The Women's Board of The Johns Hopkins Hospital
Billings Administration Building, Room 221
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GRANT APPLICATION FOR FISCAL YEAR 2024

DIRECTIONS: Please complete the entire form. If appropriate, indicate "Not Applicable" and justify. The original application plus an electronic version is due in The Women's Board office on or before 4:00 pm on Friday, January 6, 2023. Only one (1) application from each department will be accepted. Late or incomplete applications will not be considered.

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DATE: 1/6/2022
CLINICAL DEPARTMENT: Pathology
CONTACT PERSON: Jaclyn Murry, PhD
Phone: 410-955-8363
Email: jmurry3@jh.edu

TITLE OF REQUEST: Digital Polymerase Chain Reaction (dPCR) platform for detecting clinically significant gene mutations in tumor DNA.

PHYSICAL LOCATION OF PROJECT: Johns Hopkins Genomics, 1812 Ashland Ave, Suite 200, Baltimore, MD, 21205.

ABSTRACT (Non-technical overview - 150 words or less):

For patients with cancer, monitoring the residual disease after diagnosis and treatment may be crucial. Mutations causing the tumor can also be used to track disease. Technological advances enable non-invasive mutational testing via circulating tumor DNA, or liquid biopsy. It is possible to detect mutations from liquid biopsy using digital PCR (dPCR). dPCR is a method that provides high-precision and absolute quantification of target DNA sequences in a scalable manner for the detection of previously known mutations. The Molecular Diagnostics Laboratory is validating the Applied Biosystems QuantStudio Absolute Q Digital PCR System for detecting low-level gene mutations and gene copy number variants in tumor DNA for hundreds of cancer patients yearly. The requested platform will enable monitoring with greater assay sensitivity for at-risk patients. Patients with known mutations could have targeted follow-up testing, resulting in a shorter turnaround time, greater lab efficiency, cost reduction, and improved patient care.

SIGNATURE OF CLINICAL DEPARTMENT CHAIRPERSON:

(Please type) Chairperson Name: **Ralph Hruban, MD**
Chairperson Title: **Baxley Professor and Director of Pathology**
Chairperson Email: **rhruban@jhmi.edu**

NOTE: Questions 1-6 must be answered. Please be thorough and concise.

1. Impact on patient care:

Molecular pathology diagnostics routinely rely on complementary but separate assays for an initial cancer diagnosis, traditionally carried out on formalin-fixed paraffin-embedded (FFPE) specimens. Comprehensive Next-Generation Sequencing (NGS) is vital for detecting clinically significant single nucleotide variation (SNVs) and RT-PCR along with traditional fluorescence in-situ hybridization (FISH), and microarray for detecting aneuploidy, rearrangements, gene amplifications, and fusion transcripts in tumors for diagnostic and prognostic purposes. After initial diagnosis,

treatment monitoring traditionally requires invasive follow-up procedures to employ these same methodologies again, each with its strengths and limitations. Mutational tracking of the relationship between tissue and circulating tumor (ctDNA) has been successfully performed in various tumors with high sensitivity, including lung, breast, and colon cancers. ctDNA can be used to detect molecular residual disease (MRD) in solid tumors minimally invasively as part of dynamic molecular imaging assessments in a longitudinal manner. As ctDNA bearing these mutations may be low-level in patients with localized disease, high sensitivity and precision are required to detect and quantify target nucleic acid sequences (1). The development of multiple liquid biopsy molecular detection methods has sought to address these sensitivity and precision needs, mainly falling into next-generation-based methods, PCR-based methods, or combinatorial approaches (1). While comprehensive, NGS-based testing requires laborious wet lab time, sequencing and bioinformatics processing, and extensive expert review and subsequently may result in a longer turnaround time. NGS may not be cost-effective in the background of known historical mutations. Instead, targeted cancer mutation testing can provide rapid and accurate identification of known mutational events. A type of targeted mutational PCR assay, called digital polymerase chain reaction (dPCR), targets nucleic acid sequences partitioned into concurrent reactions for massively parallel quantitative PCR (qPCR), either designed as a single-locus or multiplex assay. These parallel reactions take place in microchambers on a microfluidic array plate which yields greater sensitivity due to its compartmentalization of the molecules. dPCR can be considered a 3rd generation qPCR assay because the end result allows for the absolute quantification of PCR products, an improvement over the previously indirect quantification methods associated with qPCR.

Published studies have compared results from these cross-platform methods (NGS vs. PCR) using matched samples for *EGFR* hotspot mutation detection; a limit of detection for mutation calling using digital PCR (>0.1%) and NGS (>0.2%) was established (2). Recent studies show that dPCR performance is more accurate for *EGFR* hotspot mutation detection in lung and colon cancer relative to NGS (3,4). A published systematic comparison of four PCR-based methods for the detection of *KRAS* mutations in plasma cell-free DNA demonstrated that dPCR can achieve a higher sensitivity for *KRAS* hotspot mutations and is least expensive at higher sample throughput compared to other platforms (5).

The Molecular Diagnostics Laboratory (MDL) is a CLIA-certified and CAP-accredited (highest level of regulatory standards) clinical laboratory that performs molecular and genetic testing for cancer patients at Hopkins. MDL has selected the dPCR methodology for clinical use as it provides a highly sensitive method for detecting previously known actionable mutations in cancer patients requiring longitudinal follow-up. In-house adoption of this high-throughput and non-invasive assay will provide a personalized and real-time approach to patient care, potentially detecting mutational events earlier than the standard of care.

Comparisons across the various dPCR platforms have also been published (6). MDL has selected the QuantStudio Absolute Q Digital PCR System. The platform is cost-conscious and harnesses microfluidics to ensure consistent digitization efficiency for the analyzed reaction microchambers, resulting in highly accurate and consistent quantification results within 90 minutes. This platform meets the clinical laboratory needs for achieving precision, ease of operation, and a short turnaround time (7).

This proposed assay will test key mutational events in solid tumor cancers, supporting real-time drug selection decision-making with a shorter turnaround time (Figure 1, lower panel). Future applications may also include the detection of rare alleles, fusion transcripts, or expression levels of transcripts or performing quantification of NGS sample library preparations to validate variants reducing the need to repeat a costly sequencing run (Figure 1, lower panel).

2. Number and type of patient who will benefit annually from this award:

MDL tests for clinically significant gene mutations and gene copy number changes in solid tumors and hematologic malignancies. The current monthly NGS sequencing volume for clinical patients is 180 for solid tumors. It can be time-consuming and expensive to conduct additional cytogenetics and cytogenomics tests in-house, prolonging the correlation of significant diagnostic and prognostic results as reflexive testing is often invoked to reduce the costs to the patient. The in-house NGS Solid Tumor Hotspot Panel costs $1,000 and can take up to 14 days, or the in-house Full NGS Solid Tumor Panel costs ~$2,000 and can take up to 21 days for a final result. If a cancer array is added on, the cost is $3,000 and can take up to 21 days, a karyotype can take up to 14 days costs $1000 and if FISH is added on it can cost $3000 and can take up to 10 days. This explanation is not exhaustive and does not include other existing assays that may be added on. An in-house patient-specific targeted mutational assay can offer an alternative in personalized care for patients whose prior mutations or copy number alterations profiles are known. This platform could serve at least 180 cancer patients each month, impacting care for more than 2,160 patients yearly. PCR-based assays generated will provide

customized and sensitive mutation or copy number alteration profiles for monitoring minimal/molecular residual risk of disease at Hopkins.

3. Significance

The dPCR assay design is based on the same gold-standard TaqMan chemistry used in real-time PCR and is cited in greater than 200K research publications (8). Predesigned Absolute Q PCR assays consist of a forward primer, one or more target-specific probes, and a reverse primer; the assay is pre-mixed and requires no additional testing or optimization (Figure 1, upper left). The proprietary probe has a minor groove binder (MGB) moiety at the 3' end that serves to increase the melting temperature (T_m) of the probe, improving sequence discrimination and flexibility to accommodate more targets in a single reaction (Figure 1, upper right) (9).

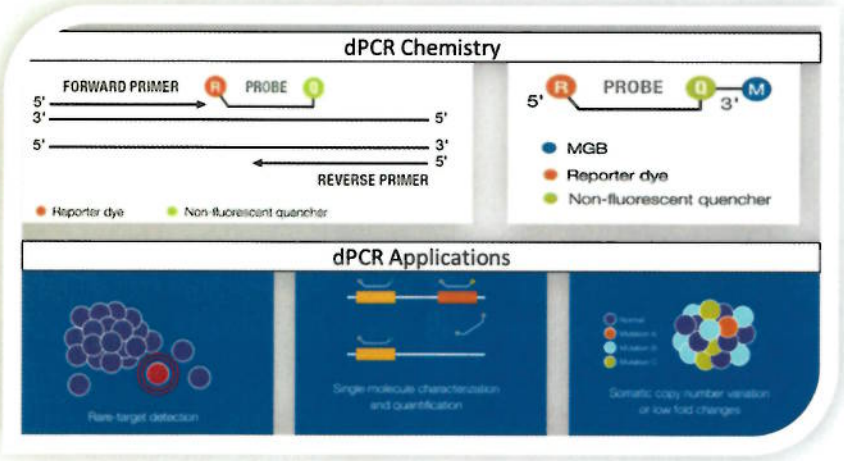

Figure 1. Predesigned Absolute Q PCR assays (upper left) are premixed together. After adding the DNA and reagents, the performance guaranteed assay will take 90 minutes. The probe design (upper right) is based on the gold-standard TaqMan chemistry and is extensively cited in the literature. The proprietary probe serves to improving sequence discrimination and flexibility to accommodate more targets in a single reaction. Potential dPCR applications (bottom panel) include the ability to detect rare targets, single molecule characterization and quantification, and somatic copy number variation or low fold changes.

The Absolute Q allows for the preparation of up to 16 samples at a time, which requires up to 90 minutes for a run, with minimal hands-on time for laboratory technologists. Once a panel of probe sets is designed, the probe sets are hybridized to DNA extracted from patient plasma (or body fluids) or tumor specimens (FFPE), after which droplets are generated by microfluidic array plate (MAP) technology, PCR is performed and dPCR data analysis is carried out to detect positive molecules in a single system with no manual transfer steps required (Fig. 2) (10,11).

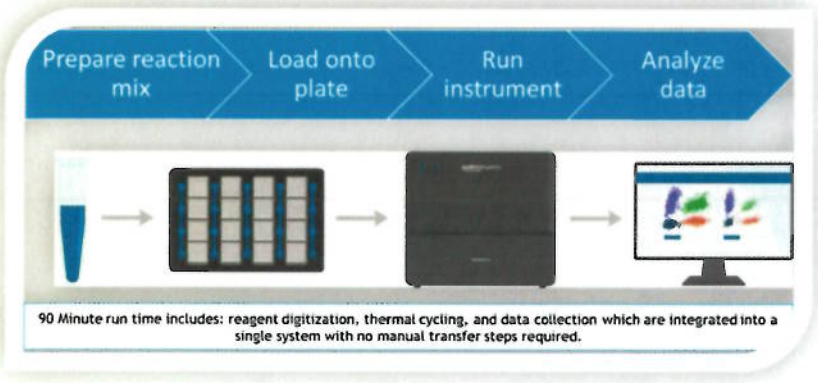

Figure 2. The Absolute Q platform workflow. DNA, primers, and probes are added along with reagents. Next, samples are loaded onto plates that can accommodate up to 16 samples at a time. Then, plates are loaded onto the QuantStudio Absolute Q system where droplets are generated by microfluidic array plate (MAP) technology and the dPCR reaction is then run. Finally, QuantStudio software performs mutation detection and quantification with precision and confidence.

Based on current estimates of 180 cancer specimens monthly, a majority of specimens processed in the lab are receiving initial diagnostic results. If a subset estimated at 10% will need known mutational testing, then the monthly volume of patients needing dPCR processing is approximately 18. However, based on the practice of each solid tumor case requiring two to three separate reactions for known copy number changes and mutations, and the practice of follow-up testing at quarterly intervals, we estimate several hundred separate assays each month. Given this volume, MDL has

requested a single unit of each instrument at this time: 1 Absolute Q instrument + Desktop, installation and 1 day on-site Field Application Scientist Training, 1 year of AB assurance (extended warranty) with 1 PM (preventative maintenance by Field Service Engineer visit), an optional additional year of AB assurance, 1xMAP 16 plate kit+ Starter Kit and White Glove Shipping. Given the minimal hands-on tech time required, the short duration of the run, and the plate size of 16, this desired platform can accommodate the current repeat testing volumes. As the estimated median clinical turnaround time is days for dPCR (specimen received to final report) compared to weeks for NGS, the requested equipment will improve overall lab efficiency and reduce turnaround and associated costs for testing for the patient, resulting in improved clinical care.

4. Implications, if any, that this has to do with the Covid pandemic:
This assay does not have a direct application to Covid detection.

5. Personnel (Please note that we cannot fund grants that incorporate any salaries.)
This proposal is led by Jaclyn B. Murry, Ph.D. She is a co-investigator of this proposal. She performs research examining emerging cytogenomic and molecular genomics methods for future clinical implementation.

Ying Zou, MD, Ph.D., is an associate director of MDL and is the Cancer Cytogenetics Director at JHH and is co-investigator of this proposal. She carries out research focused on clinical cytogenomic and molecular genetics laboratory tests.

Rena Xian, M.D., is an associate director of MDL and co-investigator of this proposal. She conducts research focused on novel diagnostic techniques.

Ming-Tseh Lin, M.D., Ph.D., is a co-investigator of this proposal. He is an associate director of MDL and is a leader in molecular diagnostics.

Christopher D. Gocke, M.D., is a co-investigator of this proposal. He is the division director of Molecular Pathology, and the medical director of MDL, and an expert in cancer genomics and non-invasive diagnostics.

Jim Eshleman, M.D., Ph.D., is a co-investigator of this proposal. He is an associate director of MDL and has an active research program dedicated to the diagnosis and response monitoring of cancer patients.

Jonathan C. Dudley, M.D., is a co-investigator of this proposal. He is an associate director of MDL and his research focuses on the development of novel molecular diagnostic methods for the detection and classification of cancer from cytopathology specimens.

6. Budget: Total Request: $ 75,000.

A. Equipment - price per item and discount if applicable for multiples. Please add compelling justification if multiples are requested. (Itemize and justify):
This $75,000 request includes 1 Absolute Q instrument + Desktop, installation and 1 day on-site Field Application Scientist Training, 1 year of AB assurance (extended warranty) with 1 PM (preventative maintenance by Field Service Engineer visit), an optional additional year of AB assurance, 1xMAP 16 plate kit+ Starter Kit, and White Glove Shipping.

We recognize that the Women's Board budget may not extend to cover the full cost, so we will gratefully accept what is available and use the clinical lab budgeting process to obtain the remaining funds.

B. Supplies (Itemize and justify):
None

C. What is the out-of-pocket cost to the patient? (Itemize and justify):
None

D. Other Expenses, Hidden Costs (Please consider whether your grant proposal contains other costs that would require hospital funding, such as structural modifications for equipment installation, operating costs such as additional FTEs, training costs, etc.)*

None

7. Have you requested funds from any other source?

☐ Yes (What was the result?)

Click or tap here to enter text.

X No (Explain why)

We have not requested funding for this instrument from other sources, as funding for clinical lab instruments is not typically available through traditional funding mechanisms. We will place the balance of the cost of the instrument on the capital expenditure budget for the clinical laboratory in 2023-24. All proposed clinical assays will be validated in MDL using the existing lab budget. CPT code 81479 for Genetic Analysis Procedures may be considered once the clinical test is launched.

* If you have any concerns about additional costs of your grant to the hospital please feel free to contact the CFO Katina Williams @ kwill249@jhmi.edu. She is aware of our grant process. All grants selected for funding will eventually be submitted for final hospital approval by the Women's Board. It is not required for the departments to request approval from the hospital prior to submission on January 6, 2023.

References:

1. Chin RI, et al. Detection of Solid Tumor Molecular Residual Disease (MRD) Using Circulating Tumor DNA (ctDNA). Mol Diagn Ther. 2019;23(3):311-331.
2. Bartels S, Persing S, Hasemeier B, Schipper E, Kreipe H, Lehmann U. Molecular Analysis of Circulating Free DNA from Lung Cancer Patients in Routine Laboratory Practice: A Cross-Platform Comparison of Three Different Molecular Methods for Mutation Detection. J Mol Diagn. 2017; 19:722-32.
3. Beije N, Helmijr JC, Weerts MJ, Beaufort CM, Wiggin M, Marziali A, Verhoef C, Sleijfer S, Jansen MP, Martens JW. Somatic mutation detection using various targeted detection assays in paired samples of circulating tumor DNA, primary tumor and metastases from patients undergoing resection of colorectal liver metastases. Mol Oncol. 2016; 10:1575-84. https://doi.org/10.1016/j.molonc.2016.10.001.
4. Iwama E, Sakai K, Azuma K, Harada T, Harada D, Nosaki K, Hotta K, Ohyanagi F, Kurata T, Fukuhara T, Akamatsu H, Goto K, Shimose T, et al. Monitoring of somatic mutations in circulating cell-free DNA by digital PCR and next-generation sequencing during afatinib treatment in patients with lung adenocarcinoma positive for EGFR activating mutations. Ann Oncol. 2017; 28:136-141.
5. Vessies DCL, Greuter MJE, van Rooijen KL, Linders TC, Lanfermeijer M, Ramkisoensing KL, Meijer GA, Koopman M, Coupé VMH, Vink GR, Fijneman RJA, van den Broek D. Performance of four platforms for KRAS mutation detection in plasma cell-free DNA: ddPCR, Idylla, COBAS z480 and BEAMing. Sci Rep. 2020 May 15;10(1):8122.
6. Dong, L., Meng, Y., Sui, Z. et al. Comparison of four digital PCR platforms for accurate quantification of DNA copy number of a certified plasmid DNA reference material. Sci Rep 5, 13174 (2015).
7. https://www.technologynetworks.com/analysis/product-news/thermo-fisher-scientific-unveils-q-digital-pcr-system-for-innovation-in-genetic-analysis-355644
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11. https://assets.thermofisher.com/TFS-Assets/GSD/posters/comparing-realtime-digital-pcr-quantitation-poster.pdf.

January 6th, 2023

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To The Women's Board of The Johns Hopkins Hospital,

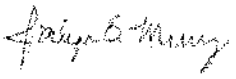
I am submitting the Molecular Diagnostics Laboratory (MDL) proposal for clinical testing development support on behalf of the Department of Pathology for review with The Women's Board of the Johns Hopkins Hospital.

We appreciate that our proposal is being considered by the Board and we hope to partner with The Women's Board so that at least 180 cancer patients each month at Hopkins can receive real-time mutational follow up results in a more rapid and cost-effective manner, resulting in improved clinical care. If selected for the award, this clinical development support would translate into an anticipated 2,180 Hopkins cancer patients receiving rapid mutation results over the next year.

We are requesting $75,000 to purchase a digital polymerase chain reaction (dPCR) instrument and desktop, installation and training, warranty, preventative maintenance, initial consumables, and white glove shipping. As we understand the budget may not extend to cover the full cost, we will gratefully accept what is available and will use the clinical laboratory budgeting process to obtain the remaining funds. Once validated in MDL, the proposed clinical assay would simplify repeated targeted mutation testing for cancer patients, thereby reducing the wet lab burden, the assay run duration, and offer a streamlined analysis and reporting approach for laboratory staff and directors that would translate into an actionable result in days rather than weeks, compared to the current in-house next-generation sequencing workflow.

We are excited for the opportunity to partner with The Women's Board of the Johns Hopkins Hospital and are looking forward to adopting this new assay platform that will allow MDL to personalize mutation testing needs for our current clinical patients in a potentially non-invasive manner by using liquid biopsy specimens. Once again, thank you for consideration of our proposal.

Sincerely,



Jaclyn B. Murry, PhD, FACMG
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Assistant Director-JHH Cytogenetics Laboratory
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Jmurry3@jh.edu