A SIMPLE NGS METHOD FOR DETECTION OF SEQUENCE VARIANTS CAUSING ALPHA AND BETA THALASSEMIA

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RESULTS FOR LIFE

BACKGROUND

Thalassemias are inherited blood disorders characterized by abnormal hemoglobin production. Depending on the type and number of variants, the symptoms can vary from none to severe forms where stem cell transplantation is the only curative treatment.

Today, many different methods are available for variant analysis of thalassemia patients. These include ARMS-PCR, restriction-enzyme PCR, GAP-PCR, Sanger sequencing, LIPA and MLPA. The use of different methods may be time-consuming and expensive, especially when analyzing less common deletions.

MATERIALS & METHODS

We have developed an amplicon based NGS method using only ONE oligo-mix to detect virtually all known variants for alpha and beta thalassemia. The method includes sequencing of the HBB, HBA1 and HBA2 genes (table 1). We have also included oligos for direct detection of the most common deletions in alpha and beta thalassemia (Gap-PCR), tables 2 and 3. In addition, we amplify regions upstream and downstream of the above-mentioned genes to find less common deletions using copy number variation (CNV) analysis (figure 1). The upstream regions also include regulatory sequences, i.e. HS-40 (HBA) and LCRB (HBB). The protocol is user-friendly, requires less than one hour of hands-on time and only 10 ng of DNA is needed.

The Devyser Thalassemia kit has been set up and tested on Illumina sequencing platforms MiSeq and MiniSeq.

Data analysis was performed using AmpliconSuite (SmartSeq®) pipeline for Devyser Thalassemia.

CONCLUSIONS

Evaluation of clinical samples show that 15% of the samples with confirmed variants have additional variants that have not been tested for. These findings demonstrate the need for a method that simultaneously can detect variants involved in both alpha- and beta thalassemia. The Devyser Thalassemia kit is simple, user-friendly and efficiently detects variants causing thalassemia.

TARGETS

Table 1. Genes and sequenced regions

Gene	No of amplicons	Covered region	Notes
HBA1	7	c.-101_c.*173	c.*19_c.*74 not sequenced
HBA2	7	c.-101_c.*141	
HBB	13	c.-290_c.*472	

Fig 1. Illustration of covered positions in the alpha and beta gene clusters

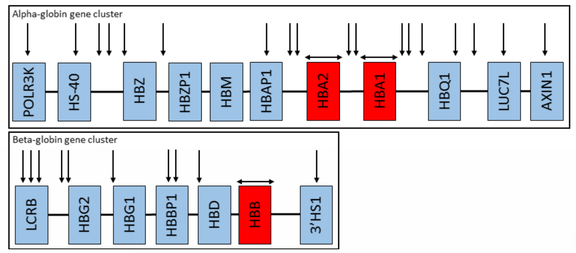


Table 2. Alpha globin deletions and mode of detection

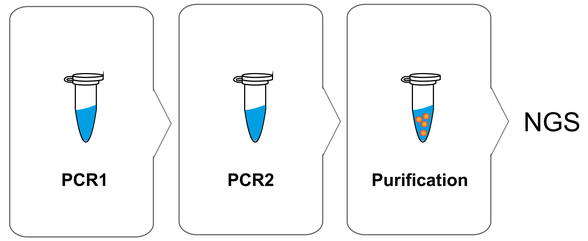
Deletion	HGVS	Mode of detection
--SEA	NG_000006.1:g.26264_45564del19301	Direct detection
--FIL	NG_000006.1:g.11684_43534	Direct detection
--THAI	NG_000006.1:g.10664_44164del33501	Direct detection
-(α)20.5	NG_000006.1:g.15164_37864del22701	Direct detection
--MED 1	NG_000006.1:g.24664_41064del16401	Direct detection
-(α)21.9	NG_000006.1:g.[14373_36299del21927; ins29bp]	Direct detection
-(α)27.6	NG_000006.1:g.9079_36718del27640	Direct detection
-(α)3.7	Exact breakpoints not known	CNV
-(α)4.2	Exact breakpoints not known	CNV
HS-40	Several deletions	CNV
Other deletions	Several deletions	CNV

Table 3. Beta globin deletions and mode of detection

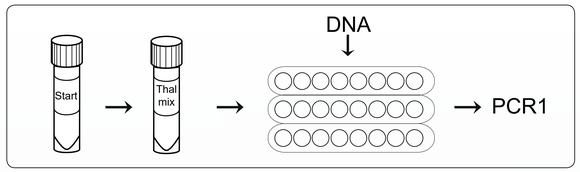
Deletion	HGVS	Mode of detection
Chinese	NG_000007.3:g.48795_127698del78904	Direct detection
Filipino	NG_000007.3:g.66258_184734del118477	Direct detection
Yunnanese	NC_000011.10: 5182847-5249973del67127	Direct detection
Taiwanese	NG_000007.3:g.69997_71353del1357	Direct detection
SEA-HPFH	NC_000011.10: 5201647-5229059del27412	Direct detection
δβ-Sicilia	NG_000007.3:g.64336_77738del13403	Direct detection
Hb-Lepore Boston	NG_000007.3:g.63632_71046del	Direct detection
Hb-Lepore Baltimore	NG_000007.3:g.63564_70978del	Direct detection
Hb-Lepore Hollandia	NG_000007.3:g.63290_70702del	Direct detection
290bp-del	HBB:c.-176_92+25del	Direct detection
LCRB	Several deletions	CNV
Other deletions	Several deletions	CNV

PROCEDURE

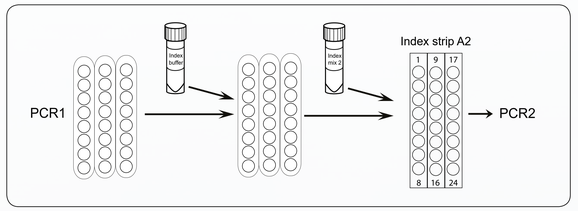
Overview of the library preparation procedure



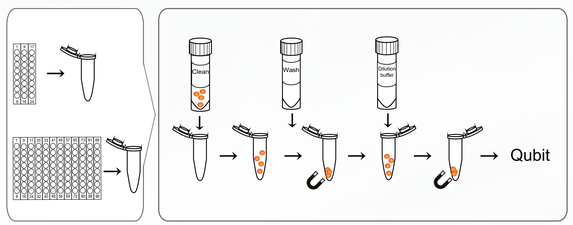
Step 1: PCR1, library generation



Step 2: PCR2, library indexing



Step 3: Pooling and purification



RESULTS

The Devyser Thalassemia kit was used to analyze 125 previously characterized thalassemia samples. All variants (SNVs and deletions) were detected with the kit. Additional variants were also found in 19/125 (15%) of the samples (Table 4).

Figure 2 shows data analysis of a sample with the alpha FIL-deletion. This deletion is detected both with direct detection (GAP-PCR) and CNV analysis.

Figure 3 shows CNV analysis of a sample with the common alpha 3.7-deletion

Table 4. Analysis of 125 precharacterized thalassemia samples

Diagnosed Disease	No of Samples	Additional Variants Found
Alpha-thalassemia	31	4 (HBB4)
Beta-thalassemia	89	15 (HBA10,HBB5)
Alpha + Beta	5	0

Fig 2. Direct detection and CNV analysis of --FIL deletion

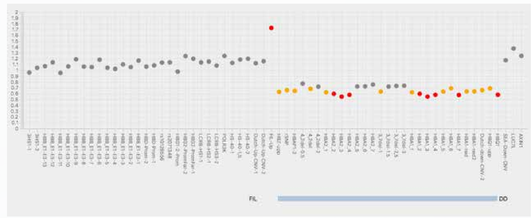
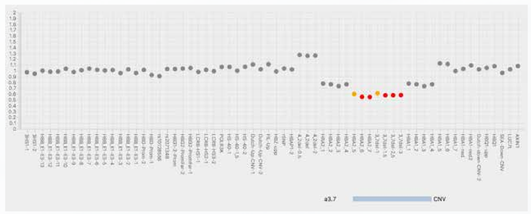


Fig 3. CNV analysis of -(α)3.7 deletion



CONTACT

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