Application Note

Mutation screening of c-kit and EGFR using the QIAxcel® system prior to Pyrosequencing®

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The QIAxcel Advanced System was successfully used to separate PCR amplicons and detect mutations in exons of c-kit and EGFR genes found in gastrointestinal stromal tumors (GIST) and non-small cell lung cancers. It reduced analysis time and provided high-quality, reproducible determination of deletions and insertions of key genes. This study shows that the QIAxcel system can be used to increase the efficiency of Pyrosequencing applications.

Introduction

Screening amplicons for mutations prior to sequencing is a critical step, but it can be tedious and time consuming. It is therefore important to develop rapid and reliable detection techniques that yield results of sufficiently high quality for downstream Sanger sequencing and Pyrosequencing applications. In this study, a method for detecting activating mutations of c-kit and EGFR genes using the QIAxcel Advanced System was assessed. It proved highly suitable for this purpose.

Activating mutations of c-kit and EGFR genes can be detected in DNA samples from gastrointestinal stromal tumors (GIST) and non-small cell lung cancers (1–3). Some of the most common mutations are the EGFR exon 19 deletion and exon 20 insertion, and the duplication of the c-kit gene within exon 9 or deletion within exon 11. The assessed QIAxcel-based technique readily detected the mutations in EGFR exon 19 and c-kit exon 11.

Materials and methods

DNA isolation and purification

Genomic DNA was isolated from formalin-fixed paraffin embedded (FFPE) samples with representative tumor cellularity. Sections with a thickness of 10 µm were taken and deparaffinized with xylene. DNA was extracted using the QIAamp® DNA FFPE Tissue Kit. The genomic DNA concentration was measured with a NanoDrop® spectrophotometer (ThermoFisher® Scientific Inc.).

* The therascreen EGFR Pyro Kit is not available in the US and Canada.
Real-time PCR

Amplification was performed using the therascreen® EGFR Pyro Kit* on a real-time PCR system. As per the manufacturer’s protocol, a 25 µl PCR mix was prepared containing 30 ng of template DNA and 8 µM of primers. The initial denaturation step was at 95°C for 15 min, followed by 42 amplification cycles of denaturation at 95°C for 20 s, annealing at 53°C for 30 s, and elongation at 72°C for 20 s. The final elongation step was at 72°C for 10 min.

Electrophoresis and DNA size estimation

After amplification, the PCR products were separated using the QIAxcel Advanced, the QIAxcel DNA High Resolution Kit, and the OM800 method. Amplicon size determination was done with the QX Alignment Marker 15 bp/600 bp and QX DNA Size Marker 25–500 bp. Data were analyzed and visualized using the QIAxcel ScreenGel® software. Pyrosequencing was then performed.

Results

PCR fragments were amplified from EGFR exon 19 and c-kit exon 11 using samples with known deletions compared to the wild type (Figure 1 and 2). Analyses using the QIAxcel Advanced System showed high accuracy in identifying wild-type and mutated DNA fragments based on the size estimation. Human EGFR exon 19 has an amplicon size of about 250 bp. When there were deletions, extra bands could be seen in the gel images (Figure 1). Human c-kit exon 11 has an amplicon size of about 220 bp. Mutations were found at various sizes (Figure 2). All of the samples with deletion mutations were detected and the corresponding deletion size was correctly scored, allowing for the exclusion of wild-type samples from subsequent Pyrosequencing.

![Figure 1. Detection of deletion mutations in EGFR exon 19. Human EGFR exon 19 has an amplicon size of 250 bp. Lanes A1 and A3 are from samples with EGFR exon 19 deletions and have extra bands, while the remaining lanes are from wild-type EGFR.](image-url)
Conclusions

- The QIAxcel system proved to be highly suitable for screening amplicons prior to Pyrosequencing using EGFR and c-kit as the model systems. All of the deletion mutants were detected and the corresponding deletion size was correctly scored, allowing the exclusion of wild-type samples from the downstream sequencing step.

- The described QIAxcel-based screening method yields robust and reproducible results of sufficiently high quality for more efficient downstream Sanger sequencing and Pyrosequencing applications.

- This method using the QIAxcel system can be applied to increase the speed and reduce the costs of deletion/insertion studies.

References


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<td>For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)</td>
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* The therascreen EGFR Pyro Kit is not available in the US and Canada.

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Discover more about the QIAxcel Advanced System at [www.qiagen.com/QIAxcel](http://www.qiagen.com/QIAxcel).

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