中文題目：應用 NanoString 數位化精準定量核酸分析儀定量血清 mRNA 之表達

英文題目：**Profiling plasma cell-free RNA (cfRNA) with the NanoString low input nCounter assay**

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Background: Liquid biopsy offers advantages over tissue biopsy for early disease diagnosis, monitoring of disease progression, and evaluation of therapeutic interventions because sample collection is rapid, easy, minimally invasive, and repeatable. In particular, measuring cell-free RNA (cfRNA) in the plasma offers the opportunity for direct monitoring of a tumor cell byproduct without the need for direct access to the tumor. However, robust cfRNA detection has been difficult historically due to weak and highly variable signal detection. The nCounter platform (Research Use Only)and low input profiling protocol have been adapted to enable robust and reproducible detection of cfRNA from plasma, which is demonstrated here in proof of concept experiments using synthetic targets and samples to quantify transcription of standard gene transcripts and fusion products.

Methods: The standard nCounter detection platform which leverages direct hybridization of optical barcodes to RNA transcripts of interest has been adapted to enable detection of rare targets by leveraging an initial targeted amplification step to increase transcript abundance with minimal bias of relative transcript abundance. Feasibility of this process to detect cfRNA was assessed with synthetic DNA oligos and commercially sourced patient-derived plasma samples. Plasma cfRNA was isolated and concentrated using commercial cfRNA kits. Relative expression of targets was benchmarked to universal reference RNA and compared between samples by both nCounter and qPCR.

Results: Using synthetic ultramer DNA oligo targets for 7 genes, the limit of detection of the assay was as low as 24 molecules per target with 100% specificity. Using cfRNA from healthy donors, the nCounter assay was shown to profile relative abundance of 40 targets. 9 genes were selected to benchmark nCounter against qPCR and showed high correlation (R^2 = 0.84) with linearity (slope = 0.97). cfRNA from healthy donors or patients with advanced CRC or NSCLC was profiled by the 770-gene PanCancer IO360 panel with a pre-amplification by the adapted nanoString low RNA input protocol. Unsupervised hierarchical clustering revealed that CRC derived cfRNA had different transcriptional profiles from healthy donors, and NSCLC cfRNA had an intermediate profile. Furthermore, a number of therapeutically relevant targets, including EGF, EGFR, PD-1, and PD-L1 could be detected in cfRNA from cancer patients but not in cfRNA from healthy donors.

Conclusion: The nCounter low input protocol can be used for transcriptional profiling of cfRNA in samples with high sensitivity and specificity. The flexible primer design strategy enables profiling of a variety of transcripts, including fusion gene products, with the possibility of profiling up to 800 transcripts from a single sample. In summary, the nCounter platform (RUO) provides a robust flexible molecular profiling tool for liquid biopsy research.