A novel digitalMLPA carrier screening assay for simultaneous detection of copy number variations and selected point mutations in challenging genomic regions

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RESEARCH

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Targeted multiplex carrier screening

Carrier screening is crucial to identify individuals at risk of having children affected by genetic disorders. Despite advancements, accurate detection of copy number variations (CNVs) and single nucleotide variants (SNVs) remains challenging, especially in complex genomic regions such as *CYP21A2*. To address these challenges, **SALSA® digitalMLPA™ Probemix D028 Carrier Screening** is in development. This multiplex PCR assay simultaneously detects CNVs and selected SNVs and is designed for use in conjunction with sequence analysis. The assay contains 216 target probes, covering nine regions (***Figure 1***) including complex genomic areas, and is suitable for high throughput use.

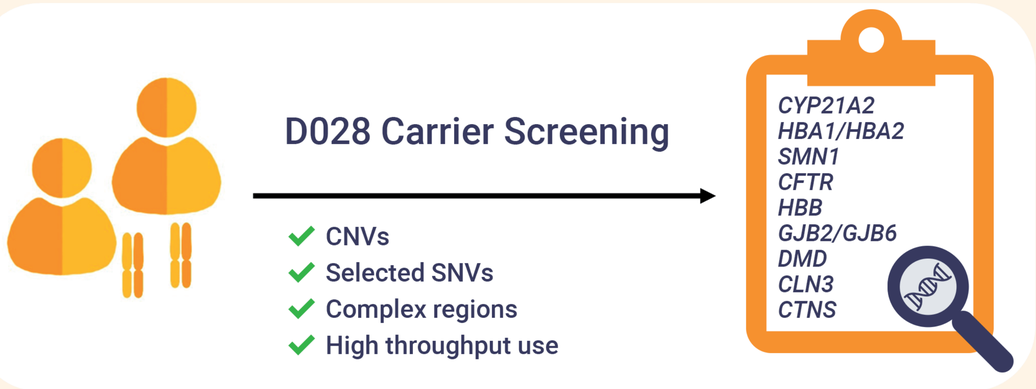

Figure 1. **Highlights of D028 Carrier Screening.**

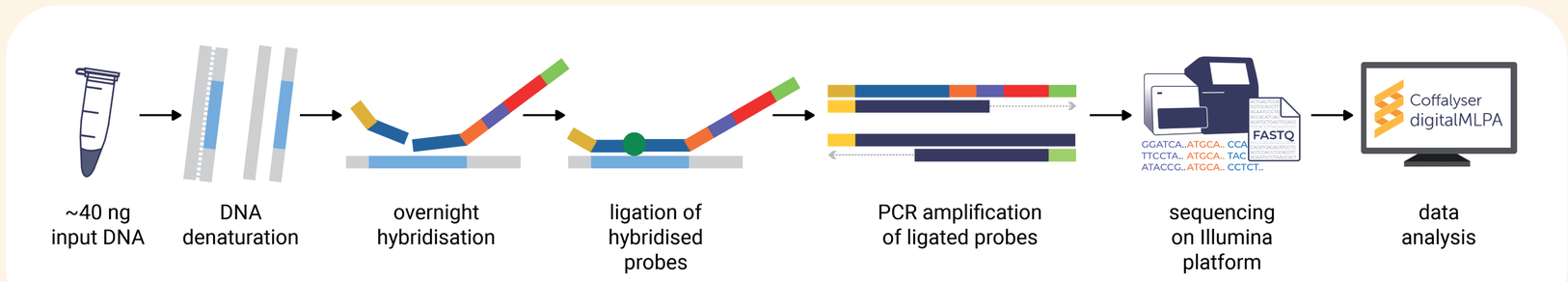

Figure 2. **digitalMLPA workflow.**

digitalMLPA experiment

D028 Carrier Screening was used to analyse **70 positive samples** (~40 ng DNA) from the Coriell Institute and previously genotyped blood-derived positive samples. The experiment was done according to the digitalMLPA workflow (***Figure 2***). Sequencing was performed using the MiSeq and NextSeq 1000 Illumina platforms.

CNV and selected SNV detection

All expected CNVs and SNVs were accurately identified for all targeted regions, across the diverse sample set tested.

Aberrations found included:

- 33 deletions
- 6 duplications
- 28 SNVs
- 3 cases with both a CNV and a SNV

Results of all 70 samples tested are shown for the *HBB* and *CFTR* genes in ***Figure 3***.

Figure 3. ***HBB* and *CFTR* CN ratio plots across all 70 samples tested.** Each data point represents the normalized CN ratio obtained for a probe in a sample.

CYP21A2 – addressing the challenges

Genotyping the functional *CYP21A2* gene poses a significant challenge due to the existence of the highly homologous and inactive pseudogene *CYP21A1P*, located closely upstream as displayed in ***Figure 4***. The great majority of the *CYP21A2* mutant alleles arise through recombination between *CYP21A2* and *CYP21A1P*. This assay can be used to accurately detect *CYP21A2* gene deletions and conversions and the majority of the most frequent pathogenic SNVs, as illustrated in ***Figure 5***.

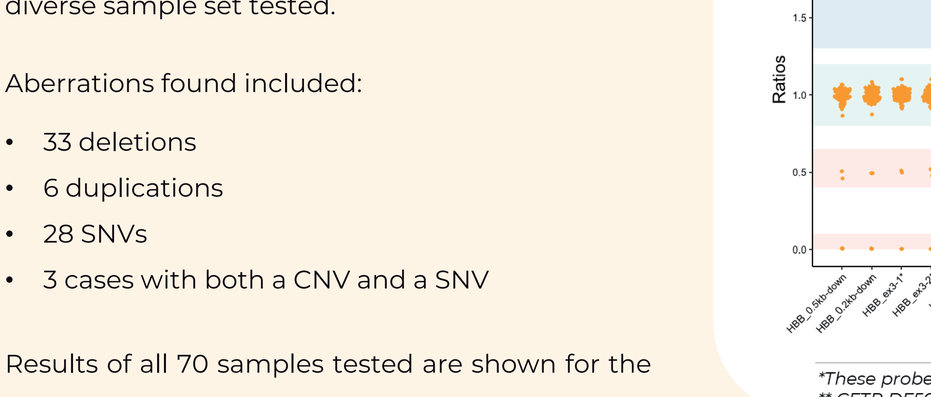

Figure 4. **Schematic overview of Chr6p21.3 region (RCCX module).** Arrows indicate direction of transcription. Genes are displayed in dark orange, and (inactive) pseudogenes in light orange.

Quite frequently, *CYP21A1P* pseudogene copies have acquired the wildtype *CYP21A2* sequence at the P31L, V282L, Q319X and/or R357W locations. This can obscure the presence of a pathogenic SNV at these sites in the *CYP21A2* gene. Hence, a **novel probe type** (marked with * in ***Figure 5***) was designed targeting the wildtype *CYP21A2* sequence at **two locations** simultaneously: one location in which the pseudogene often acquires the wildtype *CYP21A2* sequence and another location in which that very rarely happens. This novel design renders the probes specific to the *CYP21A2* gene, even when the pseudogene has acquired the wildtype sequence at one of the two targeted locations. In case of a lower ratio for one of these probes, a second probe can deduce which SNV is present as it only targets one of the two locations (pairs indicated by coloured boxes on the x-axis of ***Figure 5***).

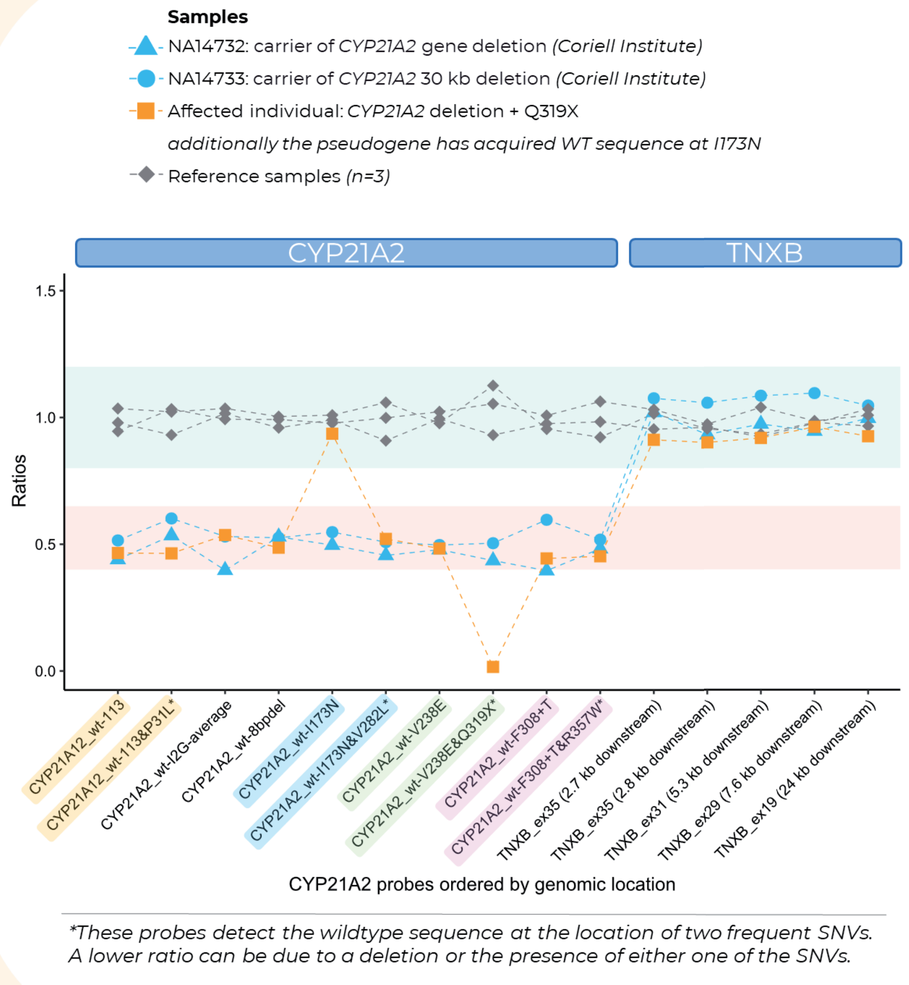

Figure 5. ***CYP21A2* CN ratio plots including 3 positive samples and 3 reference samples.** Each data point represents the normalized CN ratio obtained for a probe in a sample.

For further information, please contact
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digitalMLPA is for research use only. Not for use in diagnostic procedures.

The product described concerns a test version that is not available for general purchase.

Conclusions

- SALSA® digitalMLPA™ Probemix D028 Carrier Screening accurately detected aberrations across all targeted regions in a diverse sample set, including CNVs and selected SNVs.
- The novel probe type allows the detection of *CYP21A2* CNVs and a considerable number of the SNVs, showcasing its capability to overcome the challenges presented by complex genomic regions.
- This robust assay is intended to complement sequence analysis, with the prospect of enabling informed reproductive decision-making in the future.

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