Multiplexed Reagents and Novel Software for qPCR-Based Microchimerism Analysis

Abstract

In an effort to overcome the sensitivity and analysis shortcomings of STR-based transplant monitoring methods, and to improve upon the current state of the art in qPCR-based chimerism analysis and laboratory workflow, we have created a new assay and software suite.

We utilize a panel of 42 multiplexed qPCR research assays and software to comparatively genotype multiple samples on a single plate. The software presents the unique marker choices in their genomic context, allowing for knowledgeable informative assay decision making and use in post-transplant monitoring. The software enables numbers of combinations of samples to be comparatively genotyped, facilitating rapid multiple donor analyses. The results from the genotyping are stored by the software for recall during subsequent monitoring. Our software facilitates post-transplant monitoring by: allowing operators to customize their plate configuration or work with lab-defined template, performing all calculations necessary to execute the test, generating a printable protocol to assist laboratory work and generating templates to import into the qPCR machines. The software accepts standard qPCR data output for generating results. Reports from a monitoring test may be generated from a single time point or using the longitudinal data collected from the individual over time - providing a temporal view for better understanding rejection or relapse kinetics.

This assay system provides the highest probability of finding informative markers, as compared to other commercially available qPCR systems. The tests and software are compatible with qPCR platforms from multiple vendors. Our software solution reduces manual calculations, increases flexibility in experimental execution, generates complete protocols for use in the lab and stores/plots data.

Materials and Methods

Following the approaches described in the literature for qPCR-based post-transplant monitoring, we are building a suite of products which are poised to become best in class solutions.

We obtained DNA samples from the European Collection of Cell Cultures and the Coriell Institute for Medical Research. All samples were quantified via nanodrop.

Assay target loci were selected from public databases and on-line design tools and reference databases supported the design of oligonucleotide primers and probes against them. Primers and probes were purchased from DNA Technology (Aarhus, Denmark).

Assay systems were characterized via endpoint PCR to confirm the molecular weight of intended amplicons, the specificity of the assay and the allelic frequency of variants against European populations. When assays which met their intended specifications, probes were ordered and the systems further characterized using qPCR and the Qiagen RQG or LifeTechnologies ViiA7.

Software was developed using Visual Studio 2012 and Windows Presentation Foundation.

Workflow

The assay first involves genotyping donors and recipients to identify informative markers. This is followed by monitoring patients using assays which were positive for the recipient and negative for the donor.

For monitoring, the KMRengine software intuitively guides the operator in test set up. The software provides flexibility in plate configuration – providing the ability to leverage lab-developed templates, or allowing operators to customize the location of samples and the number of replicates for any assay within the group being used for a sample. KMRengine performs all calculations necessary to provide a printable protocol for lab execution.

Once the qPCR run has completed, the operator exports the results which are then imported into KMRengine for analysis. KMRengine analyzes the data collected during PCR and provides data and results reports. KMRengine stores the time point data and may provide a sample’s longitudinal testing history in a report.

References

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Conclusions

This new assay system provides the most automated and platform independent qPCR-based transplant monitoring research tool available to date.