

A Single Test Replaces Multiple for High-Resolution HLA Allele Profiling

Dr. Carmen Aláez reflects on her experience using the TruSight® HLA Sequencing Panel for accurate HLA typing.

Introduction

With over 20 years of experience, Dr. Carmen Aláez is a key opinion leader in the evolution of human leukocyte antigen (HLA) research. Her interest in HLA research began in 1994 when she moved from Cuba to Mexico to become the Head of the Laboratory of Molecular Biology at the Institute of Epidemiological Diagnosis and Reference (InDRE) Department of Immunogenetics. Throughout her career, Dr. Aláez has earned numerous accolades, presented over 200 papers at national and international scientific meetings, and published 43 articles in international journals about HLA.

Today, Dr. Aláez is Head of the Laboratory of Translational Medicine at the National Institute of Genomic Medicine (INMEGEN) in Mexico City. She works to identify alleles within the HLA class I and class II genes for matching organ or bone marrow donors and recipients. Recently, Dr. Aláez had the opportunity to evaluate the TruSight HLA Sequencing Panel from Illumina.

Current Challenges of HLA Typing

Sequencing HLA has been notoriously difficult due to the high levels of sequence homology and dense variability found within this region of the genome. Current attempts at deciphering this region require multiple assays and produce ambiguous results. Tedious strategies, often involving design and use of multiple sequencing primers and methods such as PCR-SSP (sequencing-specific primed PCR), increase the time, effort, cost, and expertise required to achieve confident results.

One Procedure, Highly Resolved Typing

The TruSight HLA Sequencing Panel uses proven next-generation sequencing (NGS) technology to provide accurate, phase-resolved HLA typing in a single assay. To demonstrate, Dr. Aláez performed allelic typing of 11 loci (A, B, C, DRB1/3/4/5, DQB1, DPB1, DQA1, and DPA1) for 24 DNA samples from Mexican mestizo individuals. DNA was extracted using 3 different methods (Maxwell, QIAGEN, and phenol chloroform). Following the protocol provided by Illumina, she obtained a typing result for every sample except 1, in which a novel DQB1 allele was discovered. Of the 24 samples analyzed, 12 were previously typed for HLA-A, B, C, DRB1, and DQB1 using Sanger sequencing. Typing results obtained with the



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TruSight HLA Panel were concordant with these results. The DNA extraction method did not appear to influence the ability to obtain typing results, as long as the DNA sample dilution is prepared as recommended by the protocol.

“The ability to perform allelic typing of all HLA genes necessary to establish compatibility between 2 individuals using only 1 experimental procedure represents an undeniable advantage, particularly for hematopoietic stem cell transplant programs,” states Aláez. “Using Sanger sequencing requires combining the results of several technologies and making extensive use of additional sequencing primers to establish the phase of polymorphisms. These additional steps represent hidden costs not usually considered in an initial price comparison.”

For research laboratories, Dr. Aláez notes that “the high-resolution typing required for publishing in scientific journals is obtainable directly from a single [TruSight HLA] assay, avoiding having to use too much DNA on multiple samples.”

Dr. Aláez is new to NGS. Using the MiSeq® System and following the TruSight HLA protocol, she observed no allelic loss during the amplification steps, experiencing “simple library preparation and sequencing.”

Automated Analysis

The TruSight HLA Panel uses the widely known Conexio Assign software for HLA analysis. This software provides automatic analysis of sequences and alleles, eliminating the need for a bioinformatician. Integrated tools facilitate analysis and avoid the time-consuming task of consulting multiple website to make a classification call. “Conexio Assign is recognized for its quality in the HLA community, which will undoubtedly facilitate a transition to NGS for users already using Sanger sequencing,” states Dr. Aláez.

A New Path Forward

Dr. Aláez is a leader in HLA research. Her work has provided significant insight into how the HLA system functions in various autoimmune diseases and responses, pushing the boundaries of HLA studies. After over 20 years in the field, Dr. Carmen Aláez has had experience with most of the HLA typing methods available. She summarizes her experience using the TruSight HLA Sequencing Panel with the following, “I believe that TruSight HLA is a robust product that permits the profiling of HLA alleles at a good level of resolution and represents a considerable advantage over the high-resolution HLA typing methods that currently exist on the market. Without a doubt, it is a method that I will implement and maintain in my lab.”

Learn more about the TruSight HLA Sequencing Panel at www.illumina.com/hla

Summary

Overview

Dr. Carmen Aláez is trying to identify alleles within the HLA class I and class II genes for matching organ or bone marrow donors and recipients.

Challenge

The HLA is a notoriously difficult region to study, requiring multiple tedious, time-consuming, and costly assays to obtain decent data.

Solution

Dr. Aláez evaluated the TruSight HLA Sequencing Panel for its ability to produce phase-resolved HLA typing.

Benefits

Using the TruSight HLA Panel, Dr. Aláez achieved unambiguous, accurate HLA typing of 24 samples simultaneously.

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