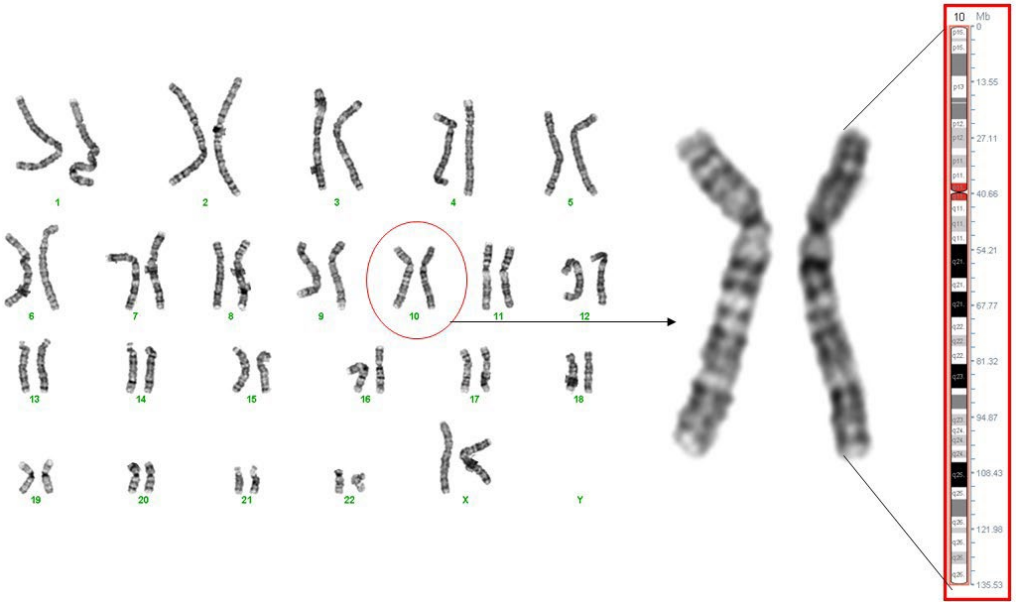
PathWest
LABORATORY MEDICINE WA

PathWest Education

Conventional Karyotype Vs Chromosomal Microarray



What is a Conventional Karyotype and What Does it Detect

- Karyotyping refers to **visualising chromosomes under a microscope**
- Special stains are used to help distinguish chromosome regions, most commonly **Giemsa staining** i.e. a **G-Banded Karyotype**
- Assesses **ALL** chromosomes and identifies the sex chromosome constitution
- Assesses for abnormalities in:
  - Total number of chromosomes (aneuploidy)
  - Size and structure of each chromosome
    - **Detects large chromosomal imbalances** (e.g. subchromosomal deletion)
    - **Detects balanced translocations**: where there is no loss of total amount of genetic material
    - **Detects unbalanced translocations**: where there is a change in the total amount of genetic material
- **Multiple cells are assessed** (in case an abnormality is not present in all cells – known as mosaicism)
- Deletions, duplications or rearrangements must be **large enough** (approximately >5 Mb) to be visualised under the microscope to be detected by conventional karyotype. Small changes will not be detected.

What is Chromosomal Microarray (CMA)

- There are two types of Chromosomal microarray:
  - **Comparative Genomic Hybridization (CGH) array**
  - **Single Nucleotide Polymorphism (SNP) Array**

- At PathWest, we do **SNP array** only: provides information on copy number variants and genotype

- CMA looks at **ALL chromosomes**: allows many regions across the genome to be analysed simultaneously

- Allows the identification of smaller copy number changes (microdeletions and microduplications) than conventional karyotype analysis, but analysis can be more complex and usually has a longer turn-around time

What Chromosomal Microarray (CMA) DOES Detect

Chromosomal Microarray is a test that searches for additional (gains) or missing (losses) segments of chromosomes. These include:

- **Most changes in total chromosome number**: i.e. trisomy (an extra chromosome) or monosomy (a missing chromosome)
- **Sub-chromosomal content changes: micro-duplications** or **micro-deletions** of segments of chromosomes: these changes may be too small to see under a microscope (i.e. by karyotype)
- **Structural chromosomal changes: unbalanced rearrangements** of chromosome structure: e.g. translocations with some loss or gain of genetic material

Other abnormalities that CMA may detect include:

- **Mosaicism** (where some cells are normal and some cells are abnormal), may be detected if the proportion of abnormal cells is approximately >5-10%
- **Duplications of the entire set of chromosomes**: e.g. triploidy (3 copies of each chromosome, resulting in 69 chromosomes per cell)
- **Regions of homozygosity** (may suggest common ancestry, an imprinting disorder, or a recessive disease)

What Chromosomal Microarray (CMA) Does NOT Detect

Chromosomal Microarray does NOT detect:

- **Point mutations**: a change in a single nucleotide base of a gene i.e. the majority of changes within individual genes
- **Tiny deletions or duplications** of DNA segments within a single gene
- **Balanced rearrangements** of chromosomes: e.g. inversions, balanced translocations, insertions
- **Low level mosaicism** i.e. when the proportion of abnormal cells is below the detection threshold of the technology

CMA is generally not the most appropriate test if there is high suspicion of a single gene disorder.

Risks Associated with Chromosomal Microarray Testing

Variants of Uncertain Significance (VOUS)

- As CMA allows wider analysis of the genome than other more specific genetic testing methods, there is a risk of finding variants of uncertain significance:
  - Testing of family members may help clarify the result
  - Future knowledge may also improve understanding of the result
  - Genetic counselling is advised in this scenario

Unsolicited Findings

- With CMA, there is also a risk of identifying variants that may be unrelated to the reason CMA was originally requested, and may have relevance for other family members
- Genetic counselling is helpful in this scenario

Parental Relatedness

- CMA may reveal parental relatedness

Families / patients should therefore be **counselled** appropriately prior to requesting chromosomal microarray

Should I request Conventional Karyotype or Microarray?

Common Conventional Karyotype Indications:

- Recurrent miscarriage or Infertility
- Suspected common aneuploidy (Trisomy 13, Trisomy 18, Trisomy 21, Turner syndrome, Klinefelter syndrome)
- Family history of a known chromosomal rearrangement detectable by karyotype analysis

Common Microarray indications:

- Postnatal*: Microarray is useful when you have a patient who has features that do not fit a specific known syndrome or when the concerns are more general and include:
  - Developmental delay
  - Intellectual impairment
  - Autism spectrum disorder
  - 2 or more congenital abnormalities
- Prenatal^: Microarray is useful when you have a fetus with:
  - 1 or more congenital abnormalities detected on ultrasound
  - Nuchal Translucency greater than 3.5mm
- Testing of fresh products of conception/fetal tissue in pregnancy loss cases, particularly where there is a history of recurrent miscarriage or fetal anomalies are present

Please see Medicare Benefits Schedule (MBS) online:
*Postnatal: Item 73292 | Medicare Benefits Schedule (health.gov.au)
^Prenatal: Item 73388 | Medicare Benefits Schedule (health.gov.au)

Karyotype vs Microarray Summary

	Conventional Karyotype	Chromosomal Microarray (SNP)
Variation type detected	Numerical and structural chromosomal anomalies >5Mb	Copy number variants (CNVs) with mean test resolution >27Kb Regions of homozygosity
Level of mosaicism detected	Generally ~20%	5-10%
Specimen Requirement	Sodium-Heparin tube	EDTA tube
Turn-around time*	5-18 days	2 weeks – 3 months
Main indications	• Infertility • Recurrent miscarriage • Suspected common aneuploidy • Suspected chromosomal rearrangement	• Developmental delay • Intellectual impairment • Autism spectrum disorder • 2 or more congenital malformations • Fetal ultrasound abnormalities

*Please note that prenatal and neonatal samples are prioritised. For non-priority samples, turn-around times may be at the longer end of the range specified

Suspected Cytogenomic Syndrome: Which Genetic Test Should I Request?

- Although conventional karyotype and microarray can detect some genetic syndromes, they cannot detect everything
- For some genetic syndromes there may be a more specific genetic test available with a higher detection rate than microarray
- Depending on the clinical features and your index of suspicion for a particular syndrome, starting with a microarray may be the most appropriate 1st step, moving onto more specific testing if the microarray result is unremarkable or does not account for the clinical features in your patient
- Suggested first line testing when you suspect a specific genetic syndrome is described in the table on the next slide

Cytogenomic Syndromes/Conditions: Which Genetic Test Should I Request First-Line at PathWest?

Suspected Syndrome or Condition	First Line Test Required	Notes
Trisomy 13	Conventional karyotype	Can also be detected on SNP microarray*
Trisomy 18	Conventional karyotype	Can also be detected on SNP microarray*
Trisomy 21	Conventional karyotype	Can also be detected on SNP microarray*
Monosomy X (Turner Syndrome)	Conventional Karyotype	Can also be detected on SNP microarray*
XXY (Klinefelter Syndrome)	Conventional Karyotype	Can also be detected on SNP microarray*
22q11.2 Deletion (DiGeorge Syndrome)	MLPA for 22q11 deletion	Can also be detected on SNP microarray*
Williams Syndrome	Microarray	
Prader-Willi Syndrome	MS-MLPA for Prader-Willi Syndrome	SNP microarray will detect approximately 80% of cases*
Angelman Syndrome	MS-MLPA for Angelman syndrome	
Silver-Russell Syndrome	MS-MLPA for Silver-Russel syndrome	
Beckwith-Wiedemann Syndrome	MS-MLPA for Beckwith-Wiedemann syndrome	
Fragile X syndrome	Fragile X genetic testing (CGG repeat analysis of *FMR1* gene)	
Spinal Muscular Atrophy	SMA genetic testing	

* N.B. if less specific phenotype and more general features present (such as developmental delay, autistic spectrum disorder or multiple congenital abnormalities): a SNP microarray may be the more appropriate first-line genetic test

Tips for Requesting Cytogenomic Testing

- Ensure the patient has been **counselled and consented appropriately**
- Please **clearly state the test** being requested on the request form
- Highlight if test is **prenatal/diagnostic/predictive/carrier testing**
- Please **provide as much clinical information as possible** on the request form
- Please be mindful of the extensive time that goes into analysing and reporting the data:
  - We suggest requesting the genetic test most likely to provide an answer to the clinical query initially
  - Consider clinical genetics service referral if the phenotype is complex or specialist genetic counselling is required
  - Turn-around times are likely to be longer than pathology tests performed by many other departments due to the complex nature of genetic analyses