Advanta NGS Library Prep Reagent Kits with TSP Sample Loading Reagent v2

July 2018

The Advanta™ family of products for genomic analysis using Fluidigm integrated fluidic circuit (IFC) technology includes a growing portfolio of predesigned panels and assays for disease research, as well as reagent kits for next-generation sequencing (NGS) library preparation using the Juno™ platform.

In an ongoing effort to offer the most flexible and robust products for targeted NGS applications, a new formulation of the Targeted DNA Seq Library Preparation (TSP) Sample Loading Reagent v2 is now a component of the Advanta NGS Library Prep Reagent Kits. The new TSP Sample Loading Reagent v2 formulation, in conjunction with a minor protocol modification (increasing the PCR sample reaction mix volume from 5 µL to 6.25 µL), helps minimize bubble generation during sample transfer, thereby improving sample transfer from 96-well plates to IFCs.

TSP Sample Loading Reagent v2 (PN 101-7633 and 101-7634) now replaces the original TSP Sample Loading Reagent (PN 101-0407 and 101-3049) in the TSP workflows to help ensure optimal sample loading and performance.

All Advanta NGS Library Reagent Kit configurations include the new TSP Sample Loading Reagent v2 and the 4X Master Mix. **Please note, the currently offered LP 192.24 and LP 48.48 IFC Targeted DNA Seq Library Reagent Kits will be discontinued after November 30, 2018.**

Summary of Changes

Sample Loading Reagent v2: The revision to the TSP Sample Loading Reagent addresses the potential for bubbles to develop when inefficient sample mixture loading has occurred. The effect was observed in a few isolated instances when running the protocol for Targeted DNA Seq Library Preparation using the LP 48.48 IFC.

To improve loading efficiency of the LP IFCs, viscosity of the TSP Sample Loading Reagent v2 has been reduced. The lower viscosity of the TSP Sample Loading Reagent v2 improves sample mix usability and enables more efficient transfer of sample mix to the IFC by reducing bubble formation. This also improves IFC loading efficiency and can provide more successful sample analysis. Table 1 summarizes the results of sample loading efficiency experiments using 20 IFCs and TSP Sample Loading Reagent v2.

Table 1: Sample Load Efficency Experiments

| IFC | Operator | IFC Type | Bubbles | | | Successful Sample Loads |
			In Sample Plate Before Spin	In Sample Plate After Spin (Updated Protocol)	In IFC	
1	A	48.48	20	0	0	47/48
2	A	48.48	0	0	0	47/48
3	B	48.48	0	0	0	48/48
4	B	48.48	0	0	0	48/48
5	C	48.48	10	0	0	48/48
6	C	48.48	14	0	0	48/48
7	D	48.48	0	0	0	48/48
8	D	48.48	0	0	0	48/48
9	C	48.48	0	0	0	48/48
10	C	48.48	0	0	0	48/48
11	D	48.48	0	0	0	48/48
12	D	48.48	0	0	0	48/48
13	C	48.48	0	0	0	48/48
14	C	48.48	0	0	0	48/48
15	D	48.48	0	0	0	48/48
16	D	48.48	0	0	0	48/48
17	C	192.24	>50	0	1	48/48
18	C	192.24	>50	0	0	48/48
19	D	192.24	0	0	0	48/48
20	D	192.24	0	0	0	48/48
		1,536 Total Samples				**1,534/1,536 (99.9%)**

Three experiments were performed comparing samples loaded into IFCs using the existing TSP Sample Loading Reagent and TSP Sample Loading Reagent v2. Extensive protocol testing demonstrated that the TSP Sample Loading Reagent v2 (PN 101-7634) resulted in comparable performance relative to samples analyzed using the current TSP Sample Loading Reagent (PNs 101-0407 and 101-3049). Tables 2–4 summarize the results from the experiments.

Table 2: TSP Sample Loading Reagent Performance Comparison with LP 48.48 and 1,508 Assays

(4 IFCs; 8 samples in replicate; 1,508 assays)

Criteria	Target Value	Average Value ± SD	
		Current TSP Sample Loading Reagent	TSP Sample Loading Reagent v2
Mapping rate to genome	>90%	99.3% ±0.08%	99.2% ±0.13%
Mapping rate to target from reads mapped to genome	>95%	97.09% ±0.60%	96.14% ±0.69%
Assay uniformity (25–65% GC)	>85%	97.03% ±0.38%	96.73% ±1.58%
Error rate	<0.5%	0.04% ±1 x 10^{-5}	0.04% ±1 x 10^{-5}
FDR at ≥5% AF	≤10^{-5}	1.08 x 10^{-6} ±2.65 x 10^{-6}	1.22 x 10^{-6} ±1.98 x 10^{-6}
Cross-contamination between samples	95% of NTCs <0.5% (no NTC >3%)	0.12%	0.07%
Mutation detection sensitivity*	≤5%	3.1% ±0.17%	3.3% ±0.35%

* Mutation detection sensitivity is defined as the minimal variant allele frequency detected at false discovery rate of 10^{-5} and confidence level of 95%.

Table 3: TSP Sample Loading Reagent Performance Comparison with LP 48.48 and 6,062 Assays

(4 IFCs; 1 sample in replicate; 6,062 assays)

Criteria	Target Value	Average Value ± SD	
		Current TSP Sample Loading Reagent	TSP Sample Loading Reagent v2
Mapping rate to genome	>90%	98.04% ±0.19%	98.3% ±0.1
Mapping rate to target from reads mapped to genome	>95%	95.76% ±0.47%	95.3% ±0.6%
Assay uniformity (25–65% GC)	>85%	90.53% ±0.79%	92.1% ±0.8%
Error rate	<0.5%	0.060% ±0.007%	0.04%
FDR at ≥5% AF	$\leq 10^{-5}$	8.43×10^{-7} ±5.77×10^{-7}	4.50×10^{-6}
Cross-contamination between samples	95% of NTCs <0.5%	0.019% ±0.025%	0.08% ±0.5%
Mutation detection sensitivity*	≤10%	3.78% ±0.16%	4.9% ±0.15%

* Mutation detection sensitivity is defined as the minimal variant allele frequency detected at false discovery rate of 10^{-5} and confidence level of 95%.

Table 4: TSP Sample Loading Reagent Performance Comparison with LP 192.24 and 2,670 Assays

(4 IFCs; 1 sample in replicate; 2,670 assays)

Criteria	Specifications	Average Value ± SD	
		Current TSP Sample Loading Reagent	TSP Sample Loading Reagent v2
Mapping rate to genome	>90%	91.34% ±0.53%	98.3% ±0.1%
Mapping rate to target from reads mapped to genome	>95%	96.39% ±0.41%	96.1% ±0.5%
Assay uniformity (25–65% GC)	>85%	89.00% ±0.93%	91.0% ±0.7%
Error rate	<0.5%	0.053% ±0.004%	0.17% ±0.01%
Cross-contamination between samples	<0.5%	0.012% ±0.014%	0.04% ±0.1%
Mutation detection sensitivity*	≤20%	6.33% ±0.43%	6.3% ±0.5%

* Mutation detection sensitivity is defined as the minimal variant allele frequency detected at false discovery rate of 10^{-5} and confidence level of 95%.

4X Master Mix: All Advanta NGS Library Prep Reagent Kits include 4X Master Mix. The 2X Master Mix component included in the current NGS Library Prep Reagent Kit offerings will be discontinued. Use of 4X TSP Master Mix in the Targeted DNA Seq Library Preparation protocol using the LP 192.24 IFC allows users to input 2 µL of samples with lower DNA concentrations when using the LP 192.24 IFC. The formulation of 4X and 2X Master Mixes is the same except for the concentration. Internal validation testing of 4X TSP Master Mix (PN 101-3050, 101-3055, 101-5786) has demonstrated equivalent performance to the TSP Master Mix (PN 101-0994).

The 4X TSP Master Mix is included in reagent kits used with LP 192.24 IFCs, thereby harmonizing protocols across LP 192.24 and LP 48.48 IFCs.

Changes to Targeted DNA Sequencing Library Preparation Protocol

In the Prepare the Sample Pre-Mix section of the protocol, the TSP Sample Loading Reagent v2 (PN 101-7633, 101-7634) replaces the original TSP Sample Loading Reagent (PN 101-0407, 101-3049). All other reagents remain unchanged. In addition, we have increased the sample mix volumes for the LP 48.48 IFC protocol from 5 µL to 6.25 µL to increase overage and make the transfer of the sample mix to IFC easier. The volume of all components in the sample mix has been adjusted to maintain the same sample-to-reagent ratio.

Below is a list of existing and new kit part numbers as a reference for future orders. The current Targeted DNA Seq Library Preparation Reagent kits will be discontinued after November 30, 2018. Refer to Table 5 for list of affected part numbers.

Table 5: Current and New Reagent Kit Part Numbers

Kits to be Discontinued		New Kits (Commercially Available from July 2018)	
Part Number	Product Name	Part Number	Product Name
101-2773	192.24 Targeted DNA Seq Library Reagent Kit—2 IFCs	101-7667	Advanta NGS Library Prep Reagent Kit—LP 192.24, 2 IFCs
101-5669	192.24 Targeted DNA Seq Library Reagent Kit v2—2 IFCs		
101-0406	192.24 Targeted DNA Seq Library Reagent Kit—10 IFCs	101-7669	Advanta NGS Library Reagent Kit—LP 192.24, 10 IFCs
101-5668	192.24 Targeted DNA Seq Library Reagent Kit v2—10 IFCs		
101-3052	Targeted DNA Seq Library Reagent Kit—48.48, 2 IFCs	101-7663	Advanta NGS Library Prep Reagent Kit—LP 48.48, 2 IFCs
101-3089	Kit, Targeted DNA Seq Library Reagent Bundle—48.48, 10 IFCs	101-7666	Advanta NGS Library Prep Reagent Bundle—LP 48.48, 10 IFCs

Related Documentation

Part Number	Document Name
101-7885	Advanta NGS Library Preparation with Access Array Protocol
101-7886	Advanta NGS Library Preparation on the LP 48.48 IFC with Access Array Quick Reference
101-7878	Advanta NGS Library Preparation with Juno Protocol
101-7879	Advanta NGS Library Preparation on the LP 48.48 with Juno Quick Reference
101-7880	Advanta NGS Library Preparation on the LP 192.24 with Juno Quick Reference

Juno and Access Array Systems and System Software

There are no changes to the Juno or Access Array™ systems, IFCs, or system software.

Suggested Actions

- Review changes in the Targeted Sequencing Preparation with Juno Getting Started Guide (PN 101-0414), Targeted DNA Sequencing Library Preparation with Access Array User Guide (PN 101-2737), and related quick references.

- Implement the new protocol to improve sample loading.

- Save this notification for your records.

To minimize any disruption of your laboratory operations, the previous versions of the Targeted DNA Seq Library Reagent Kits (PN 101-0406, 101-2773, 101-3052, 101-3089, 101-5668, 101-5669) will continue to be available until **November 30, 2018**, to accommodate your transition to the new Advanta NGS Library Prep Reagent Kits (PN 101-7663, 101-7666, 101-7667, 101-7669).

Final orders for previous part numbers PN 101-2773 and PN 101-0406 must be received by November 16, 2018, to enable final shipments by November 30, 2018.

Your Fluidigm account manager will reach out to you in the coming days to answer any questions you may have about the new Advanta NGS Library Prep Reagent Kits or the transition.

If you have questions, please contact your Fluidigm account manager or Tech Support at techsupport@fluidigm.com.