FFPE RNA Quality Evaluation (Qiagen) -- University of Minnesota TMCs

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Cellular Senescence Network (SenNet) Method Development Community

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Protocol status: Working
We use this protocol and it's working

Created: May 03, 2023

ABSTRACT

Completed prior to going through -omics and spatial technologies to evaluate FFPE blocks for their RNA quality.

BEFORE START INSTRUCTIONS

Evaluate if this is required for processing with your platform/technology. Not all require a certain DV200 to move forward with processing.

If tissue sample is scarce or rare, consider the best step -- Use some of the sample for RNA quality evaluation or move forward without it, understanding the risks and/or benefits of doing so.

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PROTOCOL integer ID: 81377

Keywords: RNA, FFPE, QC, UMN

1 **Qiagen Protocol:**

Qiagen RNeasy FFPE Handbook.pdf

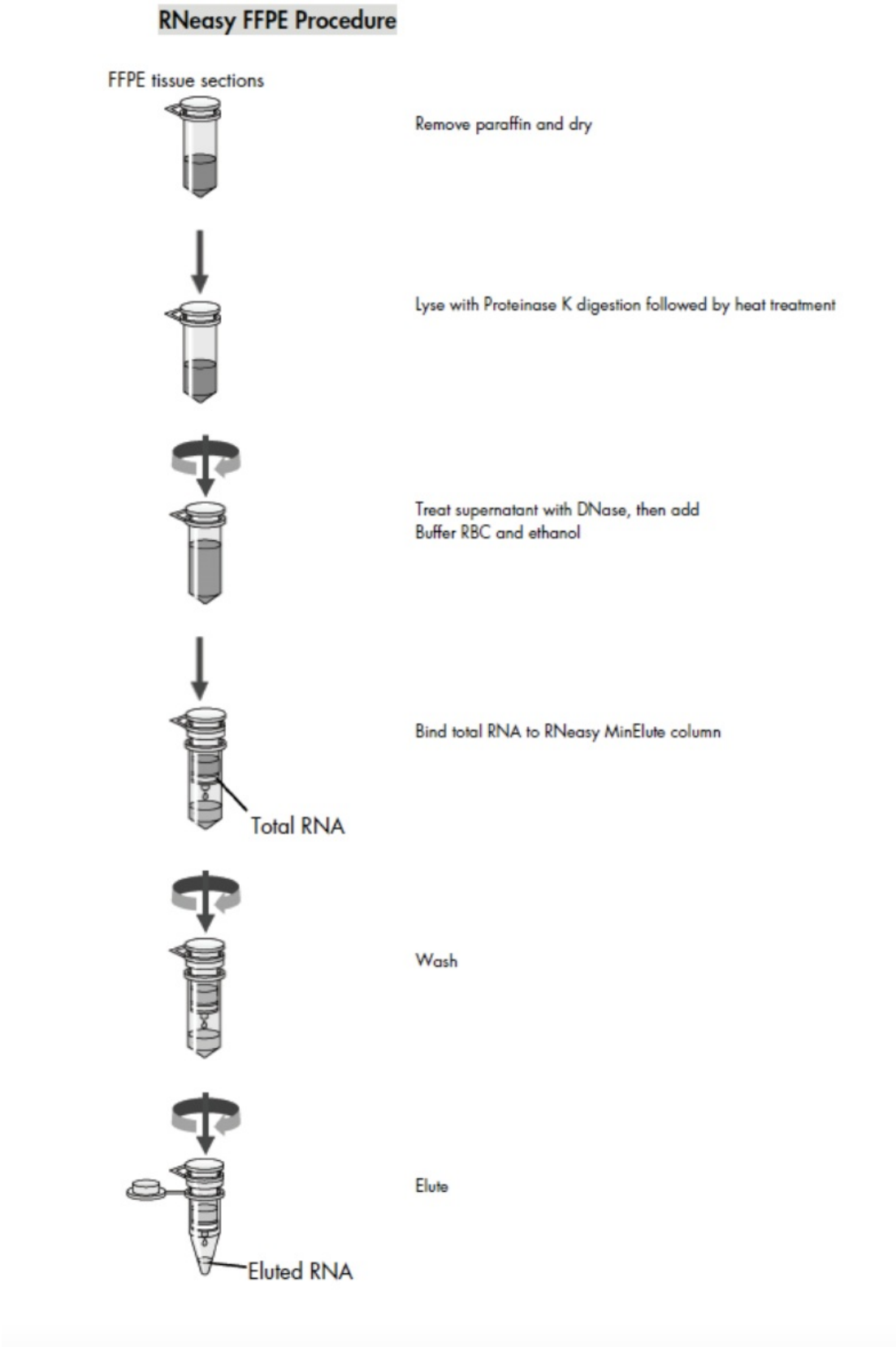
Qiagen product website: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/total-rna/rneasy-ffpe-kit

Cat. No. / ID: 73504

Qiagen Support contact form: https://www.qiagen.com/us/knowledge-and-support/~/link.aspx?_id=5402A118CD214AF7ADBAC65422659A71&_z=z

Time needed: ~2-4 hours, depending on number of samples

2



Overview of RNA isolation from FFPE

Preparation of Samples for Processing

3 Create or request curls from the FFPE blocks (typically done by histologist).
Follow recommendations as noted below.
Keep curls in a 1.5mL tube at -80ºC until RNA isolation process.

4

Starting material

Standard formalin-fixation and paraffin-embedding procedures always result in significant fragmentation and crosslinking of nucleic acids. To limit the extent of nucleic acid fragmentation and crosslinking, be sure to:

- Use tissue samples less than 5 mm thick to allow complete penetration by formalin
- Fixate tissue samples in 4–10% neutral-buffered formalin as quickly as possible after surgical removal
- Use a maximum fixation time of 24 hours (longer fixation times lead to over-fixation and more severe nucleic acid fragmentation, resulting in poor performance in downstream assays)
- Thoroughly dehydrate samples prior to embedding
- Use low-melting paraffin for embedding

The starting material for RNA purification should be freshly cut sections of FFPE tissue, each with a thickness of up to 20 µm. Thicker sections may result in lower nucleic acid yields, even after prolonged incubation with Proteinase K. Up to 4 sections, each with a thickness of up to 10 µm and a surface area of up to 250 mm2, can be combined in one preparation. More than 4 sections can be combined if the total sum of the thickness of the sections is 40 µm or less (e.g., eight 5 µm thick sections), or if less than 30% of the surface area consists of tissue and the excess paraffin is removed using a scalpel prior to starting the protocol.

For tissues with particularly high DNA content, such as thymus, we recommend using fewer sections per preparation to avoid DNA contamination of the purified RNA.

Once RNA isolation complete

5 Complete:
Nanodrop for RNA quantification
DV200 for (Eukaryotic total) RNA qualification