DATA SHEET

Illumina DNA Prep with Enrichment

Fast, integrated workflow for a wide range of target enrichment applications

- Provides a fast library preparation and enrichment workflow with a total turnaround time of ~6.5 hours
- Enhances library preparation efficiency with integrated protocols for blood and saliva
- Enables advanced study designs in cancer research, genetic disease research, and whole-exome sequencing

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Introduction

The Illumina DNA Prep with Enrichment solution combines versatile, simple, and fast library prep and enrichment functionality for targeted enrichment and exome sequencing applications. It offers extraordinary flexibility for input type and amount (Table 1), and a wide range of supported enrichment sequencing applications encompassing custom panels, fixed panels, and whole-exome sequencing from Illumina or third-party vendors.

Illumina DNA Prep with Enrichment uses innovative bead-based chemistry incorporating a simplified, single hybridization step (Figure 1). With the Illumina DNA Prep with Enrichment workflow, DNA extraction can be processed directly from fresh blood and saliva samples using the Flex Lysis Reagent Kit and Saliva Lysis Protocol, respectively, for additional time savings.

Table 1: Illumina DNA Prep with Enrichment specifications

Parameter	Specification
DNA input type	gDNA, whole blood, saliva, DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue
DNA input verified[a]	10–1000 ng
Sample multiplexing	384 unique dual indexes (UDIs)
Pre-enrichment pooling[b]	1-plex or 12-plex verified and supported
Supported sequencing systems	All Illumina systems
Total workflow time[c]	~6.5 hours

a. DNA inputs as low as 10 ng are possible, but will not provide saturation-based DNA normalization.
b. Other enrichment plexities are possible, but have not been verified. Additional optimization may be required and optimal results are not guaranteed.
c. Includes library preparation, enrichment, and library normalization/pooling steps.

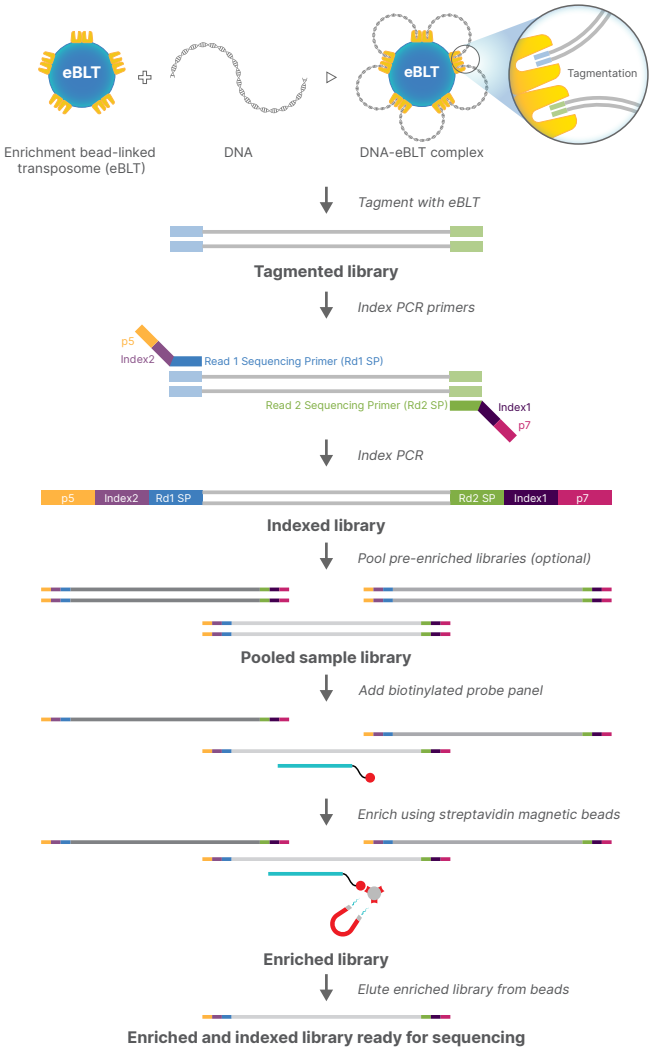

Figure 1: Illumina tagmentation chemistry—A uniform tagmentation reaction mediated by eBLTs followed by a single hybridization reaction enables a fast and flexible workflow.

Fast and flexible library preparation and enrichment workflow

A key component of the Illumina DNA Prep with Enrichment solution is on-bead tagmentation, which uses bead-bound transposomes to mediate a uniform tagmentation reaction. This strategy provides several significant advantages:

- For gDNA inputs ≥ 50 ng, accurate quantitation of the initial DNA sample is not required, as insert fragment size is not affected, saving time and costs associated with kits and reagents
- On-bead tagmentation eliminates the need for separate DNA fragmentation steps, saving time and costs associated with related consumables
- For gDNA inputs of 50–1000 ng, saturation-based DNA normalization eliminates the need for individual library quantitation and normalization steps before enrichment
- Novel 90-minute single hybridization protocol enables enrichment in less than four hours

Fast enrichment workflow

The Illumina DNA Prep with Enrichment solution supports liquid-handling systems for automated library preparation and produces a fast, streamlined workflow with few steps and low hands-on and turnaround times (Figure 2, Table 2).

Table 2: Illumina enrichment workflow

Parameter	Illumina DNA Prep with Enrichment
Integrated DNA option[a]	√
Flexible, broad DNA input range	√
Library normalization included[b]	√
FFPE compatible	√
DNA input	10–1000 ng
Total library prep and enrichment time[c]	~6.5 hr
Insert size[d]	150–220 bp
Sample index sets	384 unique dual indexes

a. Integrated lysis protocols available for blood and saliva.
b. Library normalization occurs with ≥ 50 ng gDNA input.
c. Total library prep and enrichment time includes library preparation, library normalization/pooling, and enrichment.
d. Degraded FFPE DNA may result in smaller insert sizes.

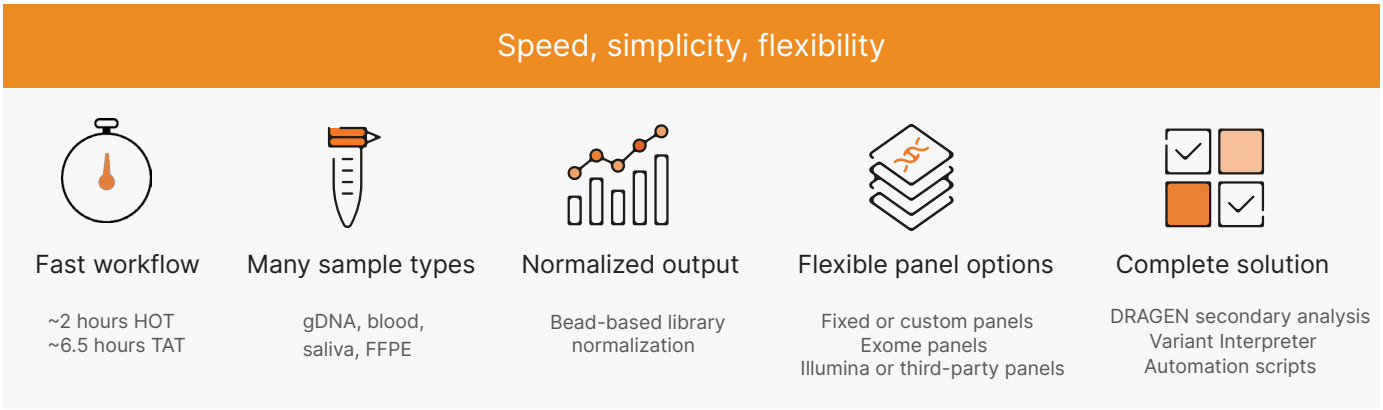

Figure 2: Illumina DNA Prep with Enrichment delivers a fast, flexible Illumina enrichment workflow—Workflow times are based on processing 12 samples at 12-plex enrichment with a manual workflow. Times may vary depending on equipment used, number of samples processed, automation procedures, or user experience. HOT, hands-on time; TOT, turnaround time; FFPE, formalin-fixed paraffin embedded tissue.

Integrated DNA Input

DNA extraction can be processed directly from whole blood or saliva samples. The optional Flex Lysis Reagent Kit, optimized and validated for using whole blood samples and Illumina DNA Prep with Enrichment, is integrated into the workflow for maximum efficiency. The lysis protocols feature bead-based reagents and require less than 30 minutes of hands-on time.

Optimized performance across Illumina sequencing systems

The robust and straightforward Illumina DNA Prep with Enrichment solution yields reliable results across all Illumina sequencing systems by providing > 90% on-target reads, > 95% uniformity, and a low PCR duplicate rate (Table 3). Illumina DNA Prep with Enrichment is compatible with various Illumina enrichment panels and is optimized for use on low-, mid-, and high-throughput systems (Figure 3, Table 4).

Table 3: Performance comparison across enrichment panels[a]

Parameter[b]	Illumina Exome Panel	Exome Panel X	Exome Panel Y
Panel size	45 Mb	39 Mb	33 Mb
Probe size	80 bp	120 bp	120 bp
Padded read enrichment (on-target)[d]	85%	91%	91%
Fragment length median	~200 bp	~200 bp	~200 bp
Coverage at 20×	93%	96%	97%
Uniformity of coverage[d]	95%	97%	98%
Read depth per sample[e]	30M CPF	25M CPF	20M CPF
SNV precision	99%	99%	99%
SNV recall	94%	94%	95%

a. Data represent example comparison data. Actual performance specifications may vary depending on read depth and sample type.
b. The analysis was run on 48 samples (all NA12878 Coriell samples) per condition. Data analysis was performed using the Enrichment BaseSpace™ App.
c. Illumina DNA Prep with Exome 2.5 Enrichment is the recommended solution for human exome sequencing.
d. See the BaseSpace™ App User Guide[2] for additional details.
e. CPF, clusters passing filter.

Figure 3: Optimized performance across Illumina sequencing systems—The Illumina DNA Prep with Enrichment solution is compatible with all Illumina sequencing systems, including the high-performance systems shown here. Low-throughput systems including the iSeq™ 100, MiniSeq™, and MiSeq™ Systems are also compatible with Illumina DNA Prep with Enrichment.

Table 4: Sample throughput per flow cell with Illumina DNA Prep with Enrichment[a]

Panel	Target coverage	iSeq 100 System	MiniSeq System		MiSeq System			NextSeq 550 System	
			Mid	High	v2	v2 Nano/Micro	v3	Mid	High
Fixed panels									
TruSight™ Cancer	395×	4	8	24	12	1/4	24	96	384
TruSight Hereditary Cancer	395×	4	8	24	12	1/4	24	96	384
Custom panels[b]									
2000 probes	450×	6	12	37	22	NR/6	37	260	384
5000 probes	370×	3	6	18	11	NR/3	18	65	200
10,000 probes	180×	3	6	19	11	NR/3	19	33	100
50,000 probes	100×	NR	2	7	4	NR	7	35	107
100,000 probes	100×	NR	NR	3	2	NR	3	17	53
300,000 probes	100×	NR	NR	NR	NR	NR	NR	6	18

Panel	Target coverage	NextSeq 2000 System			NovaSeq 6000 Series			NovaSeq X Series		
		P1	P2	P3	S1	S2	S4	1.5B	10B	25B
Custom panels[b]										
2000 probes	450×	148	593	1778	2370	6074	14,815	2370	14,815	38,519
5000 probes	370×	72	288	865	1153	2955	7207	1153	7207	18,739
10,000 probes	180×	74	296	889	1185	3037	7407	1185	7407	19,259
50,000 probes	100×	27	107	320	427	1093	2667	427	2667	6933
100,000 probes	100×	13	53	160	213	547	1333	213	1333	3467
300,000 probes	100×	4	18	53	71	182	444	71	444	1156

a. Mid, mid output; High, high output; NR, not recommended.

b. Custom panel sample calculations assume the indicated target coverage depths using 120 bp double-stranded DNA oligo panels for target capture (Illumina Custom Enrichment Panel v2) at 80% enrichment efficiency and 2 × 101 bp read length chemistry. Number of samples may vary depending on workflow handling, input sample or library quality, and actual sequencing output of each platform and flow cell.

Accurate data

Illumina DNA Prep with Enrichment produces highly uniform and consistent insert sizes across a wide DNA input range, delivering uniform and consistent library yields.[1] It also provides high coverage uniformity and padded read enrichment for custom, fixed, and exome panels (Figure 4). Illumina DNA Prep with Enrichment enables accurate single nucleotide variant (SNV) (Figure 5, Table 3) and insertion/deletion (indel) recall and precision, as compared to other Illumina enrichment solutions.

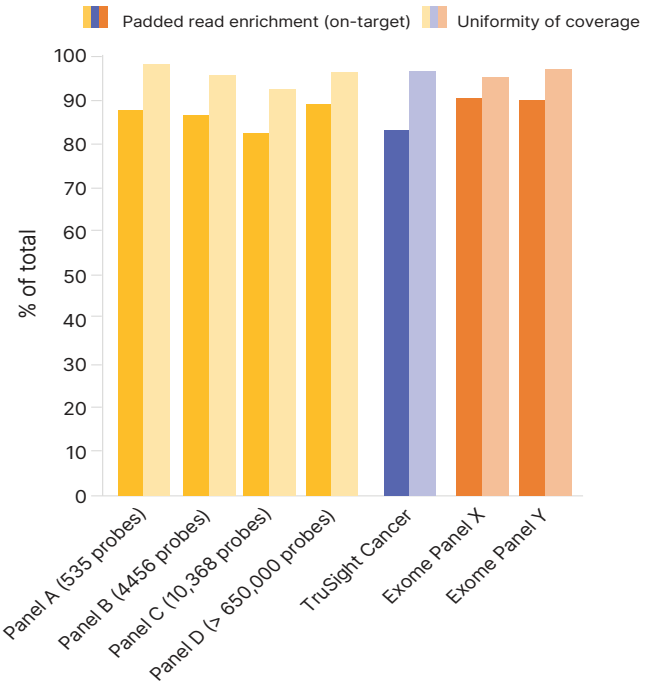

Figure 4: High coverage uniformity and padded read enrichment—Illumina DNA Prep with Enrichment provides high coverage uniformity and on-target padded read enrichment for custom (Panel A–D), fixed (TruSight Cancer), and exome panels.

Illumina custom enrichment panels

Customized enrichment panels from Illumina provide fast, flexible content for multiple targeted enrichment workflows. Design complete custom panels, spike-in panels for addition to exome or other fixed panels, or modify a panel design to suit your needs. Design content using DesignStudio™, a free online tool and create panels specific for your content of interest. Receive dynamic feedback during design to optimize coverage. Design support for non-human content is enabled through the Illumina Concierge design team. The newest probe format available, Illumina Custom Enrichment Panel v2, allows 120-bp, double-stranded probes that provide high on-target enrichment and uniformity, and are compatible with Illumina DNA Prep with Enrichment as well as other enrichment preps (Figure 6, Table 5).

DNA enrichment for a broad range of applications

By combining exceptional enrichment performance and the proven accuracy of Illumina sequencing by synthesis (SBS) and XLEAP-SBS™ chemistry, the Illumina DNA Prep with Enrichment solution supports both fixed and custom panels of varying sizes, including those designed for whole-exome sequencing, for advanced study designs in a variety of areas (Figure 7). Furthermore, Illumina DNA Prep with Enrichment is compatible with Illumina and third-party enrichment probes/panels, enabling content portability for increased flexibility.

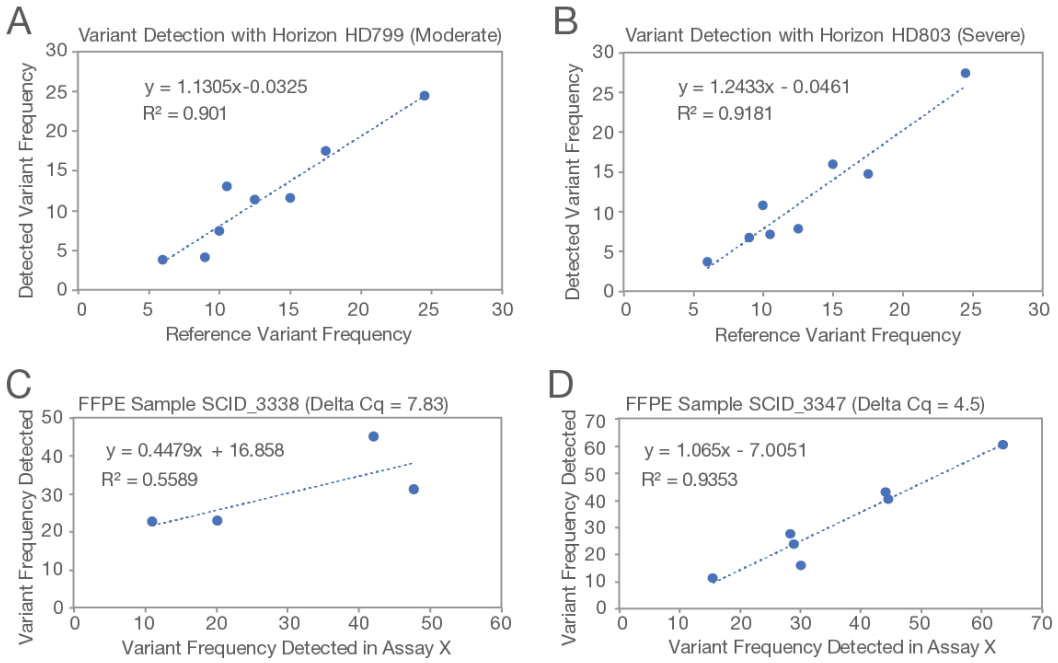

Figure 5: Accurate variant calling —Illumina DNa Prep with enrichment provides low abundance somatic variant calling for (a, B) cell line FFPe control human reference DNa samples and (c, D) real-world FFPe samples with observed variant frequency showing significant correlation with frequencies from an orthologous sequencing assay.

Table 5: Illumina custom enrichment panels

Parameter	Illumina Custom Enrichment Panel	Illumina Custom Enrichment Panel v2
Panel size	2000–67,000 probes	100–1,000,000 probes
Oligo format	80-mer single-stranded oligo probes	120 bp double-stranded oligo probes[a]
> 99.9% probes present, QC confirmed[b]	No	Yes
Compatible Illumina library preparation kits	Illumina DNA Prep with Enrichment	Illumina DNA Prep with Enrichment Illumina Cell-Free DNA Prep with Enrichment Illumina DNA Prep with Exome 2.5 Enrichment Illumina Complete Long Reads with Enrichment
Panel design tool	DesignStudio or Concierge design team	DesignStudio or Concierge design team
Turnaround time	5–7 weeks	3–6 weeks
Panel cost	$$$	$

a. 80 bp double-stranded oligo probes for microbial sequencing applications can be designed through the Concierge design team.
b. Confirmed by next-generation sequencing (NGS).

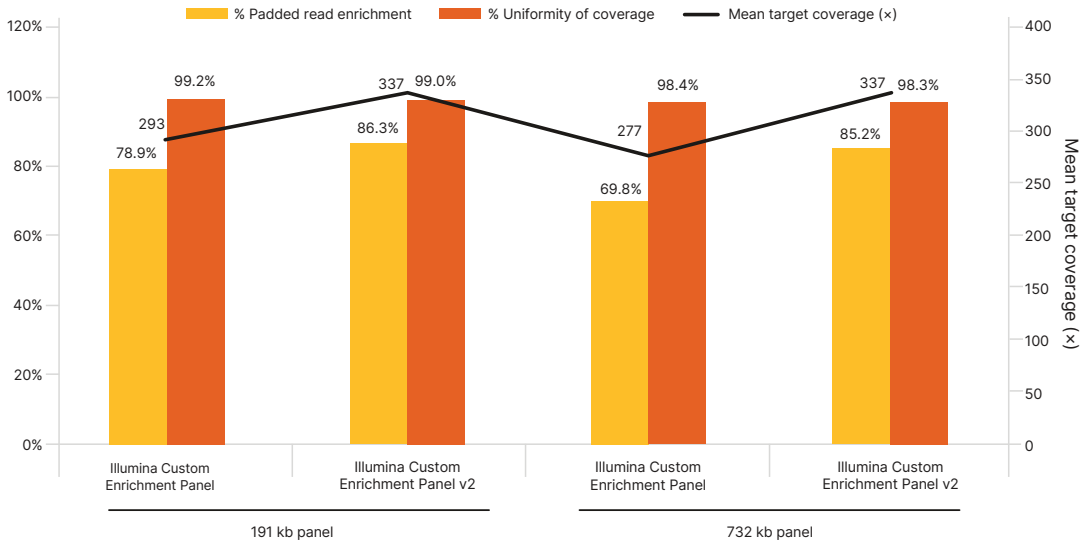

Figure 6: Illumina Custom Enrichment Panel performance with Illumina DNA Prep with Enrichment—Illumina Custom Enrichment Panel v2 with Illumina DNA Prep with Enrichment provides higher padded read enrichment compared to Illumina Custom Enrichment Panels.

Table 6: Broad range of applications with Illumina DNA Prep with Enrichment

Panel type	Application
Fixed panels	TruSight Cancer TruSight Hereditary Cancer
Custom panels	Create enrichment panels specific to your targets of interest Develop new custom panels in the DesignStudio tool Use Illumina or third-party panels between 500 and 675,000 single- or double-stranded biotinylated probes Compatible with single- or double-stranded 80-mer or 120-mer oligos Use the DesignStudio tool to order Illumina Custom Enrichment Panel and Illumina Custom Enrichment Panel v2
Whole-exome panels	Illumina Exome Panel Data sets are available for the Illumina Exome Panel and third-party exome panels in BaseSpace Sequence Hub

Summary

Illumina DNA Prep with Enrichment features the fastest workflow in the Illumina enrichment portfolio. The user-friendly, automation-compatible solution supports users of all experience levels and provides a common workflow for a variety of experimental designs, including fixed panels, custom panels, and whole-exome sequencing. On-bead tagmentation enables use of a wide range of DNA input amounts and various sample types. Illumina DNA Prep with Enrichment is compatible with Illumina and third-party enrichment probes/panels, enabling content portability. The innovative Illumina DNA Prep with Enrichment solution combined with the power of Illumina SBS chemistry provides an optimal targeted enrichment and exome sequencing experience.

Learn More

Illumina DNA Prep with Enrichment

On-bead tagmentation

Illumina Custom Enrichment Panels

DesignStudio tool

Ordering information

Product	Catalog no.
Illumina DNA Prep with Enrichment, (S) Tagmentation (96 samples)[a]	20025524
Illumina DNA Prep with Enrichment, (S) Tagmentation (16 samples)[a]	20025523
Illumina DNA Prep, (S) Tagmentation (96 samples)[b]	20025520
Illumina DNA Prep, (S) Tagmentation (16 samples)[b]	20025519
Flex Lysis Reagent Kit	20018706
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)	20091660
TruSight Cancer (8 enrichment reactions)	FC-121-0202
TruSeq Hereditary Cancer (8 enrichment reactions)	20029551
Illumina Custom Enrichment Panel v2 (32 µl, 120 bp)[c]	20073953
Illumina Custom Enrichment Panel v2 (384 µl, 120 bp)[c]	20073952
Illumina Custom Enrichment Panel v2 (1536 µl, 120 bp)[c]	20111339
Illumina Custom Enrichment Panel[c]	20025371

a. Kits include library preparation and hybridization reagents for 8 hybridization reactions at 12 samples per hybridization (12-plex). Purification beads and index adapter plate are purchased separately.
b. Kits include library preparation reagents only and should be paired with the complete library preperation and hybridization kits to support alternative hybridization plexities.
c. Custom enrichment panels for human samples can be designed through the Illumina DesignStudio tool. Design support for nonhuman content is enabled through the Illumina Concierge design team. Contact your Illumina sales representative for more information about Concierge design services.

References

1. Illumina. Illumina DNA Prep Data Sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/illumina-dna-prep-data-sheet-m-gl-10373/illumina-dna-prep-data-sheet-m-gl-10373.pdf. Updated 2022. Accessed August 17, 2023.
2. Illumina. BWA Enrichment v2.1 BaseSpace App Guide. support.illumina.com/content/dam/illumina-support/documents/documentation/software_documentation/basespace/basespace-bwa-enrichment-v2-1-app-guide-15050958-01.pdf. Updated 2016. Accessed September 22, 2023.

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