

Genomic Variation Analysis: Single Nucleotide Polymorphism

Detection of Single Nucleotide Variation Frequencies by Droplet Digital PCR

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“Our laboratory is interested in the mechanism involved in reprogramming somatic cells into induced pluripotent stem cells.”

Research Background

Several recent studies demonstrate that defined transcription factors mediating reprogramming can potentially induce or enlarge mutations in the resulting induced pluripotent stem cells (iPSCs). In our study, DNAs were extracted from several iPSC lines and their matched progenitor somatic cells, then the whole genome was sequenced using a HiSeq 2000 sequencer (Illumina, Inc.). Thousands of single nucleotide variations (SNVs) that occurred in iPSC lines and some high-frequency SNVs, including all the coding ones and randomly selected ones in noncoding regions, were verified by Sequenom genotyping.

Application

We used a QX100™ Droplet Digital™ PCR (ddPCR™) system to test whether some observed mutations pre-existed in the starting somatic cells and to further validate the high frequency of SNVs. However, we first performed our experiment using an Applied Biosystems 7500 fast real-time PCR system. We did not trust the results because of the known limited resolution of this instrument. Fortunately, with Bio-Rad's guidance, we applied genotyping using a QX100 ddPCR system, which allowed us not only to validate the high frequencies of SNVs in iPSC lines, but also to observe the low frequencies of the SNVs in matched progenitor somatic cell lines.

ddPCR Results

Using the QX100 ddPCR system, we further confirmed the high frequency of SNVs in iPSC lines validated by whole genome sequencing and Sequenom genotyping (Figure 1). Moreover, low-frequency SNVs in somatic cells were detected and the cell frequencies were estimated correctly (Figure 2).

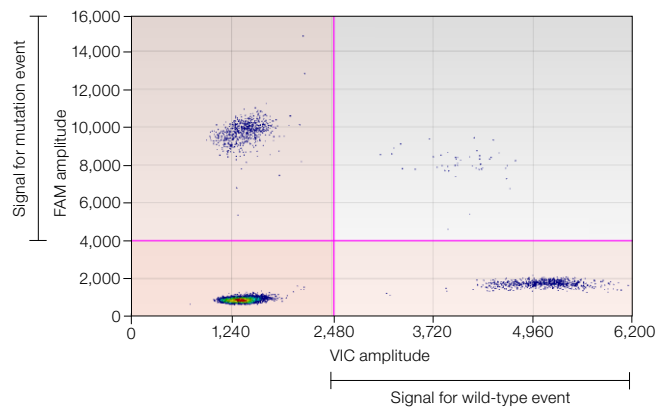


Fig. 1. Scatter plot showing high-frequency SNVs in iPSC lines.

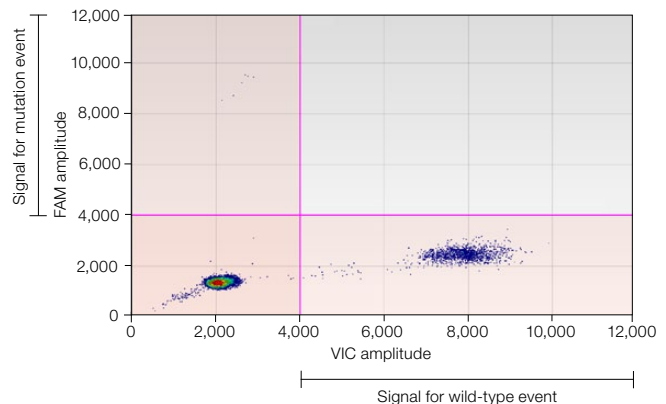


Fig. 2. Scatter plot showing low-frequency SNVs in somatic cells.

In our study, two primary SNV occurrence patterns were demonstrated by the QX100 ddPCR system. One type of SNV had a low frequency only in its corresponding matched progenitor somatic cells and occurred with high frequency in iPSC lines. Another type of SNV had no detectable frequency in parent somatic cells but could be detected in subsequent iPSC lines with high frequency. This latter type is considered a de novo SNV.

“Using the QX100 ddPCR system, we confirmed the high frequency of SNVs in iPSC lines validated by whole genome sequencing and Sequenom genotyping.”

Publications

Abyzov A et al. (2012). Somatic copy number mosaicism in human skin revealed by induced pluripotent stem cells. *Nature* 492, 438–442.

Hindson BJ et al. (2011). High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Anal Chem* 83, 8604–8610.

Pinheiro LB et al. (2012). Evaluation of a droplet digital polymerase chain reaction format for DNA copy number quantification. *Anal Chem* 84, 1003–1011.

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