

An Optimized Protocol for Microdissection of Epithelial Cells from Goat Mammary Tissue to Preserve RNA Integrity

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In order to aid in understanding how mammary cells proliferate and prepare for milk synthesis, expression profiling analyses of mammary epithelial cells (MEC) is required. However, the mammary gland is a complex tissue made of multiple cell types. To overcome such a tissue heterogeneity, cell and cell types must be isolated selectively for further analyses. Laser Capture Microdissection (LCM) has proved to allow simple, rapid and precise retrieval of target cells. The isolation of nucleic acids comprise numerous steps that may cause degradation and loss of RNA. Poor quality of RNA results in lower recovery of an already limited amount of input material.

During lactation, the mammary tissue is rich in RNA degrading enzymes. This is a critical issue to preserve RNA integrity after LCM. Classical LCM protocols, applied to mammary tissue provide tissue sections with preserved morphology. Unfortunately, the integrity of RNA extracted from captured cells (checked with the Agilent Bioanalyzer 2100: RIN*), is not reproducible. The aim of this study was to develop an alternative protocol to preserve RNA integrity without loss of tissue section morphology. Treatments of tissue sections just before staining with different RNA stabilization reagents were tested since the staining solution may promote RNA degradation.