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providing tools for target enrichment

Best-in-class PCR assays covering the human exome

Almost 1 million PCR assays for targeted resequencing of all human canonical exons of protein coding genes

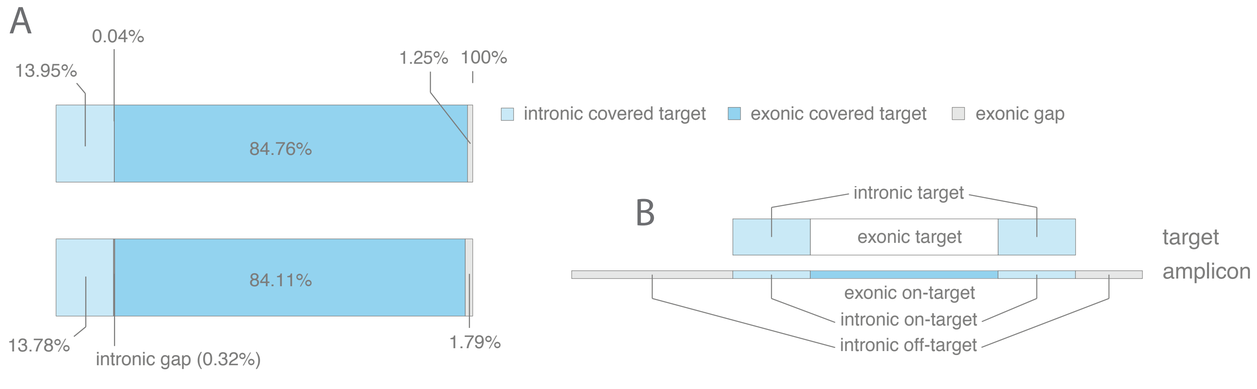
Advantages & key features

- 2 sets with uniform PCR conditions
- High success rate and 98.75% coverage of the human exome
- Uniform sequence coverage (read depth)
- Uniform amplicon length (125-275 (FFPE) or 350-750 bp) ready for most NGS library preps
- Flexible and easily expandable

Applications

- Quick confirmation of NGS findings
- Targeted resequencing of custom gene panels
- Targeted resequencing of a custom selection of disease-causing mutations
- Cell line authentication and patient sample tracking

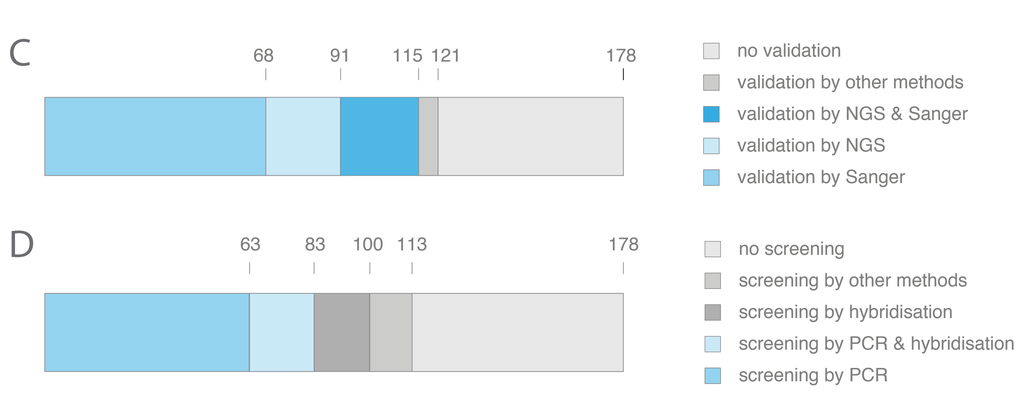
PCR assay specifications



Assay specifications of both PCR assay sets

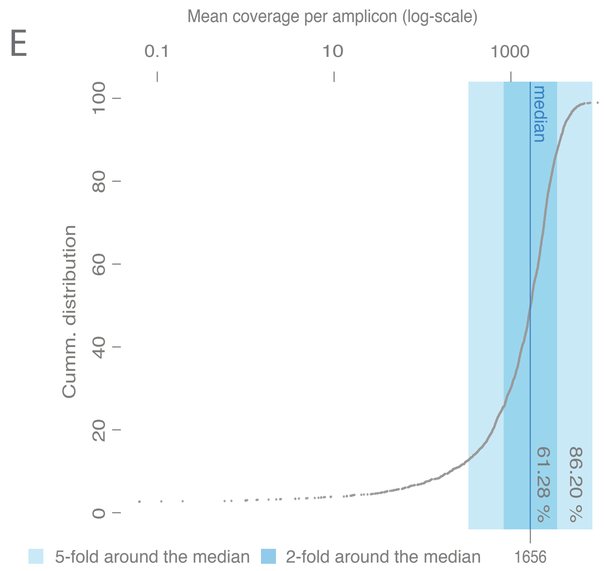
The target region for the primer design included each exon (*exonic target*) with 25 bp intronic sequence (*intronic on-target*) (**Figure B**). Both assay sets cover over 98% of the target region (**Figure A**).

PCR validation and enrichment market



178 researchers from Europe, USA and Asia were questioned about NGS variant validation and targeted gene screening (September, 2014). Approximately 68% of the respondents indicated they are currently validating their NGS findings using either NGS or Sanger sequencing (**Figure C**), while ~47% uses PCR for NGS target enrichment (**Figure D**).

PCR assay performance



Sequencing results for 2,300 assays

Figure E shows the cummulative distribution of the mean coverage per amplicon on 2,300 pooled amplicons of one normal human DNA sample. Over 61% of the assays show a mean coverage of 2-fold around the median, whereas over 86% of the assays are within 5-fold around the median. NGS library preparation was performed using the NEBNext library kit, followed by sequencing on the Illumina MiSeq (2 x 250 cycles). Data analysis was done using the CLC Genomics Workbench.

pxlence assays in practice

The pxlence assays have been extensively used for both research and diagnostic purposes. The following studies refer to the development of targeted resequencing gene panels for genetically heterogeneous disorders and cancer :

- congenital deafness : 15 genes (PubMed ID 22607986)
- cancer (NCI60) : 16 genes (PubMed ID 24612714)
- congenital blindness : 16 genes (PubMed ID 22261762)

Further, over 4,000 amplicons covering the coding regions of 200 disease genes for various monogenic disorders were used in the implementation of next generation sequencing in an ISO15189 accredited clinical genetics laboratory (Center for Medical Genetics Ghent) (PMID 25504618).