ion torrent
by life technologies™

Ion AmpliSeq™ Designer provides full flexibility to sequence genes of your choice

Simplicity

- Create custom panels targeting only your genes of interest
- Just 10 ng of input DNA—enables routine sequencing of FFPE samples
- Simple PCR-based target selection

Scalability

- 24 to 3,072 amplicons per pool—perfect for targeting a few genes to hundreds of genes
- Multiplex up to 96 samples per run
- Three Ion Chips producing up to 1 Gb of total sequence

Speed

- Automated primer design in as little as 2.5 hours with minutes of hands-on time
- Targeted library constructed in 3.5 hours
- DNA to variants in a single day with a 2-hour sequencing run

Introduction

Coupled with simple, and scalable Ion semiconductor sequencing, Ion AmpliSeq™ Technology transforms genetic research by delivering ultrahigh-multiplex PCR and a low DNA input requirement. With the ability to multiplex thousands of PCR primer pairs in one tube, researchers are no longer limited to sequencing just a few genes, but can quickly and easily analyze hundreds of gene targets. The breakthrough requirement of only 10 ng of input DNA per reaction enables researchers to analyze highly challenging DNA samples, like those derived from FFPE tissues, previously not amenable to sequencing due to limited amounts of available DNA. The Ion AmpliSeq™ Cancer Panel is the first commercial panel developed using Ion AmpliSeq™ Technology, targeting 46 informative oncogenes by multiplexing 190 PCR primer pairs in a single tube. With this panel and only 10 ng of input DNA, oncology researchers can, for the first time, go from tumor research samples to variants in a single day.

To further broaden the utility of this critical solution, we have introduced Ion AmpliSeq™ Designer, an online tool that allows researchers to create and order Ion AmpliSeq™ Custom Panels, designed for just their genes of interest. Leveraging more than a decade of expertise in custom primer design, Ion AmpliSeq™ Designer produces optimized primer designs in just hours. With only 10 ng of input DNA required per pool, researchers now have full flexibility to analyze up to hundreds of genes of their choice, such as those implicated in a particular disease state or representing specific biochemical pathways.

Simple and integrated end-to-end Ion AmpliSeq™ custom workflow

The Ion AmpliSeq™ custom workflow integrates a series of easy-to-use solutions, making targeted sequencing of hundreds of genes simple to implement in any lab. The complete process is depicted in Figure 1.

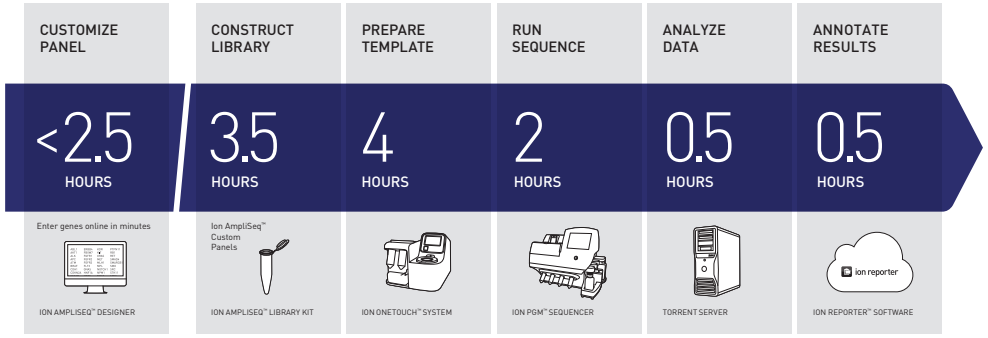

Figure 1. Ion AmpliSeq™ custom workflow using an Ion 314™ Chip and a 2-hour, 1 x 200 base sequencing run and includes the option to annotate results with Ion Reporter™ Software. Complete automation options allow the three final steps—run sequence, analyze data and annotate results—to be completed hands-free and initiated at the end of the work day.

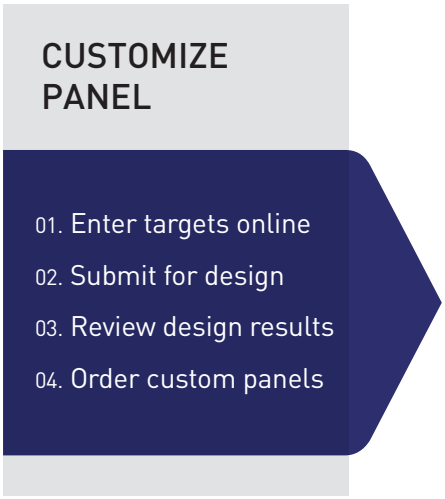

Figure 2A. Ion AmpliSeq™ custom workflow starts with custom panel creation using Ion AmpliSeq™ Designer.

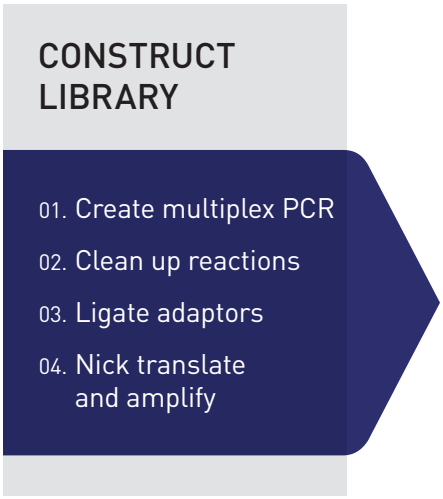

Figure 2B. Ion AmpliSeq™ libraries are constructed using multiplex PCR and standard molecular biology steps.

The Ion AmpliSeq™ custom workflow starts with panel creation and ordering using Ion AmpliSeq™ Designer. Once the custom panels are delivered, it takes just a single day to go from DNA samples to variants with a 2-hour, 1 x 200 base sequencing run.

Customize panels using the Ion AmpliSeq™ Designer online tool

Ion AmpliSeq™ Custom Panels are simple to create using Ion AmpliSeq™ Designer—our free, Web-based assay design tool that transforms your list of genes into custom primer pools to construct targeted libraries. Custom assay designs are typically one to two pools, each containing 24 to 3,072 primer pairs.

Join the Ion Community to gain free access to Ion AmpliSeq™ Designer (ampliseq.com) and begin the four-step process outlined in Figure 2A. The first step takes just a few minutes; simply enter gene symbols based on HUGO gene nomenclature, or genomic regions using coordinates based on the human reference genome hg19. In the second step, optimize for short (ideal for FFPE samples) or standard amplicons and submit targets for assay design. The third step begins with Ion AmpliSeq™ Designer notifying users when results are available for review, and is completed when you review and accept the design results (Figure 3). Typically, results for assay designs targeting 10 kb are available within 2.5 hours, while results for larger designs targeting up to 250 kb are available within 48 hours. The final step is to place an order for the Ion AmpliSeq™ Custom Panel, which is typically synthesized and shipped within 4 weeks after order confirmation.

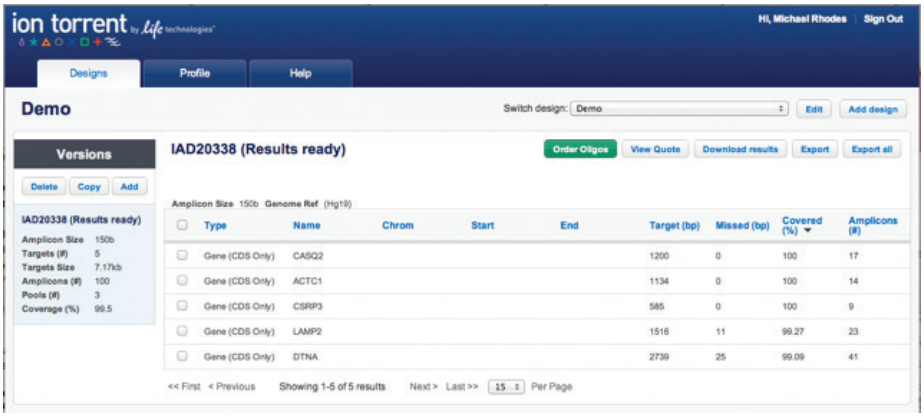

Figure 3. Ion AmpliSeq™ Designer presents results that are ready for review.

Custom panels delivered in flexible formats

Each custom primer pool is delivered as both a pre-pooled tube and as individual primer pairs plated into 384-well plates. Small orders of up to 96 amplicons per pool will contain 750 pre-pooled reactions and individual primer pairs sufficient for 1,500 reactions. Larger orders of more than 96 amplicons per pool will contain 3,000 pre-pooled reactions and individual primer pairs sufficient for 6,000 reactions.

Simple 3.5-hour library construction

Once the Ion AmpliSeq™ Custom Panel is delivered, the library construction can begin (Figure 2B). The simple workflow starts with PCR amplification of the target regions by combining DNA, an Ion AmpliSeq™ primer pool, and Ion AmpliSeq™ master mix. In the second step, the reaction is cleaned up by removing the target-specific primers. The third step is to ligate the Ion-specific primers adaptors and optional barcode adaptors for multiplexing DNA samples into a single run. The final step in library construction is a PCR amplification round, creating products that are ready for template preparation.

Because the Ion AmpliSeq™ Technology library construction uses simple PCR amplification, no special equipment or complicated procedures are involved. Multiplexing of up to 3,072 amplicons in each tube, combined with the requirement of only 10 ng of DNA per pool, enables routine sequencing of FFPE and FNB (fine needle biopsy) samples.

Ion AmpliSeq™ Designer specifications and performance

Ion AmpliSeq™ Designer is optimized to deliver primer pools with a high target design rate, producing sequence data with high coverage uniformity and resulting in a high percentage of bases that map back to the target sequence. Performance specifications for typical Ion AmpliSeq™ Custom Panels created using Ion AmpliSeq™ Designer are shown in Table 1.

Target design rate is the percentage of bases covered by the insert regions of amplicons out of the total number of target bases submitted for design. This number can be impacted by a wide range of factors such as nucleotide composition (% GC), sequence complexity, location of known SNPs, and uniqueness of regions. Ion

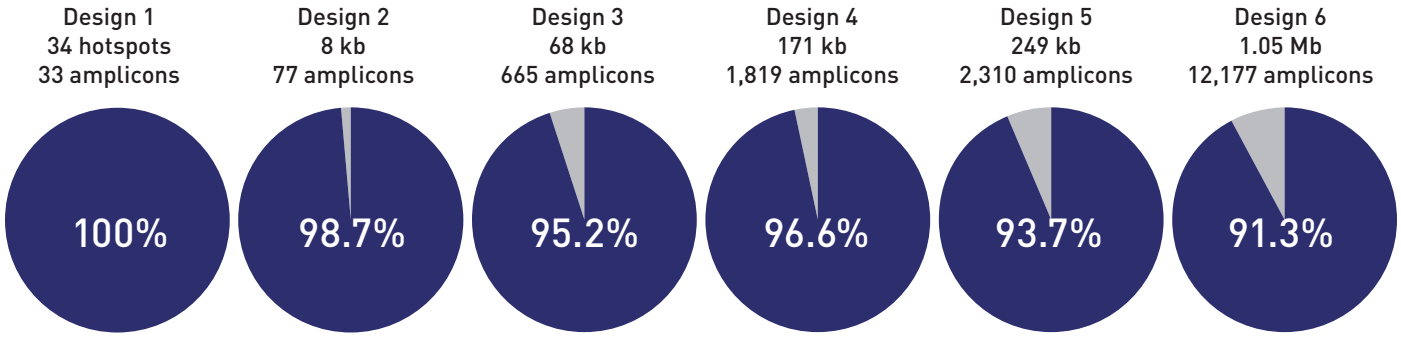

Figure 4. Target design rates for 6 custom assay designs ranging in target sizes from 1 kb to 1 Mb.

AmpliSeq™ Designer reports this metric so researchers can evaluate the success of the design prior to ordering the custom primer pools. Results from 32 assay designs indicate that the *target design rate* is routinely greater than 94%; a subset of six designs for target sizes spanning 1 kb to 1 Mb is presented (Figure 4).

Coverage uniformity is the percentage of bases from amplicons that were designed, synthesized, and pooled that also generated sequence data which achieved a depth of >20% of the mean sequence coverage. Six unique DNA samples enriched with four different Ion AmpliSeq™ Custom Panels produced *coverage uniformity* of greater than 90% (Figure 6). Samples 1–3 were amplified with the same custom panel, and samples 4–6 were selected—each with a unique custom panel. Reproducibility was measured across the same pool of 190 amplicons between two samples (DNA2 and DNA3) and produced an R^2 value of 0.989.

On-target bases is the percentage of bases from the amplicons that were designed, synthesized, and pooled that also generated sequence data that mapped back to the target regions. For the same six samples presented in Figure 5, *on-target bases* were measured at 93% or greater.

Results presented in Figures 4, 5, and 6 are based on experiments conducted by Life Technologies' scientists on DNA samples selected with Ion AmpliSeq™ Custom Panels designed using Ion AmpliSeq™ Designer. All samples were processed using released protocols.

Integration with TaqMan® Assays enables easy follow-on studies

Once variants are discovered with Ion AmpliSeq™ Panels, follow-on experiments to validate and test the variants with a wider population can easily be done using TaqMan® Assays. Sequences generated on Ion semiconductor sequencing systems are analyzed by Torrent Suite Software, which is integrated with the search portal for TaqMan® Assays. The Torrent Suite Software will display all variants detected. In cases where a detected variant is represented by a corresponding predesigned TaqMan® Assay, the software provides a link to the assay for your convenience. With 4.5 million predesigned TaqMan® SNP Genotyping Assays (for germline mutation) and TaqMan® Mutation Detection Assays, powered by castPCR™ technology (for somatic mutation)

Table 1. Ion AmpliSeq™ Designer specifications.

Specification*	Value
DNA input amount per primer pool	10 ng
Primer pairs per pool	24 to 3,072
Target size	1 kb to 1 Mb
Target design rate	>85%
Coverage uniformity	>85%
On-target bases	>80%

* Performance values will vary with each custom design.
Target design rate—bases covered by insert region of amplicons out of total target bases submitted.
Coverage uniformity—bases covered at >20% of the mean coverage.
On-target bases— bases mapped to target regions out of total mapped bases per run.

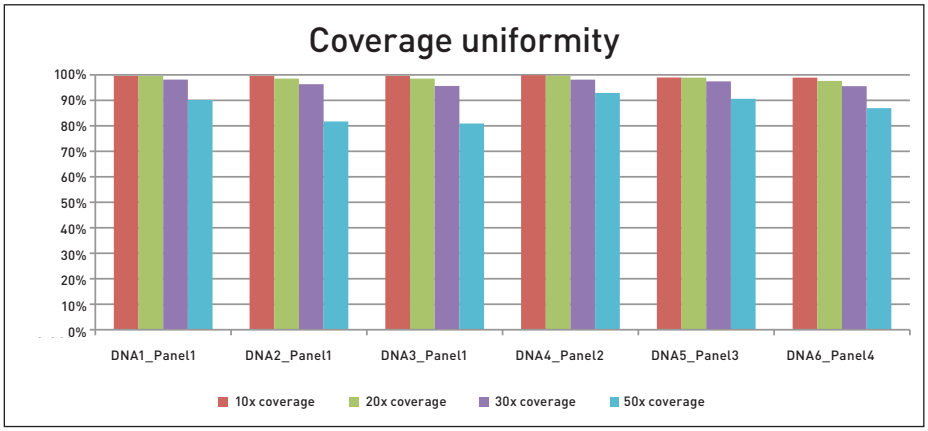

Figure 5. Coverage uniformity rates, normalized to 100x across 6 DNA samples selected using four Ion AmpliSeq™ Custom Panels. The first three samples were selected with the same custom panel; the last three were each selected using a unique custom panel. Reproducibility measured between DNA2 and DNA3 produced an R^2 value of 0.989.

to choose from, further validation of variants is simple. (Figure 7). Custom TaqMan® SNP Genotyping Assays associated with digital PCR represent an alternative solution for somatic mutation detection.

The transformative power of Ion AmpliSeq™ Technology

The introduction of the Ion PGM™ Sequencer democratized sequencing—and this fast, simple, scalable, and affordable platform is now the fastest-selling sequencer on the market. An expansion of this platform, the Ion AmpliSeq™ Cancer Panel, Comprehensive Cancer Panel, and Inherited Disease Panel, transforms oncology and disease research with extensive gene panels.

The utility of this breakthrough solution is further elevated with the introduction of Ion AmpliSeq™ Designer for creating and ordering Ion AmpliSeq™ Custom Panels. Performance specifications of Ion AmpliSeq™ Designer include *target design rate* of >85%, *coverage uniformity* of >85%, and *on-target bases* of >80%. These three performance metrics were tracked during development of Ion AmpliSeq™ Designer and Ion AmpliSeq™ Custom Panels, and results greatly exceeded specification values.

Get started today. Transform your genetic research with Ion AmpliSeq™ Custom Panels.

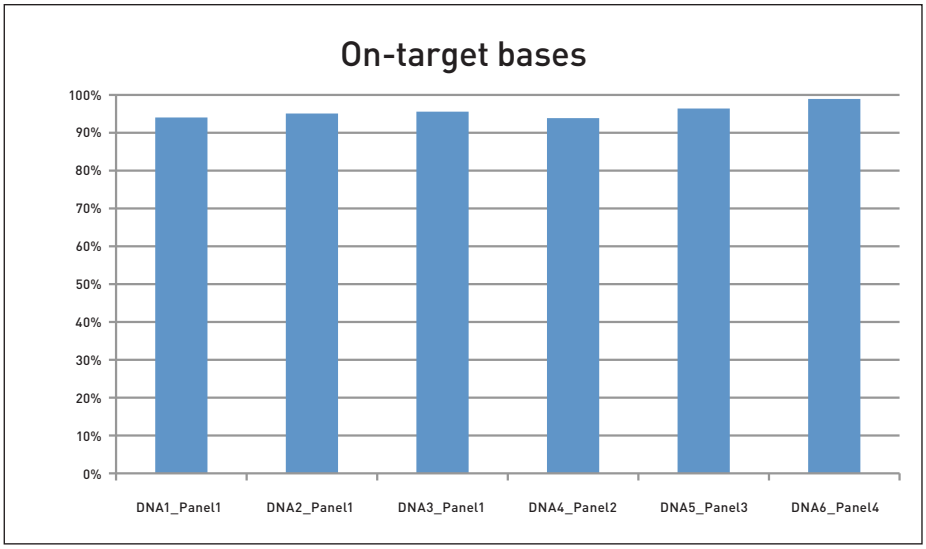

Figure 6. On-target bases for the same six samples depicted in Figure 5.

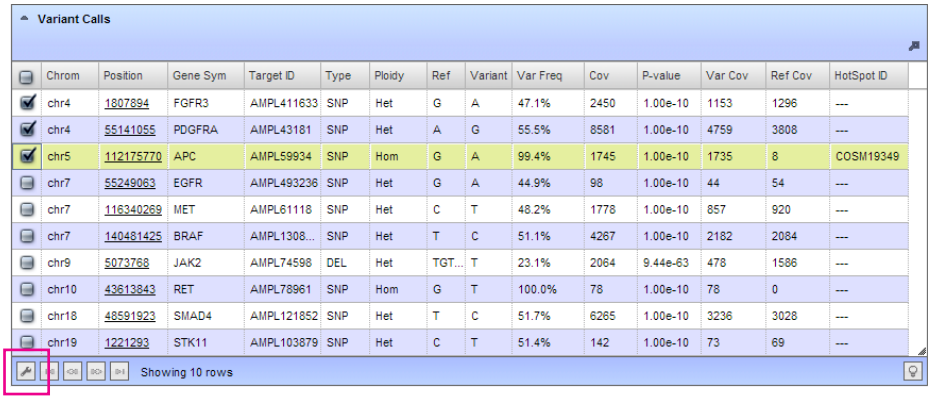

Figure 7: Torrent Variant Caller Plugin, a component of Torrent Suite Software links the variants identified to the corresponding TaqMan® Assays in a simple click using the highlighted icon.

Ordering information

Ion AmpliSeq™ Custom Panels are ordered via the Ion AmpliSeq™ Designer (www.ampliseq.com).
Custom panels are delivered in two formats—tubes of pre-pooled multiplexed primers in ready-to-use concentration and 384-well plates in which each well contains individual primer pairs in concentrated format.

Additional Ion AmpliSeq™ Products	Cat. No.
Ion AmpliSeq™ Cancer Panel v1 (primer pool)	4471262
Ion AmpliSeq™ Comprehensive Cancer Panel (primer pool)	4477685
Ion AmpliSeq™ Inherited Disease Panel (primer pool)	4477686
Ion AmpliSeq™ Library Kit 2.0 (8, 96, 384 reactions for both PCR amplification and library construction)	4475345, 4478378, 4478379
Ion Xpress™ Barcode Adapter Kits	4474517, 4471250, 4474009, 4474518, 4474519, 4474520, 4474521

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