Automated DNA extraction from FFPE tissue using a xylene-free deparaffinization method on QIAsymphony®

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Introduction

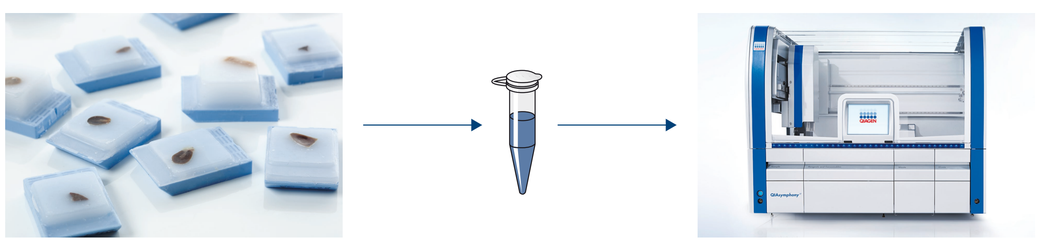
Formalin-fixed paraffin-embedded (FFPE) tissue samples are routinely used for immunohistochemistry and molecular analysis in cancer research. However, many methods for DNA extraction from FFPE tissue sections are manual procedures that are not standardized, time consuming and often involve the use of hazardous materials like xylene. Recently we introduced an automated solution for the DNA extraction from FFPE tissue using the QIAsymphony SP instrument in combination with the QIAsymphony DNA Mini kit. So far deparaffinization of the FFPE tissue sections is achieved by manual xylene/ethanol pretreatment followed by proteinase K digestion. Afterwards, lysates are transferred into QIAsymphony sample tubes and placed on the instrument for further processing.

In order to reduce hands-on time and to avoid use of xylene/ethanol we developed an alternative deparaffinization method that can be used for automated DNA extraction on the QIAsymphony SP instrument. The new method enables parallel deparaffinization and lysis of the FFPE tissue material without use of hazardous substances. The manual transfer and centrifugation steps were minimized and the risk of losing the sample material was eliminated. The handson time was significantly reduced resulting in a total processing time of approximately 6h for 96 samples.

Material and methods

FFPE tissue sections were cut freshly from FFPE tissue blocks. Deparaffinization and lysis of the tissue sections was performed in parallel using the QIAGEN Deparaffinization solution and proteinase K. DNA was extracted automatically using the QIAsymphony SP and the QIAsymphony DNA Mini kit in combination with the Tissue Low content protocol. Performance was compared to commonly used xylene/ethanol deparaffinization and manual DNA extraction using the QIAamp DNA FFPE Tissue kit as well as an automated DNA extraction on the QIAsymphony SP instrument. Yield and purity of extracted DNA was analyzed by UV spectroscopy. Linearity of extraction was assessed by using increasing amounts of FFPE tissue sections. Quality of extracted DNA was tested by whole genome amplification and an 8-Plex end-point PCR as well as a real-time PCR for detection of biomarkers.

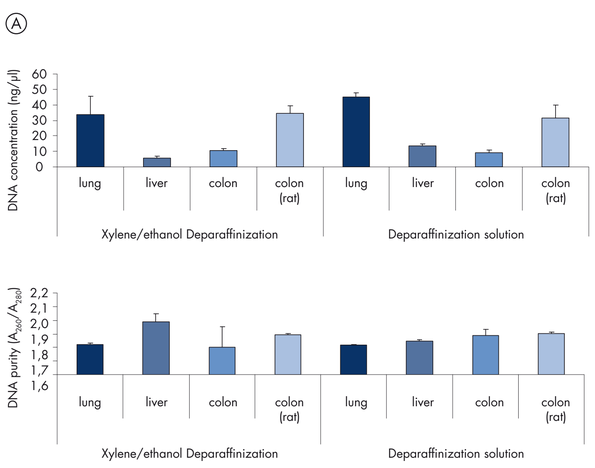
Single-tube preparation



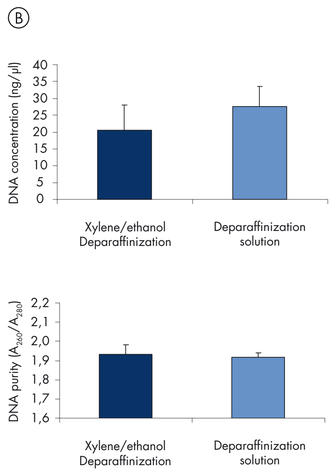
- Cut up to 8 FFPE tissue sections into sample tube
- Add Deparaffinization solution
- Lysis buffer and proteinase K
- Lyse and heat
- Transfer tube on the QIAsymphony SP

DNA yield and purity

Xylene/ethanol deparaffinization vs Deparaffinization solution method

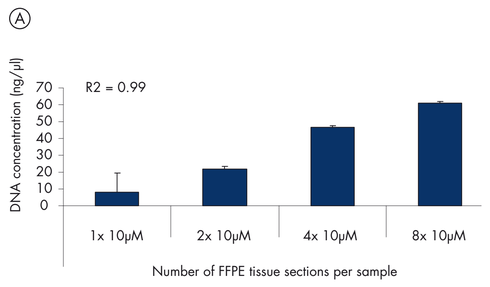


A. Concentration and purity of DNA obtained from five different FFPE tissue samples. DNA was isolated from FFPE tissue sections of human lung, liver and colon as well as sections from rat colon. For DNA extraction 2x10µM FFPE sections were used per preparation with 4 replicates per tissue block. Deparaffinization was performed by using either xylene/ethanol or the QIAGEN Deparaffinization solution. The DNA was extracted automatically on the QIAsymphony instrument. Concentration and purity of obtained DNA was found to be comparable.

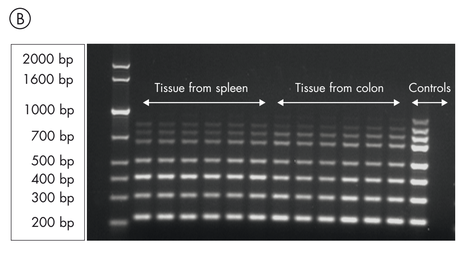


B. Concentration and purity of DNA obtained from 24 replicate samples. DNA was isolated from 24 replicate samples of 2x10µM rat colon FFPE tissue sections. Deparaffinization was performed using xylene/ethanol or Deparaffinization solution. Results for DNA concentration and purity of DNA were comparable.

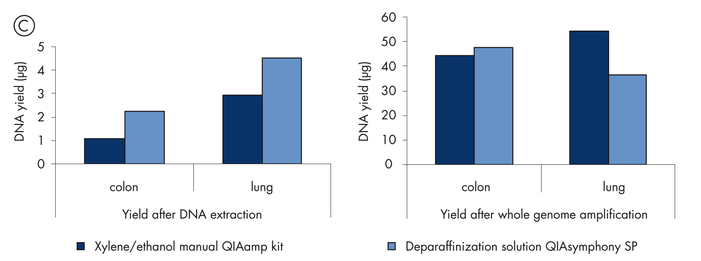
Performance and quality of extracted DNA



A. Linearity of DNA extraction from FFPE tissue sections. Samples with one to eight 10µM FFPE tissue sections from rat colon were used for DNA extraction (4 replicates each). Extraction was performed by using the QIAGEN Deparaffinization solution and the QIAsymphony SP. Linearity of DNA yield versus number of FFPE tissue sections was demonstrated by a regression coefficient of 0.99.



B. Agarose gel electrophoresis of human 8Plex PCR. DNA was extracted from human FFPE tissue (1x5µM) using the QIAGEN Deparaffinization solution and the sample preparation on QIAsymphony SP. PCR was performed with 20ng of obtained DNA as template using the QuantiTect Multiplex PCR Master Mix. Integrity of DNA was demonstrated by amplicon sizes of up to 845bp.



C. Whole genome amplification of DNA extracted from FFPE tissue. 3x10µM FFPE tissue sections from human colon and lung were used for sample preparation. DNA extraction was performed in parallel using either the QIAGEN Deparaffinization solution in combination with the QIAsymphony SP or manually using xylene/ethanol and the QIAamp DNA FFPE Tissue Kit. Whole genome amplification was performed with 100ng of extracted DNA (REPLI-g FFPE Kit). The total DNA yield after PCR was between 35 to 55 µg.

Analysis of mutational status of biomakers

Sample	Assay	CT Target	CT IC	ΔCT*
NTC	Control		32,75	
	12ALA		32,65	
	12ASP		32,69	
	12ARG		32,86	
	12CYS		32,35	
	12SER		32,76	
	12VAL		32,41	
	13ASP		32,26	
Standard	Control	25,95	32,73	
	12ALA	26,39	32,29	
	12ASP	26,54	32,15	
	12ARG	26,35	32,14	
	12CYS	26,31	32,47	
	12SER	26,5	32,34	
	12VAL	25,8	31,92	
	13ASP	27,09	32,54	
Deparaffinization solution QIAsymphony SP FFPE tissue from human colon	Control	24,94	31,98	
	12ALA		32,42	
	12ASP		32,73	
	12ARG		33,05	
	12CYS		32,74	
	12SER	29,11	32,34	**4,17**
	12VAL		32,81	
	13ASP		33,2	
Xylene/ethanol QIAamp DNA FFPE Tissue Kit FFPE tissue from human colon	Control	26,78	32,32	
	12ALA		32,51	
	12ASP		32,89	
	12ARG		32,89	
	12CYS		32,49	
	12SER	30,61	32,8	**3,83**
	12VAL		32,8	
	13ASP		33,45	

* ΔCT = mutation CT – control CT

Real-time PCR analysis of mutational status of the KRAS gene. DNA was extracted from 3x10µM FFPE sections of human colon. Sample preparation was performed manually using the QIAamp DNA FFPE Tissue Kit (xylene/ethanol) or the QIAsymphony SP (Deparaffinization solution). Eluates were analyzed using a real-time PCR for KRAS mutation detection, which identified a mutation in codon 12 (Gly → Ser) as demonstrated by delta CT values < 8.00. The result was identical for DNA extracted with the QIAamp DNA FFPE Tissue Kit or the QIAsymphony SP.

Sample	Pyro result (% mutation)
Control	Wildtype
12ALA	66.3
12ALA	69.7
12ASP	73.8
12ASP	70.1
12ARG	87.1
12ARG	97.4
12CYS	97.4
12CYS	96.0
12SER	99.4
12SER	100
12VAL	100
12VAL	100
13ASP	53.4
13ASP	49.1

Pyrosequencing analysis of mutational status of the KRAS gene. DNA was extracted from 1x10µM sections of K-RAS FFPE process control material. Sample preparation was performed automatically on the QIAsymphony SP in combination with the Deparaffinization solution. Eluates were analyzed using a KRAS specific Pyrosequencing method. Analysis showed that all mutations were identified.

Summary and conclusions

The QIAGEN Deparaffinization solution in combination with the QIAsymphony SP and the QIAsymphony DNA Mini Kit enables automated extraction of high-quality DNA from FFPE tissue.

- Reduced hands-on time (96 FFPE tissue samples in 6h)
- No risk of losing sample material
- DNA purity with ratios A_{260}/A_{280} of >1.8
- Performance was found to be equivalent to standard xylene/ethanol pretreatment and DNA extraction using manual QIAamp DNA FFPE Tissue Kit

The QIAsymphony DNA Mini Kit, the Deparaffinization solution and the described PCR assays are for research use only. Not for use in diagnostic procedures.

For up-to-date licensing information and product- specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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