

## Targeted resequencing:

### Design of next-generation sequencing (NGS) panels

NXTGNT, the interdisciplinary platform for next generation genome analysis and Biomarked, the UGent valorization consortium for validation of biomarkers, are seeking partners interested in development of PCR based NGS diagnostic tests.

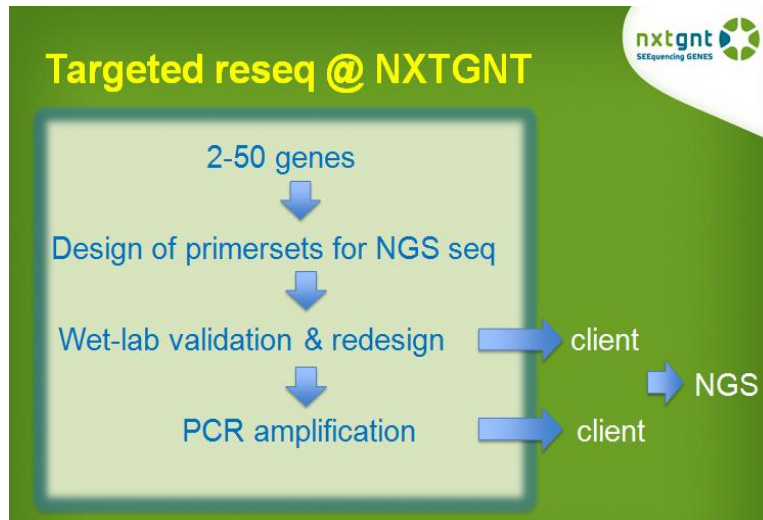
#### Introduction

Targeted resequencing allows researchers to obtain statistically significant data sets for their regions of interest (ROI) from a large number of samples while avoiding the expense and time required for whole genome sequencing. Focusing on a subset of the genome is generally done to reduce costs, although there may be certain cases, such as in a clinical setting, where it is important to sequence only particular regions. By focusing the sequencing on a small region of the genome, it is possible to detect low levels of variation that might have otherwise been missed. Researchers use targeted resequencing for genome-wide association studies (GWAS) instead of arrays as it is better suited for measuring rare alleles. Two main strategies are followed for targeted resequencing, capturing by hybridizing with complementary oligonucleotides or amplification with long range PCR.

NXTGNT, has developed several tests for multigene, multisample analysis either in house or in collaboration with UGent researchers based on the PCR approach. A first molecular test was developed for autosomal recessive non-syndromal hearing loss (ARNSHL). Six hundred and fourty six amplicons covering the coding regions of fifteen genes including their splice junctions are sequenced by NGS technology. Targeted resequencing workflows have also been tested and validated on hundreds of samples in aorta aneurysms (Baetens et al., Hum Mutat. 2011).

#### Technology

The NGS technology offers fast and cost-effective molecular diagnostic testing for genetically heterogeneous diseases. Next-generation sequencing (NGS) panels with amplicon size (NGS panels) maximum up to 1000 bp are targeted. UGent in-house primer design pipeline is used for target enrichment of a selection of genomic regions. Regions with known SNPs are excluded as primer binding sites and secondary DNA structures are avoided. Primers sets will be validated before handling to the customer supplied in tubes or in plates. Also possible is handling pooled PCR products to the customer which can be used as input for library preparation.



### Applications

Molecular diagnostic tests, for genetically heterogeneous diseases, to be designed are typically a set of selected genes (5-50 genes) with amplicon size maximum up to 1000 bp. All exons or a set of selected exons can be included for this gene panel. Design is flexible and can be adapted on request. Customers can use the designed panels for multigene, multisample next generation sequencing. Tools and expertise enable development of PCR based screening panels for additional molecular diagnostic tests in a short turnaround time.

### Advantages

- Smallest shift for genetic labs to NGS testing
- Design flexibility towards # samples and # regions or combinations
- Low cost for relatively small regions
- Fast turnaround time for design
- Coverage uniformity: lowest drop out; 80% of the amplicons within coverage of 2x
- Coverage width: optimal capturing of target region: dependent on sequence context , very high on-target specificity
- Minimal amount of DNA input: 2ng/amplicon
- Low amount of genomic DNA contamination
- Broader and more equimolar coverage: all exons are covered
- Very good reproducibility: robustness of PCR, uniform conditions and temperature

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