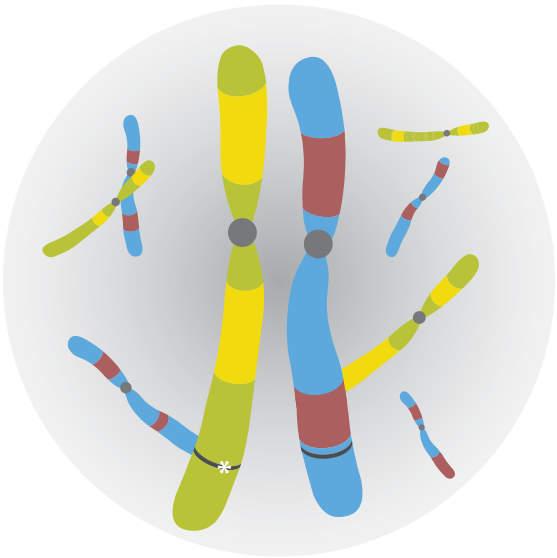
Karyomapping: A Rapid PGD Solution for Single-Gene Disorders

The ability to decipher DNA sequences is providing new insights into human health and helping us enter a new era of genomics-based healthcare. Among the many areas benefitting from this increase in knowledge is reproductive health, particularly preimplantation genetic diagnosis (PGD). In PGD, embryos are assessed for a specific genetic condition before transfer into the uterus. The goal of PGD is to prevent couples with a known risk of transmitting an inherited genetic disorder of having children affected with that disorder.

PGD relies on the use of genetic markers within the genome to assess the likelihood of an embryo carrying a mutated version of the gene involved in a severe single-gene disorder. This is often performed through short tandem repeat (STR) analysis. This method has been successful, but involves extensive patient-specific work-up and is labor-intensive to perform. An alternative method, karyomapping[1], uses linkage-based genome-wide mapping to provide a high-density view of chromosomes and insight into their parental origin. Karyomapping creates a complete map of the chromosomes inherited by the embryo for accurate assessment of the presence of severe single-gene disorders from a single embryonic cell and can be performed with a 24-hour protocol.

Illumina technology provides the accuracy, reliability, and ease of use required for karyomapping. The HumanKaryomap-12 BeadChip targets the most informative markers in the genome for efficient genome-wide coverage. The result is the single most informative assay available at the single-cell level, providing insight into the inheritance of single-gene defects, genome-wide.



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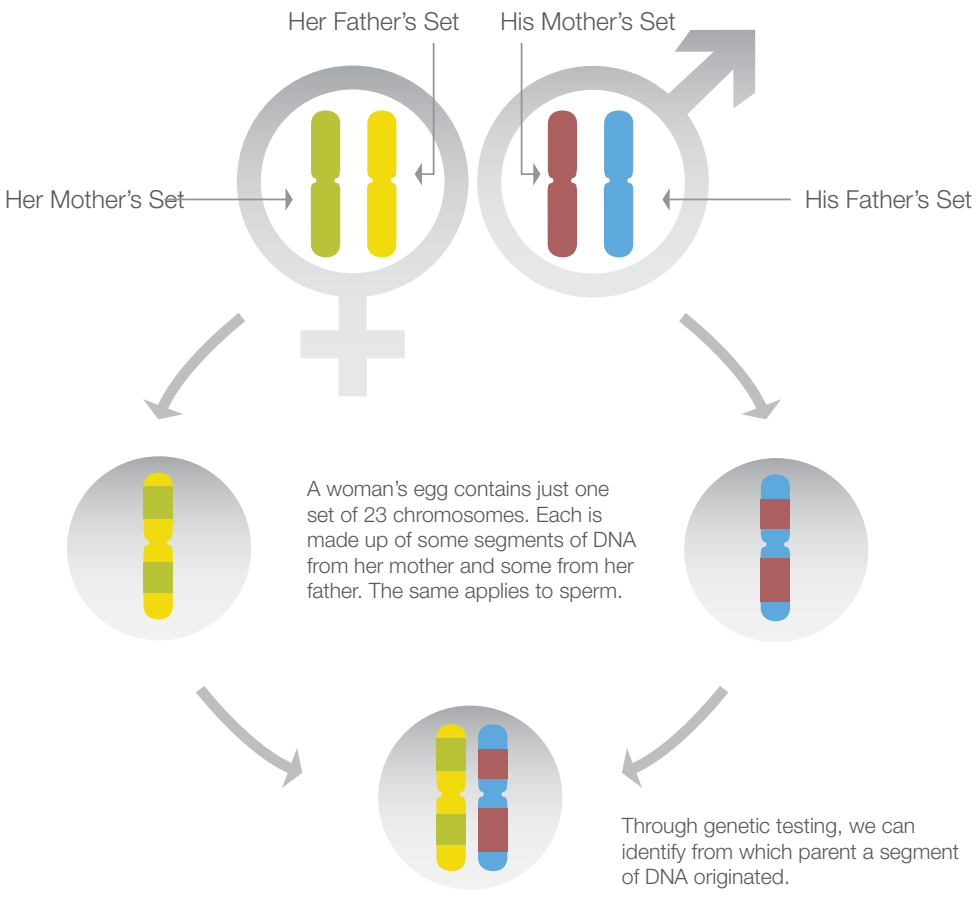
Human genetic variation and PGD.

Human cells contain two sets of chromosomes, one inherited from the mother and one from the father, to make a total of 46. Egg and sperm cells are the exception; each containing just 23 chromosomes. When the egg and sperm cells fuse at fertilization, the correct number of chromosomes is restored. During egg and sperm development, chromosomes undergo recombination—a process in which a pair of chromosomes, one inherited from the grandmother and one from the grandfather (relative to the embryo) exchange genetic material. This recombination means that an embryo does not always inherit an exact copy of a paternal chromosome and an exact copy of a maternal chromosome. In fact, the embryo inherits a mixture of the two paternal chromosomes and a mixture of the two maternal chromosomes (Figure 1). This process helps to generate genetic variation in the human population.

PGD, either with karyomapping or STR analysis, identifies genetic loci on the inherited chromosomal segments to assess the genetic status of each embryo. These methods require genetic information from the parents, and a "reference", which is a close relative of known disease status. The determination of the disease status of the embryo works by establishing whether the embryos have inherited the same chromosome segments from the parents as the reference or not.

Figure 1: Generation of Human Genetic Variation



More informed PGD.

Two methods of PGD are currently in use: STR analysis and karyomapping (Table 1).

STR analysis

Current PGD methods rely on examining STRs adjacent to specific disease loci to identify a gene mutation for a particular disorder. STRs are repeating sequences of 2–6 base pairs of DNA; a common STR is the sequence CA. STRs often differ in repeat number between alleles and between individuals. When used for PGD, each STR test for a genetic disorder must be developed individually, resulting in a time-consuming process that can only be accessed at a few specialist laboratories. Each test examines only a few STR markers and can produce inaccurate results due to recombination events.

To determine the source of parental chromosomes in the embryo, a laboratory performing STR analysis searches for microsatellite regions surrounding the gene of interest that contains specific STRs. If microsatellites can be identified, it is possible to use PCR to calculate the size of each of microsatellite region. If the sizes of these regions differ between each parent, it is then possible to identify the origin, maternal or paternal, of the chromosome region the child has inherited.

STR analysis has a number of inherent challenges:

1. Microsatellite regions must be on both sides of the gene of interest to make sure that a recombination event has not occurred within that region.

2. Microsatellite regions may not be informative if, for example, the parents happen to have the same number of STRs in the microsatellite region of interest.

3. Microsatellite regions can be a significant distance from the gene, reducing confidence that a recombination event has not ‘swapped’ the affected gene into that region without affecting the flanking microsatellite regions.

4. PCR probes must be designed for each different condition, may need to be specific to the family being studied, and may not always work.

5. Interpreting STRs is a skilled, time-consuming, and sometimes subjective, process.

Karyomapping

Karyomapping analysis uses genome-wide linkage to reveal the inheritance of genetic disease loci present in one or both parents. Instead of STRs flanking a gene of interest as markers, karyomapping uses single nucleotide polymorphisms (SNPs). A SNP is a DNA sequence variation occurring when a single nucleotide in the genome differs between individuals. They are easily identified using existing genome-wide SNP technologies. Karyomapping compares SNPs from an embryo to those of a reference to establish the likelihood of the embryo having inherited the mutated gene.

Karyomapping offers a number of advantages over STR analysis, including:

1. SNPs are fairly evenly distributed over the entire genome.
2. The HumanKaryomap-12 BeadChip provides a large number of data points, reducing the risk of missing a recombination event in the genomic region of interest.
3. Karyomapping does not require custom design of probes for each inherited single-gene disorder, resulting in significant time savings.
4. Karyomapping is amenable to automated analysis using software for consistent results.

Table 1: A Comparison of STR Analysis and Karyomapping

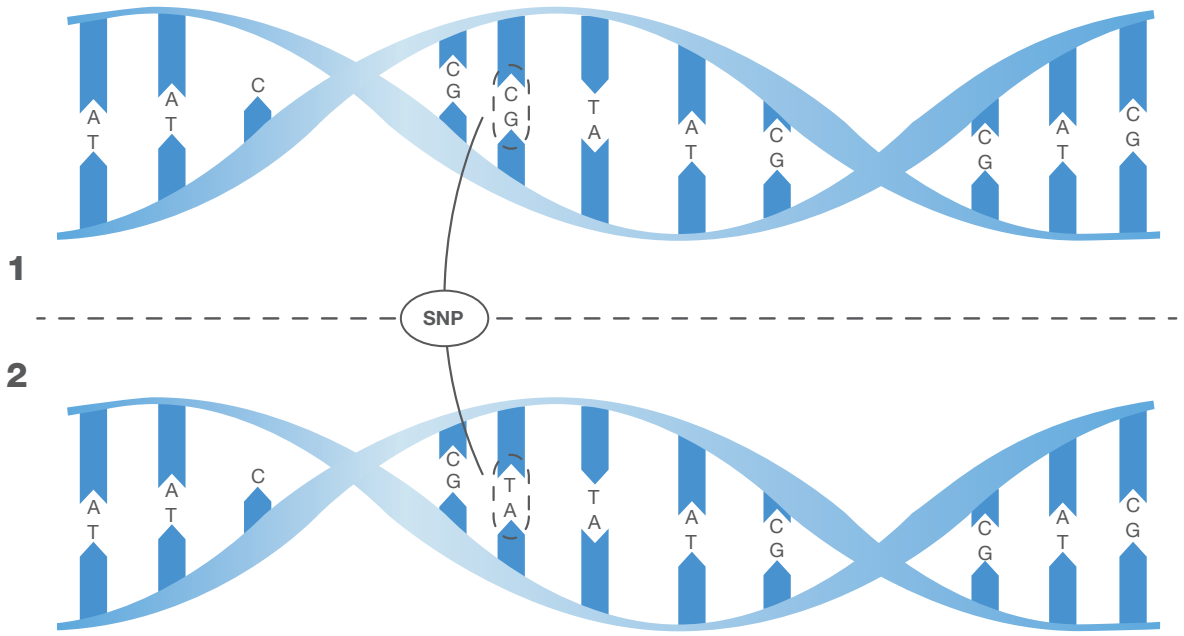
	STR Analysis	Karyomapping
Description	Uses DNA repeats of 2–6 base pairs adjacent to a specific locus to identify a defective gene	Uses SNPs and genome-wide linkage data to inform the presence or absence of a defective gene
Markers	Multiallelic; measures variation in repeat length	Biallelic; measures variation at a single base
Coverage	Limited to a single locus in each set of STR markers	Able to screen multiple loci in parallel
Location	Requires knowledge of the location of the affected gene	Requires knowledge of the location of the affected gene
Preparation Time	Typically 3–6 months to work up and validate multiple STR markers	None; off-the-shelf solution
Workflow	Customized set of primers for each case	Standard workflow for all studies
Linkage Analysis	Performed manually	Automated data interpretation using BlueFuse Multi analysis software
Aneuploidy	Not available	Not currently offered

The power of SNPs

Karyomapping relies on genetic base differences at particular sites in the DNA sequence, known as SNPs, to identify chromosome segment inheritance. SNPs can differ from one chromosome to another, generating different genetic alleles. By referring to the different allele types in a chromosome pair as allele ‘A’ and allele ‘B’, the karyomap shows hetero- or homozygosity at a specific location and helps to identify parental origin (Figure 4).

The 1000 Genomes Project (1kGP) recently identified ~35M SNPs across the human genome. To generate a karyomap, it is not necessary to look at all 35M SNPs. Illumina uses a process of intelligent marker selection to identify tag SNPs that can serve as proxies for many other SNPs in the genome. The use of tag SNPs greatly improves the power of a study as the same information from a larger number of SNPs can be gathered by reviewing only a subset of the loci. To this end, the Illumina HumanKaryomap-12 BeadChip is able to produce a genome-wide map of chromosomal abnormalities and markers for single-gene disorders using ~300,000 SNPs.

Figure 2: Single Nucleotide Polymorphisms (SNPs)

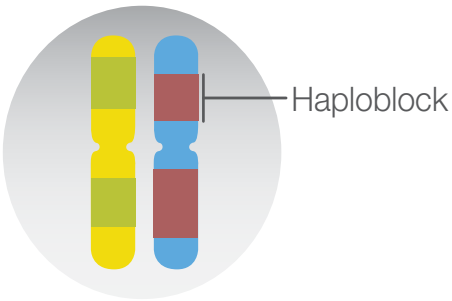


Two chromosomes (1 and 2) showing a SNP. Biallelic SNPs are generically referred to as ‘A’ and ‘B’. Homozygous: AA or BB; Heterozygous (as shown): AB

About karyomapping.

Karyomapping uses SNP genotyping data from the parents and reference to create a comprehensive map of the parental origin of chromosome segments inherited by the embryo. These chromosome segments are called haploblocks (Figure 2). By establishing the inheritance of haploblocks surrounding the region of interest, it is possible to infer the disease status of each embryo—affected, carrier, or unaffected (Figure 3)—by comparing it to the disease status of a reference.

Figure 3: Inherited Haploblocks



A pair of chromosomes in an embryo with their predicted haploblocks. The green and yellow chromosome has come from the mother, the red and blue chromosome has come from the father.

Figure 4: Identifying the Inheritance Status of an Embryo

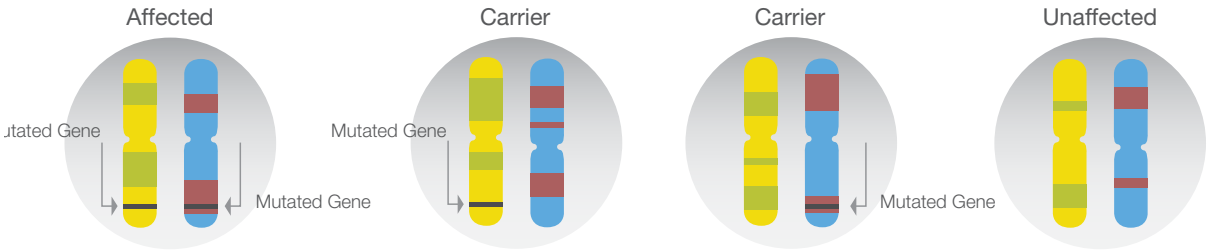


Illustration of the inheritance possibilities for four embryos from a karyomapping case with a recessive disorder. By identifying which haploblocks have been inherited, the status of the embryo (affected, carrier, or unaffected) can be established.

Glossary

1000 Genomes Project (1kGP)

An international collaboration to produce an extensive public catalog of human genetics variation, including SNPs; learn more at www.1000genomes.org

Haploblock

Intact chromosome segment between recombination sites that is inherited from one of the parental chromosomes

In vitro Fertilization (IVF)

A complex procedure that involves removal of eggs, fertilization of the eggs in a laboratory dish, and transfer of an embryo 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)

Microsatellite

Simple DNA sequence repeats of 2 to 6 bases that can differ between individuals and chromosomes

Preimplantation Genetic Diagnosis (PGD)

The genetic testing of embryos before implantation in the uterus

Recombination

The process by which two chromosomes exchange genetic information during meiosis

Reference

The sibling, or other closely related relative, used to identify the affected haplotype

Short Tandem Repeat (STR) Analysis

A method for comparing specific DNA loci from two or more samples

Single Nucleotide Polymorphism (SNP)

A DNA sequence variation that occurs at a single nucleotide

Tag SNP

A SNP that can be used to represent a genomic region with high linkage disequilibrium

Learn more.

Karyomapping provides a faster, easier method for PGD before embryo implantation in IVF. To learn more or to find a specialist PGD laboratory, visit www.illumina.com/PGD.

Reference

1. Handyside AH, Harton GL, Mariani B, Thornhill AR, Affara N, et al. (2010) Karyomapping: A universal method for genome wide analysis of genetic disease based on mapping crossovers between parental haplotypes. J Med Genet. 47: 651–658.

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