GENDX
personalising diagnostics

High Resolution HLA Typing For Companion Diagnostics Using The Next Generation DNA Sequencing Strategy: Pyrosequencing

Introduction

Recently, requests for accurate HLA typing have been significantly raised in the area of personalized health care. Evidence has been presented by several groups worldwide that some HLA alleles are associated with diseases or alternatively with adverse effects of certain drugs.

For instance, Celiac Disease is strongly associated with HLA-DQ2 and HLA-DQ8, as the combination of the underlying HLA-DQA1 / HLA-DQB1 genotypes predispose for disease development. Another example is the association of HLA-DQA1*01:02 with a higher risk for adverse reactions to lumiracoxib, a painkiller for treatment of the bone disease Osteoporosis. On the other hand, it has also been shown that certain alleles within the groups of HLA-B*27 and HLA-B*57 slow down the development and progression of AIDS in HIV positive patients, as compared to patients without this allele.

A test, specific for these kinds of HLA typing requires in most cases a presence / absence scoring and is performed by non-HLA specialized laboratories which requires a different design compared to the tests used for tissue typing.

We have optimized the Pyrosequencing technology to enable fast, cost effective and accurate analysis of HLA sequences. Here, we will present an update on the status of our Pyrosequencing assay developments.

Materials and Methods

We recently presented a new typing strategy for the HLA-DQA1 gene using Pyrosequencing. The workflow consists of one generic HLA-DQA1 amplification, followed by 4 Pyrosequencing reactions. We have verified the performance of the assay on a panel of 40 samples (Table 1), consisting of a mix of cell line (IHWG) and clinical samples (S). Samples were tested in a blinded manner by two independent operators. Data analysis was performed using in-house developed software. Typing results obtained with Pyrosequencing were compared to typing results obtained with SBTexcellerator.

Data analysis

The in-house developed software for analyses of the Pyrosequencing data is used to:
- Load the alleles and sequences from the IMGT database
- Predict the relative peak heights based on the possible (IMGT based) alleles and genotypes
- Compare the experimental peak heights to the predicted peak heights
- Judge the quality of the experimental data
- Show genotypes with similar peak heights
- Direct the user to areas that need to be verified by the user to be able to select the right genotype

Table 1: Sample overview

Frequencies were calculated based on a compilation of historical data from the University Medical Center in Utrecht (UMCU), proficiency testing results and the results of the customer service activity of GenDx. This set represents a total of more than 900 HLA-DQA1-typed samples.

P-group			01:02P	03:01P	01:01P	02:01	01:03	04:01P	06:01P
	Freq	23.93	19.67	19.51	13.27	9.25	6.46	5.59	2.27
05:01P	23.93	IHWG9275 S0695							
01:02P	19.67	IHWG9254 S0484	IHWG9259 S0231						
03:01P	19.51	IHWG9114 S0706	IHWG9257 S0707	IHWG9364 S0310					
01:01P	13.27	IHWG9136 S0286	IHWG9237	IHWG9191	S0317				
02:01	9.25	IHWG9270	S0313	IHWG9220 S0352	IHWG9109	IHWG9047			
01:03	6.46	S0270	IHWG9366 S0273	S0205	IHWG9263 S0228		IHWG9193 S0693		
04:01P	5.59			S0685	S0240		S0226	IHWG9067 S0238	
06:01P	2.27			S0073	IHWG9227		S0209		IHWG 9199

Figure 1: Snaphot of the Pyrosequencing analysis software
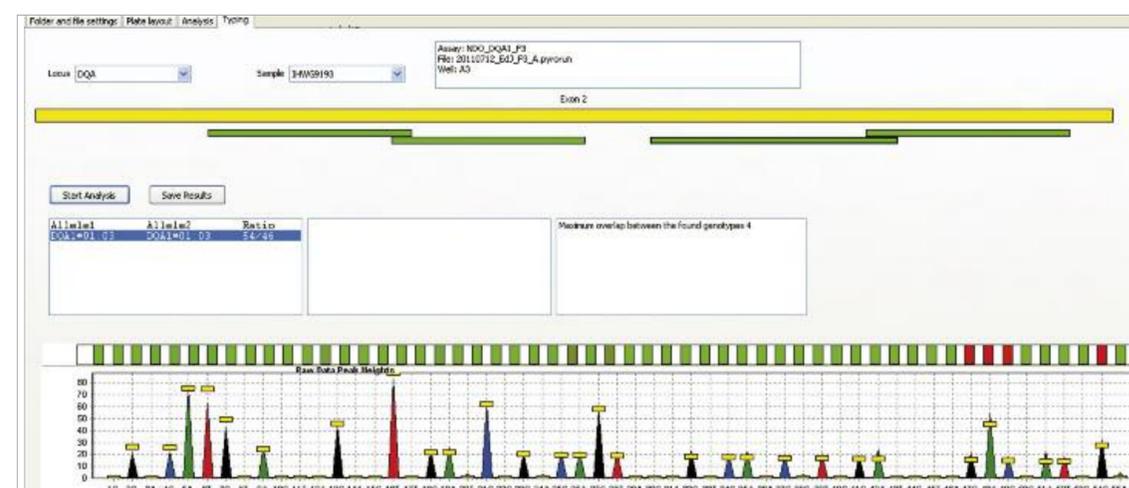
Conclusion

- By means of a generic amplification followed by a limited number of Pyrosequencing reactions the DQA1 alleles present in samples can be determined in a robust manner.
- Each assay can be performed in 4 hours.
- Pyrosequencing technology can be used to enable fast, cost effective and accurate analysis of HLA sequences.
- The in-house developed software has been developed in close collaboration with researchers and laboratory technicians. As a result, it enhances typing of unknown samples in an efficient and user-friendly manner.
- GenDx continues to develop more assays for personalized healthcare based on Pyrosequencing.

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