# Dot blotting for fibre type identification of single muscle fibres: a fast, reliable and sample-sparing method

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**Background:** Many skeletal muscle proteins are present in a cell-specific or fibre type-dependent manner. Stimuli such as exercise, aging, and disease have been reported to result in fibre-specific responses in protein abundances. Thus, fibre-specific determination of the content of specific proteins provides enhanced mechanistic understanding of muscle physiology and biochemistry compared with typically performed whole-muscle homogenate analyses. This analysis, however, is laborious and typically not performed. We present a novel dot blotting methodology for easy and rapid determination of skeletal muscle fibre type based on myosin heavy chain (MHC) isoform presence, further demonstrating a sample-sparing method of broad fibre type-dependent protein analyses that should be implemented for future human studies.

**Methods:** The study participants were healthy males, non-smokers, and performed structured exercise 2-3 times per week (n=2). Briefly, samples were obtained from the *vastus lateralis* muscle under local anaesthesia (1% Xylocaine) using a Bergström needle with suction. Samples were freeze-dried for 48 hours, brought to room temperature, and segments of individual muscle fibres (1-3mm) were collected under a microscope using jeweller's forceps in preparation for dot blotting and Western blotting analysis, as described by Murphy *et al* (2011). Following confirmation of skeletal muscle fibre type based on myosin heavy chain (MHC) isoform presence within individual fibre segments *via* dot blotting, the remaining volume of samples were analysed *via* Western blotting for the presence of sarco-endoplasmic reticulum calcium-ATPase (SERCA) isoforms, SERCA1 and SERCA2a, calsequestrin (CSQ) isoforms, CSQ1 and CSQ2, Actin, AMP-activated protein kinase-beta 2 and cytochrome c oxidase subunit 4, demonstrating fibre type-dependent protein abundance between Type I and II muscle fibres.

**Results and Conclusions:** Following the prescribed methodology, the ability to collect and analyse skeletal muscle samples for relative qualitative and quantitative measurements of proteins in broad muscle fibre types is easily accessible. Utilising dot blotting, the rapid determination of muscle fibre type of the collected segments proceeds the pooling of fibre segment samples. Subsequently, proteins can then be quantified at the pooled-fibre segment level (*i.e.* Type I or II) using an innovative Western blotting technique. Overall, the significant conclusions from this work are that *1)* qualitative determination of muscle fibre type of fibre segments from a biopsy can be performed using dot blotting, with a low volume of sample (*i.e.* small amount of protein); *2)* fibre typing ~50 fibre segments using the prescribed methodology reduces the cost ~40-fold and the experimental time ~3-fold, compared to traditional Western blotting MHC isoform analyses; *3)* breakpoints in the 95% confidence interval widths occurred between 3 and 9 pooled fibre segments, indicating the correct number of fibre segments to pool with respect to accuracy of protein quantification. The methodology presented, and our demonstrated ability to reliably measure the abundance of proteins of varying abundance in groups of only a few fibre segments, will facilitate improvements in our understanding of how muscle fibre type plays a crucial regulatory role in skeletal muscle physiology.

Murphy RM. (2011). Enhanced technique to measure proteins in single segments of human skeletal muscle fibres: fibre-type dependence of AMPK-$\alpha_1$ and $-\beta_1$. *Journal of Applied Physiology* **110**, 820-825.