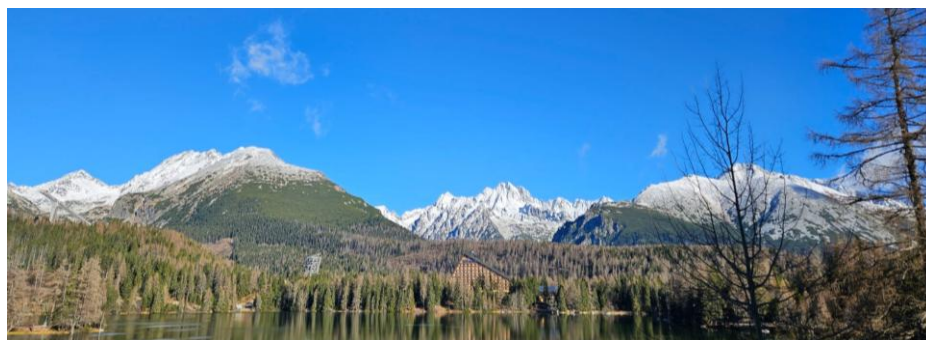
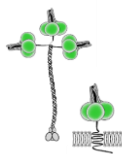




7th symposium on
**“Novel Frontiers
in Physiology and Pharmacology
of the Cholinergic System”**

Organizing Institution:
Department of Pharmacology and Toxicology
Faculty of Pharmacy
Comenius University

**15th – 19th November 2025
Štrbské Pleso**



“Novel Frontiers in Physiology and Pharmacology of the Cholinergic System”

Summary

The objective of the symposium is to discuss the latest discoveries and progress in the field of physiology and pharmacology of the cholinergic system, presented by young researchers, postgraduate students, and graduate students.

Anna Paul Hrabovská

Main Financial Support

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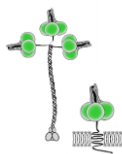
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SLOVAK RESEARCH
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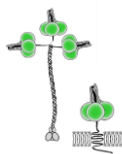
Német Lukáš

Anna Paul Hrabovská

Alexandra Zahradníková

Tomáš Fazekaš

All contributions were in the form of oral presentations. The time schedule was flexible to enable sufficient time to introduce the topic, elaborate the results, and have an engaging discussion. Presenters were encouraged present up to 25 minutes but discussions were unlimited.



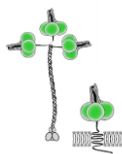
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PREFACE

The annual scientific meeting in the High Tatras once again provided a platform for dialogue, collaboration, and the exchange of research ideas among PhD students, postdoctoral fellows, and young investigators. As in previous years, the aim of the congress was to create a supportive and stimulating environment for presenting, discussing, and critically evaluating ongoing scientific work conducted by emerging researchers. The program reflected the breadth and depth of research within the community.

Tibor presented the main findings of his PhD project, which examined the involvement of butyrylcholinesterase (BChE) in metabolism and obesity. **Parsa**, a doctoral candidate, reported interim results on the role of BChE and the non-neuronal cholinergic system in the pathogenesis of heart failure, using an experimental GMO-based model. **Lukáš** explained the principles of genotyping GMO animals, which are used in these experiments, and described the laboratory work he performs as part of his research. **Anastazia**, demonstrated the application of electron microscopy and presented preliminary data from her PhD studies on pathological cardiomyocyte remodeling.

Among the postdoctoral contributions, **Julka** introduced a methodological approach for studying calcium transients and sarcomere shortening, while **Miroslava** outlined the development of a spatial transcriptomics workflow,



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intended for characterizing transcriptional changes in heart failure and for elucidating the role of cholinesterases using genetically modified models.

A new feature of this year’s meeting was the inclusion of educational lectures delivered by principal investigators. **Anna** provided an overview of the molecular forms, processing, localization, and physiological functions of cholinesterases in the heart. **Alexandra** addressed the maturation of excitation–contraction coupling in cardiomyocytes, and **Tomas** summarized the most frequent issues encountered in the statistical analysis of omics datasets.

The meeting also benefited from international expertise. Evan, an expert in sequencing and spatial transcriptomics, whose laboratory has already established these methodologies, and Eric, a recognized authority on cholinesterases, their physiology across organs, molecular properties, and variants, provided valuable insights that enriched the discussions.

This collection of abstracts reflects both the scientific rigor and the collaborative spirit that characterized the meeting. It serves as a record of the research presented and as an inspiration for further scientific exploration.

Bratislava, November 21st 2025

Anna Paul Hrabovská



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DIFFERENTIAL ROLES OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE IN NEURONAL AND NON-NEURONAL CARDIAC CHOLINERGIC SIGNALING

Anna Paul Hrabovska

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University Bratislava

Cardiac function is strongly influenced by the autonomic nervous system, and its imbalance is characteristic of cardiovascular diseases such as heart failure. While sympathetic hyperactivity is well described, recent evidence highlights the therapeutic potential of enhancing the downregulated parasympathetic system through vagus nerve stimulation or cholinesterase inhibition. Besides neuronal acetylcholine (ACh) released from parasympathetic nerves, cardiomyocytes also generate ACh as part of a non-neuronal cholinergic system, yet regulation of cardiac ACh levels remains insufficiently understood. The heart contains two cholinesterases—acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)—in several molecular forms.

Using genetically modified mice lacking specific AChE or BChE molecular forms we showed that AChE predominates in atria and is strongly enriched in the SA and AV nodes. Additionally, AChE activity is present throughout atrial myocardium and epicardium, while in ventricles it is confined to epicardial fiber-like structures and co-localizes with neurons. Functional experiments in Langendorff-perfused hearts showed that the bradycardic effect of neostigmine was abolished in AChE-deficient mice, confirming that AChE mediates the neostigmine-evoked parasympathetic response. In contrast, BChE is diffusely distributed throughout ventricular and atrial tissue, consistent with the presence of soluble monomeric forms. Single-cell RNA sequencing data indicate that BChE is produced primarily by cardiomyocytes and fibroblasts. In BChE knockout hearts, altered sensitivity to ACh and atropine, together with an exaggerated response to neostigmine, indicates disrupted ACh handling and modified muscarinic signaling.

Together, these findings indicate that AChE is part of the neuronal cholinergic system and is responsible for the bradycardic effects of cholinesterase inhibitors, whereas BChE belongs to the non-neuronal cholinergic system and regulates ACh release, potentially via presynaptic or autoregulatory mechanisms.

This project was supported by APVV-22-0541, VEGA 1/0293/25.

Keywords: Acetylcholinesterase, Butyrylcholinesterase, Cardiac cholinergic system, Parasympathetic regulation, Acetylcholine release



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FORMATION OF DYADS DURING POSTNATAL DEVELOPMENT IN THE RAT

Alexandra Zahradníková jr.^{1,2}, Simona Kazmerova³, Alexandra Zahradnikova^{1,2}

¹Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center of Slovak Academy of Sciences, Bratislava, Slovakia, ²Department of Muscle Cell Research, Institute of Molecular Physiology and Genetics, Centre of Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia, ³Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Proper contraction of cardiomyocytes requires a rapid increase in cytosolic Ca^{2+} concentration in response to an action potential, excitation-contraction (EC) coupling. In adult cardiomyocytes, this process is mediated by Ca^{2+} release from the intracellular store, the sarcoplasmic reticulum (SR). Transmission of the excitatory signal from the sarcolemma to the SR occurs in specialized structures known as dyads. Cardiomyocytes of newborn rats are not fully differentiated, and EC coupling is maintained primarily through direct Ca^{2+} influx from the extracellular space. During the maturation of cardiomyocytes, EC coupling is reorganized, and dyads are formed.

In this study, we examined the localization and colocalization of proteins involved in EC coupling (the DHP receptor Cav1.2 and the ryanodine receptor RyR2) and in dyad stability (junctophilin-2, JP-2) in fixed cardiomyocytes isolated from rat hearts at 7, 15, and 20 days of age, as well as in adulthood. We employed multiplex immunofluorescence labeling and confocal microscopy. Wheat germ agglutinin (WGA) conjugated to a fluorescent tag was used to visualize the surface membrane.

During myocyte development, we observed a translocation of sarcolemmal DHPR from the cell surface into the developing tubular system, accompanied by an apparent decrease in their colocalization with the membrane. In contrast, no change in the localization of RyR, which mediate Ca^{2+} release from the SR, was detected throughout postnatal development. Junctophilin-2, a protein responsible for maintaining dyad structure, gradually shifted from the cell surface to the cell interior, similarly to DHPR. Concurrently, colocalization of DHPR–RyR, DHPR–JP2, and RyR–JP2 progressively increased.

These findings support previous assumptions regarding the progression of dyad formation in developing rat cardiomyocytes, derived from measurements of Ca^{2+} current inactivation and the growth of the tubular system. The presence of colocalization among all three proteins in 7-day-old rats indicates that dyads are already present at the sarcolemma, and the subsequent increase in their colocalization during development is consistent with the observed enhancement of fast Ca^{2+} current inactivation.

This work was supported by APVV-22-0541.

Keywords: Excitation-contraction coupling, Dyads, Dihydropyridine receptor Cav1.2, Ryanodine receptor, Junctophilin-2



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BIOSTATISTICAL METHODS FOR INTERPRETING HIGH-DIMENSIONAL OMICS DATA: FINDING PATTERNS IN CHAOS

Tomáš Fazekaš

*Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University Bratislava,
Slovakia*

The rapid advancement of omics technologies — including genomics, transcriptomics, proteomics, and metabolomics — has led to an unprecedented influx of biological data, characterised by high dimensionality, heterogeneity, and noise. Modern analytical platforms (e.g. LC/MS, LC/MS-MS, UHPLC-QTOF, NGS, RT-qPCR) generate extensive datasets enabling the simultaneous monitoring of thousands of entities. While such “Big Data” opens new avenues for biological discovery, it also poses significant challenges for data analysis, particularly when the number of variables far exceeds the number of samples. Pattern recognition and dimensionality reduction are typically achieved through multivariate techniques (PCA, PLS, PLS-DA), advanced regression methods (LASSO, GRW), and non-linear dimensionality reduction approaches. A particularly demanding task is the integration of multi-omics datasets that span multiple biological layers.

In the applied section, we present a case study involving the analysis of high-dimensional lipidomic data obtained via mass spectrometry. The objective was to identify a minimal set of lipids capable of classifying individuals based on selected criteria (e.g. type of mutations in the BChE gene or measure of the resulting BChE activity). To support this, we developed scripts in JMP Scripting Language for automated extraction of structural information from the available mass spectrometry-based dataset for molar percentages just from listed lipid names, and also calculation of molecular properties potentially influencing BChE-mediated lipid cleavage (e.g. carbon count, double bonds, lipid class), and subsequent application of biostatistical methods to uncover biologically relevant patterns. Special attention was given to handling missing data, minimising statistical bias, and implementing robust normalisation strategies.

The outcome of this work is a tailored biostatistical workflow suitable for high-dimensional omics datasets with relatively small sample sizes, enabling the identification of molecular features that maximise subgroup discrimination among individuals.

This project was supported by APVV-22-0541, VEGA 1/0293/25, APW-22-0541.

Keywords: *Butyrylcholinesterase, Lipidomics, Multivariate Analysis, Missing Data*



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ANALYSIS OF CALCIUM TRANSIENTS AND SARCOMERE SHORTENING OF CARDIOMYOCYTES: A METHODOLOGICAL FRAMEWORK

Iuliia Baglaeva¹, Bogdan Iaparov², Alexandra Zahradníková jr.¹

¹Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center of Slovak Academy of Sciences, Bratislava, Slovakia, ²Laboratory of Bioinformatics, Institute of Experimental Endocrinology, Biomedical Research Center of Slovak Academy of Sciences, Bratislava, Slovakia

Cardiac contraction is driven by transient increases in intracellular calcium concentration that trigger sarcomere shortening and maintain proper excitation–contraction coupling. Quantitative analysis of calcium dynamics together with contractile function provides valuable insight into cardiac regulation under physiological and pathological conditions. Altered calcium handling leads to characteristic changes in the amplitude and kinetics of calcium transients and sarcomere shortening, reflecting modifications in intracellular calcium-handling mechanisms. To investigate these effects, isolated cardiomyocytes are loaded with a calcium-sensitive fluorescent dye, electrically stimulated, and observed under a confocal or epifluorescence microscope. Changes in fluorescence intensity, corresponding to fluctuations in intracellular calcium concentration, are recorded as line-scan images. Transient signals are then extracted from these images using TransientVisualizer (github.com/IuliiaBaglaeva/TransientVisualizer) and analyzed with TransientAnalyzer (github.com/IuliiaBaglaeva/TransientAnalyzerUI). Because multiple recordings originate from individual cells and animals, data are analyzed using linear mixed models (LMM) implemented in R to account for hierarchical variability.

This methodological framework provides a reproducible and statistically robust approach for evaluating calcium-handling dynamics and contractile function across diverse experimental conditions.

This project was supported by APVV- 22-0541.

Keywords: Calcium transients, sarcomere shortening, cardiomyocytes, excitation–contraction coupling (ECC), linear mixed models (LMM), analysis of transients



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SPATIAL TRANSCRIPTOMICS AS A NOVEL APPROACH FOR THE ASSESSMENT OF THE CARDIAC CHOLINERGIC SYSTEM

Miroslava Molitorisová, Anna Paul Hrabovská

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University Bratislava

The cardiac cholinergic system, encompassing both neuronal and non-neuronal components, plays a crucial role in the regulation of heart rate, conduction, contractility, and cardioprotection. Despite increasing evidence for the importance of cholinergic pathways in the heart, their spatial organization within cardiac tissue remains poorly characterized.

To overcome the current gaps in existing knowledge, we decided to implement spatial transcriptomics as a novel methodological approach in our laboratory. Spatial transcriptomics is a cutting-edge technique that measures gene expression while preserving the spatial context within tissue samples.

In our workflow using the Visium CytAssist platform (10x Genomics), mouse cardiac tissue cryosections are first placed on glass slides and stained with hematoxylin and eosin (H&E) to assess tissue morphology. After imaging and destaining, probes complementary to the whole transcriptome are hybridized and ligated on the tissue section. The cardiac tissue is then transferred to a special Visium capture slide in the Visium CytAssist instrument, where the ligated probes are released and captured by spatially barcoded spots on the Visium slide. Spatially-barcoded ligation products are amplified and indexed by using qPCR to generate final library molecules. The libraries are subsequently cleaned up by using SPRISelect, assessed on a Bioanalyzer, quantified by qPCR, and finally sequenced on an Illumina platform to generate transcriptomics maps, providing a detailed overview of gene expression patterns across different regions of the heart.

The implementation of spatial transcriptomics in our laboratory will provide new insights into cardiac cholinergic pathways and their contribution to cardiac physiology and pathology.

This project was supported by APVV-22-0541, VEGA 1/0293/25.

Keywords: cholinergic system, heart, spatial transcriptomics



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ROLE OF BUTYRYLCHOLINESTERASE IN HELP TO COPE WITH HIGH-CALORIC DIET

Tibor Hodbod¹, Tomáš Fazekaš², Anna Paul Hrabovská¹

¹Department of Pharmacology and Toxicology, ²Department of Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University Bratislava

Butyrylcholinesterase (BChE) has been associated with metabolic regulation through its effects on ghrelin and energy-balance pathways, particularly in mouse high-fat diet models. Although high-caloric (HC) diets typically raise BChE activity, the biological purpose and metabolic consequences of this response are not yet fully elucidated, and may differ in rats, which are the focus of the present study. Male Wistar rats (8 weeks old) were fed standard chow or a high-fat/high-fructose diet for 16 weeks, with parallel subgroups receiving the selective BChE inhibitor isoOMPA (3 mg/kg/day). Body weight and caloric intake were monitored weekly. Rats were sacrificed under fasting conditions and serum, liver samples were collected. Glycemia, triacylglycerols, ketone bodies, and plasma concentrations of total and active ghrelin, leptin, and insulin were determined. Serum BChE activity was assessed by a modified Ellman's method, and hypothalamic mRNA expression of BChE, MBOAT4, GHSR, and c-Fos was quantified by RT-qPCR. HC feeding significantly increased serum BChE activity, but BChE inhibition did not affect food intake, body weight, glycemia, plasma ghrelin, or insulin. Leptin increased only due to adipose accumulation. HC feeding also reduced hypothalamic c-Fos expression, suggesting decreased neuronal activation, while BChE, MBOAT4, and GHSR expression remained unchanged. As expected, HC diet elevated β -hydroxybutyrate (BHB); however, when HC feeding was combined with BChE inhibition, this increase was abolished and BHB levels remained comparable to both standard diet groups. Both HC intake and BChE inhibition influenced thermoregulation, supporting a role of BChE in temperature maintenance during stress conditions. In conclusion, increased HC intake accompanies BChE activity, but does not alter feeding behaviour, glucose handling, or hypothalamic signalling indicating a compensatory response to metabolic load. Instead, BChE inhibition prevents the expected rise in ketone bodies level and potentially modifies lipid utilisation and play role in thermometabolic stability.

This project was supported by APVV-22-0541, VEGA 1/0293/25.

Keywords: Butyrylcholinesterase, High-caloric diet, β -hydroxybutyrate, Lipid metabolism, Thermoregulation



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QUANTITATIVE ANALYSIS OF CARDIOMYOCYTES AND CARDIAC MUSCLE ULTRASTRUCTURE USING ELECTRON MICROSCOPY

A. Novak¹, I. Baglaeva¹, M. Novotová¹, B. Iaparov², and A. Zahradníková Jr.¹

¹Department of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center of Slovak Academy of Sciences, Bratislava, Slovakia, ²Laboratory of Bioinformatics, Institute of Experimental Endocrinology, Biomedical Research Center of Slovak Academy of Sciences, Bratislava, Slovakia

Quantitative analysis of cellular ultrastructure is essential for understanding morphological changes in biological samples.

Morphometry focuses on 2D analysis but delivers precise quantitative data on parameters such as density area, and the number of organelles within a defined cellular region. In contrast, stereological approaches enable the reconstruction of three-dimensional (3D) characteristics of cellular components based on two-dimensional (2D) images, providing information on volume ratios and spatial organization.

Stereological and morphometric methods provide a deeper understanding of cellular architecture. They allow quantitative assessment of organelle volumes, dimensions, and the density of specific structures within cells or tissues, contributing to a better understanding of structural changes at the cellular level. Measurements will be performed using the specialized software EMAnalyzer, and analytical methods such as linear mixed models (LMMs) will be presented to ensure robust statistical interpretation.

Careful sample preparation, accurate data collection and analysis, and the integration of stereological and morphometric approaches are all essential for obtaining reliable results and a deeper understanding of structural changes at the cellular level. Neglecting any of these steps reduces the accuracy and interpretability of the findings.

This research was supported by the project: APVV-22-0541

Keywords: Morphometry, Stereology, Electron microscopy, Linear mixed models, Ultrastructure



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COMPARISON BETWEEN WILD-TYPE AND BChE KNOCKOUT DURING HEART FAILURE MOUSE MODEL

Parsa Shafieikazerooni¹, Alexandra Zahradníková jr²., Miroslava

Molitorisova¹, Iuliia Baglaeva², Anastasiia Novak², Anna Paul Hrabovska¹

¹ Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University in Bratislava, Bratislava, Slovakia, ² Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovak Republic

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) govern acetylcholine signalling in the heart, influencing autonomic regulation, contractility and stress responses. While AChE is mainly in neuronal tissue and atria, BChE predominates in ventricular myocardium, yet its role in cardiac disease remains poorly defined.

This study explored BChE function in a pressure-overload heart failure (HF) model using BChE knockout (BChE^{-/-}) and wild-type (WT) mice aged 5 months, subjected to transverse aortic constriction (TAC). After seven weeks, echocardiography and electrocardiography (ECG) were used to assess cardiac structure and function. In echo, left ventricular internal dimension (LVID), interventricular septum thickness (IVS), left ventricular posterior wall thickness (LVPW), fractional shortening (FS), and ejection fraction (EF) were measured. Subsequently, hearts were harvested for calcium-transient analysis, and blood was collected for plasma BNP measurement.

Echo data revealed that BChE^{-/-} HF mice showed a decrease LVID at end-systole compared to WT HF, and increased IVS and LVPW compared to both BChE^{-/-} controls and WT HF. EF and FS showed trends toward an alteration in BChE^{-/-} HF mice. ECG revealed increase in P and QT intervals durations in WT HF mice. Plasma BNP was elevated in the BChE^{-/-} HF group. Calcium-transient data showed increased signalling amplitude in both BChE^{-/-} HF and WT HF groups relative to controls.

Taken together, these findings suggest that BChE deficiency alters ventricular remodelling, calcium handling and biomarker activation under pressure overload, indicating that BChE contributes meaningfully to myocardial adaptation in HF.

This project was supported by APVV-22-0541, VEGA 1/0293/25.

Keywords: Heart Failure, calcium transient, Butyrylcholinesterase, Echo, ECG



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HIGH-RESOLUTION MELTING AND PCR GENOTYPING WORKFLOW FOR POINT-MUTATED AND DELETION VARIANTS OF AChE AND BChE IN GENETICALLY MODIFIED MICE

Lukáš Némec, Anna Paul Hrabovská

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University Bratislava

Genotyping is essential for the breeding and use of genetically modified mouse strains in our research. We work with two CRISPR-Cas9-generated strains carrying single-point mutations in the active sites of acetylcholinesterase (AChE; wAChE) and butyrylcholinesterase (BChE; SBChE), as well as a delAChE-liRR knockout strain containing a 250-bp deletion in intron 1 of the AChE gene. To reliably distinguish wild-type, heterozygous, and knockout genotypes, we implemented a PCR-based genotyping workflow with high-resolution melting (HRM) analysis.

DNA is obtained from ear-punch samples and extracted by alkaline lysis, followed by neutralization and storage at 4 °C. PCR amplification of the target loci is performed using locus-specific primers, and genotypes are assigned based on melting-curve profiles. HRM is used for the wAChE and SBChE strains, where single-nucleotide substitutions produce small differences in melting temperatures; therefore, precise and gradual temperature increase is required. In contrast, the 250-bp deletion in the delAChE-liRR allele yields a large melting-temperature shift, making HRM unnecessary. Fluorescent DNA-binding dyes such as SYTO 9 allow discrimination of alleles, and recent verified controls are included in each run to ensure accurate assignment of melting peaks. Wild-type and knockout samples exhibit single, distinct melting peaks, whereas heterozygotes display two peaks corresponding to each allele.

This workflow provides a rapid, reliable, and cost-effective method for routine genotyping of our engineered mouse strains.

This project was supported by APVV-22-0541, VEGA 1/0293/25.

Keywords: PCR genotyping; high-resolution melting; CRISPR-Cas9; acetylcholinesterase; genetically modified mice



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