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# LASER CAPTURE MICRODISSECTION OF PIGMENT CELLS FROM SALMONID FISH SKIN TO IDENTIFY DIFFERENTIALLY EXPRESSED GENES IN AN ENRICHED CELL POPULATION

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In order to determine the function of pigment cell types in different pigment pattern of skin, we have applied the laser capture microdissection method (LCM) for isolation of different pigment cell types from skin sections of marble trout (*Salmo marmoratus*) and brown trout (*S. trutta*), characterized with labyrinthine and spotted skin pattern, respectively. RNA was isolated from dissected pigment cell populations and used for expression analysis. Expression profiles of candidate genes, potentially involved in pigmentation, in LCM isolated pigment cell population, were more specific compared to differently pigmented skin regions, containing a heterogeneous mixture of cells, including more types of chromatophores. Moreover, they could provide important information about particular pigment cell type function and its contribution to different pigment pattern. Protocol of combined LCM and genomic approach to study single pigment cell populations can be applied in the future, but needs to be further optimized in order to yield RNA of sufficient quantity and quality required for i.e. transcriptomic analyses.

*Key words*: LCM, *Salmo trutta*, *Salmo marmoratus*, pigment cell, RNA expression

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## INTRODUCTION

Huge variety of pigmentation and pigment patterns of teleost fish skin is the result of the spatial combination and changes in six types of pigment cells or chromatophores: melanophores (black and dark brown), xanthophores (yellow), erythrophores (red/orange), iridophores (reflecting), leucophores (white) and cyanophores (blue). Pigmentation and pigment pattern have a significant role in teleost fish contributing to thermoregulation, camouflage, predator avoidance, social communication, selective mating, sex recognition, and speciation (KELSH, 2004; PROTAS and PATEL, 2008; HUBBARD *et al*., 2010).

The genetic basis of pigment production in fish and subsequent skin pigmentation is well understood, particularly in model species, e.g. zebrafish (JOHNSON *et al*., 1995; KELSH *et al*., 1996; PAICHY and TURNER, 2003; PARICHY, 2006). However, much less is known about how these patterns are generated. Recent studies have suggested the importance of interactions between chromatophores and subsequent cell-cell communication in the formation of different pigment patterns (MADERSPACHER and NÜSSLEIN-VOLHARD, 2003; NAKAMASU *et al*., 2009; WATANABE and KONDO, 2012; FROHNHÖFER *et al*., 2013; PATTERSON and PARICHY, 2013; FADEEV *et al*., 2015). Expression profiles of particular pigment cell types and single gene mutations in ion channels and cell junctions are therefore proposed to play a significant role in setting up different pigment patterning in closely related individuals. Recently, laser capture microdissection (LCM) was established as a method for isolating pure cell populations from a heterogeneous tissue (EMMERT-BUCK *et al*., 1996; BONNER *et al*., 1997). It can precisely target and capture the cells or even a single cell of interest from post-mortem material for a wide range of downstream applications (SIMONE *et al*., 1998; DATTA *et al*., 2015; FARRIS *et al*., 2017; BEVILACQUA and DUCOS, 2018), including expression analysis (qPCR of candidate genes, microarrays, NGS transcriptome sequencing) to identify differentially expressed genes in an enriched cell population. This technique could provide new insights into cell biology from cells grown in their natural tissue habitat. As such, the method could be applied for studying differential gene expression in different pigment cell populations, potentially contributing to different pigment pattern.

The aim of the study was to apply the LCM method for isolation of different pigment cell types from skin sections of brown trout (*Salmo trutta*) and marble trout (*S. marmoratus*), representatives of Salmonidae, known as one of the most phenotypically diverse fish family. While marble trout is characterized by labyrinthine skin pattern interlacing light and dark pigmented regions, spotted skin pattern with black and red spots on a light background is typical for its sister taxon, brown trout. RNA was isolated from dissected pigment cell populations and used for expression analyses. Expression profiles of selected genes were compared with expression profiles generated from RNA isolated from whole skin samples. We also aimed to evaluate the LCM method for analyzing differential expression of chromatophores in fish skin.

## MATERIAL AND METHODS

### *Experimental animals and sample preparation*

Adult marble trout individuals (2+) were collected from Tolminka fish farm in Tolmin, Slovenia, and adult brown trout (2+) from the Bled fish farm in Bled, Slovenia. With the age of two years, trout reached their sexual maturity and had already developed their adult skin pattern. Prior to skin sample collection fish were sedated in anaesthetic Tricaine-S (MS-222, Western chemical, Ferndale, USA) and killed by a blow to the neck. Treatment of the animals was carried

out following the national regulations and decision letter U34401-60/2013/4 issued by the Ministry of Agriculture and Environment for performing research activities on fish tissues.

Small pieces of skin from the lateral part of the trunk of the body (Figure 1) were obtained with 2 mm biopsy punch (Kai Group, Japan) to selectively dissect differently pigmented regions of skin in marble trout (dark (MTD) and light (MTL) region) and brown trout (light region (BTL), black (BTB) and red (BTR) spots). In addition, a piece of muscle tissue from marble trout (MTM) was collected as a control. The biopsy samples were immediately immersed in liquid nitrogen and (1) used for RNA isolation and (2) subsequently used for the preparation of cryosections for further processing. Frozen tissue blocks were made using a minimum amount of Tissue-Tek Optimal Cutting Temperature (OCT, Sakura Finetek) compound.

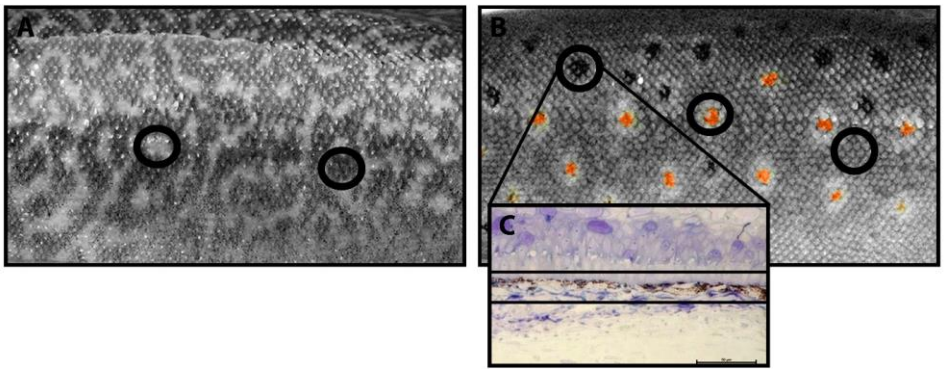


Figure 1. Biopsy samples (circles) of differently pigmented skin region of marble (A) and brown (B) trout. (C) Region of dermis used for LCM; scale bar: 50 μm.

***Slide preparation and laser capture microdissection (LCM)***

Frozen skin samples were transferred to the cryostat and allowed to equilibrate to the pre-set temperature of –20°C. The samples were cut into 11 μm sections. Sections of the dermis, containing pigment cells (see DJURDJEVIČ *et al.*, 2015) (Figure 1) were mounted onto the sterilized and cooled slides and immediately immersed for fixation and removing the OCT in ice cold, RNase-free 70% EtOH for 30 sec. The slides were then transferred to 100% EtOH. After dehydrating, all sample slides were dipped in acetone to remove residual ethanol. The sections were not stained since the pigment cells can be easily recognized based on their pigmented content. Slides were kept in sterile Falcon tubes at –80°C until LCM. Before LCM, the slides were allowed to equilibrate to room temperature and then microdissected using the PALM MicroBeam system (Carl Zeiss MicroImaging GmbH, Bernried, Germany) with UV laser and inverted ZEISS microscope. Dissected regions containing mainly particular pigment cells were gathered in 0.2 ml tubes with adhesive caps and placed in lysis buffer immediately after the LCM.

***RNA extraction and quantification, quantitative real-time PCR***

Total RNA was extracted using the PicoPure RNA isolation kit, according to the manufacturer's instructions (Arcturus BioScience, UK), with cell lysis performed immediately after laser dissection. RNA quality and concentration were evaluated with a Bioanalyser 2100 (Agilent, USA) on Agilent RNA Pico assays.

Quantitative real-time PCR (qPCR) was performed using SYBR Green PCR Master Mix (Thermo Fischer Scientific, MA USA) on Viia7 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific). Primers for selected genes (Table 1) were designed using Primer-BLAST (YE *et al*., 2012) with an amplicon size of approx. 200 bp. According to our previous results, *rps20* and *β-actin* were chosen as the most appropriate reference housekeeping genes. To verify the specificity of isolated cells, three genes were selected from the list of candidate genes expressed in pigment cells and potentially involved in pigment pattern formation: *pmel* (premelanosome protein), exclusively expressed in melanophores; *sox10* (transcription factor), one of the genes highly overexpressed in red spot in brown trout (candidate gene specific for erythrophores); *gja5* (Gap junction alpha-5 protein or connexin 40 (*cx40*)), overexpressed in red and black spots (melanophores and/or erythrophores) in brown trout (unpublished data, manuscript in preparation).

The expression of candidate genes was analyzed on five samples from differently pigmented regions (MTD, MTL, BTL, BTB, BTR; all as LCM treated and whole skin samples), with every LCM sample being a pool of two different specimens, each of them done in triplicates. A no-template-control (NTC) was used to check for potential contamination. Standard curve analysis was performed for reference and target genes to assess the amplification efficiency. The conditions for all reactions were 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, and 60°C for 1 min. At the end of every run, melting curve analysis was performed in order to confirm unique amplicon reaction. Primers used for qPCR are listed in Table 1.

*Table 1. Primers used for qPCR analysis.*

| Gene | Database code | Gene name | Forward/ reverse primer |
|---|---|---|---|
| **RPS20** | NM_001140843.1 | 40S Ribosomal Protein S20 | AGCCGCAACGTCAAGTCT/ GTCTTGGTGGGCATACGG |
| **β-ACTIN** | NM_001123525.1 | Beta-actin | CCAAAGCCAACAGGGAGAAGATGA/ TCGTAGATGGGTACTGTGTGGGT |
| **PMEL** | XM_014132714.1 | Premelanosome Protein | TTAAGCAGTATCGCCCACTGA/ CTGTCGGCTCAGAAGATTCCA |
| **SOX10** | XM_014203766.1 | Transcription Factor SOX-10 | TAGCTGGCGGGATTTCCATC/ GAGGTGCGGATACTGGTCTG |
| **GJA5** | XM_014174142.1 | Gap Junction Alpha-5 Protein | TGCCTTCTCACCCCTTCAAC/ CCGGTATGCAGTAAGGGTGG |

## RESULTS

Regions containing pigmented cells were successfully dissected from *Salmo* skin slices (Figure 2).

Short overview of laser captured tissue amount and isolated RNA quality is shown in Table 2.

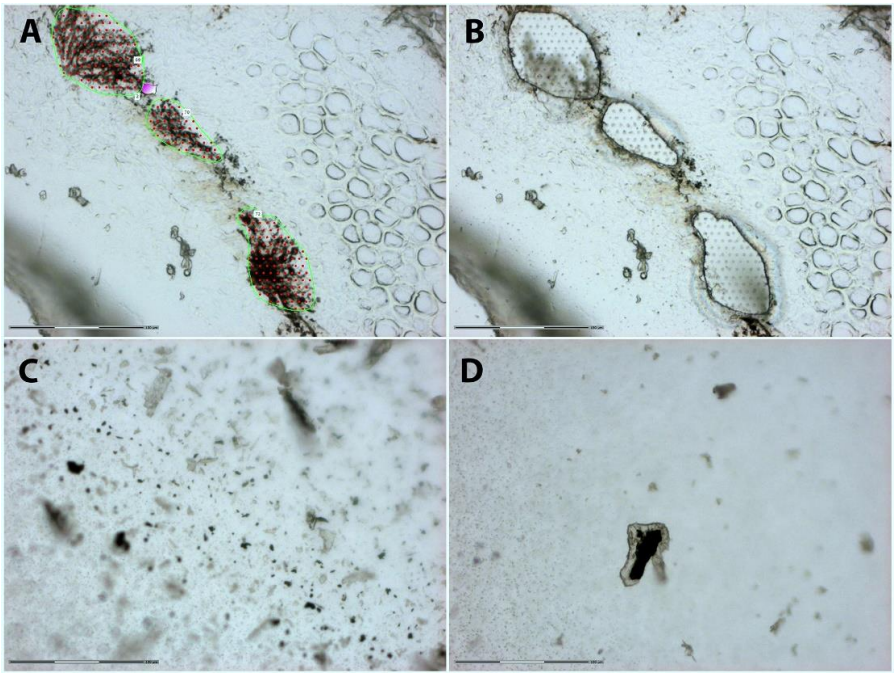


Figure 2. LCM sample collection. (A) Selected pigment cells before collection (B) after cell collection (C–D) view of collected material in the cap of the tube. Scale bars: 150 µm.

*Table 2. Within sample overview of a number of dissected elements from dermis slices, its overall area, and concentration and quality of isolated RNA.*

| Sample | No. of dissected elements | Dissected area size ($mm^2$) | RNA concentration (ng/µL) | Conc./area $mm^2$ | RIN value |
|---|---|---|---|---|---|
| lcm-MTD | 179 | 3.599 | 183 | 50.85 | 3,4 |
| lcm-MTL | 113 | 1.373 | 52 | 37.87 | 1 |
| lcm-MTM | 59 | 2.177 | 29 | 13.32 | 1 |
| lcm-BTL | 39 | 0.610 | 2.30 | 3.77 | N/A |
| lcm-BTB | 84 | 3,133 | 13.73 | 4.38 | 4,5 |
| lcm-BTR | 60 | 1,452 | 1.51 | 1.04 | 5,1 |

Concentration of isolated RNA varied between the samples and did not correlate with the amount of dissected area. Overall, the retrieved RNA from brown trout samples was of very low concentration. RIN values were low for all of the samples indicating very poor quality of isolated RNA.

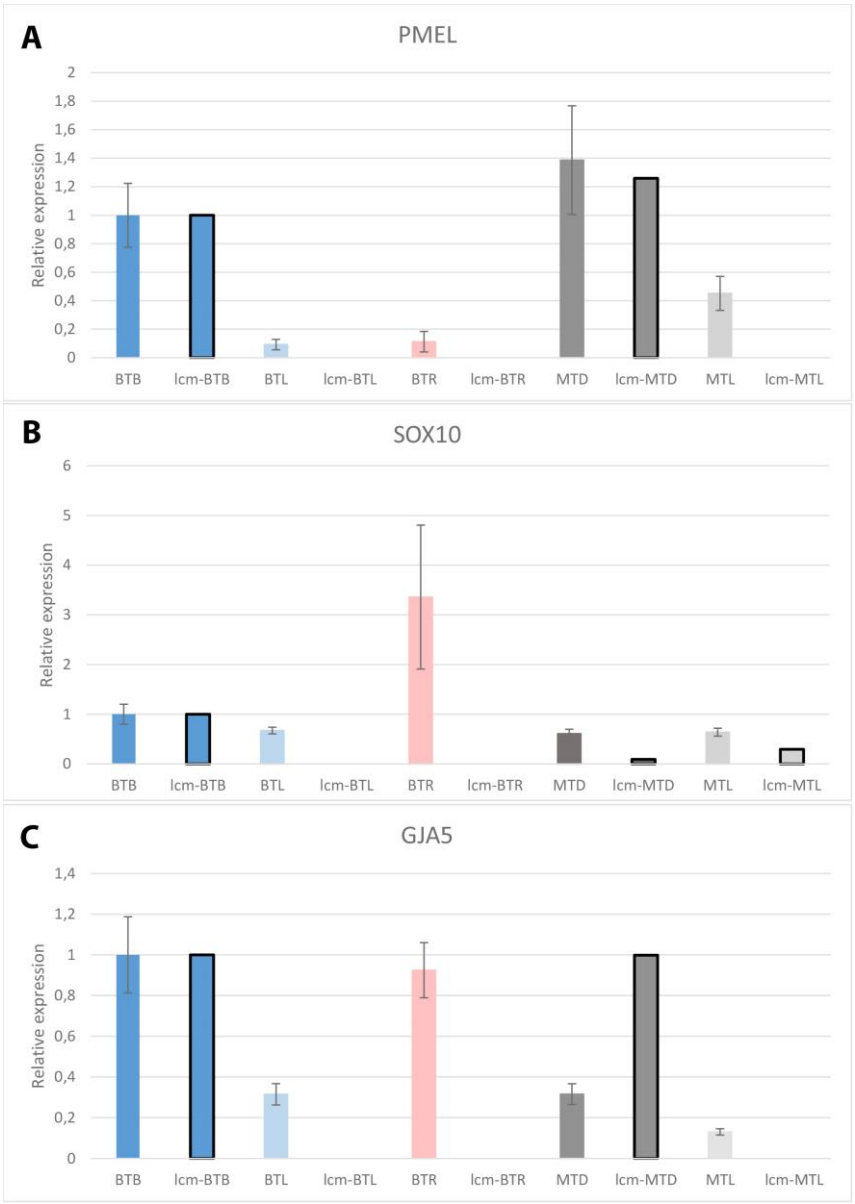


Figure 3. Relative expression of LCM samples (lcm-BTx and lcm-MTx; edged with black). Expression of a gene in differently pigmented whole skin section is also shown for comparison (BTx and MTx). (A) Relative expression of *pmel*, (B) relative expression of *sox10*, (C) relative expression of *gja5*.

All the samples were nevertheless tested with qPCR. Using different concentrations of the samples in the amplification curve, none of the amplicons had optimal efficiency. This was due to the presence of inhibitors in non-diluted samples and extremely low RNA concentration at higher dilutions of the samples disabling the amplification. Expression of housekeeping genes was detected in all samples, except for lcm-BTL (sample with no RIN value), where *rps20* could not be detected, and lcm-BTR (lowest RNA concentration), where *β-actin* was undetectable. Even though the concentration of RNA was low, some of the samples contained inhibitors, as evidenced by calibration curves, and were therefore further diluted for expression analyses.

Expression profiles of three candidate genes potentially involved in pigment pattern were analyzed. *sox10*, whose expression is not limited to pigment cells, was the only target gene expressed also in muscle (lcm-MTM, Figure 3C). Expression profile of LCM isolated pigment cell populations was compared to expression profile obtained from differently pigmented skin regions (containing mixture of chromatophores with one prevailing type; see DJURDJEVIČ et al., (2015) for more details on skin morphology) (Figure 3). *pmel* was expressed in black spot of brown trout and dark region of marble trout skin, containing exclusively melanophores (samples lcm-BTB and lcm-MTD; Figure 3), while there was no or very low expression detected in light pigmented regions (lcm-BTL and lcm-MTL) and red spot (lcm-BTR). *sox10* was expressed in almost all samples except for lcm-BTL and lcm-BTR. The highest expression was expected in the lcm-BTR sample. The highest expression of *gja5* was expected in black and red spots in brown trout, but again we could not detect expression of this gene in lcm-BTR. *gja5* gene was also not expressed in cell population isolated from light skin regions (lcm-BTL and lcm-MTL).

## DISCUSSION

Laser capture microdissection is a microscopy-based technique developed to isolate selected cell populations or even a single cell from complex, heterogeneous tissue (FARRIS *et al.*, 2017). LCM combined with cell expression studies has been applied in numerous experiments to study molecular characteristics of particular cell populations, especially in medical applications, for instance in comparing carcinoma cells with neighboring tissue cells (e.g., WANG *et al.*, 2010; LAMBERT *et al.*, 2014; CASTRO *et al.*, 2016). The range of application is growing, but to the best of our knowledge, this methodology has not yet been applied to pigmentation and pigment pattern studies in animals. Here, we present the potential application of LCM to study expression profiles of dissected pigment cell populations from differently pigmented region in the skin of brown and marble trout.

Sample preparation and optimal laser beam are very important for successful capture of cells by LCM. Following workflow for PALM MicroBeam system (PALM protocols, 2011) including some optimization steps, the capture of pigment cells from fish skin was successful. The captured area (Table 2) was in the range suggested by FARRIS *et al.*, (2017) to yield sufficient quantity of RNA for downstream analyses, except for the sample lcm-BTL. Nevertheless, the concentration of isolated RNA was very low, particularly for BT samples. All the samples were further characterized by very low RIN values, indicating low quality and degradation of isolated RNA. Isolation of small cell populations using LCM typically results in minimal RNA yields, that is affected primarily by quality of sample, time, and type of preservation (preferably at −80°C), fixation method, and efficiency of microdissection (less than an hour) (DATTA *et al.*, 2015). Taking into account that microdissections on one slide were completed in less than half an hour and that all the samples were processed, preserved, and fixed

using the same protocol, the quality of the sample might have a significant influence on RNA in our case. The fish skin represents a very effective barrier between the fish and its surroundings. It's tougher than internal organs and covered with mucus and RNases (HILLWIG *et al*., 2009) as a part of its protective function. These reasons make RNA isolation from fish skin a challenging task. Furthermore, a study comparing the use of different lasers for LCM and different kits for RNA isolation was recently performed by FARRIS *et al*., (2017). The study has indicated worse performance of UV lasers compared to IR lasers, and significant influence of the RNA isolation procedure, with QIAGEN Lysis (minimal digestion time and temperature) yielding higher quantity and quality of RNA isolated from the same amount of tissue compared to PicoPure Lysis (longer digestion times at higher temperature). The choice of PicoPure kit, standard in LCM, could therefore also be a factor that has influenced the RNA quantity and quality in our case.

Despite low quantity and quality of RNA, qPCR could be performed for almost all of the samples. The most problematic was sample lcm-BTR with the lowest RNA concentration, resulting in no amplification of housekeeping *β-actin* gene and also no amplification of candidate genes, even though the highest expression in this sample was expected for *sox10* and *gja5*. Therefore, the results obtained from lcm-BTR sample should not be taken into account, and hypothetic specificity of *sox10* expression in erythrophores could not be verified. On the other hand, we confirmed specific expression of *pmel* in melanophores. While the gene was expressed also in light pigmented region of both brown and marble trout skin, that besides iridophores and xanthophores contain also rare melanophores (DJURDJEVIČ *et al*., 2015), no expression was detected in cell population isolated from light region with LCM (no melanophores dissected). Higher expression of *gja5* was hypothesized to be connected to spots present in brown trout (unpublished data, manuscript in preparation). While there was no expression detected in light pigmented regions (lcm-BTL and lcm-MTL), the expression of this gene in dark pigmented region of marble trout (lcm-MTD) was as high as in black spots of brown trout (lcm-BTB), pointing to specific expression of *gja5* in melanophores of both species. Due to low quality of RNA isolated from cells in the red spot, the synergistic expression of *gja5* in melanophores and erythrophores contributing to spotted pattern could not be confirmed.

As already mentioned by ESAKI et al., (2015), our study also encountered some limitations of the technology used: (1) LCM is a labor-intensive method, usually allowing analyses of small number of samples; (2) due to small amount of tissue used, the quality of a sample, its storage, and processing are of great importance, and all possible measures should be taken to reduce the possibility of RNA degradation; (3) even though the technique is straightforward, a lot of optimizations in all the steps (sample preparation and storage; LCM; RNA isolation) should be required so that it could be applied in studies of non-model species, especially when using problematic tissue samples.

## CONCLUSIONS

We could conclude that expression profiles of LCM isolated pigment cell populations are more specific compared to differently pigmented skin regions, containing a heterogeneous mixture of cells, including more types of chromatophores. In future studies, we propose to apply LCM in order to verify expression profiles of particular chromatophore type, compare expression profiles between chromatophore types, and infer interactions between chromatophores contributing to the formation of different pigment patterns. Combined LCM and genomic

approach to study single pigment cell populations can in the future also be applied in case of difficult fish skin tissue, but the protocol needs further optimization in order to yield RNA of sufficient quantity and quality required for i.e. transcriptomic analyses.

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REFERENCES

BEVILACQUA, C., B. DUCOS (2018): Laser microdissection: A powerful tool for genomics at cell level. Mol. Aspects Med., *59*: 5-27.

BONNER, R.F., M. EMMERT-BUCK, K. COLE, T. POHIDA, R. CHUAQUI, S. GOLDSTEIN, L.A. LIOTTA (1997): Cell sampling: laser capture microdissection: molecular analysis of tissue. Science, *278*: 1481–1483.

CASTRO, N.P., A.S. MERCHANT, K.L. SAYLOR, M.R. ANVER, D.S. SALOMON, Y.G. GOLUBEVA (2016): Adaptation of Laser Microdissection Technique for the Study of a Spontaneous Metastatic Mammary Carcinoma Mouse Model by NanoString Technologies. PLoS ONE, *11* (4): e0153270.

DATTA, S., L. MALHOTRA, R. DICKERSON, S. CHAFFEE, C.K. SEN, S. ROY (2015): Laser capture microdissection: Big data from small samples. Histol. Histopathol, *30* (11): 1255-1269.

DJURDJEVIČ, I., M.E. KREFT, S. SUŠNIK BAJEC (2015): Comparison of pigment cell ultrastructure and organisation in the dermis of marble trout and brown trout, and first description of erythrophore ultrastructure in salmonids. J. Anat., *227* (5): 583-95.

EMMERT-BUCK, M.R., R.F. BONNER, P.D. SMITH, R.F. CHUAQUI, Z.P. ZHUANG, S.R. GOLDSTEIN, R.A. WEISS, L.A. LIOTTA (1996): Laser capture microdissection. Science, *274*: 998–1001.

ESAKI, H., D.A. EWALD, B. UNGAR, M. ROZENBLIT, X. ZHENG, H. XU, Y.D. ESTRADA, X. PENG, H. MITSUI, T. LITMAN, M. SUAREZ-FARINAS, J.G. KRUEGER, E. GUTTMAN-YASSKY (2015): Identification of Novel Immune and Barrier Genes in Atopic Dermatitis by Laser Capture Micro-dissection. J. Allergy Clin. Immun., *135* (1): 153–163.

FADEEV, A., J. KRAUSS, H.G. FRȍHNHOFER, U. IRION, C. NUSSLEIN-VOLHARD (2015): Tight junction protein 1a regulates pigment cell organisation during zebrafish colour patterning. eLife, *4*: 1711.

FARRIS S., Y. WANG, J.M. WARD, S.M. DUDEK (2017): Optimized Method for Robust Transcriptome Profiling of Minute Tissues Using Laser Capture Microdissection and Low-Input RNA-Seq. Front Mol Neurosci, *10*: 185.

FROHNHȍFER, H.G., J. KRAUSS, H.-M. MAISCHEIN, C. NŰSSLEIN-VOLHARD (2013): Iridophores and their interactions with other chromatophores are required for stripe formation in zebrafish. Development, *140*: 2997-3007.

HILLWIG M, L. RIZHSKY, Y. WANG, A. UMANSKAYA, J.J. ESSNER, G.C. MACINTOSH (2009): Zebrafish RNase T2 genes and the evolution of secretory ribonucleases in animals. BMC Evol. Biol., *9*: 170.

HUBBARD, J.K., J.A. UY, M.E. HAUBER, H.E. HOEKSTRA, R.J. SAFRAN (2010): Vertebrate pigmentation: from underlying genes to adaptive function. Trends Genet., *26*: 231–239.

JOHNSON, S.L., D. AFRICA, C. WALKER, J.A. WESTON (1995): Genetic-control of adult pigment stripe development in zebrafish. Dev. Biol., *167* (1): 27-33.

KELSH, R.N., M. BRAND, Y.J. JIANG (1996): Zebrafish pigmentation mutations and the processes of neural crest development. Development, *123* (SI): 369-389.

KELSH, R.N. (2004): Genetics and evolution of pigment patterns in fish. Pigm. Cell Res., *17*: 326–336.

LAMBERT, S.R., N. MLADKOVA, A. GULATI, R. HAMOUDI, K. PURDIE, R. CERIO, I. LEIGH, C. PROBY, C.A. HARWOOD (2014): Key differences identified between actinic keratosis and cutaneous squamous cell carcinoma by transcriptome profiling. Brit. J. Cancer, *110* (2): 520–529.

MADERSPACHER, F., C. NÜSSLEIN-VOLHARD (2003): Formation of the adult pigment pattern in zebrafish requires leopard and obelix dependent cell interactions. Development, *130*: 3447-3457.

NAKAMASU, A., G. TAKAHASHI, A. KANBE, S. KONDO (2009): Interactions between zebrafish pigment cells responsible for the generation of Turing patterns. PNAS, *106*: 8429–8434.

PALM Protocols – RNA handling. 2011. Carl Zeiss MicroImaging GmbH, Jena, Germany. https://applications.zeiss.com/C1257A26004B6E67/allBySubject/759EA2E3DC5A630AC1257AF50043114B

PARICHY, D.M. (2006): Evolution of danio pigment pattern development. Heredity, *97*: 200–210.

PARICHY, D.M., J.M. TURNER (2003): Temporal and cellular requirements for Fms signaling during zebrafish adult pigment pattern development. Development, *130*: 817–833.

PATTERSON, L.B., D.M. PARICHY (2013): Interactions with iridophores and the tissue environment required for patterning melanophores and xanthophores during zebrafish adult pigment stripe formation. PLOS Genet., *9*: 1–14.

PROTAS, M.E., N.H. PATEL (2008): Evolution of coloration patterns. Ann. Rev. Cell Dev. Biol., *24*: 425–446.

SIMONE, N.L., R.F. BONNER, J.W. GILLESPIE, M.R. EMMERT-BUCK, L.A. LIOTTA (1998): Laser-capture microdissection: opening the microscopic frontier to molecular analysis. Trends Genet., *14* (7): 272-6.

WANG, S., L. WANG, T. ZHU, X. GAO, J. LI, Y. WU, H.G. ZHU (2010): Improvement of tissue preparation for laser capture microdissection: application for cell type-specific miRNA expression profiling in colorectal tumors. BMC Genomics, *11*: 163.

WATANABE, M., S. KONDO (2012): Changing clothes easily: connexin41.8 regulates skin pattern variation. Pigm. Cell Melanoma R., *25*: 326–330.

YE, J., G. COULOURIS, I. ZARETSKAYA, I. CUTCUTACHE, S. ROZEN, T.L. MADDEN (2012): Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics, *13*: 134.

# LASERSKA MIKRODISKCIJA PIGMENTNIH ĆELIJA IZ KOŽE SALMONIDNE RIBE ZA IDENTIFIKACIJU RAZLIČITO EKSPRIMOVANIH GENA U ĆELIJSKOJOJ POPULACIJI

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Izvod

U cilju određivanja funkcije pigmentnih ćelija kod različitih šara na koži, primenili smo metod mikrodisekciju laserskog hvatanja (LCM) za izolaciju različitih tipova pigmentnih ćelija iz kožnih sekcija mramorne (*Salmo marmoratus*) i braon pastrmke (*S. trutta*), koje karakteriše lavirintska, odnosno tačkasta šara na koži. RNK je izolovana iz seciranih pigmenata ćelijske populacije i korišćena za ekspresionu analizu. Eksprimovani profili kandidat gena, potencijalno uključenih u pigmentaciju, u LCD izolovanoj ćelijskoj populaciji, bili su specifičniji u odnosu na različito obojene delove kože, i sadržali su heterogenu mešavinu ćelija, uključujući više tipova hromatofora. Pored toga, oni su dali značajne informacije o posebnim funkcijama pigmentnih ćelija i njihovom doprinosu različito obojenim šarama. Protokol o kombinovanom LCM i genomskom pristupu u proučavanju pojedinačnih ćelijskih populacija može biti primenjen u budućnosti, ali je potrebna dalja optimizacija da bi se dobila dovoljna količina i kvalitetna RNK za, npr. transkriptomičnu analizu.

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