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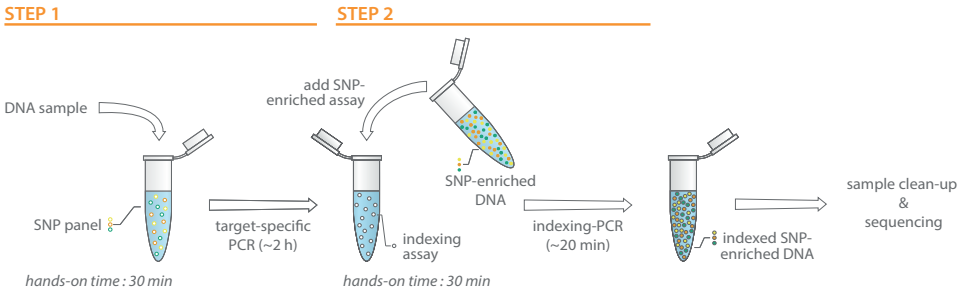
Multiplex PCR for sample identity confirmation in a whole exome sequencing workflow

Human Sample ID Kit

Gene panel, exome and genome sequencing are routinely used in molecular diagnostics and research. Because of the complexity of such workflows, DNA samples are prone to sample mix-ups. It has been estimated that such sample mix-ups occur in up to 1 % of the cases, underscoring the need for an independent method for sample identity confirmation.

pxlence's Human Sample ID Kit is an easy, flexible and cost-efficient multiplex assay that targets 44 polymorphic SNPs and 6 gender markers, creating a highly specific intrinsic genetic label for each sample. Using two simple PCR steps, sequencing-ready libraries are generated.



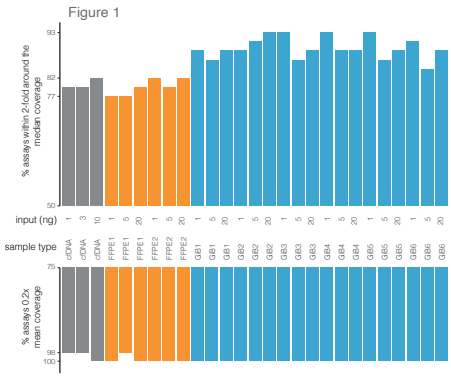
- √ 44 polymorphic SNPs
- √ 6 gender markers
- √ 1 h hands-on time
- √ 3.5 h total time
- √ Amplicon lenghts of 61 - 101 bp
- √ Compatible with Illumina platforms

Performance

The pxlence Human Sample ID Kit was applied on 6 different Genome in a Bottle (GiB) reference samples of the Ashkenazim and Asian trios. We also included a cell-free DNA reference sample (derived from huAA53E0, SensID) and DNA isolated from FFPE tissue.

A superior uniform coverage is achieved for all samples, with 88.6 % of the SNPs having a coverage within 2-fold of the median for the high-quality GiB samples, and 80.3 % and 79.5 % for the cfDNA and FFPE samples, respectively (Figure 1). The lower percentages for the latter can be attributed to the more fragmented nature of these samples. Data shows that the degree of coverage uniformity is largely independent from the input amount. Importantly, all 44 polymorphic SNPs show a coverage above 20 % of the mean coverage. Only at very low DNA input amounts (1 - 5 ng) of the cfDNA and FFPE samples a single SNP occasionally dropped slightly below the 20 % of the mean coverage threshold (Figure 1).

Figure 1



Our Kit is highly specific with a mean on-target rate of 95.3 %. A slight difference between male (95.7 %) and female (93.8 %) DNA samples could be observed attributable to the gender markers present in the kit (Figure 2).

The pxlence Human Sample ID Kit allows high-confident variant calling for all SNPs in all samples, resulting in unambiguous sample discrimination and identification using publically available genotype information for the GiB samples (Figure 3). The correlation heatmap clearly shows that the Kit is able to confirm the genetic relationship between the family members of both trios, while providing excellent discrimination power to distinguish the individual family members.

Figure 3

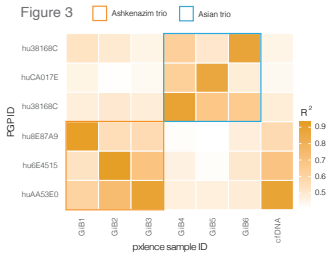
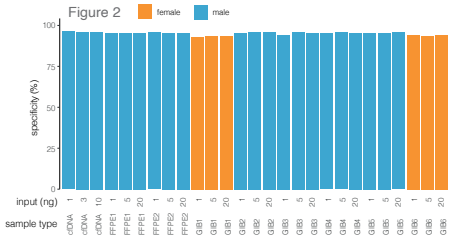


Figure 2



Advantages

- Superior coverage uniformity
- Easy integration in WES and WGS workflows
- Simple and flexible workflow
- Low DNA input requirement, down to 1 ng DNA

Key Features

- Works with high-quality, FFPE and liquid biopsy samples
- Ambient kit shipment
- Maximum compatibility with established DNA enrichment workflows
- High discrimination power >1:85,000

Contact

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