Title: Dr

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Background and aims:

Over the last three years, our team have developed a robust method that enabled us to generate good quality targeted and whole genome sequencing data from limited amounts of input DNA. The method was designed to study genomic changes in relatively small populations of cells or microanatomical structures (e.g. colonic crypts, prostatic acini and endometrial glands) which were obtained through laser capture microdissection (LCM). This approach allows us to explore genomic and evolutionary landscapes of different tissues while preserving precise phenotypic and spatial information. It also circumvents whole genome amplification and many of the associated issues such as incomplete genome coverage and amplification-induced errors.

Our initial work based on this method had provided valuable insights into to the impact of ageing on the accumulation of somatic mutations, including cancer-associated driver events, in a variety of histologically normal tissues¹⁻³. This success has led to the expansion of our research programme aiming to answer similar questions in new organ systems as well as disease states. The expansion of experimental work meant that additional histology equipment, including a second microtome, were required to keep up with the increased demand for tissues slides from which LCM microbiopsies would be generated. In addition, we aimed to explore the transcriptomic consequences of genomic changes that we observe in normal tissues and planned to develop a matching low-input LCM RNA-sequencing pipeline. We therefore applied for funding to purchase another microtome.

Equipment Purchased (full description):

Leica Manual Mechanical Rotary Microtome (total cost £7,781.03; contributed by the Pathological Society - \pounds 6,871.00)

Results of representative research work:

The second microtome allowed us to continue low input DNA work across various tissue types, which has led to a number of high impact publications (these are outlined in the 'Outputs' section). It has also helped us in the development of a matching low input RNA sequencing method to explore transcriptomic changes in those tissues (**Figure 1**).



Figure 1 | Preliminary results from our recently developed low input RNA sequencing pipeline for LCM-based experiments. These graphs show that we can extract and sequence endogenous RNA across various tissues, including testis (normal and tumour), liver (normal and cirrhotic) and kidney (tumour).

Conclusions:

The purchase of the second Leica Microtome has significantly contributed to further expansion of the LCM – based genomic studies. Furthermore, it facilitated the development of a matching RNA-sequencing pipeline. We thank the Pathological Society for this equipment grant.

How Closely Have the Original Aims been Met:

All original aims were met.

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

Peer-reviewed publications and preprints

Moore L*, Cagan A, Coorens T*, Neville DC, Sanghvi R, Sanders MA, Oliver TRW, Leongamornlert D, Ellis P, Noorani A, Mitchell TJ, Butler TM, Hooks Y, Warren AY, Jorgensen M, Dawson K, Menzies A, O'Neil L, Latimer C, Teng M, van Boxtel R, Iacobuzio-Donahue C, Martincorena I, Heer R, Campbell PJ, Fitzgerald RC, Stratton MR†, Rahbari R†. The landscape of somatic mutations in normal human cells. Manuscript under review in *Nature*.

Coorens T*, **Moore L***, Robinson PS, Sanghvi R, Christopher J, Hewinson J, Cagan A, Oliver TRW, Neville DC, Hooks Y, Noorani A, Mitchell TJ, Fitzgerald RC, Campbell PJ, Martincorena I, Fitzgerald RC, Rahbari R, Stratton MR. Extensive phylogenies of human development reveal variable embryonic patterns. Manuscript under review in *Nature*.

Ellis P*, **Moore L***, Sanders MA*, Butler TM*, Brunner SF, Lee-Six H, Osborne RJ, Farr B, Lawson ARJ, Cagan A, Stratton MR, Martincorena I, Campbell PJ. Reliable detection of somatic mutations in solid tissues by laser-capture microdissection and low-input DNA sequencing. *Nature Protocols* https://doi.org/10.1038/s41596-020-00437-6

Garcia-Alonso L[†], Handfield LF[†], Roberts K[†], Nikolakopoulou K[†], Fernando RC, Gardner L, Woodhams B, Arutyunyan A, Polanski K, Hoo R, Sancho-Serra C, Li T, Kwakwa K, Tuck E, Kleshchevnikov V, Tarkowska A, Porter T, Icoresi-Mazzeo C, van Dongen S, Dabrowska M, Vaskivskyi V, Mahbubani KT, Park J, Jimenez-Linan M, Campos L, Kiselev V, Lindskog C, Ayuk P, Prigmore E, Stratton MR, Saeb-Parsy K, Moffett A, **Moore L**, Bayraktar OA, Teichmann SA*, Turco MY*, Vento-Tormo R*. Mapping the temporal and spatial dynamics of the human endometrium *in vivo* and *in vitro*. Manuscript under review in *Science*.

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Brunner SF, Roberts ND, Wylie LA, Moore L, Aitken SJ, Davies SE, Sanders MA, Ellis P, Alder C, Hooks Y, Abascal F, Stratton MR, Martincorena I, Hoare M, Campbell PJ (2019) Somatic mutations and clonal dynamics in healthy and cirrhotic human liver. *Nature* 574, 538-542.

Lee-Six H, Olafsson S, Ellis P, Osborne RJ, Sanders MA, Moore L, Georgakopoulos N, Torrente F, Noorani A, Goddard M, Robinson R, Coorens T, O'Neill, Alder C, Wang J, Fitzgerald RC, Zilbauer M, Coleman N, Saeb-Parsy K, Martincorena I, Campbell PJ, Stratton MR (2019) The landscape of somatic mutation in normal colorectal epithelial cells. *Nature* 574, 532-537.