Application Note

Molecular Characterization of Immune Gene Rearrangement Profiles in Acute Lymphoblastic Leukemia (ALL) using QIAxcel® Advanced

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Introduction

Clonally rearranged immunoglobulin- (IG) and T-cell receptor (TR) genes are key molecular targets for Minimal Residual Disease (MRD) diagnostics in B- and T-cell Acute Lymphoblastic Leukemia (ALL). MRD is used primarily to monitor course of disease during and after therapy for sensitive remission assessment, early detection of relapse and as a tool to guide therapy. Currently, MRD diagnostics entails the analysis of follow-up samples using patient-specific, real-time quantitative PCR (ASO-RQ-PCR) to monitor course of disease. An extensive marker screening panel of multiplex PCR assays targeting IG/TR gene rearrangements of a primary diagnosis sample is used to identify tumor-specific IG/TR rearrangements. To discriminate malignant clonal rearrangements against a polyclonal background, PCR fragments from IG/TR PCR assays are analyzed. The most frequently used methods for this fragment analysis are GeneScan® or dHPLC, followed ▷

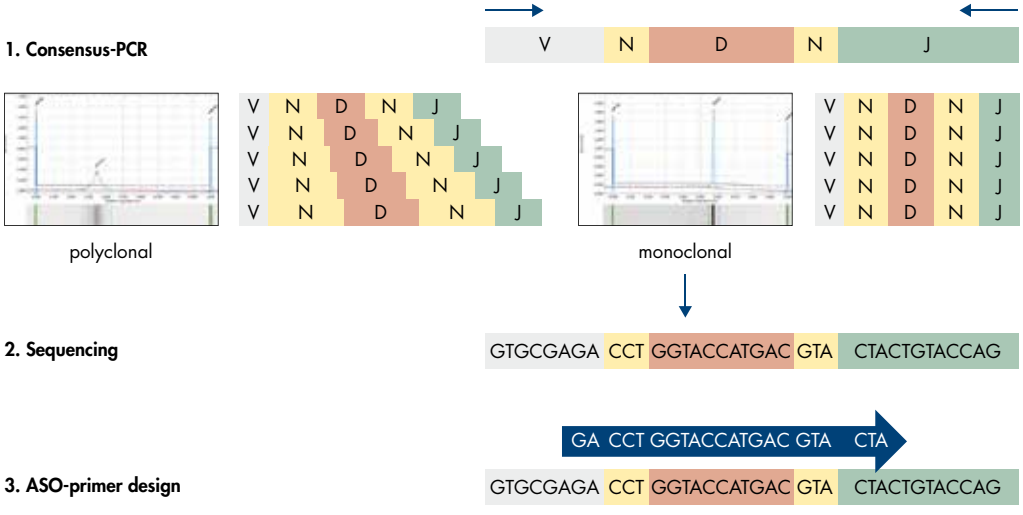

Figure 1. Standard process for Minimal Residual Disease diagnostics. IG/TR rearrangements are amplified in samples and monoclonal rearrangements are distinguished from a polyclonal background by PCR fragment analysis. The separated and purified fragments are then sequenced and primers for subsequent monitoring are designed.

by heteroduplex (HD) analysis to separate and purify fragments before sequencing (1–5; Figure 1). To improve cost effectiveness and automated high-throughput performance of MRD analysis, we tested the high-resolution capillary electrophoresis system QIAxcel Advanced as an alternative method for fragment analysis. We compared the performance of QIAxcel to GeneScan and dHPLC, specifically focusing on the retention of sensitivity.

Materials and methods

Ten B-lineage samples taken from ALL patients at initial evaluation were analyzed using a standardized panel of 15 multiplex PCR assays targeting different immune gene rearrangements (6 reactions for *IGH*, *IGL*, and *IGK* and 9 for *TRB*, *TRG*, and *TRD*; 1–3). The resulting 150 PCR products were subsequently analyzed on QIAxcel Advanced, using the 12-capillary High-Resolution Gel Cartridge and the OM800 method in the QIAxcel ScreenGel® software. A custom alignment marker 100/600 bp was run simultaneously with the samples and the QX DNA Size Marker 25–500 bp was used for size estimation. The same samples were analyzed by GeneScan (Applied Biosystems® ABI3500, POP7, 50 cm array, ROX-Size Standard 35–500 bp and dHPLC (Transgenomic® 4500HT, DNASepHT Cartridge). All identified clonal IG/TR rearrangements were isolated by HD analysis and verified by Sanger sequencing. To test sensitivity, clonal samples or monoclonal cell line DNA were diluted in polyclonal buffy coat DNA (6 dilutions ranging from 50% to 1%) and analyzed for all IG/TR targets.

Results and discussion

All amplifiable clonal IG and TR rearrangements (61/150) and all polyclonal signals (89/150) of the 10 evaluated ALL primary samples were as accurately detected with QIAxcel Advanced as with GeneScan and dHPLC (Figure 2).

GeneScan/dHPLC	QIAxcel: clonal	QIAxcel: polyclonal
clonal	**61**	**0**
polyclonal	**0**	**89**

Figure 2. All amplifiable clonal and polyclonal products were accurately detected by QIAxcel Advanced. All amplifiable products (1–3) from 10 ALL samples for initial evaluation were identified as accurately by QIAxcel Advanced as by GeneScan and dHPLC.

Depending on the individual target, the detection limit of clonal cells within a polyclonal background was 5–10% using QIAxcel Advanced (Figure 3). Figure 4 summarizes the detection limit of QIAxcel versus dHPLC for all 15 multiplex PCR assays (1–3).

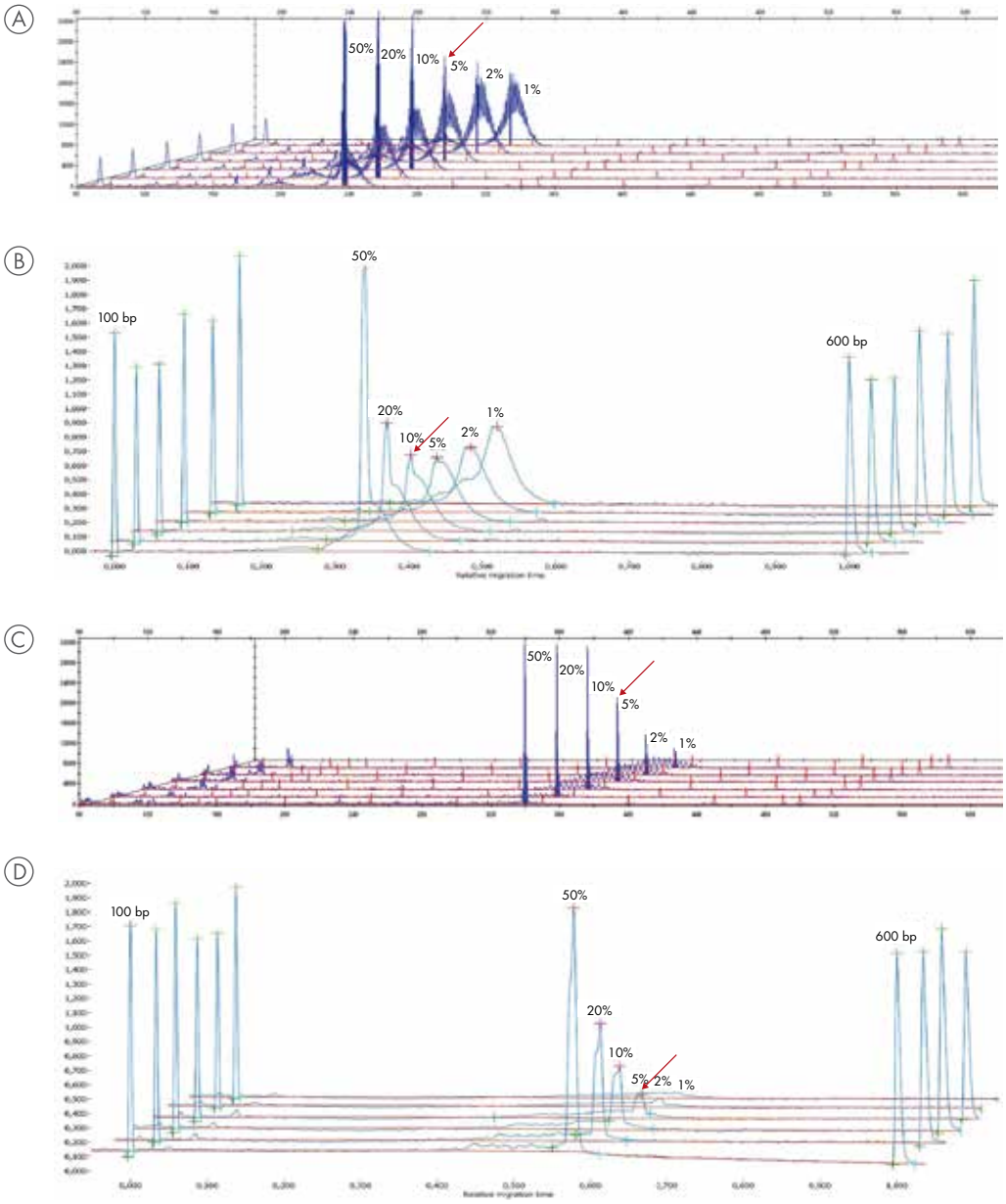

Figure 3. Detection with QIAxcel Advanced affords sufficient sensitivity. Monoclonal DNA isolated from the RAMOS (**A** and **B**) or JURKAT (**C** and **D**) cell lines was diluted (50, 20, 10, 5, 2 and 1%) in polyclonal buffy coat DNA and analyzed with GeneScan or QIAxcel Advanced. The lower detection limit (red arrow) for products of a PCR targeting *IGH-FR1* was 5% for GeneScan (**A**) and 10% for QIAxcel (**B**). The lower detection limit for products of a PCR targeting *TRG2* was 5% for both GeneScan (**C**) and QIAxcel (**D**).

QIAxcel					PCR	dHPLC				
50%	20%	10%	5%	1%	← Dilution step →	1%	5%	10%	20%	50%
	•	•			*TRB* Tube A[1]				•	
•	•	•	•		*TRB* Tube B[1]		•	•	•	•
	•	•			*TRB* Tube C[1]				•	•
	•				*TRG* Tube 1[2]			•	•	
	•	•	•		*TRG* Tube 2[2]		•	•	•	
	•	•	•		*TRG* Tube 3[2]			•	•	•
					TRD Tube A[1]					
		•			*IGH* VJ Tube A[1]		•	•	•	
		•	•		*IGH* DJ Tube D[1]		•	•	•	
					IGH DJ Tube E[1]		•			
	•	•	•		*IGL* Tube A[1]		•	•	•	
		•	•		*IGK* Tube A[1]		•	•	•	
	•	•	•		*IGK* Tube B[1]			•		
					TRD/A Tube A[1]					
				•	*TRD/A* Tube B[1]	•	•	•	•	

Figure 4. The lower detection limit of QIAxcel is comparable to results from dHPLC. The dilution series of monoclonal DNA in polyclonal buffy coat DNA was analyzed on QIAxcel Advanced (left) and by dHPLC (right). Green squares indicate a dominant clonal signal, yellow squares a weak clonal signal, and red squares indicate no detectable clonal signal. A dot (•) indicates detection of the polyclonal background.

Conclusions

- The performance of QIAxcel Advanced to identify clonal IG/TR rearrangements in Acute Lymphoblastic Leukemia using standardized multiplex PCR is comparable to dHPLC. However, GeneScan analysis should be used when extraordinary sensitivity is necessary.
- The automation afforded by QIAxcel Advanced reduced handling time and processing errors. Furthermore, the integrated QIAxcel GelScreen software enabled easy electronic documentation and immediate visualization of results.
- QIAxcel is a reliable, fast, cost-effective, and accurate tool for high-throughput IG/TR marker characterization in ALL as basis for clone-specific molecular disease monitoring.

References

1. van Dongen, J.J.M. et al. (2003) Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia **17**, 2257.
2. Kneba, M., Bolz, I., Linke, B., Bertram, J., Rothaupt, D. and Hiddemann, W. (1994) Characterization of clone-specific rearrangement T-cell receptor gamma-chain genes in lymphomas and leukemias by the polymerase chain reaction and DNA sequencing. Blood **84**, 574.
3. Szczepanski, T., van der Velden, V.H., Hoogeveen, P.G., de Bie, M., Jacobs, D.C., van Wering, E.R. and van Dongen, J.J. (2004) Vdelta2-Jalpha rearrangements are frequent in precursor-B-acute lymphoblastic leukemia but rare in normal lymphoid cells. Blood **103**, 3798.
4. Langerak, A.W. et al. (2012) EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia **26**, 2159.
5. Brüggemann, M. et al. (2007) Powerful strategy for polymerase chain reaction-based clonality assessment in T-cell malignancies: report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia **21**, 215.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced Instrument	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel software, and 1-year warranty on parts and labor	9001941
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High-Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QX DNA Size Marker 25–500 bp	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µl	929560

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1094538 04/2015 PROM-8023-003