

Ion Torrent

Personal Genome Machine™ Sequencer to QC Libraries and Validate Results for Next Generation Sequencing

Introduction

Each run of a second generation sequencing system involves significant investment of time and money. It is a frustrating experience for sequencing users to wait for weeks and spend a considerable amount of money, just to realize that their libraries are not of good quality. Moreover, second generation sequencing systems generate large numbers of variant calls from whole genome or whole exome runs. Validation with a fast, high throughput system that uses an orthogonal technology (independent chemistry) is key to ensure the scientific accuracy of the project.

Speed and a unique, simple chemistry makes the Ion Torrent Personal Genome Machine (PGM™) Sequencer the only available platform in the market that is capable of performing both rapid quality control of sequencing libraries, and fast, accurate and high throughput variant validation (Figure 1).

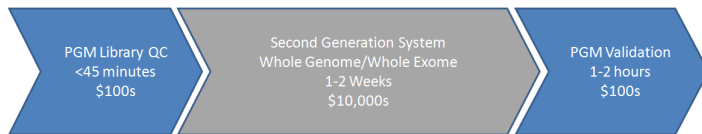


Figure 1. The Ion Torrent Personal Genome Machine™ is ideal for validation.

Qualification of library composition

Researchers at the Broad Institute have developed a procedure to quickly identify whether a pooled Illumina® library has even representation of the different barcoded individual samples. Fusion primers were designed to amplify the barcode region of the Illumina library fragments. These fusion primers contain sequences of Illumina adapters tailed with the Ion Torrent sequencing adapters sequences (Figure 2). Using this approach, they generated an Ion

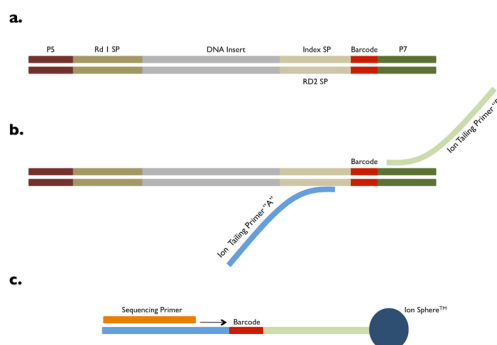


Figure 1. (a) General structure of a barcoded and enriched Illumina library. (b) Fusion primer tiling scheme used to amplify barcodes from a library pool. Primers are complementary to conserved regions of the Illumina library adapters, with tails that are complementary to the Ion library adapters. (c) Sequencing scheme for amplified Ion Torrent library.

Figure 2. Fusion Primers.

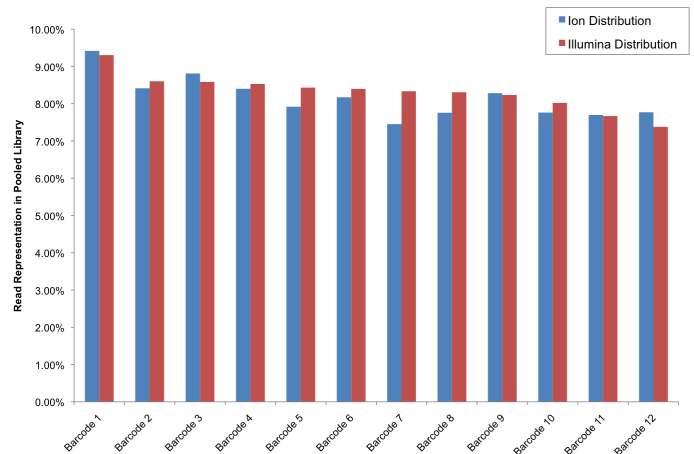


Figure 3. Ion Torrent representation Vs. Illumina representation

Torrent library with representation of all the barcodes present in the Illumina® library. The relative number of reads obtained for the different barcodes with the Ion PGM™ Sequencer matches the relative representation obtained after the same library is sequenced on the Illumina system (Figure 3).

The PGM™ Sequencer could also be used to test the complexity of other types of libraries such as whole exome or RNA libraries, before sequencing them on complex and relatively slow second generation sequencing systems.

The Ion Xpress™ workflow allows preparation of up to 8 samples in less than 6 hours. The Ion OneTouch™ automated workflow allows preparation of templates in less than 3 hours with just minutes of hands on time. Sequencing runs take under 45 minutes and the data is available in 40 minutes.

The Ion Torrent PGM™ Sequencer offers a fast and simple sequencing workflow, ideal to assess the quality of sequencing libraries prior to a lengthy, full scale run on other high throughput sequencers, such as Illumina® or SOLiD™ Systems. In less than 45 minutes, the PGM™ Sequencer starts generating data that can be used to make decisions about the quality of the sequencing library.

Validation of second generation system sequencing results

Second generation sequencing systems generate large numbers of variant calls from whole genome or whole exome sequencing runs. It is imperative for researchers to discriminate between true biological variants and errors made by the sequencing systems. Light-based sequencing technologies have some degree of systematic bias. The chemistry and the primary data analysis used by the sequencing systems are typically major contributors to this bias. Validation of newly discovered variants is essential to uncover

Select References

Technology-specific Error Signatures in the 1000 Genomes Project Data, Michael Nothnagel, Alexander Herrmann, Jochen Hampe, et. al., *Human genetics* DOI: 10.1007/s00439-011-0971-3.

Genome Remodeling in a Basal-like Breast Cancer Metastasis and Xenograft, Li Ding, Matthew J. Ellis, Elaine Mardis et. al., *Nature*. 2010 April 15; 464(7291): 999–1005.

Global Analysis of Disease-related DNA Sequence Variation in 10 Healthy Individuals: Implications for Whole Genome-based Clinical Diagnostics, Barry Moore, Hao Hu, Mark Yandell, et. al., *Genetics in Medicine*: March 2011; 13 (3): 210-217.

meaningful biological results.

The research community and scientific journals demand the use of best practices to validate next generation sequencing results. Validation MUST be done with a DIFFERENT TECHNOLOGY (independent chemistry) than the technology used to generate the original results. Several landmark publications of second generation sequencing data illustrate the practice of using different technologies to generate and validate results

Some next generation system providers have smaller versions of their flag-ship systems. These systems share the same chemistry and primary data analysis procedures as their larger counterparts,

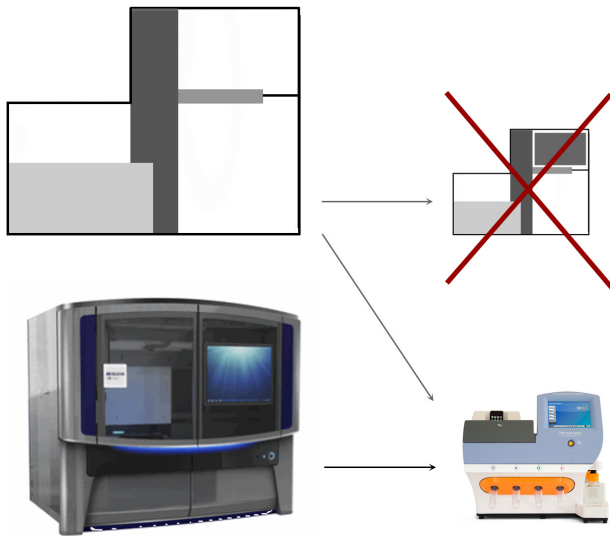


Figure 4. Next generation systems

making them unacceptable to cross-validate the results (Figure 4).

The Ion Torrent PGM™ Sequencer enables researchers to obtain highly accurate sequence (> 99.99% consensus accuracy and

>99.5% raw accuracy) in record time using a simple and unique chemistry. Unlike other technologies, Ion Torrent does not use modified nucleotides, enzyme cascades or light-based detection methods that lead to much greater bias. The PGM™ Sequencer has the fastest sequencing workflow making it ideal for validating your results by shortening the hypothesis to results and publications cycle.

There are a few easy steps needed to validate whole genome, whole exome or targeted resequencing results (Figure 5). After the amplicon library is prepared (detailed instruction on the Amplicon Sequencing Application Note), the library undergoes the template preparation procedure. The Ion Xpress™ workflow allows preparation of up to 8 samples in less than 6 hours. The Ion OneTouch™ automated workflow allows preparing templates in less than 3 hours with just minutes of hands on time. The sequencing runs will take from under 45 minutes to about 2 hours depending on the desired read length. Based on throughput requirements, researchers can choose between different Ion Semiconductor Sequencing chips and produce from 10Mb to more than 1Gb of sequence.

The fast sequencing workflow, flexible throughput and unique simplicity of its sequencing chemistry makes the PGM™ Sequencer the

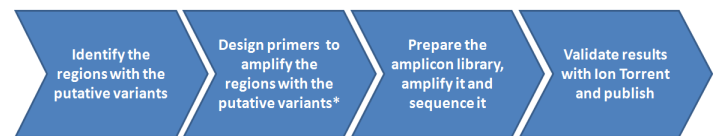


Figure 5. Validation workflow.

* Please refer to the Amplicon Sequencing Application Note

ideal system for validating results obtained with other next generation sequencing technologies.

Making sure that the quality of the sequencing libraries is optimal and cross-validating the results obtained from whole exome and whole genome sequencing runs are essential steps to maintain an efficient sequencing pipeline that generates accurate results. A single system that can achieve these important goals before and after a large sequencing run must be fast, simple and use a different chemistry than the second generation sequencing system uses for the primary run. The PGM™ Sequencer is the optimal companion for any second generation sequencing system and the perfect tool to increase productivity by saving both time and money for high throughput sequencing users.

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