Improved Performance of Gene Expression BeadChips Using the Updated Wash Procedure

Introduction

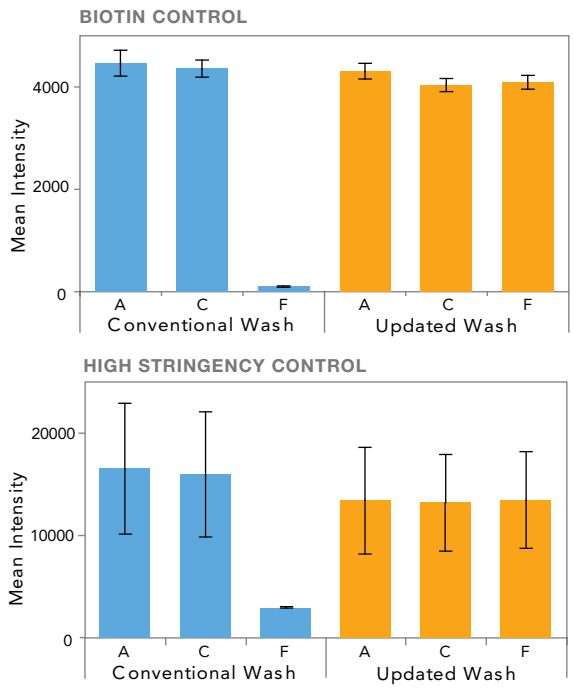
Illumina Whole-Genome Gene Expression Profiling BeadChips accommodate loading six to 12 samples onto a single BeadChip. The IntelliHybTM seal permits multiple independent samples to be hybridized accurately and reproducibly on the same array. After hybridization, the seal is removed and all samples on the BeadChip are stained in parallel. Under certain laboratory conditions, using the conventional seal removal procedure results in a gradient of sample evaporation across the BeadChip. Data quality and consistency is improved by using an updated BeadChip wash procedure1.

Comparison of Wash Procedures

According to the conventional BeadChip wash procedure, the IntelliHyb seal is removed from the BeadChip after hybridization. At this point, the surface of the BeadChip is briefly exposed to air before it is submerged in wash buffer. This brief air exposure may result in uneven evaporation across the surface of the BeadChip and could cause the sample loaded closest to the BeadChip barcode to display reduced signal intensity for the biotin, hybridization, and stringency controls. Likewise, hybridization signals from samples loaded on the last BeadChip positions may correlate poorly with replicates loaded at other positions.

In the updated wash procedure, the BeadChip is submerged in a dish containing wash buffer, and then the seal is removed from the submerged BeadChip1. Removing the seal under buffer eliminates the possibility of evaporation across the BeadChip surface. After the seal is removed, array processing continues as in the conventional procedure.

Figure 1: Positional Dependence of Control Signal for Conventional Wash Compared to Updated Wash



Positional dependence of control signal intensity for conventional (blue) and updated (orange) wash procedures. The mean signal intensities of the biotin (top) and high stringency (bottom) controls are shown for SUMR reference RNA samples hybridized to positions A, C, and F of Mouse-6 Expression BeadChips.

Analysis of Data Quality and Consistency

To evaluate the effectiveness of the updated wash procedure, a reference RNA sample was hybridized to positions A, C, and F of four Mouse-6 Expression BeadChips. Two BeadChips were washed with the conventional protocol, and two were washed with the updated procedure. BeadChips that underwent the conventional wash had decreased signal from the biotin and hybridization controls in position F. By contrast, there was no positional dependence of control intensities for the samples washed with the updated procedure (Figure 1).

To determine whether data obtained from the two wash procedures are comparable, data correlation within and between wash procedures was calculated and compared (Figure 2). Pairwise linear r2 for all probe signals hybridized to reference RNA replicates showed strong correlation between all replicates, except those loaded in position F of the Mouse-6 Expression BeadChip (Figure 2, shaded cells). The correlation between replicate samples using either the conventional or updated washes was greater than 0.99, similar to the correlation of replicates using the same wash protocol (Table 1).

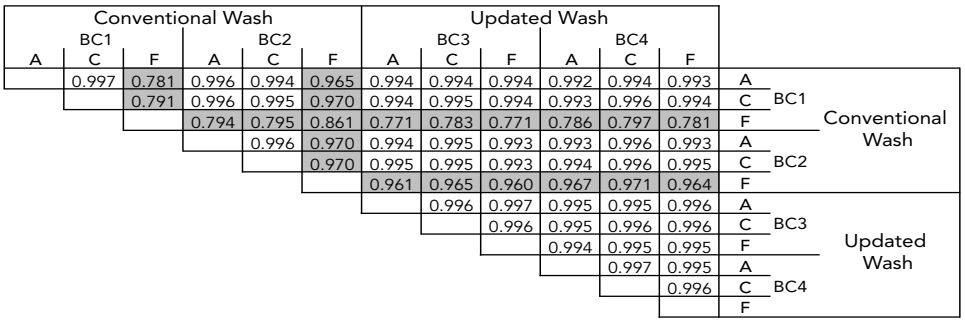
Results From Beta Test Sites

In addition to the testing conducted by Illumina, six beta test sites evaluated the updated wash procedure. More than 100 RatRef-12, Human-6, and HumanRef-8 Gene Expression BeadChips were tested. BeadChips washed with the updated procedure exhibited 100% success, and no significant positional dependence of signal intensity was observed.

Conclusions

The updated BeadChip wash protocol improves the consistency and data quality of samples loaded on the last two sections of the BeadChip. Furthermore, data generated using the updated procedure are highly correlated with data generated using the conventional wash procedure.

Figure 2: Correlation Between Wash Procedures



	BC1 A	BC1 C	BC1 F	BC2 A	BC2 C	BC2 F	BC3 A	BC3 C	BC3 F	BC4 A	BC4 C	BC4 F		
BC1 A		0.997	0.781	0.996	0.994	0.965	0.994	0.994	0.994	0.992	0.994	0.993	A	Conventional Wash
BC1 C			0.791	0.996	0.995	0.970	0.994	0.995	0.994	0.993	0.996	0.994	C	
BC1 F				0.794	0.795	0.861	0.771	0.783	0.771	0.786	0.797	0.781	F	
BC2 A					0.996	0.970	0.994	0.995	0.993	0.993	0.996	0.993	A	
BC2 C						0.970	0.995	0.995	0.993	0.994	0.996	0.995	C	
BC2 F							0.961	0.965	0.960	0.967	0.971	0.964	F	
BC3 A								0.996	0.997	0.995	0.995	0.996	A	Updated Wash
BC3 C									0.996	0.995	0.996	0.996	C	
BC3 F										0.994	0.995	0.995	F	
BC4 A											0.997	0.995	A	
BC4 C												0.996	C	
BC4 F													F	

Linear r2 correlation measurement between hybridization replicates on Mouse-6 Expression BeadChips. Only replications in position F under conventional wash conditions show low correlation values of r2 < 0.99 (shaded cells).

Table 1: Correlation Between Wash Procedures

	Conventional vs Updated	Conventional vs Updated	Updated vs Updated
Minimum r^2	0.786	0.766	0.994
Median r^2	0.970	0.994	0.996
Maximum r^2	0.996	0.996	0.997

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