Abstracts
QIAGEN Evening Meeting 2024 in Kyoto
February 5 2024 at Hotel Okura Kyoto

Tips and trick in dPCR

Mikael Kubista
Institute of Biotechnology, Czech Academy of Sciences, University of Gothenburg & MultiD Analyses AB, Sweden

The world changed one Friday night in the spring 1983 at mile marker 46.7 on Highway 128 heading toward Mendocino, California, when Kary Mullis, then a chemist with the biotech company Cetus, conceived a chemical reaction to reproduce DNA virtually an infinite number of times. PCR was born. In 1993 Russ Higuchi, then at Roche, invented serendipitously real-time PCR. A year earlier Alec Morley at Flinders University described quantification by limited dilutions, which we know today as digital PCR.

In my talk I will present key inventions and strategies for implementing reliable dPCR analysis in diagnostics and the development of cell and gene therapies, including the MIQE and dMIQE guidelines, CEN and ISO standard documents and the Good Laboratory Practice (GLP) ongoing implementations. I will also compare qPCR and dPCR discussing the advantages and limitations of the two technologies recommending when one or the other is preferred.

digital PCR applications for cell and gene therapy – standardization for a high-quality process development

Dr. Francesca Di Pasquale
QIAGEN, Director R&D • dPCR Applications Development

Cell and gene therapies seek to target previously untreatable diseases at their source using individualized treatments. However, developing safe and effective cell and gene therapies requires strict monitoring at all stages of the development process. Adeno-associated virus (AAV) has turned into a primary modality for efficient gene therapy applications. The process of generation and purification of the viral vectors require precise quality control to enable safe and reliable dosing during clinical studies or patient care. The ability to accurately and reproducibly quantify vector titers is essential for safe and effective AAV-based gene therapies. Digital PCR enables absolute quantification with unprecedented precision and a higher tolerance towards inhibitors without the need for any standards. Additionally, more than one region of interest can be quantified at once leading to further information on genome intactness.

Besides AAV genome quantification, the determination of potential impurities is crucial. Manufacturers of biologics use different approaches to show, for example, low residual host cell DNA (HCD) or Mycoplasma contaminants throughout the production process and final substance. Levels of HCD must not exceed levels established by regulatory authorities, hence, HCD monitoring is an important step in the process of manufacturing since potential carryover poses a safety concern. Here we layout a dPCR-based streamlined workflow for impurity quantification with increased precision and robustness.

Abstracts
QIAGEN Evening Meeting 2024 in Kyoto
February 5 2024 at Hotel Okura Kyoto

QIAcuity を用いた CRISPR/Cas3 ゲノム編集の検出と医療応用検討

佐伯涼太　氏
C4U 株式会社　研究開発部　研究開発センター

C4U 株式会社は CRISPR/Cas3 システムの産業応用を目指すバイオテックである。当社の CRISPR/Cas3 システムは、一般的なゲノム編集(CRISPR/Cas9)と比較してゲノムを一方向に数 kb 削るという特徴を有する。

ゲノム編集による遺伝子治療法の開発では、有効性をゲノム編集効率から定量的に説明することが求められている。当社では、CRISPR/Cas3 システムによる広範なゲノム切断と、最大 5 色の蛍光プローブを用いてゲノムコピー数を定量可能な QIAcuity との親和性に注目し、種々の検討を行った。

本セミナーでは、QIAcuity を用いた CRISPR/Cas3 ゲノム編集様式のキャラクタライゼーションの結果に加え、医療応用に向けたケーススタディとして異常ヘモグロビン症を対象とした試験結果を紹介する。