

## Application Note

# Rapid and accurate detection of Y chromosome microdeletions

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Y chromosome microdeletions and rearrangements that are relatively common causes of aberrant sperm profiles were detected using the QIAxcel<sup>®</sup> system. Samples were analyzed directly without prior manipulation, and the type of deletion was detected by automatic fragment-size determination. Use of the QIAxcel system significantly accelerated research on male infertility.

## Introduction

The 3 azoospermia factor (AZF) regions of the Y chromosome accommodate genes required for spermatogenesis. The most distal region, AZFc, is one of the most genetically dynamic regions in the human genome (1, 2).

Some AZF rearrangements are responsible for marked spermatogenic defects. For example, men with deletions in AZF regions exhibit drastically reduced sperm concentrations (<1 million sperm/ml compared to normal levels of >20 million sperm/ml) (3).

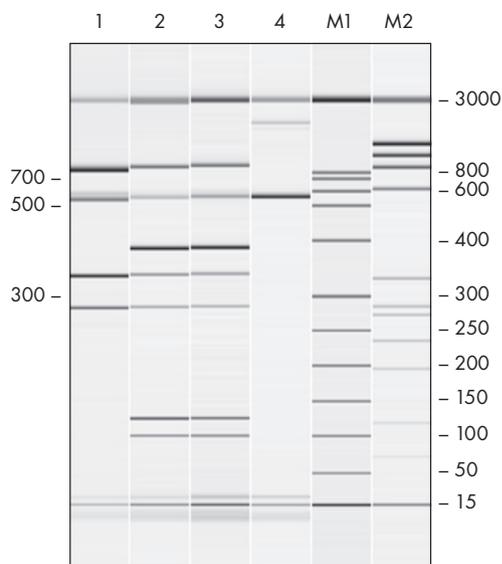
A rapid and reproducible method for determining AZF deletions would support effective research on the mechanisms of Y chromosome microdeletions. To develop an optimal screening strategy for male subfertility, a large database of microdeletion data was constructed and analyzed (4, 5). Most of the published Y chromosome microdeletions (85.6%) can be detected by PCR using a set of 6 genetic markers: sY84, sY127, sY152, RBM1, sY147 and sY254-DAZ as well as for the ZFY/ZFX gene as an internal control for the multiplex PCR reaction (5).



The QIAxcel system.

## Materials and methods

After routine PCR amplification of Y-chromosome-specific, sequence-tagged site (STS) markers as described in (5), samples were placed directly into the QIAxcel system and analyzed using the QIAxcel DNA High Resolution Kit and the QX Alignment Marker 15 bp/3 kb. The analysis included the QX DNA Size Marker 50–800 bp and the QX DNA Size Marker FX174/*Hae*III. Analysis was performed with the OM500 method at 5 kV voltage and a 500 second separation time. The alignment marker was injected at 4 kV for 20 seconds and samples at 5 kV for 10 seconds.



## Results

Results of separation of specific amplicons from the AZF regions of the Y chromosome are presented in Figure 1. In lane 3 all fragments were present: sY147 (100 bp), sY152 (125 bp), sY127 (274 bp), sY84 (326 bp), sY254-DAZ (350 bp), ZFY/ZFX (495 bp) and RBM1 (800 bp).

Using the QIAxcel DNA High Resolution Kit, 12 samples can be analyzed in less than 10 minutes. Results can be displayed as a gel-like image as well as an electropherogram.

The analysis of PCR products was simplified by the sharp banding pattern achieved with the QIAxcel system. The automated data acquisition enhanced analysis reliability and eliminated error in sample identification.

No manipulation of the PCR samples was required before analysis on the QIAxcel system, saving time and minimizing human error.

Since the QIAxcel capillary electrophoresis system uses only minute amounts of DNA through electrokinetic injection, the samples are retained for downstream procedures, such as sequencing.

**Figure 1. Identification of Y chromosome microdeletions.** PCR was prepared with primers for detecting Y chromosome microdeletions and analyzed directly on the QIAxcel system using the QIAxcel DNA High Resolution Kit. **1:** male exhibiting deletions sY152, sY147, and sY254-DAZ; **2:** unknown sample exhibiting no deletions; **3:** male control; **4:** female control; **M1:** QX DNA Size Marker 50–800 bp; **M2:** QX DNA Size Marker FX174/*Hae*III.

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## Conclusions

- The QIAxcel capillary electrophoresis system is highly suited for reproducibly detecting Y chromosome microdeletions and rearrangements in a fast and reliable manner.
- Using the QIAxcel system with the QIAxcel DNA High Resolution Kit, Y chromosome microdeletions were detected by direct analysis of PCR products: prior manipulation of the samples was not necessary.
- Results of this research, described in (4), facilitated the construction of a minimal set of markers to be used for detecting AZF microdeletions. The use of these markers will assist the research on the effect of AZF mutations on male fertility.

## References

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2. Navarro-Costa, P., Gonçalves, J., and Plancha, C.E. (2010) The AZFc region of the Y chromosome: at the crossroads between genetic diversity and male infertility. *Hum. Reprod. Update* **16**, 525; first published online March 18, 2010 doi:10.1093/humupd/dmq005.
3. Simoni, M., Tuttmann, F., Gromoll, J., and Nieschlag, E. (2008) Clinical consequences of microdeletions of the Y chromosome: the extended Munster experience. *Reprod. Biomed. Online* **16**, 289.
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5. Peterlin, B., Kunej, T., and Hristovski, D. (2004) Diagnostic test for Y chromosome microdeletion screening in male infertility. *Genet. Testing* **8**, 45.

## 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
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAxcel DNA Screening Kit (2400)	QIAxcel DNA Screening Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929004
QIAxcel DNA Fast Analysis Kit (3000)	QIAxcel DNA Fast Analysis Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX DNA Size Marker 50 bp – 1.5 kb, QX Alignment Marker 15 bp/3 kb, 12-Tube Strips	929008
QIAxcel RNA QC Kit v2.0 (1200)	For 100 runs of 12 samples: QIAxcel RNA Quality Control Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, QX RNA Alignment Marker, QX RNA Size Marker 200–6000 nt, QX RNA Denaturation Buffer, 12-Tube Strips	929104
QX DNA Size Marker FX174/HaeIII (50 µl)	DNA size marker with fragments of 72, 118, 194, 234, 271, 281, 310, 603, 872, 1078, and 1353 bp; concentration 100 ng/µl	929551
QX DNA Size Marker 50–800 bp (50 µl)	DNA size marker with fragments of 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, and 800 bp; concentration 100 ng/µl	929556
QX Alignment Marker 15 bp/3 kb (1.5 ml)	Alignment marker with 15 bp and 3 kb fragments	929522

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