Fast and accurate SNVs and CNVs screening in Parkinson's Disease patients using a Next-Generation Sequencing approach.

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Background

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting millions of people. Genome-wide association studies (GWAS) have found >25 genetic risk factors and at least 15 loci directly associated with PD. Recent advances in Next-Generation DNA Sequencing technologies, such as the semiconductor-based Ion Torrent platform, making multigene sequencing cheaper, faster, and more reliable.

Objectives

Our objective was to test the power of this Next-Generation Sequencing technology to analyze large samples of PD patients by screening the majority of the most relevant PD-related genes known for single and compound mutations.

Methods

To achive a rapid,robust, and cost-effective genetic analysis of a PD cohort, we designed a multiplex, polymerase chain reaction (PCR)-based primer panel to amplify and sequence coding exons of 42 PD-associated genes (Fig.1). We conducted parallel sequencing using the Ion Torrent Personal Genome Machine (PGM) to detect mutations in 42 blood DNA samples of PD patients from Southern-Italy. Torrent Suite (TS) version 5.10 was used to process data from PGM Ion Torrent runs for alignment and variant calling. The annotation was made using Annovar and the variants were priorized using a standard filtering pipeline.

Results

After bioinformatics analysis and filtering, 98% coverage of the targeted regions was obtained with at least >200-fold mean depth (Fig.2). We detected 74 coding nonsynonymous variants (indels, single-nucleotide variations-SNVs and frameshift variants) whose reported allelic frequency was very low or not even reported. Of these 74 variants, 10 were identified in *PARK2*, 13 in *LRRK2* and 1 in *PINK1*. The remaining variants were found in other sequenced genes involved in the pathogenesis of PD or associated with increased disease risk (*ATXN2, ATP13A2, COMT, CSF1R, DNAJC13, EIF4G1, FBXO7, GBA, GCH1, GIGYF2, PLA2G6, POLG, PRKRA, SNCAIP, SYNJ1*). The results revealed 51 previously documented variants, with 15 imputated as pathogenic. We also discovered 23 novel SNPs, 20 of which have an in silico prediction of being pathogenic. A total of 154 copy number variations-CNVs (52 amplifications and 102 deletions) were revealed. Among these CNVs, we detected 1 amplification in *SNCA*, 1 deletion in *PARK2*, 1 deletion in *PARK7*, 1 amplification and 7 deletions in *LRRK2*. Other sequenced genes presented genomic rearrangements that can have a pathogenic or susceptibility impact on PD pathobiology.

Conclusions

Next-Generation Sequencing is a powerful method for genetic screening of PD. Our results indicated that it yielded a high frequency of discovery of SNVs e CNVs variants in carriers from an enriched PD sample.

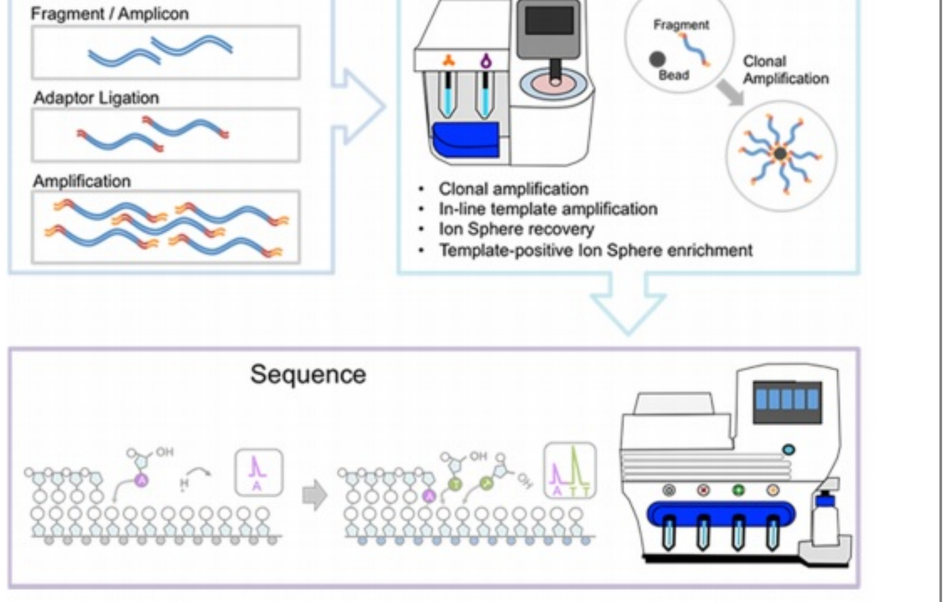

Fig.1: Ion torrent workflow

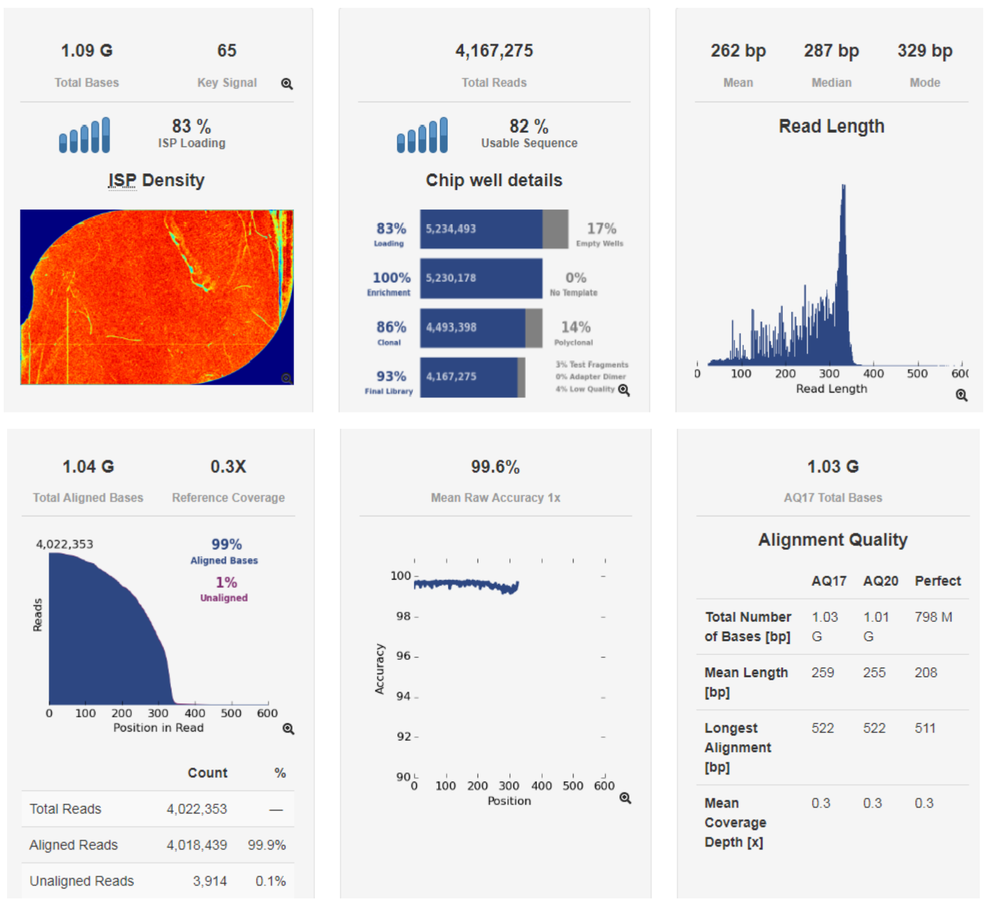

Fig.2: Sequencing quality metrics

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