Comparison of qNPA vs qPCR on FFPE Tissue
Matt Rounsefell, Klaus Pechhold, Debra Thompson, Mark Schwartz, Bruce Seligmann
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INTRODUCTION
Accurate measurements of gene expression in fixed tissue and cells are required for clinical diagnostic tests and re- \nexpression analysis of studied samples. We evaluated the \nmeasurement of gene expression by qPCR and qNPA (HTG Molecular Diagnostics) using FFPE samples. qNPA uses qPCR to measure RNA expression on FFPE tissue and qNPA's multiplexed technology uses RNA extracted from fixed tissue. qPCR results indicate a high percentage of gene expression is observed in FFPE samples.

FIXATION AND ISCHEMIC TIME IMPACT
4 h 24 h 48 h 72 h
- Same tissue dissected and fixed for indicated times in neutral-buffered formalin.
- Samples lysed.
- qNPA housekeeper assay run in parallel. 
- Fixation times did not significantly impact the qNPA results.

EXPERIMENTAL OVERVIEW:
- Mouse lung tissue dissected and placed into fixative at 4 °C, and fixed for indicated times.
- Correlation plots show that 10 genes were affected by time and ischemic time. Other genes were not significantly affected. In contrast, qPCR returned a “present call” of 32 of the 35 genes in FFPE lysate, with a p-value of 0.01.

CONCLUSIONS:
- qNPA and qPCR performed well on RNA from frozen tissues.
- qNPA on FFPE samples was relatively insensitive to RNA degradation, and had a 95% present call percentage.
- Multiplexed qNPA used for fixed less than fixed tissue.
- TaqMan performed poorly on RNA isolated from fixed tissue.

FEPE PERFORMANCE

Transcription Aligned Pairs

DETAILED ANALYSIS

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RESULTS

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Measurement of mRNA from Cells Prepared for Flow Sorting

#4133

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Quantitative polymerase chain reaction (qPCR) is a method for genetically characterizing a sample. qPCR is a highly sensitive and specific technique for detecting and quantifying DNA or RNA sequences. The technology is based on the ability of DNA polymerase enzymes to replicate DNA from a single molecule, a process known as polymerase chain reaction (PCR). By measuring the increase in the amount of DNA during PCR, qPCR can be used to quantify the amount of DNA present in a sample. qPCR is widely used in molecular biology and genetics to study gene expression, analyze genetic variations, and diagnose genetic diseases.