Frauke Coppieters[1,2], Thalia Van Laethem[3], Matthias De Smet[1], Paul Coucke[1,3], Kathleen Claes[1,3], Elfride De Baere[1,3], Björn Menten[1,3], Jo Vandesompele[2,3], Steve Lefever[2,3]

1 Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium
2 pxlence bvba, Dendermonde, Belgium
3 Center for Medical Genetics Ghent, Ghent University, Ghent, Belgium

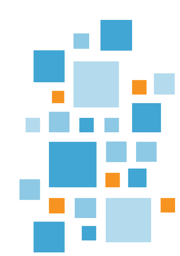
pxlence
www.pxlence.com

Multiplex PCR sample tracking in a whole exome sequencing workflow

Product portfolio

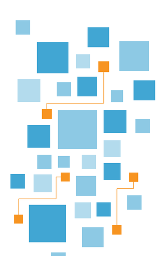
Our offering comprises best-in-class PCR assays for targeted resequencing of all human canonical exons of protein coding genes (both Sanger and massively parallel sequencing)

WGS/WES variant validation



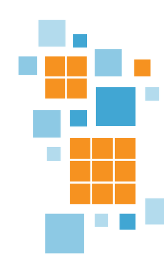
1 million PCR assays, of which over 10,000 have been validated for variant confirmation

Sample tracking



An optimized 30-SNP multiplex panel to identify your samples in a single PCR reaction

Targeted resequencing



54 validated gene panels totalling up to 223 genes and 4008 assays

NGS gap filling

Extend your preferred enrichment method with our assays to fill low coverage regions

All our assays have been thoroughly validated *in silico*, resulting in an off-the-shelf PCR success rate of >97%. The optimized design parameters allow uniform PCR conditions while ensuring uniform sequencing coverage.

- ✓ Avoid SNPs in primer annealing sites
- ✓ Maximize assay specificity
- ✓ Minimize secondary structures
- ✓ Optimize GC content, annealing temperature and other parameters

Ready-to-use SNP panels for sample tracking

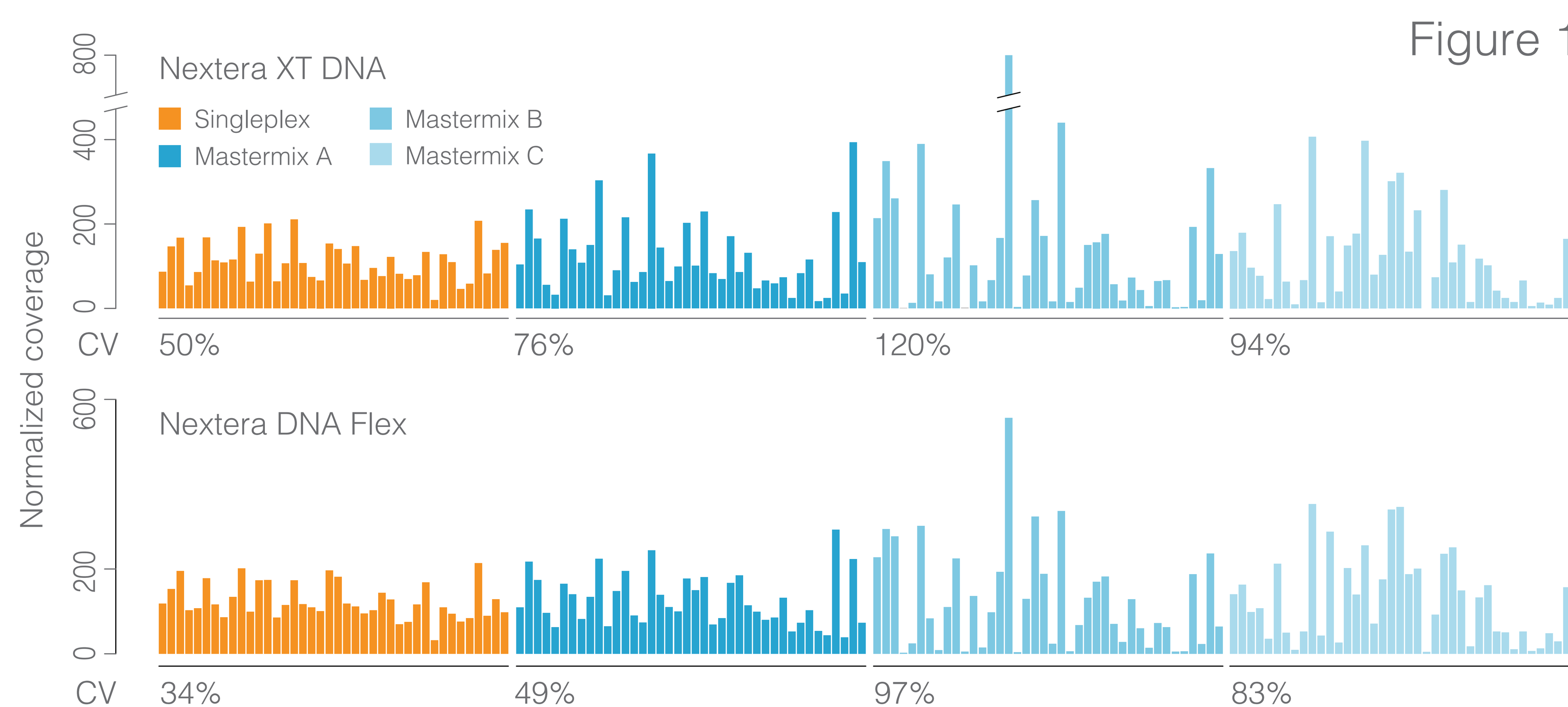


Figure 1

SNP panel A for high-molecular weight DNA

Assays were designed with a size range of 300-387 bp and amplified in a multiplex PCR using three different mastermixes. Library preparation was performed using the Illumina Nextera XT DNA or the Nextera DNA Flex kits, followed by sequencing on a MiSeq instrument (PE250 cycles). Results show that the type of mastermix has a significant impact on coverage uniformity.

Multiplex amplification of out-of-the-box pxlence assays using mastermix A results in a highly uniform sequencing coverage (CV_{XT} = 76%, CV_{Flex}= 49%), approaching singleplex conditions (Figure 1).

The pxlence SNP panel A - in combination with mastermix A and Nextera XT DNA library preparation - was applied on 194 cases for sample tracking during a diagnostic exome workflow. For over 86.29% of the SNPs a uniform coverage within 2-fold of the mean was observed. Distances between SNP median and overall mean is within acceptable range for lower covered SNPs (Figure 2).

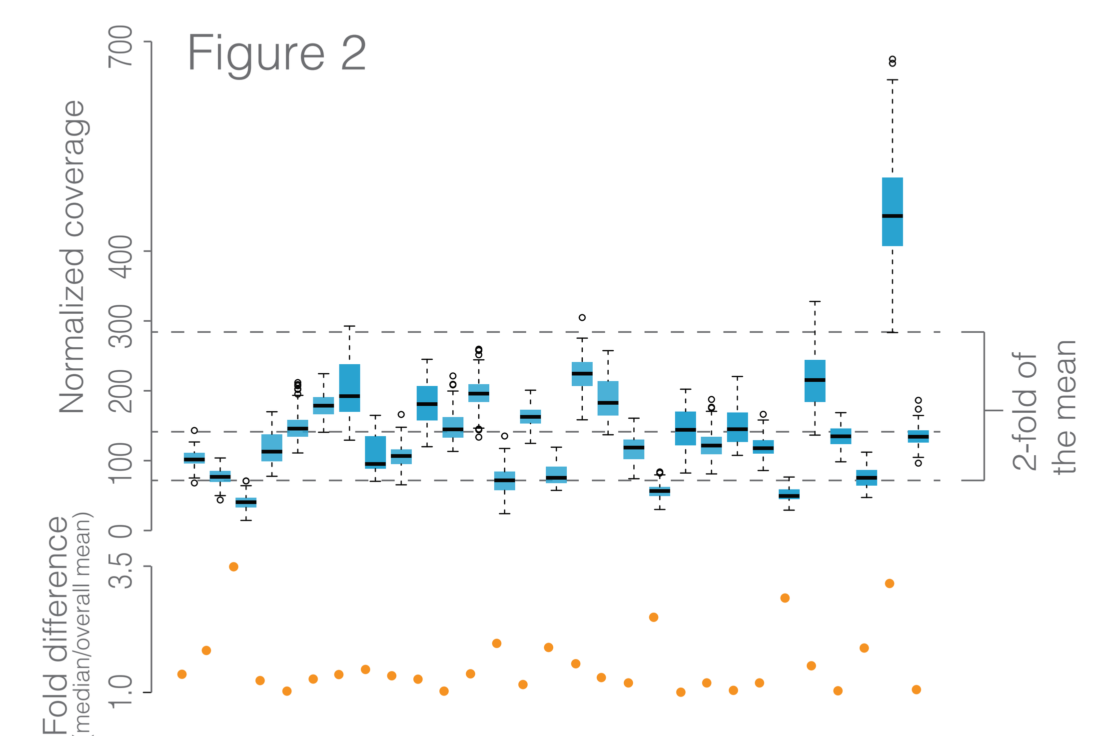


Figure 2

SNP panel B for degraded DNA

DNA from FFPE material or liquid biopsies requires a specialized approach. We developed a second SNP panel with amplicons ranging from 60-100 bp.

Figure 3 shows the normalized coverage of each assay following multiplex PCR with mastermix A and library preparation using the Nextera DNA Flex kit. No optimization of primer concentrations was performed.

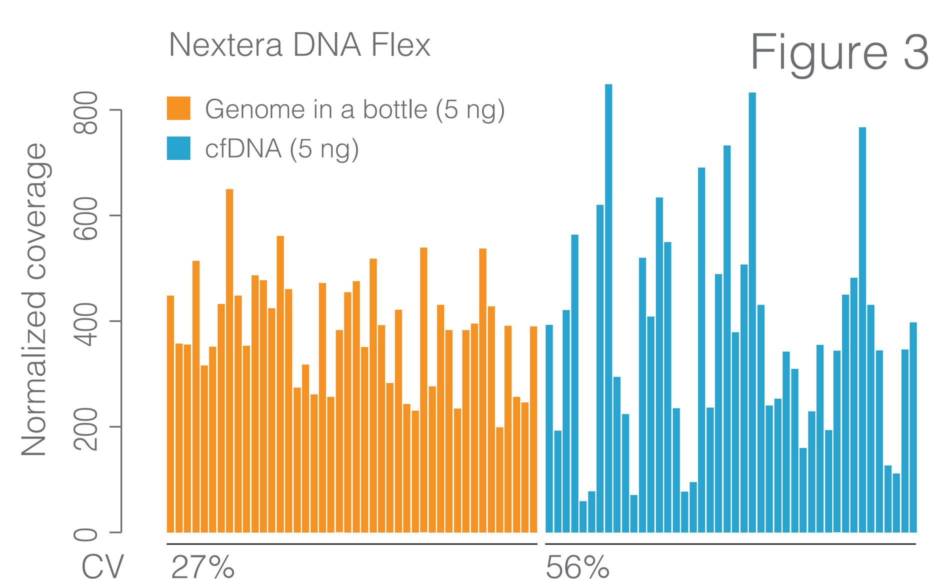


Figure 3

Again, a uniform sequencing coverage was obtained for a 5 ng high-quality DNA sample (GiB), whereas the amplification of a plasma cell-free DNA sample proved to be more challenging, yet feasible.

High-confidence calls could be generated for all SNPs in both the plasma cell-free DNA and the GiB sample.

A G A A C G A C A C C A C C C A A G G A C T C A A A C A C A C A T G T T C C A C A A G A
G G C T T G G C A T C A C C C A G T G G T T C G A G T A C G C A T G T T T C A T G A G C

SNP calls plasma cell-free DNA sample

Contact

www.pxlence.com
Frauke Coppieters, PhD - frauke.coppieters@pxlence.com