Isolation of skeletal muscle stem cells by fluorescence-activated cell sorting.

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Public Summary:
The prospective isolation of purified stem cell populations has dramatically altered the field of stem cell biology and has been a major focus of research across tissues in different organisms. Muscle stem cells are now among the most intensely studied stem cell populations in mammalian systems and the prospective isolation of these cells has allowed cellular and molecular characterizations not dreamed of a decade ago. In this protocol, we describe a method to isolate muscle stem cells from limb muscles of adult mice based on the specific and unique markers that these cells possess on their surface. We provide a detailed description of the physical and enzymatic dissociation to release all cells in the muscle, a procedure that is essential to maximize cell yield. We also describe a method that is used subsequently to separate muscle stem cells from the other types of cells using specific markers. The protocol has been proven efficient and reliable and broadly used in laboratories around the world.

Scientific Abstract:
The prospective isolation of purified stem cell populations has dramatically altered the field of stem cell biology, and it has been a major focus of research across tissues in different organisms. Muscle stem cells (MuSCs) are now among the most intensely studied stem cell populations in mammalian systems, and the prospective isolation of these cells has allowed cellular and molecular characterizations that were not dreamed of a decade ago. In this protocol, we describe how to isolate MuSCs from limb muscles of adult mice by fluorescence-activated cell sorting (FACS). We provide a detailed description of the physical and enzymatic dissociation of mononucleated cells from limb muscles, a procedure that is essential in order to maximize cell yield. We also describe a FACS-based method that is used subsequently to obtain highly pure populations of either quiescent or activated MuSCs (VCAM(+)CD31(-)CD45(-)Sca1(-)). The isolation process takes approximately 5-6 h to complete. The protocol also allows for the isolation of endothelial cells, hematopoietic cells and mesenchymal stem cells from muscle tissue.