

QuantiGene FFPE

Formalin Fixed, Paraffin-Embedded Tissue Reagent System

Introduction

Over 400 million human tissue samples, with well-annotated clinical outcome, are stored in numerous facilities around the world. The majority of these specimens, archived as formalin-fixed paraffin-embedded (FFPE) tissue, represent a vast resource for retrospective studies, in fields such as biomarker discovery and gene-disease association.

The Challenges Inherent to FFPE Samples

RNA extracted from FFPE samples is often of poor quality and low yield, because of degradation and chemical modification that occur during tissue collection, processing and storage. As a result, FFPE samples are not easily assayed by methods that require RNA extraction followed by reverse transcription or amplification.

Built on QuantiGene bDNA Assay technology, the QuantiGene FFPE Reagent System overcomes these significant problems, and delivers superior analytical outcomes.

Highlights

No RNA Purification—

Work directly from tissue homogenates

No Reverse Transcription—

Eliminate biases against messages that do not reverse transcribe well

No Target Amplification—

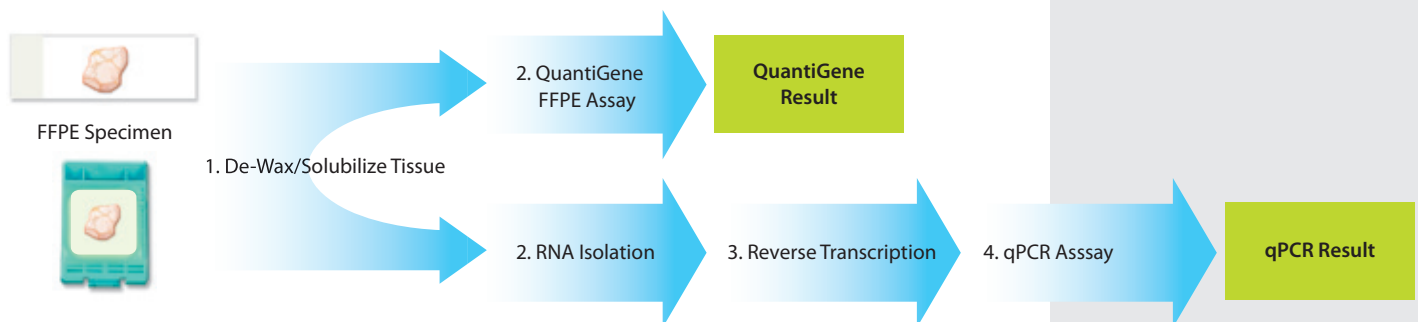
Eliminate biases against messages that do not amplify proportionately due to either random events or sequence composition

Insensitive to—

- RNA degradation
- Chemical modifications of RNA
- RNA cross-linking with proteins

"Simple methods for preparation of FFPE tissue homogenates—NO RNA PURIFICATION"

"Housekeeping probe set panels and improved methods for normalization"



"Parallel QuantiGene FFPE assays for sample quantity and RNA quality assessment"

"Optimized probe set designs for quantitation of highly degraded RNA"

“The QuantiGene® FFPE bDNA assay permits measurements of RNA in tissue homogenates without RNA isolation and provides better reproducibility, accuracy and sensitivity than PCR-based methods.”

— Beatrice Knudsen, Ph.D, M.D.
Fred Hutchinson Cancer Center

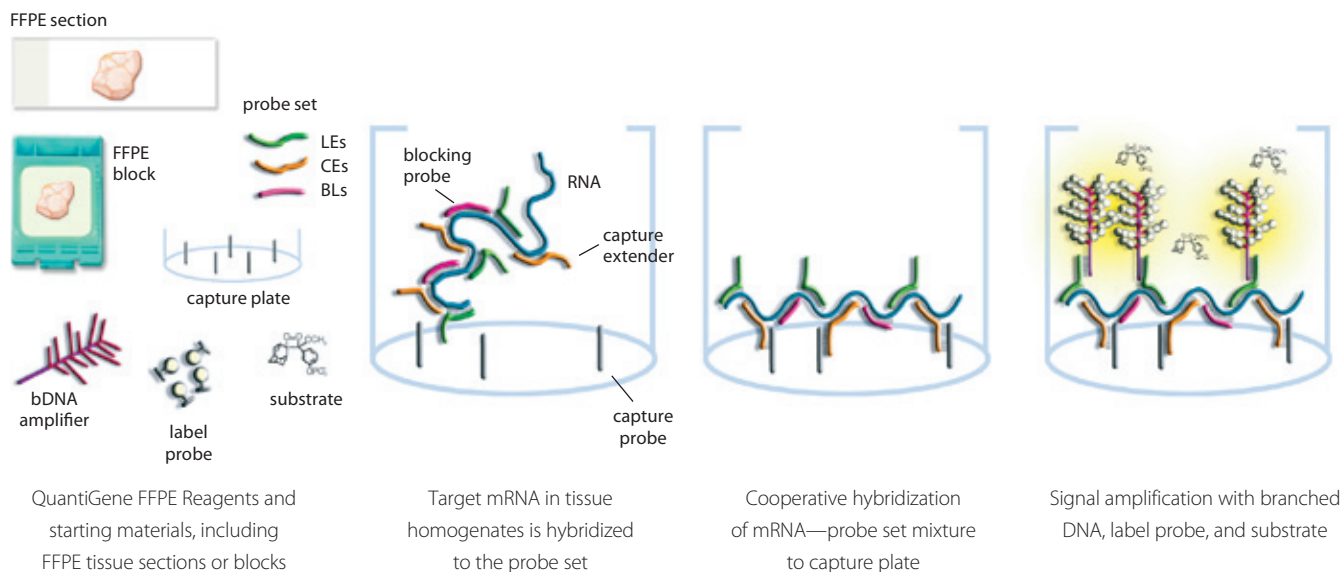
Features of the QuantiGene FFPE Reagent System Include:

- 1. Simple Workflow:** Tissue homogenates are prepared in two easy steps for direct use in QuantiGene FFPE Assays. No RNA purification is required.
- 2. Sample Quantity and RNA Quality Controls:** Because tissues are heterogeneous, and the degree of RNA degradation in FFPE samples may vary, we have developed parallel QuantiGene FFPE assays for assessment of tissue homogenates:
 - **18S Ribosomal DNA Assay**—measures sample quantity independent of RNA quality
 - **28S Ribosomal RNA Assay**—indicates sample RNA quality
- 3. Housekeeping Probe Set Panels** for improved data analysis.
- 4. Optimized Probe Set Design** for efficient capture of even highly degraded RNAs.

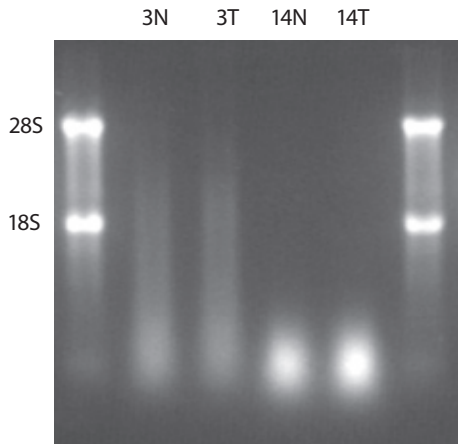
QuantiGene FFPE bDNA Technology Overview

Branched DNA technology is a sandwich nucleic acid hybridization assay that provides a unique approach for RNA detection and quantification by amplifying the reporter signal rather than the target sequence. By measuring RNA directly from the

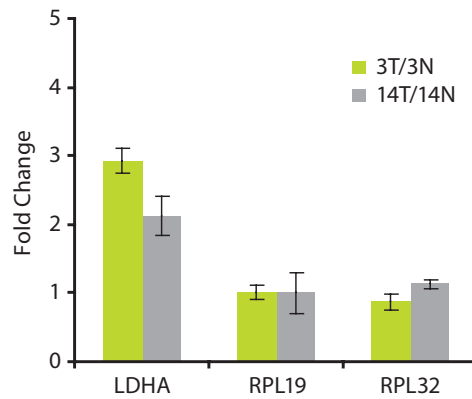
sample source, the assay avoids variations or errors inherent to extraction and amplification of RNA. Branched DNA technology is the basis of a clinically proven viral load tests commercialized by Bayer Corporation as VERSANT® HIV-1 3.0, HCV 3.0 and HBV and has been in use for over a decade.



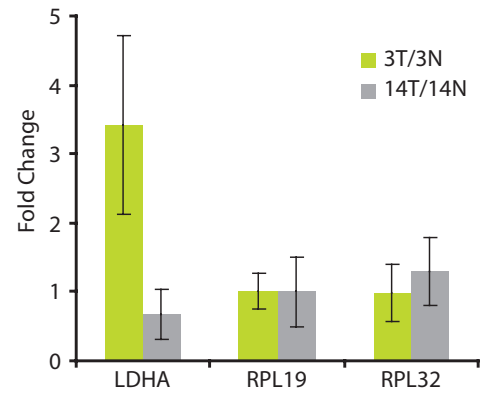
Quantification of 2-Fold Gene Expression Changes in 14 Year Old FFPE Samples



RNA from 3 and 14 year old FFPE samples of tumor and adjacent normal tissues from lung cancer patients as visualized by agarose gel electrophoresis. The positions of the 28S and 18S rRNAs are indicated.



In agreement with the literature (Beer et al 2002), QuantiGene detected 2-fold induction of LDHA RNA in tumor relative to normal tissue even in highly-degraded 14 year old samples.



In contrast, qPCR signals for LDHA RNA from 14 year old samples were below limit of quantitation ($C_t > 35$) and therefore, no induction was observed for this sample.

QuantiGene FFPE Assays Deliver Expected Clinical Outcomes

FFPE Studies to Date:

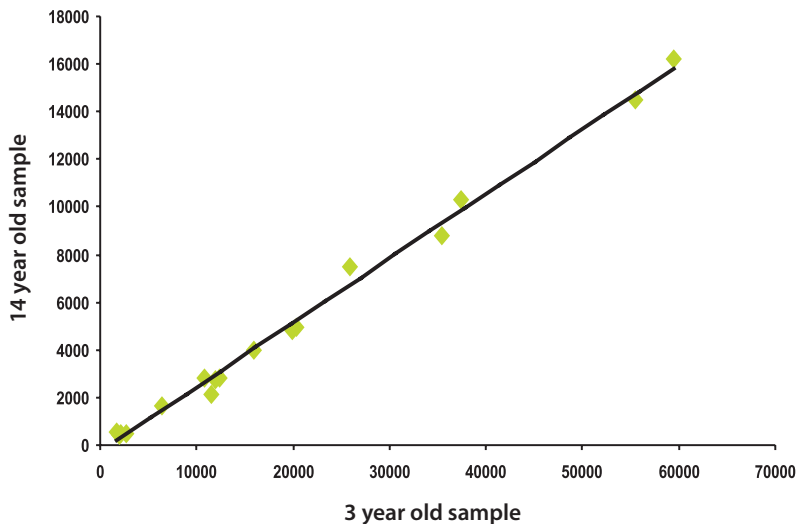
- 11 Independent Studies
- 3 Species
- 7 Tissue Types
- 400 Samples Analyzed
- 200 Genes Quantified

Outcome:

Study 1: Successfully predicted drug resistance in lung and prostate tumors based on expected gene expression profiles.

Study 2: Demonstrated expected antisense knockdown of target RNA in mouse lung.

Study 3: Differentiated Stage I and II lung tumors in concordance with Beer et al. 2002. Nat. Med. 8(8):816-824, Epub 2002 July 15.



Excellent correlation of signals for 12 housekeeping RNAs ($R^2=0.9938$) from 3 and 14 year old FFPE lung tissue samples from different patients.

Assay Specifications

Assay sensitivity: 5,000 (intact)–20,000 (degraded) RNA molecules

Assay CV: <10% intra-run, <15% inter-run

Dynamic Range: >3 logs

Compatible Sample Types:

Fresh, frozen or FFPE animal tissues or total RNA isolated from any of these sources

Average Targets/Sample*: 100

Assay format: 96-well plate

Targets/well: 1

Hardware requirements:

Microplate Luminometer

* Typical sample inputs: 25–100 mm² x 100 micron (pooled) tissue sections, 25–50 mm³ tissue block or 10–50 mg fresh or frozen tissue

For pricing and more information visit our website at www.panomics.com or call us at 1.877.726.6642.

Ordering Information

The QuantiGene FFPE Reagent System, comprised of 4 modules, is a comprehensive solution for gene expression profiling of FFPE samples. Each of the modules is sold separately and is available in multiple sizes.

For new users, Proficiency and Evaluation Kits are available.

Product	Size	Catalog No.
QuantiGene FFPE Assay Kits		
QuantiGene FFPE Assay Kit	2-plate	QF0001
QuantiGene FFPE Assay Kit	10-plate	QF0002
QuantiGene FFPE Assay Kit	5 x 10-plate	QF0003
QuantiGene FFPE Sample Processing and Assessment Kits		
FFPE Animal Tissue Sections	25 samples	QF0100
FFPE Animal Tissue Sections	100 samples	QF0101
FFPE Animal Tissue Blocks	25 samples	QF0102
FFPE Animal Tissue Blocks	100 samples	QF0103
Fresh or Frozen Animal Tissue	25 samples	QF0104
Fresh or Frozen Animal Tissue	100 samples	QF0105
Total RNA*	25 samples	QF0106
Total RNA*	100 samples	QF0107
Universal**	25 samples	QF0108
Universal**	100 samples	QF0109

* Total RNA isolated from fresh, frozen or FFPE tissues.

** Universal kits are designed for users working with multiple sample types including FFPE animal tissue sections or blocks, fresh or frozen animal tissues and/or total RNA isolated from fresh, frozen or FFPE tissues.

Product	Size	Catalog No.
QuantiGene FFPE Housekeeping Probe Set Panels		
Set of 3 Housekeeping Probe Sets*	50 rxns each	QF0070
Set of 3 Housekeeping Probe Sets*	200 rxns each	QF0071
Set of 3 Housekeeping Probe Sets*	1,000 rxns each	QF0072
Set of 3 Housekeeping Probe Sets*	5,000 rxns each	QF0073
Set of 5 Housekeeping Probe Sets*	50 rxns each	QF0074
Set of 5 Housekeeping Probe Sets*	200 rxns each	QF0075
Set of 5 Housekeeping Probe Sets*	1,000 rxns each	QF0076
Set of 5 Housekeeping Probe Sets*	5,000 rxns each	QF0077

* Selected from a list of >20 FFPE Housekeeping Genes, visit our website at www.panomics.com to view the complete list.

QuantiGene FFPE Target-Specific Probe Sets		
QuantiGene FFPE Probe Sets, Catalog	Multiple	See Website
QuantiGene FFPE Probe Sets, by Request	1,000 rxns	QF0051
QuantiGene FFPE Probe Sets, by Request	5,000 rxns	QF0052

QuantiGene FFPE Products for New Users

QuantiGene FFPE Proficiency Kit*	2-plates	QF0004
QuantiGene FFPE Evaluation Kit**	2-plates	QF0005

* Designed and packaged for new users to evaluate the QuantiGene FFPE technology using provided control FFPE samples.

** Designed and packaged for new users to evaluate the QuantiGene FFPE technology using their own sample materials.



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