Challenging biological samples: stabilization and simultaneous purification of DNA and RNA

October 10, 2012

Stefanie Schröer

- **Requirements for analysis of precious samples**
  - **Precious and inhomogeneous samples**
  - **Different analytes from the same sample**
- Stabilization of RNA and DNA
- Simultaneous purification of different analytes
  - Simultaneous analysis of DNA, RNA, and protein
  - DNA, RNA, and miRNA from challenging starting material
  - Analysis of DNA and RNA from FFPE samples
- Downstream applications
- Summary

Challenging biological samples

Precious and nonhomogeneous samples

- Clinical samples that are irreplaceable
- Samples of limited quantity
- Tumor samples that often nonhomogeneous
- Highly variable starting material (e.g., different tissue types)
- Samples from biobanks

Requirements for analysis of biological samples

- Requirements for analysis of precious samples
  - Reliable comparison of mRNA/miRNA expression profiles
  - Reliable comparison of methylation status of samples
  - Different analytes from the same sample
- Stabilization of RNA and DNA
  - Stabilization is required in biomedical research
  - QIAGEN offers RNA*later* and Allprotect products

Need for reliable stabilization and parallel purification of nucleic acids from even the most challenging samples

- Requirements for analysis of precious samples
  - Precious and inhomogeneous samples
  - Different analytes from the same sample
- **Stabilization of RNA and DNA**
- Simultaneous purification of different analytes
  - Simultaneous analysis of DNA, RNA, and protein
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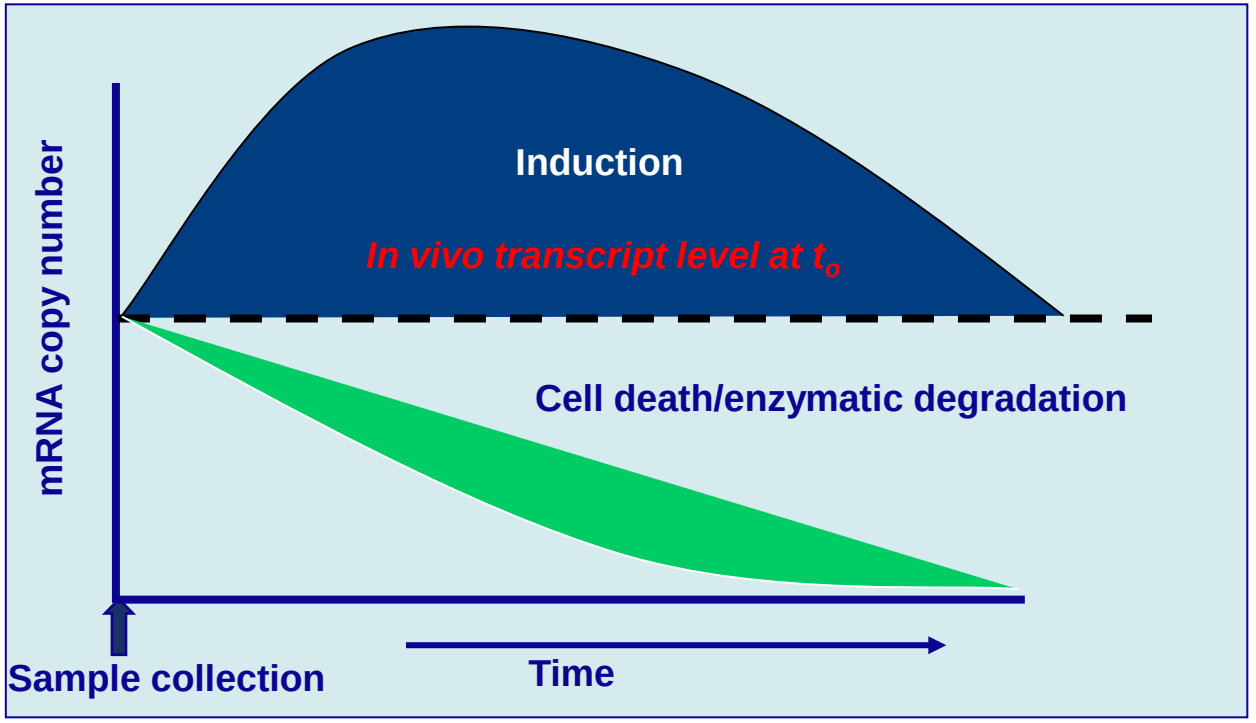
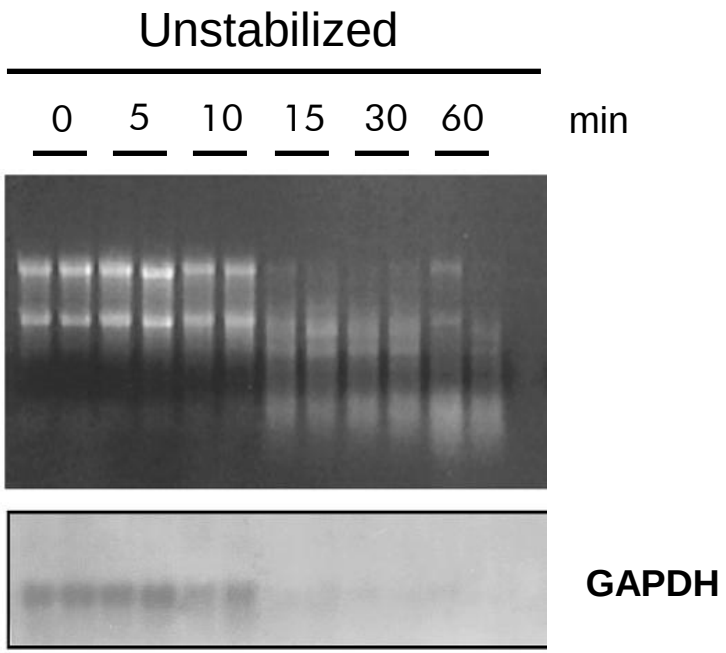
Stabilization of RNA and DNA

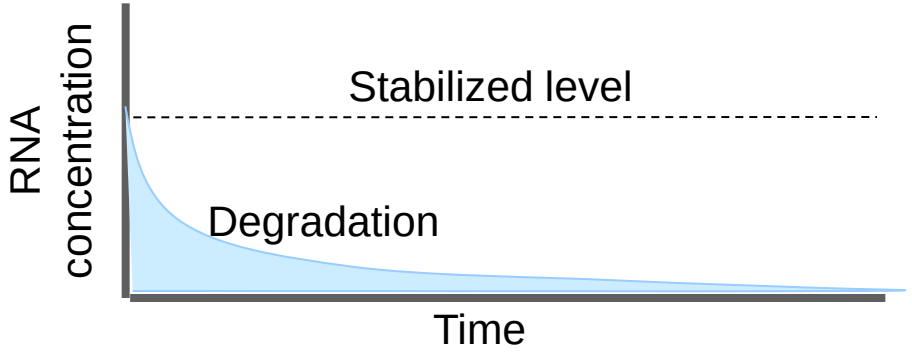
Stabilization:

- Protection of RNA from degradation
- Prevention of induction or downregulation of mRNA
- Prevention of degradation of genomic DNA



Degradation of RNA without stabilization





Degradation of RNA

Guidelines for handling RNA

- Work in an RNase-free environment
- Always wear latex or vinyl gloves
- Only use RNase-free water and buffers
- Only use new tips
- Use sterile, disposable plasticware

Prevent introduction of RNases into the RNA sample during or after isolation procedure

RNA stabilization

Stabilization reagents:

- Depends on analyte (DNA, RNA, protein)
- Depends on sample type

Products:

- Tissue: Allprotect Tissue Reagent (DNA/RNA/protein),
  RNA*later* RNA Stabilization Reagent (RNA)
- Cells: RNAprotect Cell Reagent
- Bacteria: RNAprotect Bacteria Reagent
- Saliva: RNeasy Protect Saliva Mini Kit
- Animal blood: RNeasy Protect Animal Blood System

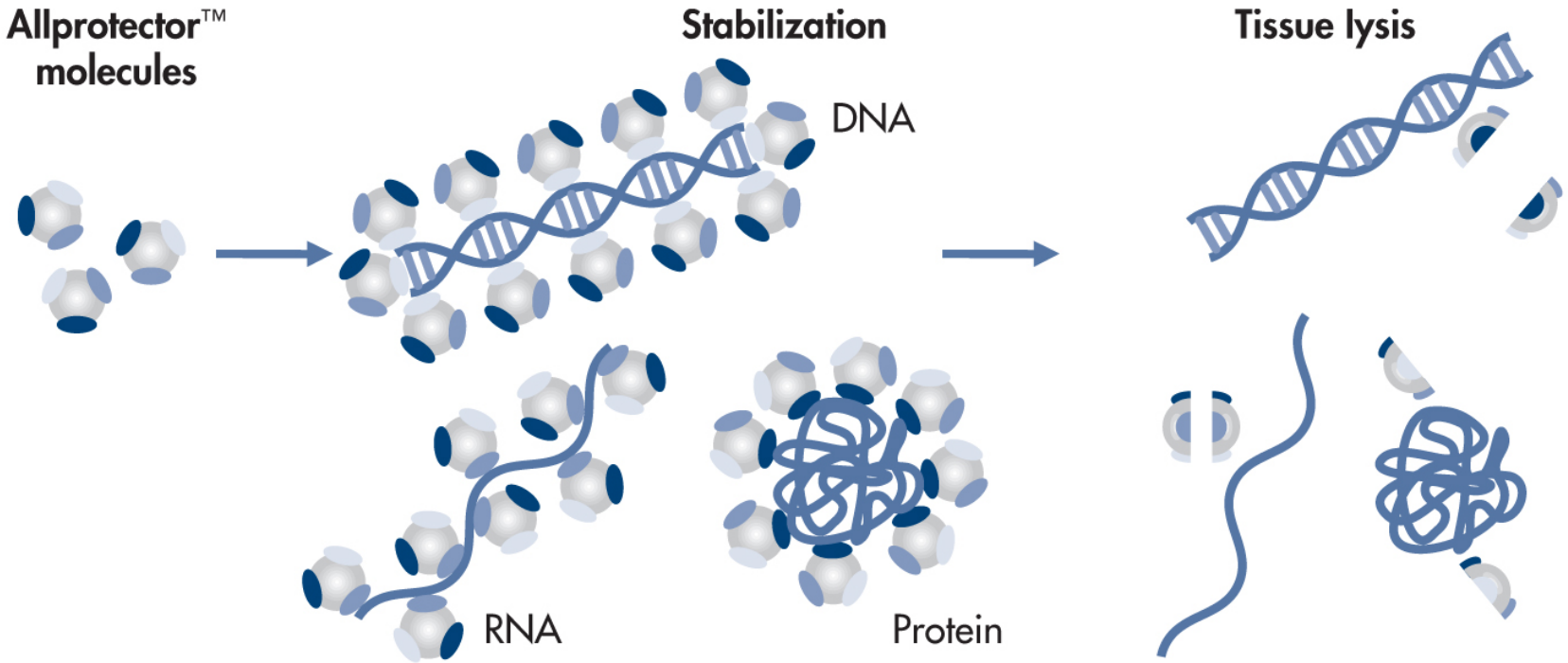
Allprotect Tissue Reagent

Allprotect Tissue Reagent ensures simultaneous stabilization of DNA, RNA, miRNA and proteins in tissue samples

- Immediate, convenient stabilization of harvested tissue
- Streamlined workflow with no need for liquid nitrogen or phenol
- No toxicity
- No degradation, induction, or modification
- Storage up to 1 day at 37°C, 7 days at 25°C, and 1 year at 2–8°C
- Long-term storage of samples at –20°C or –80°C
- Conveniently used in combination with AllPrep Kits

Allprotect Tissue Reagent

Principle: Allprotector molecules

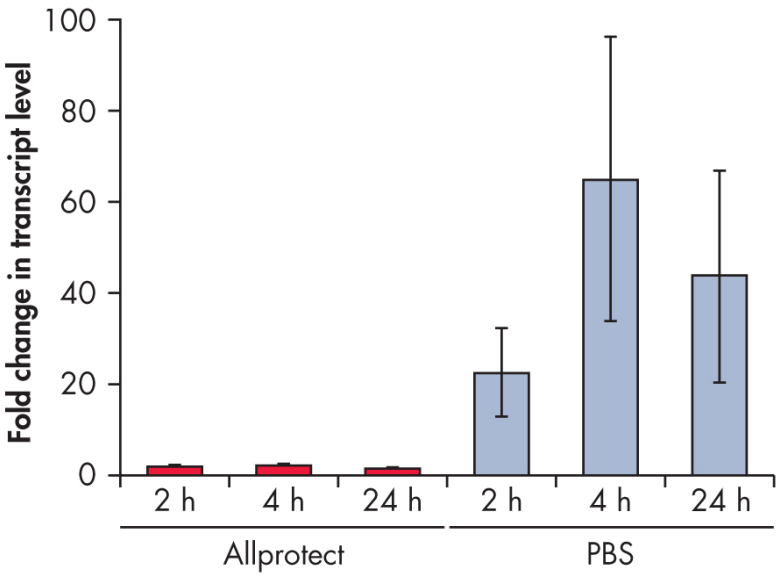


- Allprotector molecules with contact points to envelop and protect DNA, RNA, and protein
- Upon lysis, Allprotector molecules diluted and release of DNA, RNA, and protein

Allprotect stabilization of RNA

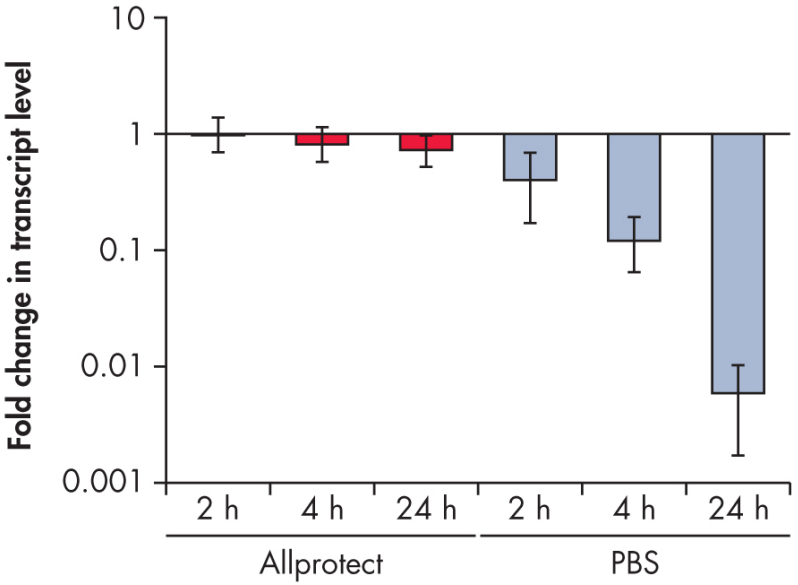
$\Delta\Delta C_T$ analysis of transcriptional induction

Transcript levels of c-fos relative to N_2



➢ Prevention of induction

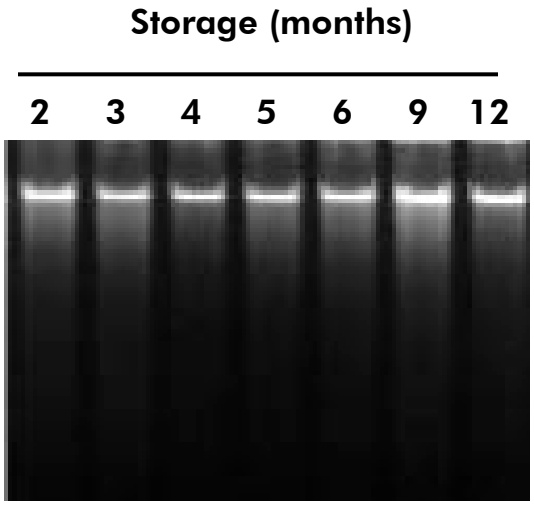
Transcript levels of Madh7 relative to N_2



➢ Prevention of degradation

Allprotect: Long-term storage of RNA , DNA at 2–8°C

Genomic DNA



- Rat intestine tissue (10 mg) analyzed
- EZ1 RNA Tissue Mini Kit on the BioRobot EZ1
- Agarose gel

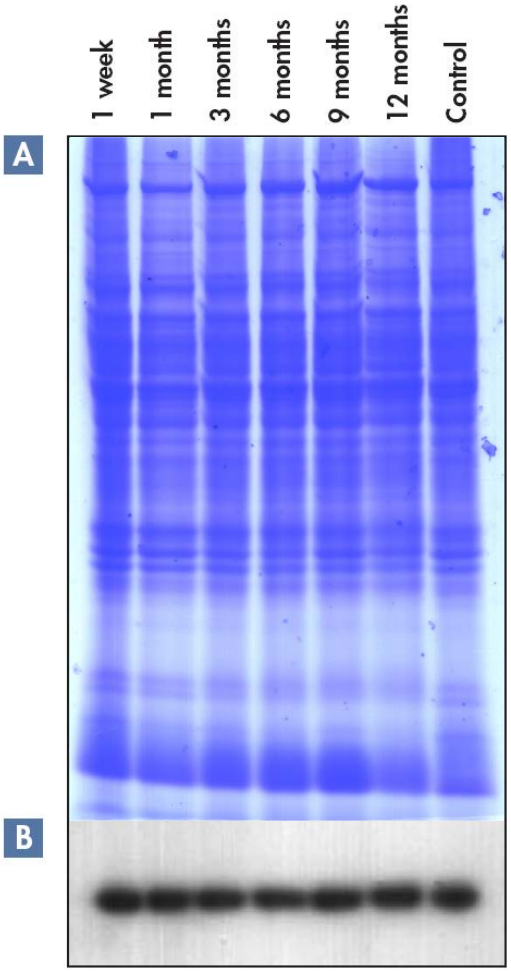
RNA

Storage (months)	RIN value		
	Liver	Intestine	Brain
1	9.4	9.3	8.5
2	9.2	9.3	8.6
4	9.4	9.3	8.4
6	9.2	9.5	8.6
9	9.4	9.7	8.2
12	9.4	9.3	8.4

- Rat tissue (10 mg) analyzed
- RNeasy, RNeasy Lipid Tissue
- Agilent BioAnalyzer

Stabilization of nucleic acids for up to 12 months without freezing

Allprotect: Long-term storage of protein at 2–8°C



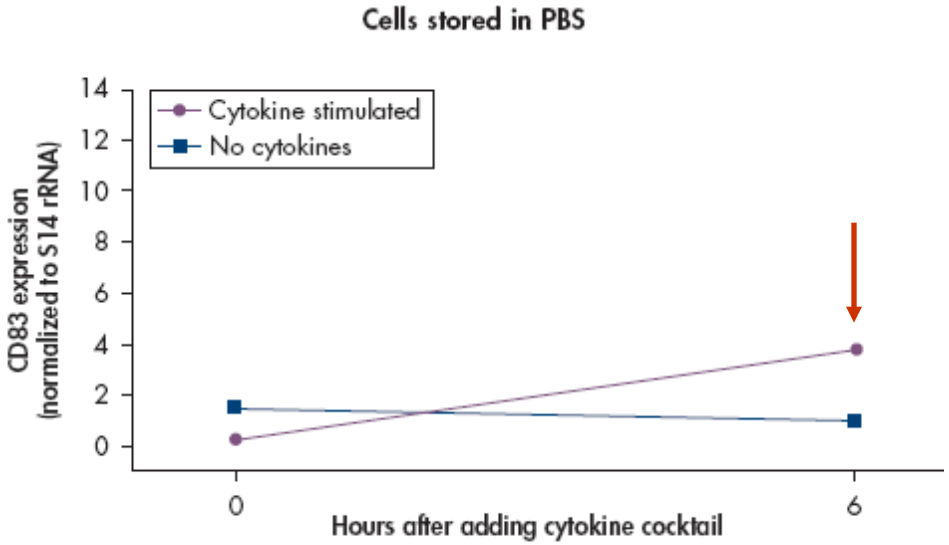
- Rat liver
- Total protein purified with Qproteome Mammalian Protein Prep Kit
- ERK2 analysis by SDS PAGE and western blotting
- Control: Liquid nitrogen

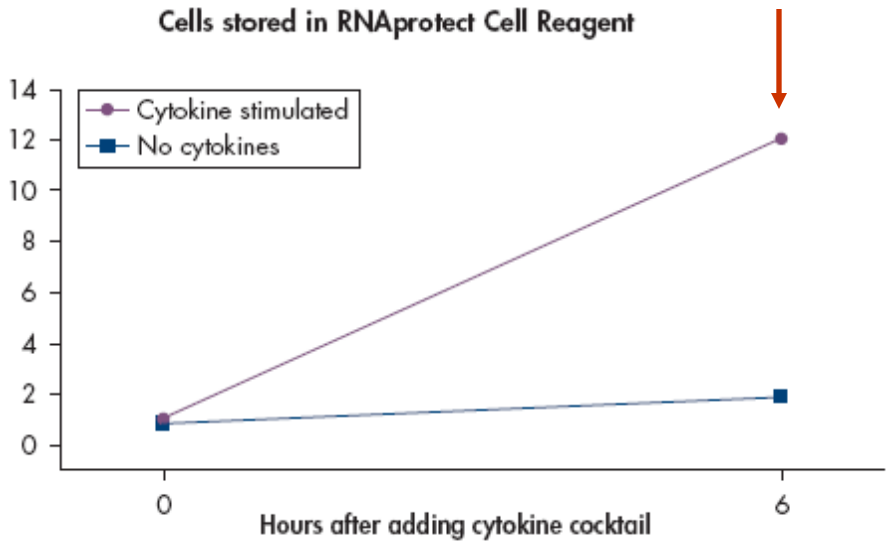
Stabilization of proteins for up to 12 months without freezing

RNA stabilization

RNAprotect Cell Reagent:

- Dendritic cells stimulated with cytokines
- Stored in PBS or RNAprotect immediately at –20°C for 6 hours
- Quantification of CD83 expression
- Normalization to S14 rRNA





Data kindly provided by Dr. Alexander Prechtel, University Hospital Erlangen, Germany.

- Requirements for analysis of precious samples
  - Precious and inhomogeneous samples
  - Different analytes from the same sample
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Multiple analytes from the same sample

All information derived from the same sample:

- Precious, nonhomogeneous samples
  - All analytes from exactly the same part of the sample
- Complete picture needed

AllPrep DNA/RNA Mini Kit

AllPrep DNA/RNA Micro Kit

AllPrep DNA/RNA 96 Kit

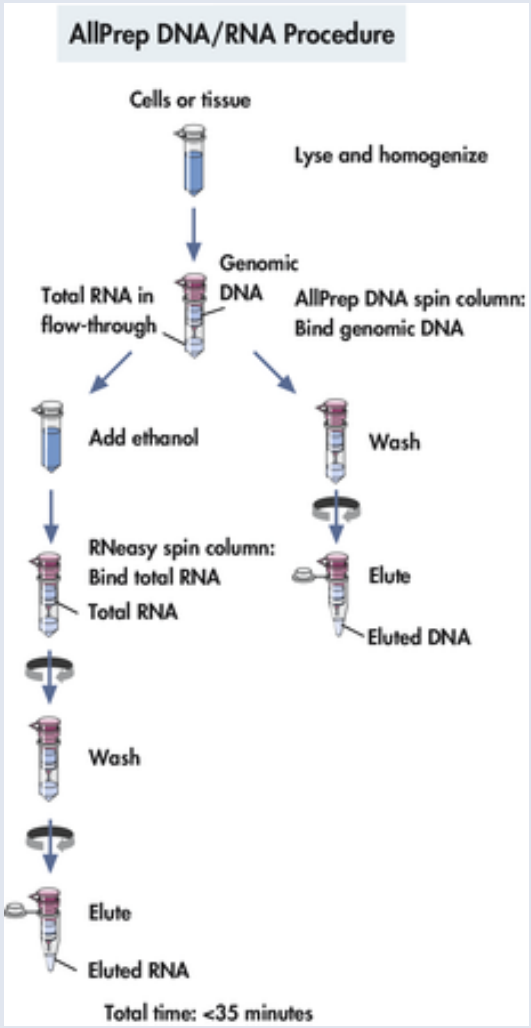
AllPrep DNA/RNA FFPE Kit

AllPrep DNA/RNA/Protein Kit

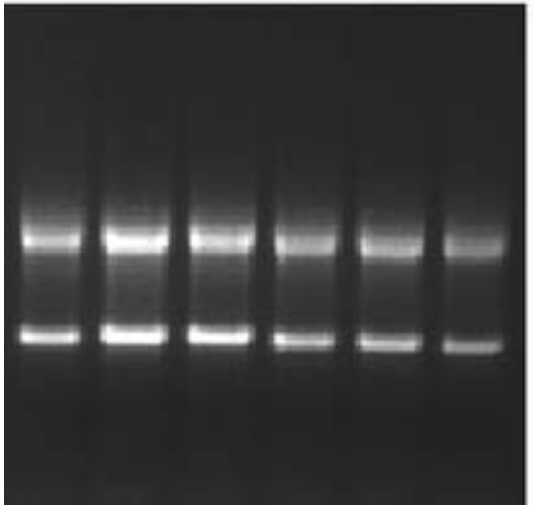
AllPrep RNA/Protein Kit

AllPrep gDNA/RNA/miRNA Universal Kit new

AllPrep DNA/RNA Mini Kit

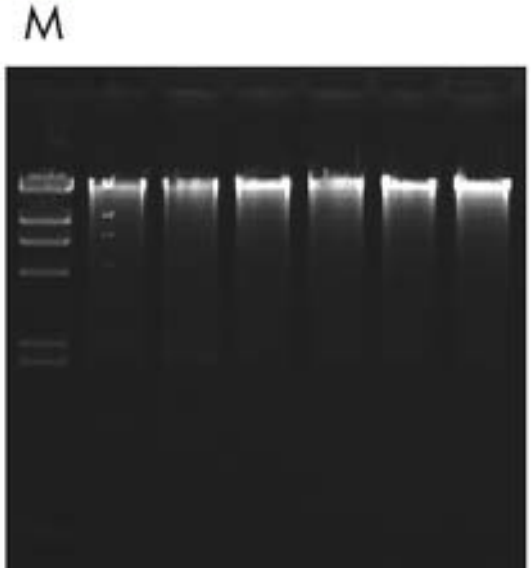


A RNA



- Rat liver (10 mg) analyzed
- 6 independent samples
- Analyzed on 1.2% formaldehyde agarose gel

B Genomic DNA

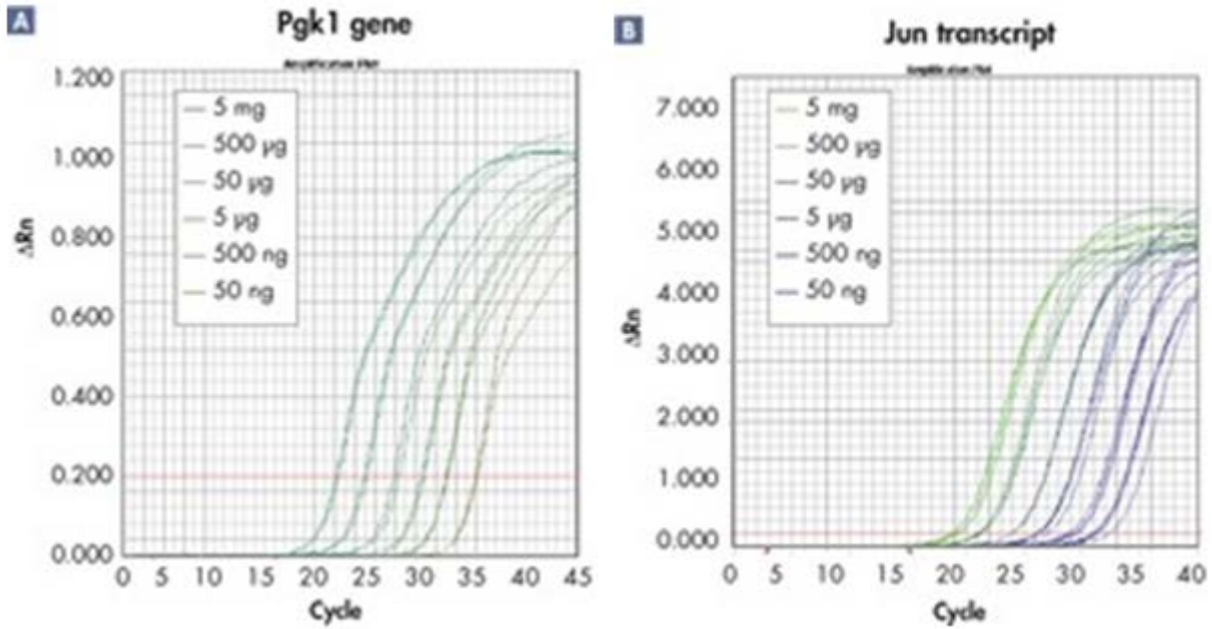


- Rat liver (10 mg) analyzed
- 6 independent samples
- Analyzed on 0.8% agarose gel
- M: Size marker

High-quality of RNA and gDNA with high reliability

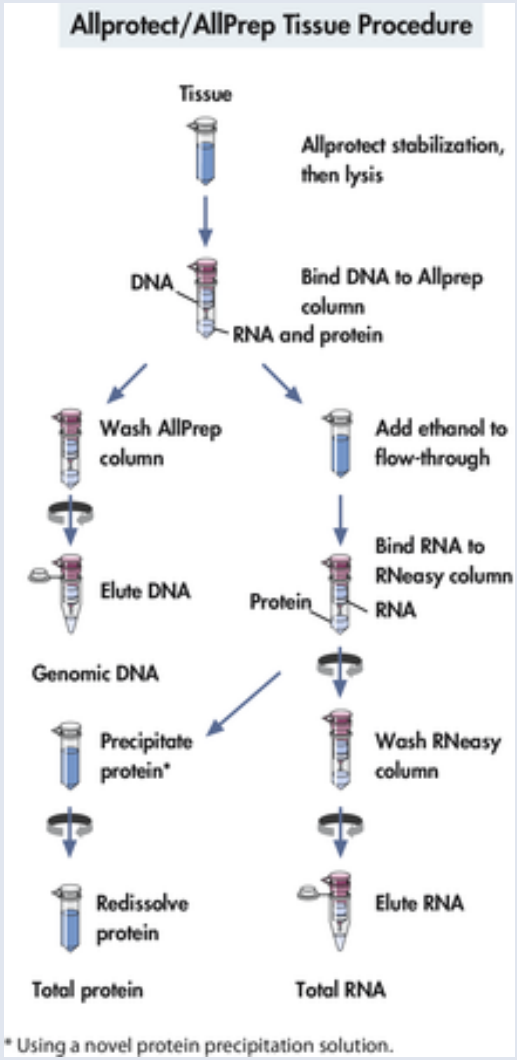
AllPrep DNA/RNA Micro Kit

Serial dilutions of rat kidney homogenate

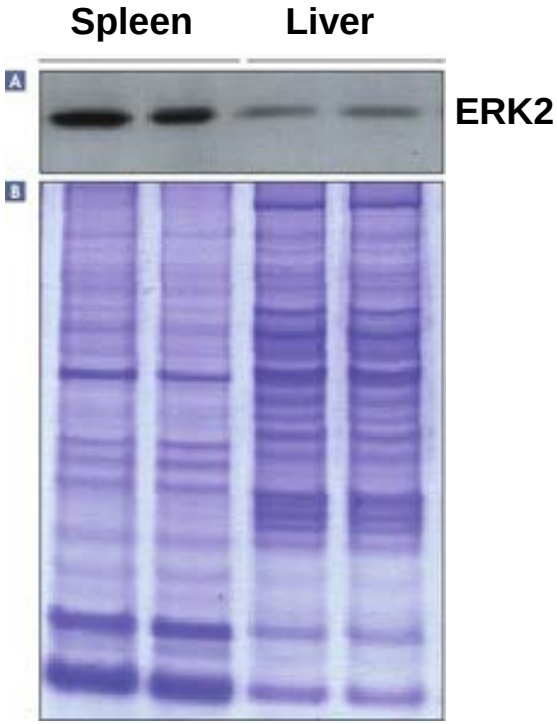


Wide dynamic range also for small sample sizes

AllPrep DNA/RNA/Protein Mini Kit



- Technology is based on the proven Allprep DNA/RNA technology
- Additional aqueous protein precipitation solution
- miRNA isolation possible



Reliable western blotting and SDS-PAGE results

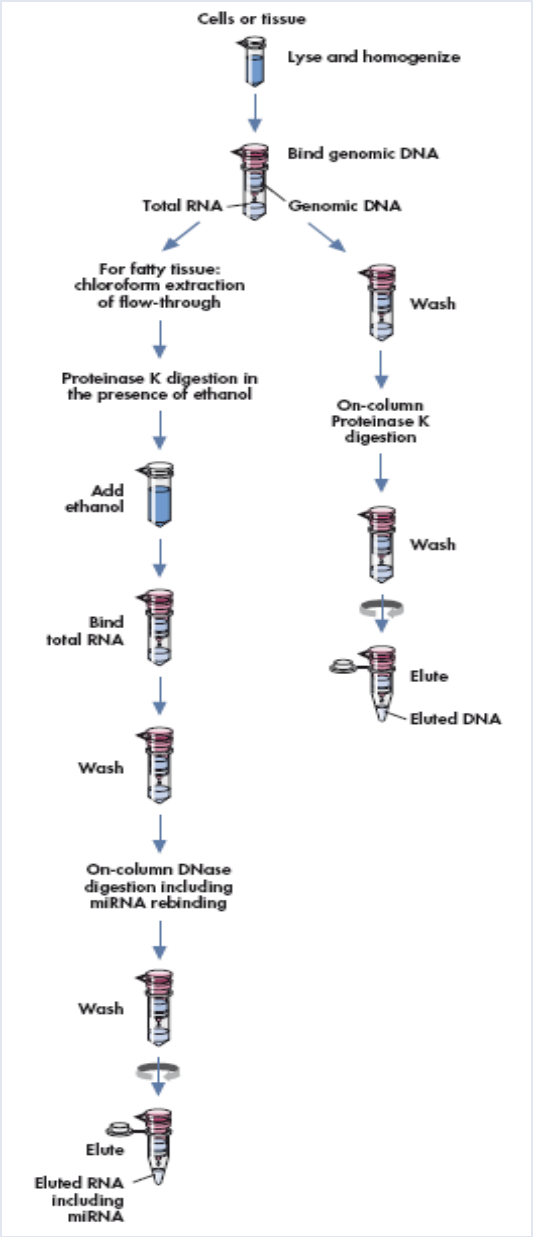
AllPrep DNA/RNA/miRNA Universal Kit

Kit optimized for various difficult-to-lyse starting materials

- Suitable for lipid-rich tissues (e.g., brain, adipose tissue)
- Suitable for fiber-rich tissues (e.g., muscle, heart)
- Suitable for isolated blood cells
- Lipid- and fiber- rich tissues are often very scientifically relevant
- Isolation of nucleic acids from these samples is highly difficult
- Difficult to obtain sufficient yields and quality with existing kits

New Allprep DNA/RNA/miRNA Universal Kit

AllPrep DNA/RNA/miRNA Universal Kit



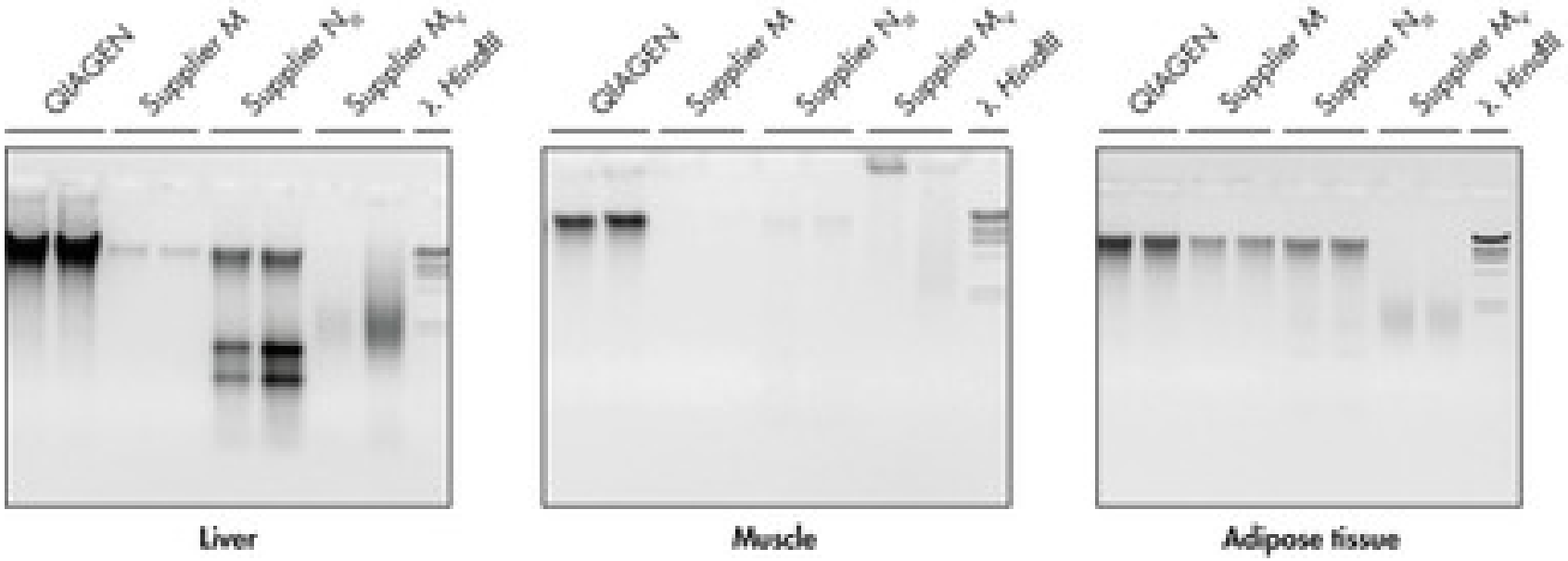
Protocol and technology

- Based on well-established AllPrep DNA/RNA technology
- Optimized proteolytic digestion steps for RNA and DNA
- No phenol required
- Co-purification of miRNA

High-quality results from precious and challenging samples

AllPrep DNA/RNA/miRNA Universal Kit

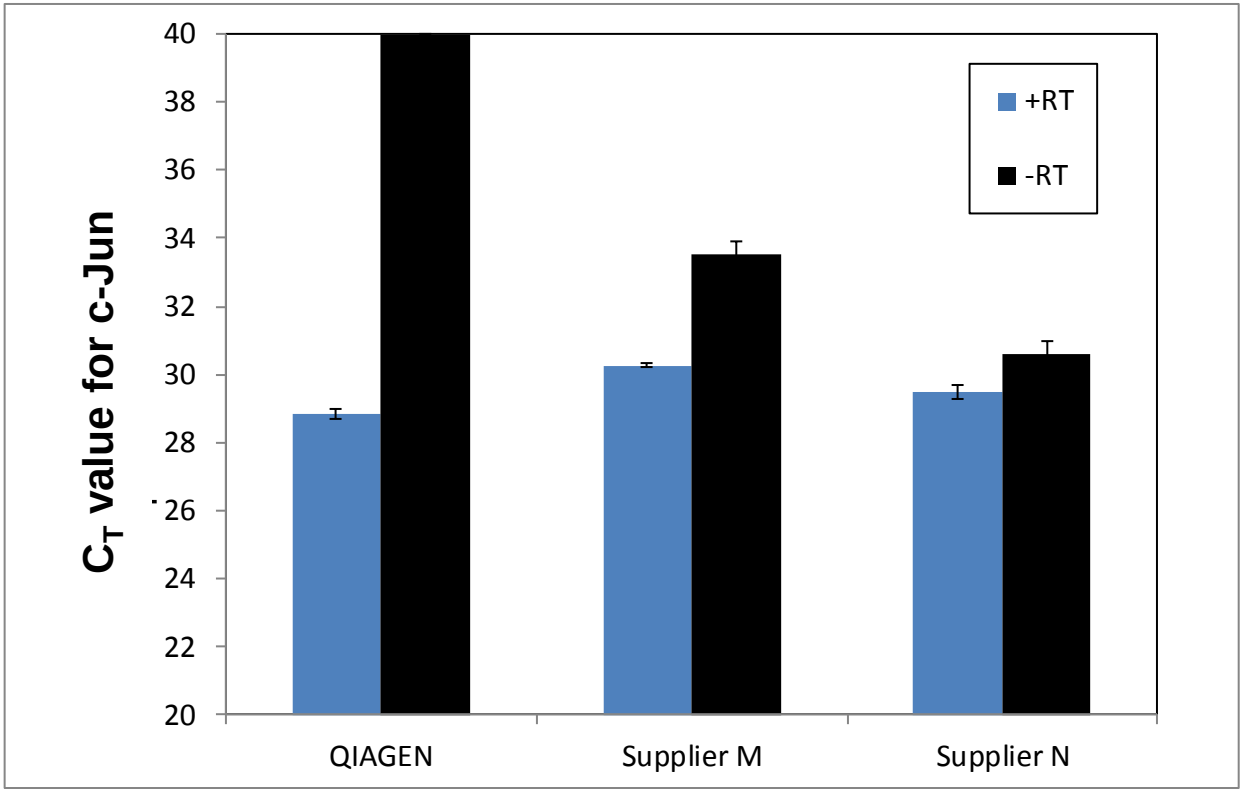
Purification of high yields of DNA from difficult-to-lyse tissues



- Intact genomic DNA from most challenging tissue types

AllPrep DNA/RNA/miRNA Universal Kit

Rat muscle tissue: c-Jun expression

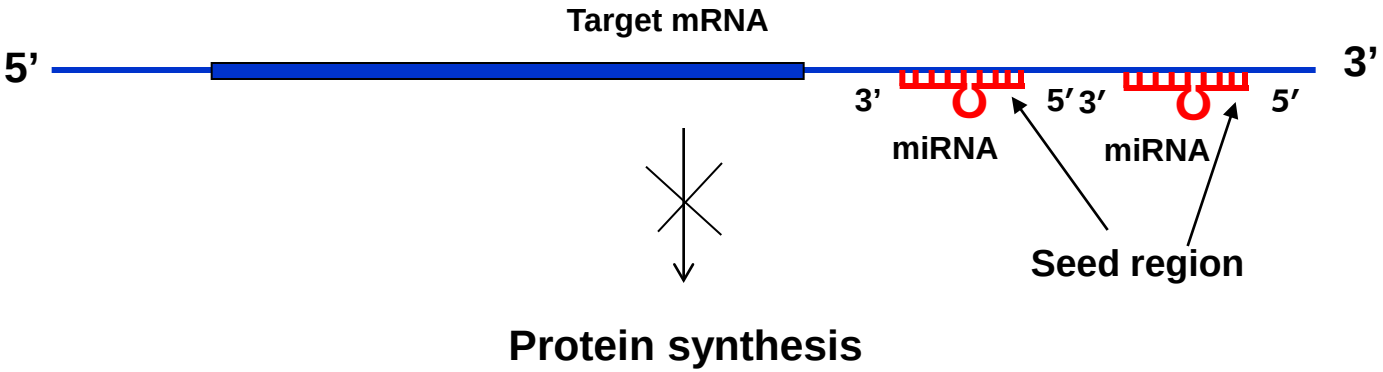


Complete removal of genomic DNA from RNA fraction

microRNA

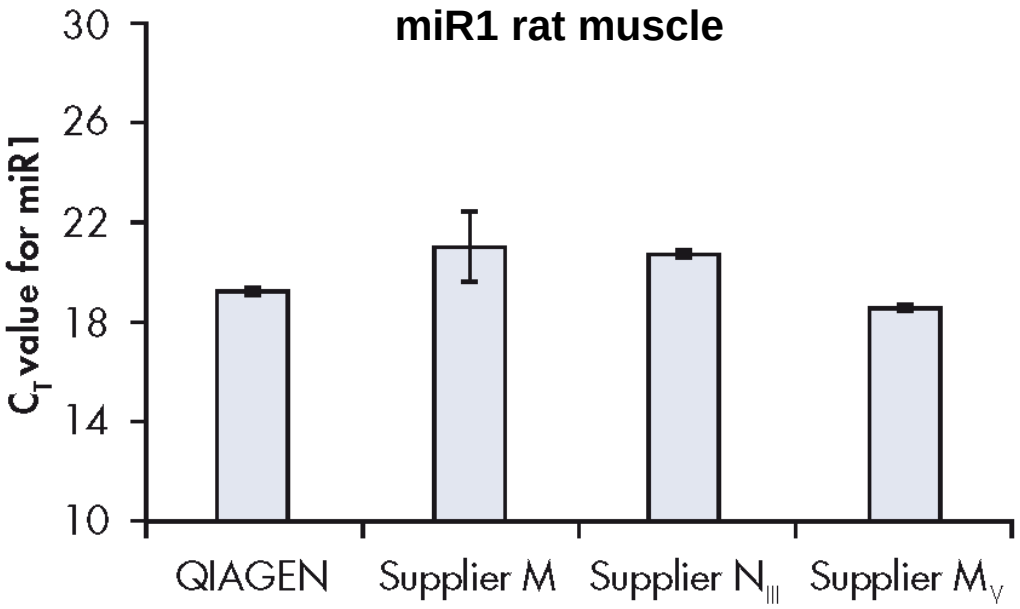
microRNAs — micromanagers of gene expression

- Naturally occurring, endogenous small RNA
- Mature miRNA approximately 22 nt long
- Regulate at least 1/3 of protein encoding genes
- miRNA binding sites typically at 3'-UTR of target transcripts
- Imperfectly base pair to target resulting in translational repression
- > 1700 miRNAs in human (miRBase V17.0)



AllPrep DNA/RNA/miRNA Universal Kit

Purification of miRNA from difficult-to-lyse tissues



- High yields of miRNA also from difficult-to-lyse tissues

Analysis of DNA and RNA from FFPE samples

Formaldehyde-fixed paraffin-embedded samples

- Formaldehyde fixation resulting in cross linking of:
  - Protein– protein
  - Protein–nucleic acid
  - Nucleic acid–nucleic acid
- Paraffin embedding resulting in:
  - Nucleic acid fragmentation
  - Deparaffinization required prior to nucleic acid preparation
- Quality of nucleic acids from FFPE samples compromised by:
  - Remaining crosslinks
  - Chemical modifications of analytes
  - Fragmentation

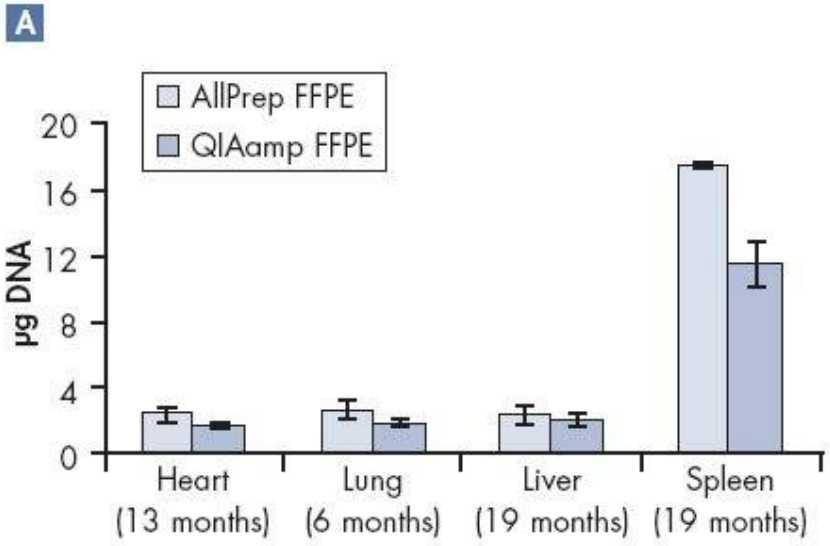
Consequences

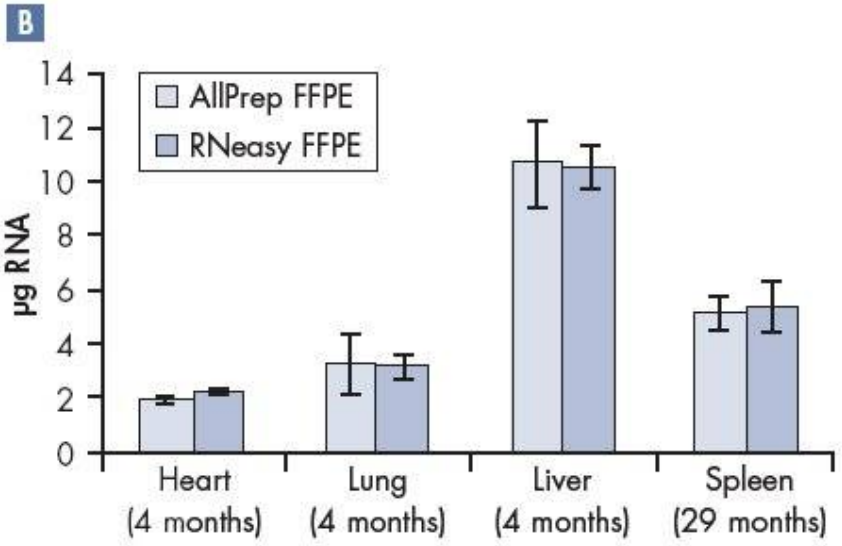
- For effective lysis, harsh conditions required
- Poor yield (especially due to overfixation)
- Interference with enzymatic assays

Analysis of DNA and RNA from FFPE samples

AllPrep DNA/RNA FFPE Kit:

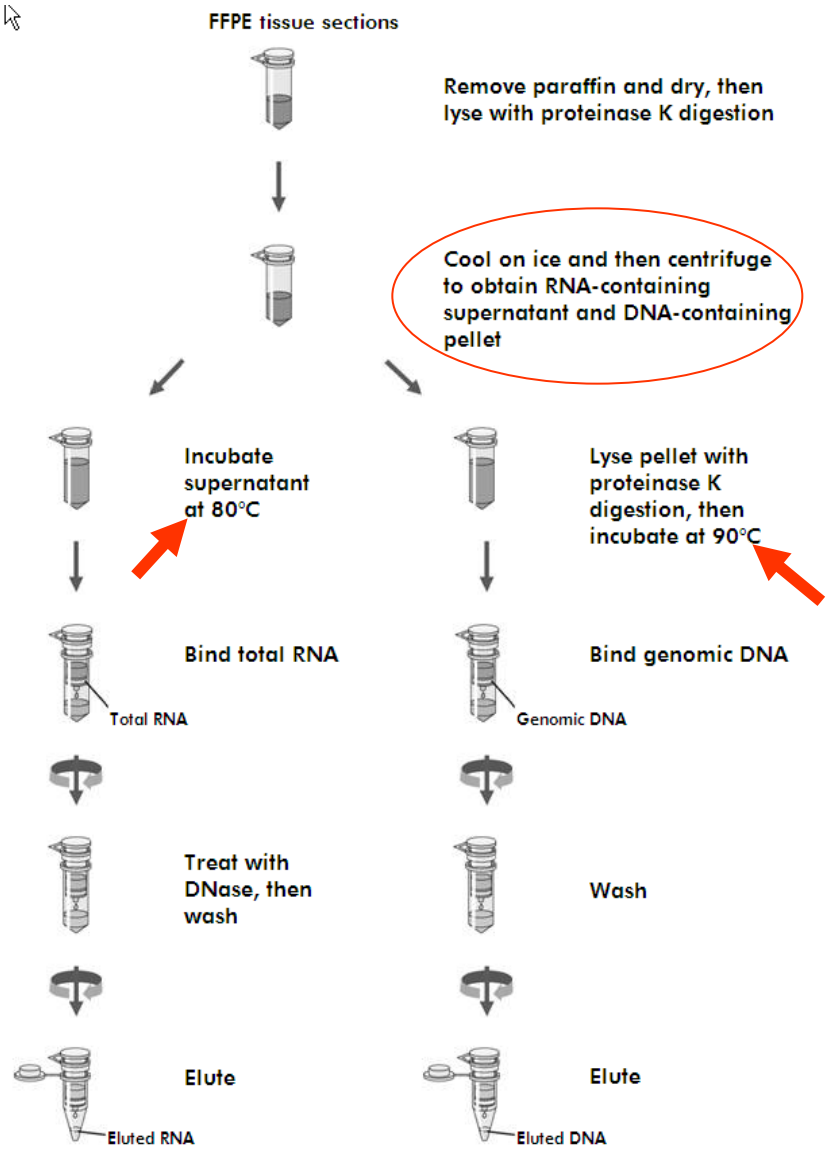
- Simultaneous purification of DNA and RNA from FFPE samples
- Differential solubilization methodology
- Efficient removal of crosslinks
- Yield/quality comparable to that obtained with specialized kits
- Includes efficient genomic DNA removal
- Protocol including miRNA available



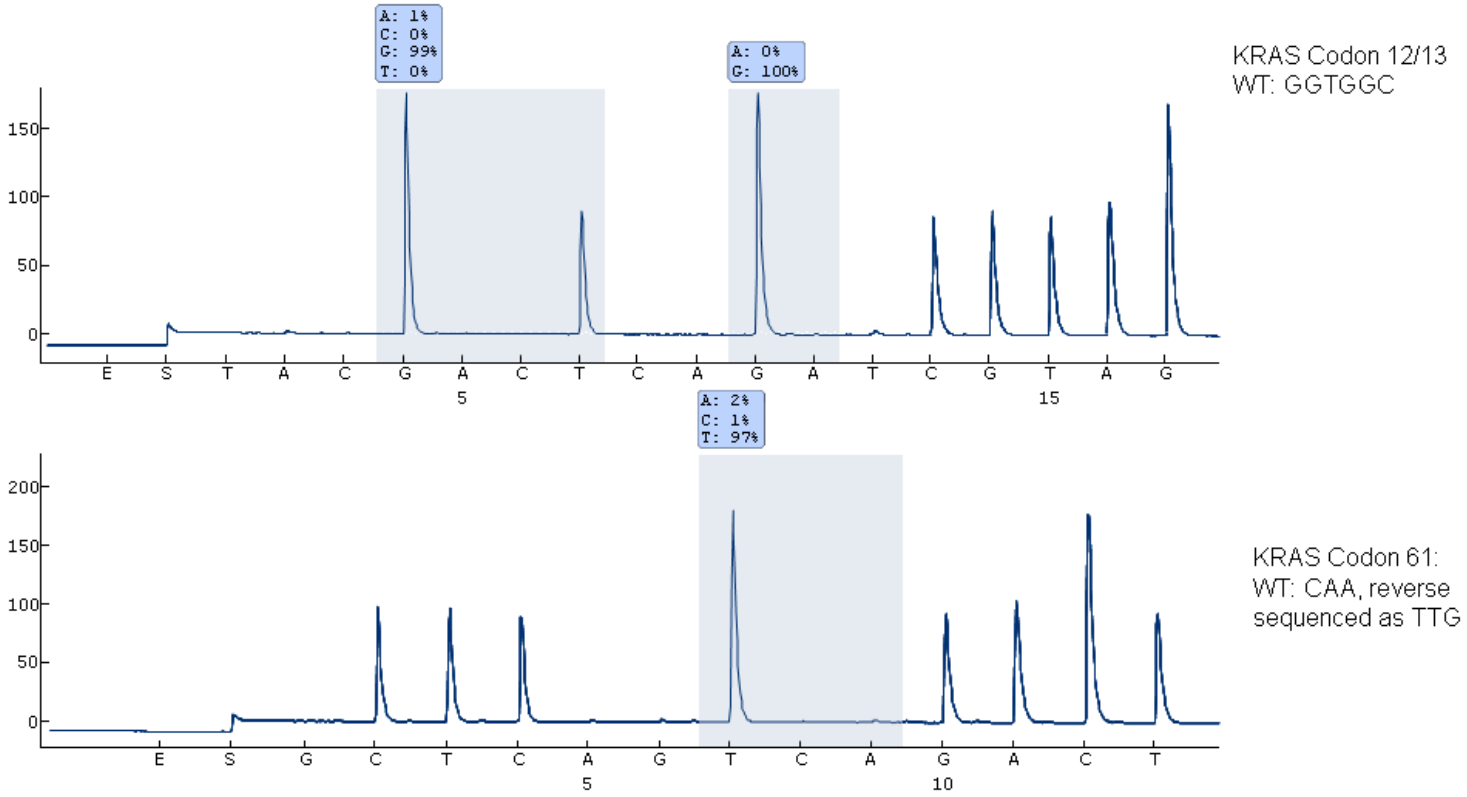


Analysis of DNA and RNA from FFPE samples

AllPrep DNA/RNA FFPE Kit - protocol



Analysis of DNA and RNA from FFPE samples



- DNA purified from breast cancer FFPE tissue using AllPrep DNA/RNA FFPE Kit
- Pyrosequencing to identify mutation in KRAS gene

  (*therascreen* KRAS Pyro Kit)

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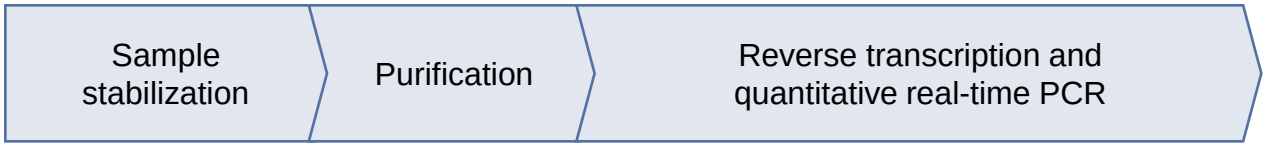
Gene expression analysis using qPCR

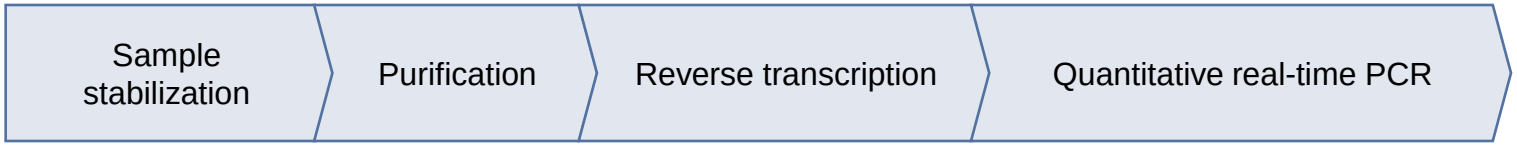
One-step or two-step RT-PCR

One-step RT-PCR



Sample stabilization	Purification	Reverse transcription and quantitative real-time PCR
Allprotect	AllPrep Kits	QuantiFast SYBR Green/Probe RT-PCR Kits Rotor-Gene SYBR Green/Probe RT-PCR Kits

Two-step RT-PCR



Sample stabilization	Purification	Reverse transcription	Quantitative real-time PCR
Allprotect	AllPrep Kits	QuantiTect Reverse Transcription Kit	QuantiFast SYBR Green/Probe PCR Kits Rotor-Gene SYBR Green/Probe PCR Kits

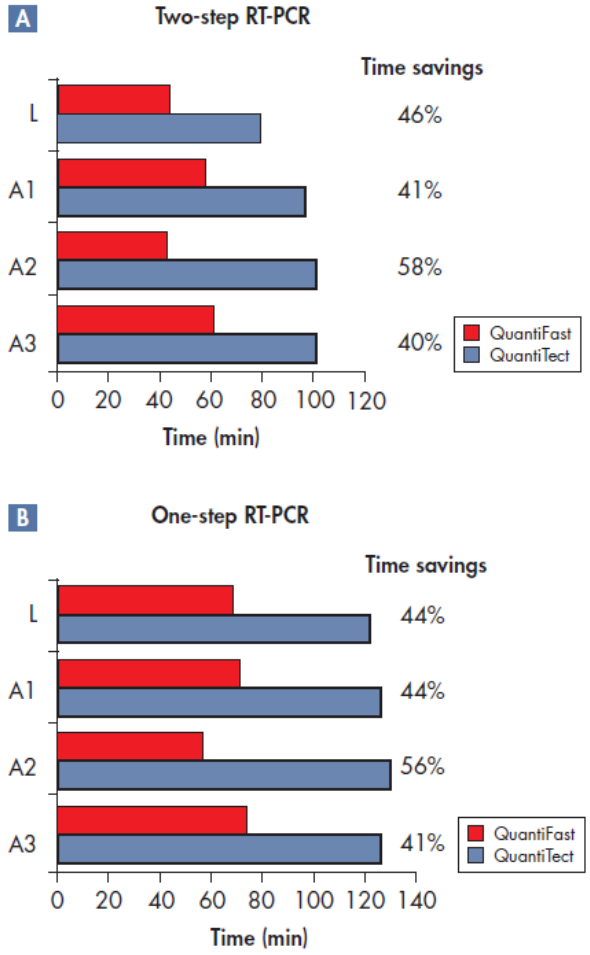
Various kits for probe-based and SYBR Green detection and one-step and two-step RT-PCR

Features of QuantiFast and Rotor-Gene Kits

Unique composition for success at the first attempt

Enzymes

- HotStarTaq *Plus* DNA Polymerase
  - Reactivation within 5 minutes
  - Unmatched specificity and sensitivity
- QuantiFast RT Mix (for one-step kits)
  - Optimized combination of Sensiscript and Omniscript
  - High sensitivity



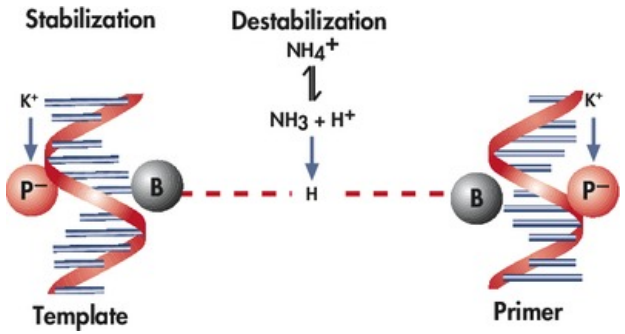
Significantly reduced qPCR times

Features of QuantiFast Kits and Rotor-Gene Kits

Unique composition for success at the first attempt

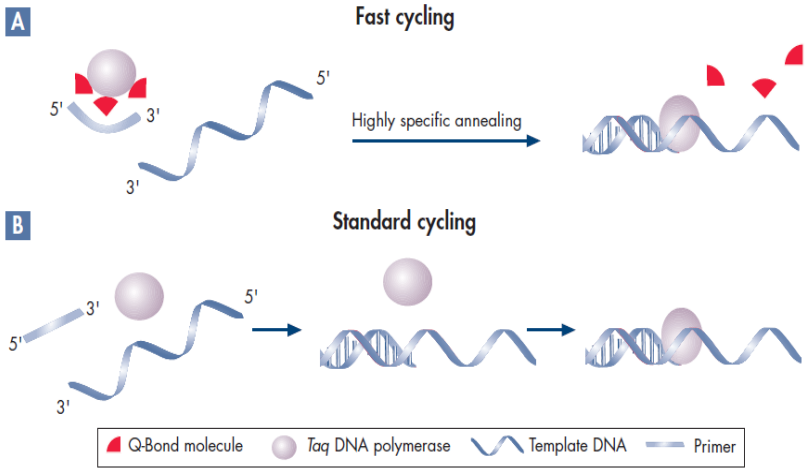
Unique qPCR reaction buffer

- Stabilizes specific bonds
- Destabilizes unspecific bonds



Q-Bond

- Increases affinity of DNA polymerase to short ssDNA
- Reduces primer annealing time to a few seconds



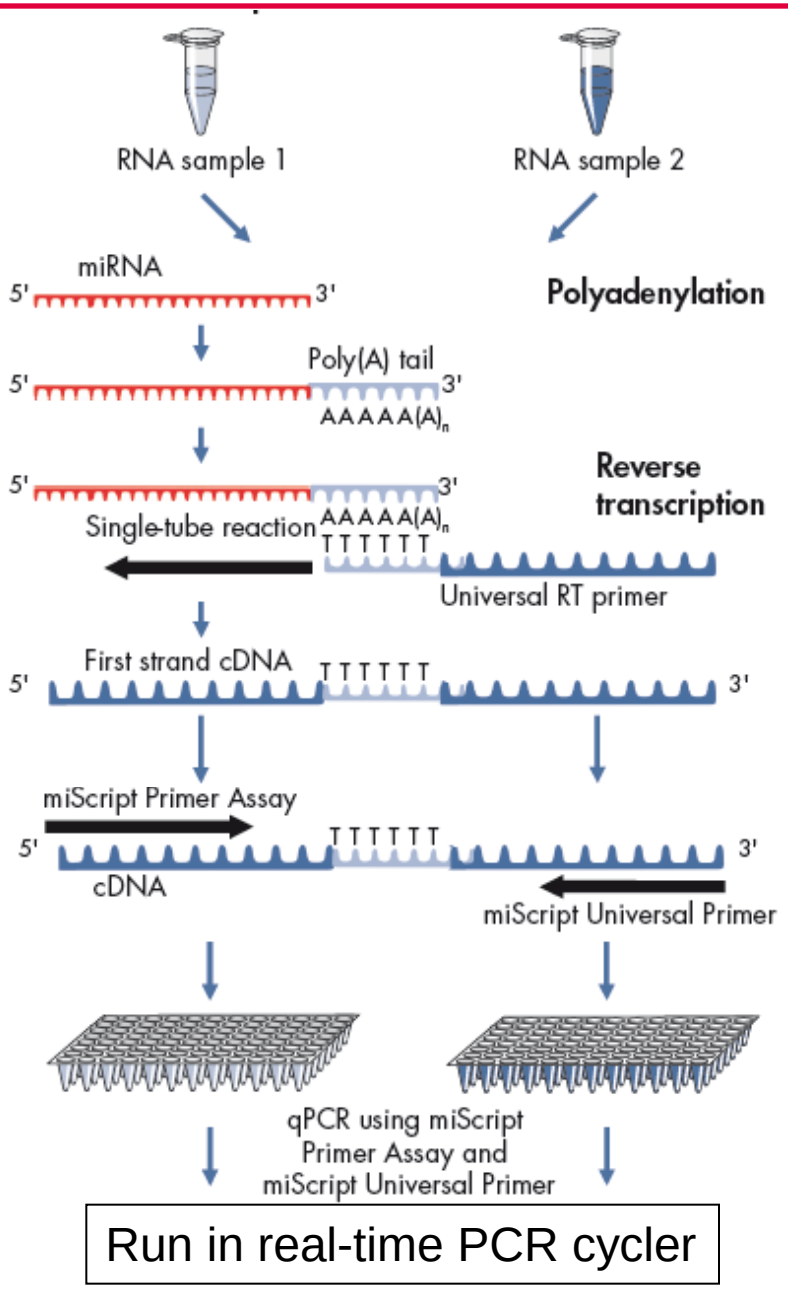
Successful qPCR analysis without optimization

miScript miRNA Detection System

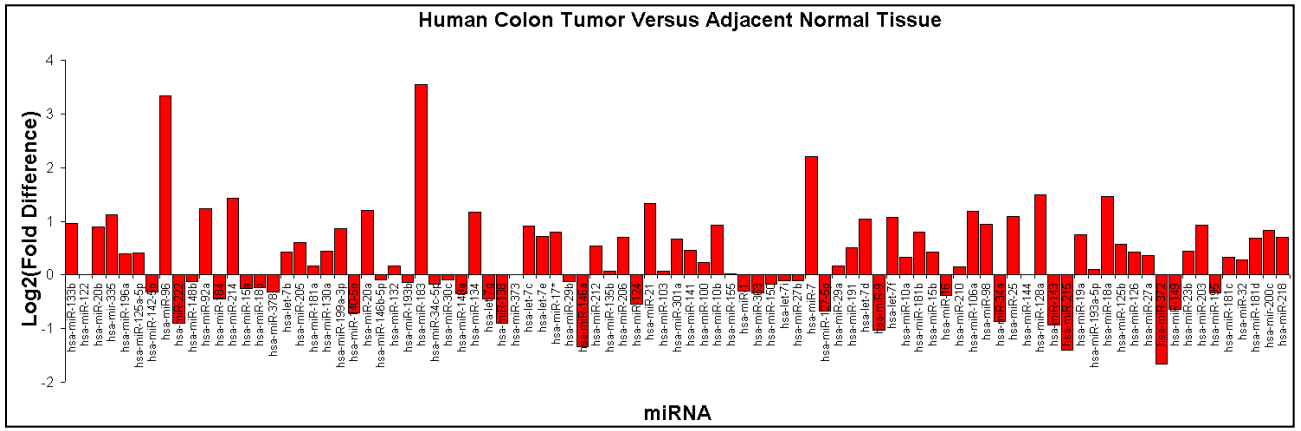
cDNA Synthesis from miRNA
1 hour

Add cDNA to master mix and load plates
2 minutes

Run 40 cycle qPCR program
2 hours



miRNA biomarker discovery: Cancer biomarkers



Many cancer-specific miRNA are upregulated in a colon tumor

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Summary

- Induction and degradation RNA: a general risk
  - Careful handling of samples and stabilization needed
- Precious samples often small or nonhomogeneous
  - Co-purification of DNA, RNA, miRNA, and protein
- Lipid- and fiber-rich tissues are challenging to purify
- FFPE samples with cross-linked and fragmented nucleic acids
  - Use of specialized technologies
- Downstream applications

Questions & answers

Thank you for your attention!

Stefanie.Schroeer@qiagen.com