A promising single DNA-based assay to test for hemoglobinopathies, congenital adrenal hyperplasia, cystic fibrosis, spinal muscular atrophy and severe combined immunodeficiency for newborn screening

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RESEARCH

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digitalMLPA for the analysis of SNVs and CNVs associated with five newborn conditions

Newborn screening (NBS) programs rely primarily on biochemical tests for the detection of severe early-onset disorders. DNA-based techniques provide opportunities for confirmatory testing and expansion of newborn screening panels. However, currently used methods (e.g. Sanger sequencing, qPCR) often only target one or a handful of regions and therefore still require a condition-specific workflow. We are developing a **SALSA® digitalMLPA™** assay consisting of ~350 probes that detect single nucleotide variants (SNVs) and copy number variants (CNVs) associated with five conditions included in many NBS programs: severe combined immunodeficiency (SCID), spinal muscular atrophy (SMA), hemoglobinopathies (HbPs), cystic fibrosis (CF) and congenital adrenal hyperplasia (CAH; **Figure 1**, **Table 1**). In this study we highlight our results on SCID and HbPs.

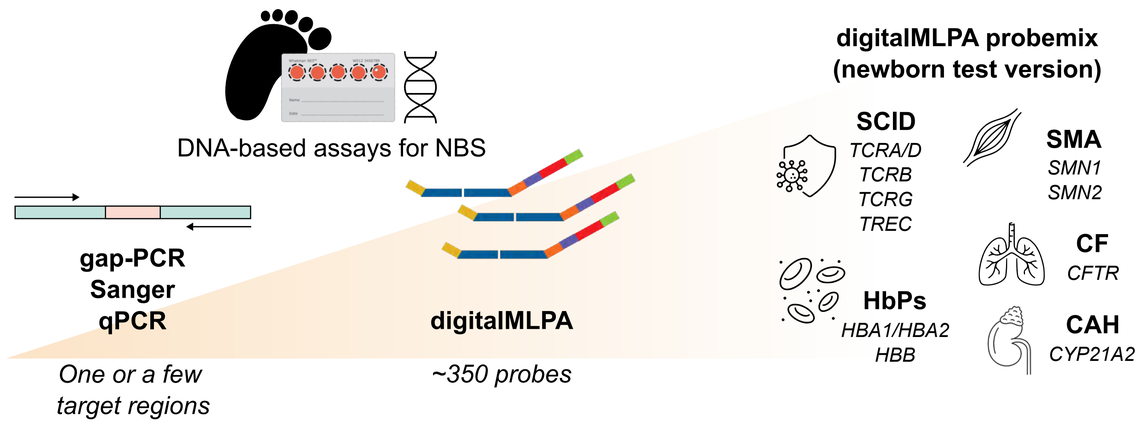

Figure 1. Left, schematic comparison of digitalMLPA to DNA-based assays currently used in NBS. Right, an overview of the targeted regions of the digitalMLPA probemix currently in development.

digitalMLPA: a two-day workflow using low amounts of DNA

digitalMLPA is a multiplex PCR-based method that uses Illumina sequencing platforms for amplicon detection. The workflow of digitalMLPA is illustrated in **Figure 2**.

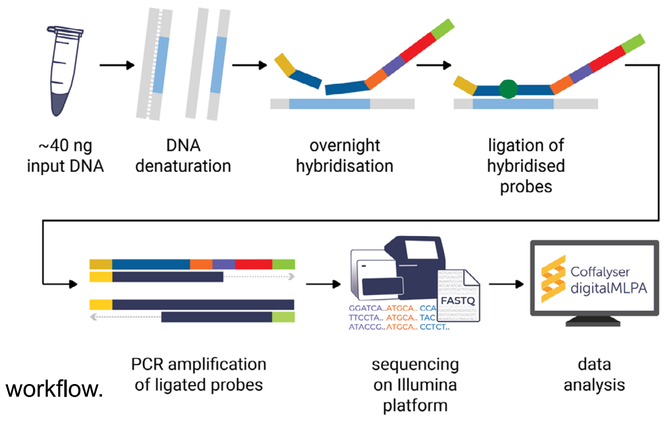

Figure 2. digitalMLPA workflow.

Table 1. Coverage of the probemix (in development)

Condition	Target	Coverage
SCID	T cell excision circles (TREC)	sjTREC and cjTREC
	TCRA, TCRB, TCRD, TCRG	Detection of rearrangements of the variable TCR regions
SMA	*SMN1/SMN2*	CNVs of *SMN1* exon 7, *SMN2* copy number determination
HbP	*HBA1/HBA2*	CNVs and 6 frequent pathogenic SNVs
	HBB	CNVs and 14 frequent pathogenic SNVs
CF	*CFTR*	CNVs and 33 frequent pathogenic SNVs
CAH	*CYP21A2*	CNVs and 11 frequent pathogenic SNVs

Detection of distinct *HBA* deletions as well as six frequent missense variants in *HBA1* and *HBA2*

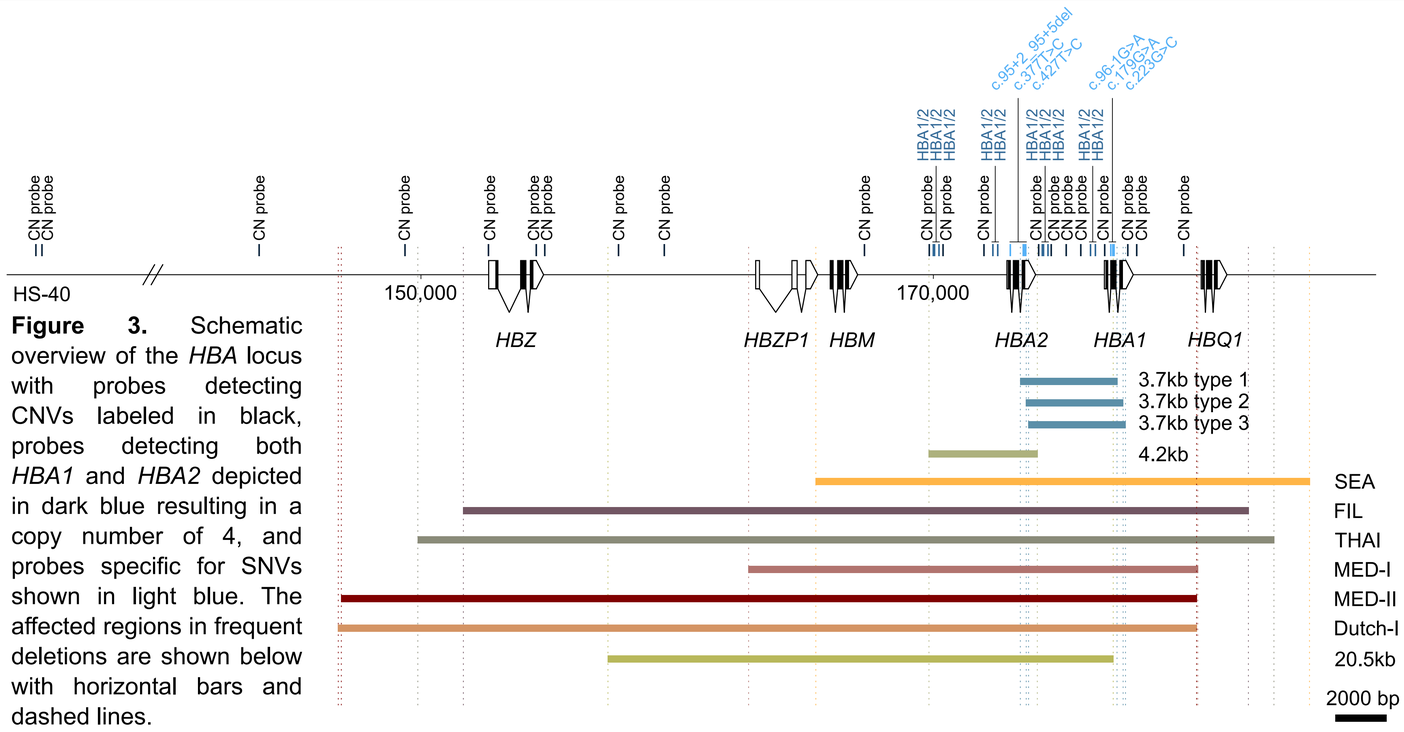

Figure 3. Schematic overview of the *HBA* locus with probes detecting CNVs labeled in black, probes detecting both *HBA1* and *HBA2* depicted in dark blue resulting in a copy number of 4, and probes specific for SNVs shown in light blue. The affected regions in frequent deletions are shown below with horizontal bars and dashed lines.

Alpha-thalassemia is caused by reduced production of the alpha-globin chain, encoded by *HBA1* and *HBA2*. The HBA locus is a complex region, with *HBA1* and *HBA2* being almost identical. About 85-90% of cases are caused by deletions of varying size in this region. Some of these deletions also affect *HBZ* (**Figure 3**), which causes embryonic lethality. The digitalMLPA probes are able to detect various of these frequent deletions (**Figure 3 and 4**).

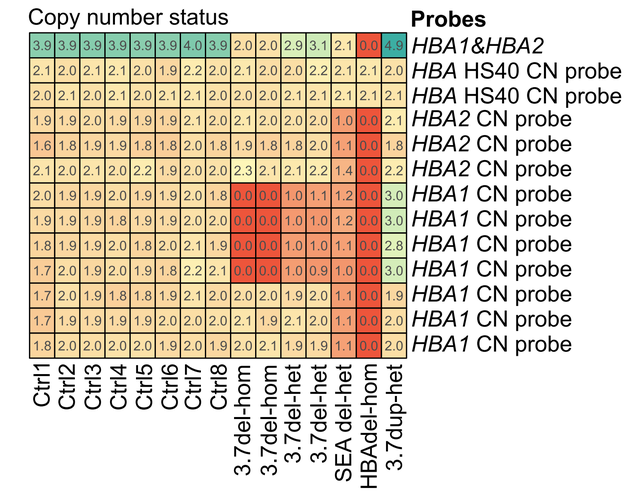

Figure 4. Heatmap indicating the copy number status of 15 samples (columns) obtained using a selection of *HBA1*/*HBA2* probes (rows). The top row shows the median signal for the probes that recognize both *HBA1* and *HBA2*. Values represent the copy number status.

Combined detection of TCR recombination and TREC circles as measure for T cells in blood-derived DNA

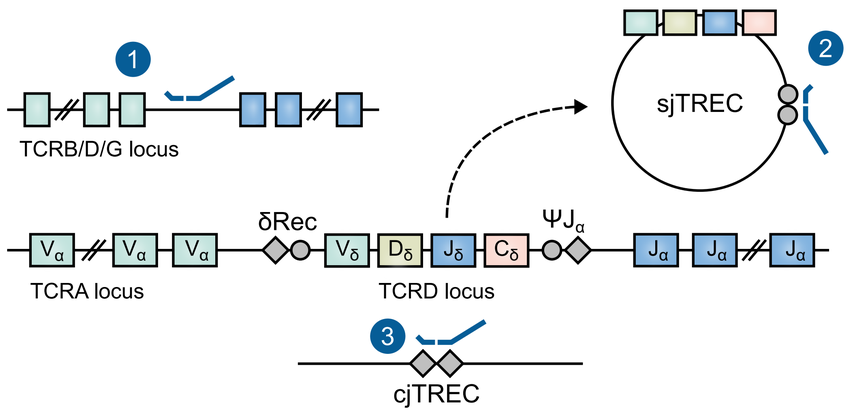

Figure 5. Overview of the detection of T cell DNA using three probe types 1) Probes located between variable rearrangement regions of the TCRs are no longer present in T cells, resulting in a decreased probe ratio in blood-derived DNA. 2) Probes recognizing the signal joint of TREC circles are specific for naive T cells. 3) Probes recognizing the coding joint in the TCR delta locus are specific for T cells.

SCID is genetically heterogeneous, and is currently tested for using a TREC assay, which indirectly assesses the amount of naive T cells in a dried blood spot sample. The digitalMLPA assay includes a set of probes, currently under development, that target the recombinatory regions of the α/δ, β and γ T cell receptors, as well as TREC (**Figure 5**) to determine the presence of T cells (**Figure 6**).

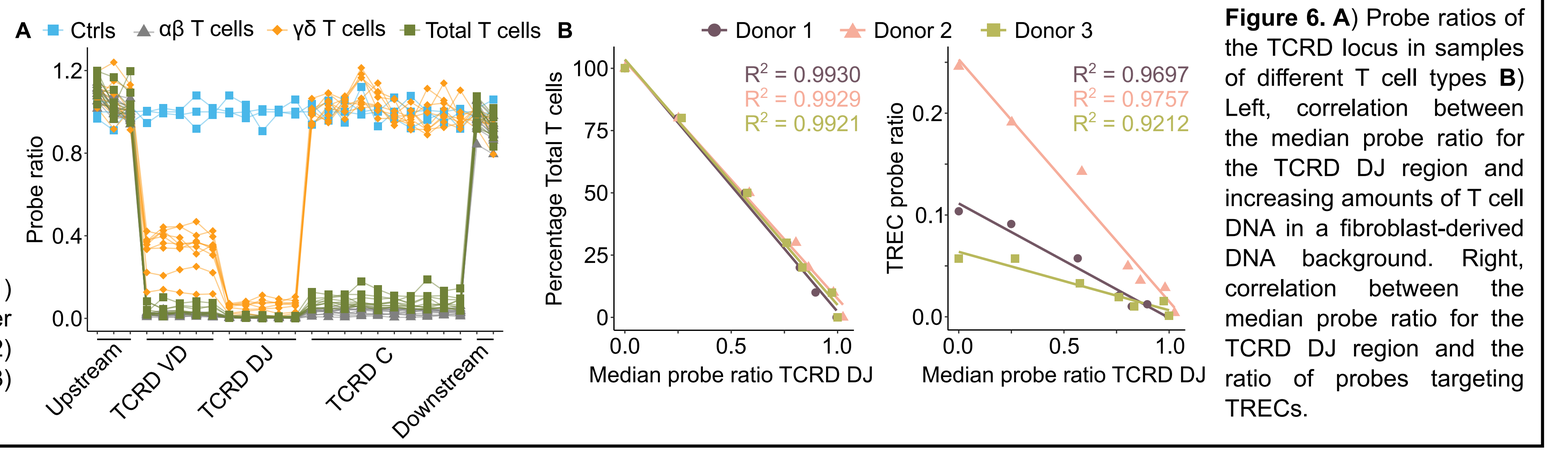

Figure 6. A) Probe ratios of the TCRD locus in samples of different T cell types **B**) Left, correlation between the median probe ratio for the TCRD DJ region and increasing amounts of T cell DNA in a fibroblast-derived DNA background. Right, correlation between the median probe ratio for the TCRD DJ region and the ratio of probes targeting TRECs.

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digitalMLPA is for research use only. Not for use in diagnostic procedures.

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

Conclusions

- ✓ This digitalMLPA assay (newborn test version) can reliably detect distinct frequent deletions in the complex *HBA* locus associated with alpha-thalassemias.
- ✓ A probe set with a two-fold approach to detect the presence of T cell DNA in a blood-derived sample holds promise as a novel approach to screen for SCID.

Taken together, this SALSA® digitalMLPA™ probemix (newborn test version) is a promising DNA-based assay for testing of multiple early-onset conditions.

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