':
     t.test(formula, data, subset, na.action, ...)

Arguments:
       x: a (non-empty) numeric vector of data values.

       y: an optional (non-empty) numeric vector of data values.

alternative: a character string specifying the alternative hypothesis,
          must be one of '"two.sided"' (default), '"greater"' or
```

```
> setwd('/home/user/rnaseq')
> getwd()
[1] "/home/user/rnaseq"
```

```
read.table(file, header = FALSE, sep = "", quote = "\"'", dec = ".",
row.names, col.names, as.is = !stringsAsFactors, na.strings = "NA",
colClasses = NA, nrows = -1, skip = 0, check.names = TRUE,
fill = !blank.lines.skip, strip.white = FALSE, blank.lines.skip = TRUE,
comment.char = "#", allowEscapes = FALSE, flush = FALSE,
stringsAsFactors = default.stringsAsFactors(), fileEncoding = "",
encoding = "unknown")
```

function	sep	dec	quote	fill
read.line	""	.	\"'	!blank.lines.skip
read.csv	,	.	\"	TRUE
read.csv2	;	,	\"	TRUE
read.delim	\t	.	\"	TRUE
read.delim2	\t	,	\"	TRUE

```
write()
write.table()

save()
save(list = ls(all=TRUE), file = "all_object.RData")
save.image()

dput()
dget()

dump()
source()

savehistory()
loadhistory()
```

```
> (a <- 1:5)
[1] 1 2 3 4 5
> (a <- c(9,4,6,7,1,2,5))
[1] 9 4 6 7 1 2 5
> a[3]
[1] 6
> (a <- vector(mode = "numeric", length = 5))
> (a <- numeric(length = 5))
[1] 0 0 0 0 0
> (a <- vector(mode = "logical", length = 5))
> (a <- logical(length = 5))
[1] FALSE FALSE FALSE FALSE FALSE
> (a <- vector(mode = "character", length = 5))
> (a <- character(length = 5))
[1] "" "" "" "" ""
```

```
> a <- 1:6
> (m <- matrix(a, nr = 3))
     [,1] [,2]
[1,]    1    4
[2,]    2    5
[3,]    3    6
> (m <- matrix(a, nr = 3, byrow = T))
     [,1] [,2]
[1,]    1    2
[2,]    3    4
[3,]    5    6
> dim(a) <- c(3, 2)
> a
     [,1] [,2]
[1,]    1    4
[2,]    2    5
[3,]    3    6
```

```
> (x <- matrix(1:9, nc = 3))

     [,1] [,2] [,3]
[1,]    1    4    7
[2,]    2    5    8
[3,]    3    6    9

> x[2, 2]

[1] 5

> x[2, ]

[1] 2 5 8

> x[, 2]

[1] 4 5 6
```

```
> x[-1, ]

     [,1] [,2] [,3]
[1,]    2    5    8
[2,]    3    6    9

> x[, -1]

     [,1] [,2]
[1,]    4    7
[2,]    5    8
[3,]    6    9

> x[-1, -1]

     [,1] [,2]
[1,]    5    8
[2,]    6    9

> x[-c(1, 3), ]

[1] 2 5 8
```

```
> x <- 1:4
> n <- 10
> (r <- data.frame(x, n))
  x  n
1 1 10
2 2 10
3 3 10
4 4 10
> (r <- data.frame(column1 = x, column2 = n))
  column1 column2
1       1      10
2       2      10
3       3      10
4       4      10
> r$column1
[1] 1 2 3 4
> r[,'column1']
[1] 1 2 3 4
```

```
> x <- matrix(1:9, nc = 3)
> y <- 1:5
> sub.list <- list(c("a", "b", "c"),
+ c(8, 5, 2, 4, 1, 3))
> my.list <- list(x, y, sub.list)
> names(my.list) <- c("r", "t", "z")
> my.list
$r
     [,1] [,2] [,3]
[1,]    1    4    7
[2,]    2    5    8
[3,]    3    6    9

$t
[1] 1 2 3 4 5

$z
$z[[1]]
[1] "a" "b" "c"

$z[[2]]
[1] 8 5 2 4 1 3
```

```
> my.list[[1]]
     [,1] [,2] [,3]
[1,]    1    4    7
[2,]    2    5    8
[3,]    3    6    9
> my.list
     [,1] [,2] [,3]
[1,]    1    4    7
[2,]    2    5    8
[3,]    3    6    9
```


dplyr
readr
purrr
tidyverse
ggplot2
tidyr
TIBBLE
www.rstudio.com

```
library(org.Mm.eg.db)
library(GO.db)
library(KEGGREST)
library(edgeR)
library(DESeq2)
library(openxlsx)
library(tidyverse)

org.db = org.Mm.eg.db

uniprot.db_sel = read_tsv('MOUSE_10090_idmapping_selected.tab',
col_names=c('UniProtKB_AC', 'UniProtKB_ID', 'GeneID_EntrezGene', 'RefSeq', 'GI',
'PDB', 'GO', 'UniRef100', 'UniRef90', 'UniRef50', 'UniParc', 'PIR',
'NCBI_taxon', 'MIM', 'UniGene', 'PubMed', 'EMBL', 'EMBL_CDS', 'Ensembl',
'Ensembl_TRS', 'Ensembl_PRO', 'Additional_PubMed'))

PTHR = read_tsv(
    'PTHR15.0_mouse',
    col_names=c('GeneIdentifier', 'ProteinID', 'SFID', 'FamilyName',
'SubfamilyName', 'MolecularFunction', 'BiologicalProcess', 'CellularComponents',
'ProteinClass', 'Pathway'),
    quote='') %>%
  separate(GeneIdentifier, c('GeneIdentifier', 'UniProt'), 'UniProtKB=')

ens2uniprot = read_tsv('Mus_musculus.GRCm38.100.uniprot.tsv', col_names=T) %>%
  rename(UniProt=xref, ens=gene_stable_id) %>%
  select(ens, UniProt) %>%
  unique()

go = read_tsv('mgi.gaf', skip=43, col_names=F,
  col_types = cols(.default = "c")) %>%
  select(2,5,7,9,10,11) %>%
  mutate(X9=case_when(X9=='C' ~ 'CC', X9=='P' ~ 'BP', X9=='F' ~ 'MF')) %>%
  rename(UniProt=1, GOid=2, evidence=3, ontology=4, GeneName=5, symbol=6)
```

```
read.counts = read_delim('featureCounts_GRCm38_100_0.txt', delim='\t',
col_names=T, skip=1) %>%
  rename_at(vars(contains('GRCm38_100_')), list( ~ gsub('GRCm38_100_', '', .)))
%>%
  rename_at(vars(contains('_trimmedAligned.sortedByCoord.out.bam')), list( ~
gsub('_trimmedAligned.sortedByCoord.out.bam', '', .))) %>%
  rename_at(vars(contains('-')), list( ~ paste0('s', gsub('-', '_', .))))

readcounts = read.counts %>%
  select(grep('arc', tolower(colnames(.)))) %>%
  as.data.frame()
rownames(readcounts) = read.counts$Geneid

sample_info = data.frame(smpl=colnames(readcounts))
rownames(sample_info) = sample_info$smpl
sample_info$grp = 'E2'
sample_info$grp[grep('OIL', sample_info$smpl)] = 'OIL'
sample_info$grp = factor(sample_info$grp)

dds = DESeqDataSetFromMatrix(
    countData = readcounts,
    colData = sample_info,
    design = ~ grp
)

dds = estimateSizeFactors(dds)

counts_raw = readcounts
colnames(counts_raw) = paste0('raw_', colnames(counts_raw))
counts_raw$ens = rownames(counts_raw)
counts_raw = as_tibble(counts_raw)

counts_normalized = as.data.frame(counts(dds, normalized=T))
colnames(counts_normalized) = paste0('norm_', colnames(counts_normalized))
counts_normalized$ens = rownames(counts_normalized)
```

```
m = as.matrix(readcounts)
counts_cpm = as.data.frame(cpm(m))
colnames(counts_cpm) = paste0('cpm_', colnames(counts_cpm))
counts_cpm$ens = rownames(counts_cpm)
counts_cpm = as_tibble(counts_cpm)

ids = rownames(readcounts)
n = 1
tmp = tibble(.rows=0, ens='', UniProt='')
for(ens in ids){
  tmp = rbind(tmp,
    tibble(ens, UniProt= uniprot.db_sel %>%
    filter(str_detect(Ensembl, ens)) %>%
    pull(UniProtKB_AC)
    )
  )
  n=n+1
  print(n)
}

ens2uniprot = rbind(ens2uniprot, tmp) %>%
  unique()

ens_uniprot_pthr = inner_join(ens2uniprot, PTHR)

tib = left_join(tibble(ens=rownames(readcounts)), ens_uniprot_pthr) %>%
  select(ens, UniProt)

tib = inner_join(tib, counts_raw)
tib = inner_join(tib, counts_normalized)
tib = inner_join(tib, counts_cpm)
```

```
dds.dif = DESeq(dds)

res = results(dds.dif, contrast=c('grp', 'E2', 'OIL'))
fix = res[tib$ens,]
tib$log2FC = fix$log2FoldChange
tib$pvalue = fix$pvalue
tib$padj = fix$padj

annot = left_join(tib, PTHR %>%
    select(UniProt, FamilyName, SubfamilyName, ProteinClass, BiologicalProcess,
CellularComponents, MolecularFunction)
)

evs = sort(unique(go$evidence))
onts = sort(unique(go$ontology))

for(ont in onts){
  for(ev in evs){
    tmp = go %>%
      filter(ontology==ont, evidence==ev) %>%
      select(UniProt, GeneName)
    if(dim(tmp)[1]>0){
      lst = split(tmp$GeneName, tmp$UniProt) %>%
        lapply(unique) %>%
        lapply(sort) %>%
        lapply(paste, collapse='\n')
      annot = left_join(
        annot,
        tibble(UniProt=names(lst), tmp=as.character(lst)) %>%
          rename_at(vars(tmp), ~ paste(ont, ev, sep='_'))
        )
    }
  }
}
```

```
lst = keggList('pathway', 'mmu')
paths = tibble(
  PATH=gsub('path:mmu', '', names(lst)),
  pathway=gsub(' - Mus musculus \\(mouse\\)', '', as.character(lst))
)

i = 1
query = keggGet(paste0('mmu', paths$PATH[i]))
kegg = as_tibble(matrix(query[[1]]$GENE, nc=2, byrow=T)) %>%
  rename(GeneID=1, descr=2) %>%
  mutate(PATH=paths$PATH[i])

for(i in 2:dim(paths)[1]){
  query = keggGet(paste0('mmu', paths$PATH[i]))
  if(!is.null(query[[1]]$GENE)){
    kegg = rbind(kegg,
      as_tibble(matrix(query[[1]]$GENE, nc=2, byrow=T)) %>%
      rename(GeneID=1, descr=2) %>%
      mutate(PATH=paths$PATH[i])
    )
  }
}

ens2entrez = read_tsv('Mus_musculus.GRCm38.100.entrez.tsv',
  col_types = cols(.default = "c"))
```

```
tmp = inner_join(
  inner_join(kegg,
    inner_join(
      tibble(gene_stable_id=rownames(readcounts)),
      ens2entrez
    ) %>%
    select(gene_stable_id, xref) %>%
    unique() %>%
    rename(ens=1, GeneID=2)
  ) %>%
  select(ens, PATH),
  paths
)

lst = split(tmp$pathway, tmp$ens) %>%
  lapply(unique) %>%
  lapply(sort) %>%
  lapply(paste, collapse='\n')
kegg_res = tibble(ens=names(lst), KEGG=as.character(lst))

annot = left_join(annot, kegg_res) %>%
  rename(Ensembl=1)

cs = createStyle(wrapText=T)
wb = createWorkbook()
addWorksheet(wb, 'ARC_with_annotation')
writeData(wb, 1, annot)
addStyle(wb, 1, style=cs, rows=-1, cols=-1)
saveWorkbook(wb, 'ARC_with_annotation_GRCm38_100.xlsx', overwrite = TRUE)
```

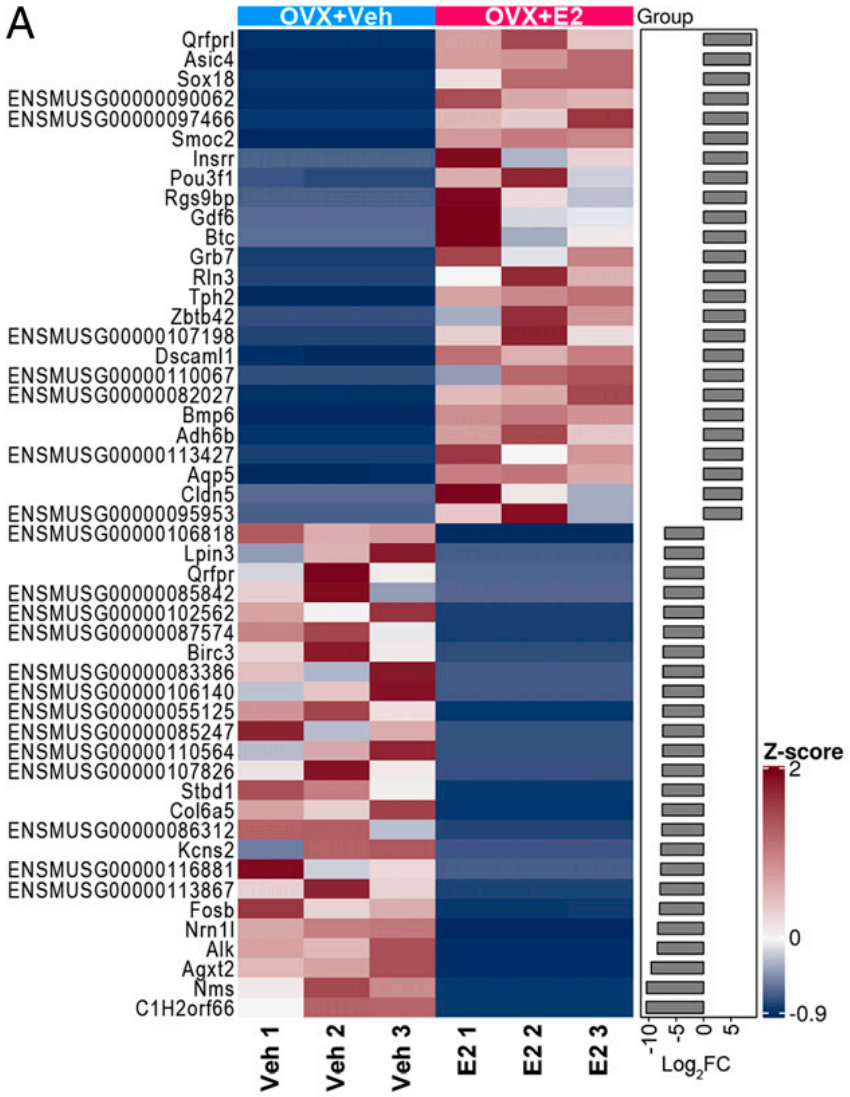
```
library(org.Mm.eg.db)
library(GO.db)
library(KEGGREST)
library(edgeR)
library(DESeq2)
library(openxlsx)
library(tidyverse)
library(pheatmap)
library(RColorBrewer)

gene_mean = counts_cpm %>% select(-7) %>% rowMeans()
sel_ens = counts_cpm$ens[which(gene_mean>10)]
sel_dds = dds[sel_ens]
sel_dds.dif = DESeq(sel_dds)

matcol = rev(colorRampPalette(brewer.pal(11, 'RdBu'))(100))

base = inner_join(
counts_normalized,
res %>% as_tibble() %>% mutate(ens=rownames(res))
)

wd = base %>%
filter(padj<=0.05) %>%
arrange(desc(log2FoldChange)) %>%
column_to_rownames('ens') %>%
select(1:6)

colnames(wd) = gsub('norm_', '', colnames(wd))
```

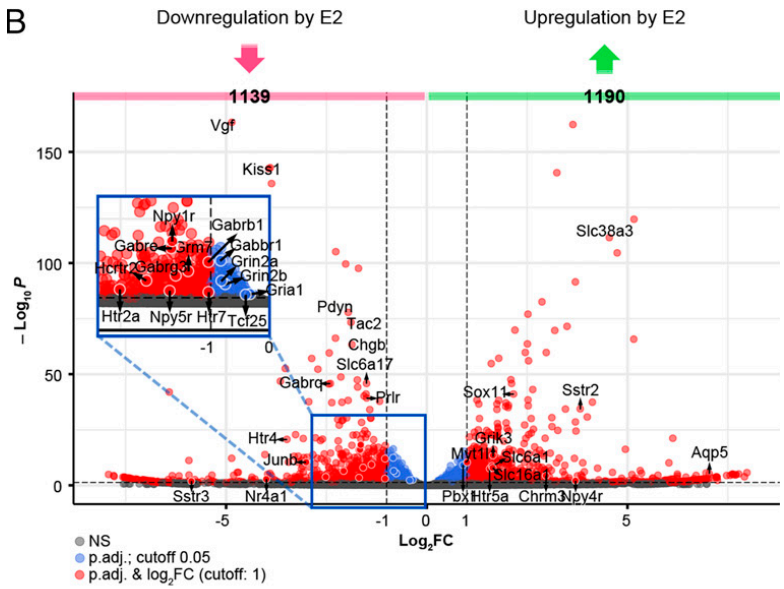
```
phann = sample_info %>%
  tibble() %>%
  rename(Group=2) %>%
  mutate(smpl=gsub('_ARC', '', smpl)) %>%
  mutate(smpl=substr(smpl,1,2)) %>%
  mutate(Group=relevel(factor(Group), 'OIL')) %>%
  column_to_rownames('smpl')

cord = rownames(phann)[c(which(phann$Group=='OIL'), which(phann$Group!='OIL'))]

ann_colors = list(Group=c(OIL="yellow",E2="firebrick"))

wd = wd[,cord]

matcol=colorRampPalette(brewer.pal(11, 'RdBu'))(100)

wd = inner_join(
counts_normalized,
res %>% as_tibble() %>% mutate(ens=rownames(res))
) %>%
filter(padj<=0.05) %>%
column_to_rownames('ens') %>%
select(1:6)

colnames(wd) = gsub('norm_', '', colnames(wd))
colnames(wd) = substr(colnames(wd),1,2)

phann = sample_info %>%
  tibble() %>%
  rename(Group=2) %>%
  mutate(smpl=gsub('norm_', '', smpl)) %>%
  mutate(smpl=substr(smpl,1,2)) %>%
  mutate(Group=relevel(factor(Group), 'OIL')) %>%
  column_to_rownames('smpl')
```

```
cord = rownames(phann)[c(which(phann$Group=='OIL'), which(phann$Group!='OIL'))]

ann_colors = list(Group=c(OIL="yellow",E2="firebrick"))

wd = wd[,cord]

fontsize = 10

wd %>%
  pheatmap(
  legend=T,
  annotation_col=phann, annotation_colors=ann_colors,
  scale='row', border_color=NA, color=matcol, width=7, height=10,
  show_rownames=F, fontfamily='sans', fontsize=fontsize*1.2, cluster_rows=F,
  cluster_cols=F, filename='figs/Fig1d.pdf'
)

library(ComplexHeatmap)
library(Cairo)

lst = mapIds(org.Mm.eg.db, ens2entrez %>% pull(xref), 'SYMBOL', 'ENTREZID')
entrez_tab = tibble(xref=names(unlist(lst)), symbol=as.character(unlist(lst)))
%>% unique()

wd = base %>% filter(padj<=0.05)

tmp = left_join(left_join(wd, ens2entrez %>% select(gene_stable_id, xref) %>%
unique() %>% rename(ens=1)),entrez_tab
) %>%
mutate(symbol=case_when(is.na(symbol) ~ ens, TRUE~symbol)) %>%
mutate(symbol=make.unique(symbol)) %>%
filter(str_detect(symbol, '\\.', negate=T))
```

```
top_up = tmp %>% filter(log2FoldChange>0) %>% arrange(desc(log2FoldChange)) %>%
slice_head(n=25)
top_down = tmp %>% filter(log2FoldChange<0) %>% arrange(log2FoldChange) %>%
slice_head(n=25)
pm = rbind(top_up, top_down) %>% arrange(desc(log2FoldChange))
pd = pm %>% column_to_rownames('symbol') %>% select(1:6)
colnames(pd) = gsub('norm_', '', colnames(pd))
colnames(pd) = substr(colnames(pd),1,2)

mat_scaled = t(scale(t(pd)))

ha = HeatmapAnnotation(df=phann,
 col=list(Group=c(OIL='yellow', E2='firebrick')),
 annotation_name_gp = gpar(fontsize = 12*1.2),
 annotation_legend_param = list(
   title_gp=gpar(fontsize = 10*1.2, fontface="bold"),
    labels_gp = gpar(fontsize = 10*1.2)
 )
)

ats = sort(c(0,round(range(mat_scaled),1)))

library(circlize)

cols = circlize::colorRamp2(ats, rev(brewer.pal(11, 'RdBu')[c(1,6,11)]))

ht1 = Heatmap(mat_scaled, col=cols, top_annotation=ha, row_names_side='left',
  name='Z-score', column_order=order(phann$Group), cluster_rows=F,
  row_names_gp = gpar(fontsize = 12*1.2),
  column_names_gp = gpar(fontsize = 12*1.2),
  heatmap_legend_param=list(at=ats, legend_height=unit(5, 'cm'),
    title_gp=gpar(fontsize = 10*1.2, fontface="bold"),
    labels_gp = gpar(fontsize = 10*1.2)
  )
)
```

```
ht_list = ht1 +
rowAnnotation(log2FC=anno_barplot(pm$log2FoldChange, width=unit(3, "cm"),
   axis_param = list(gp=gpar(fontsize=8*1.2))),
annotation_name_gp=gpar(fontsize=12*1.2))

CairoPDF('figs/Fig2b.pdf', height=11, width=9)
draw(ht_list)
graphics.off()

library(EnhancedVolcano)

ppd = left_join(left_join(res %>% as_tibble() %>% mutate(ens=rownames(res)),
  ens2entrez %>% select(gene_stable_id, xref) %>% rename(ens=1) %>% unique()),
entrez_tab) %>% mutate(symbol=case_when(is.na(symbol) ~ ens, TRUE~symbol)) %>%
mutate(symbol=make.unique(symbol)) %>% mutate(semmi='')

CairoPDF('figs/Fig2d_connected.pdf', height=10, width=13)
EnhancedVolcano(ppd, title='', subtitle='', xlim = c(-8, 8),
    lab = ppd$symbol, selectLab=labs,
    x = 'log2FoldChange',
    y = 'padj',
    pCutoff = 0.05,
    FCcutoff = 1, col = c('grey30', 'grey30', 'royalblue', 'red2'),
    pointSize = 3.0,
    legendLabels = c('NS', expression(Log[2]~FC), 'p-value',
expression(p-value~and~log[2]~FC)),
    .legend = c('NS','Log2 FC','P','P & Log2 FC'),
    caption = bquote(~Log[2]~"fold change cutoff: 1; adjusted p-value cutoff:
0.05"),
    drawConnectors=T
)
graphics.off()
```


A
OVX+Veh
OVX+E2
Group
Qrfprl
Asic4
Sox18
ENSMUSG00000090062
ENSMUSG00000097466
Smoc2
Insrr
Pou3f1
Rgs9bp
Gdf6
Btc
Grb7
Rln3
Tph2
Zbtb42
ENSMUSG00000107198
Dscaml1
ENSMUSG00000110067
ENSMUSG00000082027
Bmp6
Adh6b
ENSMUSG00000113427
Aqp5
Cldn5
ENSMUSG00000095953
ENSMUSG00000106818
Lpin3
Qrfpr
ENSMUSG00000085842
ENSMUSG00000102562
ENSMUSG00000087574
Birc3
ENSMUSG00000083386
ENSMUSG00000106140
ENSMUSG00000055125
ENSMUSG00000085247
ENSMUSG00000110564
ENSMUSG00000107826
Stbd1
Col6a5
ENSMUSG00000086312
Kcns2
ENSMUSG00000116881
ENSMUSG00000113867
Fosb
Nrn1l
Alk
Agxt2
Nms
C1H2orf66
Z-score
2
0
-0.9
Veh 1
Veh 2
Veh 3
E2 1
E2 2
E2 3
-10
-5
0
5
Log2FC


B
Downregulation by E2
Upregulation by E2
1139
1190
Vgf
Kiss1
Npy1r
Gabrb1
Gabre
Srm
Gabbr1
Grin2a
Hcrtr2
Gabrg3
Grin2b
Gria1
Htr2a
Npy5r
Htr7
Tcf25
Pdyn
Tac2
Chgb
Slc6a17
Gabrq
Prlr
Slc38a3
Sox11
Sstr2
Htr4
Junb
Grik3
Myt1l
Slc6a1
Slc16a1
Aqp5
Sstr3
Nr4a1
Pbx1
Htr5a
Chrm3
Npy4r
−Log10P
Log2FC
NS
p.adj.; cutoff 0.05
p.adj. & log2FC (cutoff: 1)

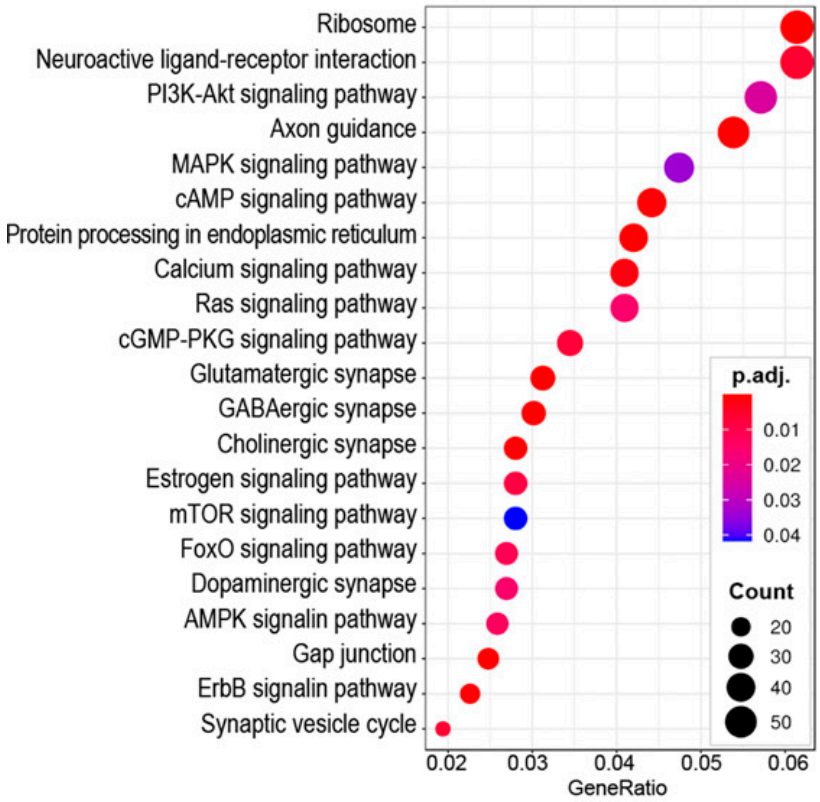
```
library(DOSE)
library(magrittr)
library(clusterProfiler)
library(org.Mm.eg.db)

tres = inner_join(res %>%
  as_tibble() %>%
  mutate(ens=rownames(res)),
  ens2entrez %>% select(gene_stable_id, xref) %>% rename(ens=1, entrezid=2) %>%
unique()
) %>%
filter(!is.na(log2FoldChange)) %>%
arrange(desc(log2FoldChange))


prb = tres %>% group_by(entrezid) %>% summarize(fc=max(log2FoldChange)) %>%
arrange(desc(fc))
ged = prb %>% pull(fc)
names(ged) = prb %>% pull(entrezid)
enrich = gseKEGG(geneList=ged, organism='mmu', minGSSize=1, maxGSSize=5000,
pvalueCutoff=1, eps=1e-20, verbose=F)
renrich = setReadable(enrich, 'org.Mm.eg.db', 'ENTREZID')

WriteXLS(as.data.frame(renrich), ExcelFileName='figs/ARC_GSEA_KEGG.xls',
SheetNames='GSEA')
```

```
geneList = tres %>% pull(log2FoldChange)
names(geneList) = tres %>% pull(entrezid)

de = tres %>% filter(padj<0.05) %>% pull(entrezid)

kk = enrichKEGG(gene=de, organism='mmu', pvalueCutoff=0.05)

pkk =
kk[
kk@result$Description=='Ribosome' |
kk@result$Description=='Protein processing in endoplasmic reticulum' |
kk@result$Description=='Axon guidance' |
kk@result$Description=='Neuroactive ligand-receptor interaction' |
kk@result$Description=='GABAergic synapse' |
kk@result$Description=='Glutamatergic synapse' |
kk@result$Description=='Cholinergic synapse' |
kk@result$Description=='Dopaminergic synapse' |
kk@result$Description=='Estrogen signaling pathway' |
kk@result$Description=='Synaptic vesicle cycle' |
kk@result$Description=='Gap junction' |
kk@result$Description=='cAMP signaling pathway' |
kk@result$Description=='Calcium signaling pathway' |
kk@result$Description=='ErbB signaling pathway' |
kk@result$Description=='cGMP-PKG signaling pathway' |
kk@result$Description=='FoxO signaling pathway' |
kk@result$Description=='PI3K-Akt signaling pathway' |
kk@result$Description=='MAPK signaling pathway' |
kk@result$Description=='Ras signaling pathway' |
kk@result$Description=='AMPK signaling pathway' |
kk@result$Description=='mTOR signaling pathway' |
kk@result$Description=='Rap1 signaling pathway',
asis=T
]
```

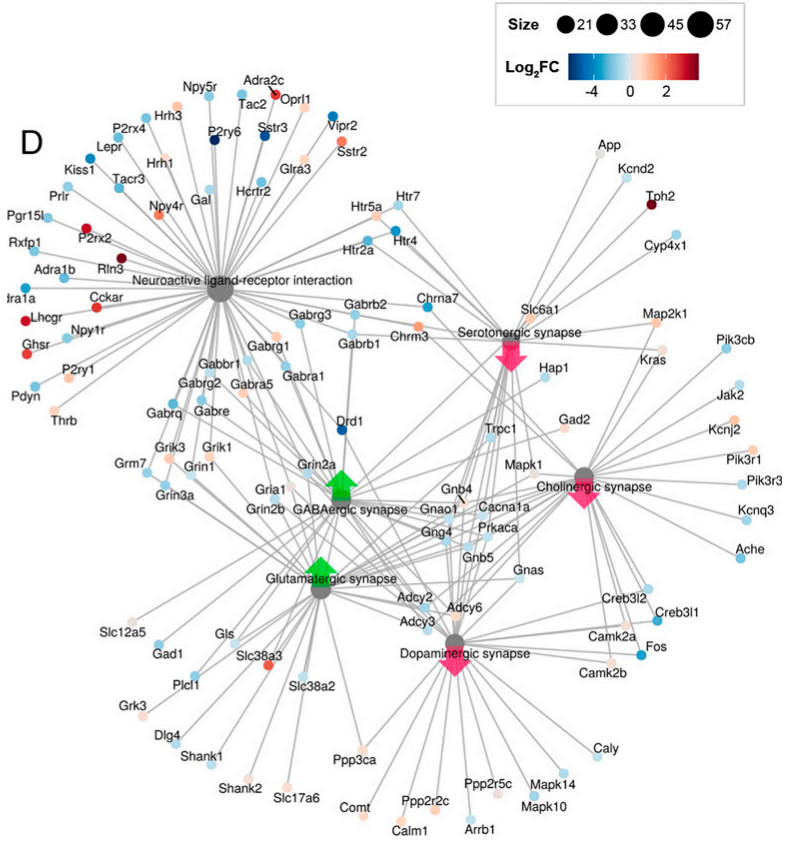
```
mpl = 1.2
CairoPDF('figs/ARC_dotplot.pdf', height=10, width=10)
dotplot(pkk, showCategory=dim(pkk)[1]) +
theme(
  axis.text.x=element_text(size=12*mpl),
  axis.text.y=element_text(size=12*mpl),
  legend.title=element_text(size=11*mpl),
  axis.title=element_text(size=12*mpl),
  legend.text=element_text(size=9*mpl)
)
graphics.off()


edox = setReadable(kk, 'org.Mm.eg.db', 'ENTREZID')

library(WriteXLS)

WriteXLS(as.data.frame(edox), ExcelFileName='figs/ARC_ORA_KEGG.xls',
SheetNames='ORA')

kka = enrichKEGG(gene=de, organism='mmu', pvalueCutoff=0.99)
edoxa = setReadable(kka, 'org.Mm.eg.db', 'ENTREZID')
```

```
CairoPDF('figs/ARC_net_7x7.pdf', height=7, width=7)
cnetplot(
edoxa[
edoxa@result$Description=='Neuroactive ligand-receptor interaction' |
edoxa@result$Description=='GABAergic synapse' |
edoxa@result$Description=='Glutamatergic synapse' |
edoxa@result$Description=='Cholinergic synapse' |
edoxa@result$Description=='Dopaminergic synapse' |
edoxa@result$Description=='Serotonergic synapse',
asis=T
],
foldChange=geneList, showCategory=6
) + theme(legend.position = c(.05, .3), legend.direction = "vertical",
legend.box = "vertical") + scale_colour_gradientn(colours = rev(brewer.pal(11,
'RdBu')[-c(6)]), name ="log2FC") +
guides(fill=guide_legend(order=0), size=guide_legend(order=1))
graphics.off()

library(ToPASeq)
library(graphite)
library(WriteXLS)

cmat = readcounts[rowSums(readcounts)>0,]
group = ifelse(as.character(sample_info$grp)=='E2', 1,0)

pwys = pathways(species="mmusculus", database="kegg")
pwys = graphite::convertIdentifiers(pwys, "ENSEMBL")

spi = SPIA(cmat, group, pwys, type="RNASeq", logFC.th=-1, test.method="DESeq2")

WriteXLS(res(spi)$results, row.names=T, ExcelFileName='figs/ARC_SPIA_KEGG.xls',
SheetNames='SPIA')
```

C


Ribosome
Neuroactive ligand-receptor interaction
PI3K-Akt signaling pathway
Axon guidance
MAPK signaling pathway
cAMP signaling pathway
Protein processing in endoplasmic reticulum
Calcium signaling pathway
Ras signaling pathway
cGMP-PKG signaling pathway
Glutamatergic synapse
GABAergic synapse
Cholinergic synapse
Estrogen signaling pathway
mTOR signaling pathway
FoxO signaling pathway
Dopaminergic synapse
AMPK signalin pathway
Gap junction
ErbB signalin pathway
Synaptic vesicle cycle
0.02
0.03
0.04
0.05
0.06
GeneRatio
p.adj.
0.01
0.02
0.03
0.04
Count
20
30
40
50

D


Size
21
33
45
57
Log2FC
-4
0
2
Neuroactive ligand-receptor interaction
Serotonergic synapse
Cholinergic synapse
GABAergic synapse
Glutamatergic synapse
Dopaminergic synapse

- PhD course, spring semester, 30 hours
- NGS:
  - metagenomics
  - de novo assembly
  - variant calling
  - RNA-seq
- problem oriented, based on the interest of students
- University of Veterinary Medicine Budapest