# Development of a Novel Cell-Scanning System (Cell Track) for Expression Cloning of The Carboxyl-Terminal Parathyroid Hormone Receptor (CPTHR)

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Parathyroid hormone (PTH), an 84-amino-acid polypeptide, is a major systemic regulator of calcium homeostasis and bone remodeling and elicits its classical functions by activating PTH/PTHrP receptors (PTHRs) on target cells. Carboxyl (C) fragments of PTH, secreted by the parathyroids in a calcium-dependent manner or generated by PTH proteolysis in the liver, circulate in blood at concentrations much higher than intact PTH(1-84) but cannot activate PTHRs. Receptors specific for C-PTH fragments (CPTHRs), distinct from PTHRs, are expressed by bone cells, especially osteocytes, in which they may regulate intercellular communication and cell survival. Cloning of the CPTH receptor is essential for characterization of its function(s) and mechanism of action(s). We are using an expression cloning approach to clone the CPTH receptor. Expression cloning requires a highly sensitive, specific and rapid technique for detecting receptor expression in single cells transfected with pools of the cDNA library of interest.

We have tested the sensitivity of a novel cell scanning system, “Cell Track”, adapted from an instrument originally designed for analysis of multiplexed short tandem repeat DNA. The system supports a glass microdevice with 16 parallel channels, each of 20 cm effective length, and double-T cross injectors. A high-speed rotatory conofocal line scanner with four-color detector enables detection of single-cell fluorescence(s) and was specially designed to accommodate the high elution rates on the microdevice. To examine the sensitivity of the Cell Track system, we tested samples in which 0.1% of cells (osteocytes) expressed CPTHRs and were pre-bound to biotinylated human [Bio85, Tyr34]PTH (23-84) and then reacted with fluorescent streptavidin (Texas Red). The remaining cells in these samples (99.9%) were COS-7 cells that are known not to express CPTHRs. The Cell Track System reliably detected the cells expressing CPTHR in these samples (0.1%), displaying data as images of single positive cells with corresponding fluorescent signal intensity. No signal was detected in control samples consisting of COS-7 cells alone. The Cell Track System, which is adaptable for analysis of 384 samples simultaneously, provides rapid, reliable and highly sensitive detection and promises to facilitate CPTHR expression cloning and other applications requiring analysis of low-frequency subpopulations of cells identifiable by fluorescent markers.