

High resolution qPCR tomography: method to study mRNA localization within biological sample

Monika Sidova^{1,3}, Radek Sindelka^{1,2}, David Svec¹, Tereza Tlapakova³ and Mikael Kubista¹

(Monika.Sidova@img.cas.cz, mikael.kubista@tataa.com)

¹Institute of Biotechnology AS CR, v.v.i., Laboratory of gene expression, Czech Republic.

²Whitehead Institute, Cambridge, USA

³Charles University in Prague, Faculty of Science, Department of Cell Biology, Czech Republic.



Introduction:

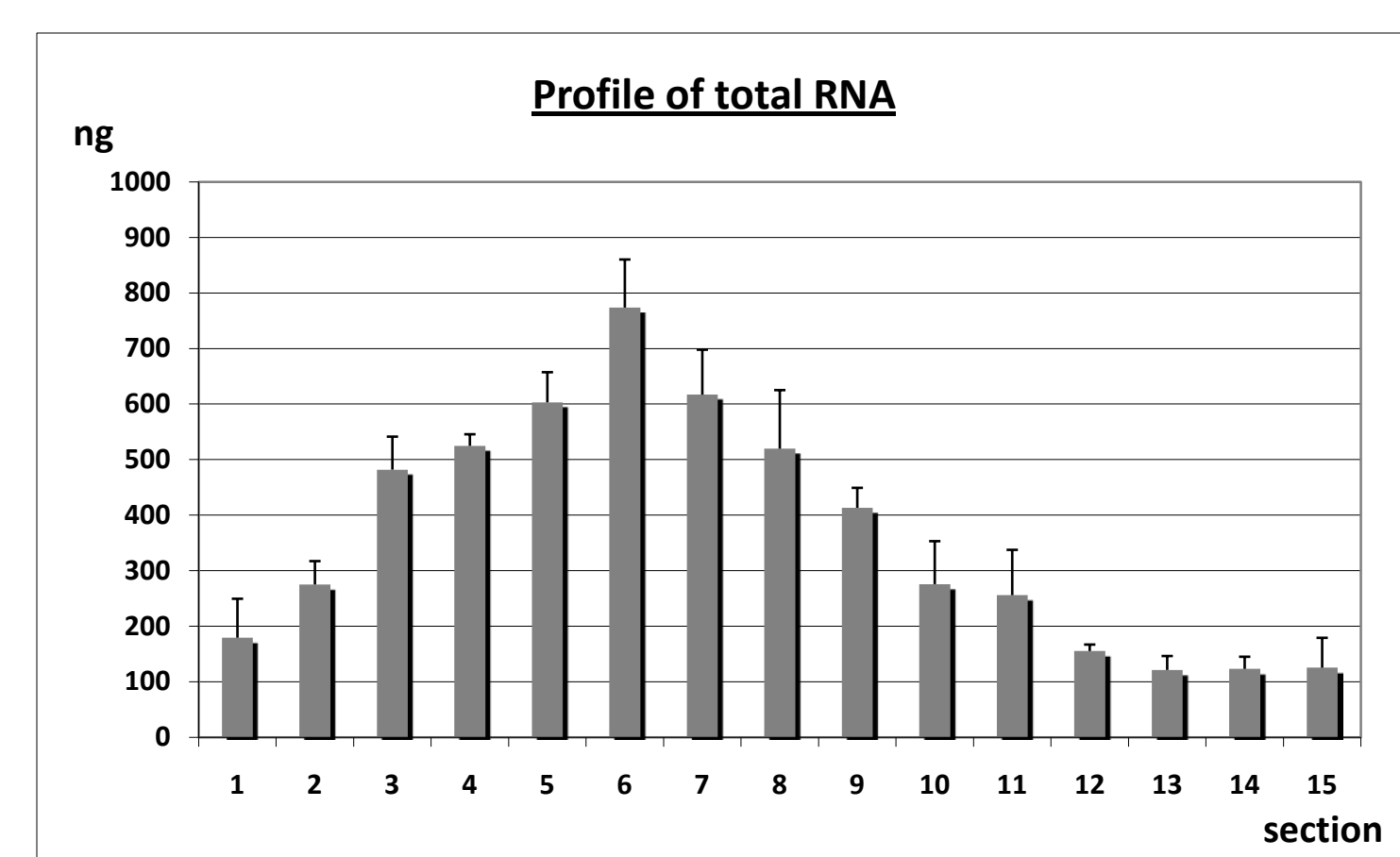
Real-time PCR tomography is a novel, quantitative method for measuring localization RNA patterns within biological samples and even single cells. We present here its usefulness by dissecting an *Xenopus laevis* oocyte into slices along animal-vegetal axis, extracting RNA and measuring the expression levels of 48 selected maternal genes. Biomark platform was used for high throughput qPCR. 48x48 dynamic array allow to analyze roughly 2,300 samples in parallel in one run. Two groups of genes were identified in *Xenopus* oocyte based on their expression patterns. Animal genes, which mRNAs are localized mainly in the animal hemisphere. Surprisingly, all available *Xenopus* reference genes showed animal localization. Second group, called vegetal, consists of genes with gradient localization from animal to vegetal pole. Further the vegetal genes can be separated into two subgroups based on high resolution qPCR tomography results. First subgroup contains genes, which are localized together with mitochondrial cloud of vegetal cortex, such as Vg1, Wnt11 and Otx1. Second subgroup consists of germ plasm determinants, such as Xcad2 and Xdazl.

This work is supported by GACR 301-09-1752.

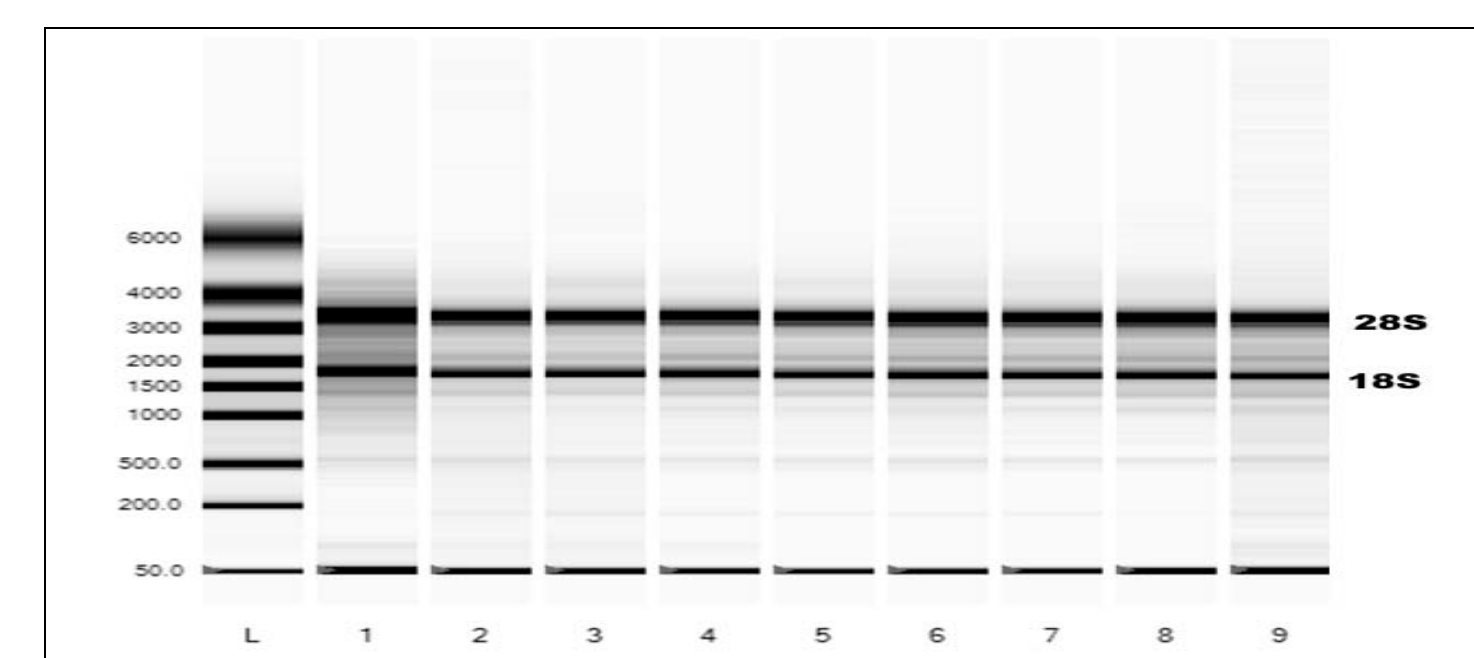
Method:

Three *Xenopus* oocytes were cryo-sectioned along animal-vegetal axis into 15 serial segments. Sample 1 contains first animal segment and sample 15 last vegetal segment. Total RNA was extracted using Micro RNeasy kit (Qiagen). Same amount of RNA was reverse transcribed to cDNA and then preamplified. Preamplified samples were loaded into dynamic array together with primers for 40 maternal genes. Expression levels in segments were recalculated to the whole oocyte (percentage portion within the oocyte as y-axis).

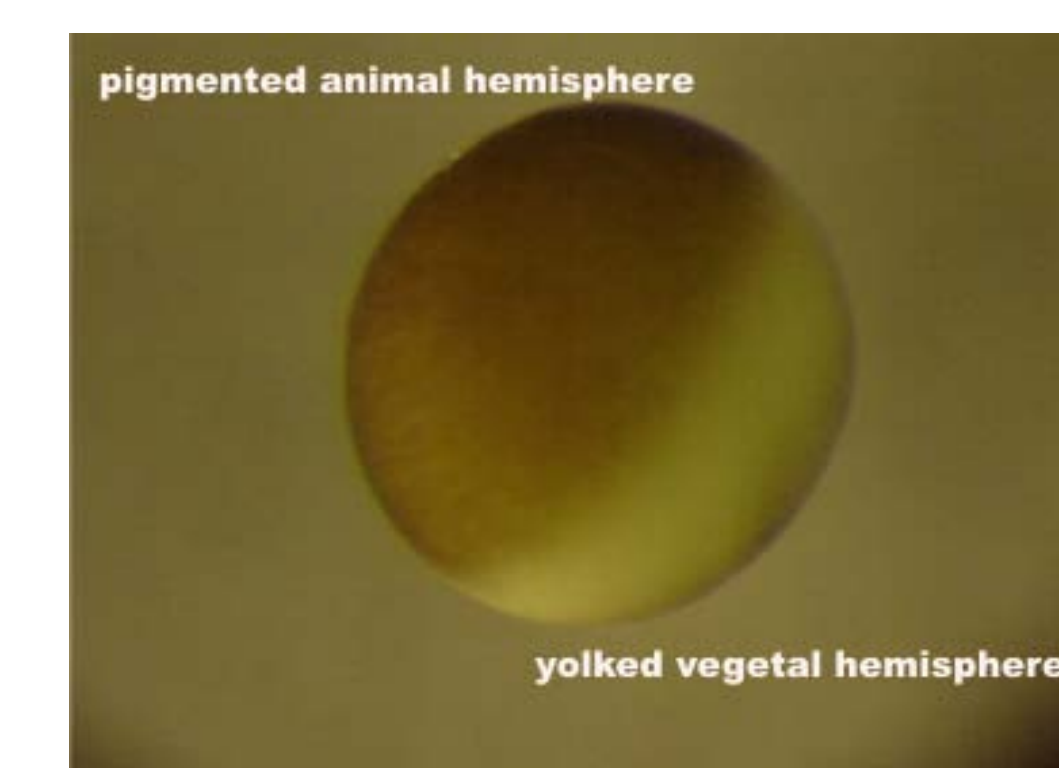
Results:



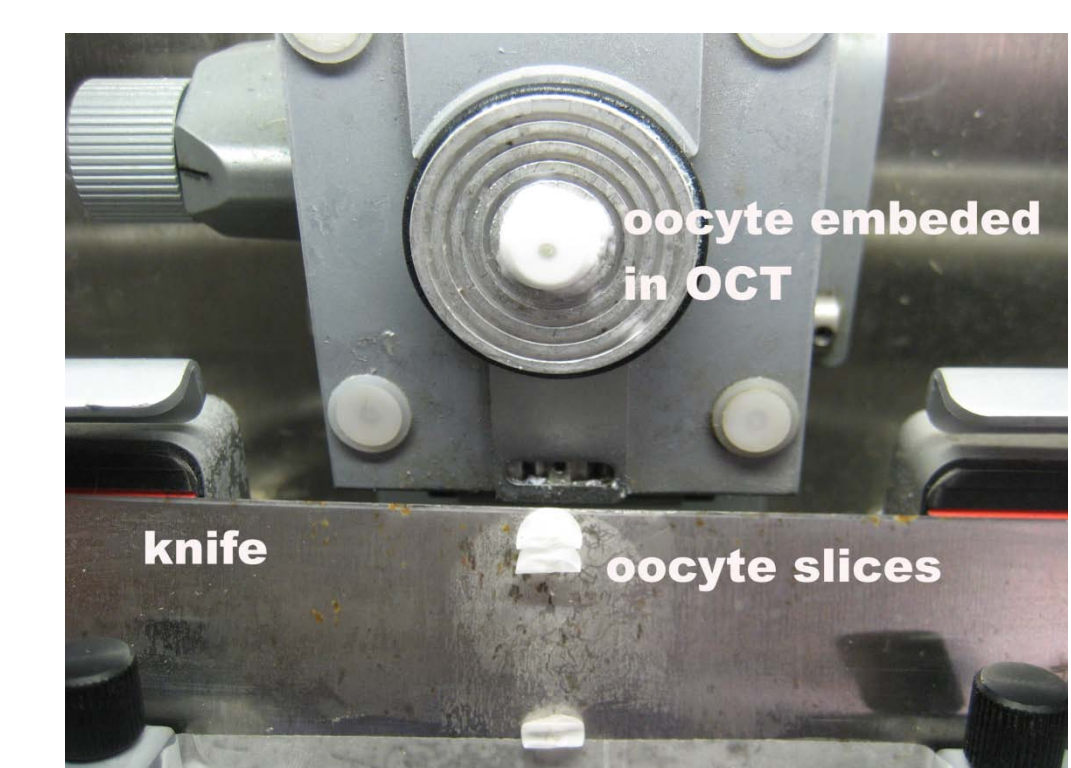
Profile of total RNA within *Xenopus* oocyte.



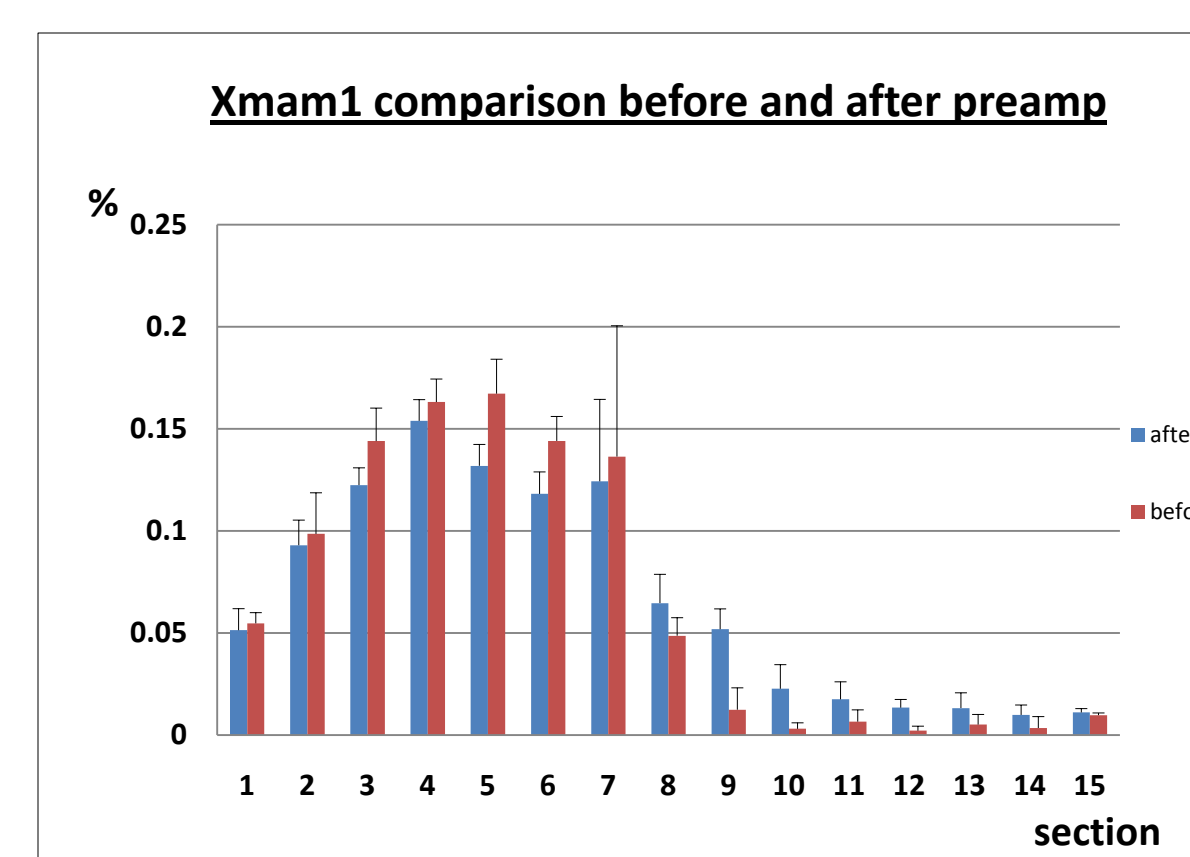
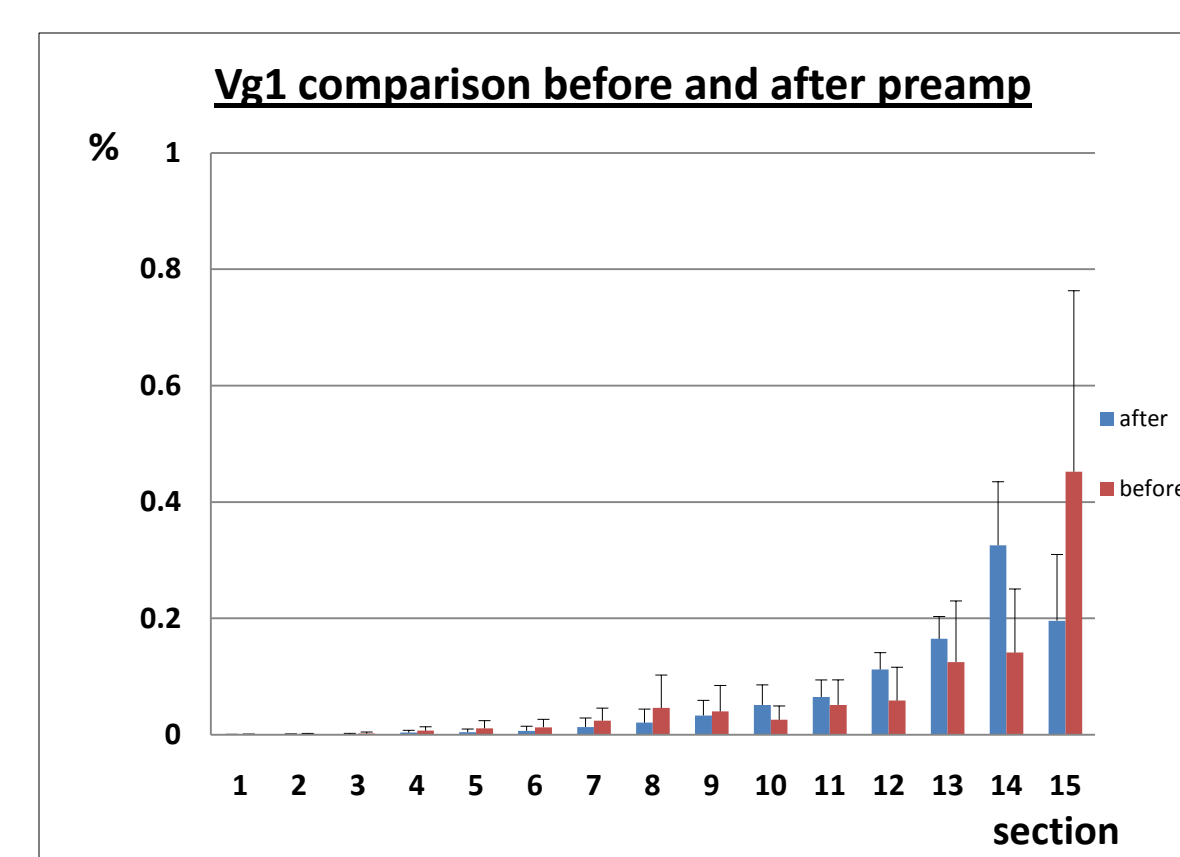
Quality control of total RNA. Integrity of ribosomal RNAs was determined with capillary electrophoresis system Experion (Bio-Rad).



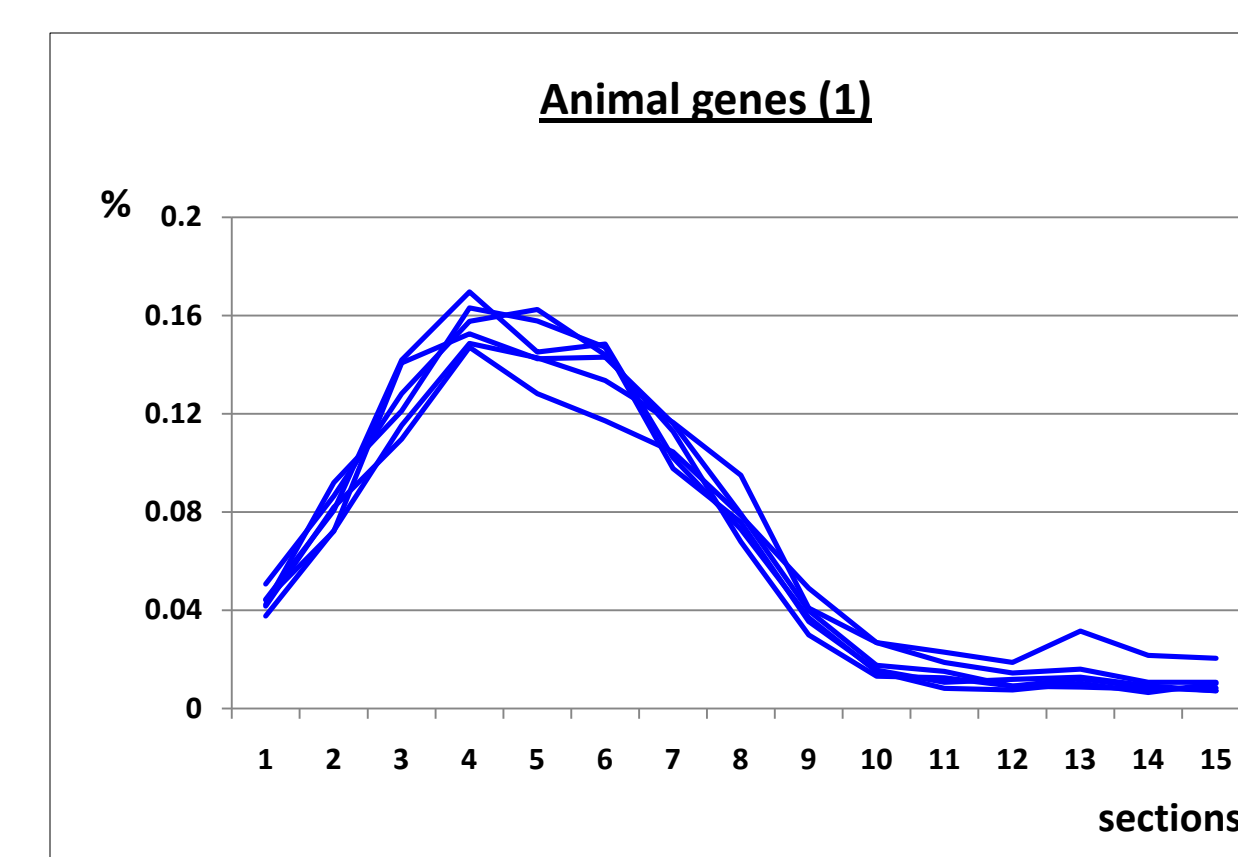
Hemisphere of *Xenopus* oocyte



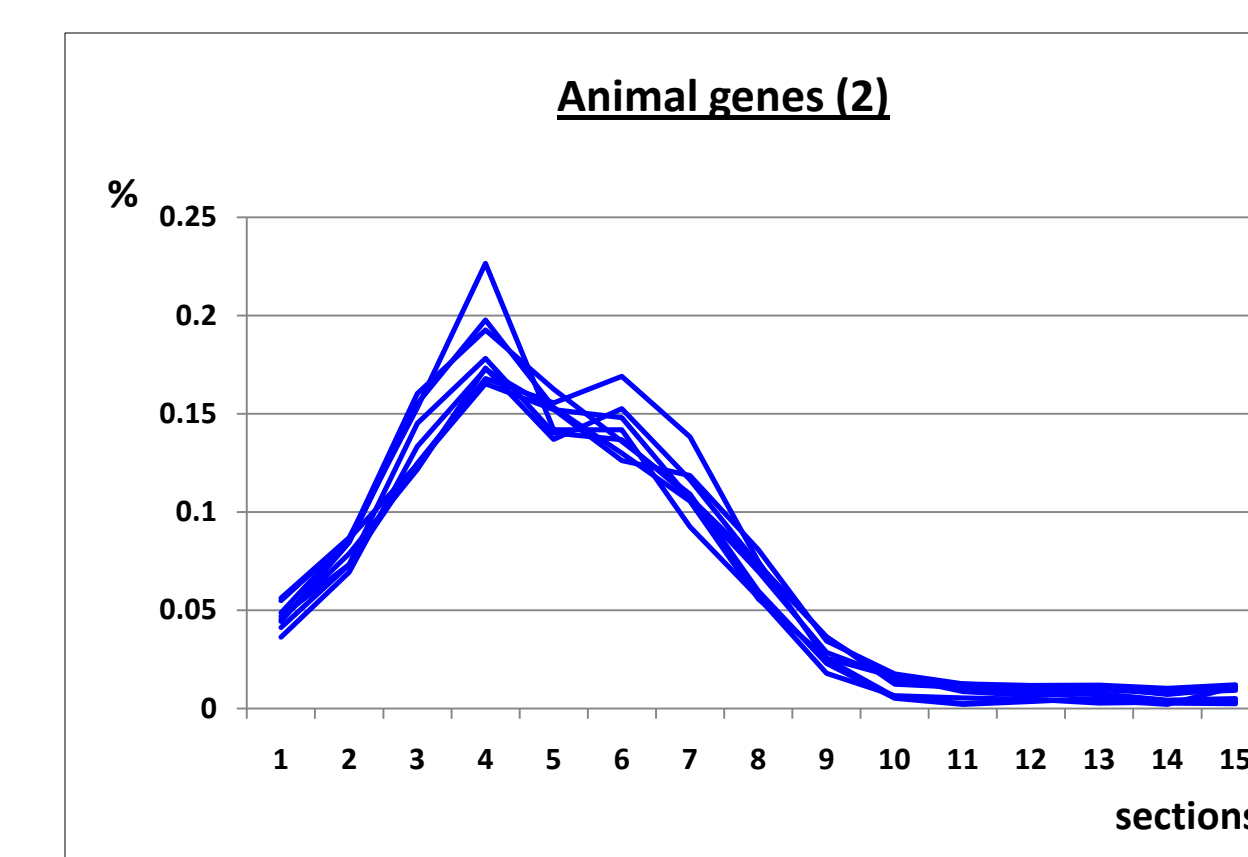
Before cutting oocyte on cryostat, the oocyte have to be embedded in optimal cut temperature compound (OCT)



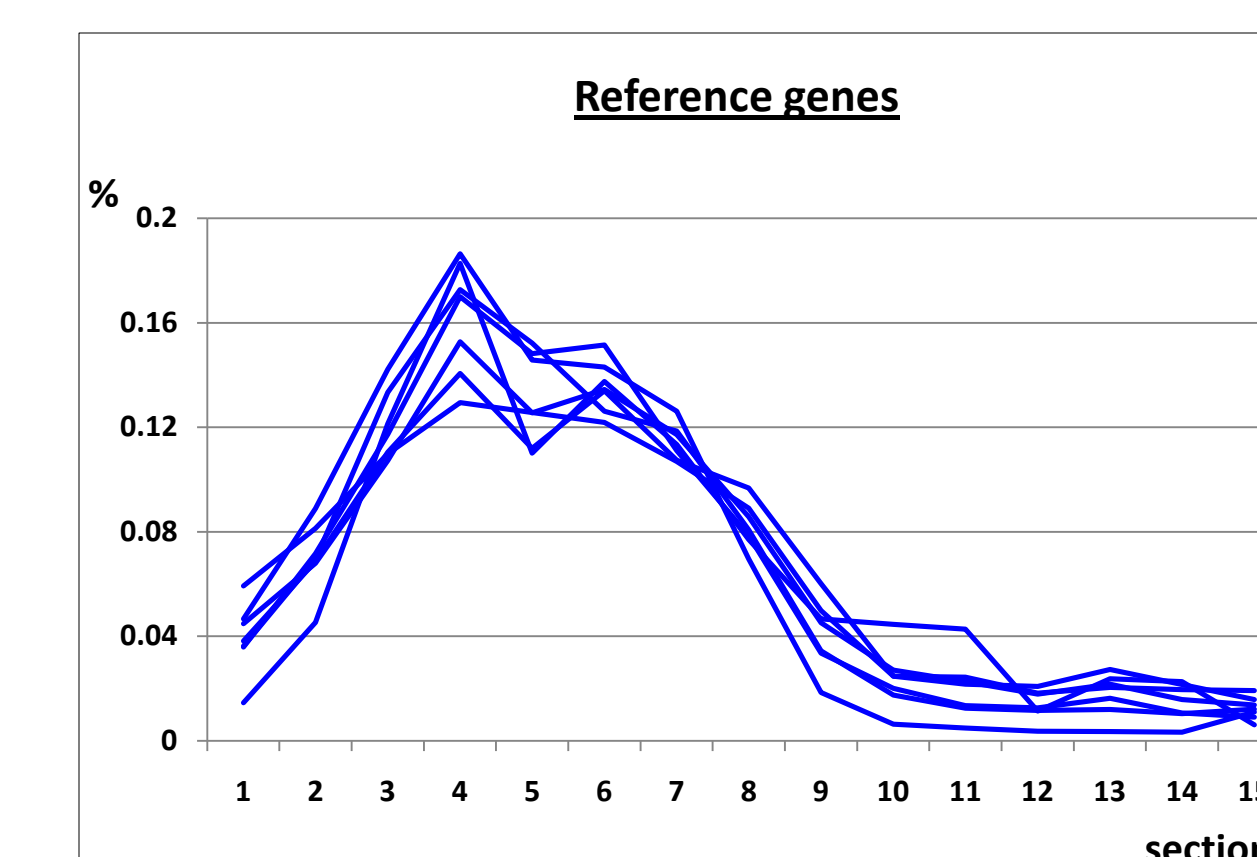
Validation of preamplification. BioMark™ dynamic array (Fluidigm) required preamplification step, because of limited amount of material. Two control genes were selected, Xmam1 as an animal gene and Vg1 as a vegetal gene. Expression patterns are compared before and after preamplification step.



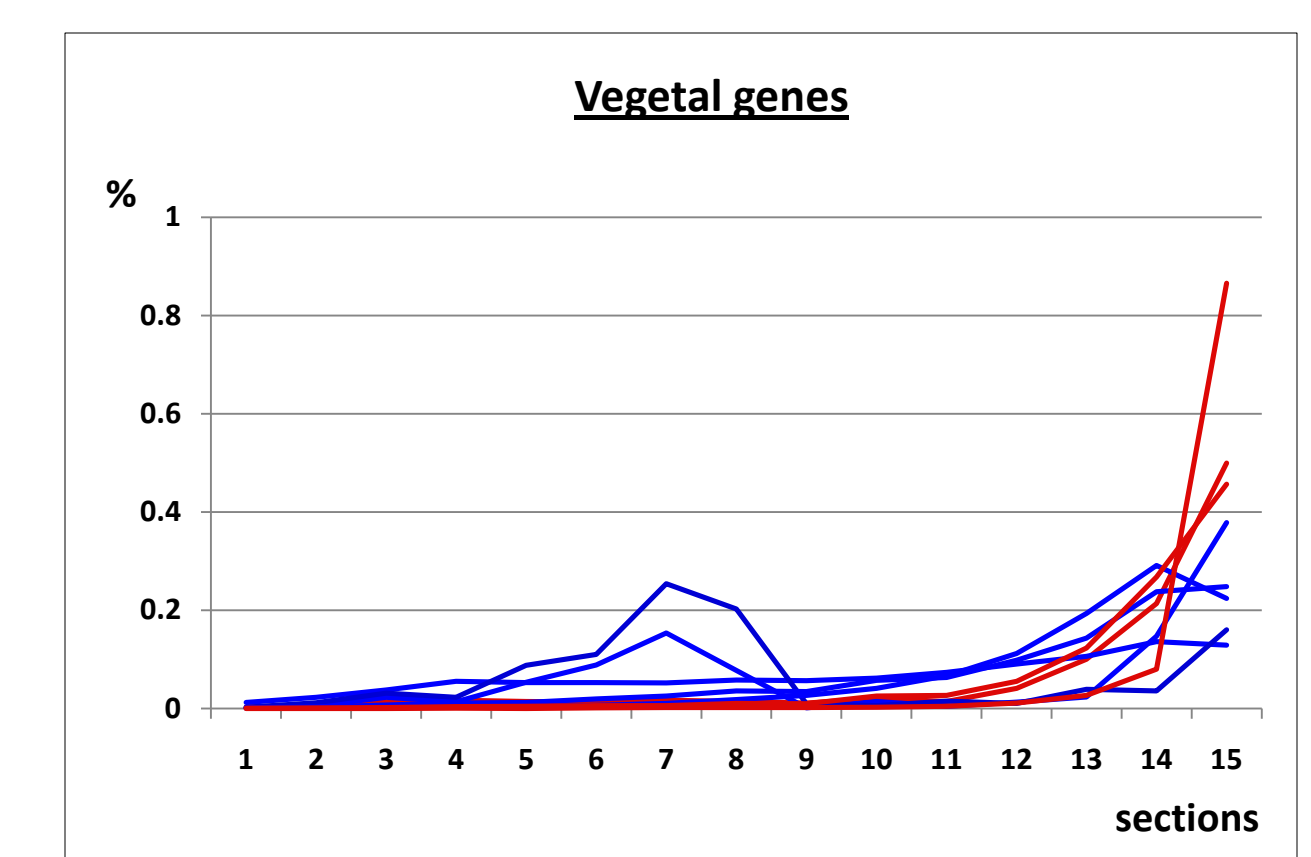
Expression patterns of animal genes (1) – ZPC, An2, APC, Est1, axin, Fz7.



(2) – Xpar1, Oct-60, Stat3, FoxH1, β -catenin, Tcf-3, Xmam1, GSK-3 β .



Distribution of mRNAs coding "reference" genes: GAPDH, mitochondrial cytochrome C, RNA polymerase, U3- snoRNA, EF-1 α , β -tubulin, α -actin.



Expression patterns of vegetal genes. Two subgroups can be identified. First subgroup contains primordial germ cell markers such as DEADSouth, Xdazl and Xcad2 (in red). Second group consists of VegT, Vg1, Wnt11 and Otx1 (in blue).

Conclusions:

- Amount of total RNA in a single cell, *Xenopus* oocyte allows us to measure mRNA expression patterns in high resolution.
- High throughput qPCR of 40 genes in 45 samples in four hours were done using Biomark platform (Prague core facility - www.img.cas.cz/ge).
- Two groups of genes with animal and vegetal expression patterns were identified. All measured "reference" genes showed animal localization.
- Yolk stored in vegetal hemisphere was found to be a strong inhibitor and therefore RNA extraction must be done carefully. Quality control is necessary.