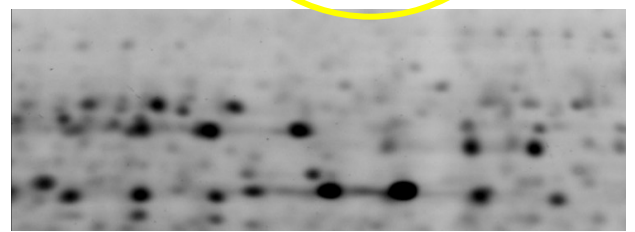
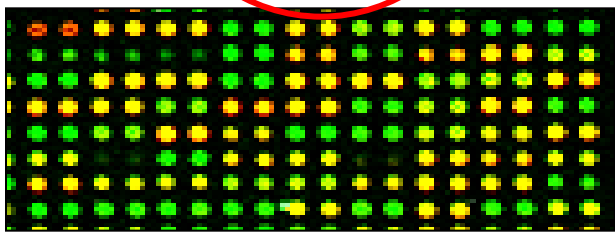
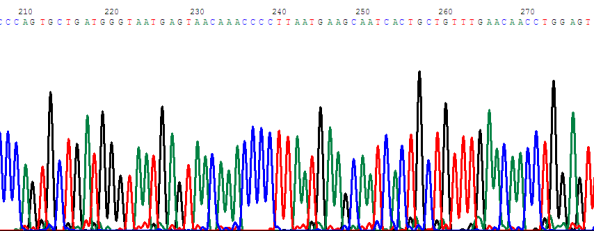
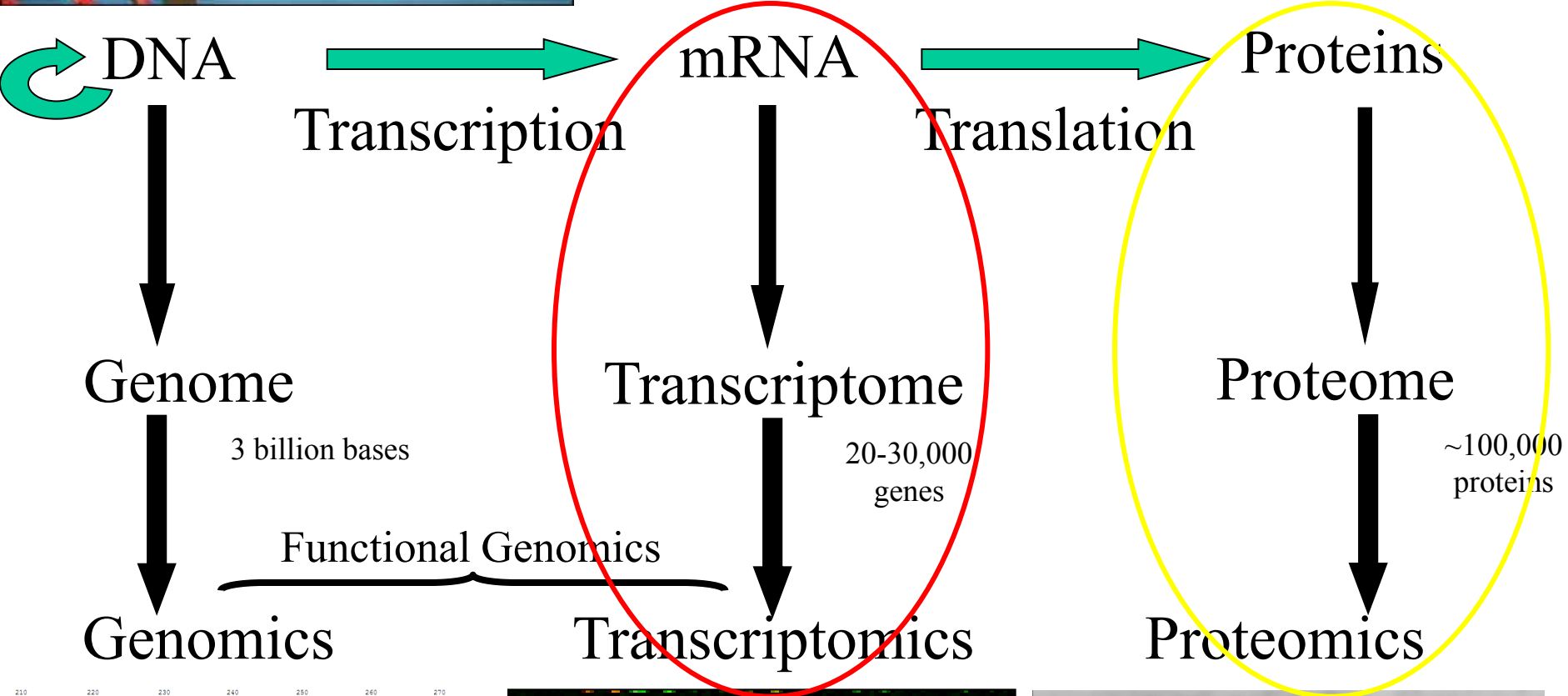
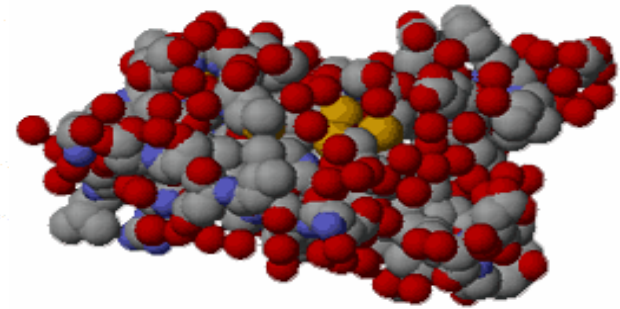
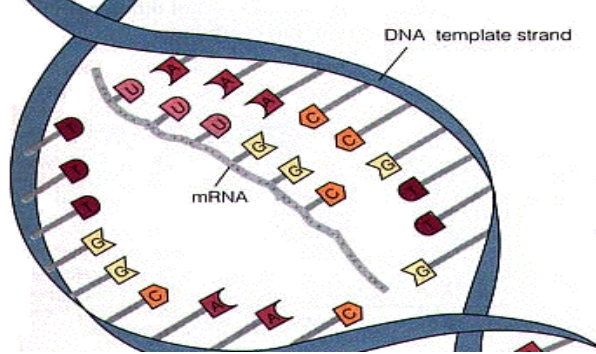
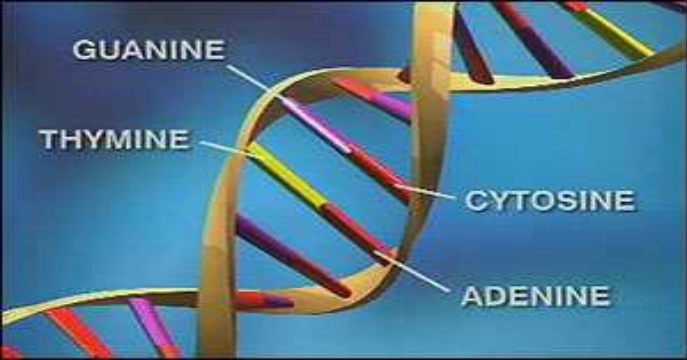


Analýza genové exprese



Rozdíly v expresi genů mohou mít velký význam

- humans and chimpanzees are 98.7% identical in their genome sequences, but large tissue-specific differences exist in expression – particularly in the brain (Enard et al. 2002)



Analýza genové exprese

(1) Kvantifikace kandidátních transkriptů

(2) Microarrays

(3) RNAseq (transkriptom)

Kandidátní geny - relativní kvantifikace pomocí standardů

- **Měření úrovně exprese** (např. v různých typech tkání nebo treatment vs. non-treatment atd.
- **mRNA → reverzní transkripce → cDNA → PCR**
- **housekeeping geny** – slouží jako standard pro měření
- stejný počet kopií ve všech buňkách
- exprimované ve všech buňkách, nezávislé na experimentu

Srovnání množství PCR produktu na elektroforéze

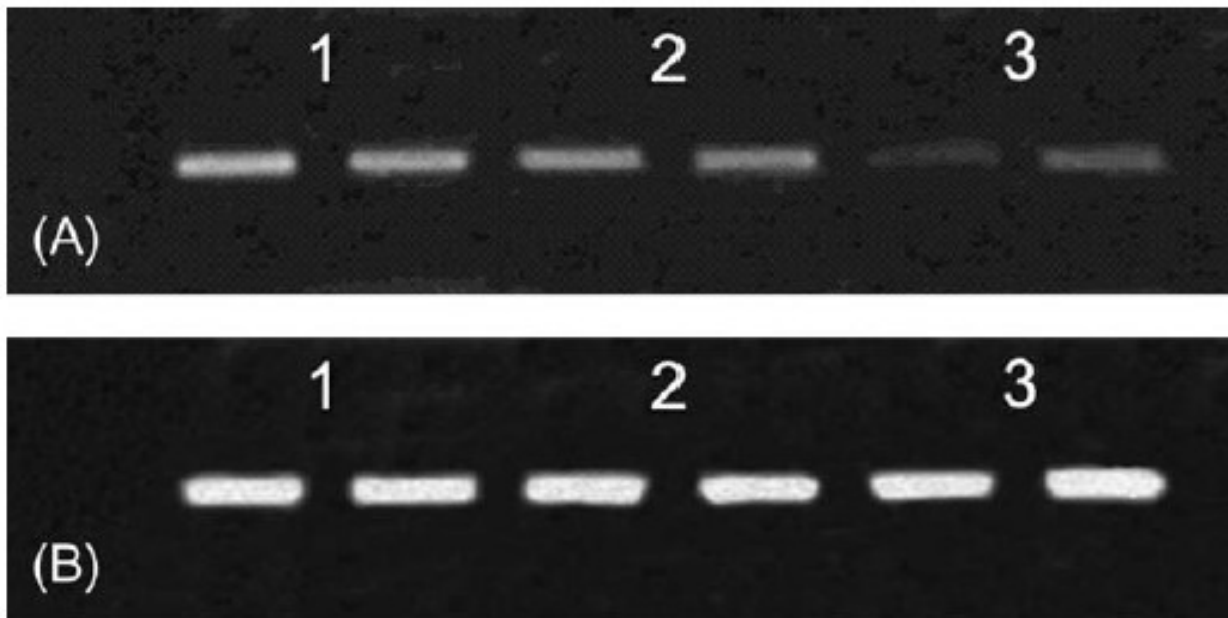
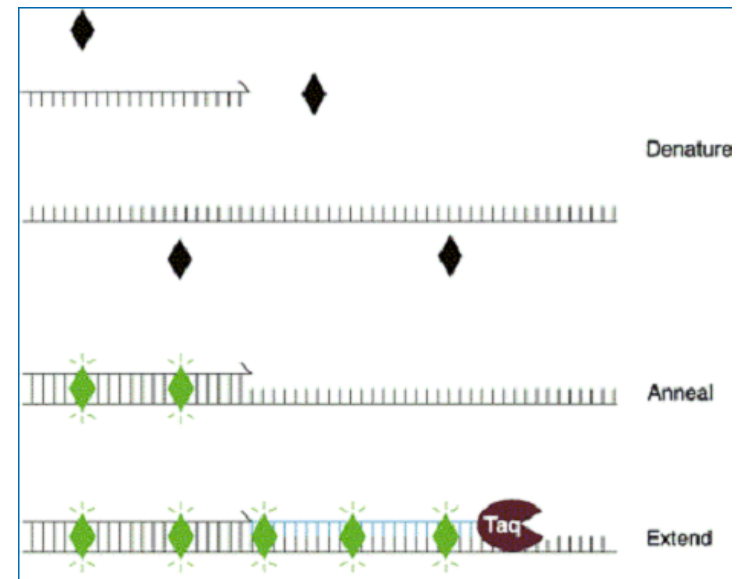
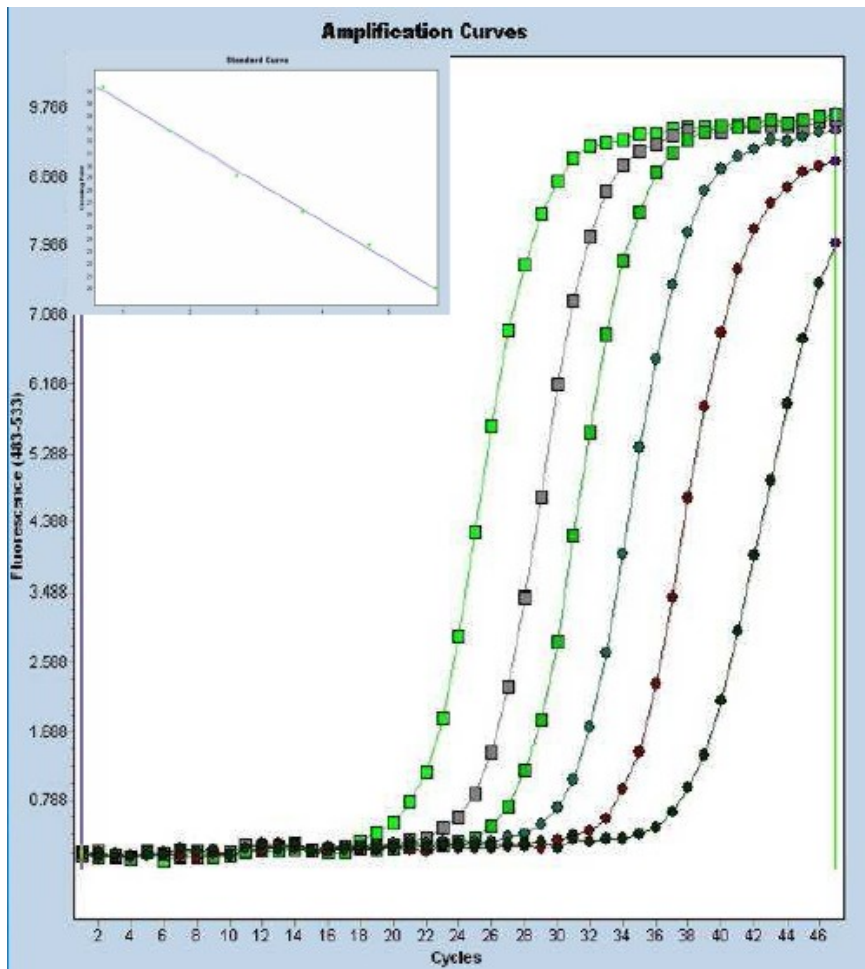


Figure 6 Lipopolysaccharide-induced expression of tgTLR4 in peritoneal macrophages (A) in comparison with 18S RNA expression (B): 1, stimulated both *in vivo* and *in vitro*; 2, stimulated only *in vitro* and 3, unstimulated.

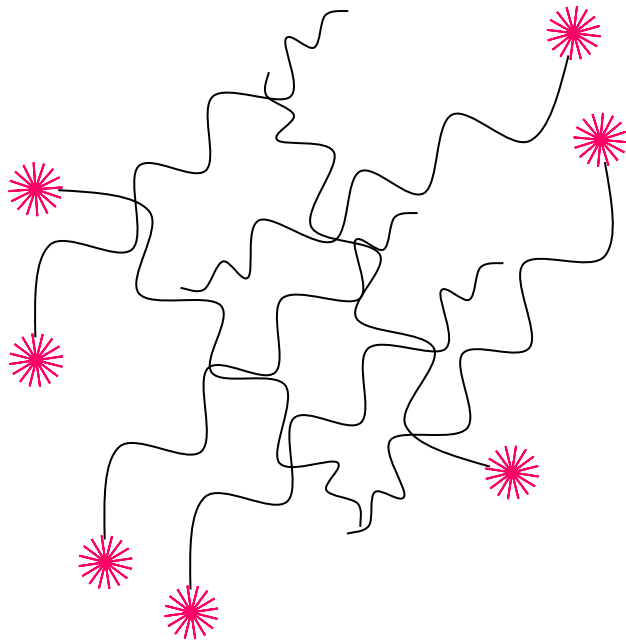
Studium kandidátních genů - qPCR



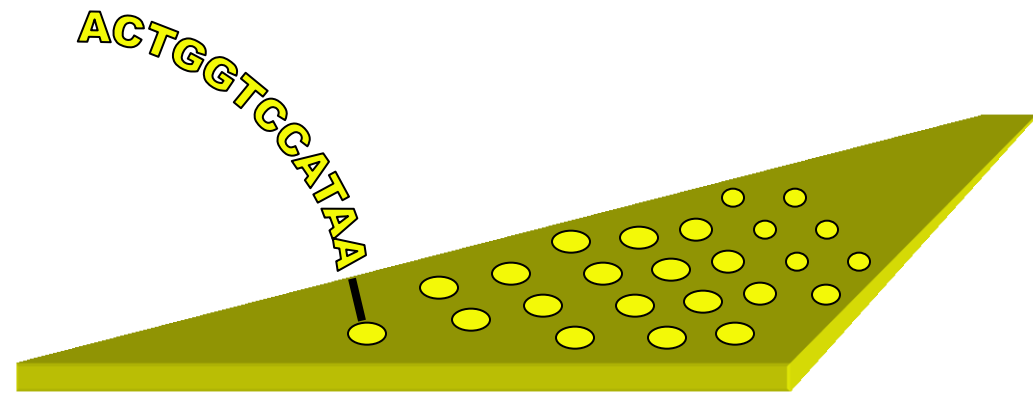
- většinou nespecifická detekce (SYBR Green, aj.)
- nutné srovnání s house-keeping geny

Microarray transcriptomics

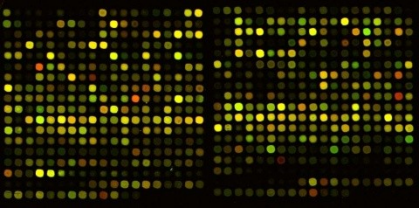
Microarray analysis of transcriptome (~ specific DNA hybridization)



Target (i.e. mix of transcripts in a form of cDNA = mRNA přepsaná do DNA reverzní transkriptázou, tj. neobsahuje introny)

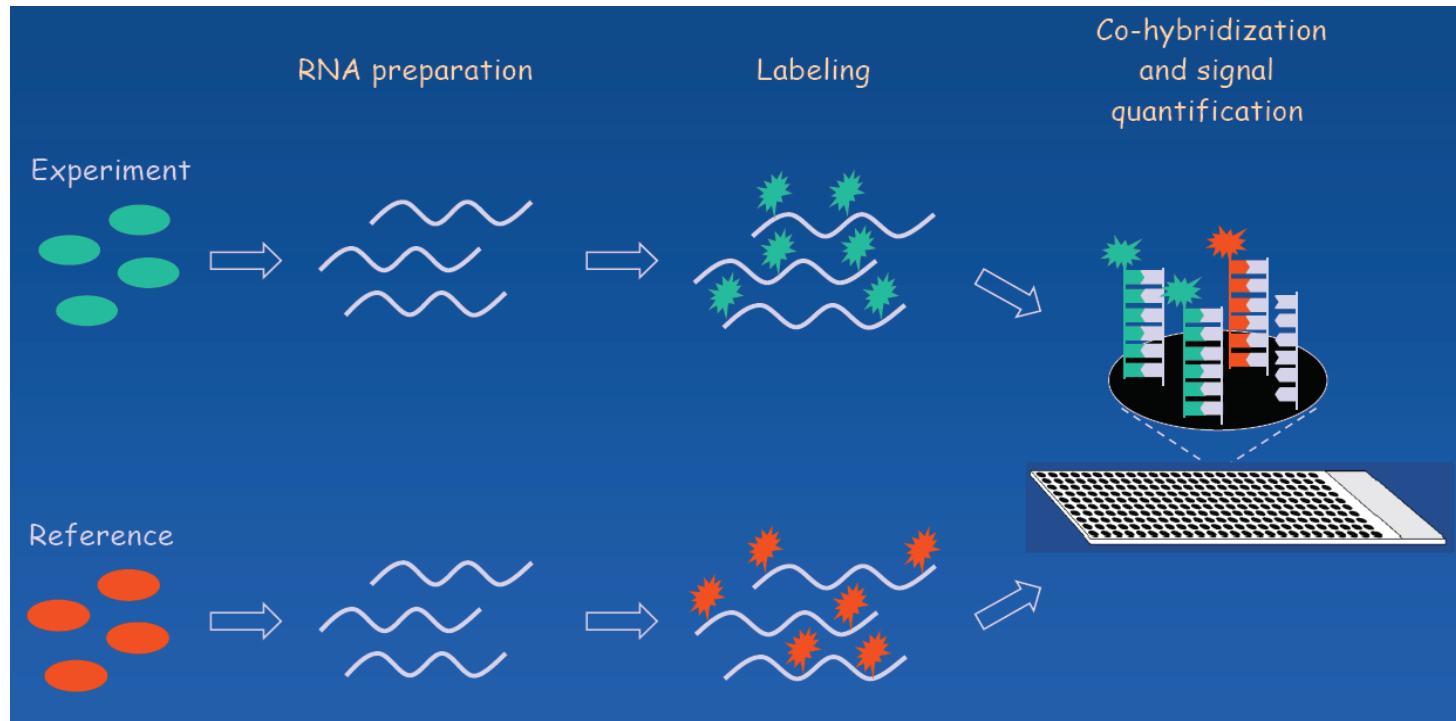


Probe
(i.e. synthesized oligonucleotides complementary to particular genes)



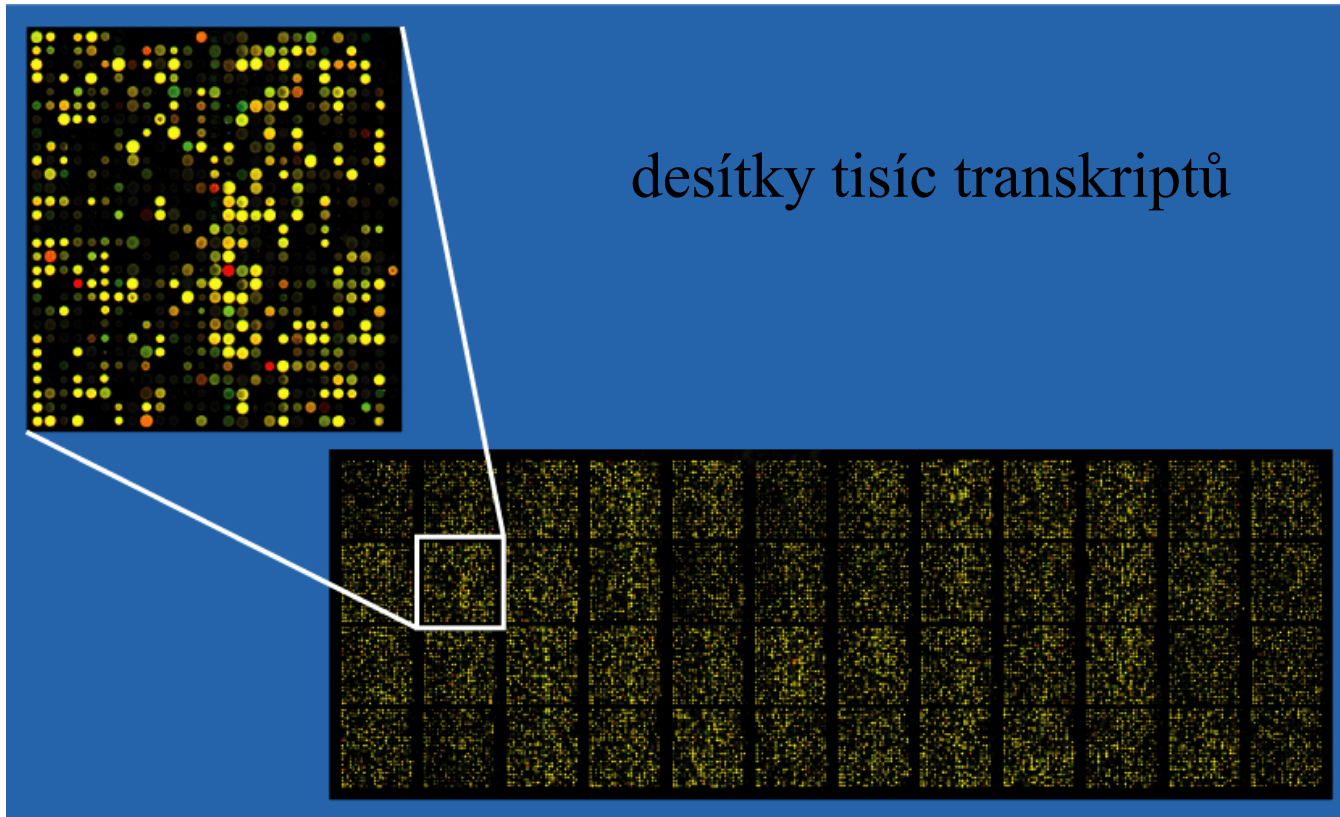
Sledování exprese genů (microarrays)

- Sledování exprese mnoha (tisíce) genů najednou
- Založeno na hybridizaci
- Sleduje se rozdíl vůči kontrole ("heterologous hybridization") = dvoukanálový experiment



Vyhodnocení chipu - analýza obrazu

(srovnání úrovně exprese mezi kontrolou a experimentem)



Komerčně dostupné pro kompletní transkriptom cca 25 druhů
(další jsou rychle vyvíjeny, i na zakázku)

Agilent	Affymetrix	Custom	
<i>Arabidopsis thaliana</i>	<i>A. thaliana</i>	<i>Acropora millepora</i>	<i>Locusta migratoria</i>
<i>Bovinae</i> spp. (bovine)	<i>Bovinae</i> spp. (bovine)	<i>Acropora palmata</i>	<i>Loxodonta africana</i>
<i>Brassica</i> spp.	<i>Caenorhabditis elegans</i>	<i>Ambrosia artemisiifolia</i>	<i>Megachile rotundata</i>
<i>C. elegans</i>	<i>Callitrichidae</i> spp. (Marmoset)	<i>Amphihura filiformis</i>	<i>Melitta cinxia</i>
<i>Canis</i> spp. (canine)	<i>Canis</i> spp. (canine)	<i>Anemania viridis</i>	<i>Melitteaea cinxia</i>
<i>Culicidae</i> spp. (mosquito)	<i>Danio rerio</i> (Zebrafish)	<i>Anopheles gambiae</i>	<i>Metarhizium robertsii</i>
<i>D. rerio</i> (Zebrafish)	<i>Drosophila melanogaster</i>	<i>Apis mellifera carnica</i>	<i>Montastraea faveolata</i>
<i>D. melanogaster</i>	<i>Equus ferus</i> (horse)	<i>Azospirillum brasilense</i>	<i>Mytilus californianus</i>
<i>E. ferus</i> (horse)	<i>Felidae</i> spp. (feline)	<i>Bruglia malayi</i>	<i>Neotoma lepida</i>
<i>Gallus gallus</i> (chicken)	<i>G. gallus</i> (chicken)	<i>Calanus finmarchicus</i>	<i>Neurospora crassa</i>
<i>Gossypium</i> spp. (cotton)	<i>Glycine max</i> (soybean)	<i>Carpodacus mexicanus</i>	<i>Neurospora tetrasperma</i>
<i>Homo sapiens</i>	<i>H. sapiens</i>	<i>Coregonus cupleaformis</i>	<i>Onthophagus taurus</i>
<i>Hordeum vulgare</i> (barley)	<i>Macaca mulatta</i> (rhesus)	<i>Crassostrea gigas</i>	<i>Ostreococcus tauri</i>
<i>Leporidae</i> spp. (rabbit)	<i>Medicago</i> spp.	<i>Crassostrea virginica</i>	<i>Petalisthes cinclipes</i>
<i>M. mulatta</i> (rhesus)	<i>Mus musculus</i>	<i>Daphnia magna</i>	<i>Picea glauca</i>
<i>Magnaporthe</i> spp.	<i>Oryza sativa</i> (rice)	<i>Daphnia pulex</i>	<i>Pimephales promelas</i>
<i>Medicago</i> spp.	<i>Ovis aries</i> (sheep)	<i>Dreissena polymorpha</i>	<i>Pinus pinaster</i>
<i>M. musculus</i> (mouse)	<i>Saccharomyces cerevisiae</i>	<i>Drosophila melanogaster</i>	<i>Pinus taeda</i>
<i>Nicotiana tabacum</i> (tobacco)	<i>Schizosaccharomyces pombe</i>	<i>Eucalyptus grandis</i>	<i>Platichthys flesus</i>
<i>O. sativa</i> (rice)	<i>Suidae</i> spp. (porcine)	<i>Falsamia candida</i>	<i>Ruditapes philippinarum</i>
<i>O. aries</i> (sheep)	<i>Taeniopygia guttata</i> (Zebra Finch)	<i>Fundulus heteroclitus</i>	<i>Ruditapes philippinarum</i>
<i>Rattus norvegicus</i> (rat)		<i>Gadus morhua</i>	<i>Salmo salar</i>
<i>S. cerevisiae</i>		<i>Gasterosteus aculeatus</i>	<i>Salvelinus fontinalis</i>
<i>Salmonidae</i> spp. (salmon)		<i>Helianthus annuus</i>	<i>Schistocerca gregaria</i>
<i>Solanum lycopersicum</i> (tomato)		<i>Karenia brevis</i>	<i>S. lycopersicum</i>
<i>Suidae</i> spp. (porcine)		<i>Lagopus lagopus</i>	<i>Solenopsis invicta</i>
<i>Triticum</i> spp. (wheat)		<i>Lagopus lagopus scoticus</i>	<i>Tribolium castaneum</i>
<i>Xenopus</i> spp.		<i>Laternula elliptica</i>	<i>Tursiops truncatus</i>
		<i>Lepeophtheirus salmonis</i>	

Commercial or custom microarrays



Agilent Technologies



RNA-seq

Differential gene expression (DE) studies in non-model species without the need for prior genomic resources

Typical RNA-seq workflow

Experimental design

Careful experimental design that considers desired power in the context of research aims, budget and biology is critical to ensure the validity of biological conclusions and the reproducibility of results.

Sample acquisition and RNA isolation

Following sample acquisition, RNA is purified from preserved samples using one of many available protocols.

Library preparation (~\$300 USD/library)

Purified RNA undergoes size and/or type selection (e.g. ribosomal RNA depletion or poly A selection) and is converted to cDNA. cDNA is fragmented and sequencing adapters are incorporated. Unique barcodes may be added to individual libraries for multiplexed sequencing of several libraries per 'lane'.

High-throughput sequencing (~\$2500 USD/lane)

Libraries are sequenced by high-throughput sequencing technology (typically Illumina), producing millions of short 'read' sequences from one (single-end) or both (paired-end) ends of the cDNA fragments.

Bioinformatics processing and analysis

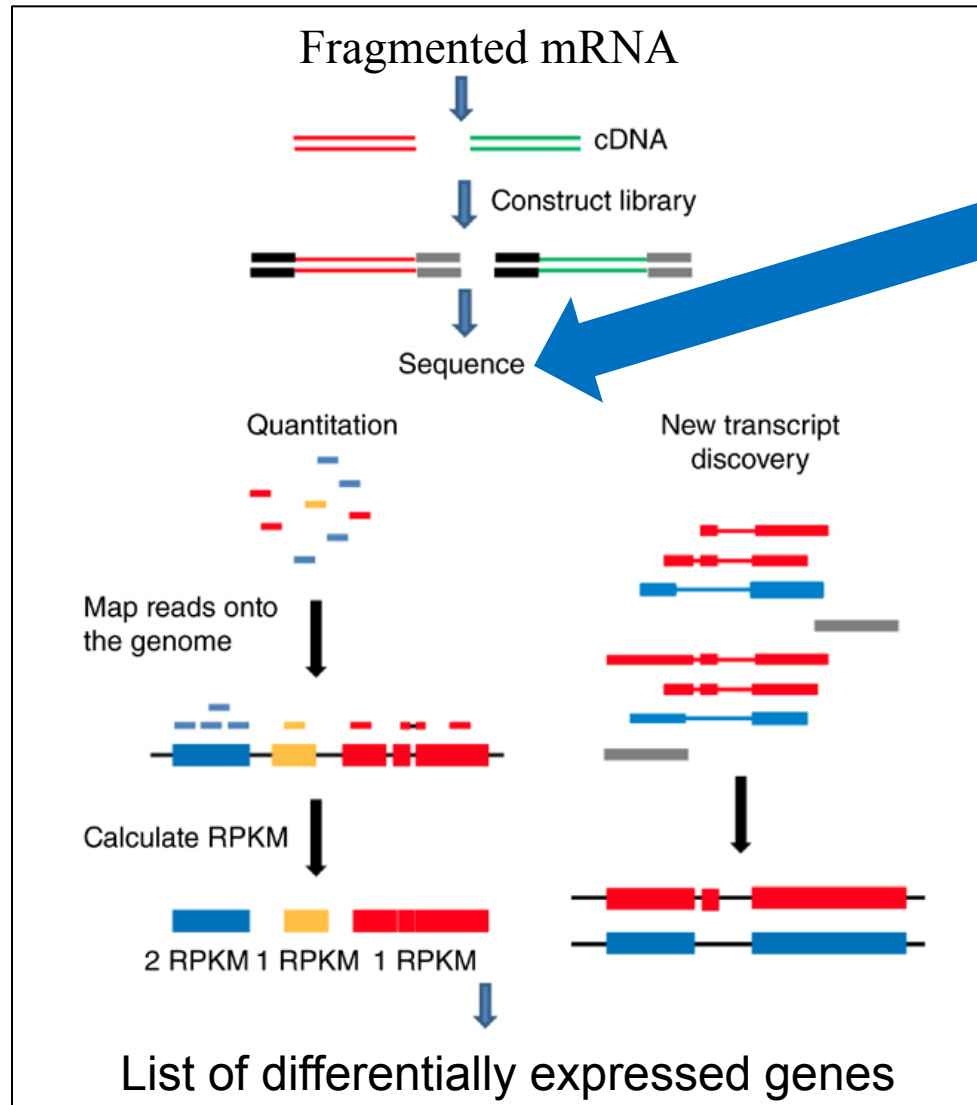
Data QC and read filtering: Raw reads are trimmed of sequencing adapters. Low-quality base trimming and filtering of short and low-quality reads may also be performed.

Read mapping: Reads are aligned to a reference genome or transcriptome for each sample separately, resulting in a matrix of counts (as expression information) for all genes/transcripts across all samples. When a suitable reference is unavailable, reads are often combined for *de novo* transcriptome assembly.

Differential expression: Tests for significant differential expression among samples representing alternative treatment conditions results in a list of candidate genes for further study.

RNA-Seq = sekvenování transkriptomu

RNA-Seq
workflow for
gene
expression
analysis



Využití NGS

RNA-Seq quantification

(RPKM = reads per kilobase per million reads)

Gene 1



Gene 2



Actual Read Alignment to Alternative Splice Variants

Transcript 1



Transcript 2



Transcript 3



Transcript 4



Musí být znám referenční transkriptom

RNA-seq commercially available from 245 USD/sample



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RNA sequencing

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New Zealand

Russian Federation

United States

RNA sequencing

We found 59 labs offering this service with prices from \$240.00 to \$900.00 per sample.

99% positive ratings across 77 reviews.



Technology Center for Genomics & Bioinformatics

University of California, Los Angeles
Los Angeles California

✓ 90 Orders Completed
★ 100% Positive

3 Endorsements

Starting at \$295.00 USD per sample

1X50 sequencing with HiSeq3000
\$295/sample pricing requires a minimum of 24 samples

\$450 for 1X75 sequencing with NextSeq500 (need at least 20 samples for this price)

STOREFRONT

REQUEST QUOTE



Quick Biology

Pasadena CA

✓ 66 Orders Completed
★ 100% Positive
3 Endorsements

Starting at \$280.00 USD per sample

RNA seq promotion (ends on Aug 30, 2016)
1, RNA seq for gene expression \$280/sample (was \$350/sample)
---10M reads, 50bp SE or 75bp SE sequencing with HiSeq4000 or NextSeq 500
---\$280/sample pricing requires a minimum of 12 samples for this price
---Including library preparation/QC

2, RNA splicing/RNA editing/Fusion... [Show more »](#)

STOREFRONT

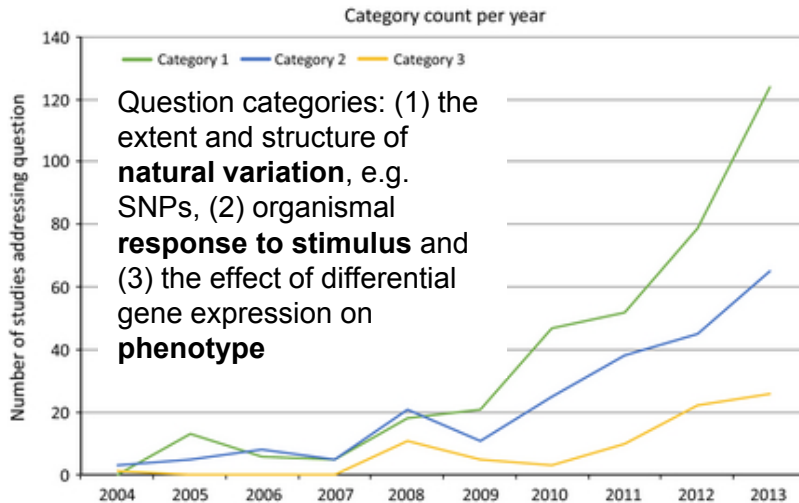
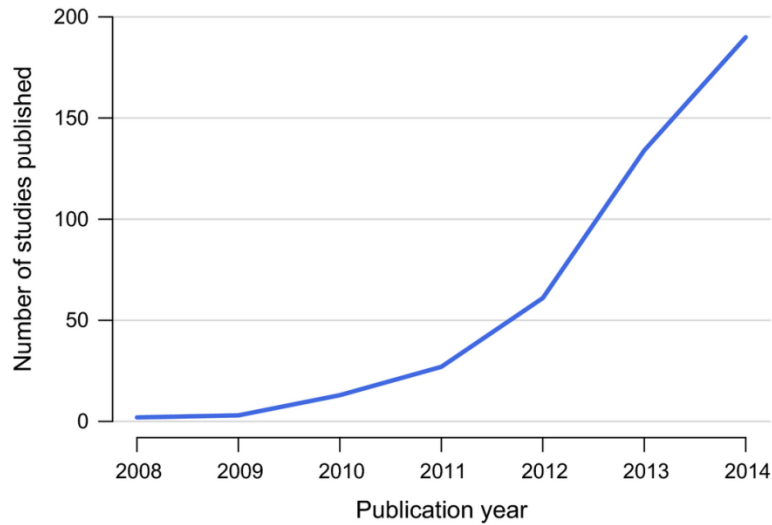
REQUEST QUOTE

Microbiome Core Facility

Starting at \$823.00 USD per sample

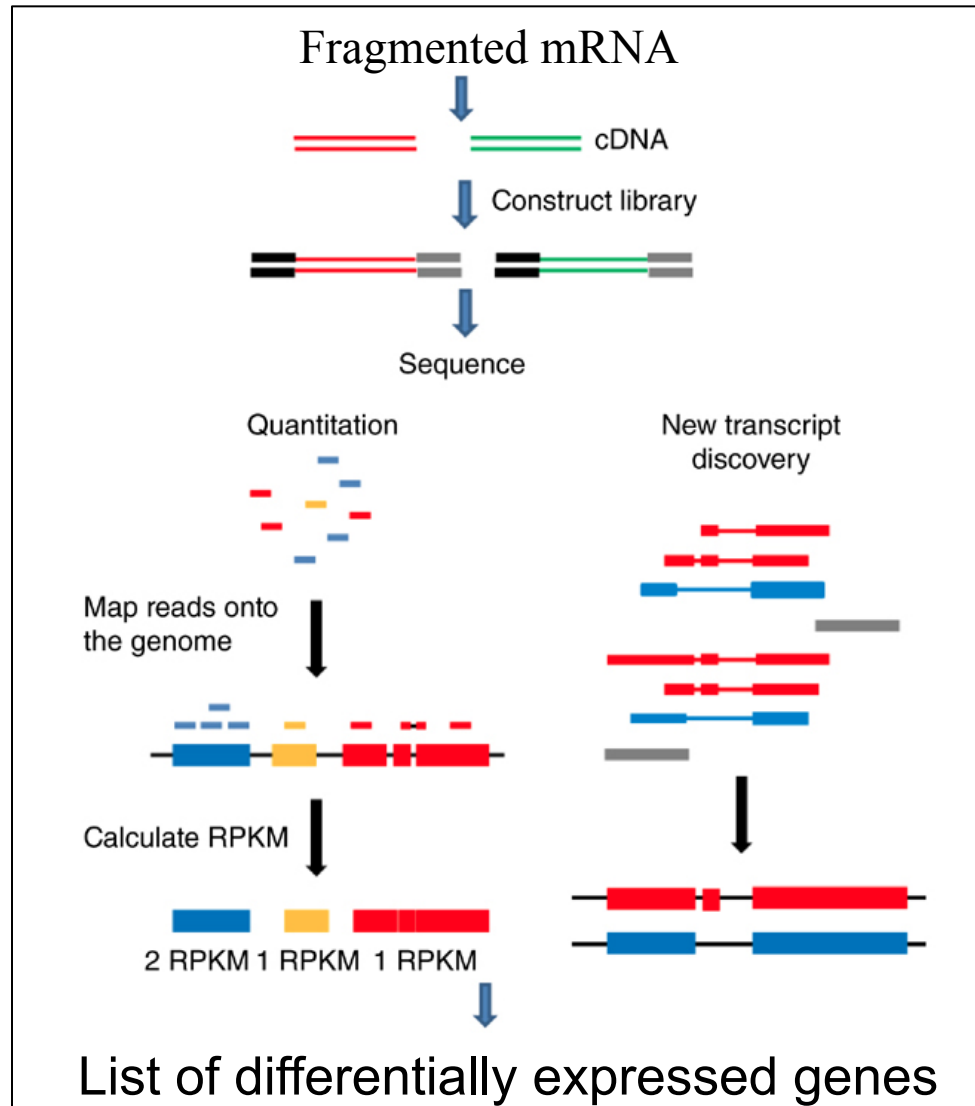
RNAseq - review

(A) RNA-seq publications by year in ecology and evolution



RNA-Seq = sekvenování transkriptomu

RNA-Seq workflow for gene expression analysis



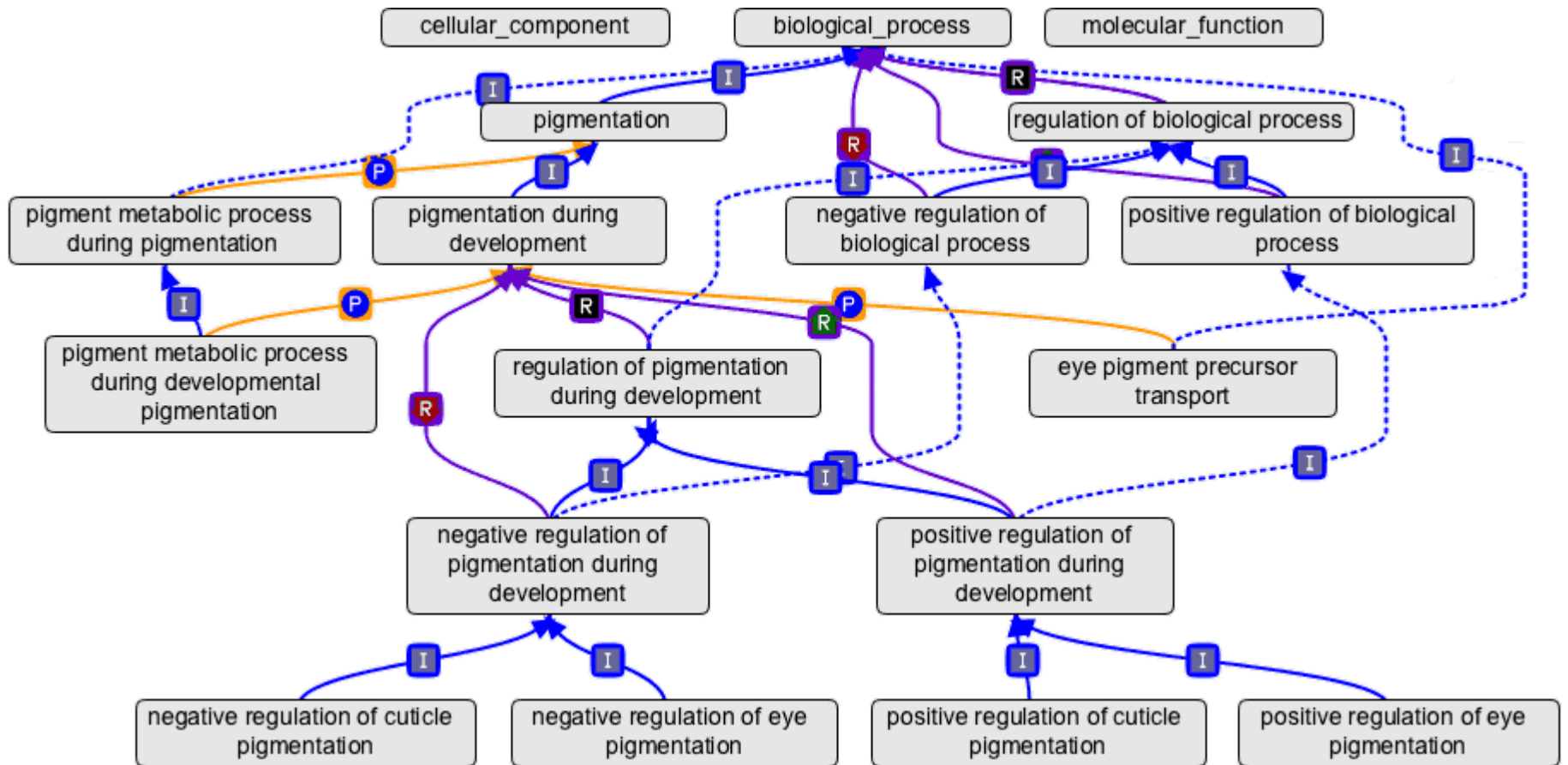
gene ontology
identifikace
funkce daného
genu

Gene ontology (<http://geneontology.org/>)

= functional annotation analysis

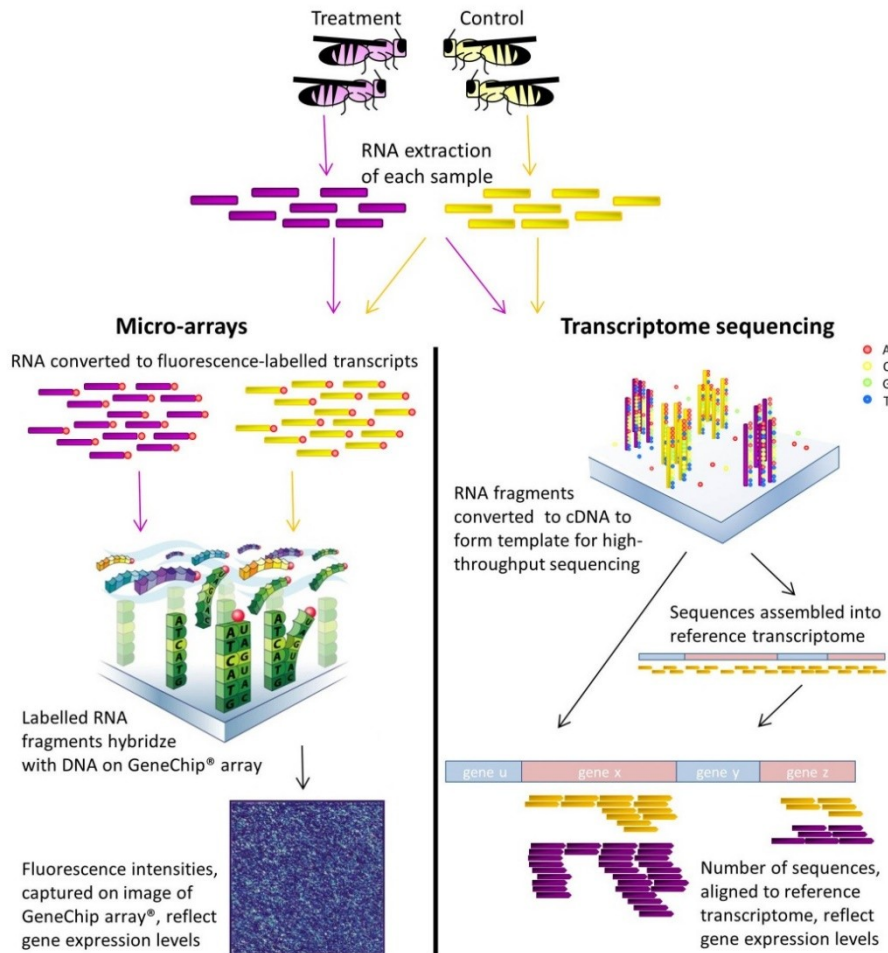
- založena na databázích dostupných anotovaných genů u modelových organismů
- **Cellular Component** - the parts of a cell or its extracellular environment
- **Molecular Function** - the elemental activities of a gene product at the molecular level, such as binding or catalysis
- **Biological Process** - operations or sets of molecular events with a defined beginning and end, pertinent to the functioning of integrated living units: cells, tissues, organs, and organisms.

Gene ontology as a graph



Example: A set of terms under the biological process node pigmentation.

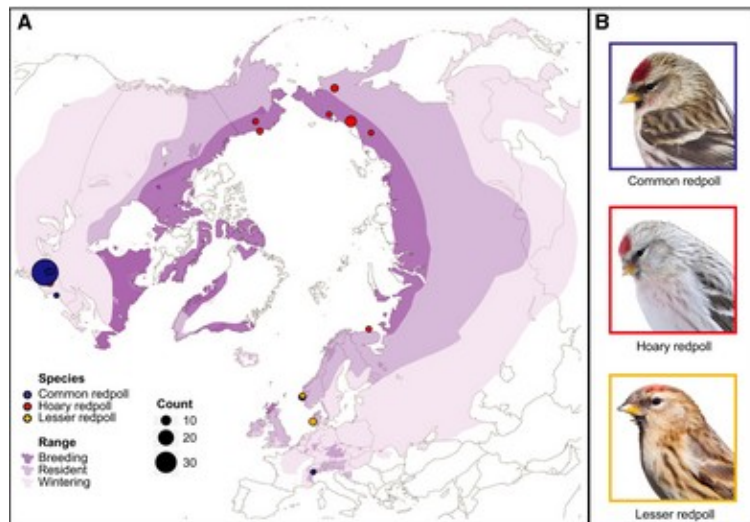
Microarrays vs. RNA-seq



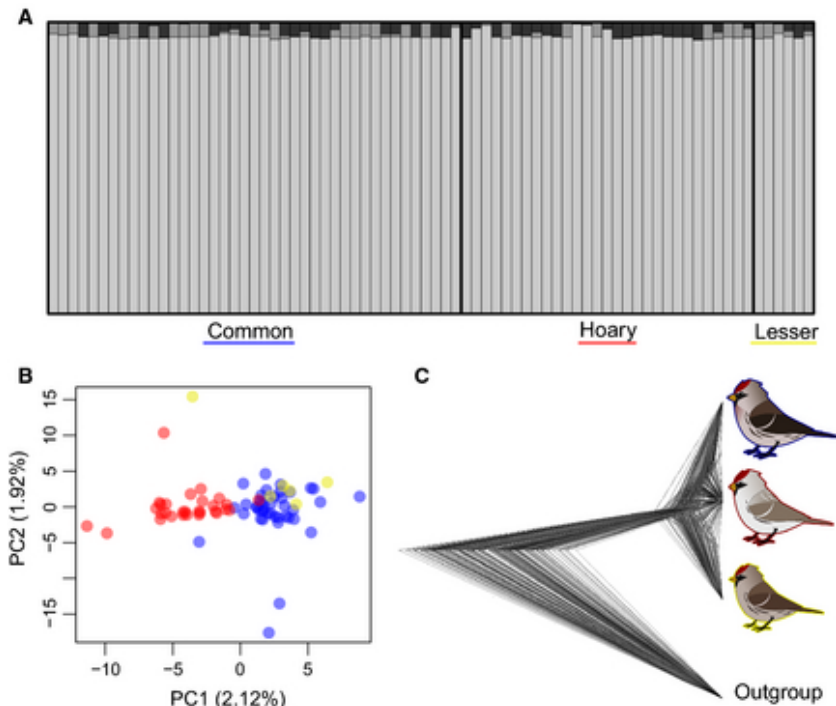
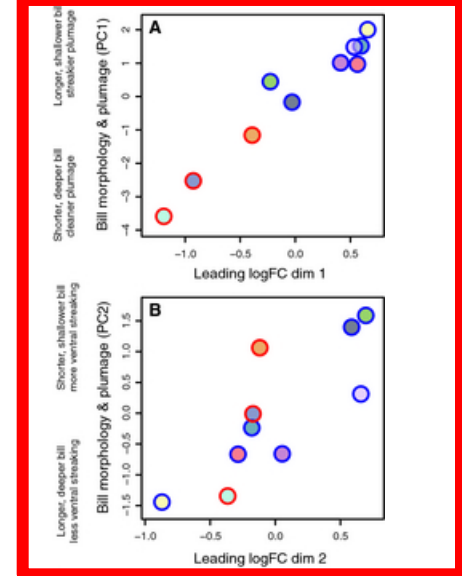
Měly by dávat stejné výsledky, ale srovnávacích studií je velmi málo

Gene ID	Transcript abundance in Treatment sample	Transcript abundance in Control sample
ABc00001	3778	3894
ABc00002	768	189
ABc00003	1087	1476

Příklady



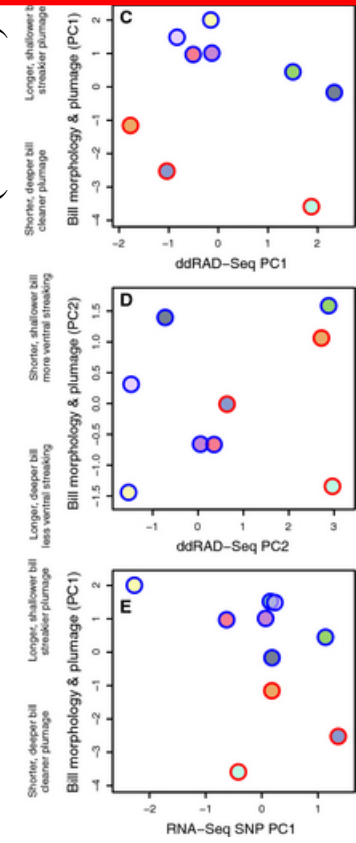
Only differentially expressed genes are responsible for morphological changes (zobák, zbarvení)



20721 SNPs (ddRAD) – no genetic difference at neutral loci

20721 SNPs (ddRAD)

215825 SNPs (RNAseq)



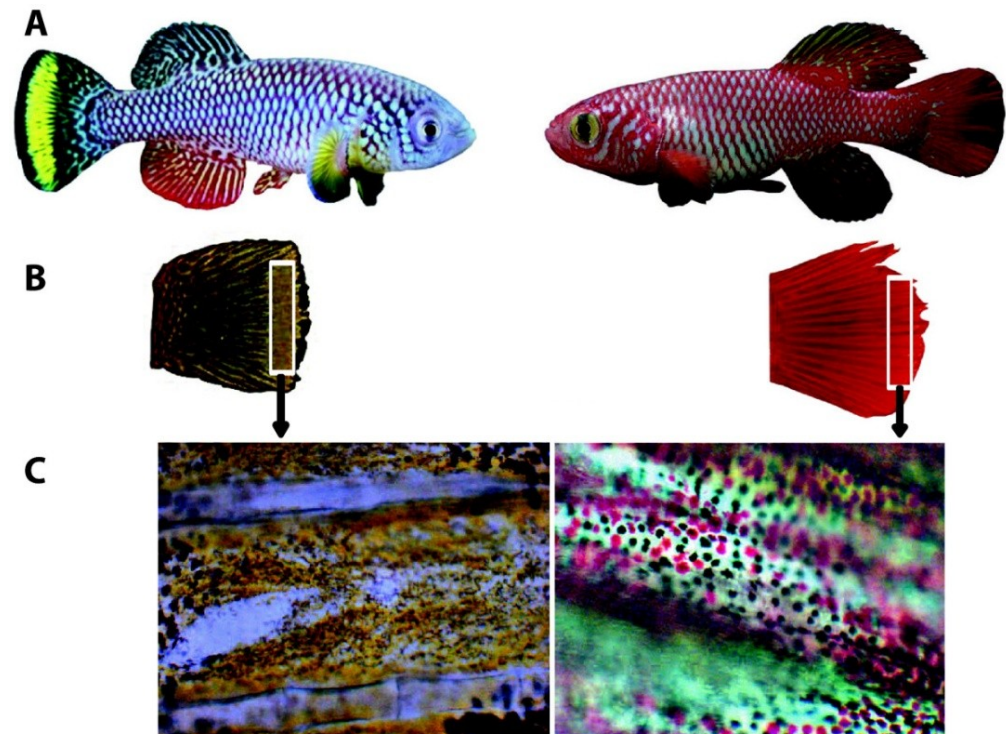
RESEARCH ARTICLE

Open Access

Transcriptome profiling of natural dichromatism
in the annual fishes *Nothobranchius furzeri* and
Nothobranchius kadleci

Enoch Ng'oma¹, Marco Groth¹, Roberto Ripa², Matthias Platzer¹ and Alessandro Cellerino^{1,2*}

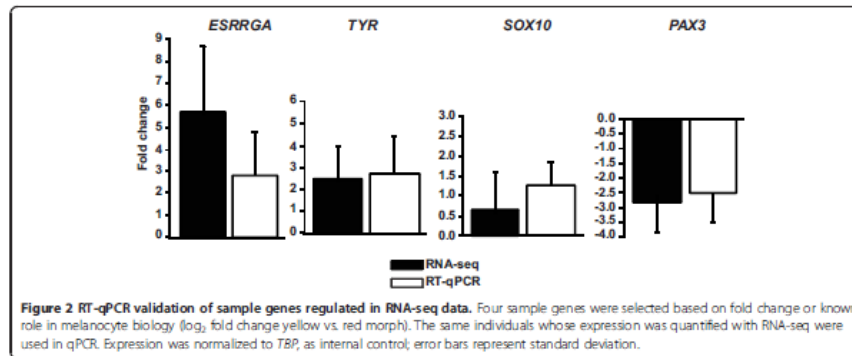
- cca 334 miliónů sekvencí („reads“); 42 mil./sample
- 210 DEGs („differentially expressed genes“) – 119 up-regulated, 91 down-regulated u žlutých jedinců



xanthophory

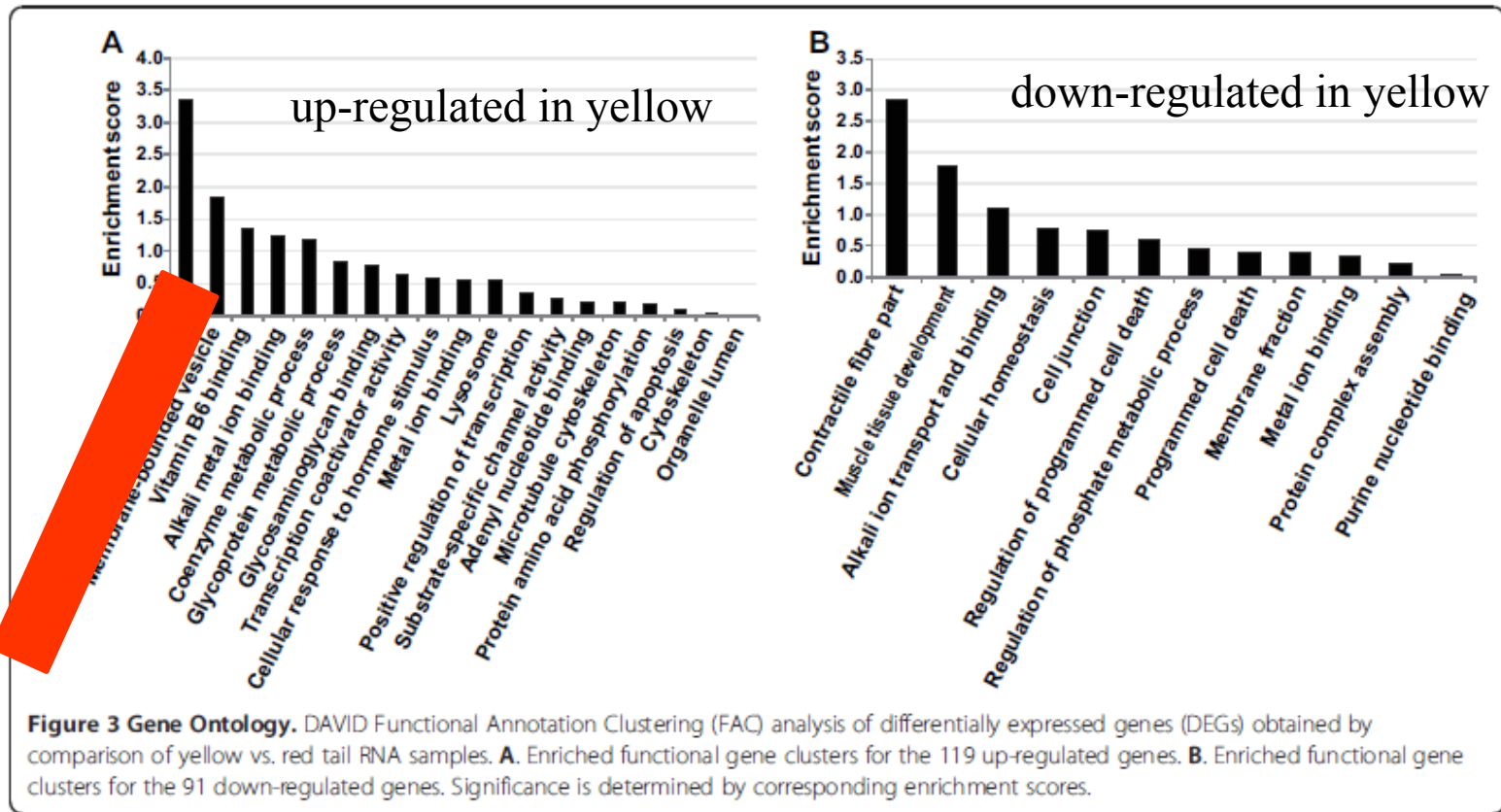
melanophory
erythrophory

Konzistence výsledků



- změny v expresi jdou stejným směrem u RNA-seq i RT-qPCR vybraných genů

Functional annotation clustering (= gene ontology)



- xanthophory u žlutých jedinců jsou asociovány s melanogenezí
- v dalším kroku je možné studovat roli jednotlivých kandidátních genů