

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: Application for a Qubit Flex DNA quantitation kit and a EVOS Core XL microscope

Name & Address: Dr Laura Thomas and Dr Gillian Conway, Institute of Life Sciences 1, Swansea University, Swansea, SA2 8PP.

Background and aims:

A greater reliance on tissue culture and genomic technologies as the basis for our research in the *In vitro* Toxicology Group (IVTG), has put pressure on existing resources and led to out-of-date technologies requiring replacement. The funding requested was sought for two pieces of equipment to be used in conjunction with one another as part of several programmes of work:

1. Characterisation of intestinal tumour drivers using 3D patient-derived organoids. Dr L Thomas.
2. Investigation of the antineoplastic efficacy of plant derived bioactive compounds in a three-dimensional (3D) *in vitro* Glioblastoma multiforme models, Dr G Conway.
3. Investigation into the effect of marine nutraceuticals on *in vitro* multi-culture osteoarthritis models, Dr G Conway & Dr S Heffernan.

These studies use a combination of advanced tissue culture and genomic analyses to establish and characterise novel disease models, with an aim of providing a greater understanding of disease pathogenesis and leading to the development of new treatments.

Equipment Purchased (full description):

Total cost: £9,357.09 (VAT exempt)
Qubit Flex DNA quantitation kit (£4,600.00),
EVOS Core XL microscope (£4,757.09)

Amount Requested: £8,357.09

Amount Contributed: £1000

Results of representative research work:

Characterisation of intestinal tumour drivers using 3D patient-derived organoids. Dr L Thomas.

A total of 36 patient derived organoids (PDOs) from duodenal adenomas (n=16) and normal duodenal mucosa (n=20), have been derived (Figure 1). These PDOs have undergone genetic, morphological and histological characterisation using the equipment funded here. This has demonstrated that the PDOs recapitulate the tumours from which they were derived. The EVOS microscope was used as part of the histological and morphological characterisation process, producing high quality images. These images will be used in a publication and will form the basis of grants to national funders. The qubit was used for the genetic characterisation of the organoids, accurately quantifying the amount of DNA and RNA

derived from the lines prior to whole exome and whole transcriptome analysis. It was also used to quantify the prepared libraries to confirm success of the process prior to undertaking costly sequencing. Our ability to grow and characterise these lines has further led to the establishment of additional organoid projects, including the development of normal duodenal and colorectal cancer and normal organoids at Swansea University.

Investigation of the antineoplastic efficacy of plant derived bioactive compounds in a three-dimensional (3D) *in vitro* Glioblastoma multiforme models, Dr G Conway.

Human neural stem cells have been grown in 2D with onward 3D development underway. Naturally derived bioactive compounds (NDBC) have been investigated in these lines to investigate changes in the PI3K–AKT–mTOR signalling pathway following treatment with NDBC. Neural cells show a dose dependent response to NDBC.

Investigation into the effect of marine nutraceuticals on *in vitro* multi-culture osteoarthritis models, Dr G Conway & Dr S Heffernan.

The EVOS XL was used in a project that is establishing a osteoporotic inflammatory *in vitro* cell culture model. The microscope was used in the characterisation of osteoblasts cultures by the identification mineral deposits using Alizarin Red staining (Figure 2). The microscope is also being used to confirm whether the mineral deposits are in fact calcium deposits using the Von Kossa Stain, a feature commonly associated with osteoblast models. The images generated from the microscope will be used for publication in the near future. In addition, the microscope is used on daily basis for routine microscopic evaluation of cell culture flasks, to determine cell confluency and over cell health.

Conclusions:

The Institute of Life Sciences, Swansea University is rapidly progressing towards 3D technologies and genomics methodologies to underpin their research in toxicology and carcinogenesis. The purchase of the EVOS microscope in conjunction with the qubit has significantly contributed to our development in this area and we sincerely thank the Pathological Society for this equipment grant.

How Closely Have the Original Aims been Met:

The scientific aims have been met. The new EVOS Core XL has not only taken the pressure off the existing older microscope but also has allowed the capture of high-quality colour images suitable for high-impact publications, thus significantly enhancing the quality of the work that we produce. The Qubit has provided greater sensitivity, specificity and accuracy for quantification of nucleic acids, particularly at lower concentrations. The addition of the Qubit has had a significant impact on our lab by increasing work efficiency, while future proofing and improving our facility.

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

Two publications from the above-described work are being prepared for submission. Images of duodenal organoid models were presented in a platform presentation at The European Hereditary Tumour Group (EHTG) meeting (Online), September 2021.

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

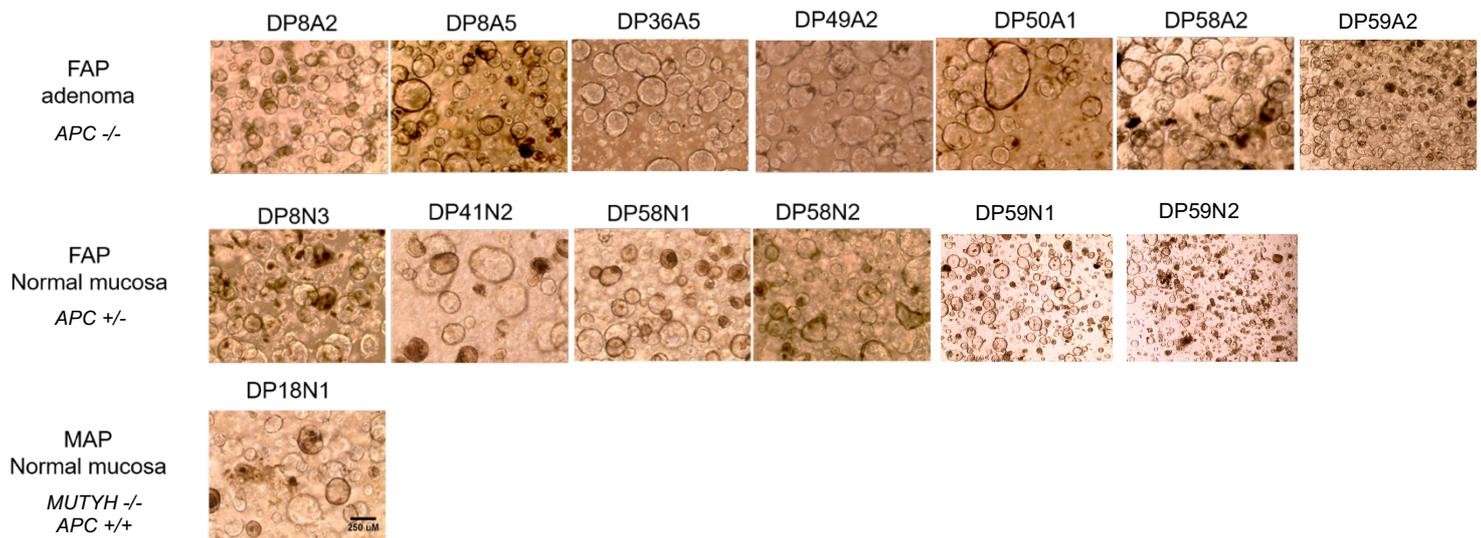


Figure 1. Representative images of patient derived organoid lines successfully established and characterised from FAP and MAP patient duodenal tissue biopsies from a total of 36 lines. Representative phase-contrast images of all organoid lines (day 10). Images were captured using a x4 objective. Scale bar = 250μM.

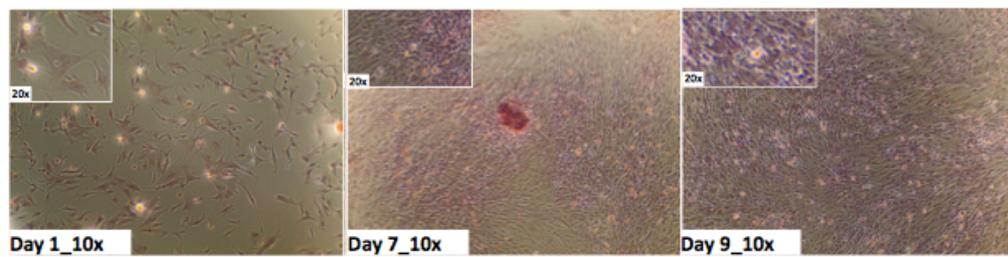


Figure 2. Alizarin Red staining showing osteoblast cell calcium deposition across days day1, 7 & 9 of cell growth. Day 9 image also shows significant confluency and cells clumping together (multiple larger bright red areas).