Structured Reports for the Pathological Society Equipment Award

Recipients of equipment awards from the Pathological Society of Great Britain and Ireland should submit a scientific report (within 12 months) detailing the equipment purchased using the award (even if only part funded), together with a brief description of representative research work undertaken using this equipment that has been partly supported by this equipment award and any outputs arising from this work. The reports should be set out using the following subheadings and should consist of:

Final report: 1 A4 page of text

Title: Miss and Mr
Name & Address:
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Background and aims:
At the time of application, two droplet digital PCR (ddPCR) droplet generators and one reader were available at Cardiff University. One droplet generator was located off-site at another faculty and therefore not available to us. The second droplet generator as well as a reader were available in a local NHS laboratory. Usage required a semi-formal application procedure, lengthy training and constant supervision from an NHS staff member. Due to diagnostic testing requirements, access to the equipment was restricted to two days a week for Cardiff University users. However, researchers from at least three different research groups across multiple divisions needed to access the droplet generator during its two days of availability; this naturally demanded rigorous adherence to a booking sheet and did not allow for any flexibility in case of unforeseen changes, leading to tension between individuals and groups.
The purchase of a ddPCR droplet generator was expected to have significant impact not only for our own projects but also for other research groups within the Division of Cancer and Genetics. For several users, sample throughput and experimental flexibility would be substantially improved, enabling research to progress far more efficiently. In addition, researchers previously unable to conform to the limited time-slots available around standard NHS procedures would be encouraged to incorporate it into their regular workflows.

Equipment Purchased (full description):
We used the £10,000 equipment grant to partially fund a QX200 droplet generator (Bio-Rad). The equipment was costed at £14,885.50 and we used internal funds to cover the exceeding expense of £4,885.50

Results of representative research work:

Felix Dobbs - Determining the mechanism of insertional mutagenesis caused by CRISPR/Cas9 Genome editing
Targeted genome editing using the RNA-guided nuclease CRISPR/Cas9 has rapidly become a routine method for generating precise mutations in mammalian cells. One major caveat of CRISPR/Cas9 technology is its ability to induce numerous off-target DNA double strand breaks (DSBs), in addition to the on-target cleavage site. For the development of CRISPR/Cas9 as a safe therapeutic modality, it is critical to be able to precisely identify the number and locations of DSBs induced throughout the genome. Recently, several NGS-based methods have been developed to identify DSBs genome-wide. Many of these methods however, suffer significant limitations, such as a high input material requirement and a lengthy low-throughput process. Others capture DSBs indirectly using oligonucleotide tags, making the study of DSBs in primary or hard to transfect cells unattainable.
To address these issues, we are developing a novel NGS-based method to detect and quantify DSBs genome-wide. We have utilised a single locus DSB quantification assay, based on droplet digital PCR, to quantify a selection of restriction endonuclease-induced DSBs at known CRISPR on/off-target sites. This data will be combined with NGS-based DSB detection

in order to improve genome-wide quantification. We will normalise read coverage across the set of DSBs measured using ddPCR to determine the relative frequencies of DSBs captured via NGS. DdPCR based library preparation will also be employed to reduce PCR amplification bias of sequencing libraries. By utilising these ddPCR based DSB capture methodologies in parallel, we aim to quantify the off-target activities induced by CRISPR/Cas9.

Conclusions:

The purchase of the QX200 droplet generator has had a significant impact for several groups within the Division of Cancer and Genetics and has removed a bottleneck that was previously limiting the use of droplet digital PCR. Access to an in-house droplet generator has vastly improved assay throughput for existing uses of ddPCR and has encouraged members of the Division who are unacquainted with the technology to consider it for their work. As a stand-alone piece of equipment, the ddPCR droplet generator has also provided a unique opportunity for the application of emulsion PCR to other non-standard applications, such as to perform single molecule PCR for the generation NGS libraries. The acquisition of the QX200 droplet generator has aided in our ability to harness omics and NGS-based applications for biomedical research. We sincerely thank the Pathological Society for this equipment grant.

How Closely Have the Original Aims been Met:

The original aims were met 100%.

Outputs (including meeting abstracts, oral presentations, original papers, review articles) from the study in which the Pathological Society has been acknowledged:

F. Dobbs: INDUCE-seq: a scalable, unbiased, and quantitative method to identify genomic DSBs in cells or tissue. Oral and poster presentation at the UKEMS Annual Meeting, Oxford, September 2018

E. Meuser: The genomic and transcriptomic landscape of duodenal adenomas from patients with familial adenomatous polyposis and MUTYH-associated polyposis. Oral and poster presentation at the 3rd meeting of EHTG, Nice, France, September 2018

E. Meuser: The genomic and transcriptomic landscape of duodenal adenomas from patients with familial adenomatous polyposis and MUTYH-associated polyposis. Oral presentation at the annual meeting of CGA-ICC, San Diego, USA, October 2018

You may include up to 1 extra page for diagrams/images/tables and references.

MJ Arends as Chair of Research Subcommittee July 2017.