www.pxlence.com providing PCR assays for target enrichment & variant validation

Product portfolio

Almost 1 million PCR assays for targeted resequencing of all human canonical exons of protein coding genes (both Sanger and massively parallel sequencing)

Advantages & key features

- Uniform PCR conditions
- Uniform sequence coverage (read depth)
- High success rate, > 98.7% coverage of the human exome
- Flexible and easily expandable
  define your own regions of interest, as many or as few as you choose
- Uniform amplicon length (125-275 (FFPE) or 350-750 bp)
  ready for most NGS library prep protocols

Applications

- Fast confirmation of NGS findings derived from gene panels, whole exome sequencing or other
- Targeted resequencing of custom gene panels
- Targeted resequencing of a custom selection of disease-causing mutations
- Much more ... design your own experiment

References

Our assays have been used in the development of a wide range of NGS gene panels, both for research and diagnostic purposes. In addition, our customers use the assays for Sanger based validation of variants.

Massively parallel sequencing for early molecular diagnosis in Leber congenital amaurosis
16 genes - 375 assays - PubMed ID 22261762

Molecular diagnostics for congenital hearing loss including 15 deafness genes using a next generation sequencing plaform
15 genes - 646 assays - PubMed ID 22607986

Target enrichment using parallel nanoliter quantitative PCR amplification
16 genes - 376 assays - PubMed ID 24612714

Flexible, scalable, and efficient targeted resequencing on a benchtop sequencer for variant detection in clinical practice
265 genes - over 4000 assays - PubMed ID 25504618

Contact

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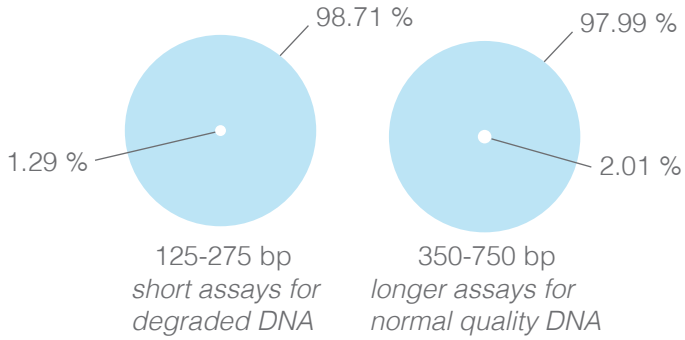
pxlence assay performance

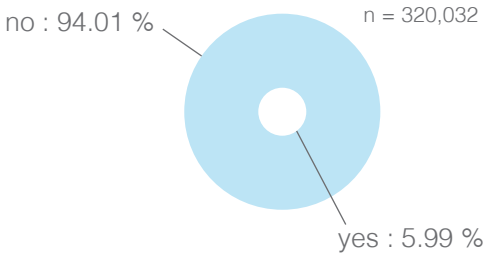


Exome coverage

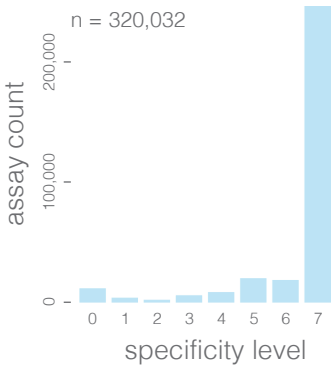
Both assay catalogues target all exons of the canonical Ensembl transcripts (hg38), including 25 base pairs up- and downstream of each exon. The long assay set (350-750 bp) covers almost 98% of these targets and is suited for normal quality DNA samples. The short set (125-275 bp) covers 98.7% of the exome and is ideally suited for degraded DNA (eg. FFPE).

SNP presence in primer annealing sites

Since single nucleotide polymorphisms (SNP) in primer annealing sites can hamper proper binding of primers to their target of interest - especially if the SNP is located at the primer 3' end - or cause allelic bias during amplification, these features have to be avoided during primer design. Over 94% of the primers in the long assay set are free of SNPs. Of the remaining assays (5.99%), the number of SNPs is limited to one in over 92% of the assays and the SNPs are outside the last 5 nucleotides (3' end) in 85% of these assays.

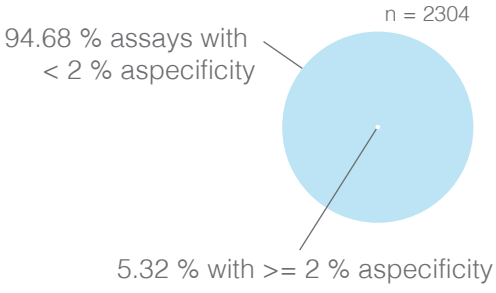


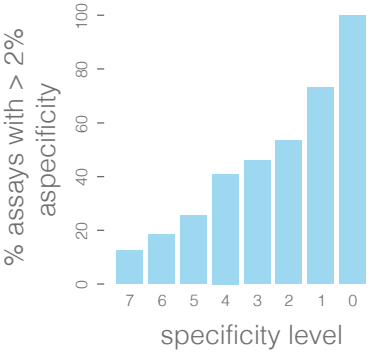
Assay specificity



All assays have been checked *in silico* to discard assays having a higher chance of aspecific amplification. This assessment includes potential primer annealing sites harboring up to three mismatches, as we have observed that assays with at least three or more mismatches in one of their primer annealing sites result in almost no amplification. Based on this, each assay receives a score between 0 and 7, depicting the number of mismatches in the aspecific hit with the highest potential towards aspecific amplification - higher scores represent a lower chance of generating aspecific amplicons. The graph on the left shows that most of our assays have the highest specificity score and should thus result in no or very limited aspecific amplification.

To test the wet-lab performance of our assays, 2304 randomly selected primer pairs from the long assay set were amplified and sequenced. The resulting reads were grouped per assay and aligned to the human genome. Aspecificity per assay was calculated by dividing the off-target coverage per amplicon by the total coverage per amplicon. Over 94% of these assays show less than 2% aspecificity, with over half (~55%) of the remaining assays having less than 10% aspecificity.





In order to assess whether the observed wet-lab specificity results were in agreement with our *in silico* predictions, we checked the correlation between both values. We observed a clear correlation between the predicted assay specificity and the sequencing off-target percentage, as the largest portion of these assays have received a low score during *in silico* specificity assessment. For this analysis we only included the assays with > 2% aspecificity (5.32% of all tested assays), to prevent bias resulting from the large number of assays having high specificity scores.