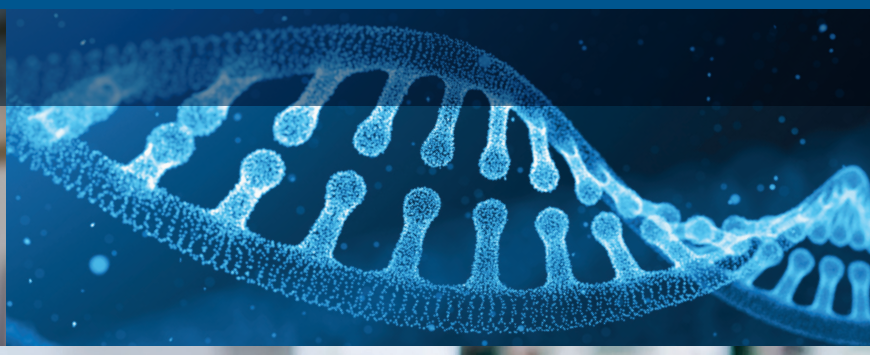
Agilent

Trusted Answers

Dynamic Duo

Agilent Avida target enrichment



Agilent Avida Targeted Profiling Workflows Designed to "Duo" More

For the first time, experience the power of DNA and methylation in one target enrichment workflow. The Agilent Avida Duo workflow enables both DNA and methylation profiling of a single sample, with no sample splitting required. The novel, high-performance workflow offers multiomics capabilities and outstanding fidelity, turnaround time, and ease of use. Unleash the full potential of every sample with the Agilent Avida workflow—designed to "duo" more.

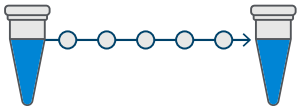
Assessing the genetic and epigenetic changes in liquid and tissue samples has become increasingly important in translational research for the potential use in cancer detection, monitoring, and therapy selection. Now, the innovative Avida duo workflow empowers you to get more information from every sample, so you can keep pace with the ever-evolving cancer landscape. With Avida, go from extracting cell-free DNA (cfDNA) from a single sample to sequencing genetic and epigenetic targets in hours, not days.

Optimized sample recovery unlocks maximum capabilities

Tne Avida proprietary workflow is purpose-built to maximize sample retention and recovery, ensuring the integrity and fidelity of your original sample is retained. With a 'no fragment left behind' approach, the Avida workflow and reagent kits are ideal for sensitive and innovative applications such as liquid biopsy and multiomics sequencing.

Avida Reagent Kits

- Avida Duo Methyl Kit
- Avida DNA Kit
- Avida Methyl Kit

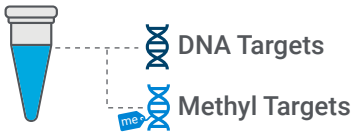


1. Optimize sample recovery

From initial library prep to indexing PCR, the Avida workflow is designed to minimize steps that lead to sample loss, such as sample transfers. And, the novel Avida interlocked hybrid capture chemistry is more efficient and faster than single, long biotinylated probes.

2. Maintain sample fidelity

Thanks to maximized sample recovery, the Avida workflow eliminates the precapture PCR step. This allows for capture of DNA and methyl targets from the original sample. As a result, sample complexity is maintained and bias is minimized.



3. Automate and streamline

The streamlined Avida workflow includes fewer sample transfers and amplification steps, combined with a more efficient hybridization method, dramatically reducing turnaround times and ensuring full automation capability.

4. Novel, multiomic application

The result? A sensitive, fast workflow ideal for low-input applications like cfDNA, and a novel, multiomic application. With the Avida Duo workflow, both DNA and methyl targets can be captured from a single sample without signal loss.

The Dynamic Duo | DNA + Methylation Enrichment

Experience the power of DNA and methylation in one target enrichment workflow with Avida Duo

By maximizing sample recovery, the Agilent Avida Duo workflow enables DNA and methylation profiling from the same low input sample. The streamlined workflow is automation compatible so you can go from extracted sample to sequencing genetic and epigenetic targets in as little as eight hours.

Applicable Kit

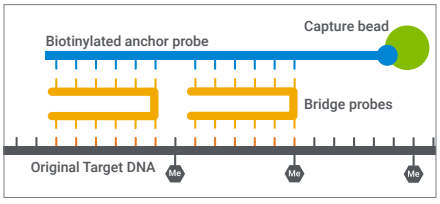
Avida Duo Methyl Kit

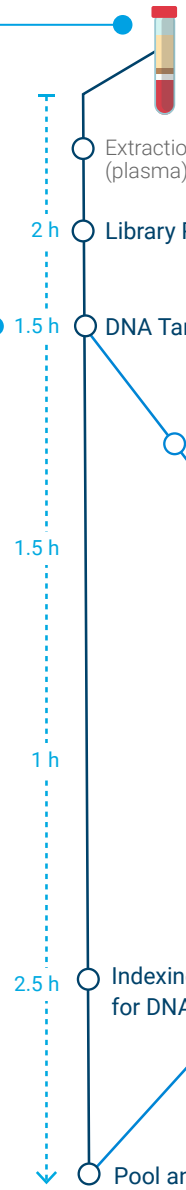
Optimized for sensitive sample types

Less required input makes the method ideal for analysis of difficult sample types like cfDNA and FFPE DNA from liquid and tissue biopsies where low input is typical.

Capturing more targets, more efficiently

A faster, more efficient hybridization reaction is made possible by a dual probe system. First, short bridge probes hybridize to target DNA. When more than one bridge probe hybridizes to the same target, they are stabilized by a biotinylated anchor probe, creating a "3D umbrella" probe system. Highly specific capture is achieved thanks to this synergetic probe structure.





Uncompromising quality from low input samples

The library preparation steps are optimized to limit loss of material, and proceeds directly from adapter ligation and library purification to hybridization capture of target regions, avoiding elution and associated sample loss. Without precapture PCR steps, researchers are ready to capture targets within two hours.

Multiomics without sample splitting

The same DNA library used to capture DNA targets is immediately used to capture methyl targets, avoiding the sample splitting and signal loss of conventional multiomic methods.

Sensitive detection can lower sequencing costs

High sample recovery translates into high sensitivity. Fewer reads required during sequencing can lower sequencing costs.

5 days	8.5 h
Total Time for Conventional DNA and Methyl NGS Profiling	Total Time for Avida Duo

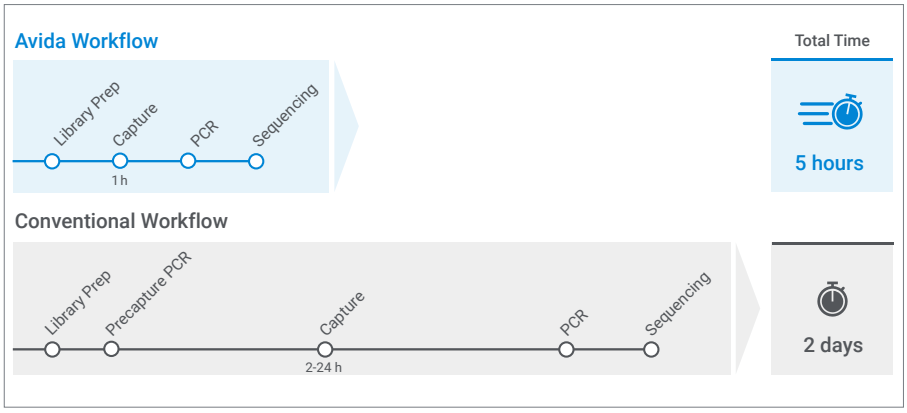
DNA Target Enrichment

Go from sample to sequencer in as little as 5 hours with the Avida DNA workflow

By eliminating precapture PCR and enabling faster hybrid capture, the Avida workflow is streamlined for high-performance with low sample inputs, and can be used for a range of inputs and panel sizes.

Applicable Kits

- Avida DNA Kit
- Avida Duo Methyl Kit



Agilent Avida DNA Workflow

- ✓ No precapture PCR
- ✓ 4x faster hybrid capture
- ✓ Sample to sequencer in a single shift

High performance across input amounts

Maximizing sample recovery leads to more uniform coverage. In turn, high uniformity and percentage of on-target fragments allows for more efficient, lower-cost sequencing.

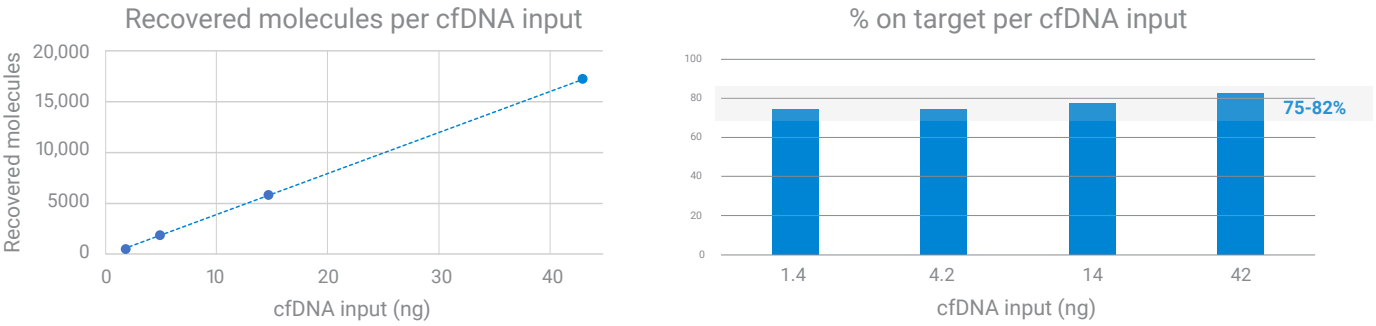


Figure 1: DNA performance for the Avida DNA Focused Cancer panel (27 kb) was assessed for human plasma cfDNA isolated from a pool of healthy donors. Data shown is the mean value between four replicates. Recovered molecules were calculated after UMI deduplication for both DNA strands.

Do It Together or Alone

In addition to the innovative Duo DNA + Methylation workflow, the Avida DNA and Avida Methylation workflows can be carried out separately. In combination or alone, the Agilent Avida targeted profiling workflows offer high performance and outstanding fidelity, turnaround time, and ease of use.

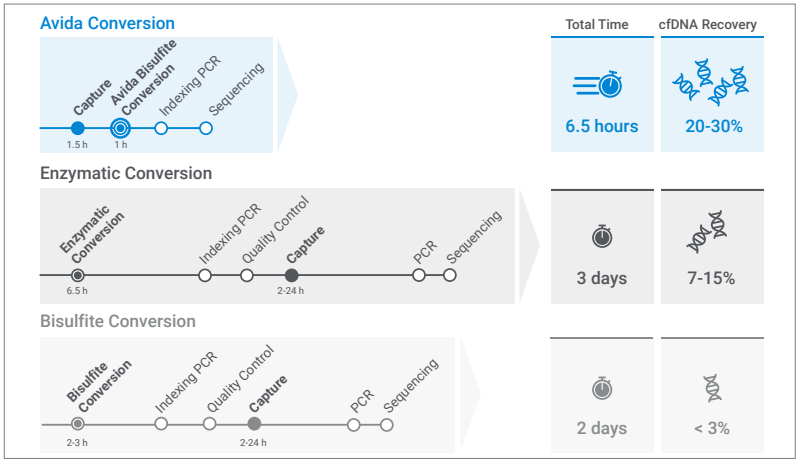
Methyl Target Enrichment

Achieve superior recovery of cfDNA and 2-3x faster turnaround time with the Avida Methyl workflow

By maximizing sample recovery, the Avida workflow enables native methylation targets to be captured before DNA conversion and amplification. In contrast, conventional workflows require precapture PCR to achieve the input requirements for methyl target capture. And, because PCR does not preserve methylation patterns, methylated DNA must be converted first. The innovative, streamlined Avida workflow makes probe design more straightforward, reduces bias from conversion and precapture PCR, and enables multiomic capabilities.

Applicable Kits

- Avida Methyl Kit
- Avida Duo Methyl Kit



Agilent Avida Methyl Workflow

- ✓ Preserve methylation signatures in low input samples
- ✓ 2-3x faster conversion
- ✓ Efficient, faster hybrid capture
- ✓ High DNA recovery

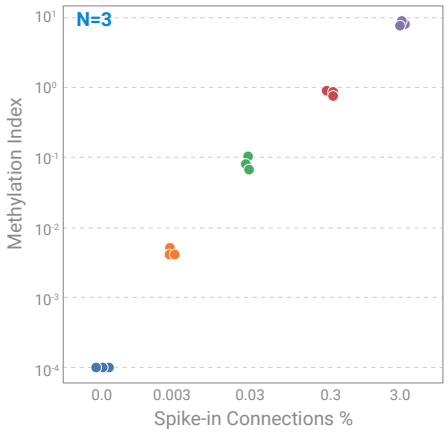


Figure 2. Analytical sensitivity was assessed by detecting known spike-in concentrations of methylated DNA in cfDNA background. A 10 ng of serial titration of Seraseq Methylated ctDNA Mutation Mix was spiked into Seraseq Unmethylated ctDNA Mutation Mix (SeraCare). Library preparation and target enrichment performed with a 10 M PE read budget using the Avida Methyl reagent kit and Avida Methyl 3400 DMR Cancer panel. Data shown is the average methylation percentage across three replicates.

* *Coverage metrics reflect the average number of unique reads per target after single-strand UMI deduplication (covering the center of the target). The average across replicates of the median of all target regions is reported.*

** *Beta version of the reagents were used to generate this data*

Unprecedented analytical sensitivity for methyl targets

Sensitivity to methylated DNA—down to 0.0025% in cfDNA background—is superior to conventional methods and SNV-based ctDNA detection.

Optimized conversion for more coverage

Combining pre-amplification capture with a proprietary, "soft" bisulfite conversion method reduces DNA damage compared to bisulfite conversion and reduces sample loss compared to enzymatic conversion, resulting in high coverage.

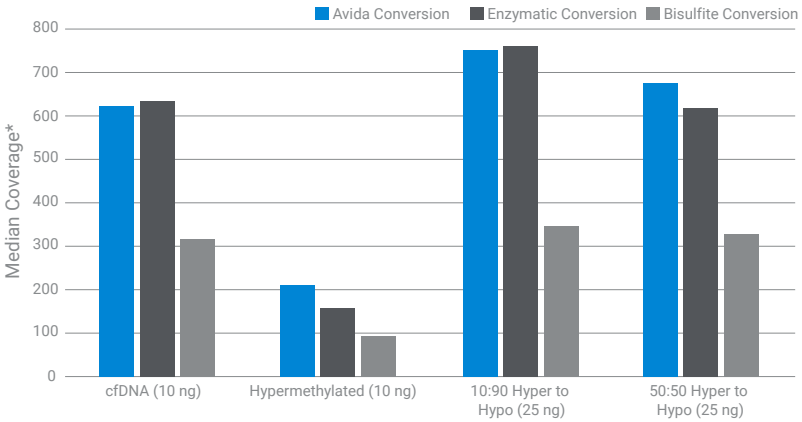


Figure 3. To assess the efficiency of the Avida conversion, Human HCT116 DKO Methylated ('Hyper', Zymo D5014-2) and Non-methylated DNA ('Hypo', Zymo D5014-1) were spiked in at various percentages with in-house control cfDNA sample. All samples (at 10 to 25 ng input) were prepared using Avida library preparation and capture with a 200 DMR subset (60 kb) of the Avida 3400 DMR cancer panel. Varying conversion methods (traditional bisulfite and enzymatic conversion) were compared alongside Avida conversion. Each sample was sequenced with 10 M paired end reads per sample.**

Avida Target Enrichment Custom Panels

Capture your specific regions of interest

Avida custom probe libraries allow you to specifically target your regions of interest and maximize use of your sequencing budget.

Custom probes can be designed for the DNA workflow, targeting genetic differences; the methyl workflow, investigating epigenetic regulation; or as complementary DNA and methyl probe sets for use in the unique Avida Duo workflow.

Custom probe libraries can also be designed to be complementary for use in an Avida Duo workflow with an Avida catalog design.

Backed by decades of experience in target capture with flexible design options

1. Capitalize on your local Agilent design experts

2. Use Agilent SureDesign

Leverage our global team of design experts to consult with you on your exact requirements or use the intuitive SureDesign platform and design wizard to guide the creation of your new Avida DNA or Methyl design.

Choose the design strategy that best meets your cfDNA needs

We know that during the design of each experiment, it is important to understand the experimental question and have a design strategy that best addresses your needs. We offer two different custom design strategies.

- All: To cover all your regions of interest, ensuring all important variants are covered, irrespective of the effect on capture statistics
- High-quality: To maximize sequencing budget, only designing for targets/ regions that lack repeats and are predicted to capture with high efficiency

The Agilent Avida custom probe workflow leverages its unique 3D probe structure, superior design algorithms, and design flexibility—making Avida custom panels the premier solution to meet your experimental needs for investigating DNA and epigenetic changes in liquid biopsy samples.

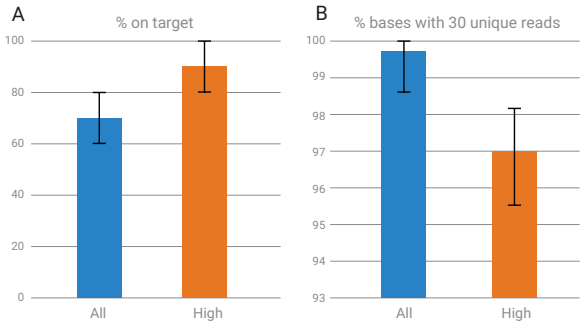


Figure 4. Highlights performance differences between the two design strategies. (A) Agilent Avida high-quality probes (orange; High) have, on average, a 22% higher on-target percentage than the Agilent Avida all probes (blue; All), as measured by Percent Selected Bases. Percent Selected Bases, computed using Picard HsMetrics, represents the percentage of all aligned bases that are on or near target. Higher values indicate a better on-target percentage.

(B) On average, Agilent Avida all probes (blue; All) achieve 30X coverage for 3% more bases after single-strand molecular barcode (MBC) deduplication compared to Agilent Avida high-quality probes (orange; High).

Applicable Kits

- Avida DNA Kit
- Avida Methyl Kit
- Avida Duo Methyl Kit

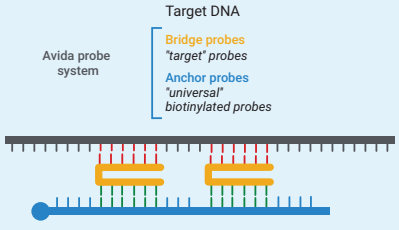
Unique features of the Avida probe system—maximizing performance in liquid biopsy workflows

The innovative Avida capture approach uses a three-dimensional umbrella probe system, combining two types of probes.

- Short Bridge probes to specifically target your regions of interest
- Universal biotinylated Anchor probes to enrich your targeted regions in an NGS workflow.

This design addresses some of the specific challenges encountered when working on liquid biopsy samples.

- Short, target-specific probes for rapid one-hour hybridization and quick turnaround time in your oncology workflow
- Anchor probes within the 3D probe system for highly specific enrich-ment of your targets
- DNA and methylation probes designed to hybridize with native gDNA for preconversion capture in the methyl workflow—ensuring no loss in target complexity
- A user-friendly, single-tube system within the workflow



The power of the Avida workflow can be paired with the high-performance panel of your choosing

Agilent Avida panels are flexible, modular and automation-ready. DNA panels may be matched with the methyl panel for use with the Avida Duo workflow.

Table 1. Avida panels for variant and methylation detection.

Panel	Targets	Description
Avida DNA Focused Cancer panel	14 genes	Catalog panel for oncology research. This highly focused panel provides hotspot or full exon coverage for 14 key cancer genes. The small panel size enables flexible and cost-effective deep sequencing to achieve sensitive detection of rare variants from key cancer genes for liquid biopsy samples.
Avida DNA Expanded Cancer panel	105 genes	Catalog panel targeting 105 cancer-associated genes with hotspot or full exon coverage. This medium-sized panel is optimized for liquid biopsy samples by balancing gene content with sequencing depth requirements for efficient sequencing.
Avida DNA Discovery Cancer panel	682 genes	Catalog panel for oncology biomarker discovery and assessment. This larger panel targets 682 genes to detect key classes of somatic mutations for translational research: single nucleotide variants (SNVs), copy number variants (CNVs), and translocations (TLs).
Avida Methyl 3400 DMR Cancer panel	3400 DMRs	Catalog panel targeting 3400 differentially methylated regions (DMRs) selected for their ability to discriminate solid tumor DNA versus non-tumoral DNA, both from tissue and liquid, allowing for the detection of ctDNA across different cancer types.
Avida Custom panel	Customer defined	Custom panel designed with customized content. Available for use with Avida DNA, Methyl, and Duo Methyl reagent kits. Contact your local account manager for more information on custom designs.

Table 2. Avida Onco LB series panels curated based on the requirements of the European oncNGS consortium.

Panel	Targets	Description
Avida DNA Onco LB panel*	164 genes	Catalog panel targeting 164 pan cancer-associated genes covering exonic regions and 26 translocation-associated targets. Curated based on the requirements for the European oncNGS project, this medium-sized panel is optimized to deliver extra sensitivity for liquid biopsy samples.
Avida DNA Onco LB Plus panel*	437 genes	Catalog panel targeting 437 pan cancer-associated genes covering exonic regions and 33 translocation associated targets. Curated based on the requirements for the European oncNGS project, this larger panel extends the Avida DNA Onco LB panel, with 273 additional cancer-associated genes, to provide sensitive variant detection for liquid biopsy samples.
Avida DNA Lymphoma panel*	86 genes	Catalog panel targeting 86 genes involved in lymphatic system cancers. Curated based on the requirements for the European oncNGS project, this medium-sized panel balances gene content and sequencing depth requirements for efficient sequencing of lymphoma samples, including those derived from liquid biopsies.

**The Agilent Avida DNA Onco LB, Avida DNA Onco LB Plus, and Avida DNA Lymphoma panels were developed as part of the OncNGS Project that has received funding from the European Union's Horizon 2020 Research and Innovation Programme.*

ONCNGS PCP

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PR7003-262

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Published in the USA, September 26, 2025
5994-7187EN

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