qPCR Service

Biomarker Assay Services

Real-time quantitative PCR

The real-time quantitative PCR (qPCR; RT-qPCR) service at Reaction Biology provides custom-tailored gene expression analysis and copy number determination including high-quality DNA and RNA isolation. Relative and absolute quantification approaches enable the detection of quantitative differences in mRNA expression of drug targets, PD markers, or predictive biomarkers. Our qPCR platform is set up to support drug discovery projects performed with cellular and animal models at Reaction Biology as well as the investigation of customer samples.

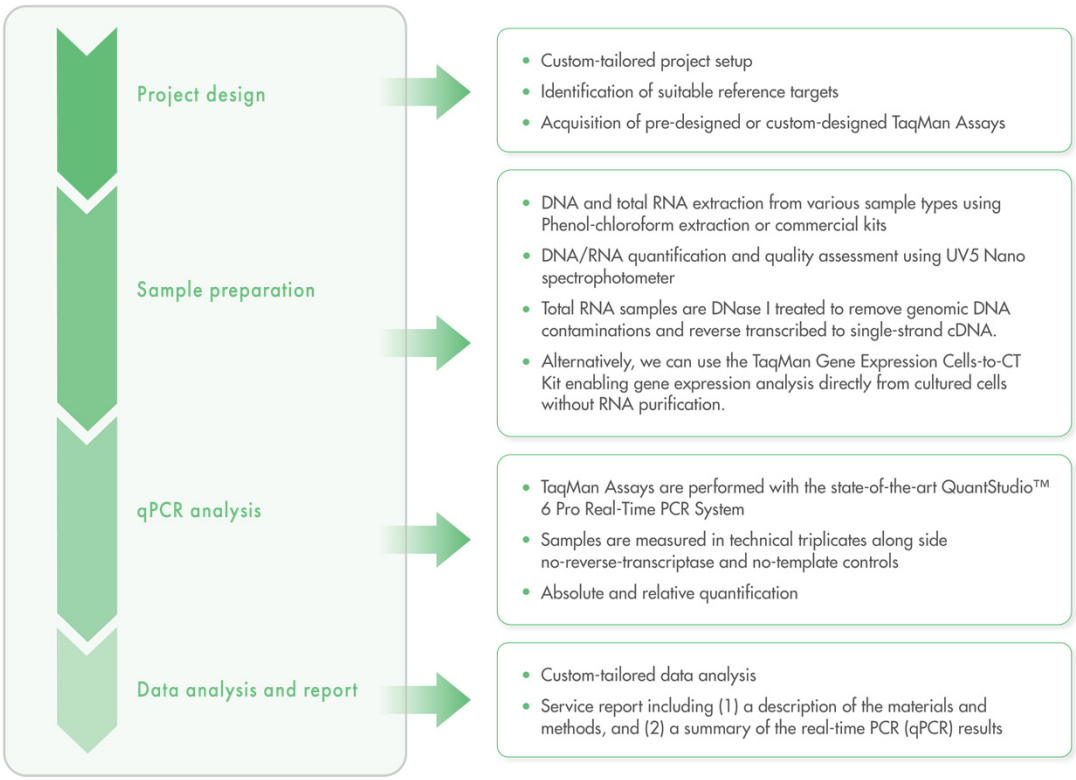
Our qPCR services support numerous applications in the drug discovery process, such as:

- Quantification of drug target gene expression for the identification of suitable tumor cell lines for drug efficacy testing
- Screening biomarkers to predict a drug's response in cellular or animal models
- Determining pharmacodynamic parameters of drugs that affect the expression of specific genes for the determination of time- or dose-dependency and mode of action analysis
- Genotyping for verification of stratification markers

Set up: Our QuantStudio 6 Pro Real-Time PCR System from Applied Biosystems allows mRNA quantification, multiplex assays, and high-throughput projects. All qPCR services are carried out in our qPCR lab with dedicated work areas for DNA and RNA extraction and handling.

Sample options: 2D and 3D cell culture, tissues including tumor material, whole blood, PBMCs, fixed-formalin paraffin-embedded (FFPE) samples, and cell-free samples such as cell culture supernatants, plasma to analyze cell-free, or circulating DNA.

Workflow



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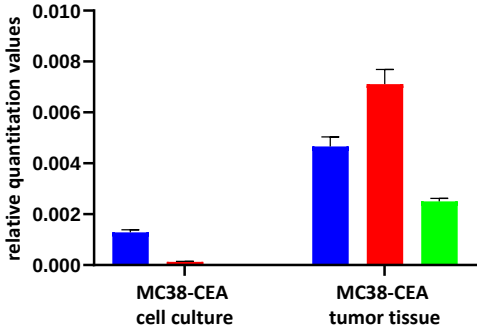
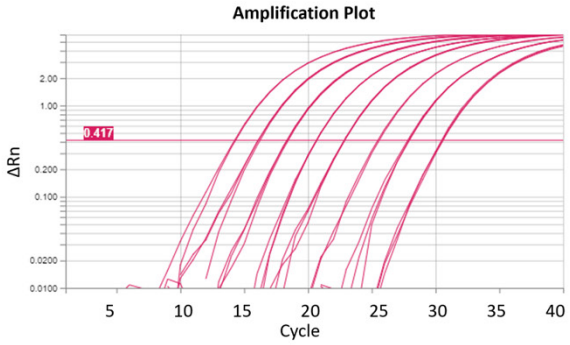
Example: Relative quantification of drug target gene expression in cell culture vs in vivo samples

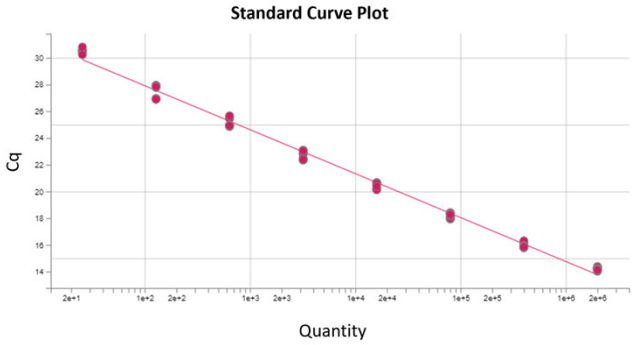
The gene expression plot displays the fold change in expression levels of three putative targets in MC38-CEA cells and MC38-CEA tumor samples relative to C57/Bl6 lung tissue.

The comparative CT method (ΔΔCT) was used. Housekeeping gene and healthy lung tissue data were used to calculate relative quantitation values (RQ).



Example: Absolute quantification of drug target mRNA



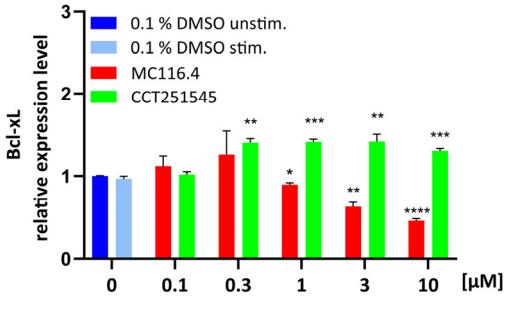


We determine the absolute drug target mRNA quantity in test samples using the standard curve method. The amplification plot displays the amplification of a 5-fold dilution series of a standard sample (recombinant plasmid).
The x-axis of the standard curve plot represents the logarithm of the diluted DNA quantity, and the y-axis represents the measured quantification cycle (Cq) value.

Example: Analysis of downstream signaling effects after drug target inhibition

The expression of CDK8/CycC-dependent genes was investigated after treatment with two CDK8 inhibitors in HCT116 colon cancer cells.

Analysis of expression levels of the CDK8/CycC-regulated Bcl-XL gene was performed by qPCR. Data were analyzed by the ΔΔCt method using a housekeeping gene for normalization. Each column represents the mean ±SE of two biological replicates.



Whereas treatment with compound MC116.4 results in significant downregulation of Bcl-XL mRNA, opposite effects were observed with compound CCT251545.