Questions Answered by Professor Maurizio Ferrari and Dr. Guilaine Boursier

Answered by Professor Ferrari:

What do you think about the unclear results for microdeletion detection in the NIPT examination of the target with cffDNA, which is due to a minimal incidence? Is this related to a database library that is lacking?

I think that the detection of microdeletions in NIPT is still an open problem. There are very consistent results on classical trisomies but for the microdeletion, it depends on the technology utilized and the database available.

Answered by Dr. Boursier:

Can you tell us what number of reads is considered as "deep." For example, you show us that one sequence was read 65X. Is that "deep enough"?

We usually consider in routine practice that 30X is enough to detect a heterozygous variant. We aim to have 95% of the regions of interest (ROI) sequenced with a horizontal coverage of 50X for panel. For WES, we don't reach this performance at this time (90% at 10X only) but we are working on it.

The national recommendations (written by the French society of molecular genetics) state that the probability to detect a heterozygous variant is 94% at 4X and 99.9 à 10X but I don't know the reference of this probabilities.

If you want to detect low-level mosaicism, you must adapt your analysis settings (we detect it when >= 2%) and assess the LoD (dilute samples with homozygous variants).

Regarding DNA sample quality....can you elaborate what methods of DNA isolation are good enough for sequencing, and what kind of quality of sample is mandatory if we want to use NGS?

To perform NGS, the extracted DNA quality is important whatever the method of extraction you use. We tested our panel with both column and beads extraction. Results are good in either cases. We check the quality of DNA sample in the same way as for Sanger sequencing --> at least 260/280 ratio >1.8 and a nice absorption curve (nanodrop technique).

According to the protocol you use (depending on reagents kits), you have to check the quality of your library at different steps of the preparation and at these points, nanodrop is not recommended --> we use Bioanalyzer or Tapesation.

How to deal with variant of unknown significance (VUS)?

VUS (assessed by ACMG classification) are the most challenging variants to interpret...in routine practice:

- if the gene in which we found the VUS suits the phenotype
  - recessive disease / one mutation : no report
  - recessive disease / a pathogenic/VUS mutation in trans : report
  - dominant disease : report for phasing but we can exclude the VUS after having tested the parents

- if the gene in which we found the VUS doesn't suit the phenotype : no report

These aren't fixed rules, it depends on our knowledge and expertise of the gene regulation, the disease and its expression (variable, incomplete penetrance, etc.), the frequency of the VUS general population, in silico prediction strength, etc.

I would like to understand more about amplicon and capture technology.

Please see this publication about these NGS technologies:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4832303/