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Review Article

Establishing a Basic Molecular Laboratory in Tertiary Cancer Centre: Practical Considerations and Implementation Challenges

Mrs Sruthi M K[1], Dr. Madan K[2]

[1] Molecular Biologist, Department of Onco Pathology, Yenepoya Medical College, Mangalore
*[2]Assistant Professor Onco Pathology & Molecular Pathologist Department of Onco Pathology, Yenepoya Medical College, Mangalore. (**ORCID iD**: 0000-0001-8052-6034)*

Corresponding Author:

Dr Madan K
Assistant Professor Onco Pathology & Molecular Pathologist Department of Onco Pathology, Yenepoya Medical College, Mangalore. *(**ORCID iD**: 0000-0001-8052-6034)*

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ABSTRACT

Recently, there is increasing incidence of cancer and the cases are going to double in the next 30 years. With improved understanding of molecular biology of cancers, there has been a revolution in the field of diagnostics and therapeutics. Identification of various molecular alterations (biomarkers) are now part of standard practice guidelines in Oncology, which is crucial for diagnosis, risk stratification, prognostication, prediction of treatment response, monitoring treatment response and recurrence. Molecular testing is routine in common solid tumor certain hematological malignancies. There is lack of accessibility of these diagnostic assays in tier2 cities and smaller places, leading to delayed treatment decisions thus adding to cancer mortality rates. Numerous diagnostic molecular and genomic platforms are available offering wide variety of assays. Among these, in spite of latest technological advances, PCR remains the basic, powerful molecular technique which is highly specific, accurate, with low turnaround time and cost-effective. Common molecular assays in oncology such as *BCR*::*ABL1*, *PML*::*RARA*, *EGFR*, *ALK*, *ROS1*, *BRAF* and *KRAS* can all be performed on real-time PCR platform, making the technique and tests sensitive and cost effective at the same time. The recent pandemic has made us realize the importance of molecular diagnostics and also this technique is relatively accessible, post pandemic.

In this context, in this review we highlight the specific requirements and key considerations in terms of infrastructure, equipment, human resource and quality assurance and certain challenges commonly faced in setting up a basic molecular laboratory consisting of real-time PCR based assays.

Keywords: *Basic Molecular Lab, Real-time PCR, Molecular Diagnostics, Oncology, Key Considerations, Challenges*

INTRODUCTION:

Based on the surveys conducted by Global Cancer Observatory, there were 3.2 million cancer cases in India for the year 2022 [1,2]. The incidence of cancer cases is going to double in the next 30 years because of improved diagnostics facilities and also because of increased lifespan in the Indian population [3]. The most common cancer types in India are head & neck and lung in males, whereas breast and cervix in females [4] .With an improved understanding of molecular biology of cancers, there has been a revolution in the field of diagnostics and therapeutics. Molecular diagnosis is now a routine practice in certain solid tumors and hematological malignancies, thanks to the technical advances in the last couple of decades. In oncology, molecular testing is crucial for diagnosis, risk stratification, prognostication, prediction of treatment response, monitoring treatment response and recurrence [5]. In the era of precision medicine, there has been an evolution in treatment options with a more personalized approach.[6] .

Molecular analysis could be based on DNA, RNA or proteins, performed from a wide variety of sample types, consisting of either germ cells or somatic cells. Various alterations of these serve as predictive biomarkers, which is now the latest armamentarium in diagnosis and management of cancer, particularly in the advanced and metastatic setup. There are predetermined sets of biomarkers for which there are recommended molecular testing methods which are now part of standard practice guidelines such as National Comprehensive Cancer Network (NCCN) and European Society of Medical Oncology (ESMO) [7] . Some of the disease specific biomarkers, commonly implicated in solid tumors in clinical oncology are, *EGFR*, *ALK*, *ROS1* in Non-Small Cell Lung Carcinoma (NSCLC); *KRAS*, *NRAS*, *BRAF* in colorectal carcinoma; *ER*, *PR*, *HER*2 in breast carcinoma, *BRAF* in Melanoma [8,9].

In India, cancer mortality rates are double in rural areas as compared to urban populations.[3] Currently, the molecular diagnostic laboratories are limited to metros and tier 1 cities. The accessibility of molecular laboratories in tier 2 and lesser cities can reduce the delay in diagnosis with timely treatment decisions. Thus, it's essential to understand the fundamentals of setting up a basic molecular diagnostic laboratory, where adequate space & layout, suitable equipment and qualified personnel are key fundamental considerations.

Currently, there are a wide variety of molecular genetics assays based on numerous platforms, such as polymerase chain reaction (PCR), Sanger sequencing, Fluorescent in situ hybridization (FISH) and Next Generation Sequencing (NGS) techniques. With all these, PCR remains the basic, yet powerful molecular technique which is highly specific, accurate, with low turnaround time and cost-effective [10].

In this context, we describe the specific requirements and consideration to establish a basic molecular laboratory, consisting of real-time PCR based assays, concentrating on Oncological diagnosis.

REQUIREMENTS:

Adequate infrastructure, appropriate equipment and qualified personnel are the key requirements in setting up a molecular laboratory. The specifications and needs vary based on assays planned, however, the general principles are supposed to be met for quality and standardization.

A) Infrastructure:

Identification of a distinct, suitable area for the laboratory is essential, consisting of separate office space as well as necessary space required for the work stations. PCR is an amplification technique, where the template copies are exponentially amplified. During the process, there is high risk of contamination, leading to false positive results. With this key consideration, it's essential to have separate work areas with specific pressure levels and maintained temperature.

A well planned molecular laboratory with real-time PCR technique must have distinct areas / rooms for various steps in the workflow. Ideally there should be 3 distinct areas for; reagent preparation (area 1, cleanest), sample preparation (area 2, clean) and amplification (area 3, dirty).

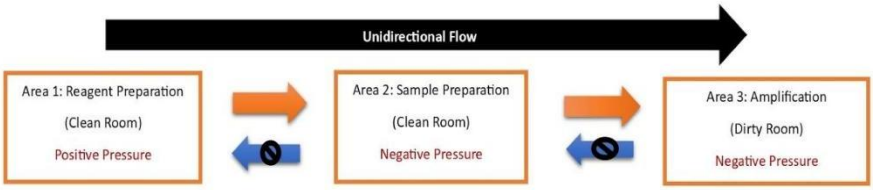

Figure 1: Workflow of an ideal real-time PCR laboratory

However, the minimum requirement is for 2 rooms especially when considering commercially available kit based assays; one clean room (pre-PCR) for reagent and sample preparation, (spatial / temporal separation) and the second dirty room (post-PCR) for amplification and analysis of PCR products. Post PCR analysis of products is bare necessities in real-time PCR laboratories, where sequencing techniques are not considered.

B) Workflow:

A unidirectional flow for samples, personnel and materials in the molecular lab is required to protect against aerosols. The direction of flow should always be from reagent preparation room where reagents are stored and handled to the low DNA / RNA concentration room i.e., sample preparation room, and then to higher concentrations of DNA which is the PCR room [11].

C) Environmental Considerations:

i. Ventilation:

PCR is an extremely high-performance technique involved in detecting very-low levels of DNA or RNA molecules in the sample. Hence, additional measures are required in contamination prevent from the air being recirculated across pre and post-PCR areas of the laboratory with separate ventilation and the air pressure system in place. The pre-PCR area should have a slight positive pressure and the post-PCR area should be at slight negative pressure as compared to the outside area. Finally, the air handlers for pre and post-PCR areas should to be connected to separate air ducts with each leading to a separate location for exhaust. In simple terms, a positive pressure prevents the transport of unwanted substances from outside to pre-PCR rooms and negative pressure in post-PCR rooms prevents air migration to the surrounding room.

ii. Temperature:

An ideal molecular laboratory must have centralized air conditioning with sensors for monitoring and recording temperature and humidity. The optimal temperature range is between 20-25°C, which helps in controlling contamination issues and also helps in maintenance and functioning of particular equipment.

iii. UV irradiation:

Sterilization of the pre-PCR area can be achieved by UV light (at ceiling or exit doors), because of the known sensitivity of nucleic acid. However, when such a method is used, certain considerations such as auto lock out facility on exit doors, ventilation system to eliminate UV generated ozone and rigid UV monitor systems need to be in place [12] .

iv. Protective clothing:

A full sleeved lab coat is recommended, which will not only provide personnel protection from chemical and biological splashes but also helps sample contamination from the personnel. Safety glasses, long pants, closed toe shoes, gloves, masks and head cap are the other personal protections required in any molecular laboratory.

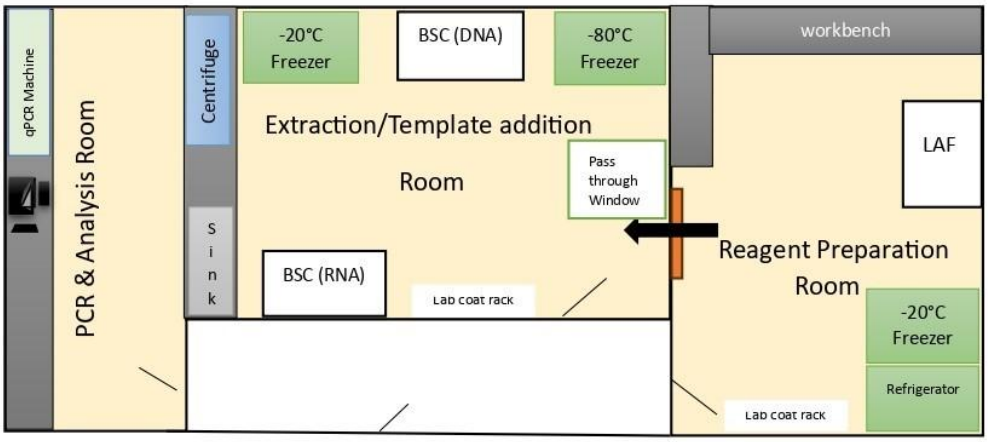

Figure 2: Laboratory layout with distinct areas as described

D) Key Aspects:

In molecular labs, contamination is a key issue that can impact the accuracy and reliability of test results [13] . Prevention of contamination can be practiced by establishing robust contamination control measures and good lab practice guidelines. Measures such as, precise laboratory layout, workflow optimization, air filtration and controlled access helps in minimizing the contamination risks. Regular monitoring of cleaning, maintenance, temperature and pressure should also be done. Hypochlorite solutions are found to be more effective than ethanol to clean the surfaces containing DNA contamination [14].

E) Other Considerations:

There are other certain key considerations; some of them are in general common for any laboratory. They are sample load, adequately trained personnel, waste management, storage space for reagents and consumables (including specialized storage cabinets for hazardous chemicals) and uninterrupted power supply. It is essential to consider these at the start of the laboratory design process for effective management.

EQUIPMENTS AND CONSUMABLES:

Molecular lab requires a variety of equipment and consumables which depends on the diagnostic platforms and the assays considered. Here we will look into the requirements for basic qPCR based laboratories. Each room / area must have its own equipment and consumables to prevent contamination. The purchase of equipment and consumables are considered based on workload, assays, sample load, sample type and most importantly the financial considerations. Tables 1 & 2 lists the variety of equipment and consumables required for a molecular laboratory highlighting their general purpose of utility.

Bio-safety cabinets (BSC), required in are selected mainly based on the type of protection required. There are three main classifications of BSC, class I to class III. A molecular lab with Oncological assays requires BSC class II, which is further divided into 4 subtypes based on their differences in exhaust system.

Table 1: List of equipment and their purpose in molecular laboratory

Equipment	Purpose of Use
Real-Time PCR Machine	Amplify and analyze DNA
Biosafety Cabinet & Laminar Air Flow	To prevent contamination
Centrifuges (Refrigerated & Non refrigerated)	Separation of components based on density
Micro Centrifuge	Spin down
Mini Centrifuge	Spin down of PCR tubes
Refrigerator	Storage of chemical kits and PCR products
-20°C & -80°C Freezer	Storage of DNA/RNA, Storage of Reagents
Vortex Mixer	Mixing of samples / reagents
Spectrophotometer / Nanodrop	Quantification and purity check of DNA/RNA
Micropipettes	Transfer and handling of precise volumes of samples / reagents
Dry bath	Incubating samples at a constant temperature

Table 2: List of consumables required for real-time PCR assay

Consumables
Micro Centrifuge Tube
Microcentrifuge Tube Rack
Microcentrifuge Tube Storage Boxes
RT PCR strip tubes / plates (as per the sample load)
PCR plate sealer & sealing Paddle
PCR Workup Rack
PCR Cooling Rack
Falcon Tubes
Falcon Tube Rack
Ethanol 100%
Molecular Grade Water
Filtered tips
Extraction and Test (qPCR assay) kits
Sodium Hypochlorite
Measuring Cylinder
Kit Requirements (If any)
Discard Jars
Personal protective equipments
Disinfectants & Cleaning Supplies
Bio Medical Waste Plastic Pedal Dustbin

Key factors while procuring equipment & consumables:
Understanding the needs and applications in terms of sample load, assays and scalability are to be considered prior to the setting up of the laboratory. Smaller labs may need only versatile, general-purpose equipment such as a real-time PCR machine, biosafety cabinet, refrigerated centrifuge, freezers and other consumables.

- **Real-time PCR machine**: Sensitivity, throughput and multiplexing.
- **Nucleic acid extraction**: Manual extraction kit with pure and high yield nucleic acid can be considered for a smaller lab.
- **Centrifuge**: Refrigerated centrifuge, rotors and buckets with specific RPM.
- **Pipettes**: Accuracy and precision is the key along a range of volume capacity and single / multichannel pipettes.
- **Freezers: (-20°C and -80°C)**: Storage of samples (DNA / RNA), adequacy of space should be considered as per workload.
- **Real-time PCR assays**: Laboratories with limited research facilities can opt for a wide variety of commercially available qPCR assays, as per the assays. Companies offering a variety of qPCR based assays in Oncology are TruPCR, Invitrogen, Genes2me and NeoDx. However, compatibility with the qPCR machine needs to be looked into.

In general, while considering equipment, quality should be given high priority and never be compromised because of budget constraints or other reasons, which affects the reliability and accuracy. Refurbished equipment with reliable service and warranty can be considered.

As per Clinical Laboratory Improvement Amendments (CLIA) guidelines, College of American Pathologists (CAP) guidelines and ICMR guidelines for good clinical laboratory practices 2021, all equipments (expect equipments like vortex mixer) should be qualified for Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ). All equipment must have working SOP and separate log books. Calibration, cleaning and maintenance should be done periodically as per maintained SOP and should be documented.

HUMAN RESOURCE:
The backbone of any molecular laboratory is their well trained qualified personnel. Expertise and the number of the trained personnel depend on molecular diagnostic platforms, assays and the sample load. However, a basic diagnostic laboratory based on a real-time PCR platform would require a minimum of two personnel consisting of a molecular pathologist / molecular scientist and a molecular biologist / technologist. The terminology, designation and the position may vary based on the institute or hospital setup. The molecular pathologist is a qualified MD pathologist with specialized training in molecular diagnostics with a good clinical acumen, whereas the molecular scientist is PhD personnel with a good hold on technical aspects. They are responsible for setting up the lab, routine reporting of cases and oversee effective lab functioning. Biologists or technologists are post graduates / graduates in biotechnology or molecular biology who should be able to perform the assays in entirety, interpret results and troubleshoot as required. They are also responsible for maintaining all the records, logs, files and certificates in various sections of the lab. Both the personnel are equally responsible for lab quality procedures and facilitating and implementing effective quality management systems.

QUALITY ASSURANCE:
Effective quality assurance enhances the accuracy and reliability of laboratory results. It secures the regulatory standards and promotes consistency of test results. Quality assessment is essential during various aspects of workflow such as pre-analytical, analytical and post-analytical steps. Most important facet in quality assurance includes:

Standard Operating Procedures (SOP): SOPs are clear and concise written documents with explanations of the task's goal, roles and responsibilities. These are controlled documents which contain descriptions of what needs to be done to complete each step and a discussion of decisions that must be made.

Quality control (QC): Helps in preventing or minimizing erroneous reports, thus provides confidence that the quality requirements are fulfilled.

Turn-around time (TAT): TAT is the time interval between a test request and release of test results timely [15]. Common goal of TAT is to report the tests within the defined time, thus aiding in timely treatment decisions of patients.

Internal quality assessment (IQA): IQA involves a set of procedures for routine monitoring and verification of testing processes within the laboratory to produce reliability and accuracy in results. "Split sampling" is one of the effective IQA measures which can be used by laboratories for monitoring the testing process [16].

External quality assessment (EQA): EQA encompasses comparison of results between laboratories for an enhanced laboratory practice [17]. This helps in identification and replacing dissatisfying methodologies of poor performing laboratories and for accreditation and regulatory requirements [17].

Accreditation process: Accreditation and participation in EQA are recognized as effective and important tools to improve the accuracy and reliability of molecular testing [18]. Variety of accreditation processes exists, both national and international. The National Accreditation Board for Testing and Calibration Laboratories (NABL) is a Government of India established constituent board for accreditation testing, calibration and clinical laboratories. It offers voluntary test-based accreditation and gives International recognition and assurance of accurate and reliable results. The College of American Pathologists (CAP) laboratory accreditation program, an internationally recognized voluntary accreditation program, which is widely accepted globally and meets the required standards from CLIA, FDA and OSHA. All the tests under the leadership of one laboratory director have been accredited by CAP as compared to NABL which accredits individual tests.

Sample Report:
According to AMP and ICMR guidelines, a test report should contain the patient particulars such as name, age, gender and unique identification number. This should also contain specimen details such as date and time of collection and sample source. Test details such as test methodology, measurement units, reference details, cut-off values, date and time of testing and the results must be incorporated and should be signed by an authorized personnel.

CHALLENGES IN ESTABLISHING MOLECULAR LAB:
Financial aspect is the first and foremost challenge faced in setting up any molecular laboratory, especially in resource constrained countries like India. Molecular diagnostic equipment and consumables are highly priced and to invest in the latest technology while remaining financially sustainable is a challenging attempt. Most of the equipment is manufactured abroad and import duties add to the already higher cost. With the government's Make in India initiative, currently there are many regional companies, thus increasing the availability and also bringing down their cost. Hence, such decisions are crucial; however, the quality and accuracy of test results are not to be compromised.

Finding molecular pathology professionals having a deep foundation in clinical practice of pathology and soft skills required to lead molecular laboratories is equally challenging [19] . Locating a trained and skilled molecular technologist who can handle all types of samples, perform tests and interpret results is also a crucial requirement. Adequate induction and training programme for the molecular laboratory professionals improves the competence and confidence while handling the assays.

Once all the key primary considerations are met, gathering all the requirements for the accreditation process is another challenge. Standardization of methods, validation, maintaining SOPs, qualifying QC, documentation, reporting within the TAT comes under accreditation requirements of CAP and NABL.

Sample issues because of storage or fixation, transportation and contamination are the other important test related issues which need to be addressed. Samples should be transported and stored at prescribed temperature, failing to which can degrade the nucleic acid. The quality of FFPE tissue samples and the quality of nucleic acid extracted depends upon many factors such as tissue dehydration, too short or prolonged fixation or a delay in fixation, fixation temperature, formalin concentration and pH, thickness of the sample, the specimen storage conditions [20]. Hence space and adequate storage conditions can be a challenge for newer centers.

Potential Solutions:
Public-Private Partnerships (PPP): Government and private sector collaboration can help in pooling resources to set up molecular laboratories. Government Initiatives: The Indian government has been encouraging investment in biotechnology and research, offering grants and incentives that can be tapped to mitigate some of these challenges.

CONCLUSION:

To provide an accurate and affordable molecular diagnosis is an important parameter when setting up a molecular laboratory especially for cancer patients where test report has a major role in treatment decisions. With the lack of such centers in tier II cities, Public – Private partnership can help to reduce the gap and also expenses incurred. Setting up a molecular lab is a daunting task and improved understanding of needs can reduce the challenges faced thus offering affordable Oncological assays in patient care.

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REFERENCES:

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021 Feb 4. doi: 10.3322/caac.21660. Epub ahead of print. PMID: 33538338.
2. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A, Bray F. Cancer statistics for the year 2020: An overview. Int J Cancer. 2021 Apr 5. doi: 10.1002/ijc.33588. Epub ahead of print. PMID: 33818764.
3. Banavali SD. Delivery of cancer care in rural India: Experiences of establishing a rural comprehensive cancer care facility. Indian J Med Paediatr Oncol 2015;36:128-31
4. Krishnan Sathishkumar, Meesha Chaturvedi, Priyanka Das, S. Stephen & Prashant Mathur. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. Indian J Med Res 156, October & November 2022, pp 598-607
5. Thenrajan T, Alwarappan S, Wilson J. Molecular Diagnosis and Cancer Prognosis-A Concise Review. Diagnostics (Basel). 2023 Feb 17;13(4):766.
6. Strianese O, Rizzo F, Ciccarelli M, Galasso G, D'Agostino Y, Salvati A, Del Giudice C, Tesorio P, Rusciano MR. Precision and Personalized Medicine: How Genomic Approach Improves the Management of Cardiovascular and Neurodegenerative Disease. Genes (Basel). 2020 Jul 6;11(7):747.
7. Wafik S. El-Deiry, Richard M. Goldberg, Heinz-Josef Lenz, Anthony F. Shields, Geoffrey T. Gibney, Antoinette R. Tan; Jubilee Brown, Burton Eisenberg, Elisabeth I. Heath; Surasak Phuphanich, Edward Kim, Andrew J. Brenner, John L. Marshall. The Current State of Molecular Testing in the Treatment of Patients With Solid Tumors, 2019. CA CANCER J CLIN 2019;69:305–343.
8. Wilbur A. Franklin WA, Aisner DL, Davies KD, et al. Chapter 15: Pathology, Biomarkers, and Molecular Diagnostics. In: Niederhuber JE, Armitage JO, Doroshow JH, Kastan MB, Tepper JE, eds. Abeloff's Clinical Oncology. 6th ed. Philadelphia, Pa: Elsevier; 2020.
9. National Cancer Institute. Biomarker Testing for Cancer Treatment. 2021. Accessed at https://www.cancer.gov/about-cancer/treatment/types/biomarker-testing-cancer-treatment on June 3, 2022.
10. Ruby Gilmor, Hashir Qamar, Nicholas Huerta, In Handbook for Designing and Conducting Clinical and Translational Research, Translational Surgery, Academic Press, 2023, Pages 15-19.
11. Dieffenbach, CW, Dveksler, GS. 1993. Setting up a PCR laboratory. PCR Methods Appl., **3**, Supplement, S2–7.
12. Mifflin TE. Setting up a PCR laboratory. CSH Protoc. 2007 Jul 1;2007:pdb.top14. doi: 10.1101/pdb.top14. PMID: 21357132.
13. Anıl AYSAL, Burçin PEHLIVANOĞLU, Sümeyye EKMEKCI, Betül GÜNDOĞDU. How to Set Up a Molecular Pathology Lab: A Guide for Pathologists. Turk Patoloji Derg 2020, 36:179-187.
14. Marie-Louise Kampmann, Claus Børsting, Niels Morling. Decrease DNA contamination in the laboratories. Forensic Science International: Genetics Supplement Series 6 (2017) e577–e578
15. Robert C Hawkins. Laboratory Turnaround Time.Clin Biochem Rev Vol 28 November 2007
16. Paul Wallace and ElaineMcCulloch.Quality Assurance in the Clinical Virology Laboratory. Encyclopedia of Virology, 4 th Edition, Volume 5.
17. Laura Sciacovelli, Sandra Secchiero, Lorena Zardo, Mario Plebani. The role of the External Quality Assessment. Biochemia Medica 2010;20(2):160–4
18. Berwouts S, Fanning K, Morris MA, Barton DE, Dequeker E. Quality assurance practices in Europe: A survey of molecular genetic testing laboratories. Eur J Hum Genet. 2012;20:1118-26.
19. Eriko G. Clements and Erin H. Graf . The Journal of Molecular Diagnostics, Vol. 24, No. 2, February 2022.
20. Steiert TA, Parra G, Gut M, Arnold N, Trotta JR, Tonda R, Moussy A, Gerber Z, Abuja PM, Zatloukal K, Röcken C, Folseraas T, Grimsrud MM, Vogel A, Goeppert B, Roessler S, Hinz S, Schafmayer C, Rosenstiel P, Deleuze JF, Gut IG, Franke A, Forster M. A critical spotlight on the paradigms of FFPE-DNA sequencing. Nucleic Acids Res. 2023 Aug 11;51(14):7143-7162.