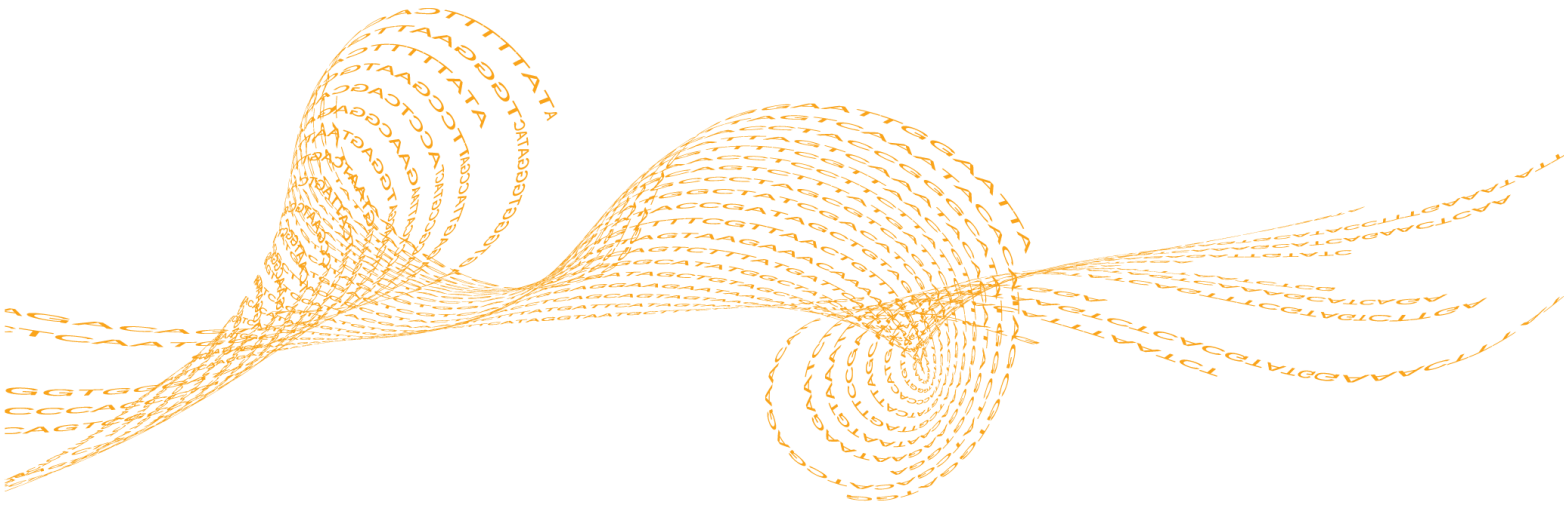
A Technical Guide to Karyomapping:
Phasing Single Gene Defects



ILLUMINA PROPRIETARY
Part # 15052496 Rev. C
January 2015

illumina®

FOR RESEARCH USE ONLY

In addition to all other terms and conditions, the use of Karyomapping products is subject to the following terms and conditions. If you do not agree to these terms and conditions you are not permitted to use these products.

1. The Karyomapping products may only be used in compliance with all applicable laws and regulations, including without limitation those laws and regulations concerning pre-implantation genetic diagnosis and pre-implantation genetic screening, privacy rights, reporting of incidental findings (as further discussed below), and the storage, transfer, and disposal of samples and materials. These laws and regulations vary significantly from jurisdiction to jurisdiction.
2. The Karyomapping products have not been cleared, approved, or otherwise reviewed by the U.S. FDA or any other governmental regulatory agency or authority for use in pre-implantation genetic diagnosis, pre-implantation genetic screening, or for any other use.
3. The Karyomapping products attempt to make a prediction of genetic phase in embryos, however, the user is responsible for examining both the supporting and contrary evidence for this predicted phase before making a determination concerning the true phase. Results obtained from the use of Karyomapping products should be confirmed using alternative methods. The Karyomapping products may generate false positives, false negatives, or inconclusive results.
4. Use of the Karyomapping products may produce incidental findings about genetic relatives used to perform the analysis. These incidental findings may include, without limitation:
   a. Paternity issues
   b. Copy number variations (CNVs) that may relate to disorders that have yet to manifest, or are of unknown significance
   c. Consanguinity between relatives
   d. Sex chromosome abnormalities

   The laws and regulations concerning the reporting of such incidental findings vary significantly from jurisdiction to jurisdiction, and it is solely the user's responsibility to ensure it uses Karyomapping products, including results obtained through their use, in compliance with all applicable laws and regulations.
5. All users of the Karyomapping products should be skilled in the areas of genetics and genetic linkage.

Revision History

Part #	Revision	Date	Description of Change
15052496	C	January 2015	Added content to the following sections: Case Level Review, Case Warnings, CNVs in Reference, and CNVs in Parents.
15052496	B	October 2014	Added content to Informative SNPs and Phasing and Embryo Phasing Review sections. Added Common Recombinations section.
15052496	A	February 2014	Initial Release

Introduction

Karyomapping is a comprehensive method for genome-wide linkage-based analysis of single gene defects. The method can be used on a single cell or small number of cells from an embryo in preimplantation genetic diagnosis (PGD)[1]. It can be used where there is a risk of severe genetic disorders being inherited from parents. A couple could have a family history of a genetic disorder or have had a child with a genetic disease. PGD screening with karyomapping can be used to identify embryos that do not carry defective genes and can be safely implanted.

PGD screening is performed by examining the inheritance of short tandem repeats (STRs) adjacent to specific disease loci. Each STR test for a genetic disorder must be developed individually. The complexity of the test means that it is expensive, time consuming and only available at a few very specialist laboratories. Karyomapping uses a genome-wide linkage-based detection of single gene defects. The method uses genome-wide SNP genotyping of parents and a close relative of known disease status (termed a trio), for example an existing child (termed the reference). For each embryo, SNPs are phased to identify the parental origin of chromosomes at all SNP loci. Karyomapping therefore provides a comprehensive method for PGD of single gene defects without the need for the development of disease or patient specific tests.

BlueFuse Multi software with a licensed karyomapping module is required for the analysis of karyomapping cases. The software is designed to assist with the visualization and interpretation of the complex data related to karyomapping cases. The software does not attempt to call whether an embryo is affected. It provides analysts with predicted phasing, combined with sufficient statistics and visualizations for each embryo, so that a manual call can be made.

The aim of this document is to provide analysts with guidance on how to call a karyomapping case. It begins by outlining the rules governing the generation of karyomapping data. It then presents a recommended calling workflow and describes important related topics, including the main patterns that can be seen in the visualizations, details of the contents of the report, reference types, and X-linked disorders.

It is important for users to note:

- Karyomapping is a research use only (RUO) product.
- Predicted phasing of embryos by the software is a best estimate. A trained analyst must confirm the predicted phasing by examining all the contextual SNP data.
- Karyomapping must only be used for PGD conditions licensed by local laws.
- Only experts in genetics, recombination, and linkage are qualified to perform karyomapping analysis.

[1]Handyside AH, Harton GL, Mariani B, et al. Karyomapping: a universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. *J Med Genet*. 2010;47:651-658

Karyomapping – In Brief

This section defines the key karyomapping concepts, many of which are the same as, or similar to, concepts associated with STR analysis methods.

Karyomapping uses the Illumina HumanKaryomap-12 BeadChip to obtain genome-wide genotypes for the trio (genomic DNA extracted from blood) and embryos (biopsy of single/few cells). The outcome of the laboratory protocol is a set of genotype calls for each SNP on the array (~300,000) – these genotypes can take 4 values: AA, AB, BB, or NC (no call). The letter A represents the nucleotides A and T and the letter B represents the nucleotides G and C in the genetic sequence.

The BlueFuse Multi karyomapping module allows data from Illumina BeadChips to be imported into a case. A prediction is then automatically made of the genetic phase of each of the chromosomes of the embryo against the chromosomes of the reference sample within the case.

Informative SNPs and Phasing

The objective of karyomapping is to determine whether each embryo in a case has inherited the same chromosomes from the parents as the selected reference. The first step is to identify **informative SNPs**, meaning that a genotype can be assigned to 1 of the chromosomes **inherited from the mother or inherited from the father**. In typical cases approximately 20%–40% of all SNPs are informative, and the remaining 60%–80% of SNPs in the assay are non-informative. Therefore in a typical karyomapping case there are approximately 60,000–120,000 phasing measurements across the genome with a spacing of 15.5 kb–31 kb. Table 1 provides examples of informative and non-informative parental SNP combinations.

Table 1 Parental genotype combinations – informative SNPs

Example	Father	Mother	Informative?
1	AB	AA	Informative for father
2	AB	BB	Informative for father
3	AA	AB	Informative for mother
4	BB	AB	Informative for mother
5	AA	AA	Not informative
6	AA	BB	Not informative
7	AB	AB	Not informative
8	BB	BB	Not informative
9	BB	AA	Not informative

For a SNP to be informative 1 parent must have a heterozygous genotype and the other a homozygous genotype. The informative allele is in orange in Table 1 and is used to phase the SNPs in the embryo against the alleles of the reference. If the embryo and the reference both inherited or both did not inherit the informative allele, then they inherited the same chromosome from that parent. If either the embryo or the reference inherited the informative allele and the other did not, then they inherited different parental chromosomes. If the embryo inherited the same chromosome as the reference it is termed **in phase**, if the embryo inherited a different chromosome than the reference it is **out of**

phase. Table 2 contains an example of phasing for the paternal chromosome of an embryo using the reference, the informative allele is shown in orange.

Table 2 The phasing of the paternal chromosome of an embryo using the informative allele.

Father	Mother	Reference	Embryo	Phase
AB	AA	AB	AB	In phase
AB	BB	BB	AB	Out of phase
AB	BB	AB	BB	Out of phase
AB	BB	AB	AB	In phase
AB	AA	AA	AA	In phase
AB	AA	AB	AA	Out of phase
AB	BB	BB	BB	In phase
AB	AA	AA	AB	Out of phase
AB	BB	BB	AB	Out of phase
AB	BB	AB	BB	Out of phase

In karyomapping, phasing is relative to the reference. The result of the phasing describes whether each embryo inherited the same chromosome as the reference at each informative SNP location. In contrast, phasing for other PGD techniques such as STR markers is absolute. These techniques separately track the inheritance of each of the 4 parental chromosomes.

A consequence of relative phasing is that recombinations in the reference cause a change of phase in all corresponding embryos. Although the embryos do not contain a crossover of biological origin, they progress from inheriting the same chromosome as the reference to inheriting a different chromosome (or the opposite way). For more information on the effect of common recombinations on data interpretation, see *Embryo Phasing Review* on page 11 and *Common Recombinations* on page 22.

Key SNPs and Non-Key SNPs

A confounding factor in genotyping is **allele drop-out** (ADO), which occurs when 1 of the alleles at a SNP fails to amplify. ADO occurs randomly along the genome and affects each allele (A or B) equally. In a worst case scenario ADO can affect up to 80% of SNPs. Karyomapping is largely immune to the effects of ADO due to the large amounts of phasing data available.

ADO can result in the loss of an informative allele, so an AB can become AA or BB. The result is 2 classes of informative SNPs: **key SNPs** and **non-key SNPs**.

1 Key SNPs are SNPs in an embryo that contain the informative allele. ADO could not have affected their phasing. Key SNPs provide strong support of the predicted phase.

2 Non-key SNPs do not contain the informative allele. There is no guarantee that they are genuinely homozygous; it is possible they have lost the informative allele through ADO and therefore had their phase altered. Non-key SNPs provide weaker support of the predicted phase.

Visualization – Haploblocks

In BlueFuse Multi, paternally and maternally informative SNPs are phased for each embryo relative to the reference and represented as colored **haploblocks** in the software.

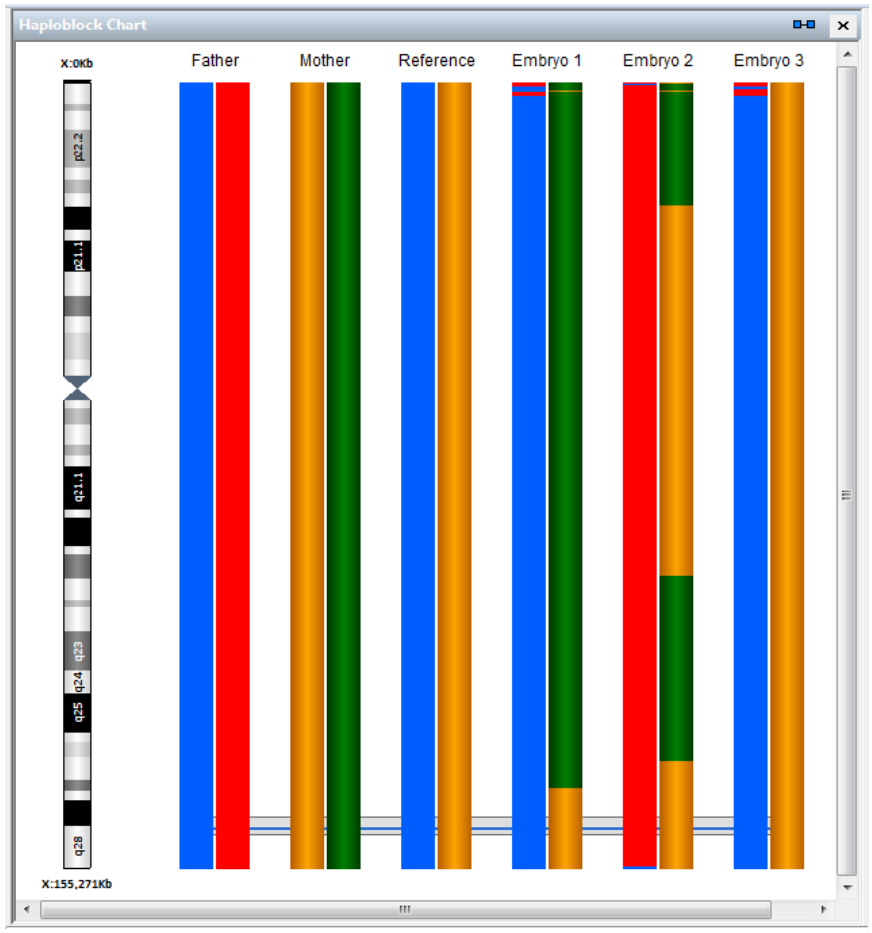
There are 2 main visualizations in BlueFuse Multi for a karyomapping case, **Haploblock Chart** and **Detailed Haploblock Chart**. (For details on creating a karyomapping case in BlueFuse Multi and the visualizations, see the BlueFuse Multi Walkthrough Guide.)

The **Haploblock Chart** shows the haploblock structure of a selected chromosome for every sample in the case: parents, reference, and each embryo. The view opens zoomed to the region of interest, but each whole chromosome can be viewed when selected from the **Karyotype Chart**. An example of this visualization is in Figure 1, which appears from left to right:

- An ideogram of the selected chromosome
- The paternal chromosome pair (P1 - blue, P2 - red).
- The maternal chromosome pair (M1 - orange, M2 - green).
- The reference chromosome pair (arbitrarily assigned as P1 - blue, M1 - orange).
- A pair of chromosomes for each embryo in the case, colored according to the phase predicted by BlueFuse Multi.

For users with the most common forms of color blindness, colors have been selected to aid discrimination between each pair of haplotypes. For further distinction between maternal and paternal haplotypes, P1 and P2 are drawn flat, whereas M1 and M2 are drawn with shading.

Figure 1 Haploblock Chart - zoomed to chromosome



The **Detailed Haploblock Chart** populates when a chromosome for an embryo is double-clicked in the Haploblock Chart View. This view opens with the reference chromosomes at the bottom of the view and the inherited haploblocks for the selected embryo stacked on top. Further information, useful for analyzing an embryo, is plotted in this view:

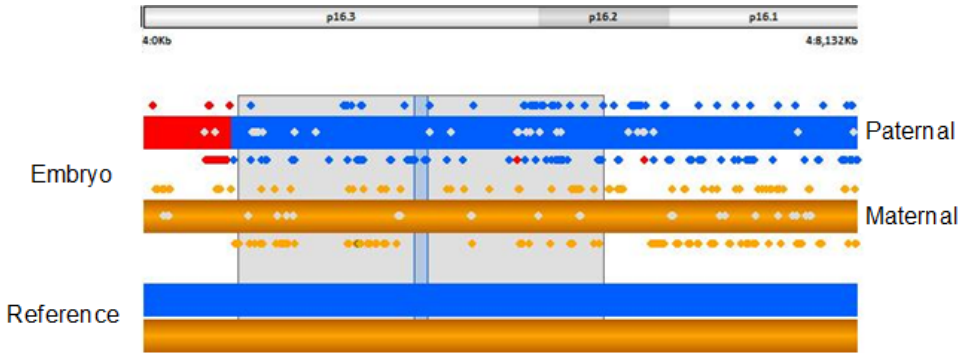
1 Key SNPs (strong evidence of phase) are plotted above the haploblock bar and are colored according to their phase (M1 or M2, P1 or P2).

2 Non-key SNPs (weaker evidence of phase) are plotted below the haploblock bar and are also colored according to their phase.

3 Informative SNPs that have not been successfully assigned to genotype (NC) are plotted down the center of the haploblocks.

An example of this visualization is provided in Figure 2.

Figure 2 Detailed Haploblock Chart



The status of the embryo is determined by examining the distribution of key and non-key SNPs in the region of interest. Decisions concerning whether the embryo is in or out of phase with the reference are then made.

Do not accept the predicted phase coloring of the haploblocks without further examination. Always examine the key and non-key SNP statistics provided in the Case Report and the distribution and phasing of the SNPs in the visualizations. The next sections provide details of a suggested workflow for calling a case and a summary of the different patterns of SNPs that can occur.

Before Starting a Karyomapping Case

The distribution of SNPs on the HumanKaryomap-12 BeadChip is not uniform, with lower coverage in the telomere and centromere regions.

Before conducting a study on a region that has not been previously karyomapped, create a dummy case in BlueFuse Multi. Then examine the case report to find out what SNP coverage the platform provides in the region of interest.

With STRs, consanguinity can prevent successful phasing. Karyomapping is able to phase consanguineous cases as long as informative SNPs are available in the region of interest. In regions or communities where consanguinity is common, create a case containing just the parents. Then evaluate whether there are sufficient informative SNPs in the region of interest for karyomapping to proceed.

Calling a Case

Karyomapping cases contain a large quantity of information that can be used to call the status of each of the embryos. It is important that all of the information is carefully assessed before decisions are made. In this section, a recommended workflow is outlined that attempts to make sure that all relevant information is fed into the decision-making process.

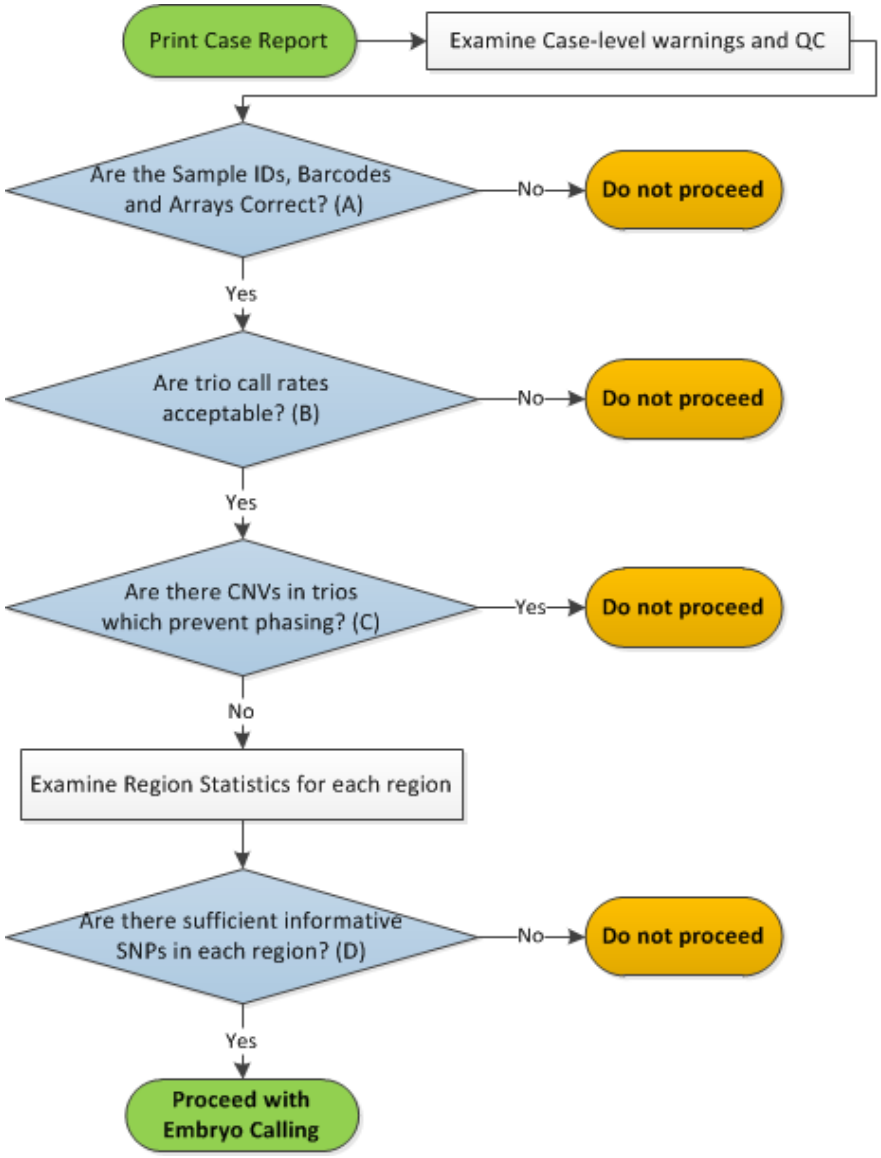
Calling Workflow

Following the creation of a karyomapping case in BlueFuse Multi (see the BlueFuse Multi Walkthrough Guide for details), work through the **Case Report** and BlueFuse Multi visualizations in a particular order. BlueFuse Multi produces a Case Report that provides a summary of the data for a case. The Case Report includes the array performance and the supporting evidence for the phase of each embryo in a case, and is the starting point for assessing a karyomapping case.

Case Level Review

The flowchart shown in Figure 3 outlines the recommended steps for conducting an initial high-level review of a karyomapping case.

Figure 3 Case Review Workflow



A **Confirm sample entry** – Compare the sample IDs, barcodes, and array locations in the Array QC table in the QC section to the sample information sheet and confirm that the samples have been entered correctly. Because incorrect sample entry can cause a misdiagnosis, Illumina recommends 2 witnesses for this step.

B **Trio SNP call rates** – If the SNP call rates (the fraction of SNPs successfully assigned a genotype) in the parents or reference are too low, BlueFuse Multi presents a warning in the Case Warnings section. It warns if the SNP call rates are less than or equal to 0.8 for either of the parents or the reference. Do not proceed with a case if the parents or reference exhibit this warning. Always examine and cross-check the detailed SNP call rates in the QC section against acceptable levels (see *SNP Call Rates* on page 16).

C **Copy Number Variants (CNVs) in Parents or Reference preventing phasing** – If a CNV in the parents or reference overlaps the region of interest, BlueFuse Multi presents a warning in the Case Warnings section. BlueFuse Multi makes CNV calls by using Log R and B-Allele Frequency (BAF) data. To investigate the size, type, and extent of any reported CNVs, select the relevant sample (chromosome icon) in the side pane of BlueFuse Multi. This action opens the standard Log R Chart and B-Allele Frequency Chart to check for CNVs easily. Informative SNP patterns in the Detailed

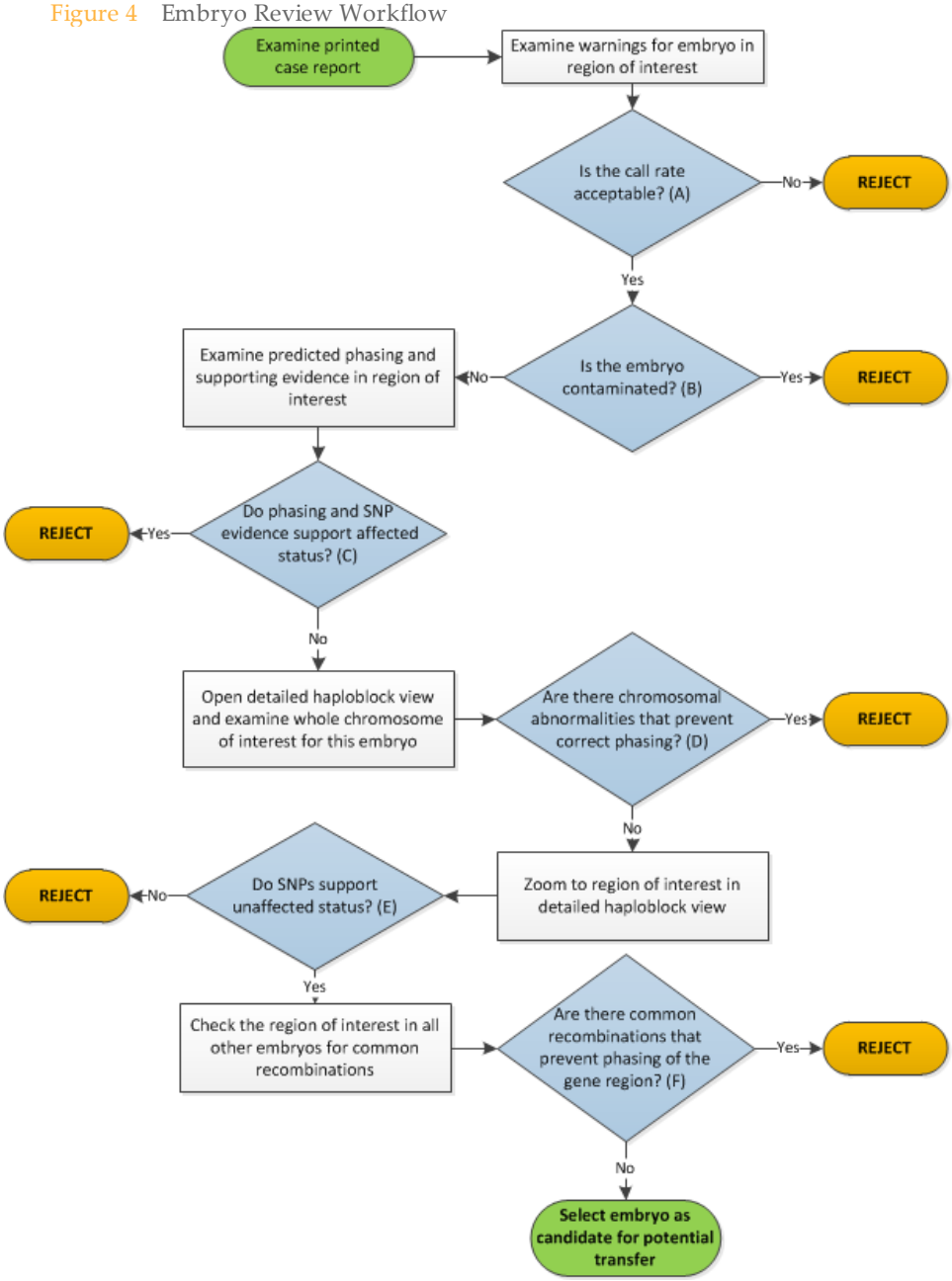
Haploblock Chart provide stronger evidence for the presence of CNVs. Always use the Detailed Haploblock Chart to confirm CNVs in the parents or reference (see *Calling Patterns* on page 18). For details on viewing CNVs in parents and the reference using BlueFuse Multi, refer to the Walkthrough guide for Illumina BeadChip Arrays that is available in the Help menu. If the overlapping CNVs are unexpected, reexamine the linkage of the disorder before proceeding with the case. There are some trio CNVs that prevent phasing of embryos (see *CNVs in Reference* on page 20 and *CNVs in Parents* on page 21).

D **Informative SNP coverage** – For each region, the report contains a Region Statistics section. This section details the number of SNPs available on the HumanKaryomap-12 inside the gene and flanking regions and how many of these SNPs are informative. Abandon the case if sufficient informative SNPs are not available in the region of interest.

Embryo Phasing Review

The flowchart shown in Figure 4 outlines the recommended steps for conducting the phasing review of each embryo in a karyomapping case.

Figure 4 Embryo Review Workflow



A **Embryo SNP call rate** – If an embryo has a SNP call rate less than or equal to a threshold of 0.6, BlueFuse Multi produces a Case Warning. This warning is repeated in the report for this embryo in each region. **Do not make any calls on an embryo for which this warning is present.** Always examine the detailed SNP call rate information for the embryo in the QC section of the report and compare it to the recommended SNP call rate statistics.

B **Contaminated embryo** – If BlueFuse Multi identifies that an embryo sample has been contaminated, BlueFuse Multi produces a case warning. Ploidy or other karyotype-wide abnormalities can trigger the warning. The warning does not by itself preclude calling the phase of the embryo. Examine the data for an embryo that presents this warning on every chromosome using the Detailed Haploblock Chart. See *Calling Patterns* on page 18 for details of how contamination presents itself in this visualization.

C **Predicted phasing** – For each embryo in each region, the case report contains a section detailing the predicted phase in the region of interest. Included with the predicted phase are details of the supporting and opposing evidence and distributions of key and non-key SNPs in each phase. If these statistics support an affected status, abandon further calling of phase.

D **Chromosomal abnormalities affecting phasing** – There are a number of chromosomal abnormalities (eg, deletions and duplications) that can produce misleading phase in the karyomapping visualizations. Many of these effects are only visible on a chromosome level. It is important to zoom the Detailed Haploblock Chart to the chromosome containing the region of interest. Then compare the resulting patterns to the results detailed in *Calling Patterns* on page 18. If an event that prevents phasing overlaps the region of interest, calling of phase is terminated and the embryo is rejected.

E **Calling status in region of interest** – Zoom the Detailed Haploblock Chart to the region of interest and review the phasing implied by the positions and phases of the key and non-key SNPs. Assess the importance of any SNPs that oppose the current estimate of phase. Issues to consider are:

1. Location of SNPs within the region and flanking regions. A key or non-key SNP with opposing phase on the very edge of a flanking region could be less significant than the one located close to the actual PGD region.
2. Distribution of SNPs across the region and flanking regions. Distribution of SNPs affects confidence in phase. For example, having only a few key SNPs at the outer edges of the flanking regions reduces confidence that the phasing across the entire region is correct.
3. Low numbers of key SNPs in the region and flanking regions. The software still attempts to estimate phase in these regions. Before deciding on the actual phase, look at the position and phase of these key SNPs and how their phase compares with any non-key SNPs.
4. When assessing each SNP, account for the overall characteristics of the sample, including SNP call rate, levels of ADO and miscalls, biopsy type, and reference type.

F **Common recombinations preventing phasing** – Recombinations in the reference appear as a change in phase in all embryos. Additionally, each embryo contains its own unique recombinations. Occasionally a recombination in the reference and a recombination in an embryo occur so closely together that only 1, 2, or no SNPs exhibit a change in phase (see *Common Recombinations* on page 22). Without knowing about the common recombination, it is possible that a small region of opposite phase might not be visible or is mistaken for noise. If this event occurs in the gene region, it can cause a misdiagnosis. By checking the region of interest in the other embryos, it is possible to identify the presence of any common recombinations. The presence of a common recombination in a case can inform the diagnosis of the embryo where it is not visible. If a common recombination has been identified in a case inside the gene region, reject all of the embryos in that case. If another reference sample is available, restart the case workflow using this alternative family member as the reference.

Case Warnings

The Case Report includes a section for warnings at the beginning of the report. The warnings displayed are a guide and require verification; review warnings as the first step in the calling process. The patterns within the data that indicate certain problems that BlueFuse Multi can identify and warn against are:

Mother/Father/Reference sample ID: Call rate ≤ non-embryo threshold (0.8)

If the father, mother, or reference have a SNP call rate of less than or equal to 0.8 (for example, 0.75), this warning is generated. Genomic DNA produces SNP call rates of > 0.95 in most cases. Poor data quality in the parents or reference affects the results from all embryos in this case. This warning is terminal for proceeding with the case. Investigate the low call rates and rerun the corresponding DNAs.

Embryo sample E1: Call rate ≤ embryo threshold (0.6)

If an embryo has a SNP call rate less than or equal to 0.6 (for example, 0.58), this warning is generated. Failed amplifications can cause low SNP call rates, in which case the genotyping algorithm can produce misleading results (indicated by a high AB call rate). Large numbers of deletions can also cause low SNP call rates. Either way, this warning is terminal for making any calls on the embryo. Embryos with this warning have their haploblocks grayed out in the Haploblock Chart and the Detailed Haploblock Chart.

Mother/Father/Reference sample ID: Check for CNVs overlapping region

This warning is generated if a region of copy number imbalance, in the trio, is suspected of overlapping the karyomapping region. BlueFuse Multi uses the LogR and BAF to call CNVs automatically. Informative SNP patterns in the Detailed Haploblock Chart provide stronger evidence for the presence of CNVs. Always use the Detailed Haploblock Chart to confirm CNVs in the parents or reference (see *Calling Patterns* on page 18). This warning is not necessarily terminal for proceeding with the case (see *CNVs in Reference* on page 20 and *CNVs in Parents* on page 21). However, always double-check understanding of the disease origin and the linkage in the family.

Simple calling has been triggered in the following samples: Mother, Father, Reference

BlueFuse Multi switches from standard CNV calling to Simple Calling (whole chromosome calling) when data are noisy (Log R Dev > 0.3). This warning indicates that simple calling has been triggered for one or more of the trio samples. Simple calling only attempts to identify whole-chromosome copy-number imbalances in a sample and does not identify CNVs in a sample.

Embryo sample E1: Maternal/Paternal/Both chromosomes possibly contaminated

If BlueFuse Multi suspects that the maternal, paternal, or both chromosomes have been contaminated, this warning is generated. The occurrence of large numbers of haploblocks across the genome, along with poor SNP statistics, generates this warning. It is not necessarily terminal to calling the phase of the sample. For example, this warning can be generated if an aunt or uncle (who can have regions where they appear unrelated to the embryo) is being used as a reference. This warning would also be generated if an embryo contained many duplications or if a labeling error has led to the inclusion of an unrelated sample. See *Contamination* on page 20 for details on how to determine if contamination has occurred.

Mother/Father sample ID or Reference sample ID: Entered as female/male but appears to be male/female

If BlueFuse Multi assesses that the sex of the sample does not match the sex specified in the Case Wizard, these warnings are generated. For example, if a sample has been entered as the father but appears to be female, this warning is generated. If 1 of these warnings is present, confirm that the correct GTC file was entered for the corresponding sample. Also confirm that no labeling errors occurred during the laboratory protocol before proceeding with any further analysis.

SNP Call Rates

The QC section in the Case Report includes metrics that can be used to consider the quality of the karyomapping data. The important metrics to consider are **call rate**, **AB rate**, **ADO**, and **Mis-Call rate.** The sample type has an influence on these values. The recommended values for good quality genomic DNA often obtained from parental and reference samples (blood), and for amplified DNA from embryo biopsies (trophectoderm and blastomere biopsies) are listed in Table 3.

Table 3 Recommended SNP statistics

Sample type	Call rate	AB rate	ADO§	Mis-Call rate
Blood	95–99%	25–29%	~0%	~0%
Blastomere biopsy	75–95%	~15%*	0–80%	<5%
Trophectoderm biopsy	85–99%	20–30%	0–80%	<5%

*Can be lower if the case has a good quality reference.

§The software is designed to account for ADO. A high ADO rate alone does not stop the calling of a case.

Predicted Phase

The region statistics for each embryo are listed in the Case Report and provide both supporting and opposing evidence for the automatically predicted phase. For each embryo, there are 2 sections (shown in Figure 5):

1 The predicted phase – M1 or M2, P1 or P2, listed with the supporting and opposing evidence.

2 The evidence that supports the predicted phase broken down into the number of key (strong evidence) and non-key (weaker evidence) SNPs for each phase.

Use this presented evidence in combination with that provided in the visualizations to decide on the actual phasing of the embryo in the region of interest.

BlueFuse Multi reports if it is unable to predict a phase for either chromosome in an embryo. This event can occur for a number of reasons:

1 Breakpoint in Karyomapping region. If there is a change in haplotype phase inside the flanking regions, BlueFuse Multi does not try to predict a phase.

2 No relevant information from reference. For example, if a reference is used that is only related to the mother, no phasing is predicted for the father. See *Reference Type* on page 22 for more details.

3 Low SNP call rate in the embryo. BlueFuse Multi does not predict a phase for samples that generate a low SNP call rate warning.

4 Potential contamination. BlueFuse Multi, does not predict a phase for samples it suspects are contaminated.

It is important to note that in all of these cases, it is still possible to produce a call on the phasing of an embryo using the available SNP and haploblock information. To aid the analyst, any Case warnings relevant to the embryo in this region are repeated here.

Figure 5 Case Report – SNP phasing

Region RB1, Sample E2

Predicted Phase	**M2, P2**			
Supporting Evidence	31 key SNPs support M2			
	56 key SNPs support P2			
Contrary Evidence	0 key SNPs oppose M2			
	0 key SNPs oppose P2			
Maternal SNPs	**Maternal-M1**		**Maternal-M2**	
Region	**Key**	**Non Key**	**Key**	**Non Key**
5'	0 / 23	0 / 18	17 / 18	23 / 23
Main	0 / 0	0 / 0	0 / 0	0 / 0
3'	0 / 15	0 / 15	14 / 15	15 / 15
Paternal SNPs	**Paternal-P1**		**Paternal-P2**	
Region	**Key**	**Non Key**	**Key**	**Non Key**
5'	0 / 0	10 / 44	26 / 44	0 / 0
Main	0 / 0	0 / 4	4 / 4	0 / 0
3'	0 / 0	14 / 43	26 / 43	0 / 0

Calling Patterns

Examination of the calling patterns is part of the case checking required to make sure that the SNP evidence supports the predicted phase. It is important to note that many of these patterns are not evident when zoomed in on a small region. Always examine the data at the chromosome level as part of the calling process. Different patterns that occur in karyomapping profiles can indicate cases where phase is not necessarily indicative of inheritance of a chromosomal region but actually indicate a different biological phenomenon. The patterns can reflect how the function of the karyomapping algorithm works rather than actual underlying phase. This section illustrates the patterns that can be identified in BlueFuse Multi and what they are likely to indicate.
Common karyomapping calling patterns are shown in Table 4, Table 5, and Table 6. Atypical calling patterns are shown in Table 7 through Table 10. Someone with background knowledge of a particular case, and having checked the warnings within BlueFuse Multi, can expect to see some of the calling patterns indicated.

Table 4 Common Calling Pattern - Normal Case

	Description	Interpretation
Key and non-key SNPs	Predominantly a single phase, supported by key and non-key SNPs.	What you expect to see in a normal karyomapping case.
Profile for 1 parental chromosome		

Table 5 Common Calling Pattern - Duplication in Parental Chromosome

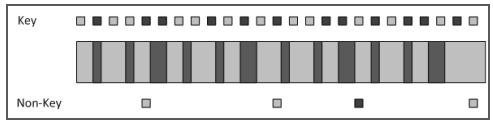
	Description	Interpretation
Key and non-key SNPs	Predominately key SNPs with a few non-key SNPs. Key SNPs vary phase 'randomly,' producing many different phase haploblocks.	Region is likely to be the result of a duplication in the parental chromosome. Interpretation is independent of phase of SNPs. NOTE Only those regions where the duplication involves material from different haplotypes are detectable through karyomapping.
Profile for 1 parental chromosome		

Table 6 Common Calling Pattern - Deletion in Parental Chromosome

	Description	Interpretation
Key and non-key SNPs	Predominately non-key SNPs with a few key SNPs. Non-key SNPs vary phase 'randomly.'	Region is likely to be the result of a deletion in the parental chromosome. Interpretation is independent of phase of SNPs.
Profile for 1 parental chromosome		

Table 7 Atypical Calling Pattern - Contaminated or Mislabeled Sample

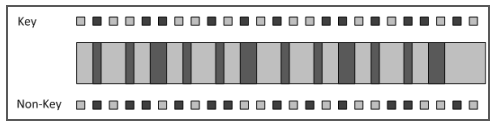
	Description	Interpretation
Key and non-key SNPs	Key and non-key SNPs are equally balanced AND phase. Key and non-key SNPs vary phase 'randomly' producing many different phase haploblocks.	Sample is likely to have been contaminated or the sample was mislabeled.
Profile for 1 parental chromosome		

Table 8 Atypical Calling Pattern - Deletion in Reference

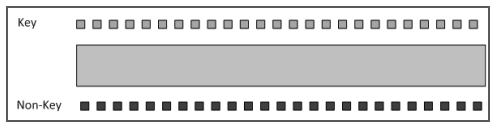
	Description	Interpretation
Key and non-key SNPs	Key and non-key SNPs are equally balanced AND key SNPs are out of phase with the reference sample.	Reference is likely to have a deletion in this region. NOTE Examine the BAF chart for the reference to confirm this finding.
Profile for 1 parental chromosome		

Table 9 Atypical Calling Pattern - Duplication in Reference

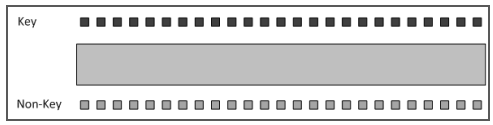
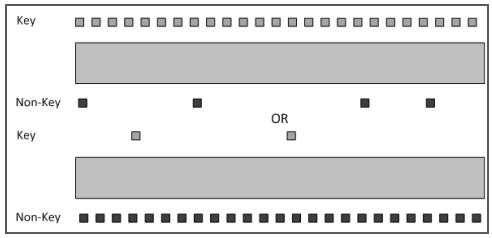
	Description	Interpretation
Key and non-key SNPs	Key and non-key SNPs are equally balanced AND key SNPs are in phase with the reference sample.	Reference is likely to have duplication in this region OR the reference might have uniparental heterodisomy. NOTE Examine the BAF chart for the reference to confirm this finding.
Profile for 1 parental chromosome		

Table 10 Atypical Calling Pattern - Parents Share Chromosome Region

	Description	Interpretation
Key and non-key SNPs	Either only key or only non-key SNPs that are either all in phase or all out of phase for each parent.	Parents share this chromosome region and possibly share their inheritance. The phasing in this situation is still an accurate representation of this inheritance. This behavior will mask the presence of any duplications or deletions.
Profiles for parental chromosomes		

Contamination

Contamination of a sample can occur on the maternal chromosome, the paternal chromosome, or both chromosomes. It can occur as part of the biopsy process or during the laboratory protocols. When contamination occurs, it affects all of the 23 chromosomes used in karyomapping.

If an embryo is contaminated on the maternal chromosome, paternal chromosome, or both, the software tries to detect contaminated samples and produces a warning in the case report.

The following situations would be indicative of a contamination event:

- The "in phase and out of phase" pattern outlined in the *Calling Patterns* summary.
- Duplications on 1 parent and deletions on the other across all 23 chromosomes.
- Poor data quality across all chromosomes despite good SNP call rates and AB rates.

CNVs in Reference

Duplications and deletions in the reference can produce misleading information about the phase of an embryo. The presence of a CNV in the reference forces the presence or absence of the informative allele in the reference, restricting the phases that key and non-key SNPs in the embryo can take. If a CNV in the reference is present, the phasing of the embryos in this region is not accurate. See *Calling Patterns* on page 18 to assess the patterns expected when there is a deletion or duplication in the reference.

Because the reference is taken from genomic DNA, we can find CNVs using the standard BeadArray processing in BlueFuse Multi. However, CNV detection performance is lower than with standard cytogenetic assays due to the optimization for PGD laboratory workflows and are secondary to the assessment of the phasing pattern. If any of the regions of interest overlap these CNV regions, the software produces a warning in the case report.

BlueFuse Multi uses simple (whole chromosome) calling for particularly noisy samples. If simple calling is used, a case warning is listed in the Case Report (see *Case Warnings* on page 14). Simple calling can mask a CNV in the parents or reference. Always use the Detailed Haploblock Chart to look for patterns associated with CNVs in the trio because they can also affect phasing (see *Calling Patterns* on page 18).

CNVs in Parents

A deletion in a parent produces a simple pattern: no informative SNPs for that parent. If a parent only has 1 chromosome, it cannot possibly be heterozygous at any of the deleted loci.

For duplications in the parents, phasing using karyomapping is still possible although the data can be of poorer quality than with a euploid parent.

BlueFuse Multi makes automatic copy number calls from the Log R and BAF data, which are less robust than the SNP patterns. Always examine these patterns in detail (see *Calling Patterns* on page 18).

Consanguinity

Consanguinity refers to a degree of relatedness between 2 individuals. Consanguinity can affect the patterns produced in a karyomapping case when it results in shared genetic material between the chromosomes of the parents.

If a parent's 2 chromosomes are identical in a certain region because of a consanguineous relationship between their parents, this region exhibits no informative SNPs for that parent because they are homozygous at every locus.

If there is 1 chromosome that both parents share, a particular set of patterns are produced. Because all embryos inherit from the same consanguineous parents, they all exhibit 1 of these patterns, which are listed in Table 11. Whether their SNPs are all key or all non-key depends on which chromosome they have inherited in this region and their phasing depends on the inheritance of the reference. In these situations, the SNPs still reflect the chromosomal inheritance and calling of phase can still proceed. In consanguineous regions, duplications and deletions no longer produce abnormal patterns in the detailed Haploblock Chart and are not visible.

Table 11 Effects of embryo and reference inheritance from consanguineous parents

Inheritance	Pattern
Embryo inherits shared chromosome	Embryo only contains non-key SNPs
Embryo inherits unique chromosome	Embryo only contains key SNPs
Reference inherits shared chromosome	All key SNPs are out of phase with reference All non-key SNPs are in phase with reference
Reference inherits unique chromosome	All key SNPs are in phase with reference All non-key SNPs are out of phase with reference

Reference Type

With recessive disorders, the parents might have become aware that they were carriers of the disease only when they had their first affected child. Here we can use the affected child as a reference.

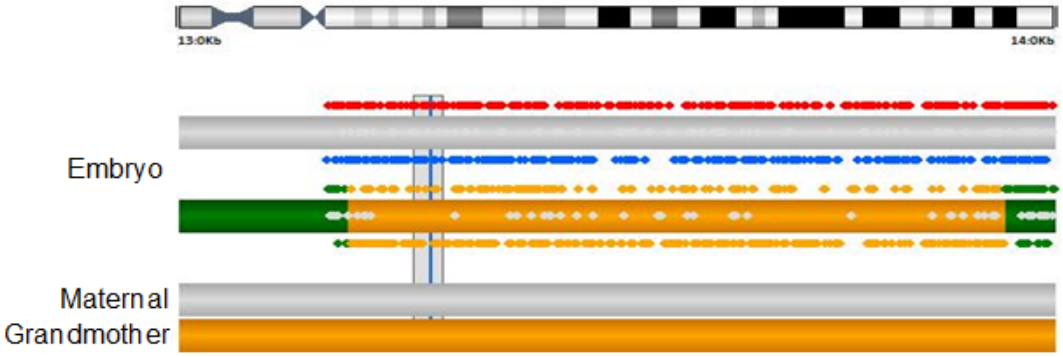
For dominant disorders, the affected parent is already aware of their status. The couple can choose to have their first child using karyomapping to make sure that it is born unaffected. A close relative of the affected parent can be used as a reference.

Non-Sibling Reference

When using a non-sibling reference, at every locus there is a haplotype in the reference that the corresponding parent has not inherited. Therefore the haplotype is not present in any of the embryos. This haplotype can interfere with the phasing of the embryos. To avoid this interference, only informative SNPs where the reference is homozygous are included in the analysis when the reference is not an existing child. Approximately half of the informative SNPs are excluded from the analysis so the data are more sparsely distributed.

Because the software is aware of the relationship status of the reference, it grays out any chromosomes for which there is no information. For example, if the reference is a maternal grandparent, the paternal chromosome is grayed out, illustrated in Figure 6.

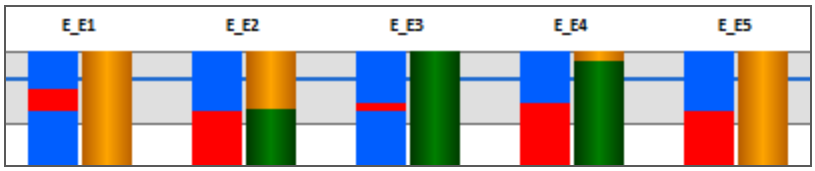
Figure 6 Detailed Haploblock Chart for an embryo processed with a maternal grandparent as a reference. Note the grayed out paternal chromosome.



Common Recombinations

Unlike other PGD techniques, phasing in karyomapping is relative to the reference. A recombination in the reference produces a change in phase in any embryo phased against it. These recombinations are called common recombinations because they appear in all of the embryos in a case. Figure 7 shows a section of the haploblock chart for 5 embryos phased against the same sibling reference. A breakpoint exists at approximately the same location in every embryo. A recombination in the reference causes this breakpoint. The precise location of the estimated breakpoint differs slightly from embryo to embryo due to differences in key and non-key SNP distributions.

Figure 7 Section of a haploblock chart where the reference contains a recombination on the paternal chromosome. The resulting common recombination is visible in all 5 embryos.



Two recombinations occurring close together in the embryo is highly unlikely. In karyomapping, small haploblocks can be produced if a recombination in the embryo and the common recombination in the reference occur close together. In Figure 7, embryos E1 and E3 have recombinations that are close to the reference recombination. Common recombinations in the gene region have implications for embryo calling. For more information, see *Calling Patterns* on page 18.

X-Linked Disorders

When dealing with disorders on the X chromosome, there are a number of situations where the determination of the status of the embryo requires knowledge of the sex of the embryo. For example, disorders where the father is affected.

To determine the sex of the embryo, BlueFuse Multi contains 2 complementary sources of information that must always be used together.

- Log ratio and BAF data. The overall quality of the BeadChip profiles for embryo biopsies is not sufficient for CNV calling. However, deletions are often visible in the BAF profile and nullisomies are visible in the log ratio data. This information can be used to check the copy number of X and for the presence of a Y chromosome.
- X chromosome heterozygous call rate and Y chromosome SNP call rate. When a region has been entered that is on the X chromosome, the Case Report presents 2 call rates: the fraction of SNP calls on X that were heterozygous and the overall fraction of SNP calls on Y. For male samples, X has zero heterozygous SNP calls and some SNP calls on Y. For female samples, one-third of SNP calls on X are heterozygous and there are almost no SNP calls on Y.

Incidental Findings

Be aware of local legislation and legal agreements with customers regarding the disclosure of incidental findings. Such incidental findings include:

- CNVs
- Paternity
- Consanguinity
- Sex of the embryos

Glossary

Allele Drop-Out (ADO) – The random non-amplification of 1 of the alleles present in a heterozygous allele; a phenomenon specific to single-cell amplification techniques.

Common recombination – A change in phase that is common to all embryos in a case and is the result of a recombination in the reference.

Haploblock – A region of continuous phase.

Haplotype – A group of alleles at adjacent loci that are inherited together.

Informative SNP – A SNP where the parental origin of a chromosome can be identified and where the 2 haplotypes belonging to that parent can be distinguished.

Informative Allele – The allele that exists on only 1 of the parental chromosomes at an informative SNP locus.

In Phase – When the embryo inherits the same chromosome region as the reference.

***In vitro* Fertilization (IVF)** – A complex procedure from removal of eggs, fertilization, and transfer of an embryo back into the uterus.

Karyomapping – A comprehensive method for genome-wide linkage-based analysis of single gene defects that can be used on a single cell or small number of cells from an embryo in preimplantation genetic diagnosis (PGD).

Key SNP – A SNP whose phase ADO cannot have altered; provides strong evidence for the underlying phase.

Microsatellite – Simple DNA sequence repeats.

Non-key SNP – A SNP whose phase ADO might have altered; provides weaker evidence for the underlying phase.

Out of Phase – When the embryo inherits a chromosome region different from that region of the reference.

Phasing – The process of identifying the haplotype of a chromosome or chromosome region.

Preimplantation Genetic Diagnosis (PGD) – The genetic profiling of embryos before implantation.

Recombination – The process during which 2 chromosomes exchange genetic information during meiosis.

Reference – The sibling, or other close relative of known disease status, used to identify the affected haplotype.

Short Tandem Repeat (STR) Analysis – A method for comparing specific DNA loci from 2 or more samples.

Single Nucleotide Polymorphism (SNP) – A DNA sequence variation that occurs at a single nucleotide.

Supporting Documentation

Additional support for karyomapping includes:

- *Infinium Karyomapping Assay Protocol Guide (part # 15052710)* – How to set up a laboratory and perform a karyomapping assay.
- BlueFuse Multi Karyomapping Walkthrough Guide – How to create a karyomapping case in BlueFuse Multi.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Table 12 Illumina General Contact Information

Website	www.illumina.com
Email	techsupport@illumina.com

Table 13 Illumina Customer Support Telephone Numbers

Region	Contact Number	Region	Contact Number
North America	1.800.809.4566	Italy	800.874909
Australia	1.800.775.688	Netherlands	0800.0223859
Austria	0800.296575	New Zealand	0800.451.650
Belgium	0800.81102	Norway	800.16836
Denmark	80882346	Spain	900.812168
Finland	0800.918363	Sweden	020790181
France	0800.911850	Switzerland	0800.563118
Germany	0800.180.8994	United Kingdom	0800.917.0041
Ireland	1.800.812949	Other countries	+44.1799.534000

Safety Data Sheets

Safety data sheets (SDSs) are available on the Illumina website at support.illumina.com/sds.html.

Product Documentation

Product documentation in PDF is available for download from the Illumina website. Go to support.illumina.com, select a product, then click **Documentation & Literature**.

Illumina
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com