Structural Variation Analysis Using Nanochannel Genome Mapping to Evaluate Genome Integrity after Induction of Pluripotency of Human Fibroblasts

Abstract

Four key genes, POLUI1, SOX2, KLF4, and MYC, are commonly used for reprogramming human fibroblasts into stem cells. Induced pluripotency of human fibroblasts into stem cells currently relies on three different transfection methods of these key genes: retroviral, Sendai virus, and mRNA. The latter two methods are non-integrating methods, while the former method integrates into the genome. Retroviral transfection to induce pluripotency is currently the most efficient method, but due to the potential damaging effects of viral integration into the chromosomes, it is thought to be of high risk for future clinical applications.

To investigate whether structural changes result during the process of induction of pluripotency in stem cells we used nanochannel genome mapping technology from BioNano Genomics (BNG) to compare structural differences among cell lines derived by the three reprogramming methods and the parental genome. By producing de novo genome map assemblies of each genome, comparing to a reference genome, and cross comparing all four assemblies, we determined sample-specific SV calls for each of the three methods. After automated assembly and comparison, followed by manual verification, 1 sample-specific SV was found in the mRNA induced sample, with respect to the Hg19 human reference genome. Examination of this structural variation found a potentially malignant X-linked deletion.

Methods

1) Long molecules of DNA is labeled with IrysPrep™ reagents by 1) incorporation of fluorophore labeled nucleotides at a specific sequence motif throughout the genome. 2) The labeled genomic DNA is then linearized in the IrysChip™ nanochannels and single molecules are imaged by Irys. 3) Single molecule data are collected and detected automatically. 4) Molecules are labeled with a unique signature pattern that is uniquely identifiable and useful in assembly into genome maps. 5) Maps may be used in a variety of downstream analysis using IrysView™ software.

Conclusions

The BioNano Genomics Irys platform was used to visualize long (>150 kb) DNA molecules for direct characterization of complex structural events in the genomes of three pluripotency-induced stem cell lines and their parental control. Approximately 50x coverage of each cell line was collected on the Irys instrument and analyzed with the standard Irys de novo assembly and structural variation analysis workflow. And expanded workflow was developed to identify cell line-specific structural variations. From this analysis, one high-confidence deletion was determined to be specific to sample 3 from the list of insertions and deletions detected by BNG Irys platform, a ~228kb deletion in the GRch37 chromosome X.

Structural Variation Confirmed by Visualization

The sample-specific call was further confirmed by looking at the single-molecule pileup that is output from the Irys platform. There are long molecules supporting the variant allele on genome map. It is important to note that this is a heterozygous variant, as there are molecules supporting the GRch37 allele.

References