Technote

Analytical Validation of the EmbryoMap Solution

Vitrolife, Cambridge, UK

Overview

The EmbryoMap solution has been analytically validated for the detection of whole- and sub-chromosomal imbalances with highly reproducible copy-number. Genomic DNA and cell line isolates with known karyotypes, and trophectoderm samples from *in vitro* embryos were used to evaluate analytical performance. Samples were tested across multiple conditions to ensure reproducibility and stability of the EmbryoMap kit. These included reagent lots, shelf life, freeze-thaw stability, operators, sequencing instruments, and workflow stopping points.

Accurate Detection of Known Karyotypes

Karyotype detection was validated using genomic DNA (n=383; 30-60pg samples) and cell line isolates (n=674; 3-8 cell samples) from 8 different reference cell lines with known karyotypes (Coriell Institute for Medical Research, NJ, USA). Whole- and sub-chromosome imbalances were reported as full copy-number change (aneuploid) if the copy-number deviation (gain or loss) was ≥0.7 from the expected diploid state. The eMap software automatically annotates imbalances at the 10-bin resolution with high sensitivity and specificity (Table 1), where each 'bin' describes read counts per ~1 Mb, variable.

Level	Sample Type	Sensitivity	Specificity	Concordance
Sample	Genomic DNA	100%	100%	100%
	Cell isolates	98.94%	99.51%	99.11%
Chromosome	Genomic DNA	100%	99.99%	99.99%
	Cell isolates	98.94%	99.98%	99.94%

Table 1: EmbryoMap performance for detection of aneuploid (≥70% gain or loss) whole- and sub-chromosome imbalances ≥10-bins (~10 Mb).

The high region-level sensitivity of cell line copy-number imbalances is further described in Table 2. The cell line model also demonstrated a low incidence of putative mosaicism and/or copy-number artefacts at the 10-bin resolution. Six out of 565 (1.1%) expected-aneuploid chromosomes were observed in the putative high-level mosaic range (≥0.5 and <0.7 copy-number deviation), accounting for the reduced sensitivity in some cell isolates.

Region	Type	Start bp	Stop bp	Size Mb	Sample Type	*n*	Sensitivity
3(s) x1	Segment	10,040	11,043,930	11.03	Genomic DNA	48	100%
					Cell Line	112	100%
18(s) x4	Segment	10,018	15,400,006	15.39	Genomic DNA	55	100%
					Cell Line	151	100%
10(s) x1	Segment	47,443,970	73,847,829	26.40	Genomic DNA	48	100%
18(s) x1	Segment	10,018	15,400,006	15.39	Genomic DNA	97	100%
18(s) x3	Segment	21,499,982	80,263,176	58.76	Genomic DNA	97	100%
13 x3	Whole-Chromosome	18,900,004	114,354,148	95.45	Genomic DNA	64	100%
21 x3	Whole-Chromosome	5,030,718	46,699,882	41.67	Genomic DNA	47	100%
					Cell Line	207	97.6%
X x3	Whole-Chromosome	10,167	156,030,877	156.02	Genomic DNA	47	100%
					Cell Line	95	99.0%

Table 2: Cell line samples tested.

In addition to evaluation of cell lines cultured *in vitro*, sub-chromosome imbalance detection by eMap software was further validated using an *in silico* model. Briefly, 574 regions between 10 Mb and 20 Mb were identified in the ClinVar database. Simulated sequencing result files were constructed from euploid read distributions, with addition or subtraction of EmbryoMap read data in defined genomic regions. The simulated result (*.bam) files were analysed using eMap software and automatic annotations were recorded (Figure 1). The eMap software algorithm automatically detected 573 regions as abnormal with a sensitivity of 99.48% in smoothed data.

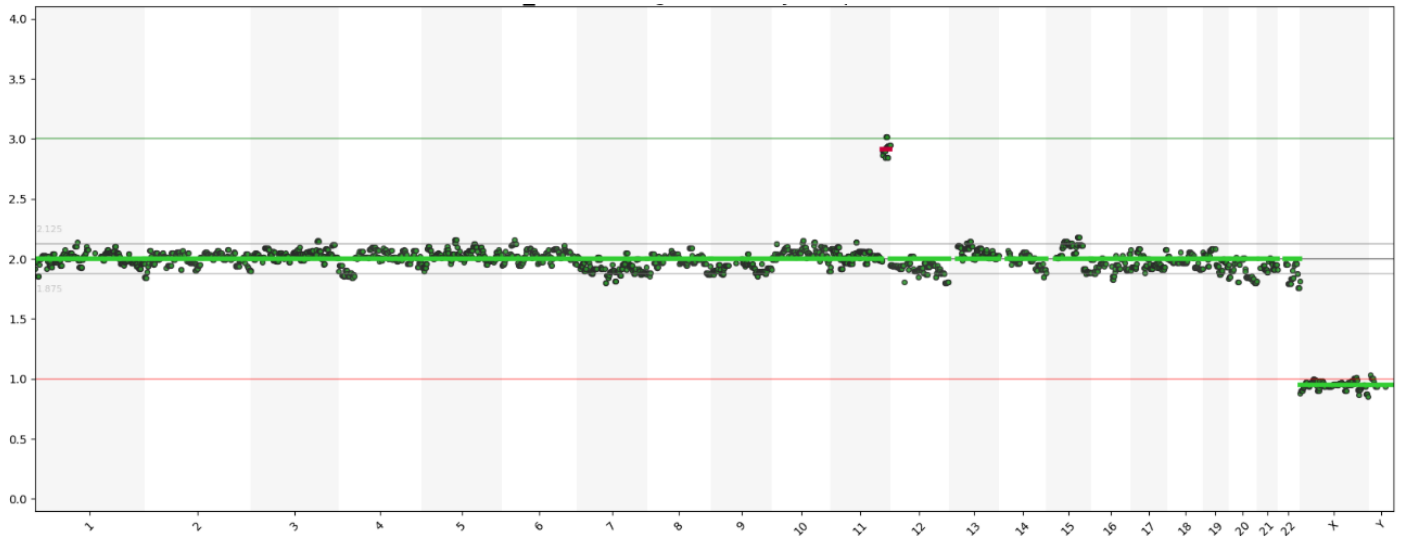

Figure 1: Example analysis results of correctly re-produced 18.1 Mb gain *ClinVar 170915* at 11q23.3-25

Accurate Copy-Number Quantitation

EmbryoMap was evaluated for copy-number quantitation using a cell line mixture model to analyse copy-number imbalances in increments of ±0.1 (10%). A series of 10-cell samples were created from two cell lines:

(46,XX,del(3)(p25).arr 3p26.3p25.3(35333-10305377)x1 and 47,XY,+21[24].arr(21)x3) (Figure 3A, 3B, 3C).

A strong correlation between the input mixture and the observed copy-number was reported (Figure 2; R^2 = 0.991). The absolute difference between expected and observed copy-number values was within 0.09 at the upper-bound 95% confidence interval (Gain 0.09; Loss 0.06; No-Change Autosomes 0.07), demonstrating the suitability of EmbryoMap to report intermediate copy-number states with high accuracy.

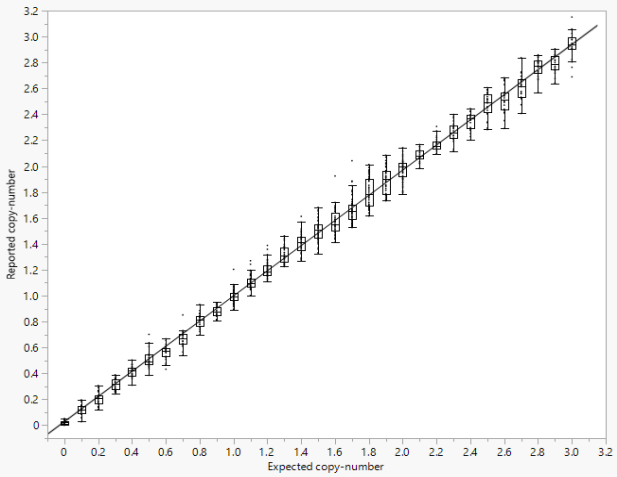

Figure 2: Correlation between expected and observed copy-number values for chromosomes 3p, 21, X and Y (R^2 = 0.991).

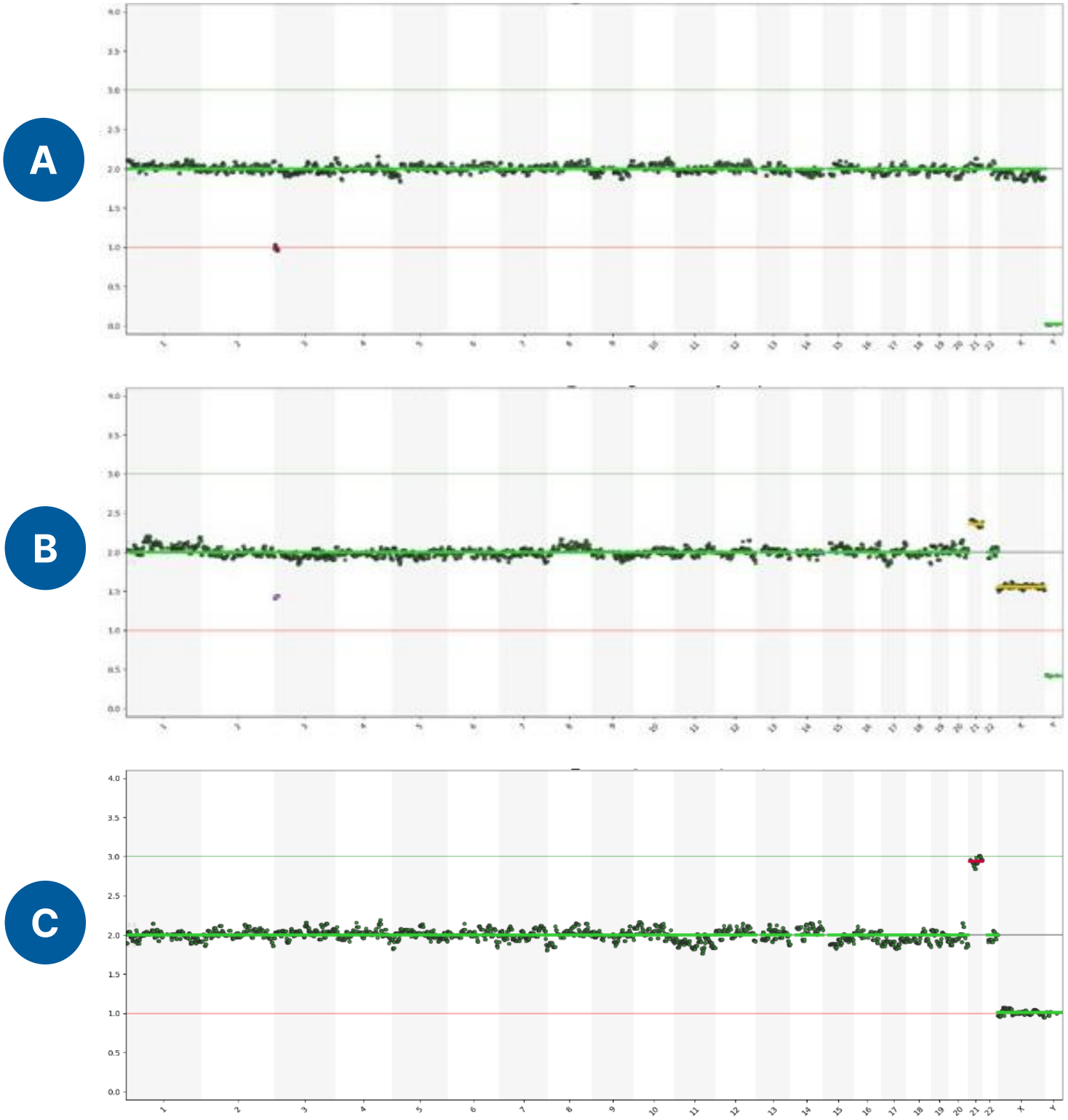

Figure 3: Cell line mixture models between 46,XX,-3p and 47,XY,+21 in the ratios **A)** 10:0, **B)** 6:4, and **C)** 0:10 respectively.

Whole-chromosome aneuploidy detection in trophectoderm biopsies

A comparison between EmbryoMap (test) and the existing VeriSeq PGS solution (reference) was performed using trophectoderm biopsies from *in vitro* embryos. Dual trophectoderm biopsies from 295 discarded embryos were donated by individuals from 10 different centres. At random, one biopsy was tested with VeriSeq PGS (24-plex NGS), and the paired biopsy was tested with EmbryoMap (48-plex NGS) according to the published platform user guides.

Copy-number imbalances were annotated automatically by eMap software* (EmbryoMap) according to criteria in Table 3. BlueFuse Multi software (VeriSeq PGS) automatic abnormality calls were manually reviewed and sub-categorised as 'whole-chromosome aneuploid' or 'putative high-level mosaic' according to matched criteria because automatic intermediate copy-number calling is not a feature of the BlueFuse Multi software. Chromosomes with a copy-number median deviation <0.5 were reported as normal.

Abnormality type	Copy-number deviation (gain or loss) from 2.0 for autosomes and X chromosome
Aneuploid	≥0.7
Putative high-level mosaic	≥0.5 and <0.7

Table 3: Copy-number imbalance calling criteria

Concordance analysis for only whole-chromosome aneuploidy was performed to control for karyotype discordances between biopsies from the same embryo, due to putative errors in mitosis. However, chromosome mis-segregation in early mitotic divisions may still lead to discordances between biopsy samples. Samples (n=15) and chromosomes (n=42) with only putative mosaic calls in either biopsy were excluded from concordance analysis. The VeriSeq PGS data was considered 'truth' for sensitivity and specificity calculations.

Level	Sensitivity	Specificity	Concordance
Sample	99.40%	97.37%	98.57%
Chromosome	99.16%	99.93%	99.90%

Table 4: Comparison to VeriSeq PGS at the sample and chromosome level.

** eMap software also supports manual annotation of copy-number chart data for experienced users.*

High-Quality Data with Increased Sequencing Throughput

Sequencing QC data from a subset of *in vitro* embryo biopsies, that were analysed with EmbryoMap (n=205) and VeriSeq PGS (n=138) were compared after controlling for the MiSeq instrument used for sequencing. EmbryoMap data were generated at 48-plex per run, and VeriSeq data were generated at 24-plex per run. Individual samples failing platform QC criteria were excluded from the comparison to remove sample quality bias.

The improved EmbryoMap sample preparation chemistry, custom sequencing recipe and advanced analytical tools allows up to 48 samples per sequencing run with no loss of resolution or increase in noise when compared to VeriSeq PGS (Table 4; Figure 4).

	VeriSeq PGS	EmbryoMap
Flow cells	8	8
Cluster Density K/mm^2	1252 (274)	1393 (114)
Clusters PF %	83.0 (4.9)	85.9 (2.3)
Reads %≥Q30	90.2 (2.6)	95.1 (0.9)
Mapped Reads %	81.8 (1.3)	97.8 (0.3)
Filtered Reads % for CN analysis	62.7 (2.1)	83.5 (0.6)
Overall noise DLR	0.18 (0.03)	0.17 (0.03)

Table 4: Mean (S.D.) flow cell and sample-level metrics comparison between EmbryoMap and VeriSeq PGS platforms.

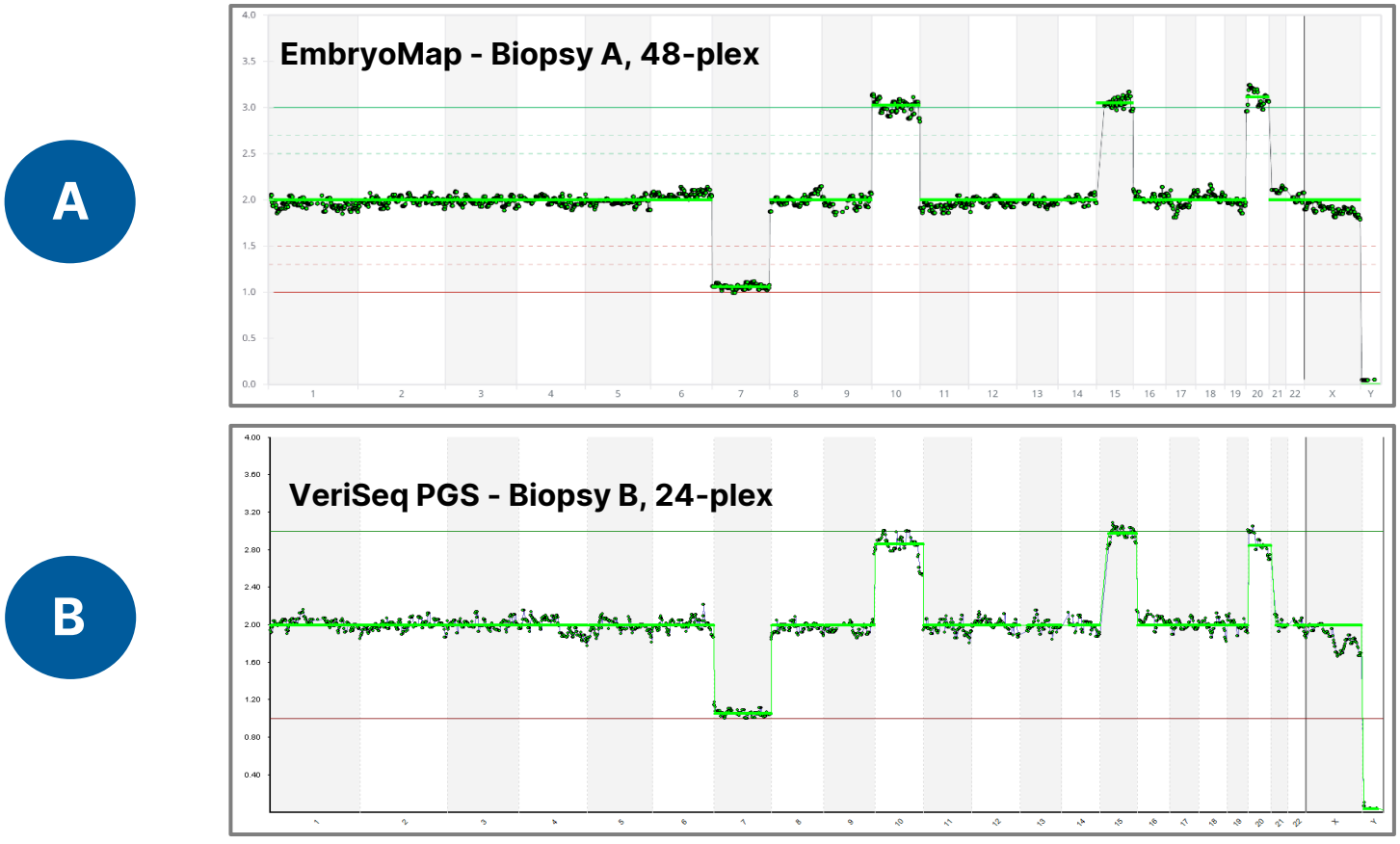

Figure 4: Representative copy-number charts for paired TE biopsies from the same embryo, analysed with **A)** EmbryoMap at 48-plex per run, and **B)** VeriSeq PGS at 24-plex per run. Samples are concordant for sex and aneuploidy in 4 autosomes.

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Summary

The EmbryoMap Solution provides a combination of improved assay chemistry, sequencing strategy, and advanced analytical tools, to enable:

- **Increased throughput** allowing up to 48 samples per sequencing run with no loss of resolution or increase in noise compared to VeriSeq PGS at 24-plex.
- **High resolution, reproducible karyotypes** with >99% karyotype concordance in cell line models of known karyotype, including detection of sub-chromosomal imbalances ≥10 bins (~10 Mb).
- **Highly accurate copy-number quantitation** in cell line mixture models, indicating suitability to detect intermediate copy-number changes at user-defined thresholds.
- **Analysis of embryo trophectoderm biopsies** with whole-chromosome karyotype concordance >98.5% when compared to independent, matched biopsies processed with VeriSeq PGS.
- **Improved QC metrics** and automatic assessment in eMap software.