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Evaluation of two highly-multiplexed custom panels for massively parallel semiconductor sequencing on paraffin DNA.

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Abstract

BACKGROUND:

AIM:

Massively parallel sequencing (MPS) holds promise for expanding cancer translational research and diagnostics. As yet, it has been applied on paraffin DNA (FFPE) with commercially available highly multiplexed gene panels (100s of DNA targets), while custom panels of low multiplexing are used for re-sequencing. Here, we evaluated the performance of two highly multiplexed custom panels on FFPE DNA.

METHODS:

Two custom multiplex amplification panels (B, 373 amplicons; T, 286 amplicons) were coupled with semiconductor sequencing on DNA samples from FFPE breast tumors and matched peripheral blood samples (n samples: 316; n libraries: 332). The two panels shared 37% DNA targets (common or shifted amplicons). Panel performance was evaluated in paired sample groups and quartets of libraries, where possible.

RESULTS:

Amplicon read ratios yielded similar patterns per gene with the same panel in FFPE and blood samples; however, performance of common amplicons differed between panels ($p<0.001$). FFPE genotypes were compared for 1267 coding and non-coding variant replicates, 999 out of which (78.8%) were concordant in different paired sample combinations. Variant frequency was highly reproducible (Spearman's rho 0.959). Repeatedly discordant variants were of high coverage / low frequency ($p<0.001$). Genotype concordance was (a) high, for intra-run duplicates with the same panel (mean±SD: 97.2±4.7, 95%CI: 94.8-99.7, $p<0.001$); (b) modest, when the same DNA was analyzed with different panels (mean±SD: 81.1±20.3, 95%CI: 66.1-95.1, $p = 0.004$); and (c) low, when different DNA samples from the same tumor were compared with the same panel (mean±SD: 59.9±24.0; 95%CI: 43.3-76.5; $p = 0.282$). Low coverage / low frequency variants were validated with Sanger sequencing even in samples with unfavourable DNA quality.

CONCLUSIONS:

Custom MPS may yield novel information on genomic alterations, provided that data evaluation is adjusted to tumor tissue FFPE DNA. To this scope, eligibility of all amplicons along with variant coverage and frequency need to be assessed.