ISSN: 3066-716X
Research Article
Journal of Clinical and Biomedical Advances

Laser Capture Microdissection (LCM) of Chromosomes from the Species Myotis Daubentonii (Chiroptera: Vespertilionidae) for NGS Sequencing

António Jorge Correia[1]*, Jorge Claúdio da Costa Pereira[2], & Maria Filomena Lopes Adega[3]

[1]Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[2]RISE-Health, UTAD, Vila Real, Portugal
[3]BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Portugal

***Corresponding author:** António Jorge Correia, Centre for Functional Ecology, Associate Laboratory TERRA, Department of Life Sciences, University of Coimbra, Coimbra, Portugal.

Submitted: 09 July 2025 **Accepted:** 15 July 2025 **Published:** 21 July 2025

https://doi.org/10.63620/MKJCBA.2025.1048

Citation: *Correia, A. J., Pereira, J. C. da C., & Adega, M. F. L. (2025). Laser Capture Microdissection (LCM) of Chromosomes from the Species Myotis Daubentonii (Chiroptera: Vespertilionidae) for NGS Sequencing. J of Clin Bio Med Adv, 4(4), 01-09.*

Abstract

Laser Capture Microdissection (LCM) is an automated technique that allows the separation of cell subgroups, specific cells, or chromosomes from a heterogeneous population. Using an inverted microscope, a laser, and a sample collection system, LCM enables microscopic observation of cells, precise sectioning of regions of interest, and subsequent sample collection in a single operation. This technique is widely used in various scientific fields, particularly in cytogenetics, to isolate specific chromosomes or chromosomal regions. However, due to the small amount of DNA obtained, amplification is necessary. One of the amplification methods used was the commercial GenomePlex WGA4 Single Cell kit. In this study, chromosomes were isolated using LCM and amplified using the whole genome amplification (WGA) methodology, with the aim of using them as chromosome probes (Whole Chromosome Paints - WCP) in molecular cytogenetic analyses.

Keywords: Laser Capture Microdissection, WGA, WCP.

Introduction

Laser Capture Microdissection (LCM) Technique

Laser capture microdissection (LCM) is a procedure primarily designed for the collection of specific and pure cell populations from different tissue sections for subsequent DNA and RNA extraction. Inevitably, LCM has been adapted for other types of isolation and collection, such as the isolation of individual chromosomes or specific regions of these chromosomes, such as centromeres. This is only possible due to the adaptive capacity of this method, allowing microscopic scanning of slides specific for this methodology, as well as laser cutting of regions of interest (ROIs) and subsequent sample collection, all in a single operation [1]. Table I presents some of the advantages and disadvantages of LCM in relation to other microdissection methodologies.

Table 1: Advantages and Disadvantages of Laser Capture Microdissection [6].

Advantages	Disadvantages
Contactless tissue capture, which	Becomes less effective when ROIs narrow to cellular or subcellular resolution
reduces the likelihood of contamination	Damages tissue surrounding the ROIs
Allows for isolation of cells in paraffin or	Requires staining steps that can directly affect subsequent analysis

frozen samples	Requires the assistance of an imaging system

Different Types of Laser Capture Microdissection (LCM)

The concept of laser capture microdissection was first proposed in 1986 for the purpose of microdissecting/isolating human chromosomes [2].

In 1996, Emmert-Buck and his team developed the IR-LCM (Infrared Laser Capture Microdissection) system to separate specific target cells from tissue samples using an 810 nm infrared laser [3].

Later, the Palm MicroBeam and Leica LMD laser microdissection systems were developed, presenting significant technological advances over IR-LCM [1]. Nowadays, LCM is a widespread and universal technology with several systems available, mainly associated with different microscopy and imaging companies, such as Zeiss (ZEISS Group), Leica (Leica Microsystems) among others [4, 5]. Currently, four different types of laser capture microdissection are used (figure I) that allow for the precise separation of ROIs from diferentes tecidos. Estes diferem bastante no que diz respeito aos dispositivos de colheita, fontes de laser e técnicas de isolamento [1].

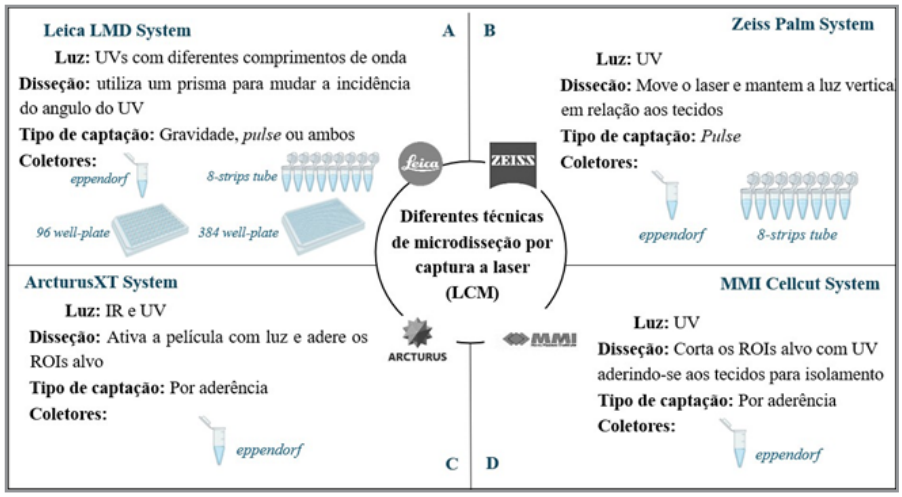

Figure 1: Illustration of the schematics of four currently used Laser Capture Microdissection (LCM) systems. A: Leica LMD System; B: Zeiss Palm System; C: ArcturusXT System; D: MMI Cellcut System [1].

Laser Capture Microdissection and its Uses

LCM has emerged as an efficient, contact-free method for isolating specific cells from different organs or tissues through microscopy, becoming an essential tool for preparing samples with omics information for subsequent analysis. Therefore, proper sample preparation is crucial for successful capture [1].

This technique can be applied to histological models, live cells and cell cultures, plant material, chromosome preparations, formalin-fixed and/or paraffin-embedded (FFPE) or frozen tissues, stained and unstained tissues, among others (Datta et al., 2015). The use of LCM for omics studies has proven to be highly versatile, due to the continuous development of recording techniques, analytical methods, and resolution systems [1], with applicability in genomics, epigenomics, transcriptomics, proteomics, and metabolomics [6-10].

LCM and its Applicability in Cytogenetics

LCM has become a powerful tool in many scientific fields, such as cytogenetics, as it allows the isolation of individual chromosomes or even specific intrachromosomal regions. The subsequent procedure and molecular analysis are very challenging, since the amount of DNA obtained is significantly low, requiring amplification [11]. Several methods have been tested to amplify DNA obtained through LCM, including DOP-PCR (Degenerated Oligonucleotide Probe-PCR).

It has proven to be a proficient technique for amplifying unknown DNA sequences, such as those obtained with this procedure, chromosomal regions, and entire chromosomes from different species, especially for the construction of whole chromosome probes (WCPs). More recently, commercial kits optimized for single-cell amplification, such as the GenomePlex WGA4 Single Cell kit and the Repli-G Single Cell kit, have been used previously through next-generation sequencing (NGS) [12, 13].

Objectives

The Objectives of this Practical Work were as Follows

1. Literature review on laser capture microdissection (LCM) of chromosomes.
2. Preparation and acquisition of chromosome suspensions through in vitro culture of cell lines of the study species, Myotis daubentonii (MDA), previously determined by the host laboratory.
3. Application of the microdissection technique to obtain isolated chromosomes of Myotis daubentonii (MDA).
4. Amplification of Myotis daubentonii (MDA) chromosomes using the Whole Genome Amplification (WGA) method.
5. Labeling of the chromosomes obtained by PCR and molecular cytogenetic analysis of one of these probes in fixed chromosome preparations of the same species, Myotis daubentonii (MDA).

Material and Methods

To carry out this practical work related to this internship, the following was carried out: Work flow:

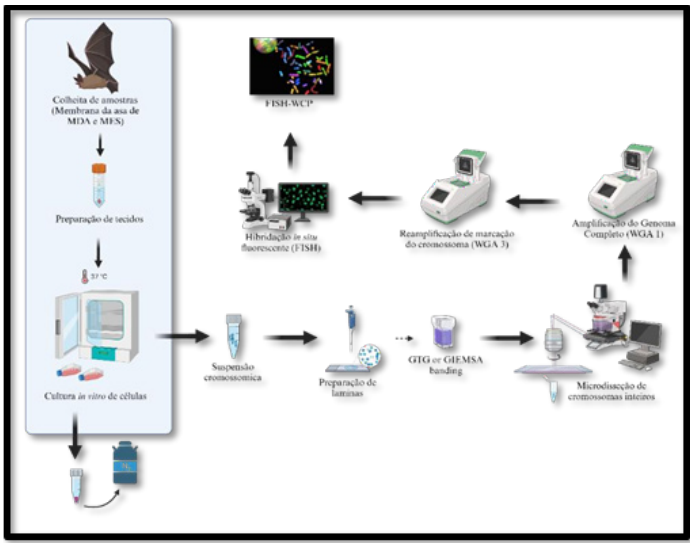

Figure 2: Workflow of the practical work carried out, which included: in vitro cell culture, obtaining chromosome preparations, laser capture microdissection, chromosome amplification, and fluorescent in situ DNA hybridization

In Vitro Cell Culture

This practical work used two cell lines corresponding to two bat species: MDA (Myotis daubentonii) and MES (Myotis escalerai). These cell lines were previously established in the Cytogenomics Laboratory. During this internship, the cell lines of both species under study were maintained in in vitro culture, to be later used in the laser capture microdissection technique.

Protocol 1: Establishment of in Vitro Culture from Cryopreserved Cells

These two cell lines were cryopreserved in Freezing Medium (containing DMSO and FBS) in liquid nitrogen at a temperature of -196°C.

1. Remove the aliquots containing the cells from the cryopreservation container and thaw them by touching them with your palms (±37°C). Note: This step is crucial to promote heat shock and healthy recovery of cellular activity.
2. Transfer the cells to a 15ml Falcon tube and add 4ml of complete culture medium (Table II). Note: At 37°C, some components of the cryopreservation medium can be toxic to the cells, compromising cell recovery.
3. Centrifuge the tube at 1200 rpm for 5 minutes.
4. Discard the supernatant and add 4ml of complete culture medium previously warmed to a temperature of ±37°C.
5. Transfer the cells to a properly labeled T25 culture dish (25cm²).
6. Incubate the cells at 37°C with 5% CO2.

Table 2: Constituents and Respective Concentrations of the Complete Culture Medium.

Complete Culture Medium
1% L-Glutamine (200 mM)
1% PNS (5 mg/ml Penicillin; 5 mg/ml Streptomycin; 10 mg/ml Neomycin)
5% AmnioMAX Complete Supplement
10% Fetal Bovine Serum (FBS)
DMEM Basal Medium

Protocol 2: Subculture

When the cells reach confluence values of approximately 70-80%, they must be subcultured to prevent a slowdown in growth and, consequently, ensure their maintenance in in vitro culture.

1. Remove the culture medium and then wash the cells with HBSS.
2. Add 1 ml of TrypLE™ Express Enzyme (1X) and incubate for 1 minute at 37°C.
3. Add 3 ml of FBS to inhibit the action of trypsin.
4. Transfer the suspended cells to a new culture dish and add the required volume of complete medium.
5.Place the culture dishes at 37°C.

2.2 Obtaining chromosome preparations. When the culture dish reaches the required confluence (70%-80%), cells are harvested to obtain chromosome preparations. These preparations will later be used in the laser capture microdissection (LCM) procedure to obtain chromosomes.

Protocol 3: Chromosome Preparations

1. Add 80 µL of colcemid (10µg/ml) to the culture dish with 8 ml of culture medium and incubate for 1 hour at 37°C. Note: During the colcemid period, visualize the cells under a microscope and check for an increase in metaphase cells, checking for the presence of round cells or cell doublets.
2. After one hour, collect the dividing cells by shake-off and/or trypsinization. Note: If using trypsin, wash with HBSS and add TrypLE™ Express Enzyme (1X) for 1 minute at 37°C. Observe the action of the trypsin under a microscope. When the cells are all circular, lift them from the box mechanically (by tapping the

box on the table or tapping the side of the box with your hand).
3. Stop the trypsin action by adding complete culture medium to the cell box and mixing the cells well with a micropipette. Remove approximately ¾ of the cells in a 15 ml falcon. Add more complete medium to the remaining volume and place in an incubator at 37°C.
4. Centrifuge the 15 ml falcon containing the cells at 1800 rpm for 10 minutes.
5. Discard the supernatant and resuspend the pellet in a hypotonic solution (Table II). 6. Incubate the tube at 37°C for 12-16 minutes.
6. Centrifuge at 1800 rpm for 10 minutes.
7. Discard the supernatant and resuspend the pellet in freshly prepared fixative (Table III). Top up to 8 ml with fixative.
8. Centrifuge the Falcon at 1800 rpm for 10 minutes.
9. Repeat steps 7 and 8 twice.

Store the Chromosome Suspension at -20°C

Table 3: Composition and Respective Proportions of the Hypotonic Solute and Fresh Fixative

Hypotonic Solute	Fresh Fixative
0.075M potassium chloride solution	3:1 Methanol:Glacial Acetic Acid

Laser Capture Microdissection

The LCM system used in this study is the Zeiss PALM System, which consists of an inverted microscope, ultraviolet laser, sample collection and placement system, software, and imaging system.

Protocol 4: Preparation of slides for microdissection
1. Sterilize the MembraneSlide 0.17 PET slides in UV for 15 minutes in an open-lid Petri dish and in UV for 15 minutes in a closed box. Note: The slides used were "MembraneSlide 0.17 PET," consisting of a slide and coverslip set with specifications that differ from conventional coverslips and slides used in cytogenetics laboratories.
2. Resuspend, pipette, and drop a drop of the chromosome suspension onto the coverslip of the MembraneSlide 0.17 PET set. Note: Ensure that the material has been spread when performing the drop. In addition to spreading, it is important to verify that the number of metaphases is satisfactory for chromosome isolation by LCM.
3. Staining of chromosome preparations in 4% Giemsa for 25 minutes. Note: In a container, add 91 ml of water, 4 ml of Giemsa, and 5 ml of phosphate buffer. Silver is placed around the container to block light to obtain 4% Giemsa.

Protocol 5: Laser Capture Microdissection of Chromosomes

1. Clean the microscope with 100% ethanol and wear sterile gloves.
2. Turn on the power supply, laser, and computer (by turning the switches inserted into them).
3. Turn on the computer screen (press "ENTER" only).
4. Ensure that the microscope is set to the 10x objective. 5. Open the "Pablorood" window on your computer.
5. Select the "No" option.
6. The program will display an error; press "Ok" until it opens.
7. Turn on the microscope. Note: If a green background appears, this indicates successful operation.
8. Install the Eppendorf in the appropriate location to store the area to be catapulted.
9. Examine chromosomes with the 10x objective, focusing on the drop location, progressively increasing the objective and its focus (10x, 40x, and 100x). Note: When using the 40x and 100x objectives, add immersion oil.
10. Take photos, if necessary, in the upper right corner of the open program.
11. Delimit the area of interest with the "Pencil" tool, and press the "ENTER" key to perform the section.
12. Close the Eppendorf to store the catapulted contents.
13. After completing the desired views, place the microscope on the 10x objective, wipe off excess immersion oil, and turn off all systems. Note: Since the laser capture microdissection microscope has three slide holders, we can switch between views by clicking the "View navigator window" in the upper left corner. When changing objectives, select the objective in the upper right corner to adjust the cursor speed relative to the microscope view.

Whole Genome Amplification (WGA)

The amplification method for the microdissected chromosome was performed using a commercial kit: GenomePlex® Whole Genome Amplification (WGA1), which allows for 500-fold representative amplification of genomic DNA. This kit uses a three-step amplification technology: random fragmentation of genomic DNA, OmniPlex library preparation, and PCR amplification. In this study, the chromosome to be amplified using the previous methodology was chromosome 3 (HSA3). Note: When amplifying the chromosome obtained by microdissection, a sample of Human Genomic DNA Control included in the kit was added, serving as a positive control.

Fragmentation

1. Add 1µl of 10x fragmentation buffer to 10µl of DNA/chromosomes (1ng/µl) in a PCR tube.
2. Place the tube in a thermocycler at 95°C for exactly 4 minutes.
3. Immediately after step 2, place the sample on ice and centrifuge it to consolidate the contents.

Library Preparation

1. Add 2µl of 10x Library Preparation buffer to each sample.
2. Add 1µl of Library Stabilization Solution. 3. Centrifuge the sample and place it in a thermocycler at 95°C for 2 minutes.
3. Immediately place the sample on ice and centrifuge it.
4. Add 1µL of library preparation enzyme and centrifuge briefly.
5. Place the sample in a thermocycler and incubate as follows:

Table 4: Temperatures and Respective times Indicated for the Different Stages in the Thermocycler

	Temperature	Time
1º	16ºC	20 minutes
2º	24ºC	20 minutes
3º	37ºC	20 minutes
4º	75ºC	20 minutes
5º	4ºC	PAUSE

7. Remove the samples from the thermal cycler. They can be amplified immediately or stored at -20°C for 3 days.

Amplification

1. Add the following reagents to the sample tube for the reaction:

Table 5: Reagents and Respective Volumes of the Main Amplification Reaction

Reagent	Volumes
Amplification Master	7,5µl
Water for Thawing	47,5µl
WGA DNA Polymerase	5µl
Total Main Reaction Volume	15µl

2. Centrifuge and place in the thermocycler with the following program:

Table 6: Temperatures and Time Required for the Respective Programs in the Thermal Cycler

Programs	Temperature	Time
Initial Denaturation	95°C	(3 minutes)
Run 14 cycles as follows:		
Denaturation	94ºC	15 (15 seconds)
Anneal	65ºC	5 minutos

3. After completing the program, keep the reactions at 4°C or store at -20°C until use for analysis or purification.

Protocol 5: Agarose Gel Electrophoresis

1. Prepare a gel mounting tray with a comb.
2. Weigh 0.7g of agarose into 60ml of 1x TBE in an Erlenmeyer flask to prepare a 1.2% gel.
3. Dissolve the agarose in the microwave until a completely clear solution is obtained, free of suspended particles.
4. Place the agarose solution in a glass vessel and add 20µl of Ethidium Bromide (EtBr) with a stock concentration of 0.6mg/ml. Mix thoroughly and place the agarose in the previously prepared mounting tray. 5. After gelling, remove the comb from the agarose gel and check that the wells are intact.
5. Place the gel in an electrophoresis tank and ensure it is covered with 1xTBE buffer solution.
6. Deposit the DNA samples (5µL) with deposition solution (2µL) and molecular marker (2µL).
7. Connect the electrophoresis tank to the power supply for 3 minutes at 80V.
8. View on the Gel Doc XR+ system (Bio-Rad) and photograph the gel with the Molecular Imager® Gel Doc™ XR software (Bio-Rad).

DNA Fluorescent In Situ Hybridization (DNA-FISH)

The DNA-FISH technique allows the analysis of nucleic acids based on the formation of DNA/DNA hybrids that are detected by fluorescence methods. As mentioned previously, HSA3 was amplified and labeled with biotin (indirect labeling), and in the FISH assay, we added a WCP HSA15 already directly labeled with FITC, enabling dual FISH with two HSA WCP probes. The human chromosomes used in this FISH as a chromosome suspension come from a HCE-T (Human Corneal Epithelial Cells-Transformed) cell line [14].

2.5.1 Protocol 6: DNA Fluorescent In Situ Hybridization (DNA-FISH). The FISH methodology involves 6 essential steps (Figure III): preparation of the target material (chromosome preparations) and pretreatment of the chromosomal targets, denaturation of the probes and chromosomes, hybridization of the probe to the chromosomal targets, post-hybridization washes, detection of hybridization sites (in the case of indirect labeling), staining and mounting of the slides.

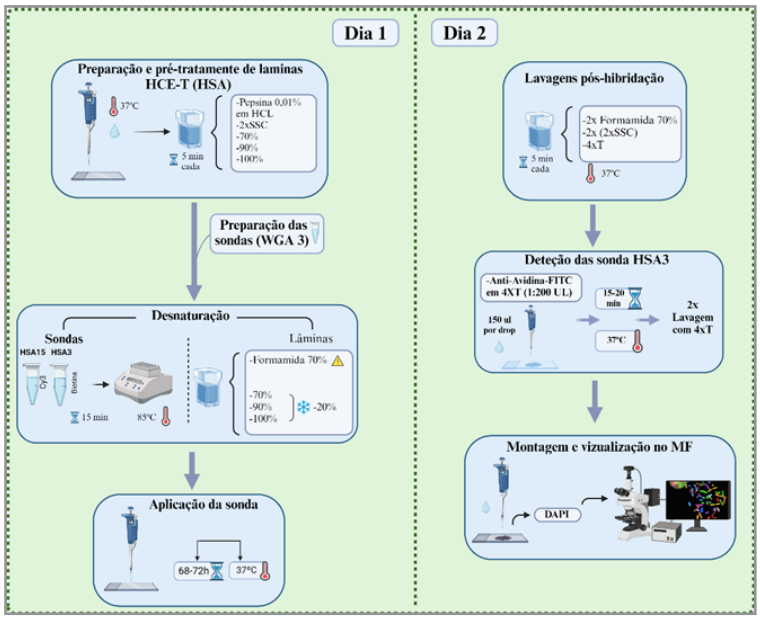

Figure 3: Workflow of the Fluorescent In Situ Hybridization (FISH) Protocol used in this Practical Work.

Analysis and Discussion of Results

In Vitro Culture of MDA and MES Cells

Preparation of species-specific WCP or genomic material for NGS sequencing from microdissection requires first obtaining chromosome suspensions of the species under study. This practical work used two cell lines from two bat species: MDA (Myotis daubentonii) and MES (Myotis escalerai). One of the objectives of this work was to establish cell lines from cells previously cryopreserved in the Cytogenomics Laboratory (Protocol 1). After establishment, it was initially observed that the growth of MDA and MES cells, compared to previous passages of these cell lines, was quite slow, as eight days after the start of the protocol, the culture dishes had not yet reached 60% confluence. This slow growth can be explained by the very low number of active cells that remained after the thawing process, and this reduced number of active cells is due to the inefficiency of the cryopreservation technique and/or the number of cells used to cryopreserve.

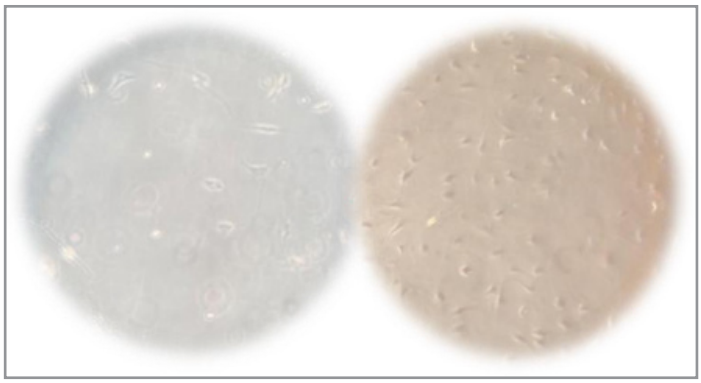

Figure 4 & 5: In the culture on the left, we can observe the growth of MDA at the end of day 5 in culture, and in the figure on the right, we can observe MDA cultures on day 8 of culture.

Comparing the growth between the two cell lines, we can also confirm that the MES culture grew substantially faster due to the greater number of active cells present in the thawed aliquot. To accelerate the growth of MDA and MES, we changed the culture medium to α-DMEM by adding 5% more AmnioMax complete, increasing cell growth in both species. These cell lines were then used to obtain metaphase suspensions.

Laser Capture Microdissection of MDA Chromosomes

One of the most important points in chromosome microdissection is the preparation of slides with complete metaphases, with individualized and non-overlapping chromosomes. The coverslip that is part of the "MembraneSlide 0.17 PET" set does not allow for natural spreading of chromosomes across the surface, so it was necessary to optimize the dropping technique for better spreading of the chromosomes in order to individualize them. Factors such as changing the fixative ratio, drop height, humidity, increasing the number of drops on the coverslip, and using fixative after dropping were tested in this practical work. Increasing the number of drops along the coverslip (one drop next to the other) combined with applying a fixative drop after dropping the chromosome suspension helped increase the number of metaphases per coverslip as well as chromosome spreading. For better visualization and capture of the chromosomes, 4% Giemnsa staining was used in Protocol 4. The execution of the protocols in point 2.3 allowed visualization of the chromosomes intended

for microdissection, but due to technical issues (the laser did not have sufficient power for cutting and collecting the specific chromosome), it was not possible to perform the cutting. Despite not obtaining the desired chromosomes through microdissection in MDA, the practical work and subsequent methodologies were possible. From this point on, we used chromosomes previously microdissected from a normal HSA cell line.

Chromosome Amplification by WGA

The isolation of specific chromosomes or even specific intra-chromosomal regions alone is not sufficient for their use in either NGS methodologies or subsequent molecular cytogenetic techniques. In addition to maintaining their three-dimensional physical structure, they also contain a significantly low amount of DNA, requiring amplification. Among the various existing methodologies, a commercial kit optimized for single-cell amplification, GenomePlex WGA4 Single Cell, was used. The protocol presented in section 2.4, despite its ease of implementation, always depends on the purity and quantity of the starting material. One way to control the yield of this methodology is to introduce a positive control, in the case of a human sample in the WGA amplification. Observing Figure VI, we can see that the samples amplified by this methodology presented smear within the desired values, as did the positive control.

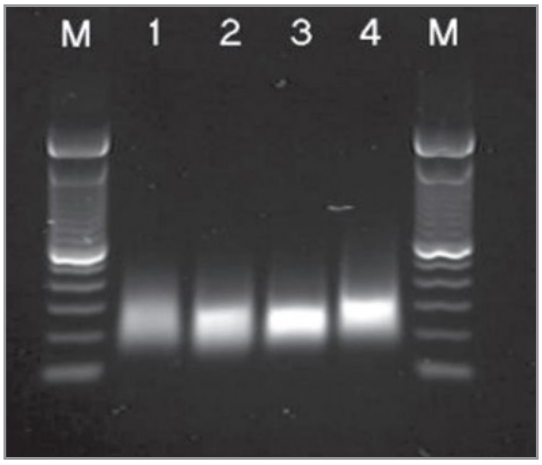

Figure 6: Agarose gel electrophoresis of DOP-PCR products after WGA protocol. M: 100bp DNA molecular marker from Invitrogen. 1: DOP-PCR product of chromosome HSA4. 2: DOP-PCR product of chromosome HSA4. 3: DOP-PCR product of chromosome HSA3. 4: DOP-PCR product of the positive control.

Fluorescent DNA In Situ Hybridization (DNA-FISH)

One of the objectives of this practical work was the isolation and amplification of chromosomes to obtain chromosome probes (WCP) using the whole genome amplification (WGA) methodology. For this to be possible, the amplified DNA must be labeled by PCR with labeling dUTPs linked to a fluorochrome. This protocol was not performed using pre-labeled WCPs. To test the construction and specificity of the WCPs, the FISH technique (point 2.6) was used, with the hybridization of the human probes, HSA3 and HSA15, on chromosomes of a human corneal cell line - HCE-T. Observing Figure VII, we see that there was hybridization of the HSA3 and HSA15 probes on the chromosomes of the HCE-T cell line. Since the objective of this work is not the cytogenetic analysis of the specific cell line, it is possible, however, to verify that the HCE-T cell line is a line with an altered karyotype both in terms of chromosome number and rearrangements. structural. This is verified by the presence of hybridization signals of chromosomes 3 and 15 in more than two pairs of complete chromosomes

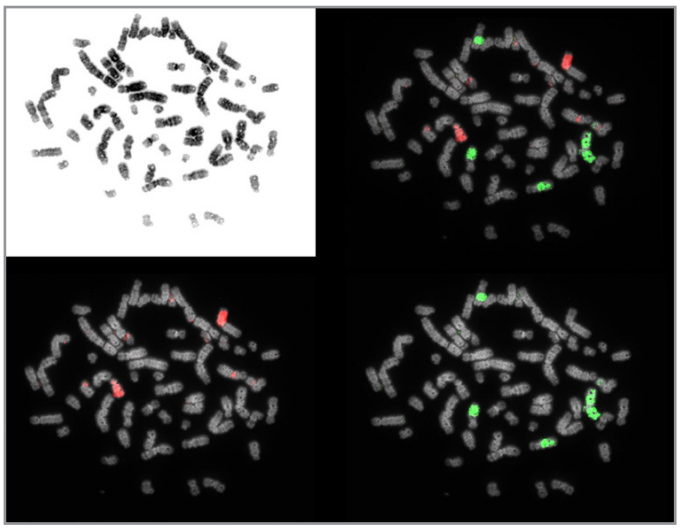

Figure 7: Hybridization of chromosomes HSA3 (red arrows) and HSA15 (green arrows) on the chromosomes of the HCE-T cell line. a) Metaphase with reverse DAPI banding. b) Dual-FISH of the HSA3 (red) and HSA15 (green) probes. c) FISH with the HSA3 probe. d) FISH with the HSA15 probe.

The laser capture microdissection technique for obtaining WCP chromosome probes has already been successfully used in several studies in humans and other animals [14, 15],namely for the isolation of derivative chromosomes and for use in evolutionary studies [16]. One of the advantages of using laser microdissection in conjunction with WGA amplification is that it is possible to obtain chromosome-specific probes by isolating a small number of copies of the target chromosome [14].

General Conclusions and Future Prospects

The general objective of this practical work was initially to isolate chromosomes by laser capture microdissection (LCM) and amplify them using the whole genome amplification (WGA) method, so that they could be labeled with fluorochromes and used as chromosome probes in molecular cytogenetic analyses [17].

We conclude that the use of the laser capture microdissection (LCM) technique depends on many factors that must be considered, the most important of which will be cell culture and the preparation of chromosome suspensions that allow for the production of slides with sufficient chromosome number and spread for their use. The choice of amplification methodology that allows for the construction of the WCP is also important, particularly given the amount of starting material obtained with microdissection.

Regarding the chromosomal probes obtained, FISH analysis revealed that they were specific to the chromosomes obtained, despite their use in a cell line with an altered genome.

From a personal perspective as a student, completing this internship was very exciting, not only because of the novelty of the first exposure to the laboratory routine, but also because of the number of methodologies and their troubleshooting that I was able to observe and absorb, which will be important for my professional and academic career in the future.

References

1. Guo, W., Hu, Y., Qian, J., Zhu, L., Cheng, J., Liao, J., & Fan, X. (2023). Laser capture microdissection for biomedical research: Towards high-throughput, multi-omics, and single-cell resolution. Journal of Genetics and Genomics, 50(9), 641-651. https://doi.org/10.1016/j.jgg.2023.07.011
2. Monajembashi, S., Cremer, C., Cremer, T., Wolfrum, J., & Greulich, K. O. (1986). Microdissection of human chromosomes by a laser microbeam. Experimental Cell Research, 167(1), 262-265. https://doi.org/10.1016/0014-4827(86)90223-5
3. Emmert-Buck, M. R., Bonner, R. F., Smith, P. D., Chuaqui, R. F., Zhuang, Z., Goldstein, S. R., …& Liotta, L. A. (1996). Laser capture microdissection. Science, 274(5289), 998-1001. https://doi.org/10.1126/science.274.5289.998
4. Espina, V., Heiby, M., Pierobon, M., & Liotta, L. A. (2007). Laser capture microdissection technology. Expert Review of Molecular Diagnostics, 7(5), 647-657. https://doi.org/10.1586/14737159.7.5.647
5. Thennavan, A., Sharma, M., Chandrashekar, C., Hunter, K., & Radhakrishnan, R. (2017). Exploring the potential of laser capture microdissection technology in integrated oral biosciences. Oral Diseases, 23(6), 737-748. https://doi.org/10.1111/odi.12578
6. Casasent, A. K., Schalck, A., Gao, R., Sei, E., Long, A., Pangburn, W., …& Navin, N. E. (2018). Multiclonal invasion in breast tumors identified by topographic single cell sequencing. Cell, 172(1), 205-217.e12. https://doi.org/10.1016/j.cell.2017.12.007
7. Nelson, O., Sowamber, R., Milea, A., Considine, M., Dodds, L., De Castro, V., …& George, S. (2018). Integrative transcriptome analyses of the ampulla and fimbria of the human fallopian tube. SSRN Scholarly Paper. https://doi.org/10.2139/ssrn.3288210
8. Zhao, Y., Gao, Y., Xu, X., Zhou, J., & Wang, H. (2021). Multi-omics analysis of genomics, epigenomics and transcriptomics for molecular subtypes and core genes for lung adenocarcinoma. BMC Cancer, 21, 257. https://doi.org/10.1186/s12885-021-07888-4
9. Gordon, A., Kannan, S. K., & Gousset, K. (2018). A novel cell fixation method that greatly enhances protein identification in microproteomic studies using laser capture microdissection and mass spectrometry. Proteomics, 18(11), 1700294. https://doi.org/10.1002/pmic.201700294
10. Zhu, Y., Dou, M., Piehowski, P. D., Liang, Y., Wang, F., Chu, R. K., …& Kelly, R. T. (2018). Spatially resolved proteome mapping of laser capture microdissected tissue with automated sample transfer to nanodroplets. Molecular & Cellular Proteomics, 17(9), 1864-1874. https://doi.org/10.1074/mcp.TIR118.000686
11. Kubickova, S., Cernohorska, H., Musilova, P., & Rubes, J. (2002). The use of laser microdissection for the preparation of chromosome-specific painting probes in farm animals. Chromosome Research, 10(7), 571-577. https://doi.org/10.1023/A:1020914702767
12. George, P., Sharma, A., & Sharakhov, I. V. (2014). 2D and 3D chromosome painting in malaria mosquitoes. Journal of Visualized Experiments: JoVE, (83), e51173. https://doi.org/10.3791/51173
13. Liehr, T. (2022). Cytogenetics and molecular cytogenetics. CRC Press.
14. Murphy, S. J., Cheville, J. C., Zarei, S., Johnson, S. H., Sikkink, R. A., Kosari, F., …& Vasmatzis, G. (2012). Mate pair sequencing of whole-genome-amplified DNA following laser capture microdissection of prostate cancer. DNA Research, 19(5), 395-406. https://doi.org/10.1093/dnares/dss021
15. Rubelowski, A.-K., Latta, L., Katiyar, P., Stachon, T., Käsmann-Kellner, B., Seitz, B., & Szentmáry, N. (2020). HCE-T cell line lacks cornea-specific differentiation markers compared to primary limbal epithelial cells and differentiated corneal epithelium. Graefe's Archive for Clinical and Experimental Ophthalmology, 258(3), 565-575. https://doi.org/10.1007/s00417-019-04563-0
16. Shim, S.-H., Kyhm, J.-H., Chung, S.-R., Kim, S.-R., Park, M.-I., Lee, C.-H., & Cho, Y.-H. (2007). Generation of FISH probes using laser microbeam microdissection and application to clinical molecular cytogenetics. Journal of Microbiology and Biotechnology, 17(7), 1079-1082.
17. Krylov, V., & Tlapakova, T. (2015). Xenopus cytogenetics and chromosomal evolution. Cytogenetic and Genome Research, 145(3-4), 192-200. https://doi.org/10.1159/000406550

18. Universidade de Trás-os-Montes e Alto Douro. (n.d.). Protocolo 5: Microdissecção por Captura a Laser de cromossomas [Video]. https://photos.google.com/album/AF1QipPW71hkliFBiFmQ_5et3B9ZcLpPpMPgfsL7wgOM/photo/AF1QipO4kZES8gCoOp1e-zicZFqm2862s41Xn6jnSwyI