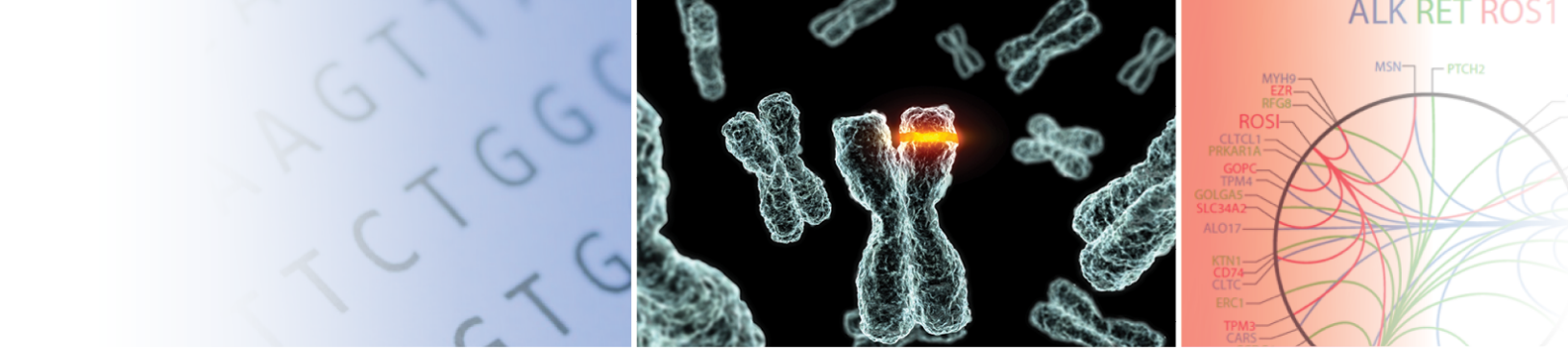


Product Note

Archer™ Assay Designer for Custom Panel Creation

PN-MKT-0011

Archer Assay Designer

The Archer Assay Designer allows users to create custom targeted sequencing panels to detect and identify known and novel fusion transcripts via next-generation sequencing. Used in conjunction with the Archer Universal RNA Reagents, and Molecular Barcode (MBC) Adapters, a custom assay generates next-generation sequencing libraries from specific transcripts of interest. Following sequencing on either Illumina® or Ion Torrent™ platforms, FASTQ files are analyzed and the results of fusion detection and identification are reported via the Archer Analysis Pipeline.

AMP™ Technology Overview

Anchored Multiplex PCR (AMP™), the enrichment chemistry used in Archer FusionPlex™ assays, amplifies regions of interest using an anchored gene-specific primer on one end of the cDNA, and a universal primer complimentary to the ligated adapter on the other end of the fragment. Unlike other PCR techniques, AMP enables enrichment of a target region with knowledge of only one of its ends. The precise design of Archer gene-specific primers prevents non-specific target amplification, thus creating libraries highly enriched for targeted transcripts. For additional information on the AMP chemistry, please visit http://enzymatics.com/archer.

After sequencing, the Archer Analysis Pipeline identifies fusion transcripts, wild-type isoforms, and splicing variants based on the identity of the sequenced fragments. Because exon-exon boundaries are used to identify fusion candidates, FusionPlex assays require the RNA from total nucleic acid input material to be of sufficient length and quality to generate accurate results.

Assay Designer Process

The Archer Assay Designer is web-based, (http://assay.enzymatics.com) and allows users to upload target lists or search for genes of interest. Target exons, with or without untranslated regions (UTRs), are defined by the user. Primers can be designed to interrogate fusion partners upstream or downstream of the target exons by indicating 5′ or 3′ directionality.

After submission, each design is reviewed by an expert to verify the following criteria:

- Gene-specific primers (GSPs) only target exons; no intronic regions are targeted

- GSP placement, coverage, and directionality is verified.
- Primers have optimal GC and Tm ranges, and no primer dimers are present
- Primers include GC clamp. A GC clamp ensures at least one G or C base will be present in the last 3 bases of the primer
- Only one GSP per fusion pair is present, preventing the possibility of generating fragments not suitable for sequencing.
    - For example, only one GSP is needed to identify BCR-ABL or EML4-ALK fusions. If GSPs have been designed to both partners in an opposing fashion (e.g., 3′ EML4 primer and 5′ ALK primer) then one of the primers would be removed from the pool.
    - The exon target should be chosen based on interest and efficiency (e.g., if ALK exon 20 is fused to exons from 10 partner genes, ALK exon 20 should be the target, rather than the exons in the 10 other partners.)

Assay Deliverables

After a design is reviewed and approved, the user may order the custom assay. The target primers are then synthesized and pooled at equimolar concentrations into two GSP cocktails, which are used during the PCR1 and PCR2 steps of the Archer Universal RNA Reagent workflow. Custom assays are not functionally tested prior to shipment, but this service is available by request (see below for more information).

Assay Performance

As an example of the range of performance seen with the custom assay designer, 337 gene-specific primers targeting translocations in solid tumor fusions were designed, synthesized, pooled at equimolar concentrations, and run on DNA templates in order to gauge relative performance of each primer.

To illustrate the variability in performance that one might observe in different custom panels, randomly chosen sets of 25, 50, 100, and 250 primers were sampled 30 times, and statistical analysis on functional data was performed. On-target reads for each GSP were assessed and >95% percent of reads mapped to the intended target. Functionality and performance of GSPs were also assessed (Table A and Figure B respectively).

Table A: Primer Functionality

Number of Designs	On-target (%)	Functionality (%)
25	97.0%	99.9%
50	96.9%	99.9%
100	97.0%	99.9%
250	96.9%	99.9%

***Table A**: Shows primer functionality normalized by sample and percentage of on-target primers. On-target is defined as the % of reads for a given GSP2 that map to the intended gene target. The number shown is the average for all the GSPs in a subset. Functionality measures the percentage of primers in the subsampled pool that were observed to produce on-target reads using DNA.*

Figure B: Primer Performance

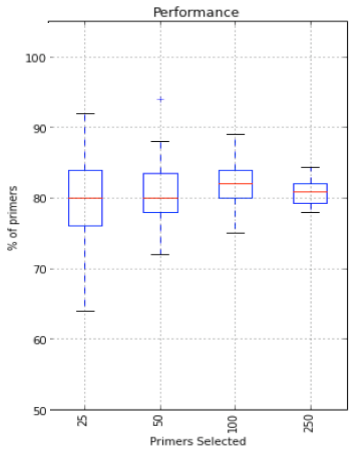
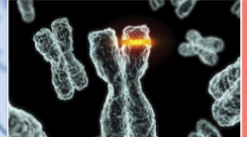


***Figure B:** Shows primer performance normalized by sample. Performance is defined by whether a primer produces at least 20% of the median read depth in DNA libraries. The number shown is the % of primers in the subsampled pool that meet this definition.*



It is worth noting that not all genes are expressed in all tissues, and that RNA abundance (as well as quality) can vary largely from sample to sample. In order for fusions to be detected, target RNA must both be present and of sufficient length to be amplified and accurately mapped to exon-exon boundaries. Archer™ assays are designed to be FFPE compatible, and we have demonstrated that several commercial RNA extraction kits produce sufficient yield and quality RNA, even from older FFPE samples. Quantity and quality of FFPE-derived RNA is generally indicative of how well the original tissue was fixed in formalin. For additional information on recommended extraction kits, see FAQs (http://www.enzymatics.com/archer/faqs-archer-products/).

Additional Service Options

The Assay Designer does not currently support automated DNA-based assay design, nor does it allow for users to rebalance individual GSPs in their assays. DNA-based panel design, wet-lab validation, and rebalancing are available as services. If interested, please contact assay@enzymatics.com to discuss your project with one of our experts.

In addition to fusion transcript detection and identification, additional NGS assays are available. Our experts can help you design a custom DNA panel that will provide hot-spot or full-exon coverage enabling detection of DNA mutations including point mutants (SNPs or SNVs) or larger insertions/deletions (InDels) as well as changes in copy number (CNVs).

Partnering with Enzymatics

Enzymatics has extensive experience partnering with customers to build sequencing assays, and is actively seeking new partnerships. Enzymatics is one of few companies in the industry with a vertically integrated supply chain that includes assay development, enzyme production, and kitting/supply chain services, all under ISO-13485 quality guidelines. With manufacturing scales ranging from 100 to 350,000 kits per year, Enzymatics excels at developing and producing commercial-grade molecular biology kits for research and diagnostic applications.

Limitations of Use

For Research Use Only. Not for use in diagnostic procedures.

This product was developed, manufactured, and sold for in vitro use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.

For more information please visit http://www.enzymatics.com/archer

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