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 0M800 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.

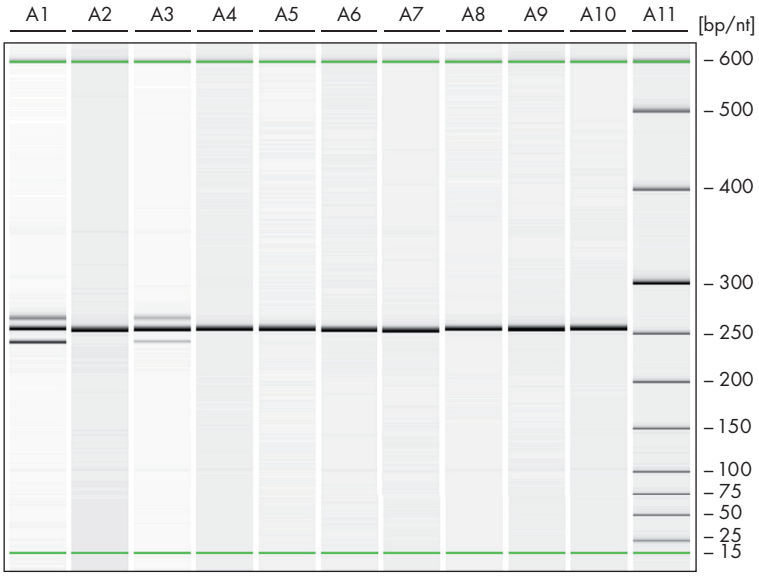

Figure 1. Detection of deletion mutations in *EGFR* exon 19. Human *EGFR* exon 19 has an amplicon size of 250 bp. Lanes A1 and A3 are from samples with *EGFR* exon 19 deletions and have extra bands, while the remaining lanes are from wild-type *EGFR*.

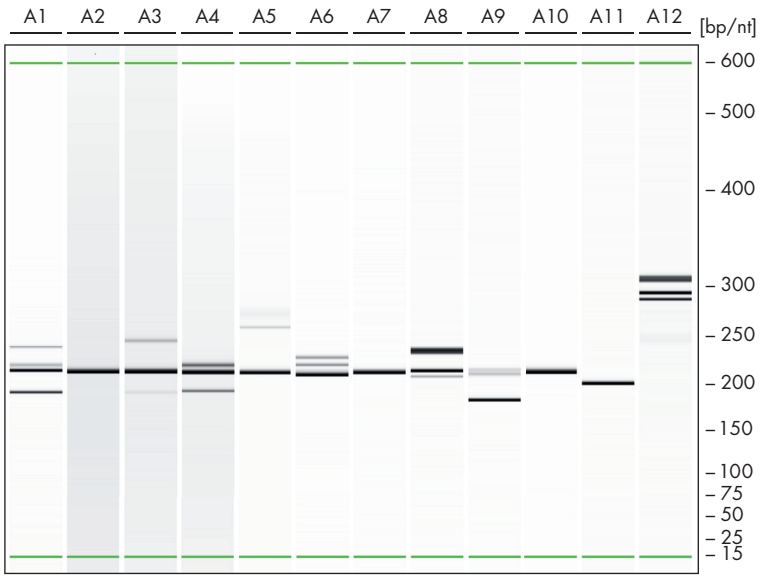

Figure 2. Deletion and duplication mutations in *c-kit* exon 11. Human *c-kit* exon 11 has an amplicon size of about 220 bp. **Lane A11**: sample with an exon 11 homozygous deletion. **Lanes A2, A7, and A10**: the wild-type *c-kit* exon 11 allele. **Lane A12**: sample with an exon 9 duplication (p.A502 Y503dup).

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

1. Willmore, C., Holden, J.A., Zhou, L., Tripp, S., Wittwer, C.T., and Layfield, L.J. (2004) Detection of *c-kit*–activating mutations in gastrointestinal stromal tumors by high-resolution amplicon melting analysis. Am. J. Clin. Pathol. **122**, 206.
2. Hirota, S. and Isosaki, K. (2006) Pathology of gastrointestinal stromal tumors. Pathol. Int. **56**, 1.
3. Lopes, L.F. and Bacchi, C.E. (2007) *EGFR* and gastrointestinal stromal tumor: an immunohistochemical and FISH study of 82 cases. Mod. Pathol. **20**, 990.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device, including computer and QIAxcel ScreenGel Software, 1-year warranty on parts and labor	9001941
QIAamp DNA FFPE Tissue Kit (50)	For 50 DNA preps: 50 QIAamp MinElute Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	56404
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
therascreen EGFR Pyro Kit (24)*	For 24 reactions: Sequencing Primers, PCR Primers, Unmethylated Control DNA, PyroMark PCR Master Mix, CoralLoad Concentrate, and therascreen Buffers and Reagents	971480
QX Alignment Marker 15 bp/600 bp	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25–500 bp (50 µl) v2.	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µl	929560

* 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**.