A new workflow for nucleic acid extraction from FFPE samples

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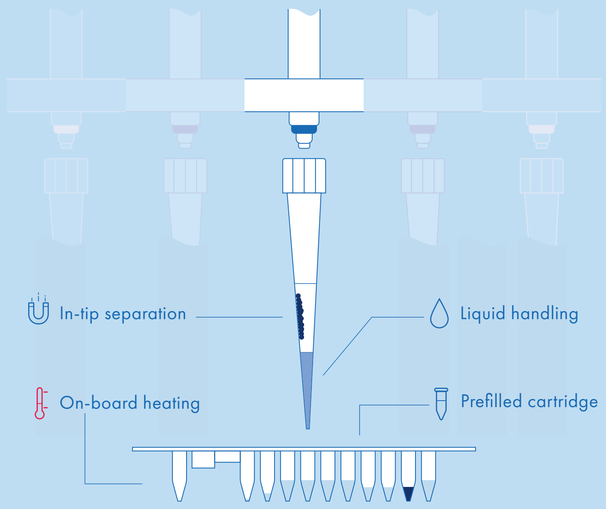
Removing tediousness in FFPE sample preparation

Formalin-fixed, paraffin-embedded (FFPE) tissue samples are often the only source of nucleic acids from tumor tissue. This makes them a valuable sample type for molecular biomedical research.

However, they are challenging to work with. The extraction of DNA, RNA or both from an FFPE slice usually requires a lot of manual interactions.

Here we present new workflows with large degrees of automation for high convenience and reproducibility in downstream analyses, such as (RT-)qPCR, NGS and digital PCR (dPCR)-based biomarker research.



High degree of automation. In-tip magnetic-bead-based separation, on-board heating, prefilled cartridges, automated cartridge piercing and liquid handling enable a high degree of automation for FFPE sample preparation.

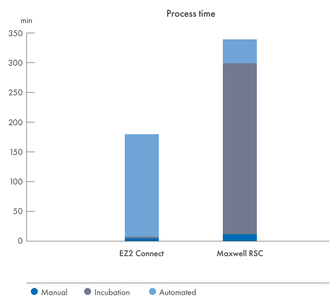
High degree of automation

For the separate extraction of DNA or RNA from FFPE samples, every step except deparaffinization is automated on the EZ2® Connect system. This reduces not only manual handling time but overall processing time as well.

Step	EZ2 Connect	Maxwell® RSC
Deparaffinization	✋	✋
First lysis	⚙	✋
Decrosslinking	⚙	✋
RNase/DNase treatment	⚙	✋
Binding	⚙	⚙
Washing	⚙	⚙
Elution	⚙	⚙

✋: manual ⚙: automated

Fewer manual steps. The EZ2 Connect instrument, used with the EZ1&2™ DNA FFPE Kit or the EZ2 RNA FFPE Kit to purify DNA or RNA, respectively, from FFPE, requires only one step (i.e., deparaffinization) to be implemented manually.

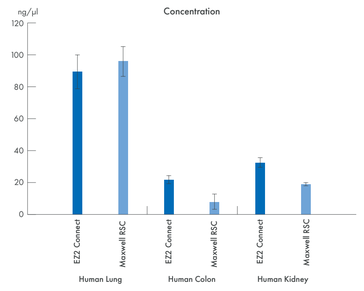


Shorter process time. Based on protocols described in their respective instructions for use, the EZ2 Connect system requires less manual handling time and drastically less overall process time to purify DNA from FFPE samples, compared to the Maxwell RSC system (1).

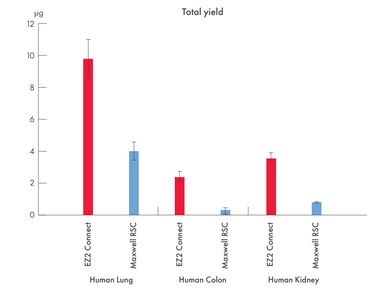
Efficient nucleic acid preparation

DNA was isolated from human lung, colon and kidney using the EZ2 Connect system (specifically, the EZ1&2 DNA FFPE Kit, fully automated on the EZ2 Connect instrument) versus the Maxwell RSC DNA FFPE Kit and its prescribed protocol.

Although DNA content varies among different organs, the EZ2 Connect system consistently yielded more DNA than the Maxwell RSC system, regardless of sample type.



Measured concentration. DNA concentration was measured on the QIAxpert® UV-vis spectrophotometer.

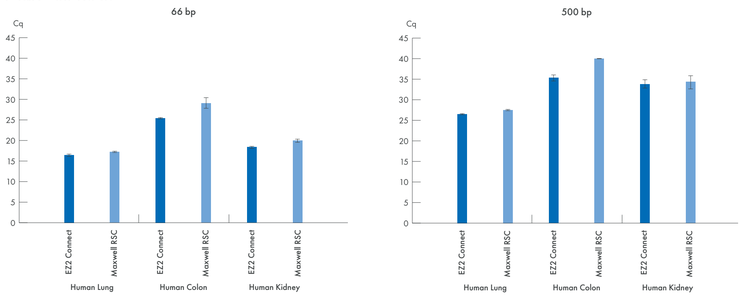


Greater DNA yield from EZ2 Connect. Total yield was calculated based on concentration and measured elution volume. Yield from the EZ2 Connect system was found to be consistently greater than that of the Maxwell RSC system.

Optimized for PCR performance

DNA was isolated from FFPE tissues of human lungs, colon, and kidney using the fully automated protocol of EZ2 Connect and the semiautomated protocol of Maxwell RSC. qPCR with a short (66 bp) and a longer (500 bp) amplicon was then performed on equal volumes of diluted eluate.

Regardless of tissue source or amplicon size, DNA extracted with the EZ2 Connect protocol performed better in qPCR than that extracted with Maxwell RSC.

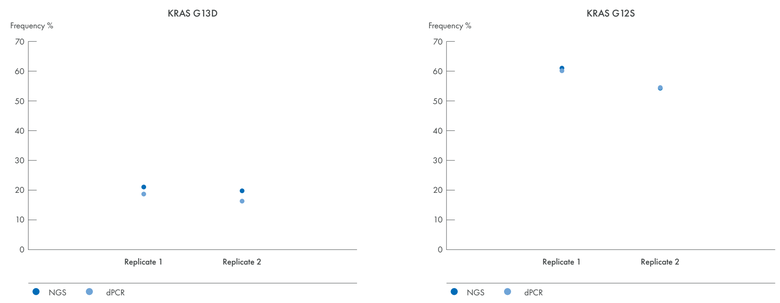


Consistently lower Cq values. DNA was extracted using either the EZ2 Connect system or the Maxwell RSC system. Equal volumes of diluted eluate were then subjected to qPCR. Samples processed with EZ2 Connect had consistently lower Cq values than those processed with Maxwell RSC.

Reliability in NGS and digital PCR

The EZ2 Connect system was used to isolate DNA from FFPE samples of human colon carcinoma. KRAS mutations G13D and G12S were measured and compared using dPCR with a QIAcuity® instrument and NGS on a NextSeq® 500 device.

Similar mutation levels were detected with both methods.

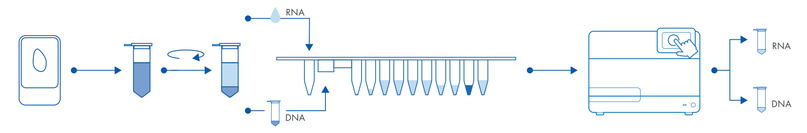


Reproducible results in dPCR and NGS. DNA was isolated from FFPE human colon carcinoma using the EZ1&2 DNA FFPE Kit fully automated protocol on an EZ2 Connect instrument. The frequencies for KRAS mutations G13D and G12S were measured using the QIAcuity system for dPCR and the NextSeq 500 with the QIAseq® Targeted DNA Panel for NGS. Replicate experiments were conducted to validate results.

Automated, simultaneous DNA and RNA purification with few manual steps

When both DNA and RNA are needed for analysis, they are commonly extracted separately, so the work has to be done twice – once for DNA extraction and the other for RNA extraction. This translates to a tedious workflow with many manual steps.

With the EZ2 AllPrep system, DNA and RNA are purified simultaneously from a single sample, using a single workflow, a single cartridge and a single run. The bind, wash and elute steps are fully automated. The result is a drastically shortened workflow with few manual steps.



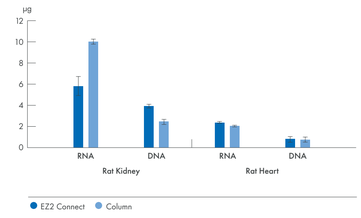
DNA and RNA from the same sample in a single run. Using the EZ2 AllPrep DNA/RNA FFPE Kit workflow, one FFPE sample is placed in a tube, where it is deparaffinized and lysed. The tube is then centrifuged to separate the DNA-containing pellet from the RNA-containing supernatant. The supernatant (RNA) is transferred into the EZ2 Connect cartridge; the tube with the pellet (DNA) is placed into the same cartridge. That cartridge is then loaded into the EZ2 Connect instrument, and the run is started. When the run is finished, a tube with the RNA eluate and another tube with the DNA eluate will be ready for use in downstream applications.

Highly suited for parallel genomics and transcriptomic profiling

Because tumor tissue is heterogenous, cutting a tumor tissue in half and then extracting DNA from one section and RNA from another means the two nucleic acid sources will have local differences. This could result in a disconnect between mutational status and transcriptome.

The EZ2 Connect system uses the EZ2 AllPrep DNA/RNA FFPE Kit on an EZ2 Connect instrument to extract both DNA and RNA from exactly the same cell population with exactly the same properties.

Compared to standard column procedure, the EZ2 AllPrep system still extracts a good amount of DNA and RNA from the same population, making it highly suited for parallel genomics and transcriptomic profiling.



Good RNA and DNA yield from exactly the same cell population DNA and RNA were extracted from FFPE rat kidney tissue and FFPE rat heart tissue, either separately, using the standard column procedure, or simultaneously, using the EZ2 AllPrep DNA/RNA FFPE Kit on an EZ2 Connect instrument. RNA was quantitated with the Qubit™ RNA HS Assay Kit; DNA, with the Qubit dsDNA BR Assay Kit. In all extractions except RNA from rat kidney, yield from the EZ2 Connect system was greater than from the column procedure.

Conclusion

With the new automated workflow, the EZ2 Connect system processes FFPE samples with:

- Shorter processing times
- Drastically less manual handling time
- Reliable compatibility with (RT-)qPCR, dPCR and NGS
- No need to divide FFPE slices to extract both DNA and RNA from the same sample

In addition, compared to the Maxwell RSC system, the EZ2 Connect system resulted in:

- Greater DNA yield
- Lower Cq values in downstream qPCR

These data show that the EZ2 Connect workflow simplifies the extraction of DNA and RNA from FFPE tissue samples without compromising the yield and quality of extracted nucleic acids for use in downstream applications.

Reference: 1. Maxwell RSC DNA FFPE Kit Technical Manual. November 2017.

Sample to Insight