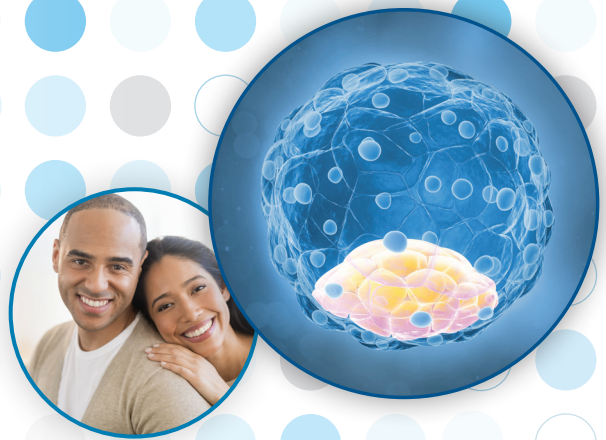




GENETISURE PRE-SCREEN KIT FAQ



Q *For which sample types can the GenetiSure Pre-Screen Kit be used?*

A The GenetiSure Pre-Screen Kit is optimized to analyze single cells from day 3 embryos or from day 5 embryos.

Q *Which type of genetic information will the GenetiSure Pre-Screen Kit reveal?*

A The GenetiSure Pre-Screen Kit was developed to identify aneuploidies in single cells and to detect genomic gains and losses in the 5–10Mb range. The Kit can also be used for the targeted analysis of known genomic aberrations in the 1 Mb range, and to identify known translocations.

Q *Can the GenetiSure Pre-Screen Kit detect smaller aberrations compared to BAC (Bacterial Artificial Chromosome) based methods?*

A Yes, He *et al.* (PLoS One. 2014 Jan 7;9(1):e85207) reported that the smallest size that the Agilent array can detect is 2.38 Mb which is smaller than the detectable size on the Illumina BAC platform.

Q *What is included in the GenetiSure Pre-Screen Kit?*

A The GenetiSure Pre-Screen Kit contains CGH (Comparative Genomic Hybridization) microarrays as well as the necessary reagents for whole genome amplification and labeling of single cells. The Microarrays are compatible with Agilent's CytoGenomics software (v3.0 or higher) for data analysis.

Array design

Q *How many probes do the GenetiSure Pre-Screen microarrays contain and how were the probes selected?*

A The 8x60K GenetiSure Pre-Screen microarrays contain ~55,000 unique biological probes with a median probe spacing of ~50 kb. The 4x180K GenetiSure Pre-Screen microarrays contain ~83,000 biological probes in duplicate with a median probe spacing of ~31 kb. All 60-mer oligo probes were empirically optimized for use with the multiple displacement-based WGA (Whole Genome Amplification) method. Both formats offer genome-wide probe coverage, with increased probe density on chromosomes 13, 18, 20, 21, 22, X and Y. These chromosomes are commonly associated with aneuploidies and/or are smaller chromosomes.

Q *Can I detect smaller aberrations with the 4x180K microarrays compared to the 8x60K microarrays?*

A No, although the probe spacing of the 4x180K is slightly lower compared to the 8x60K microarrays, the effective biological resolution is the same for both microarray formats. The 4x180K microarrays are intended to be used as a more cost-effective way to run fewer samples.

Q *How many samples can I analyze per microarray and per glass slide?*

A The capacity of one 8x60K microarray glass slide is 14 unknown samples and 2 reference samples, and one 4x180K microarray glass slide is 6 unknown samples and 2 reference samples. Only the GenetiSure Pre-Screen microarrays, and not the standard CGH microarrays, can be used and analyzed in the software in this manner.



Protocol

Q What is the recommended reference sample?

A Because CGH data is less noisy when the unknown sample and the reference sample quality, amount, etc. are as similar as possible, we recommend using lymphocytes isolated from human blood as reference sample. Because the required volume of blood is so small, blood can be collected by using a finger stick.

Q What is the recommended amplification method?

A The reagents for the amplification step are included in the GenetiSure Pre-Screen Kit. We chose an MDA (Multiple Displacement Amplification) based amplification method. MDA is a non-PCR based DNA amplification technique. In MDA, DNA is amplified isothermally by using the Φ 29 enzyme. Besides being successfully used for CGH, this method has become a promising method for single cell genome NGS (Next Generation Sequencing).

Q How do I know the amplification reaction was successful?

A Amplified DNA can be quantified with the Qubit dsDNA BR Assay Kit. A successful amplification reaction starting with 2–3 single cells yields 29 μ l amplified DNA at 50–200 ng/ μ l.

Q What is the recommended labeling method?

A The reagents for the labeling step are included in the GenetiSure Pre-Screen Kit. We chose a Klenow based labeling method. After the labeling the Cy3 and Cy5 labeled amplified DNA are pooled and cleaned-up by reverse spinning columns. The columns are also included in the GenetiSure Pre-Screen Kit.

Q What is the optimal hybridization time for the GenetiSure Pre-Screen assay? Do longer hybridization times result in better data?

A Hybridization times of 2 and 6 hours have been tested by Agilent R&D. No significant differences in the resulting data could be detected between 2 and 6 hours.

Q Which scanner can I use to scan the GenetiSure Pre-Screen microarrays?

A Supported scanners are the Agilent Microarray Scanner (Agilent p/n G4900DA) and the Agilent C-scanner (Agilent p/n G2565CA). The GenetiSure Pre-Screen microarrays have not been tested on Innopsys or other 3rd party scanners.

Analysis

Q How do I analyze data generated with the GenetiSure Pre-Screen Kit?

A Single cell analysis methods have been implemented in Agilent CytoGenomics software v3.0 or higher, available for download free-of-charge from www.agilent.com/genomics/cyto_software.

Q What are the differences between the single cell analysis methods in the Agilent CytoGenomics software?

Single Cell Analysis Method	Aberration size filter setting	Log Ratio filter setting	When to use
Single Cell Recommended	> 5 Mb	Deletion = 0.45 Amplification = 0.35	This is the recommended default method
Single Cell Small Aberration	> 1 Mb	Deletion = 0.45 Amplification = 0.35	To look for small known aberrations: for example balanced translocation in the parents, known microdeletions
Single Cell Long Low Aberration	> 20 Mb	Deletion = 0.25 Amplification = 0.20	To look for very large aberrations in mosaic samples

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