Application Note

DNA isolation with the MagDEA Dx SV kit for qPCR-based detection of genes involved in celiac disease

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Introduction

Gluten intolerance, or celiac disease, is an inherited autoimmune disorder. Celiac disease patients suffer from chronic bowel inflammation and malabsorption caused by an immune response towards gluten, a protein often found in wheat and other cereals.

Variations of the HLA-DQ2 and HLA-DQ8 genes have been detected in almost all celiac patients. These genes encode for two HLA class II proteins located on the cellular surface of antigen presenting cells. The two proteins form a complex that binds gluten peptides and presents them to T-cells, causing an immune response. Affected patients can reduce the severity of symptoms by avoiding gluten-containing foods in their diet.

The PharmGenomics HLA DQ 2 + 8™ Real-Time PCR Kit contains a set of primers optimized to detect the alleles most relevant to the disease: HLA-DQ2 (HLADQA1*05 and HLA-DQB1*02) and HLA-DQ8 (HLA-DQB1*03:02). The assay has been established using genomic DNA isolated with a column-based kit from Supplier Q. The goal of this study is to evaluate whether other commercially available methods for automated, magnetic bead-based DNA purification are suitable alternatives.

Materials & Methods

Blood samples were collected from three celiac patients into EDTA tubes. DNA was purified from each sample using three different protocols: A manual, spin column-based protocol using a kit from Supplier Q, and two automated, bead-based protocols using the MagDEA Dx SV kit on the magLEAD 6gC instrument, both provided by Precision System Sciences, or a comparable kit/instrument combination from Supplier R.

DNA quantity and quality of all nine samples were assessed by measuring absorbance at 280, 260, and 230 nm. DNA concentrations were normalized, and real-time PCR reactions were performed using primers provided in the PharmGenomics HLA DQ 2 + 8 Real-Time PCR Kit, following the instructions in the kit handbook (1).

Real-time PCR reactions were set up using DNA isolated by one of the three purification methods, and a negative template control. Primer pairs included the alleles HLA-DQA1*05, HLA-DQB1*02, and HLA-DQB1*03:02 (FAM-labeled), and an internal positive amplification control (Yakima Yellow-labeled).

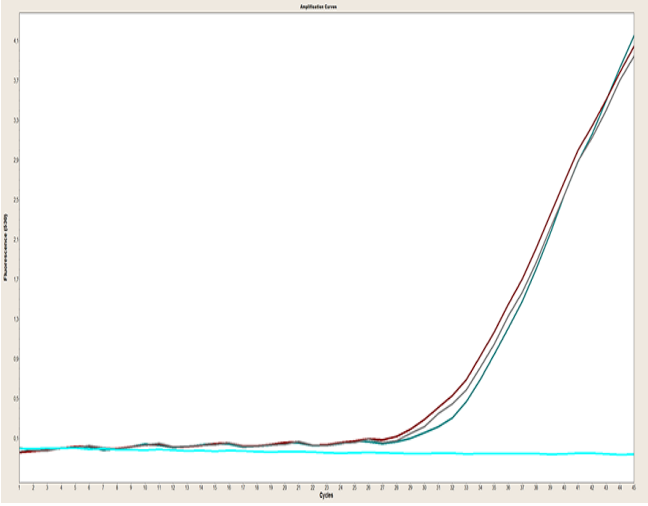

Figure 1: In real-time PCR, similar amplification curves are obtained using DNA samples isolated by all three methods. DQB1*02 amplification plot, patient #2. Turquoise: Negative control; Dark Red: PSS, Gray: R; Blue: Q.

Results

DNA was successfully isolated using all three methods. DNA quantity and quality in all samples were comparable. Results obtained in real-time PCR experiments for each patient and gene variant were similar between the three purification methods. Results were confirmed by end-point PCR followed by gel electrophoresis (MutaGEL® HLA-DQ 2+8 kit, Immundiagnostik AG, Germany).

		Patient #1			Patient #2			Patient #3		
		Q	PSS	R	Q	PSS	R	Q	PSS	R
DQ2	HLA-DQA1*05	26.81	27.97	28.20	27.43	27.81	28.06	NA	NA	NA
DQ2	HLA-DQB1*02	NA	NA	NA	31.82	31.34	31.69	NA	NA	NA
DQ8	HLA-DQB1*03:02	NA	NA	NA	NA	NA	NA	27.16	26.32	25.50

Figure 2: Similar CT values were obtained in real-time PCR reactions using DNA isolated by all three purification methods. Rows contain values for the three HLA variants. Numbers indicate C_T values obtained with DNA normalized to 10 ng/µL.
NA: HLA template not amplified, but amplification of internal control was successful (data not shown).

Conclusions

All three DNA isolation methods, the PSS MagDEA Dx SV kit run on the PSS MagLEAD 6gC instrument, a comparable bead-based, automated procedure provided by Supplier R, and a manual, column-based procedure provided by Supplier Q, yielded comparable, reliable results and can be recommended for use with the PharmGenomics HLA DQ 2 + 8 Real-Time Kit.
Automated, magnetic bead-based DNA isolation procedures using pre-filled reagent cartridges such as provided in the PSS MagDEA Dx SV kit reduce hands-on time and error rates, and therefore provide excellent alternatives to manual DNA purification methods.

Product information

Product name	Product No.	Quantity
MagDEA Dx SV Reagent kit	E1300	48 tests
magLEAD Consumable kit	F4430	50 tests
magLEAD 6gC Instrument	A1060	1 device
magLEAD 12gC Instrument	A1120	1 device

Reference:
(1) PharmGenomics HLA DQ2+8 real-time PCR kit handbook, version 2.1, February 2018

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