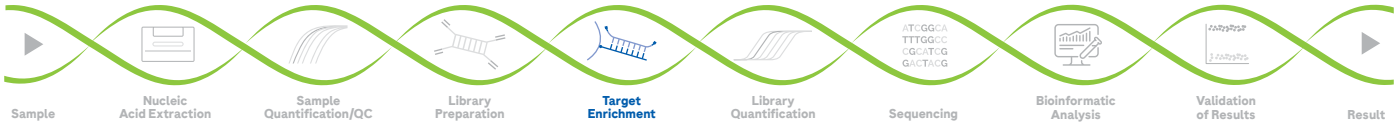
KAPA HyperPETE

The performance of hybrid-capture target enrichment with the speed & simplicity of amplicon workflows

KAPA HyperPETE (Primer Extension Target Enrichment) is a novel hybrid-capture technology designed to employ primer extension reactions to specifically capture and release target library molecules for sequencing. It is designed and **optimized to detect all major somatic variant types**, including SNVs, short indels, CNVs, MSI, and fusion transcripts (known and novel). KAPA HyperPETE is **compatible with a wide variety of sample types, including challenging samples**—such as cfDNA and FFPET-derived DNA and RNA.

The **KAPA HyperPETE Portfolio** includes readily available fixed-design panels for hereditary oncology, oncology hotspots, pan-cancer variants (with an MSI module), and lung cancer fusion variants. In addition, custom panels can be designed using HyperDesign, our easy-to-use online design tool.

- **Save valuable time with an efficient, single-day, automatable workflow**
- **Achieve superior performance and coverage uniformity**
- **Uncover critical genomic information from a wide variety of sample types,** including FFPET and cfDNA
- **Reliably enrich challenging, previously inaccessible genomic regions**



Primer Extension Target Enrichment

A new way of enriching DNA or RNA targets

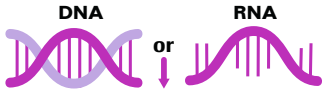
PETE is a novel NGS hybridization capture technology *designed to employ primer extension reactions to specifically capture and release target library molecules for sequencing.*

Superior performance and coverage uniformity

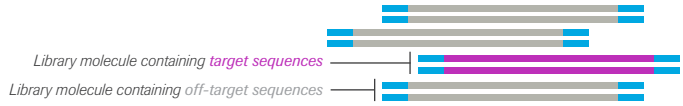
Fewer manual steps

Faster workflow

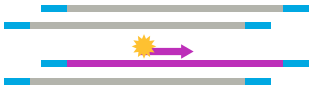
Start with DNA or RNA



1. **Prepare indexed libraries:** Prepare libraries with KAPA DNA or RNA Library Prep Kits and truncated, universal adapters (▬) with or without UMIs).



2. **Anneal target-specific capture primers:** Heat-denature libraries and hybridize to biotinylated target-specific capture primers (✸➡); for simplicity, only one strand of each denatured library molecule is shown.

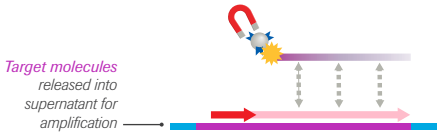


3. **Perform capture primer extension:** Library molecules containing target sequences will form biotin-labeled capture-ready extension products, while off-target library molecules will not.

4. **Capture and wash target library molecules:** Use paramagnetic streptavidin beads (✸) and a magnet (∩) to capture and immobilize target molecules, and then wash away off-target molecules. The remaining library will be greatly enriched for target sequences.

5. **Anneal target-specific release primers:** Hybridize captured library molecules to target-specific release primers (➡); the binding sites for these primers are upstream of the capture primer sites.

6. **Perform release primer extension:** Primer extension releases the target molecules into the supernatant to be collected for amplification; the biotin-labeled molecules remain behind on immobilized beads.



7. **Amplify target library molecules:** Use universal library amplification primers (➡) to amplify the released, target-enriched library molecules, and then perform cleanup.

Finish with a sequencing-ready, target-enriched library

For more information, please visit: *go.roche.com/KAPAHyperPETE*

Project: KAPA HyperPETE, Pleasanton, CA 2021

Save valuable time with an efficient, single-day, automatable workflow

- Take hours off of total workflow time compared to typical hybridization capture, with time requirements similar to amplicon and anchored multiplex methods **(Figure 1)**
- Enrich for long contiguous regions, using fewer tubes per sample compared to amplicon technologies

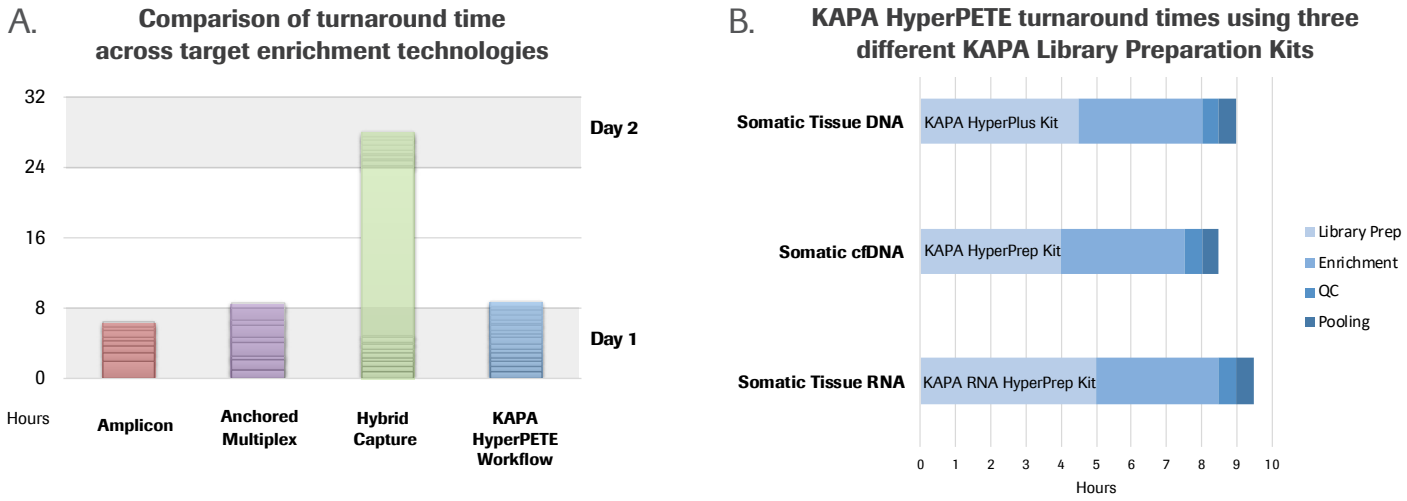


Figure 1. The turnaround time (TAT) for KAPA HyperPETE target enrichment is similar to the TAT for amplicon-based workflows. (A) While most hybridization-based workflows take two days to complete, KAPA HyperPETE workflows can be completed in one day. **(B)** Differences in the TAT for various applications of KAPA HyperPETE are dependent on the library preparation kit used, as each kit requires slightly different completion times. However, once the libraries are created, the enrichment workflow is the same across applications.

Achieve superior performance and coverage uniformity

- Obtain more uniform coverage compared to anchored multiplex PCR **(Figure 2)**
- Avoid over- or under-representation of PCR-based target regions; KAPA HyperPETE technology uses primers to capture the regions of interest, rather than amplify them, greatly reducing amplification bias
- Streamline bioinformatics pipelines and increase your sequencing efficiency by eliminating the need to remove primer binding-site sequences from your sequencing data

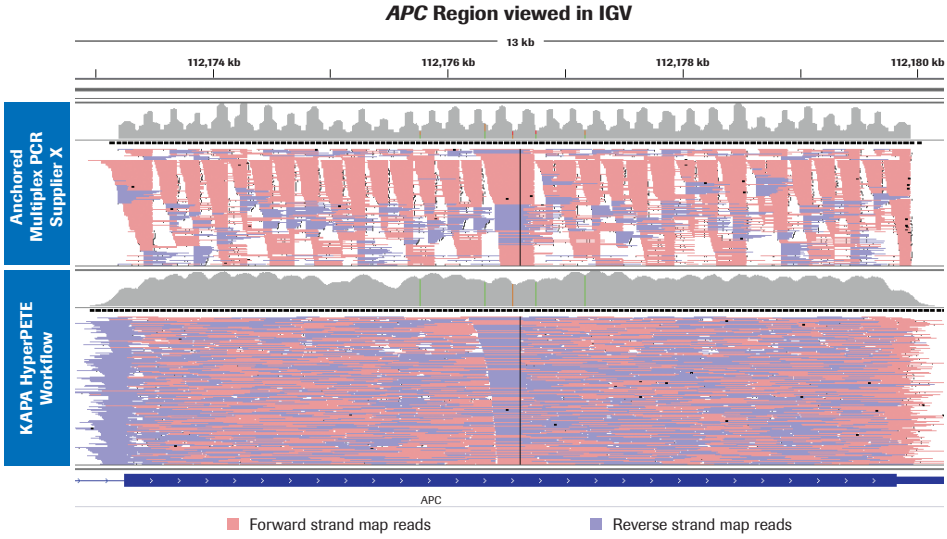


Figure 2. KAPA HyperPETE achieves greater uniformity across the *APC* region compared to anchored multiplex PCR. In this IGV (Integrative Genomics Viewer) image, white space indicates a lack of coverage. In the anchored multiplex PCR workflow, the amplification primers (which contain adapters) are fixed on one end and flexible on the other end, reducing coverage uniformity. In the KAPA HyperPETE Workflow, the primer extension and subsequent steps are used to capture full-length library molecules, which are then sequenced; this leads to improved coverage uniformity.

Project: KAPA HyperPETE, Pleasanton, CA 2021

Uncover critical genomic information from difficult sample types: Tissue RNA (FFPET)

- Achieve highly specific enrichment from a broad range of FFPET RNA input amounts **(Figure 3)**
- Detect all fusions present in reference cell line samples **(Table 1)**

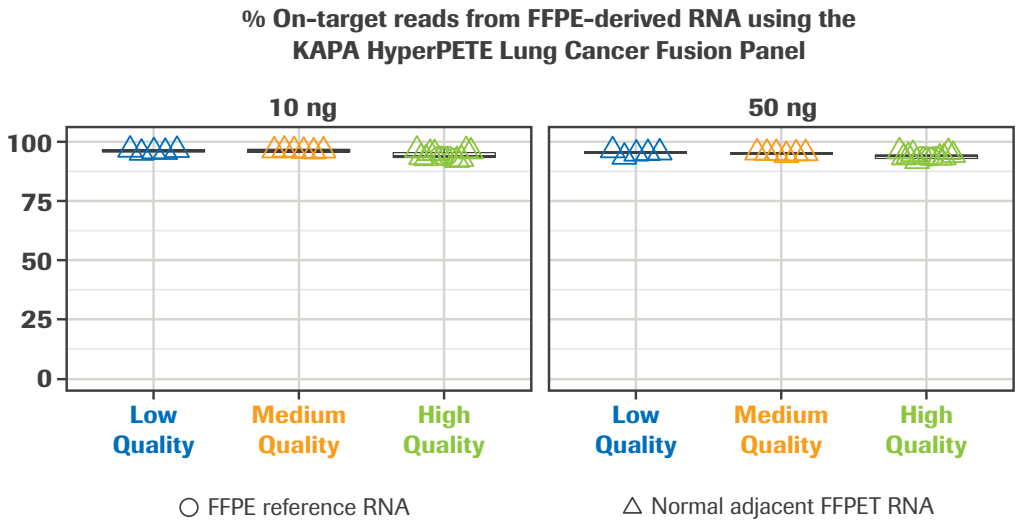


Figure 3. The KAPA HyperPETE Lung Cancer Fusion Panel achieved 92%–97% reads on-target from 10 ng and 50 ng of all FFPET samples tested. High specificity was demonstrated by the high percent of reads on target when starting from 10 ng or 50 ng of various quality (low, medium, high) input FFPE RNA. On-target rate was 92% to 97% (includes housekeeping and fusion targets) with good performance across all sample input amounts, qualities, and types. The on-target rate was calculated following rRNA read removal (0.8% to 11% of all reads). Two (2) cell-line samples (in duplicates) and fourteen (14) normal adjacent FFPET samples were used to assess performance. RNA was extracted with the Roche High Pure FFPET RNA Isolation Kit, and quality was determined with the DV200 score using the Agilent RNA 6000 Pico Assay on the Bioanalyzer. The KAPA HyperPETE Workflow for Tissue RNA Fusion Transcript Preparation using the KAPA HyperPETE LC Fusion Panel was followed. Libraries were generated using the KAPA RNA HyperPrep Kit in combination with the KAPA Universal UMI Adapter and either 10 ng or 50 ng of RNA, while adjusting PCR cycles based on the input amount and DV200 score. Libraries were captured using the KAPA HyperPETE Reagent Kit and sequenced on an Illumina NextSeq™ 550 System. Total read pairs (2 x 150 bp) per sample ranged from 3.6M to 17M.

Table 1. Fusion detection performance using tissue RNA (FFPET)

Variant type	RNA input amount	Total replicates	Expected variants	True positive rate
Fusion	10 ng	6	62	100%
Fusion	50 ng	6	62	100%

All fusions (100%) were detected in the reference cell line samples at both 10 ng and 50 ng RNA input amounts. Two (2) Seraseq® RNA Fusion FFPE samples and one Horizon Discovery RNA Fusion FFPE sample, each run in duplicate, were used to assess fusion detection performance. The EGFR-SEPT14 variant in Seraseq® Fusion RNA Mix v4 was manually curated as an *EGFR* partner that has a homologous sequence to *SEPT14*. Comparable variant detection results were achieved when down-sampling to 1M read pairs (2 x 150 bp, data not shown).

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Uncover critical genomic information from difficult sample types: Plasma cfDNA

- Achieve high % reads on-target and high exon coverage (at ≥1000X) for a range of cfDNA input amounts **(Figure 4)**
- Detect variants with high sensitivity, from a range of plasma input cfDNA amounts **(Table 2)**

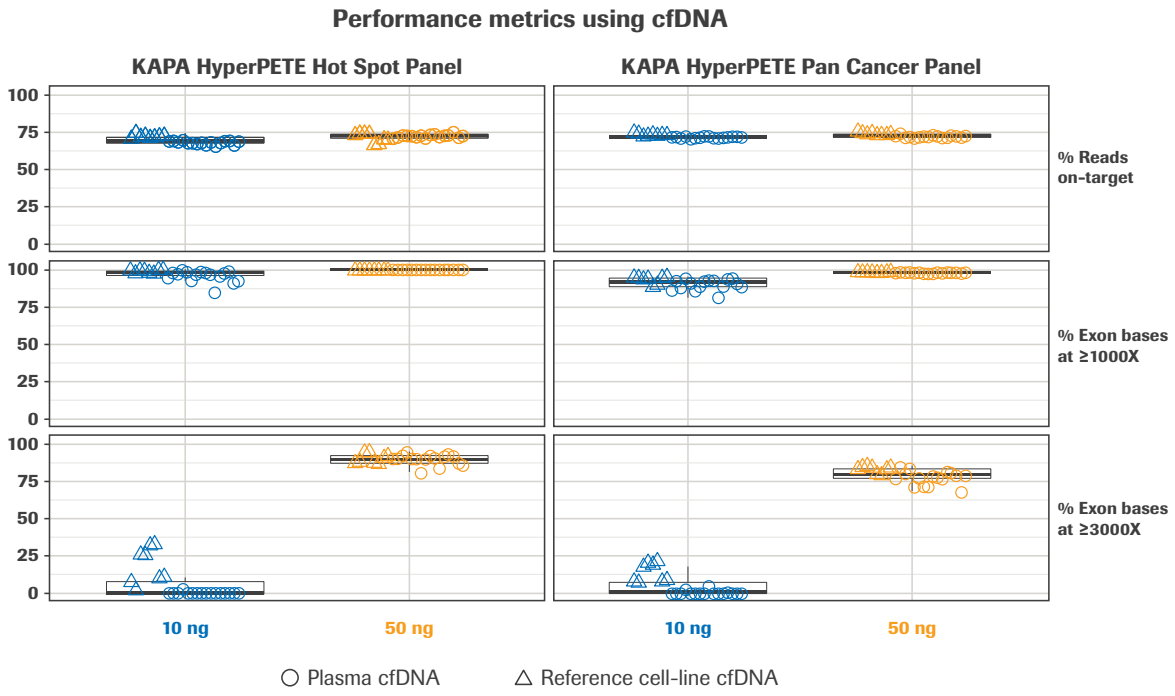

Figure 4. The KAPA HyperPETE Hot Spot Panel and KAPA HyperPETE Pan Cancer Panel provide high-quality results for plasma cfDNA (10 ng and 50 ng input). High specificity with deep and broad unique target coverage was demonstrated using the KAPA HyperPETE Workflow for Somatic Plasma cfDNA Preparation across all sample input amounts and types. On-target rate was 65% – 73% for the KAPA HyperPETE Hot Spot Panel and 70% – 73% for the KAPA HyperPETE Pan Cancer Panel. At 50 ng cfDNA input, the percent of panel exon bases covered at ≥1000X of unique depth (PCR duplicates removed) was >99% for the KAPA HyperPETE Hot Spot Panel and 97% – 98% for the KAPA HyperPETE Pan Cancer Panel. The respective percentages at ≥3000X unique depth were 80% – 94% and 67% – 85%. At 10 ng of cfDNA input, the unique depth was concordant with the available unique genome equivalents (~3300 in 10 ng of DNA) and the percentages at ≥1000X unique depth were 83% – 99% and 80% – 94% per panel respectively. Libraries were generated using the KAPA HyperPrep Kit and the KAPA Universal UMI Adapter from either 10 ng or 50 ng of plasma cfDNA or fragmented reference cell-line DNA as input, and individually captured using the KAPA HyperPETE Reagent Kit and the respective panel. Final libraries were sequenced on an Illumina NextSeq™ 550 System with 6M – 14M (median: 8M) high-quality read pairs (2 x 150 bp) allocated per sample for the KAPA HyperPETE Hot Spot Panel, and 55M – 81M (median: 66M) high-quality read pairs allocated per sample for the KAPA HyperPETE Pan Cancer Panel. Two (2) Seraseq® ctDNA Mutation Mix samples in duplicates and sixteen (16) healthy donor plasma cfDNA samples were used to assess the performance.

Table 2. Variant detection performance using reference cell-line cfDNA

Panel	Input (ng)	Allele frequency	Expected variants	Detected variants	True positive rate
KAPA HyperPETE Hot Spot Panel	10	1.0%	56	56	100%
	50			56	100%
	10	0.5%	50	47	94%
	50			50	100%
KAPA HyperPETE Pan Cancer Panel	10	1.0%	70	70	100%
	50			70	100%
	10	0.5%	64	62	96.9%
	50			63	98.4%

High true positive detection rates were demonstrated for short variants (Single Nucleotide Variants—SNVs and Indels) at low variant allele expected frequencies across all reference cell line samples (two Seraseq® ctDNA Mutation Mix samples in duplicates). From both input amounts (10 ng and 50 ng) 100% of short variants (SNVs and Indels) were detected at 1% allele frequency using either of the two KAPA HyperPETE Panels. At 0.5% allele frequency from 10 ng input, the true positive detection rate was 94% and 96.9% using the KAPA HyperPETE Hot Spot Panel and the KAPA HyperPETE Pan Cancer Panel, respectively. At 0.5% allele frequency from 50 ng input, the true positive detection rate was 100% and 98.4% using the KAPA HyperPETE Hot Spot Panel and the KAPA HyperPETE Pan Cancer Panel, respectively.

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Uncover critical genomic information from difficult sample types: Additional sample types

Table 3: Overview of sample types compatible with KAPA HyperPETE

Panel	FFPET		Plasma (cfDNA)	Human cell line gDNA	High-quality human gDNA from		High-quality human RNA from	
	DNA	RNA			Blood	Fresh Tissue	Blood	Fresh Tissue
KAPA HyperPETE Choice/Explore	✔		✔	✔	✔	✔		
KAPA HyperPETE Choice/Explore RNA		✔					✔	✔
KAPA HyperPETE Pan Cancer Panel	✔		✔	✔	✔	✔		
KAPA HyperPETE Hot Spot Panel	✔		✔	✔	✔	✔		
KAPA HyperPETE Hereditary Oncology Panel				✔	✔	✔		
KAPA HyperPETE Lung Cancer Fusion Panel		✔					✔	✔

Reliably enrich challenging, previously inaccessible genomic regions

Generating custom probe pools for targeted enrichment of NGS libraries can be a daunting endeavor. Many traditional design tools are clunky, rely on poorly optimized design algorithms, or lack the support of a live person with years of design experience—leaving you wondering if you have the best design to capture your specific regions of interest.

Roche's online design tool, **HyperDesign**, is here to address those concerns. HyperDesign is a user-friendly online probe design tool that takes advantage of Roche's extensive experience with *in silico* probe design. HyperDesign's proprietary design algorithm has been optimized to achieve deeper and more uniform downstream sequencing coverage with fewer sequencing reads—even for difficult-to-capture regions.

Start your new custom design

Visit **www.HyperDesign.com**, log in to your homepage, click on "Create new design," and ***follow these 4 simple steps...***

1. **Select your organism** of interest and name your design
2. **Add your targets** by uploading gene names, bed files or genomic coordinates; or choose from a broad list of commonly used gene identifiers
3. **Fine-tune your inputs,** review your targets, and confirm your results
4. **Submit** your design for selection

...then let our advanced algorithm do the work. Once probe selection is complete, you'll be able to review the coverage results across your target regions.

Learn more at
go.roche.com/KAPAHyperPETE

Design a KAPA HyperPETE panel at
www.HyperDesign.com

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Ordering Information

KAPA HyperPETE Catalog Panels

Product Name	Capture reactions	Catalog No.
KAPA HyperPETE Pan Cancer Panel	24	09329161001
	96	09329196001
	384	09329226001
KAPA HyperPETE Hot Spot Panel	24	09329234001
	96	09329277001
	384	09329307001
KAPA HyperPETE Hereditary Oncology Panel	24	09329315001
	96	09329340001
	384	09329374001
KAPA HyperPETE Lung Cancer Fusion Panel	24	09329471001
	96	09329501001
	384	09329536001

KAPA HyperPETE Choice Panels: Custom panels (human) designed by customers using HyperDesign

Product Name	Capture reactions	Catalog No.
KAPA HyperPETE Choice 75Kb	96, 384,1536, or 10,0000	Varies
KAPA HyperPETE Choice 150Kb		
KAPA HyperPETE Choice 250Kb		
KAPA HyperPETE Choice RNA 50Kb		

KAPA HyperPETE Explore Panels: Custom panels (human) designed by the Roche Expert Designer Service

Product Name	Capture reactions	Catalog No.
KAPA HyperPETE Explore 75Kb	96, 384,1536, or 10,0000	Varies
KAPA HyperPETE Explore 150Kb		
KAPA HyperPETE Explore 250Kb		
KAPA HyperPETE Explore RNA 50Kb		

Learn more at
go.roche.com/KAPAHyperPETE

Design a KAPA HyperPETE panel at
www.HyperDesign.com

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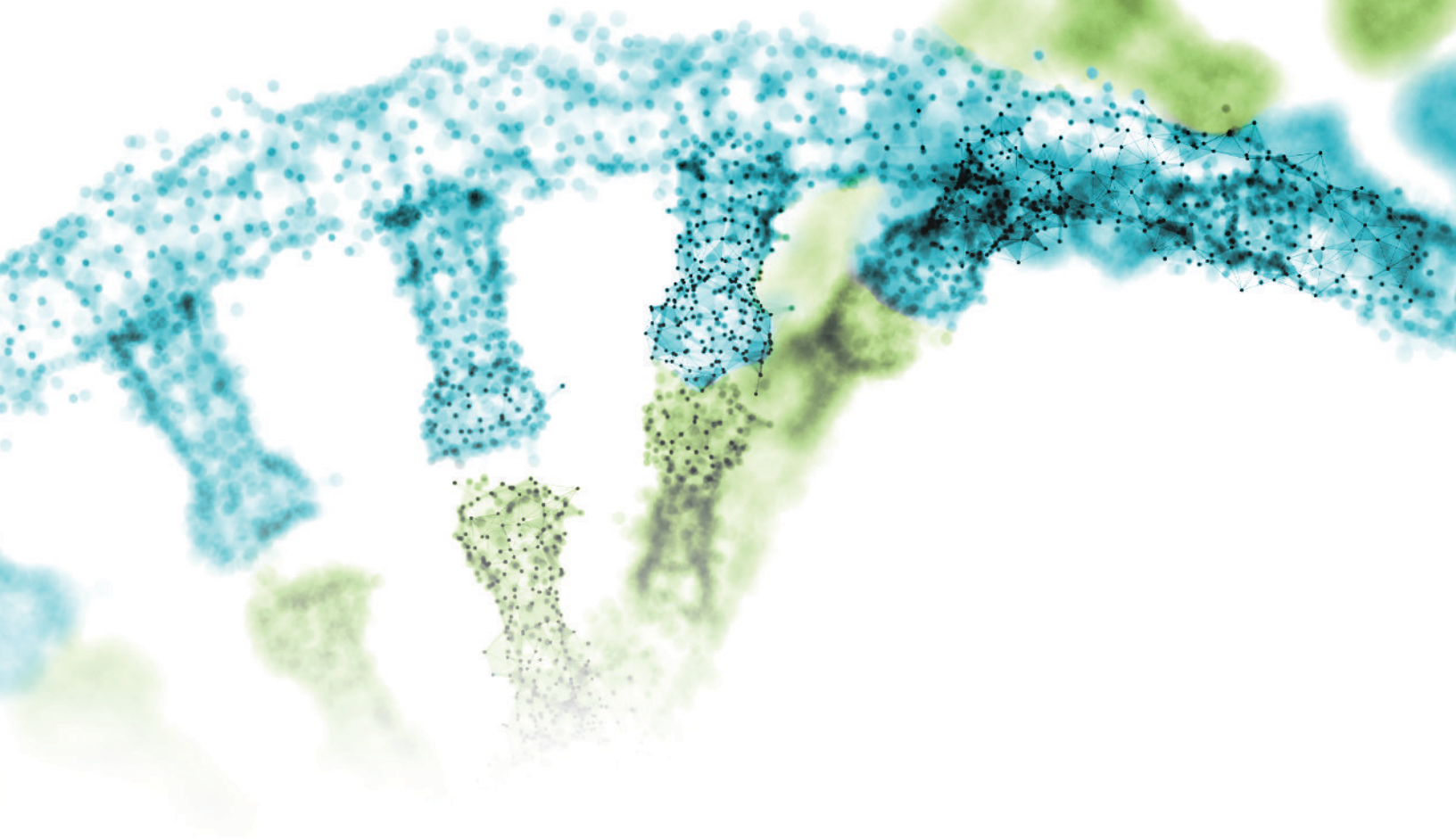

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