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Title:	Combination of Immunofluorescence and Quantitative Fluorescence In-situ Hybridization for Analysing Differential Gene Expression in the Niche Cells of the Drosophila Lymph Gland						
Authors:	Ramesh, Parvathy (/jspui/browse?type=author&value=Ramesh%2C+Parvathy) Ghosh, Sushmit (/jspui/browse?type=author&value=Ghosh%2C+Sushmit) Mandal, Lolitika (/jspui/browse?type=author&value=Mandal%2C+Lolitika)						
Keywords:	Combination of Immunofluorescence Quantitative Fluorescence In-situ Hybridization Analysing Differential Gene Expression the Niche Cells of the Drosophila Lymph Gland						
Issue Date:	2022						
Publisher:	Bio-protocol						
Citation:	Bio-Protocol, 12(2), 4290.						
Abstract:	The Drosophila larval haematopoietic organ or lymph gland consists of multiple cell types arranged in zones. The smallest stem cell compartment consists of 40-45 cells that constitute the haematopoietic niche. In order to analyse the haematopoietic niche, it needs to be labelled with a specific antibody to differentiate it from the other cell types. To characterise a phenotype, it is often necessary to investigate the expression of a gene in a particular stem cell compartment within the lymph gland. In such a situation, in-situ hybridization is performed, as it indicates the localization of gene expression. Although chromogenic in-situ hybridization enables us to compare the signal and tissue morphology simultaneously, it fails to harness the information related to the degree of gene expression. Dual immunofluorescence and in-situ hybridization (IF-FISH) serves as the powerful technique that helps to visualize both protein and mRNA expression within the same cetype. This technique also provides reliable quantification regarding mRNA expression levels. When dealing with a few cells within the organ, like the niche of the larval lymph gland, fluorescently labelled riboprobes allows us to localize and assess the magnitude of gene expression within the niche cells, which are also immunolabelled with a niche-specific marker, to distinguish them from the adjoining cell types.						
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