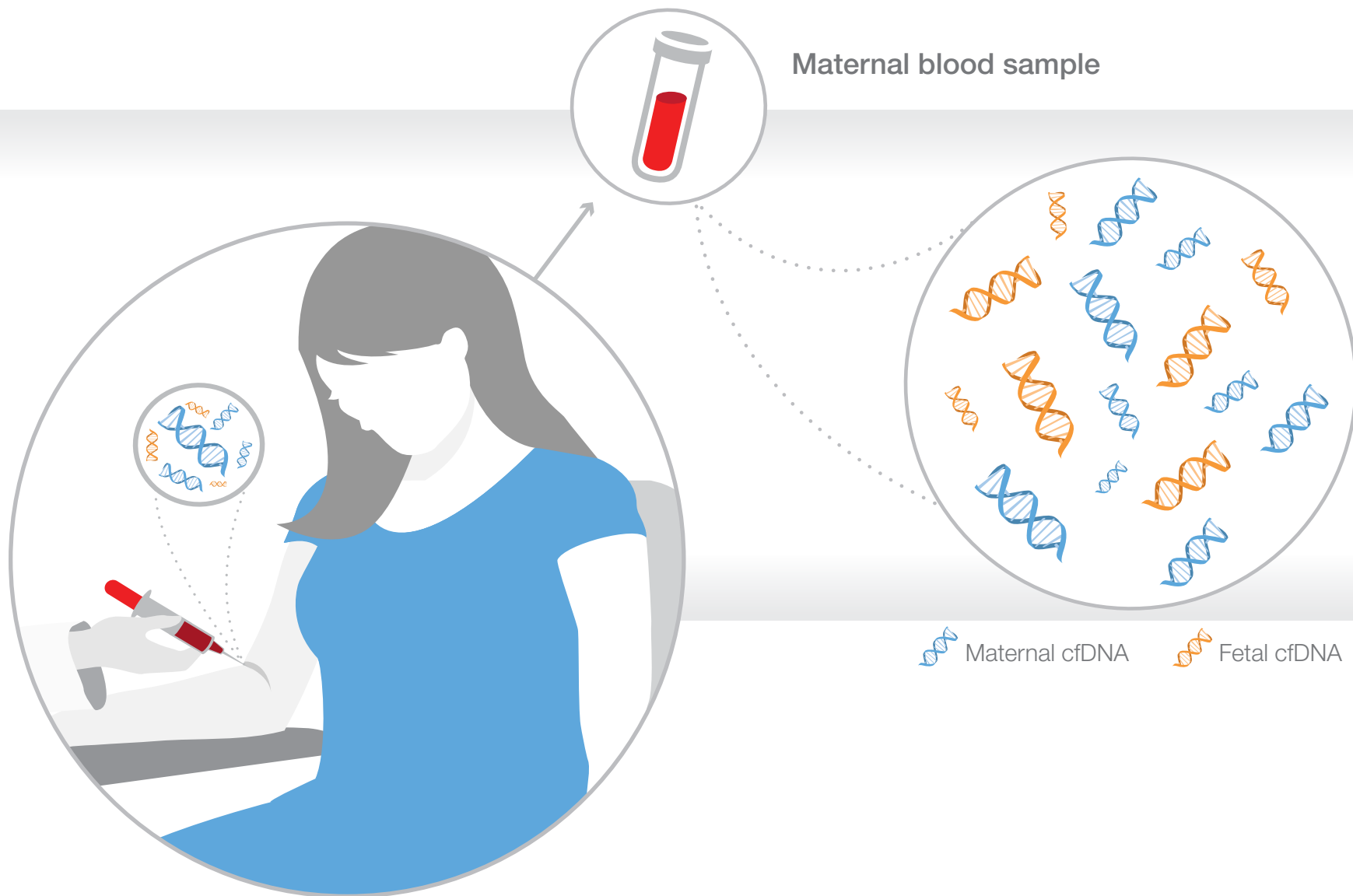




Noninvasive prenatal
testing (NIPT).



NIPT uses cell-free DNA.

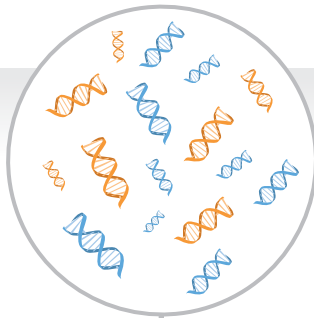


NIPT uses cell-free DNA.

- The maternal blood sample contains a mixture of both maternal and fetal cfDNA.
- Both maternal and fetal fragments are sequenced and counted in NIPT.
- Aneuploidies are detected by comparing the number of sequence counts of the chromosome of interest against a set of reference chromosomes.

How NIPT is performed.

Maternal and fetal cfDNA



CCCTTAGCGCTTTAACGTACGTAAAACCTT
AACGTACGTAAAAACGGGGTCAAAGGTTCCC
GACTTAAAATCGGAATCGATGCCCAAACCTT
AATCGATGCCCAAACGGGGTCAAAGTCCC

cfDNA sequenced via massively
parallel sequencing (MPS) and then
aligned to a reference genome

Counting

Unaffected
pregnancy

Affected
pregnancy

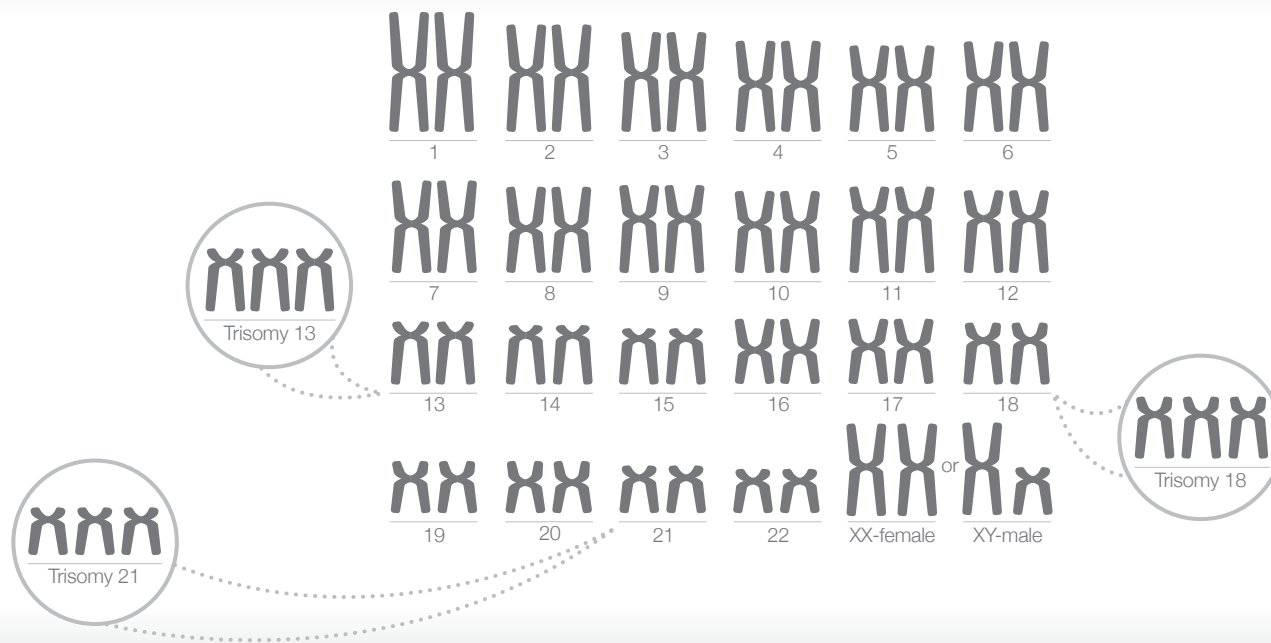


How NIPT is performed.

- Maternal blood sample contains a mixture of both maternal and fetal cfDNA.
- Massively parallel sequencing (MPS) analyzes cfDNA fragments from a maternal blood sample.
- Sequences are aligned to a reference genome to identify the chromosomal origin of the fragment.
- Aneuploidies are detected by comparing the number of sequence counts of the chromosome of interest against a set of reference chromosomes.

Screening for fetal chromosome abnormalities.

Chromosomes

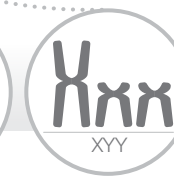


Screening for fetal chromosome abnormalities.

- NIPT screens for trisomies 21, 18, and 13.
- Additional screening for aneuploidies of other autosomes may be available.

Screening for sex chromosome abnormalities.

Chromosomes



Screening for sex chromosome abnormalities.

- NIPT can screen for sex chromosome aneuploidies including 45,X, 47,XXX, 47,XXY, and 47,XYY.
- Screening for the presence or absence of Y in twin pregnancies is available with some NIPTs.

PPV and NPV calculations.

Percentages calculated using sensitivity > 99.9% and specificity of 99.8%

Prevalence	Positive predictive value	Negative predictive value
1/10	98.2%	> 99.9%
1/100	83.5%	> 99.9%
1/250	66.7%	> 99.9%
1/500	50.0%	> 99.9%
1/1000	33.3%	> 99.9%

* Positive predictive values and negative predictive values are dependent on sensitivity and specificity as well as prevalence of disease.

To calculate PPV using a fixed sensitivity of 0.999[†] and specificity of 0.998[†], insert prevalence of the condition for your patient in decimal form.

$$\text{PPV} = \frac{0.999 \times \text{prevalence}}{(0.999 \times \text{prevalence}) + (0.002^* \times (1 - \text{prevalence}))}$$

* 0.002 = 1 – specificity

[†] Sensitivity and specificity are both test and chromosome dependent.

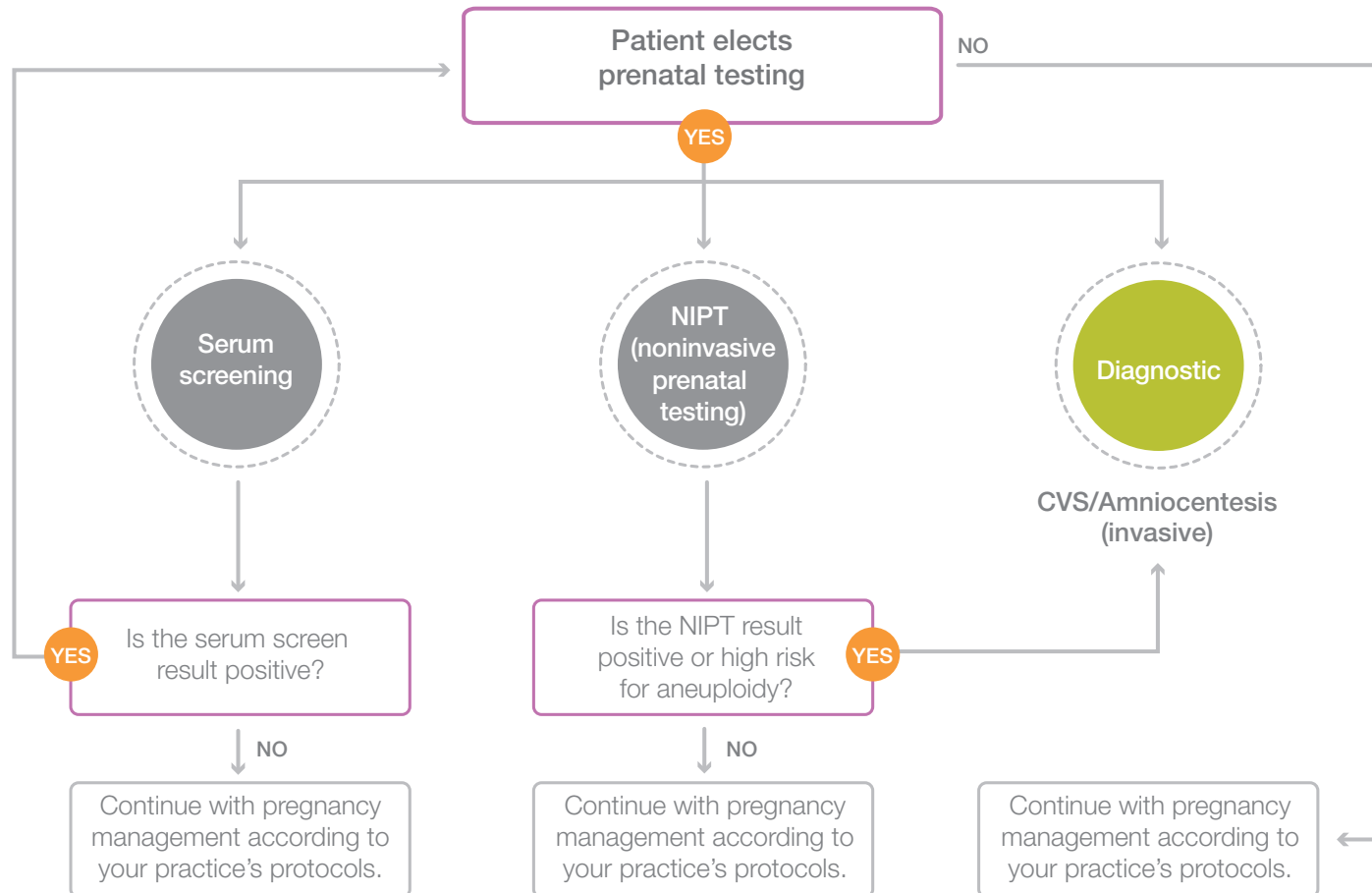
GC calculations:

Definitions.

	Definition	Formula
Sensitivity	The proportion of patients WITH a particular condition that test positive for that condition.	$\frac{\text{True positives}}{\text{True positives} + \text{false negatives}}$
Specificity	The proportion of patients WITHOUT a particular condition that test negative for that condition.	$\frac{\text{True negatives}}{\text{True negatives} + \text{false positives}}$
Positive Predictive Value (PPV)	Probability of the condition among patients with a positive result.	$\frac{\text{Sensitivity} \times \text{prevalence}^*}{\text{Sensitivity} \times \text{prevalence}^* + (1 - \text{specificity}) \times (1 - \text{prevalence}^*)}$
Negative Predictive Value (NPV)	Probability of no condition among patients with a negative result.	$\frac{\text{Specificity} \times (1 - \text{prevalence}^*)}{(1 - \text{sensitivity}) \times \text{prevalence}^* + \text{Specificity} \times (1 - \text{prevalence}^*)}$

* Prevalence is equal to the proportion of people who have a particular condition at a specified point in time.

Options for prenatal aneuploidy testing.



Options for prenatal aneuploidy testing.

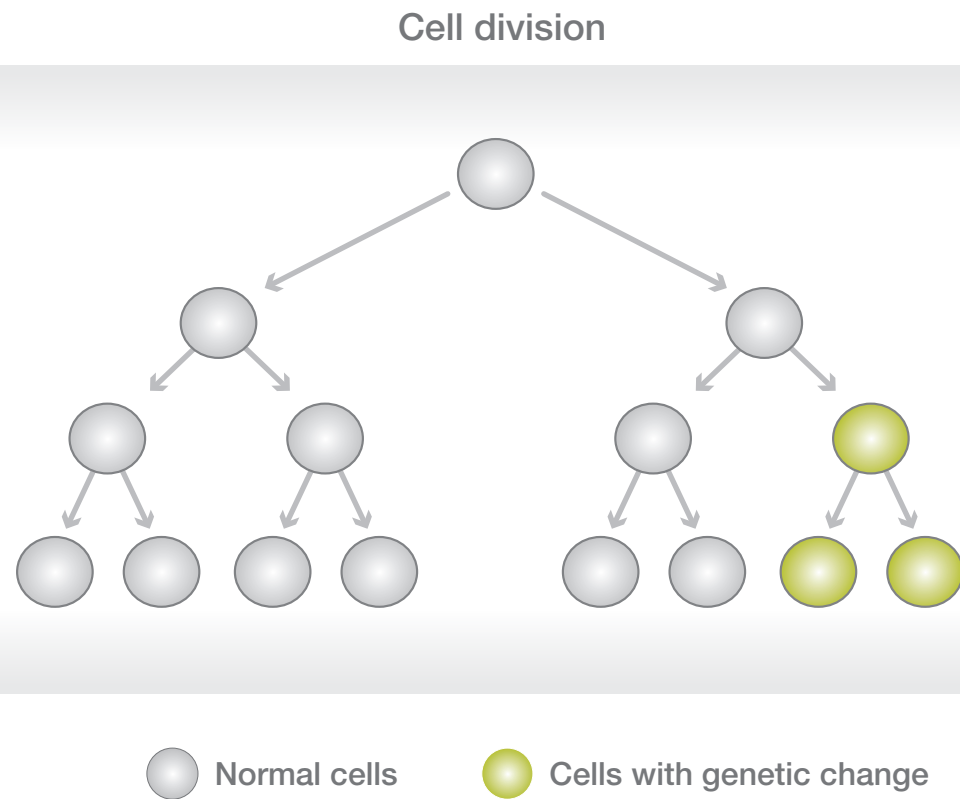
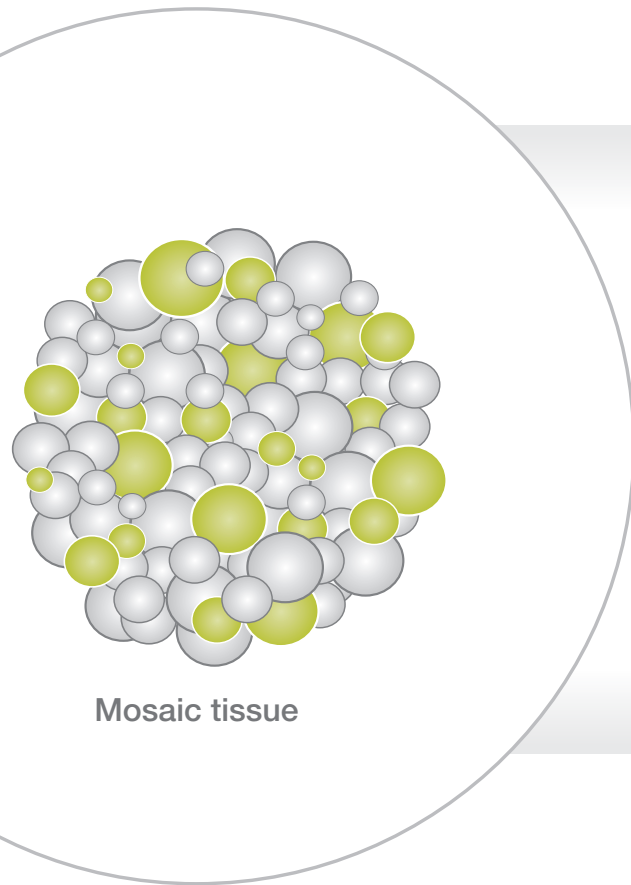
Pre-test counseling considerations

- Any test offering should be based on clinical context.
- NIPT should be an informed choice.
- NIPT screens for aneuploidies of chromosomes 21, 18 and 13. Sex chromosome aneuploidy screening may also be available.
- Screening for additional autosomal aneuploidies and microdeletions is also available with some NIPT offerings.
- CVS and amniocentesis remain options for all pregnant women.

Post-test counseling considerations

- All results should be reviewed in the context of the clinical, medical, and family history.
- Positive NIPT results should be confirmed prenatally by CVS or amniocentesis or postnatally.
- It is recommended that no irreversible action should be made based on NIPT results alone.
- Open neural tube defect screening is not part of NIPT and should be offered separately.

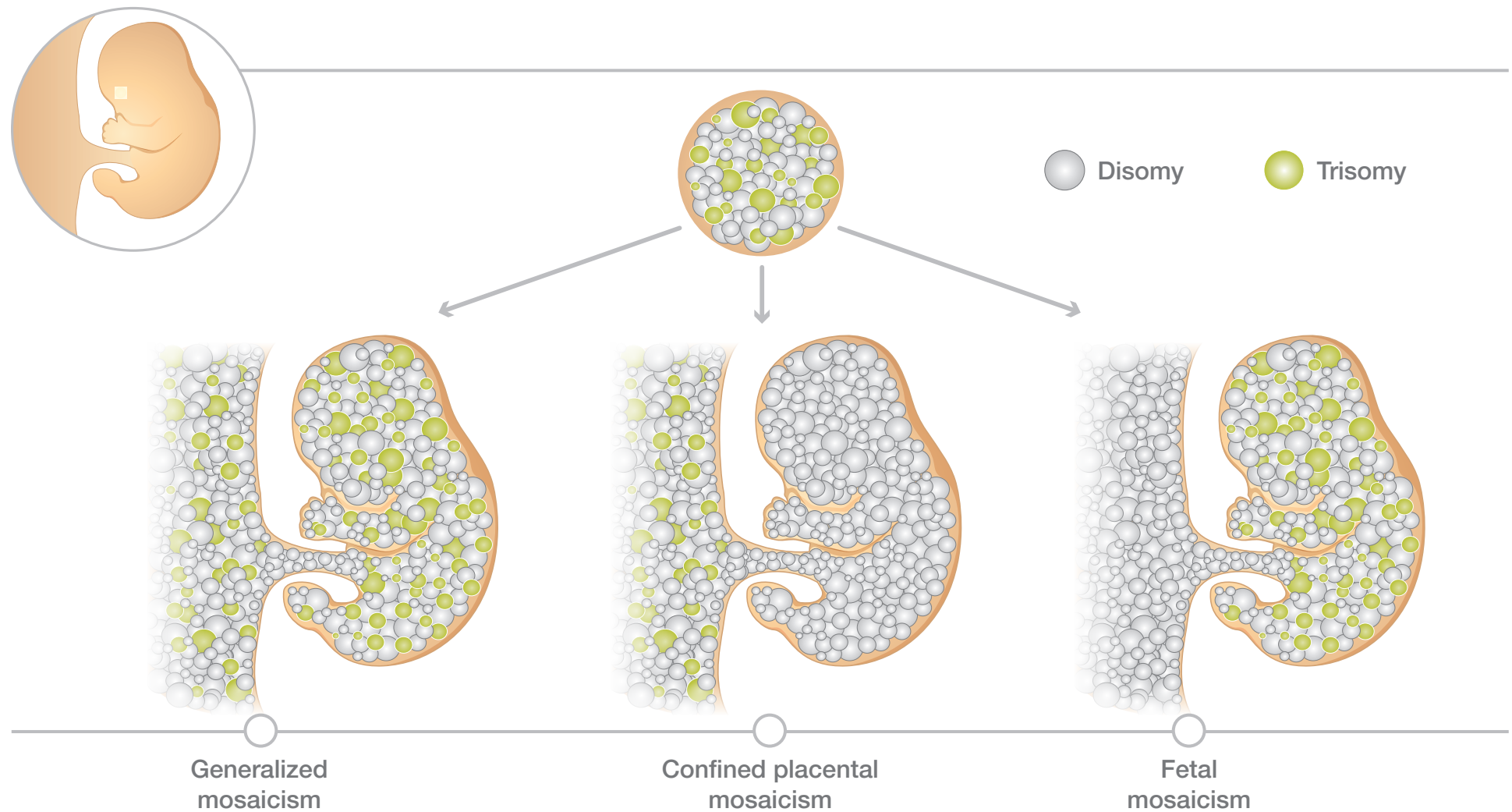
What is mosaicism?



What is mosaicism?

- Mosaicism is a biological phenomenon which results from the presence of cells with differing genetic compositions in the same individual. It usually occurs during the process of mitotic cell division.
- Mosaicism can be an underlying biological cause of discordant NIPT results.

Types of mosaicism.

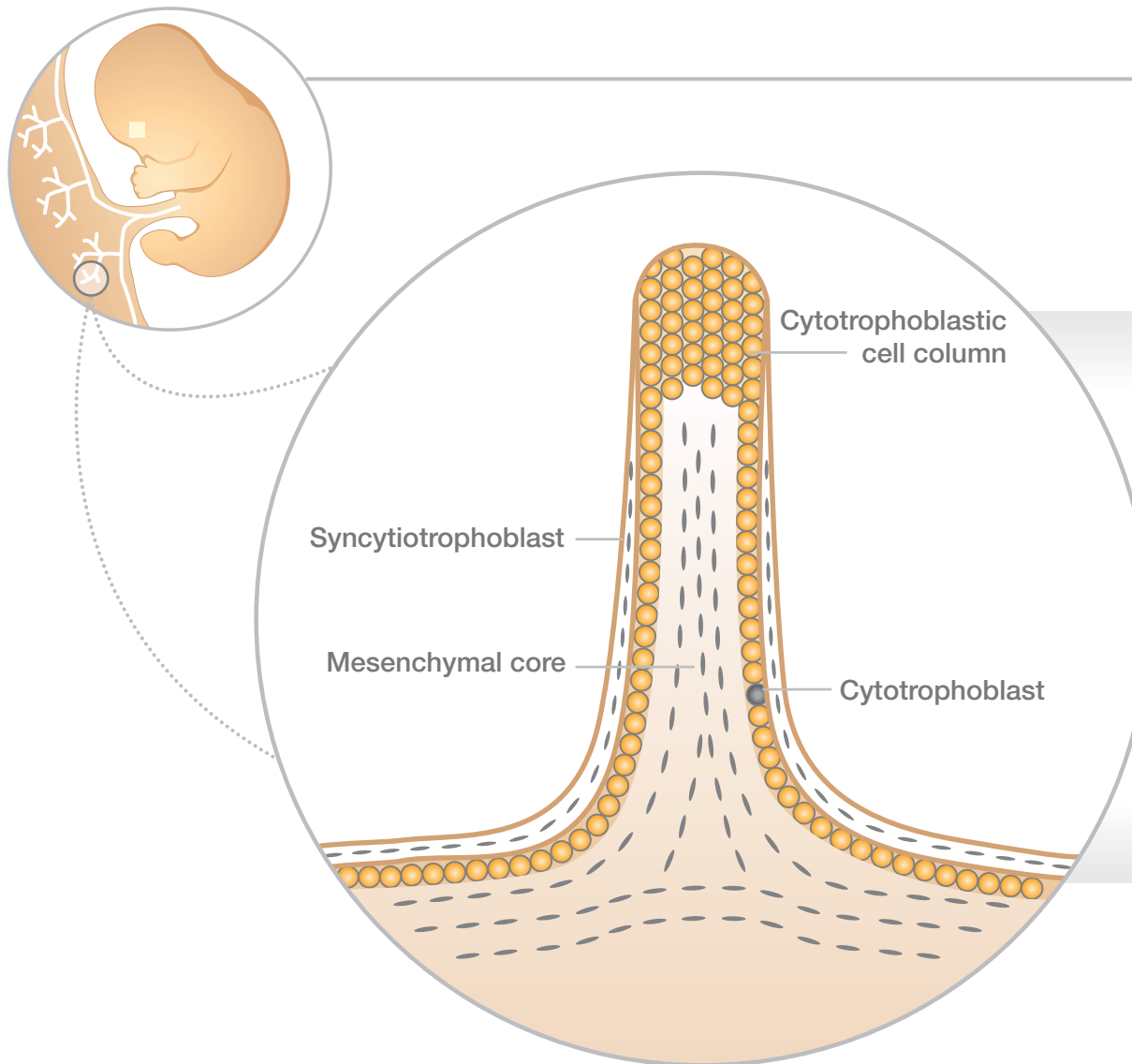


Types of mosaicism.

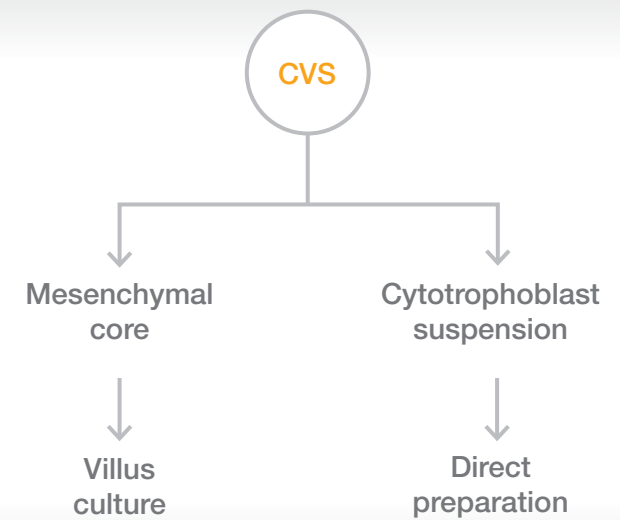
There are three types of mosaicism:

- **Generalized mosaicism:** Presence of two or more karyotypically different cell lines in both the placenta and the fetus. In cases of generalized mosaicism, there is a possibility of a false positive or a false negative NIPT result depending on the origin of the cfDNA.
- **Confined placental mosaicism:** Presence of two or more karyotypically different cell lines that are confined to the placenta and not present in the fetus. In cases of confined placental mosaicism, there is a possibility of a false positive NIPT result.
- **Fetal mosaicism:** Presence of two or more karyotypically different cell lines that are present in the fetus but not present in the placenta. In cases of fetal mosaicism, there is a possibility of a false negative NIPT result.

Confined placental mosaicism.



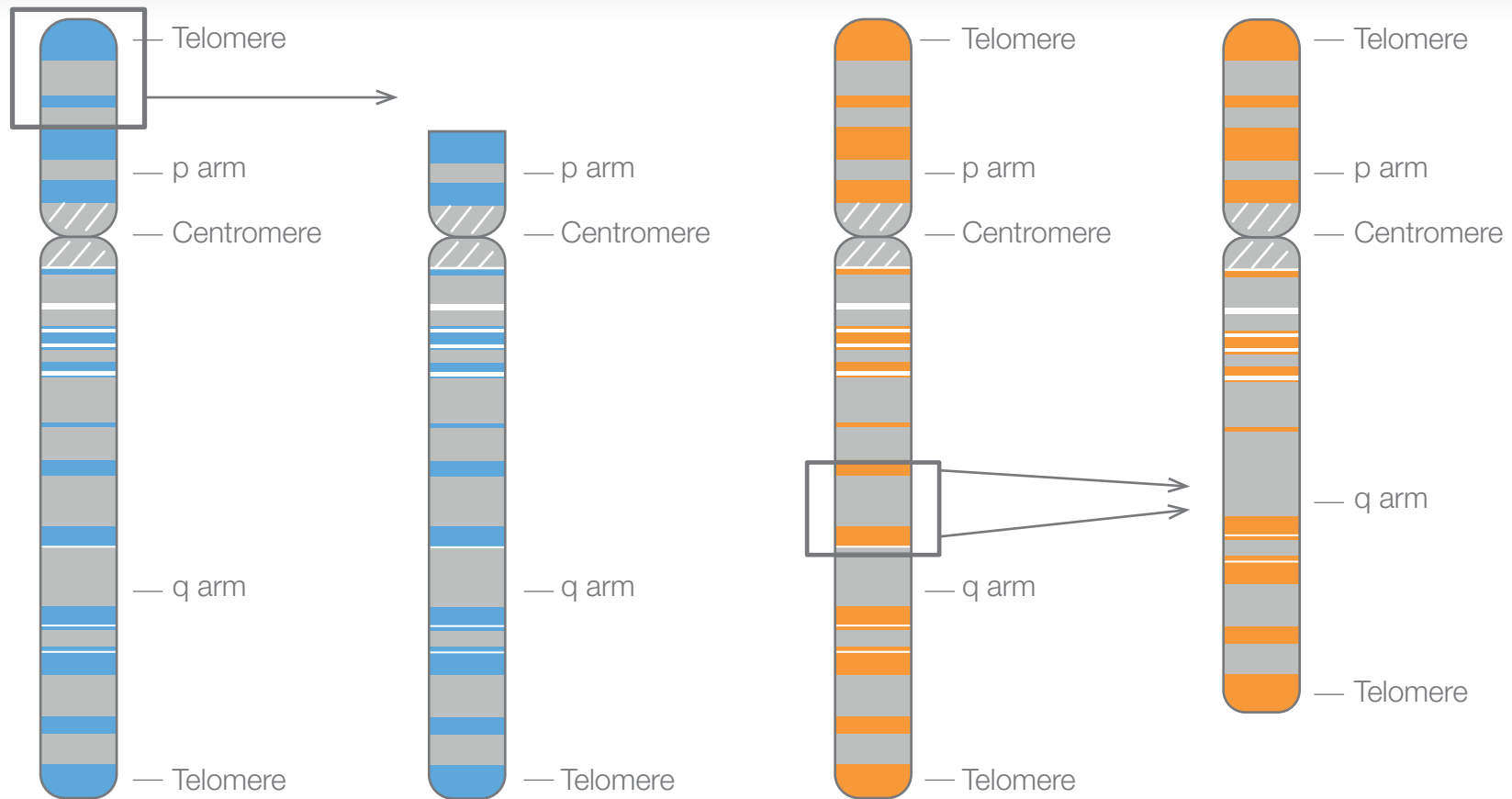
Laboratory techniques used for chorionic villus sampling (CVS)



Confined placental mosaicism (CPM).

- CPM is defined as the presence of two or more karyotypically different cell lines that are confined to the placenta and not present in the fetus.
- The cfDNA that is analyzed in NIPT arises from the cytotrophoblasts; thus an NIPT result is most similar to a CVS direct preparation result.
- **Type 1 CPM:** Mosaicism is confined to trophoblastic cells.
- **Type 2 CPM:** Mosaicism is confined to the cells from chorionic villus stroma.
- **Type 3 CPM:** Mosaicism is present in both: trophoblastic and stroma cells.
- **Type 1** and **Type 3 CPM** could result in false positive NIPT results.

Chromosome deletions and microdeletions.



Chromosome deletions and microdeletions.

- Chromosome deletions and microdeletions disorders are caused by small missing pieces of chromosome material.
- Screening for microdeletion and deletion syndromes is available with some NIPT offerings.
- Similar to NIPT for whole chromosome aneuploidies, positive predictive value is dependent on the prevalence of the microdeletion/deletion syndrome.

