DEVELOPMENT OF A DNA OLIGO-CAPTURE METHOD TO STUDY CANCER CELL METASTASIS IN ANIMAL MODELS

Roberta Pocevičiūtė[1], Šarūnas Tumas[1], Anna Malkova[2], Michael Sherman[2]

[1]Cureline Baltic, Lithuania
[2]Faculty of Natural Sciences, Department of Molecular Biology, Ariel University, Israel
roberta.poceviciute@cureline.com

DNA barcoding is a method that allows to track and identify specimens over time using short, specific DNA sequences, which are integrated into the genome by lentiviral tranduction. It is widely used to study organ development and cancer progression. Traditional cancer treatments are less effective against metastasis and there is a lack of effective cancer metastasis models for testing new cancer drugs on. Cell DNA barcoding is a valuable tool for evaluating potential cancer drugs on primary tumors and metastasis, allowing simultaneous tracking of differently barcoded cell lines, which allows to compare the drug effects across different cell populations in the same animal using next generation sequencing. Standard sequencing library preparation relies on 2 rounds of PCR amplification, which is time consuming and can lead to PCR artifacts.

This project aims to develop the protocol for DNA barcode capture by streptavidin-coated magnetic beads from mouse organs. Although PCR is mostly utilized for barcodes extraction, hybridization-based technique is more sensitive, less time consuming and cost-effective. This technique captures in hybridization formed complex made of targeted region (barcode) and biotinylated probe, which is later pulled with magnetic bead. Results of the capture were analyzed using gel electrophoresis and qPCR.

To validate this method, the capture system was optimized in vitro in the absence of any nonspecific DNA sequences and in the presence of nonspecific DNA. Optimal probe length, probe biotinylation, hybridization time and temperature were determined. A major improvement to the capture system was achieved when 5' biotinylated probe was changed to an internally biotinylated probe which captures barcodes 24 times more efficiently. Moreover, $1,31 * 10^9$ of barcode copies were isolated, which is enough for capturing all unique barcodes in a mice organ, considering that cells were transfected with $5 * 10^6$ of unique barcodes. Unspecific DNA had no impact on capture efficiency and barcodes fraction had no significant amount of unspecific DNA residues. Further steps are to optimize the extraction of barcodes from cell and organ lysates and compare the developed protocol with the PCR-based protocol.