ICE*Plex*®, an automated, high multiplex molecular analysis platform

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Abstract

Quantitative PCR using probe-based real-time detection is restricted to few targets within a single reaction. The ICE*Plex* system was developed to offer an automated, high multiplexing solution. ICE*Plex* combines PCR thermal cycling with dynamic, sequential amplicon separation and multi-color quantitative detection by capillary electrophoresis (CE) in a single integrated system. Unlike probe-based qPCR, ICE*Plex* directly measures amplicon accumulation through incorporation of labeled primers.

Here we demonstrate that the ICE*Plex* system is capable of microRNA and mRNA expression analysis from frozen and FFPE samples, as well as detecting methylation and SNP status from gDNA. Using an 10-plex reaction from the Onco*type* DX® panel we show that the ICE*Plex* instrument can provide expression profiles for all mRNAs tested whether from flash frozen or FFPE tissue. In addition, we show that the ICE*Plex* instrument can provide expression profiles of the highly conserved miRNAs let-7a and let-7b, and further that this analysis can be done simultaneously with mRNA assays, demonstrating the multi-modal potential of the system. Finally, using gDNA as template, we illustrate that the ICE*Plex* system is capable of distinguishing between methylated and unmethylated DNA.

The ICE*Plex* is an automated bench top instrument that has overcome many of the limitations of traditional real-time PCR based multiplexing. Here we have demonstrated that the system has broad capabilities and provides a solution to many previously difficult problems in molecular analysis.

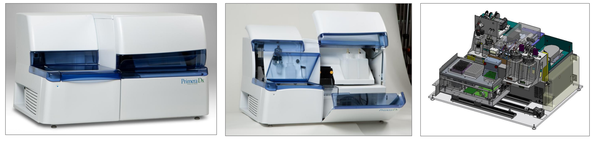
Technology Overview

The ICE*Plex* System is comprised of several vital elements (Figure 1), including a PCR thermal cycler that accommodates a standard 96-well PCR plate, and a capillary electrophoresis (CE) system with replaceable CE cartridge.

The system computer controls a three-axis robotic system with X, Y and Z motors, the fluidic pumps and valves necessary to fill capillaries with gel and to pump CE buffer and system decontamination solutions. All of these functions are preprogrammed and occur without operator intervention. The system monitors fluid levels and alerts the operator to the need to replenish consumables and empty the waste reservoir when necessary.

The CE system itself consists of a prefilled 384-well CE plate, a capillary cartridge, a gel and buffer filling system and a high voltage power supply. The system also houses two solid state lasers (488 nm and 639 nm excitation) and a spectrophotometer with CCD camera for simultaneous laser-induced fluorescence detection of two dye colors in each capillary.

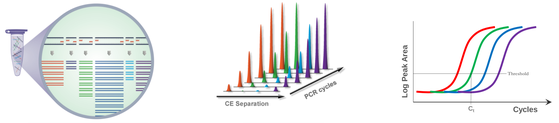
Figure 1



The ICE*Plex* System employs sequential sampling and analysis of fluorescently labeled PCR products (amplicons) by means of capillary gel electrophoresis (CE). Separation of the amplicons is facilitated by appropriate primer design allowing for accurate discrimination by size and fluorescent signal after CE separation. The coupling of PCR and CE allows for a high level of multiplexing in a single reaction tube, with quantification accuracy similar to real-time PCR methods (Figure 2).

All targets (including sample template, controls and internal calibration standards) are independently assessed within a single reaction tube. Three calibration standards are seeded in the reaction at three concentrations. These calibrators are used to perform size calibration of each of the amplifications in each individual test. Real-time sampling of the amplicons allows for the construction of amplification curves and calculation of threshold cycle (C_t) similar to other Real-time PCR methods. At specific PCR cycles, a capillary and an electrode of capillary electrophoresis module are introduced into the PCR reaction and voltage is applied for a predetermined time to force negatively charged DNA molecules to enter the capillary – a process known as electrokinetic injection. The capillary and electrode are then moved and immersed in a CE buffer, voltage applied, and capillary electrophoresis separation is performed. The PCR cycling and CE separation are timed to match one complete CE separation with two PCR cycles.

Figure 2

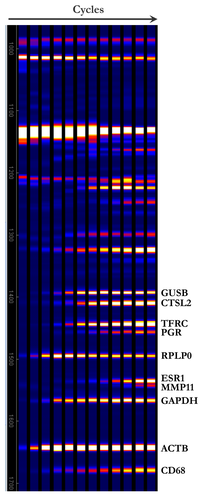


High Multiplex PCR | CE Separation | Analysis

Results

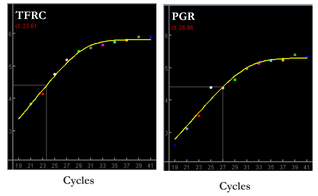
Gene Expression Analysis

Figure 3A



Breast Cancer RNA

Figure 3B

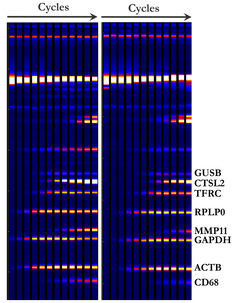


RNA isolated from Breast Cancer tissue was analyzed using the ICE*Plex* system. We chose ten individual assays from the Onco*type* Dx® panel and ran them in multiplex. The gel view in Fig-3a clearly shows the differential expression of the ten transcripts. In Fig-3b we have provided the corresponding amplification curves that were automatically generated by the ICE*Plex* software for representative transcripts. In the Data Viewer of the ICE*Plex* software C_t is computed (TFRC = 23.6 & PGR = 26.96).

These results demonstrate that the ICE*Plex* is able to multiplex ten different assays for gene expression analysis.

Gene Expression Analysis from Frozen & FFPE Tissue

Figure 4



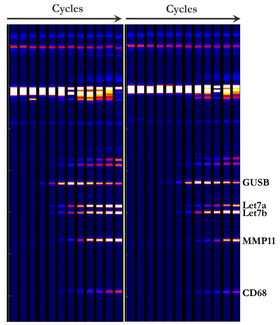
Frozen | FFPE

RNA was isolated from cell lines that were either flash frozen or formalin fixed in paraffin (FFPE), total RNA was extracted and analyzed using the ICE*Plex* system. We chose eight individual assays from the Onco*type* Dx® panel and ran them in multiplex. The gel view in Fig 4 clearly shows the differential expression of the eight transcripts, from frozen and FFPE treated cells.

These results demonstrate that the ICE*Plex* can quantitatively analyze the expression of eight transcripts in one multiplex reaction with little difference between frozen and FFPE treated cells.

MicroRNA Analysis

Figure 5



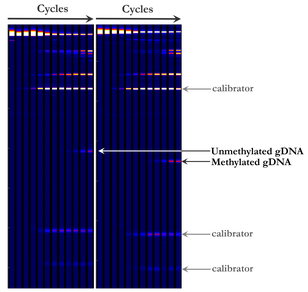
Frozen | FFPE

RNA was isolated from cell lines that were either flash frozen or formalin fixed in paraffin (FFPE), total RNA was extracted and analyzed using the ICE*Plex* system. We chose three individual gene expression assays and two microRNA assays and ran them all as a multiplex. The gel view in Fig 4 clearly shows the differential expression of the eight transcripts, from frozen and FFPE treated cells.

These results demonstrate that the ICE*Plex* can simultaneously analyze the expression of mRNAs and microRNAs in one multiplex reaction with little difference between frozen and FFPE treated cells.

Methylation-Specific qPCR

Figure 7



FFPE samples are treated with a bisulfite reagent that converts unmethylated DNA, producing methylation-state specific sequences. Converted material goes through amplification using designed PCR primers. ICE*Plex* can distinguish between the methylated and unmethylated sequences by amplicon sizes (figure 7). Methylated DNA, unmethylated DNA and calibrators are labeled.

Conclusions

1. The ICE*Plex* instrument automatically integrates PCR and CE to provide a unique multiplex qPCR platform.
2. The ICE*Plex* system can quantify 10 separate transcripts in one well (PrimeraDx has shown that 60 analytes can be multiplexed using the ICE*Plex* system).
3. Comparable gene expression analysis results from frozen cells and FFPE samples.
4. Simultaneous analysis of microRNA with mRNA targets in one tube, providing a multimodal multiplex qPCR solution.
5. Methylation-specific qPCR analysis.