Forensic Science International 203 (2010) 76–82

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Postmortem mRNA profiling II: Practical considerations☆

Marielle Vennemann [a,b,*], Antje Koppelkamm [a]

[a] Institute of Legal Medicine, University of Freiburg, Albertstr. 9, 79104 Freiburg, Germany
[b] Centre for Forensic Science, Strathclyde University, Royal College, 204 George Street, Glasgow G1 1XW, Scotland, UK

ARTICLE INFO

Article history:
Available online 17 August 2010

Keywords:
mRNA profiling
Postmortem tissue
Sample handling and processing
Data interpretation

ABSTRACT

Using human postmortem tissues for gene expression studies is particularly challenging. Besides the problem of impaired RNA one has to face a very high degree of biological variance within a sample set. Variations of individual parameters like age, body mass, health, but also the cause and circumstances of death and the postmortem interval lead to a rather inhomogeneous collection of samples.

To meet these problems it is necessary to consider certain precautions before starting a gene expression project. These precautions include the sample collection and the determination of the RNA integrity, the number of replicates needed and the methods used for reverse transcription and quantitative polymerase chain reaction, but also the strategy for data normalisation and data interpretation.

In this article practical issues are discussed to address some of the problems occurring in the work with postmortem human samples obtained during medico-legal autopsy.

1. Introduction

Studying gene expression patterns in human postmortem tissue is becoming an ambitious field in molecular forensic research. For the isolation of RNA from autopsy tissue it is necessary to strictly avoid any further degradation during handling and processing of the samples. Thus, certain considerations concerning the organisation of the workplace have to be made to ensure an RNase-free environment. The quality of RNA should be as high as possible, but nevertheless, a certain degree of degradation cannot be avoided when working with postmortem samples. Thus, the knowledge of RNA integrity and its impact on quantitative gene expression data is indispensable [1].

Besides partial degradation, one is confronted with the problem of a rather inhomogeneous sample set. When working with animal models it is possible to control the conditions before death and to minimise the biological variance within a sample collection. In humans, the samples comprise a number of highly varying parameters; including age, body mass, health, fitness and life style. Additionally, different causes and circumstances of death and varying postmortem intervals further add to the biological variances. These problems have implications for the sample collection, the number of biological and technical replicates needed and the data normalisation strategy.

Finally, the interpretation of quantitative gene expression data obtained from postmortem tissue should be performed very carefully. It is necessary to keep in mind the possibility of impaired and adulterated results, which might occur due to low RNA integrities or a high biological variance within the sample set.

In this article we aimed to discuss several practical considerations to address some of the above mentioned parameters.

2. Avoiding RNase mediated degradation

Working with RNA requires some special precautions to avoid further RNA degradation during handling and processing of the samples. The main focus should be on a strictly RNase-free environment. RNases are omnipresent and are produced by all organisms. In contrast to DNases, they do not need any co-factors, like Mg^{2+}, and are extremely stable, which explains their extraordinarily high reactivity [45]. Contact between samples/extracts and RNases via contaminated surfaces, tubes, glassware or pipette-tips should be avoided consequently. Since RNases show extreme stability, they cannot be destroyed by conventional surface cleaning and disinfection using for example detergents and alcohol. Additionally, autoclaving plastic and glassware is not sufficient to inactivate RNases. Further precautions are necessary to avoid RNase contamination in the first place and to destroy or inactivate RNases that are already present in consumables, buffers or within the sample itself.

A workplace dedicated exclusively to RNA handling including an extra set of pipettes, racks, tubes and pipette-tips is useful for the creation of a nearly RNase-free environment. It is not recommended to use a workplace for extraction of RNA close to

☆ This paper is part of the special issue entitled: Molecular Pathology in Forensic Medicine, Guest-edited by Burkhard Madea and Pekka Saukko.

* Corresponding author.
E-mail address: marielle.vennemann@strath.ac.uk (M. Vennemann).

0379-0738/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2010.07.007

the working area for extraction of DNA from crime scene stains. On the one hand, tissue samples contain high amounts of genomic DNA and thus, DNA contamination might occur. Additionally, at the end of RNA extraction, a DNase treatment of samples is necessary to eliminate residual genomic DNA. Thus, there should be no contact between any items that might be used for DNase handling and DNA extracted from stains.

Additionally, thorough cleaning of working surfaces in the RNA handling area and RNase inactivation is necessary. The most common method for RNase inactivation in water and buffers is treatment with diethylpyrocarbonate (DEPC), which is added to the solution and incubated overnight before it is autoclaved. DEPC binds primary and secondary amines (e.g. histidine) and builds covalent bindings, which inactivates RNases [2,3]. In aqueous solutions, DEPC is hydrolysed to CO_2 and ethanol, a reaction which is greatly accelerated by Tris (2-amino-2-hydroxymethyl-propane-1,3-diol) and other primary amines, which themselves can be destroyed in this process. Thus, DEPC is not suitable for the treatment of buffers containing amines [45]. Glassware and consumables can be baked at 200 °C for 2 h or rinsed with hydrogen peroxide. An easy-to-use alternative are commercially available RNase inactivation solutions that can be used to clean surfaces as well as plastic and glassware (e.g. ''RNaseAway'' from Molecular Bio Products, ''RNaseZap'' from Applied Biosystems, ''RNase-Off'' from PureBioTech or ''RNase-ExitusPlus'' from AppliChem). Additionally, the use of RNase-free aerosol-resistant pipette-tips can avoid the transfer of RNases through the pipettes.

3. Sample collection

When working with postmortem human tissues one is confronted with a rather heterogeneous sample set. The influence of parameters like age, gender [40,43], body mass and of course cause of death as well as specificities of the agonal phase [4,5] on the expression and the half-lives of certain gene transcripts is still widely unknown [6,7,43]. Thus, a careful selection of samples is crucial and within a sample set the above mentioned parameters should show a variance as low as possible.

To ensure comparability, samples should always be taken consistently from analogous areas from all individuals included in a study. Additionally, samples should be taken from undamaged areas without macroscopic signs of putrefaction.

Furthermore, when targeting biomarkers for the analysis of certain causes of death or specificities of the agonal phase, like hypoxia, it is crucial to have strict inclusion criteria. In general, results from a very well defined, but rather small group of individuals can be expected to provide more reliable data compared to those obtained from a large but rather heterogeneous group.

It is helpful to obtain as much information about the sample source as possible. By doing so, the influence of certain parameters, other than the one the study aimed to analyse, can be identified. Thus, besides the cause and circumstances of death and the postmortem interval the main results of the autopsy as well as body mass, height, gender and age at death need to be recorded. Further important forensic data may be the location in which the body was found, its temperature and clothes/covers and the time of storage at ambient and low temperature.

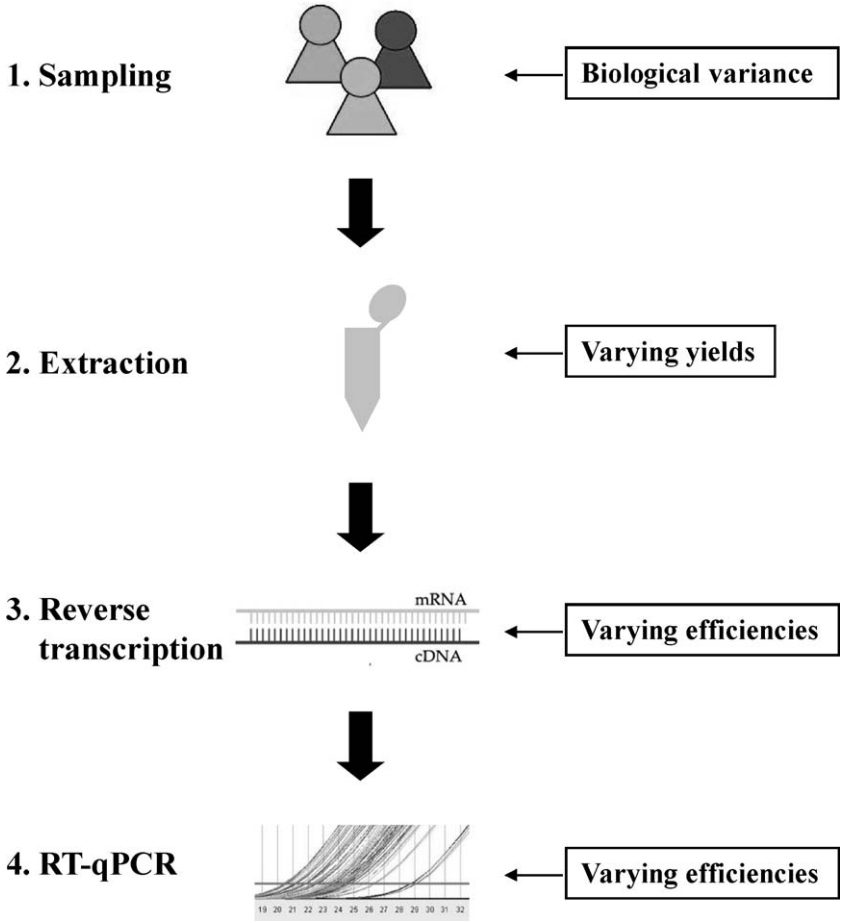

Fig. 1. Overview of sources of variation that occur throughout the workflow.