Application Note

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Mosaicism Detection Sensitivity in Preimplantation Genetic Testing for Aneuploidy (PGT-A)

Estimating mosaicism levels based on log2ratio values on the Agilent GenetiSure Pre-Screen 8x60K microarray platform

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Abstract

This application note evaluates the mosaicism detection sensitivity of the Agilent GenetiSure Pre-Screen 8x60K microarray platform for Preimplantation Genetic Testing for Aneuploidy (PGT-A). To simulate varying mosaicism levels, samples with known chromosomal aberrations were mixed with normal reference DNA. Results show that the GenetiSure Pre-Screen microarray assay reliably and reproducibly detects mosaicism down to 15%. A reference table correlating observed log2ratio with mosaicism level is included, providing users the ability to estimate mosaicism levels present in samples.

Introduction

Preimplantation Genetic Testing for Aneuploidies (PGT-A) is used to screen the chromosomal composition of embryos produced by assisted reproduction technology (ART). The goal is to identify embryos without abnormalities to increase successful implantation rates and live birth rates, reduce miscarriage rates and abnormal pregnancies, and shorten time to pregnancy.[1]

The PGT-A process typically involves embryonic biopsy on the fifth day postfertilization, during which five to eight cells are removed from the trophectoderm for further downstream analysis. However, the chromosomal composition of these cells is not always uniform. Mosaicism, defined as the presence of more than one chromosomally distinct cell line in an embryo, has been a well-known phenomenon for more than three decades.[2]

The impact of mosaicism on embryo development and IVF outcomes following the transfer of mosaic embryos remains unclear and is the subject of research. Several professional societies have issued guidelines for managing chromosomal mosaicism during PGT-A.[3,4,5] In this setting, accurate estimation of mosaicism in trophectoderm biopsies is crucial for both clinical and research applications.

Commonly used methods for PGT-A include array comparative genomic hybridization (CGH) and next-generation sequencing (NGS), with NGS often cited for its higher dynamic range in mosaicism detection. However, many of these comparisons were made against older BAC-based array designs. Modern microarray platforms use oligonucleotide probes that offer higher resolution and sensitivity.

In this study, we investigated the mosaicism detection sensitivity of the Agilent GenetiSure PreScreen 8x60K oligonucleotide microarray platform and the correlation between expected and observed log2ratio values. The goal was to provide a reference for estimating mosaicism present in samples.

Experimental

Study design and samples

Analysis was performed on two sample types:

(A) DNA derived from cell lines with known chromosomal aberrations, diluted and subjected to whole-genome amplification (WGA) (Coriell Institute; Table 1, Samples NA1, NA2, and NA3).

(B) DNA from de-identified blastocyst-stage embryo biopsies with known chromosomal aberrations, also subjected to WGA (Table 2, Samples M2 and P5).

Table 1. Coriell Institute sample IDs and known chromosomal aberrations according to ISCN.

Sample ID	Known Aberration (ISCN)
Sample NA1-NA02732	Trisomy 18 (47,XY,+18)
Sample NA2-NA07312	~16 Mb loss on 13q (46,XX,del(13)(q14q21) arr 13q14.11q21.2(43649072-59785590)x1
Sample NA3-NA09189	~5.3 Mb loss on 15q (46,XX,inv(9),del(15)(q11.2q13). ish del(15)(q11.2q13)(D15S63-,ICBD3-,SNRPN-,PAR-5- ,154P1+).arr 15q11.2q13.1(21244597-26500067)x1)

Table 2. Trophectoderm biopsy samples and detected aberrations using the Agilent GenetiSure Pre-Screen 8x60k oligonucleotide microarray platform.

Biopsy Samples	Detected Aberrations
M2 (E1)	Monosomy 21 (46,XX,-21) arr[GRCh37] 21q11.2q22.3(15736609_48016000)x1
P5 (E3)	Complex aneuploidy (46,XX,+5,+8,+9,-15,+20) arr[GRCh37] 5p15.33p12(52186_45763609)x3 arr[GRCh37] 5q11.1q35.3(49713978_180014860)x3 arr[GRCh37] 8p23.3p11.1(186477_43130589)x3 arr[GRCh37] 8q11.1q24.3(47908635_144561862)x3 arr[GRCh37] 9p24.3p11.2(146194_47212321)x3 arr[GRCh37] 9q13q34.2(66286309_136661395)x3 arr[GRCh37]15q11.1q26.3(20055137_102399819)x1 arr[GRCh37] 20p13p11.1(121521_26160099)x4 arr[GRCh37]20q11.21q13.33(29431405_61955233)x3

Sample NA1 carries a trisomy of chromosome 18, sample NA2 carries a ~16 Mb interstitial loss on the q arm of chromosome 13, and sample NA3 carries an even smaller ~5.3 Mb terminal loss on the q arm of chromosome 15. Sample M2 carries a monosomy of chromosome 21, and sample P5 exhibits complex aneuploidy with multiple trisomies and monosomies.

To simulate the different mosaicism levels, we mixed sample DNA with WGA-amplified, sex-matched reference DNA (Agilent Technologies) in varying ratios. All samples were processed according to the Agilent GenetiSure Pre-Screen protocol for single-cell analysis, with minor modifications.

Agilent Female and Male reference DNA, and genomic DNA from cell lines, were diluted to 1 ng/µL prior to WGA. Diluted DNA and blastocyst-stage embryo biopsies underwent WGA using the multiple displacement amplification (MDA) method with the RepliG Single Cell kit (Agilent Technologies), following the manufacturer's protocol. DNA concentration following WGA was assessed using the Qubit dsDNA BR Assay Kit and Qubit 2.0 Fluorometer (ThemoFisher Scientific).

After WGA, each sample was mixed with sex-matched reference DNA at different percentages to represent the following mosaicism levels: 100% (100% tested sample, 0% normal reference DNA), 50% (50% tested sample, 50% normal reference DNA), 40% (40% tested sample, 60% normal reference DNA), 30% (30% tested sample, 70% normal reference DNA), 20% (20% tested sample, 80% normal reference DNA), 15% (15% tested sample, 85% normal reference DNA), and 10% (10% tested sample, 90% normal reference DNA).

For microarray washing, an additional 10-second wash step with acetonitrile was included at the end of the process. Slides were scanned using the Agilent SureScan Dx microarray scanner (G5761AA), and resulting TIFF images were imported into Agilent CytoGenomics software v5.4 for feature extraction and downstream analysis.

Analysis method

For single-cell applications, CytoGenomics v5.4 provides three default analysis methods. These methods use different aberration filter thresholds, allowing users to customize analysis to requirements.

The main parameters of the analysis settings include:

(1) minimum aberration size filter that excludes putative aberrations (amplifications, gains, losses, deletions) smaller than the specified value.

(2) logratio filter that excludes putative aberrations if the average log2ratio within the region is less than the specified value. Regions with lower-than-expected average log2ratios can indicate the presence of mosaicism and also serve as a metric for the reliability of each call.

The first two default analysis methods (Single Cell Recommended and Single Cell Small Aberration) are designed to detect aberrations with log2ratios of 0.35 or higher for gains and –0.45 or lower for losses, corresponding to a mosaicism detection sensitivity threshold of approximately 50%.

The third available analysis method (Single Cell Long Low) allows for calls with log2ratios down to 0.2 for gains and –0.25 for losses if the aberration size is equal to or exceeds 20 Mb, corresponding to a mosaicism detection sensitivity of ~30%.

To detect lower mosaicism levels and investigate the true sensitivity of the platform, the default analysis parameters were further modified to allow the detection of aberrations with lower log2ratio values and smaller aberration sizes (Table 3).

Table 3. Aberration filter values of the Single-Cell Recommended Analysis Method and the Small Very Low Modified method in the Agilent CytoGenomics software.

Single Cell Analysis Method	Recommended Default	Small Very Low Modified Method
Minimum Size (kb) of Region for AMP/GAIN	5000	1000
Minimum Size (kb) of Region for LOSS/DEL	5000	1000
Minimum Absolute Average Log Ratio of Region for AMP/GAIN	0.35	0.065
Minimum Absolute Average Log Ratio of Region for LOSS/DEL	0.45	0.065

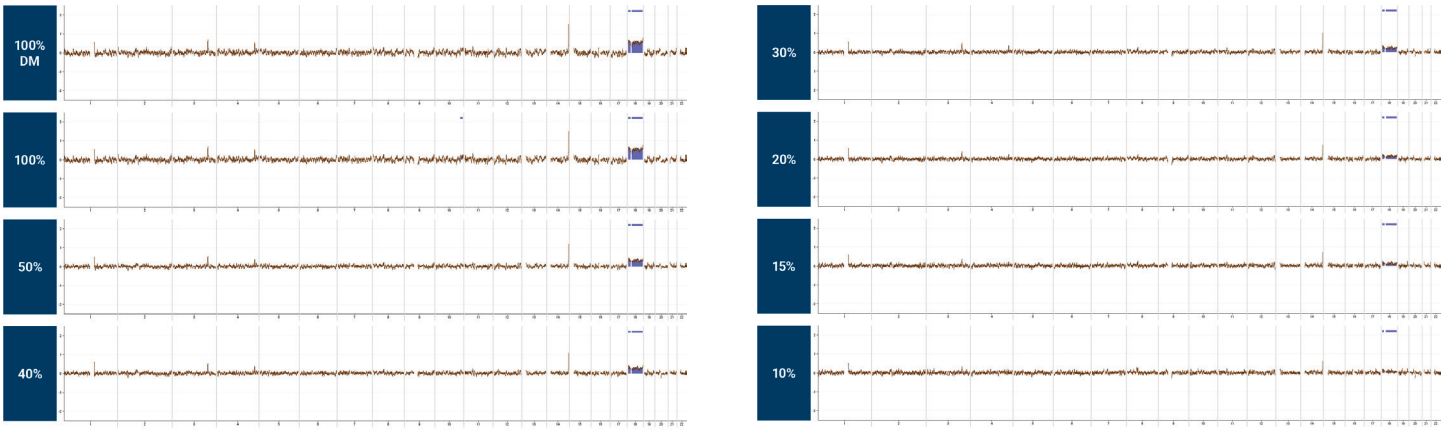
TIFF images were analyzed in Cytogenomics v5.4, using the modified method, Small Very Low Modified.

Results

First, we tested the three samples from Coriell Institute (NA1, NA2, and NA3) with known aberrations (Table 1).

For sample NA1 we were able to detect simulated mosaicism on chromosome 18 down to 10% (Figures 1A and 1B).

(A)



(B)

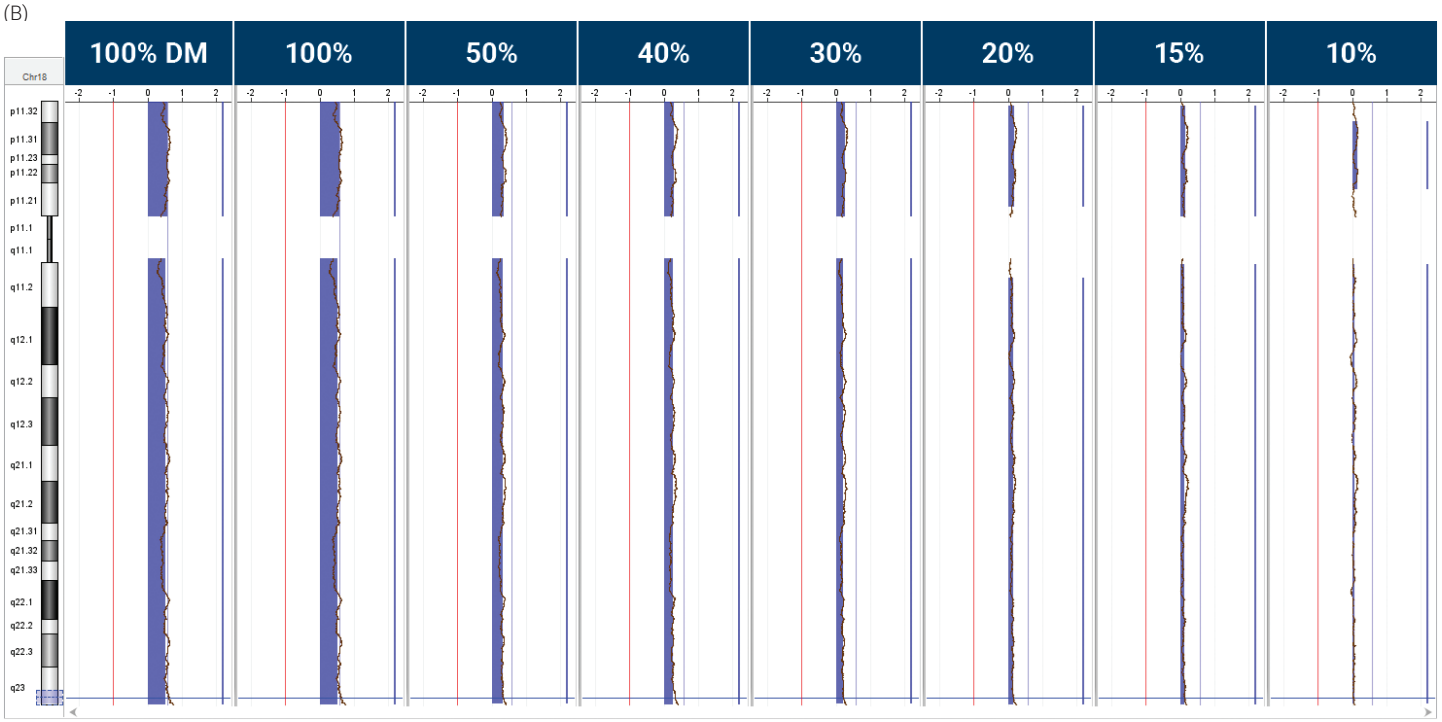


Figure 1. Screenshots from Agilent Cytogenomics software showing (A) the genome view and (B) the chromosomal view of chromosome 18 for Sample NA1. Analysis was performed using both the Default Single Cell Recommended Method (DM) and the modified method on DNA diluted from 100 to 10%. Amplification of chromosome 18 was detectable down to the 10% dilution level.

Similarly, we have identified the 16 Mb loss on the q arm of chromosome 13 in sample NA2 down to 15% (Figures 2A and 2B).

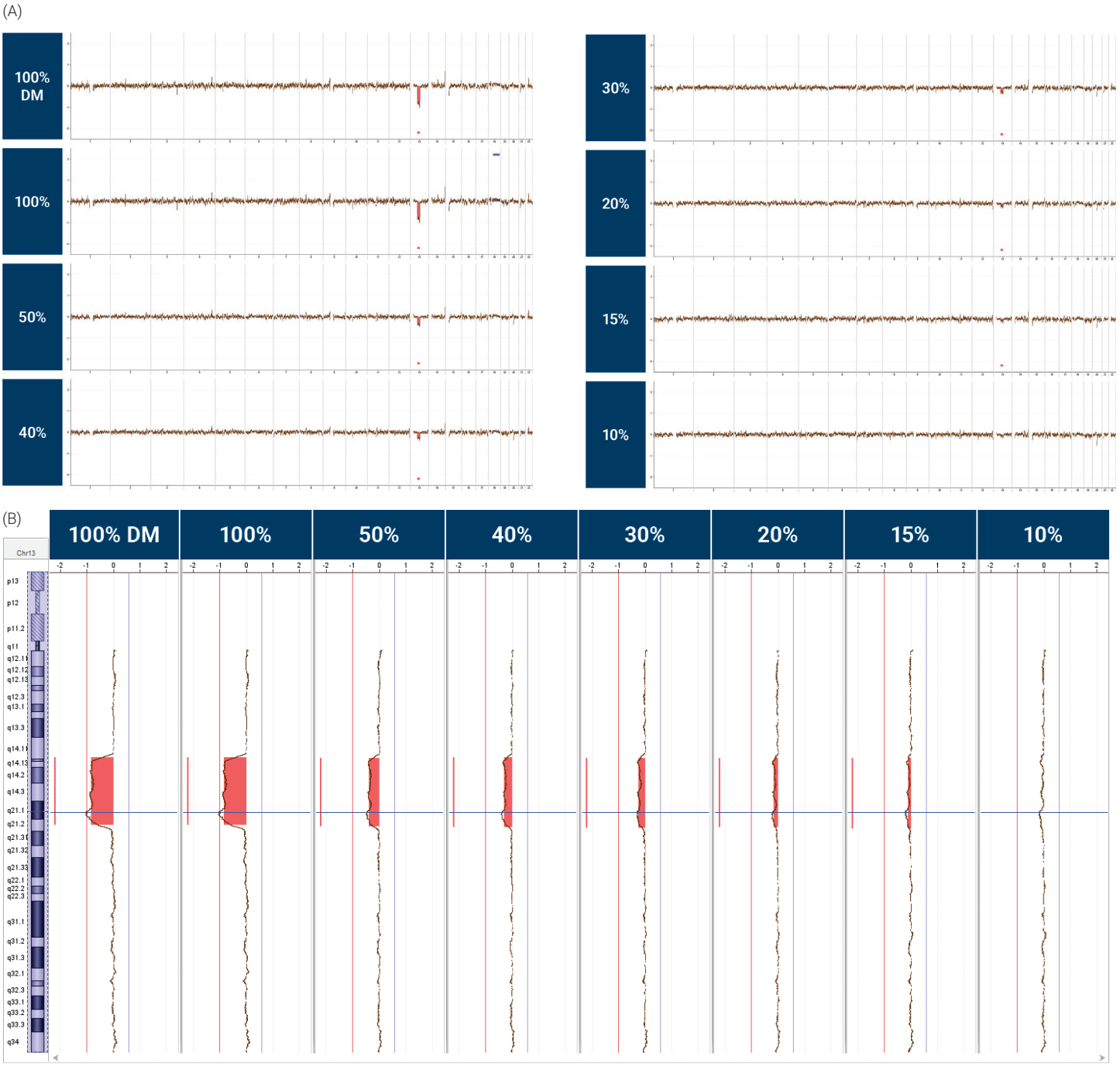
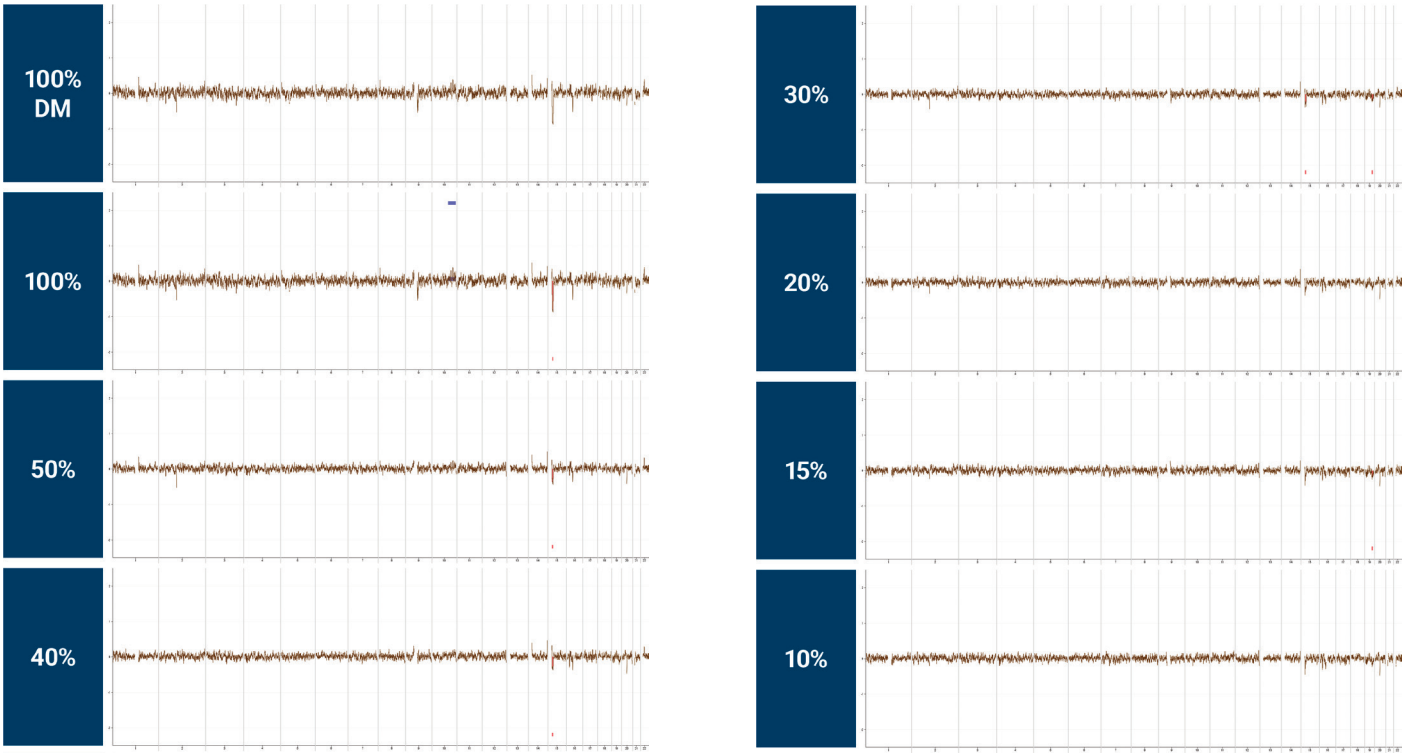


Figure 2. Screenshots from Agilent Cytogenomics software for (A) the genome view and (B) the chromosomal view of chromosome 13 for Sample NA2. Analysis was performed using both the Default Single Cell Recommended Method (DM) and the modified method for DNA diluted from 100 to 10%. The 16 Mb loss on 13q was detectable down to the 15% dilution level.

For sample NA3, the ~5.3 Mb terminal loss on chromosome 15 was detected down to 30% level (Figures 3A and 3B).

(A)



(B)

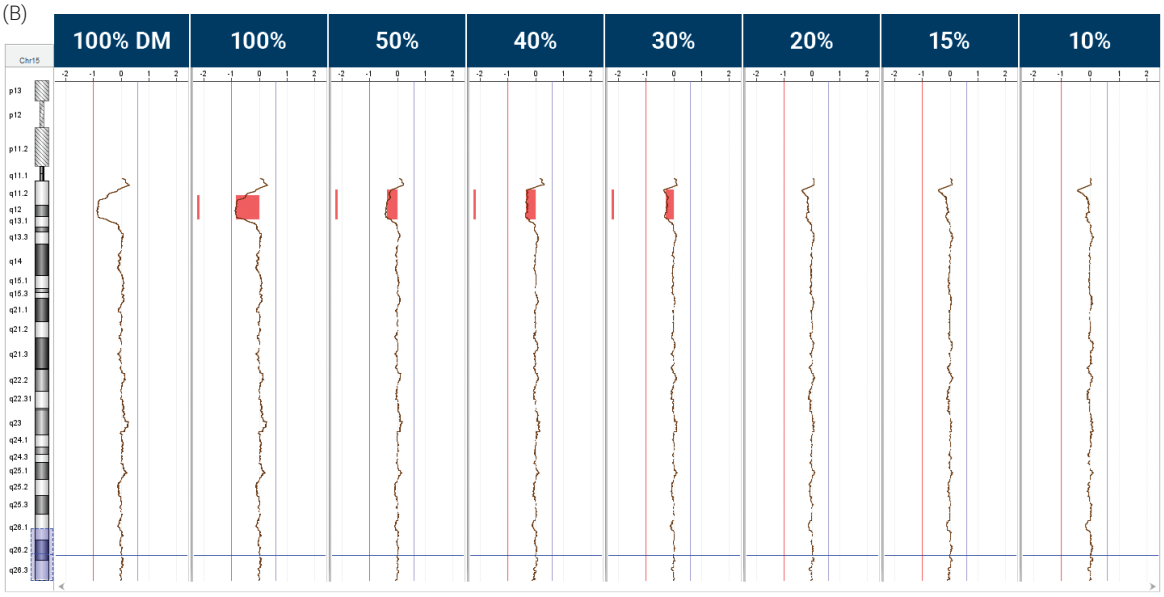
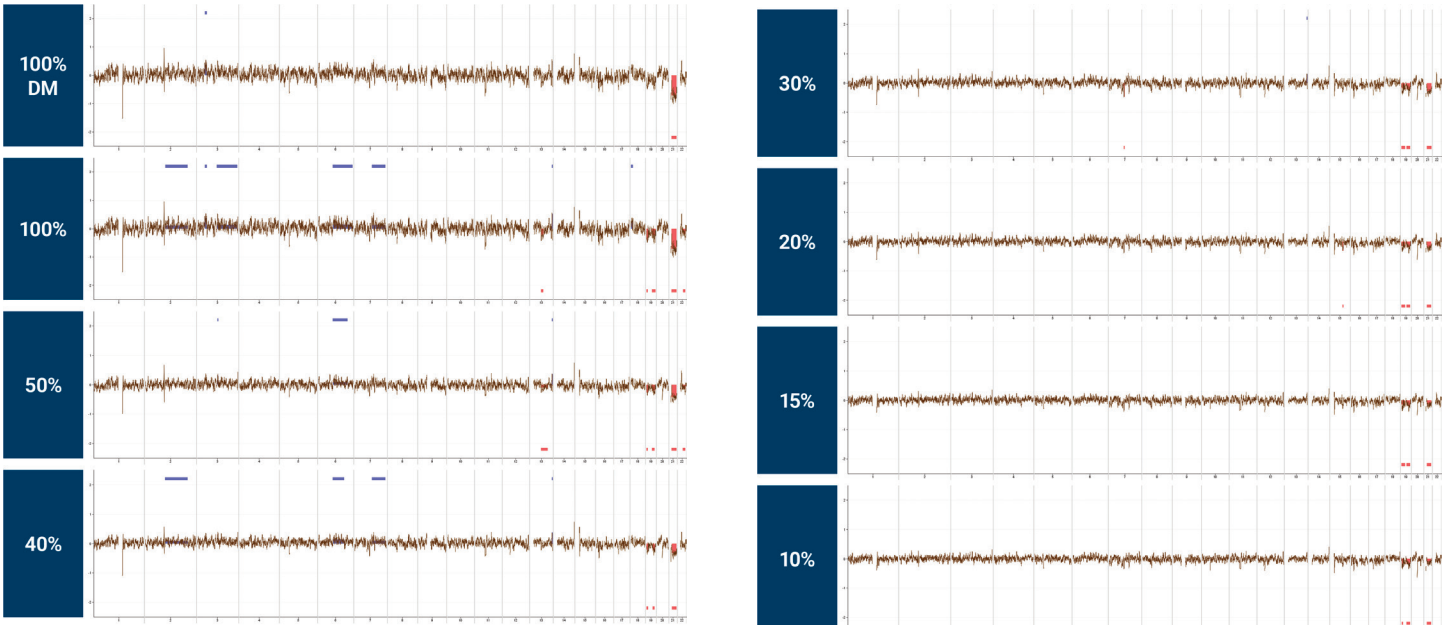


Figure 3. Screenshots from Agilent Cytogenomics software for (A) the genome view and (B) the chromosomal view of chromosome 15 for Sample NA3. Analysis was performed using both the Default Single Cell Recommended Method (DM) and the modified method for DNA diluted from 100 to 10%. The ~5.3 Mb terminal loss on chromosome 15 was detectable down to the 30% level. This call was not detected using the default method, where the minimum length of the aberration equals the size of the aberration. Additionally, one call was detected in the original non-mixed sample (100%) in chromosome 10 using the modified method. This finding is likely due to lowering the logratio threshold, and attributable to a higher noise level in this region, or to a low-level mosaicism event not previously detected.

Second, to better simulate conditions during PGT-A, two blastocyst embryo biopsies were included in the study. Aberrations in these embryos were previously identified using the GenetiSure Pre-Screen 8x60K microarray platform following the previously described protocol. A list of the detected aberrations is provided in Table 2. After WGA, similar mixing steps were performed with sex-matched Agilent reference DNA. As shown in Figures 4A and 4B, monosomy of chromosome 21 was detected down to 10%.

(A)



(B)

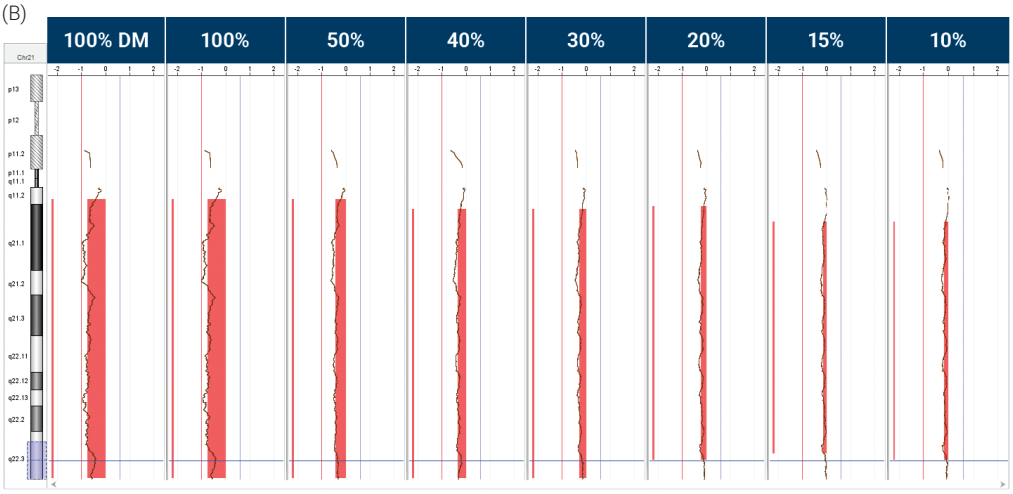
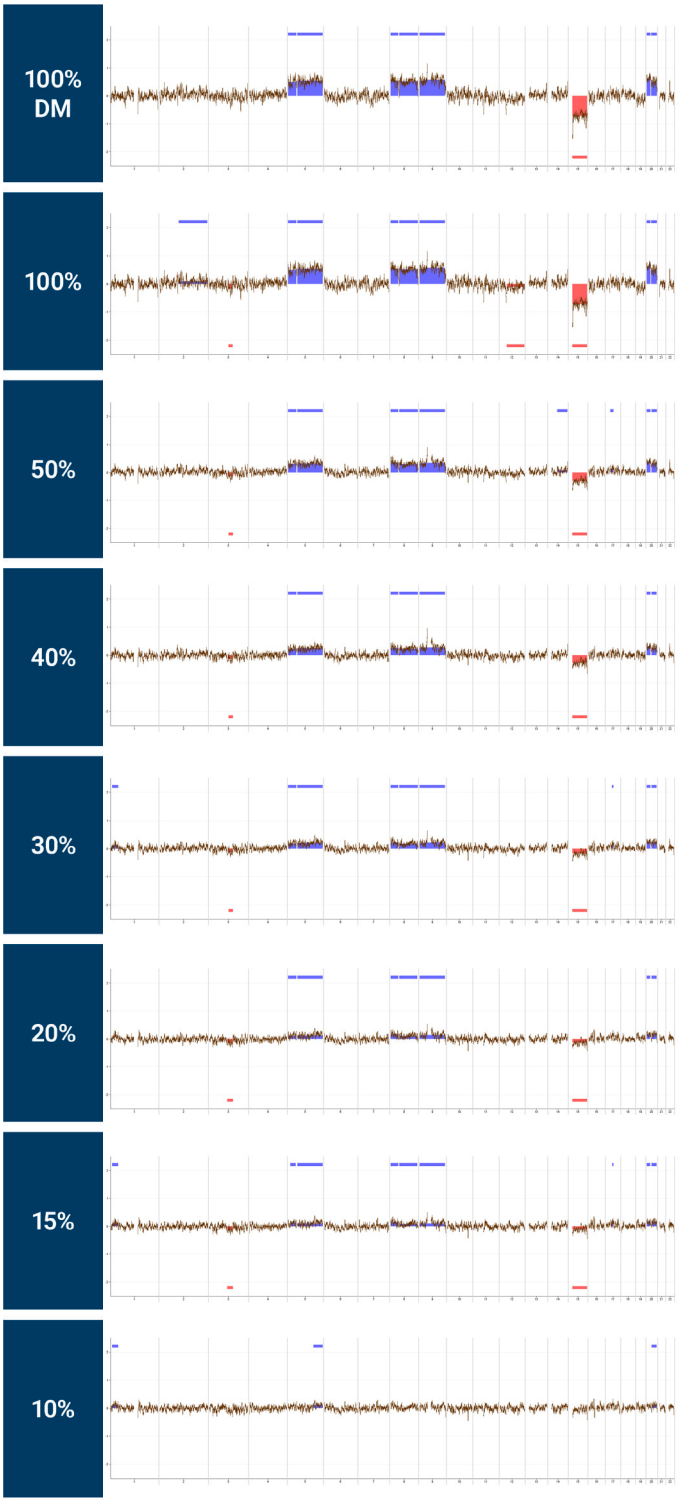


Figure 4. Screenshot from Agilent Cytogenomics software showing (A) the genome view and (B) the chromosomal view of chromosome 21 for Sample M2. Analysis was performed using both the Default Single Cell Recommended Method (DM) and the modified method on DNA diluted from 100 to 10%. The monosomy on chromosome 21 was detected down to the 10% level. Additional calls were detected under multiple simulated mosaicism levels on chromosomes 2, 3, 6, and 7, using the modified method. This result is the consequence of lowering the logratio threshold, and likely attributable to a higher noise level in this region, or to a low-level mosaicism event not previously detected.

Similarly, in Sample P5 (Figures 5A and 5B), aberrations were identified down to 15%. Certain aberrations were partially detected down to the lowest 10% level.

(A)



(B)

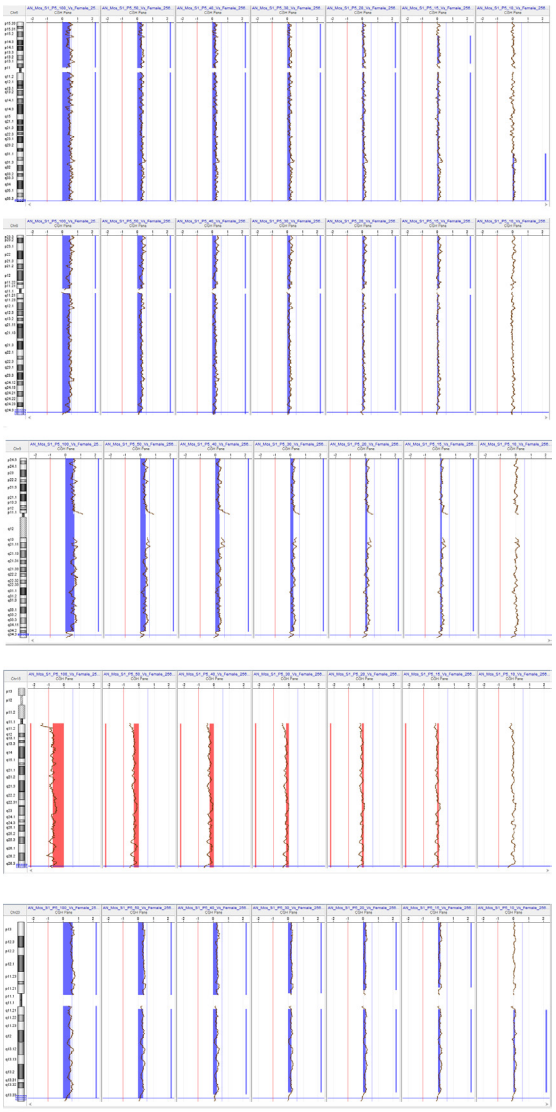


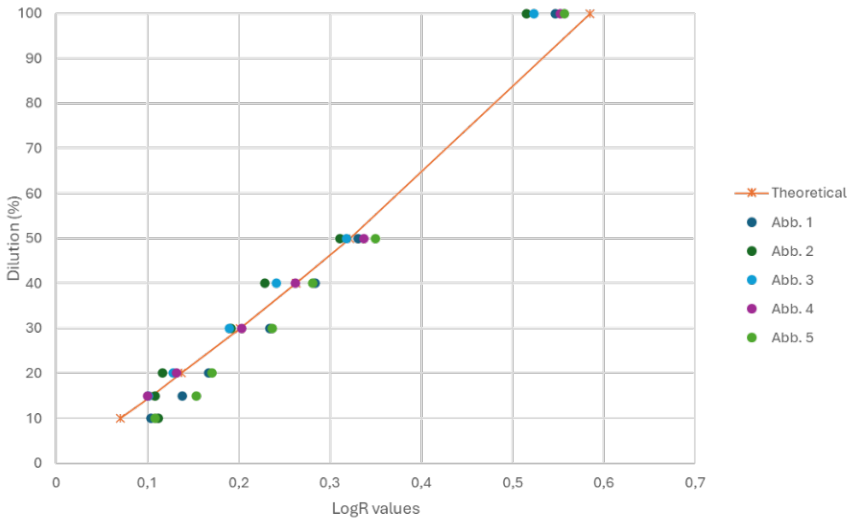
Figure 5. Screenshot from Agilent Cytogenomics software showing (A) the genome view for Sample P3 and (B) the chromosomal view of chromosomes 5, 8, 9, 15 and 20. Analysis was performed using both the Default Single Cell Recommended Method (DM) and the modified method on DNA diluted from 100 to 10%. Amplifications of chromosomes 5, 8, 9, and 20 were detected down to the 15% level and partially detected down to 10% (chromosome 5 and 20). The monosomy of chromosome 15 were detected down to 15%. Additional calls were observed on chromosomes 1, 3, and 12 using the modified method. These extra calls are the consequence of lowering the logratio threshold, and likely attributable to a higher noise level in this region, or to a low-level mosaicism event not previously detected.

To evaluate the method's effectiveness in detecting aberrations at low-level dilutions, a separate analysis of gain and loss aberrations was performed, each assessed independently against theoretical expectations of the logR. For "gain" aberrations, five distinct aberrations were analyzed across the different dilutions (Figure 6A). Similarly, four "loss" aberrations were evaluated (Figure 7A). At each dilution level, both theoretical and observed logR values were plotted, enabling a visual assessment of deviations (Figures 6B and 7B). A strong correlation was observed between the theoretical and measured logR values across all aberrations (Figures 6C and 7C).

(A)

	Sample	"Gain" Aberration
Abb1	NA1	Trisomy 18 (47,XY,+18)
Abb2	P5	arr[GRCh37] 5q11.1q35.3(49713978_180014860)x3,arr[GRCh37] 5q11.1q35.3(49713978_180014860)x3
Abb3	P5	arr[GRCh37] 8p23.3p11.1(186477_43130589)x3,arr[GRCh37] 8q11.1q24.3(47908635_144561862)x3
Abb4	P5	arr[GRCh37] 9p24.3p11.2(146194_47212321)x3, arr[GRCh37] 9q13q34.2(66286309_136661395)x3
Abb5	P5	arr[GRCh37] 20p13p11.1(121521_26160099)x4,arr[GRCh37]20q11.21q13.33(29431405_61955233)x3

(B)



(C)

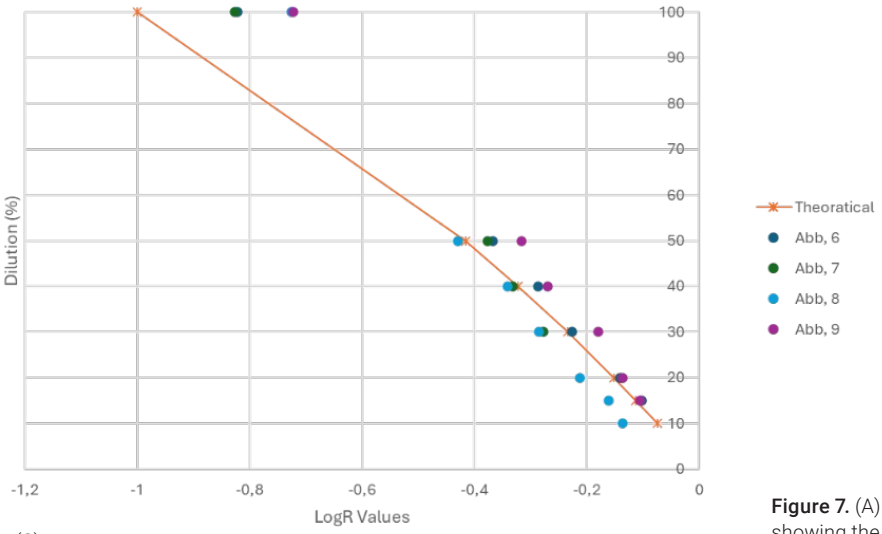
Correl ThVsAbb1	Correl ThVsAbb2	Correl ThVsAbb3	Correl ThVsAbb4	Correl ThVsAbb5
0.999026	0.992662	0.99777	0.997119	0.998321

Figure 6. (A) List of the five "gain" aberrations. (B) Graphic representation showing the theoretical logR values and the observed logR values for "gain" aberrations 1 to 5 across the dilutions 10, 15, 20, 30, 40, 50, and 100%. (C) Correlation table between theoretical and observed logR values for Abb1 to 5. The correlation coefficient metrics show a strong concordance between theoretical and observed logratio values.

(A)

	Sample	"Loss" Aberration
Abb6	NA2	~16 Mb loss on 13q (46,XX,del(13)(q14q21) arr 13q14.11q21.2(43649072-59785590)x1
Abb7	NA3	~5.3 Mb loss on 15q (46,XX,inv(9),del(15)(q11.2q13).ish del(15)(q11.2q13)(D15S63-,ICBD3-,SNRPN-, PAR-5-,154P1+).arr 15q11.2q13.1(21244597-26500067)x1)
Abb8	M2	Monosomy 21 (46,XX,-21) arr[GRCh37] 21q11.2q22.3(15736609_48016000)x1
Abb9	P5	arr[GRCh37]15q11.1q26.3(20055137_102399819)x1

(B)



(C)

Correl ThVsAbb6	Correl ThVsAbb7	Correl ThVsAbb8	Correl ThVsAbb9
0.999266	0.998409	0.989257	0.999178

Figure 7. (A) List of the four "loss" aberrations. (B) Graphic representation showing the theoretical logR values and the observed logR values for "loss" aberrations 6 to 9 across the dilutions 10, 15, 20, 30, 40, 50, and 100%. (C) Correlation table between theoretical and observed logR values for Abb 6 to 9. The correlation coefficient metrics show a strong concordance between theoretical and observed logratio values.

Additionally, for each observed logR, we calculated the corresponding estimated dilution level. We then averaged these estimates for all "gain" and "loss" aberrations at each dilution level (Table 4). The results show that the average estimated dilution level fell within a narrow range around the expected value (± 5% in most cases), with the spread between minimum and maximum values typically around 10% (Table 4).

Table 4. Minimum, average, and maximum calculated dilution level based on observed logR values for "gain" and "loss" aberrations, compared with expected dilution levels. At 10%, fewer than three aberrations were detected, so only the average is reported.

(A) **Gain**

Expected	Minimum	Average	Maximum
100%	86%	91%	94%
50%	48%	51%	55%
40%	34%	39%	44%
30%	28%	32%	36%
20%	17%	21%	25%
15%	14%	17%	22%
10%	NA*	9%	NA*

(B) **Loss**

Expected	Minimum	Average	Maximum
100%	79%	83%	87%
50%	39%	45%	51%
40%	34%	38%	42%
30%	23%	31%	36%
20%	18%	21%	27%
15%	14%	16%	21%
10%	NA*	18%	NA*

Taken together, these data clearly demonstrate that the PGT microarray platform can reliably assess different levels of mosaicism.

Discussion and conclusion

This application note evaluated the mosaicism detection sensitivity of the Agilent GenetiSure Prescreen 8x60K microarray platform for PGT-A.

Although the impact of mosaicism on the developing embryo is not fully understood, studies indicate that transferring mosaic embryos can result in healthy pregnancies. However, these transfers are associated with increased implantation failure and miscarriage rates, with higher levels of mosaicism associated with less favorable outcomes.5 Current guidelines suggest classifying embryos as euploid when less than 20% abnormal cells are included in the biopsy sample, aneuploid when greater than 80% abnormal cells are present, and mosaic when the percentage of abnormal cells in the sample is between 20 and 80%, with the latter being further divided into high- and low-level mosaicism.

To define these categories, cut off values of 40% (for example, abnormal cells between 20 and 39% for low-level and between 40 and 79% for high-level mosaicism) and 50% (20 to 49% low and 50 to 79% high) have been evaluated.4 Our data suggests that the GenetiSure Pre-Screen microarray platform can reliably detect mosaic aberrations down to 30%, enabling confident estimation of mosaicism levels for classification into high- and low-level mosaic categories and subsequent ranking of embryos for transfer. In many instances, mosaicism was detected at levels as low as 10%.

Other factors considered when ranking mosaic embryos for transfer include the perceived viability of different autosomes in the aneuploid state, risk of placental dysfunction, fetal growth restriction, and risk of a known syndrome associated with uniparental disomy (UPD).3

Mosaicism detection sensitivity varies across microarray platforms, depending not only on sample quality, size, and underlying genomic architecture of the region involved, but also on the number and distribution of probes in the specific region. Most modern platforms, including the one used in this study, apply increased probe densities on specific chromosomes and/or genomic regions of clinical importance. Therefore, sensitivity to mosaicism is expected to vary by probe density, with higher densities improving detection. The Agilent GenetiSure Pre-Screen microarray platform has increased probe concentrations on chromosomes 13, 18, 20, 21, 22, X, and Y.

This pattern was evident in the data. In sample NA2, a 16 Mb loss in chromosome 13 was detected down to 15%, with the specific region covered by 525 probes. In contrast, a 5.3 Mb loss in chromosome 15 in sample NA3, covered by 120 probes, was detected only down to 30%. This difference in detection sensitivity reflects both the larger aberration size in sample NA2 and higher absolute number of probes involved, as well as the higher probe density in chromosome 13.

The same data also clearly showcase the microarray's ability to detect mosaicism in segmental deletion or duplication events—an important consideration for ranking, since complete trisomies and monosomies can lead to different outcomes than segmental events.

At lower mosaicism levels, we observed only parts of the aneuploid chromosome being detected as aberrant. For example, in sample P3, partial monosomy of chromosome 20 was detected at the 10% mosaicism level. This phenomenon, also seen in constitutional chromosomal microarray analysis, derives from segmentation-based data analysis. Therefore, when identifying low-level segmental aberrations, the possibility of a larger aberration or aneuploidy should be considered.

Additionally, the data showed strong concordance between observed and expected log2ratio values for all aberrations, supporting confident estimation of mosaicism levels from arrayCGH data. However, log ratio compression levels will vary depending on sample quality and experimental parameters.

The goal of this study was to demonstrate the sensitivity of oligonucleotide microarrays for detecting mosaicism in the PGT-A setting, and to assess the reliability of low log2ratio values for estimating very low-level mosaicism events. To achieve this, the analysis method was modified to allow detection of low-level mosaic events. It must be noted that lowering the log2ratio and aberration size thresholds can increase false positive calls and should be avoided. This effect was evident in our data, where extreme threshold reduction (logratio down to 0.0065) resulted in additional false positive calls in some embryo biopsy samples.

For this reason, recommended default analysis methods in the Agilent CytoGenomics software are locked for editing by the user, limiting mosaicism detection to ~58% for gains and ~45% for losses to minimize the number of false positive calls.

In conclusion, the Agilent GenetiSure PreScreen microarray platform demonstrates high sensitivity for mosaicism detection. Combined with strong concordance between expected and observed log2ratio values supports confident embryo ranking for transfer when needed.

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