TECHNICAL NOTE

Qualification update for Infinium™ BeadChips

Functional testing showed comparable performance in BeadChips fabricated using either single-sided or double-sided polished wafers

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Introduction

Infinium arrays use silica microbeads as a substrate for oligonucleotide probes to assay millions of genotypes simultaneously with high sensitivity.[1] These silica beads, coated with multiple copies of locus-specific probes, are housed in microwells etched on the surface of each BeadChip (Figure 1). Raw silicon wafers, polished with a mirrored finish on one or both sides, are used to fabricate BeadChips. Wafer polishing is highly effective for removing surface damage and ensuring atomic flatness, allowing flexible, scalable, and customizable manufacturing of Infinium BeadChips.

Currently, Infinium arrays use double-sided polished (DSP) wafers for BeadChip fabrication. To simplify the use of BeadChips while maintaining the high-performance standards of Infinium arrays, all Infinium BeadChips will be moving from DSP to single-sided polished (SSP) wafer material. This change will only affect the flip side of the BeadChip, which will appear unpolished, while the functional side of the array with etched microwells will be polished. The distinct mirrored appearance of the SSP array allows users to identify the bead-loaded side of the BeadChip easily, minimizing the potential for user error during flow-through chamber assembly.

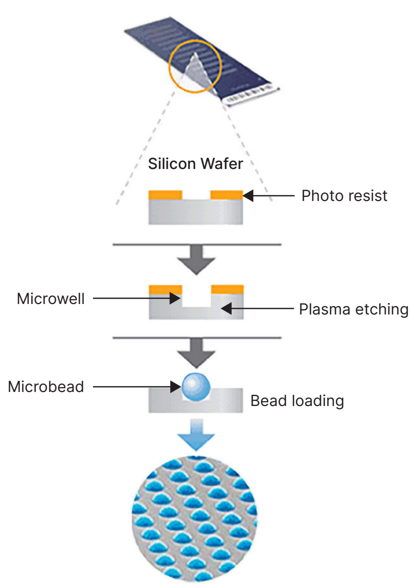

Figure 1: Infinium BeadChip construction—Illumina microarray technology uses silica microbeads that self-assemble in microwells on planar silica slides fabricated using raw silicon wafer material.

This technical note details the validation testing performed on SSP wafer material and demonstrates comparable performance between BeadChips built with SSP and DSP wafers. SSP qualification data are presented for Infinium Global Diversity Array with Enhanced PGx, Infinium Global Screening Array-24, Infinium XT 96-Sample QC Array, Infinium CytoSNP-850K v1.2, and Infinium MethylationEPIC BeadChips. Data are available on file.[2]

SSP qualification for Infinium Global Diversity Array with Enhanced PGx

Two lots of five BeadChips were built for Infinium Global Diversity Array with Enhanced PGx as DSP controls. SSP test BeadChips were built from two raw wafer suppliers, Company S (SSP-S) and Company A (SSP-A). One lot of 15 BeadChips from each supplier was tested. The same bead pool rack (BPR) was used for test and control arrays. All BeadChips followed the current array production process to image production build. Functional performance of DSP control and SSP test BeadChips was compared using the following
GenomeStudio™ Software metrics:

- Percentage of loci that a genotype was called for in a sample (call rate)
- Deviation in the normalized intensity value (LogR Dev)
- Mean probe intensity (R Avg)

Both test and control BeadChips met the product specifications for Infinium Global Diversity Array with Enhanced PGx. The overall yield for both BeadChips was similar, at 93.3% for DSP and 96.7% for SSP BeadChips. Decoded yield was 99.9% for DSP and 96.7% for SSP BeadChips. Real-time metrics and decoded metrics for SSP BeadChips were comparable to the DSP BeadChip performance with no statistically significant difference observed.

The BeadChip built using SSP raw wafer met the decoded metric specification requirement for Infinium Global Diversity Array with Enhanced PGx. Metrics, including percent bead (Pbead) and percent bead in used (Pused), were improved over control DSP data (Table 1).

Table 1: Summary of decoded metrics for Global Diversity Array with Enhanced PGx

Product	Substrate type	Pbead	Pused (≥ 65%)	Pbt (≥ 99.5%)	Miscall (≤ 0.004)
Infinium Global Diversity Array with Enhanced PGx	DSP Control	99.48%	86.40%	99.950%	0.000588
	SSP Test	99.83%	92.70%	99.950%	0.000563

Pbead indicates percent bead, Pused indicates percent bead in used, Pbt indicates percent bead type

This difference is potentially due to the 8-inch size of the SSP wafer, which provides better stability during the manufacturing process than 6-inch DSP wafers. This stability translates to improved ‘fit’ of the beads within microwells, enabling efficient handling in microfluidic systems without bead detachment. There was no significant difference between DSP and SSP percent bead type (Pbt) and miscall performance. Functional assay metrics were assessed for all probes, targeted gene amplification (TGA) probes, and copy number variation (CNV) probes (Figure 2). The cut-off for call rate was ≥ 0.99 and for LogR Dev was < 0.30.[3] All SSP and DSP BeadChips met acceptance criteria for functional metrics. The mean call rate for DSP wafer BeadChips was 0.9985 compared to 0.9993 for SSP wafer BeadChips. Mean LogR Dev for DSP BeadChips was 0.1327 compared to 0.1232 for SSP BeadChips. PGx-specific analyses support no decrease in the performance from DSP control to SSP test BeadChips.

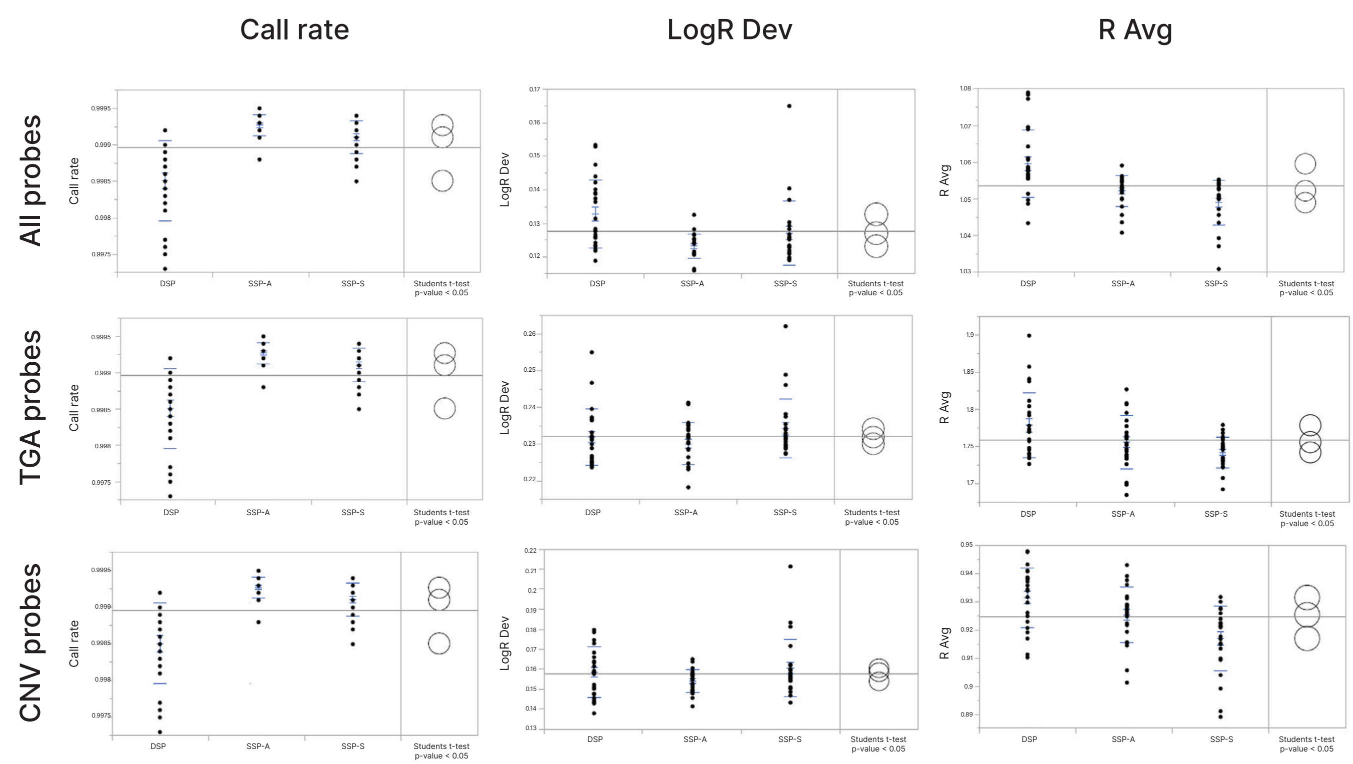

Figure 2: Functional performance of Infinium Global Diversity Arrays with PGx fabricated using DSP control and SSP test BeadChips—Functional performance of DSP control and SSP test BeadChips was compared using three key GenomeStudio Software metrics. Data were generated for call rate, LogR deviation (LogR Dev), and average probe intensity (R Avg). There was no statistically significant difference between functional metrics tested in DSP and SSP BeadChips.

SSP qualification for Infinium Global Screening Array-24

DSP control BeadChips were built from three lots of 20 BeadChips for Infinium Global Screening Array-24. SSP test BeadChips were built from three raw wafer suppliers, Company S (SSP-S), Company A (SSP-A), and Company O (SSP-O). One lot of 20 BeadChips from each supplier was tested. BeadChips were built over two days with the same BPR used for test and control samples on each individual build day. All BeadChips tested followed the current array production process to image production build.

The overall yield and decoded yield for both DSP and SSP BeadChips was 99.9%. Real-time metrics and decoded metrics for SSP BeadChips were comparable to the DSP BeadChip performance with no statistically significant difference observed. Both test and control BeadChips met the product specifications for Infinium Global Screening Array-24 (Table 2).

Maintaining an optimal temperature on the BeadChip surface is crucial for hybridization to occur. BeadChip surface temperature was assessed after exposing one DSP control and three SSP test BeadChips to a Watson heater stage for 15 minutes. Measurements were taken across five Watson stages and the same instrument was used to measure temperature for DSP and SSP BeadChips. Temperature comparison between SSP and DSP BeadChips showed that SSP does not affect thermal conduction from the Watson heater stage to the BeadChip. Surface temperature was within the tolerance limit of 40±1°C for all BeadChips tested (Figure 3). These findings are applicable to all Infinium BeadChips because the primary factor affecting thermal conduction from the Watson stage heater is the total surface area of the BeadChip in contact with the flow cell carrier, which is constant across all BeadChips.

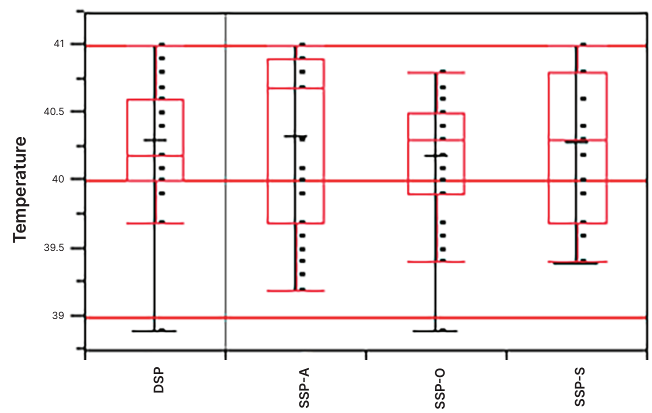

Figure 3: Thermal conduction for Infinium Global Screening Array fabricated using DSP control and SSP test BeadChips—Temperature was measured for BeadChips built using DSP and SSP wafer material following 15-minute exposure to a Watson stage heater. One DSP and three SSP BeadChips were tested, and a total of 23 temperature readings were taken for this assessment.

All BeadChips tested passed the call rate criteria of > 0.9900 with an average call rate of 99.5%. There was no significant difference between call rates for SSP test and DSP control BeadChips. For LogR Dev, SSP test BeadChips were superior to DSP control BeadChips, with significantly lower LogR Dev values compared to DSP BeadChips. All samples passed LogR Dev product specification criteria of < 0.3000. All BeadChips being tested passed the reproducibility criteria of > 99.9% with a mean value of 99.90%.

Table 2: Summary of decoded metrics for Infinium Global Screening Array-24

Product	Substrate type	Pbead	Pused (≥ 65%)	Pbt (≥ 99.5%)	Miscall (≤ 0.004)
Infinium Global Screening Array	DSP control	99.90%	97.55%	99.998%	0.000064
	SSP test	99.92%	97.50%	99.995%	0.000082

Pbead indicates percent bead, Pused indicates percent bead in used, Pbt indicates percent bead type

A high degree of correlation (R^2 > 99%) was observed between theta values for DSP control and SSP test BeadChips, confirming that SSP wafer material does not affect BeadChip performance (Figure 4).

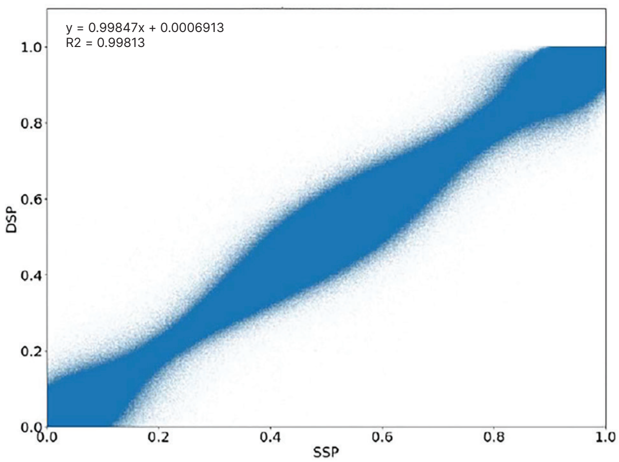

Figure 4: Correlation between assay test data obtained from BeadChips fabricated using DSP and SSP wafer material—Theta AA, theta AB, and theta BB were plotted for DSP and SSP BeadChips showing a correlation > 99%. This high degree of correlation demonstrates that SSP wafers material does not affect BeadChip performance.

SSP qualification for Infinium CytoSNP-850K v1.2 BeadChip

DSP control BeadChips were built from two lots of six BeadChips for Infinium CytoSNP-850K v1.2. SSP test BeadChips were built from two raw wafer suppliers, Company S (SSP-S) and Company A (SSP-A). One lot of six BeadChips from each supplier was tested. The same BPR was used for test and control arrays. All BeadChips tested followed the current array production process to image production build. BeadChip performance was assessed on the iScan™ and NextSeq™ 550 systems.

All BeadChips tested passed the call rate criteria of > 0.9900 with an average call rate of 99.9%. There was no significant difference between call rates for SSP test and DSP control BeadChips. SSP test BeadChips were superior to DSP control BeadChips for LogR Dev values. All samples passed LogR Dev product specification criteria of < 0.3000 with a mean LogR Dev value of 0.1875. All BeadChips being tested passed the reproducibility criteria of > 99% with a mean value of 99.99%.

These results demonstrate that functional assay performance was unaffected by the SSP wafer material used for fabricating Infinium CytoSNP-850K v1.2 BeadChips.

SSP qualification for Infinium XT 96-Sample QC Array

DSP control BeadChips were built from two lots of 15 BeadChips for Infinium XT 96-sample QC Array. SSP test BeadChips were built from a single raw wafer supplier, Company S (SSP-S). One lot of four control DSP and eight test SSP BeadChips was tested. The same BPR was used for test and control arrays. All BeadChips tested followed the current array production process to image production build.

The overall yield and decoded yield for both DSP and SSP BeadChips was 93.3%. Real-time metrics and decoded metrics for SSP BeadChips were comparable to the DSP BeadChip performance with no statistically significant difference observed. Both test and control BeadChips met the product specifications for Infinium XT 96-Sample QC Array (Table 3).

All BeadChips tested passed the call rate criteria of > 0.9900 with an average call rate of 99.7%. There was no significant difference between call rates for SSP test and DSP control BeadChips. For LogR Dev, SSP test BeadChips were comparable to DSP control BeadChips, with LogR Dev values of 0.1692 for DSP controls and 0.1706 for SSP test BeadChips. All samples passed the LogR Dev product specification criteria of < 0.3000. All BeadChips being tested passed the reproducibility criteria of > 99.9% with a mean value of 99.99%.

Overall, BeadChips built from SSP raw wafer material met all product specification requirements for Infinium XT 96-Sample QC Arrays, demonstrating that the change to SSP wafers did not affect the functional performance of BeadChips.

Table 3: Summary of decoded metrics for Infinium XT 96-Sample QC Array

Product	Substrate type	Pbead	Pused (≥ 65%)	Pbt (≥ 99.5%)	Miscall (≤ 0.004)
Infinium XT 96-Sample QC Array	DSP control	99.93	96.64	99.982	0.000094
	SSP test	99.92	95.15	99.981	0.000103

Pbead indicates percent bead, Pused indicates percent bead in used, Pbt indicates percent bead type

SSP qualification for Infinium MethylationEPIC BeadChips

Four lots of eight BeadChips were built as DSP controls for Infinium MethylationEPIC BeadChips. SSP test BeadChips were built from two raw wafer suppliers, Company S (SSP-S) and Company A (SSP-A). Four lots of eight BeadChips from each supplier were tested. The same BPR was used for test and control arrays. All BeadChips followed the current array production process to image production build.

The number of CpG sites detected for DSP control and SSP test BeadChips was assessed using the iScan System. The mean of all samples passed the CpG coverage requirement of ≥ 96% for the standard protocol and ≥ 90% for the formalin-fixed paraffin embedded (FFPE) sample protocol at a detection threshold p-value ≤ 0.05. The mean CpG coverage for SSP test and DSP control BeadChips was 99.91% (Figure 5).

All BeadChips being tested passed the reproducibility criteria of ≥ 98% for both the standard and FFPE protocols, with a mean value of 98.9% for SSP test and 99.0% for DSP control BeadChips.

A high degree of correlation (R^2 > 99.93%) was observed between beta values for DSP control and SSP test BeadChips, confirming that SSP wafer material does not affect BeadChip performance (Figure 6).

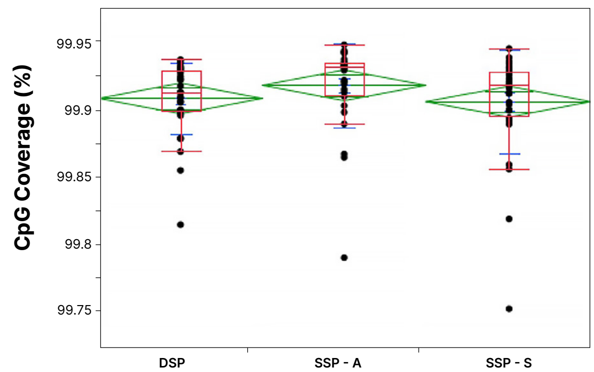

Figure 5: CpG site coverage for Infinium MethylationEPIC BeadChips fabricated using DSP and SSP raw wafer material—The number of CpG sites detected at p-value ≤ 0.05. There was no statistically significant difference between BeadChips built using SSP and DSP wafer material.

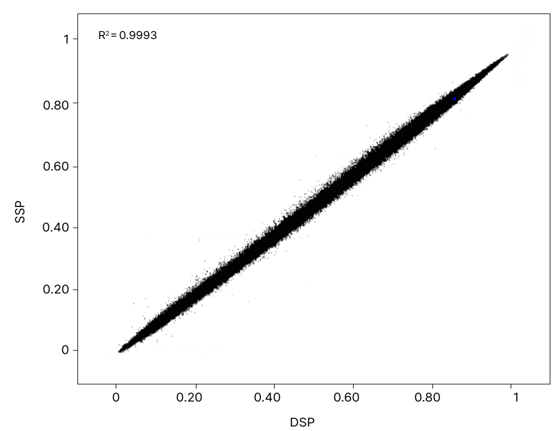

Figure 6: Correlation between assay test data obtained from BeadChips fabricated using DSP and SSP wafer material—Beta coefficients were plotted for DSP and SSP BeadChips showing a correlation > 99%. This high degree of correlation demonstrates that SSP wafers material does not affect BeadChip performance.

Summary

The data presented in this technical note confirm that functional performance for all tested metrics was not significantly different between BeadChips fabricated with either DSP or SSP raw wafer material. Decoded metrics for SSP BeadChips were improved over historical DSP BeadChip metrics, possibly due to greater stability of the SSP raw wafer material during the fabrication process. In conclusion, BeadChips built using SSP raw wafer material provide a flexible, scalable, and easy-to-use platform for Infinium arrays.

Learn more

Illumina Microarray Technology, illumina.com/science/technology/microarray

Illumina Microarray Solutions, illumina.com/techniques/microarrays

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