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Technical note

Fetal gut laser microdissection in combination with RNA preamplification enables epithelial-specific transcriptional profiling ☆

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ABSTRACT

Laser microdissection (LMD) technology enables highly specific gene expression analyses of biologically relevant questions at cell- or tissue-specific resolution. Nevertheless, specific cell types are often limited in quantity (i.e. fetal tissue), making high quality RNA extraction and subsequent gene expression approaches via common reverse transcriptase-quantitative PCR (RT-q-PCR) challenging. In the case of fetal gut epithelia representing immune modulatory interphases gene expression analysis with common RT-q-PCR is limited to a few genes (<10). To circumvent these limitations we provide a workflow using laser microdissection of 1.5 Mio μm^2 dissected area of murine fetal intestinal epithelial cells (IEC) from fetal ileum and colon with subsequent RNA isolation, whole transcriptome preamplification (WTA) and gene expression analysis by microarray and quantitative PCR (qPCR). This workflow allows simultaneous analyses of global (microarrays) and targeted gene expression (qPCR) and consequently increases the number of measurable genes up to 25-fold by qPCR. It is suitable for cryosections from many tissues and species in order to evaluate in utero biological effects on specific effector sites.

Introduction

The importance to evaluate biological mechanisms on single cell resolution is most evident, especially with regard to the analysis of human disease-relevant interfaces, such as clinical specimens. Laser microdissection with subsequent microarray analysis and reverse transcriptase-quantitative PCR (RT-qPCR) is the most suitable investigative tool for gene expression analysis in basic and clinical research (Cohen et al., 2002; Espina et al., 2006; Lotz et al., 2006). Nevertheless, limited RNA yields and qualities of laser microdissected tissue make gene expression analysis unreliable. Especially, when addressing the analysis of global transcriptional programs from limited tissues (e.g. during organogenesis) isolation of sufficient cells via LMD with adequate RNA yields and qualities is very critical. Gene expression approaches (transcriptome profiling, RT-qPCR) often do not consider quality loss. Limited RNA amounts of laser microdissected samples (Mikulowska-Mennis et al., 2002) make whole transcriptome amplification (WTA) indispensable (Vannucci et al., 2013; Zheng et al., 2013) for subsequent analysis. Consequently, RNA purity and quality are essential and therefore, we established a LMD-based protocol for fetal tissue that is applicable to be implemented for the analysis of a variety of different species and tissue types. This protocol provides a complete workflow of prenatal breeding, dissection of the fetal intestine including laser

☆ Notes: None of the material has been published or is under consideration elsewhere, including the internet. The animal experiments were performed according to the German guidelines for animal care.

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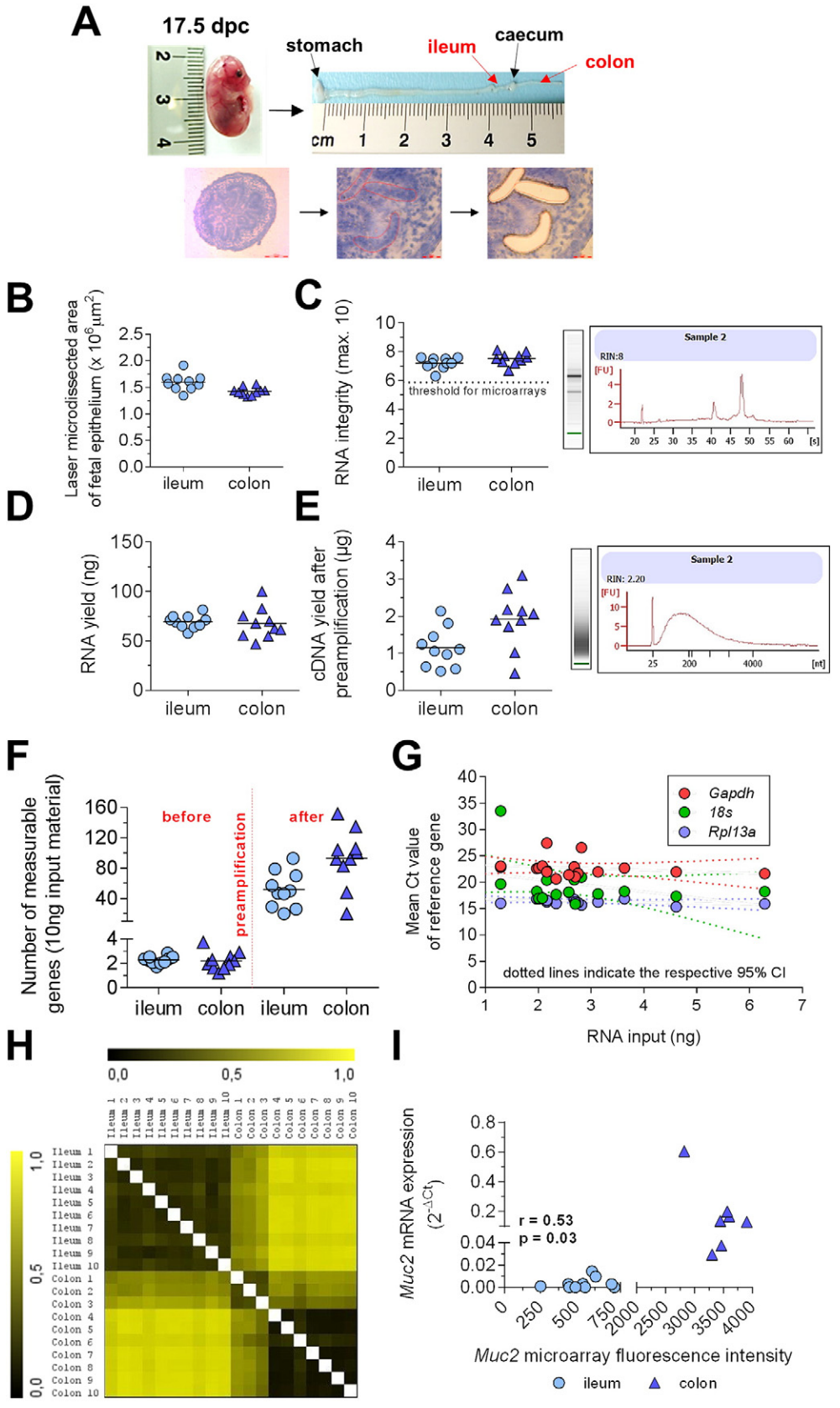
A
17.5 dpc
stomach
ileum
caecum
colon
cm 1 2 3 4 5
B
Laser microdissected area of fetal epithelium (x 10^6 µm^2)
ileum
colon
C
RNA integrity (max. 10)
threshold for microarrays
Sample 2
RIN:8
[FU]
[s]
D
RNA yield (ng)
E
cDNA yield after preamplification (µg)
Sample 2
RIN: 2.20
[nt]
F
Number of measurable genes (10ng input material)
before
preamplification
after
G
Mean Ct value of reference gene
Gapdh
18s
Rpl13a
dotted lines indicate the respective 95% CI
RNA input (ng)
H
I
Muc2 mRNA expression (2^-ΔCt)
r = 0.53
p = 0.03
Muc2 microarray fluorescence intensity
ileum
colon