Development of quantitative targeted RNA-seq methodology for use in differential gene expression

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QIAGEN’s universal workflow allows you to go
From sample to insight, regardless of the sample type and qPCR- or NGS-platform.
QIAGEN Assay Portfolio - Ready-to-use and wet-bench tested

Proof your concept, validate your results, see how good your idea is – Your solution is there

Your Concept

QIAGEN’s ready-to-use Assays for qPCR, NGS, Pyro

mRNA & IncRNA \(\text{(qPCR, RNAseq}^{\text{Coming soon}})\)

microRNA (miRNA) \(\text{(qPCR, RNAseq Serv.)}\)

DNA Mutation (SNP,CNV) \(\text{(qPCR, NGS, Pyro)}\)

DNA Methylation (CpG, ChIP) \(\text{(qPCR, Pyro)}\)

microbial Testing \(\text{(qPCR)}\)

Your Model System

&

Create Insights

QIAGEN Data Analysis & Interpretation tools

Human
Mouse
Rat
Rhesus macaque
Drosophila
Dog
Pig
Cow
Chicken
Horse
Zebrafish
Rabbit
CHO (Chinese hamster ovary)
C. elegans
Types of RNA-Seq

- Whole transcriptome sequencing
  - Every transcript: all mRNA, miRNAs, IncRNAs, snoRNAs; more about discovery

- mRNA sequencing
  - All polyA mRNA sequencing; comparative expression analysis; some discovery

- Small RNA sequencing
  - All miRNAs, IncRNAs; comparative expression analysis; discovery

- Targeted RNA sequencing
  - Differential gene expression of known genes
QIAseq Targeted RNA Panel: Sample to Insight

Sample

Sample Isolation

Targeted Enrichment

Library Construction

NGS Run

Data Analysis

Insight

Sample QC

Library QC

Variant Confirmation

RNeasy Mini Kit

QIAseq targeted RNA panels and Indexes

Any Sequencer

GeneGlobe Data Analysis Center

IPA

RNeasy FFPE Kit

exoRNeasy Serum/Plasma Kit

MiSeq

NextSeq

HiSeq

Ion PGM

Ion Proton
Making Digital Sequencing Possible - Leverage Qiagen content know-how for NGS

- Use 2-stage PCR based enrichment and library preparation
- Molecular barcoded primers
- Optimized for all sample types including FFPE samples
- NGS Platform agnostic
- Internal Controls: (GDC for sample purity and confidence call / normalization controls)
- Catalogue ready-to-use: Disease and pathway specific collections
- Modified panels and fully custom gene content
QIAseq RNA library construction workflow

RNA sample
  ↓
cDNA Synthesis
  ↓
Primer extension/molecular barcoding
  ↓
Mag bead cleanup
  ↓
PCR enrichment
  ↓
Mag bead cleanup
  ↓
Library Preparation & Sample Indexing
  ↓
Mag bead cleanup
  ↓
Library Quantification

8 hours; 96 well-plate compatible and automatable

Random tagged GS Primer

GS 1
MT
RS

GS= Gene specific
MT= Molecular tag
RS= Universal reverse sequence
FS= Universal forward sequence
Value of QIAseq molecular barcode

- Problem:
  - sequence-dependent amplification bias and noise

- Solution:
  - Molecular barcode, count unique barcodes instead of number of raw reads

In a well designed, well optimized, and well executed experiment, data from barcodes and reads ~ same. But with sample variation, FFPE, oversampling, etc, barcodes give a distinct advantage in precision and reproducibility.
Wide range of input amount 1ng to 20ng
- multiplex from 12 to 1000 genes, 1 to 96 samples

Sequencing specificity > 97%

Flexible and high performance library construction
Molecular barcodes can efficiently remove PCR amplification variation

CV computed on the basis of barcode counts vs raw read counts

ERCC standards spiked into Universal Human Reference RNA

QIAseq Assay design and validation performance

Initial validation of qRNAseq assay design on gDNA reference

384 Plex

1000 Plex

- Assays with intra-exon design tested on genomic DNA (5000 assays).
  - Rigid control of design specifications

- QC criteria: pass >20% of the mean (number of MT/gene).
  - 384 Gene Plex:
    - 99% @ 20% of mean.
  - 1000 Gene Plex:
    - 98% @ 20% of mean.
Relative gene expression changes between UHRR and UBRR samples (determined by 384plex NGS vs singleplex qRTPCR assays)

1. qPCR data was normalized by $\Delta$Ct (GOI-HKG).
2. QIAseq data was normalized to total number of molecular barcodes.
3. Fold change (Log 2) compared between two reference RNA samples.
4. NGS required 5 ng total RNA, qPCR 1200 ng (384 well PCR in triplicate)
Excellent correlation of relative gene expression changes by real-time qPCR and QIAseq Targeted RNA sequencing

Fold change between determined by both qPCR and QIAseq
Low read depth caused “dropping out” of low expressing genes (<10 tags/gene)

MiSeq multiplexing initial recommendations:
- Moderate read depth (dynamic range 3-4 logs): 8 samples X 1000 genes, 96 samples X 96 genes
- High read depth (dynamic range 4-5 logs): 12 samples X 96 genes
Benchmarking sensitivity of QIAseq with ERCC RNA standards

- ERCC standards spiked into total RNA samples at 86 to 705,500 copies
- QIAseq primers were designed to ERCC standards
- Enrichment was performed using QIAseq Targeted RNA Kit in technical triplicates
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Reliable quantification is possible down to ~100 copies or lower of an RNA target in 20ng total RNA or ~0.1 copy per cell
QIAseq Targeted RNAseq System

- Extremely sensitive expression profiling, <1 copy per cell
- Highly flexible in design, from 12 to 1000 or more targets, 1 to 96 samples
- High specificity, ~98-99% maintained through all panels
  - minimum 50bp internal sequence read
- Extremely high read uniformity ~0.98 at 20% mean
- Complete integrated workflow from sample to insight
- Molecular barcode enables digital sequencing
QIAseq Targeted RNA Panel Portfolio

QIAseq Targeted RNA Kit (12 or 96 samples)
Kit containing reagents for first strand synthesis, molecular tagging, and gene-specific amplification for targeted RNA sequencing
• Catalog
• Modified
• Custom

QIAseq sample Indexing (12-plex or 96-plex) for Ion & ILMN

Initial Content: 250 – 500 gene panels

Pan Cancer
Molecular toxicology

Immunity and Inflammation
Signal Transduction

Stem Cell
Cell Death
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