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PCR based target enrichment for NGS panels and Sanger variant confirmation

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

- Works under universal PCR conditions
- High wet-lab success rate and 98% 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

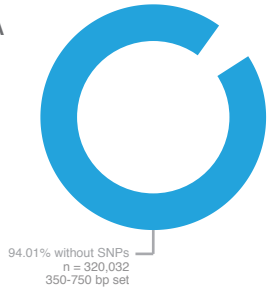
Targeted resequencing of a custom selection of disease-causing mutations

Cell line authentication and patient sample tracking

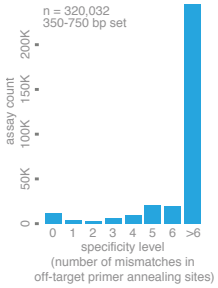
PCR assay specifications

PCR assays have been generated to cover all exons of all Ensembl canonical transcripts. The exome coverage for the long (350-750 bp) and short (125-275 bp) dataset is 97.99% and 98.71%, respectively. In 94.01% of these assays, no SNPs are present in the primer annealing sites. For the remaining assays, 85.74% contain SNP(s) outside the critical 5 bp 3' region, whereas 92.47% contain only a single SNP (Fig. A). For each assay, an *in silico* specificity score - determining the likelihood of non-specific product generation - has been calculated. This score represents the number of mismatches with the most homologous off-target region. Fig. B shows that the majority of the assays (> 77%) have the highest score, and are thus predicted to have minimal non-specific product generation.

A

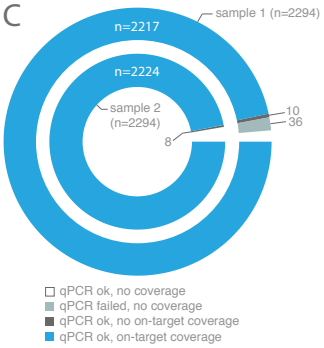


B

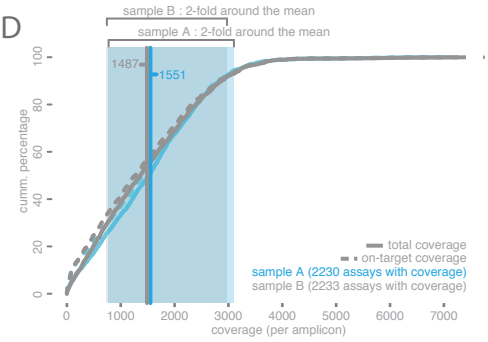


PCR assay performance

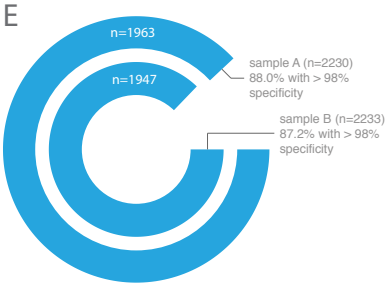
C



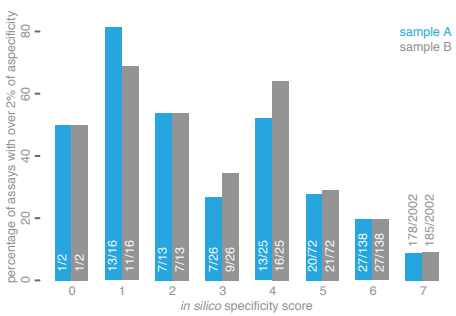
D



E



F



x/*y* with *x* the number of assays having a certain *in silico* specificity score with over 2% aspecificity and *y* all assays having that score

From the 350-750 bp dataset, 2294 assays covering 169 diagnostically relevant diseases genes were randomly selected. Using these assays, singleplex amplification was performed on two pooled samples containing male and female DNA. (q)PCR and sequencing combined success rates for both samples exceeded 96.6% (Fig. C). Coverage uniformity per assay was high, with 60% and 58.8% of the assays having a coverage within 2-fold of the mean for sample A and B respectively (Fig. D). End-point fluorescence values showed highly uniform product equimolarity, obviating the need for product normalization prior to sequencing. Coverage specificity per assay, determined as the ratio between the number of on-target reads and all reads linked to that assay, indicated that less than 12.8 % of the assays had over 2% of its reads mapping to off-target regions (Fig. E). For each score, the percentage of assays with over 2% off-target was calculated. Fig. F shows a correlation between the percentage of assays having a higher degree of aspecificity and the *in silico* specificity score, confirming the predictive value of the latter with respect to off-target sequencing coverage. In addition, 1900 out of 2294 pxlence assays were also subjected to Sanger sequencing using a universal amplification and sequencing protocol. The overall success rate of pxlence assays on LabChip GX is 95.77%. Subsequent Sanger sequencing of 1900 assays passing LabChip QC revealed a Sanger success rate of 88.63%.

Contact

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Coppieters *et al.*, Targeted resequencing and variant validation using pxlence PCR assays, Biomolecular Detection and Quantification, 2015, *in press*