

Methodological aspects of gene expression in high-speed flow sorted stem cells (side population cells) from mouse bone marrow

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Since little is known of gene expression in hematopoietic stem cells related to time, and in particular as compared to the total bone marrow population, this has been addressed in B6 D2f black mice. The aim was therefore to establish if clock genes are expressed in stem cells - side population (SP) cells and if they exhibit a rhythmic pattern in the same way as other cell types. We have therefore done a comparison between the expression of clock genes in the whole marrow and in mice kept under standardized conditions. Bone marrow was obtained from the femurs of every 4 hours for 24- hours periods. Following Hoechst 33342 fluorescent labelling, the SP containing early progenitor cells was highly enriched by the ultra-speed flow cytometric cell sorting using the MoFlo instrument. The transcription of 7 different genes Per 1, Per 2, Bmal1, Clock, Cry 1, Rev-erb alpha and Cyclin D3 was measured with real-time PCR and compared to several reference genes (18S, GAPDH and 36B4). Some preliminary data on clock genes expression are discussed.