International Agency for Research on Cancer

World Health Organization

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PURCHASE OF SCIENTIFIC EQUIPMENT

1. In line with the IARC Medium-Term Strategy (2010–2014), which highlighted the paramount importance of performing interdisciplinary research, investments have been made during the last three years to reinforce the interaction between laboratory-based and epidemiology research. Constant upgrade and acquisition of scientific instruments are essential to support this strategy.

2. These investments allowed the establishment of three centralized platforms for studies on genetics, biomarkers and carcinogenic mechanisms (next-generation sequencing (NGS), mass spectrometry platform, and the platform for the detection of multiple infectious agents), and the acquisition of equipment for DNA extraction and of a slide scanner for tumour validation.

3. To complement these investments, additional support is required to maintain and upgrade IARC capacity to handle and process large numbers of biological samples and to acquire a pyrosequencing system. The four liquid handling systems in particular represent routine pieces of equipment which are difficult to obtain from extra-budgetary sources.

4. The optimal utilization of IARC platforms and the increasing workload linked to the development of epidemiology projects require specialized robotics that would automatically and efficiently perform sample preparation and extraction to provide high-quality data at a reduced labour cost. These needs concern the immunoassay platform for biomarker measurement; the Luminex platform for pre-PCR sample handling and the NGS platform for post-PCR applications.

5. The pyrosequencing system is an essential tool of the epigenetic platform to conduct quantitative and sensitive analysis of DNA methylation in high-throughput settings. This system has been instrumental in establishing collaborations with groups internally and externally. The current model is no longer cost effective or adapted to the low sample volumes commonly required.

6. The annual maintenance costs of the requested equipment will be covered by the regular budget as well as by collaborative programmes through grant applications.

7. The Scientific Council is requested to advise the Director and Governing Council on the proposed request to use funds from the Governing Council Special Fund to purchase the following equipment:

a. Pyrosequencing system 96-well PyroMark Q96 MD (Qiagen)

b. Liquid handling system for pre-PCR procedures

c. Liquid handling system for immunoassays

d. Liquid handling system for PCR products

e. Liquid handling system for ChiP assays

a) Pyrosequencing system 96-well PyroMark Q96 MD (Qiagen)

8. Pyrosequencing is an essential tool for quantitative, sensitive and high-throughput analysis of DNA methylation, and for validation of array-based and NGS-based methylation profiling. The current pyrosequencing system which is located within the Epigenetics Group (EGE) has been instrumental in establishing numerous collaborations both within IARC (EGE, Molecular Pathology (MPA), Molecular Mechanisms and Biomarkers (MMB) and Infections and Cancer Biology (ICB) Groups) and outside. However, it is seven years old and is no longer cost effective because of a high level of reagent usage and slow run times. In addition, the quality of the results is becoming frequently unsatisfactory.

9. The proposal is to replace the Q96 ID instrument with the Q96 MD system that can run with about half of the volume of reagents, provide considerable savings and uses significantly less starting sample material. It will respond to the increasing need for methylation analyses through internal and external collaborations.

b) Liquid handling system for pre-PCR procedures

10. The activities of ICB comprise the development and validation of diagnostic assays for infectious agents with high specificity and throughput that can be used for epidemiological studies.

11. A highly sensitive and specific platform for the detection of more than 100 different infectious agents is fully operative at IARC. These assays encompass extraction of the DNA from human specimens followed by multiplex polymerase chain reaction and analysis with the Luminex reader.

12. Although ICB already uses a robot-based platform for automatic DNA extraction, all pre-PCR steps are still manually performed, which significantly impedes the analytical performance and throughput. ICB processes approximately 5 000 specimens each year in the context of internal and external epidemiological collaborative studies and over 10 000 human specimens are expected to be processed next year.

13. A new high-throughput robot to perform the pre-PCR procedures would allow standardizing the process, minimizing the risk of human contamination and increasing the throughput of analyses to limit time spent on this task by technical staff.

c) Liquid handling system for immunoassays

14. The IARC Biomarkers Group (BMA) analyses sex steroids, growth factors, insulin, inflammatory cytokines and adipocytokines in biological samples from epidemiological studies using specific and validated immunoassays (ELISA, RIA, IRMA). These analytes are assayed at the same time to avoid repeated thawing and refreezing of samples and a robot is needed to handle large numbers of samples in a limited time. These studies involve BMA, the Nutritional Epidemiology Group (NEP), ICB and a number of external collaborators as part of large collaborative projects on diverse cohorts in Europe, North America and Latin America.

15. The two robots currently being used are more than 10 years old, and are running inefficiently with significant mechanical and informatic problems. The operating programs will also soon become obsolete. We propose to replace these robots by one new one having higher performance (higher precision in pipetting small volumes, faster speed of pipetting, double throughput).

d) Liquid handling system for PCR products

16. Preparation of NGS libraries for targeted sequencing, including distribution of PCR mix, pooling of PCR products, equimolar pooling of bar-coded samples and purification of PCR products using magnetic beads require the use of liquid handling robotics for high throughput operation.

17. The Genetics Sequencing Platform (GSP) currently uses a 15-year old Qiagen 9600 (2-needle robot). The robot which currently serves for post-PCR liquid handling purposes in many GSP workflows for transfer of small volumes from 96- or 384-well plate formats to similar plates or "cherry-picking", is obsolete both in terms of software and hardware and has limited functions. More complicated tasks involved in post-PCR NGS library preparation are beyond its capacity.

18. We propose to replace the Qiagen 9600 instrument with a more flexible laboratory robot that includes every aspect of liquid handling (including pipetting, dilution, dispensing and integration) in an automated system that is powerful, efficient and economical. In addition it will provide increased throughput (eight needles instead of two), higher pipetting accuracy and flexibility for the more complicated pipetting tasks now needed by GSP.

e) Liquid handling system for ChiP assays

19. Chromatin Immunoprecipitation (ChIP) is a widely used technique to investigate the interaction between proteins and DNA in cells and tissues. ChIP is routinely used by several IARC groups (including EGE, ICB and MMB) in mechanistic studies aiming to determine whether specific proteins (such as transcription factors, chromatin modifiers and viral proteins) are associated with specific genomic regions. ChIP also allows to profile the genome-wide location that different histone modifications are associated with.

20. Because ChIP assays are currently performed manually, hands-on time is rather long (typically over two days). Importantly, during this process technical variability is often introduced. These limitations could be addressed by an automated system for ChIP and ChIP combined with deep sequencing (ChIP-seq). This system would allow generation of highly reliable cutting-edge genome-wide data in understanding aberrant cellular programmes in cancer.

21. Several basic research projects across IARC benefit from the systematic sample preparation to study protein binding, protein-protein and protein-DNA interactions as well as chromatin modifications on a genome-wide scale. This includes research intended to understand the pattern of important transcription factor binding in cancer, histone modifications, and gene expression control in general.

22. The proposed automated system is intended to replace manual, error-prone steps of immunoprecipitation with a reliable, highly-consistent and automated process. It uses a magnetic-bead technology that allows great reproducibility and can process up to 16 samples in parallel. The system would be integrated within the SOLID sequencer already available, and will significantly reduce hands-on time and technical variability. The system provides robust sample enrichment of targets using very small numbers of cells

23. Applications include many techniques for genome-wide analysis of histone modifications (ChIP, Re-ChIP), DNA methylation (MeDIP, MethylCap), and RNA binding proteins (RNA-IP), as well as library preparation for massive parallel sequencing platforms.

Requested budget

	Quantity	**Approximate price (€)**	**Annual maintenance costs (€)**
Pyrosequencing system 96-well PyroMark Q96 MD (Qiagen)	1	120 215	7 750
Liquid handling system for pre-PCR procedures	1	106 450	9 300
Liquid handling system for immunoassays	1	74 000	8 000
Liquid handling system for PCR products	1	109 630	9 300
Liquid handling system for ChiP assays	1	75 000	9 000
Total		485 295	