Comprehensive solution for single gene disorders with complex molecular genetics

Comprehensive Analysis of Thalassemia Alleles

Introduction

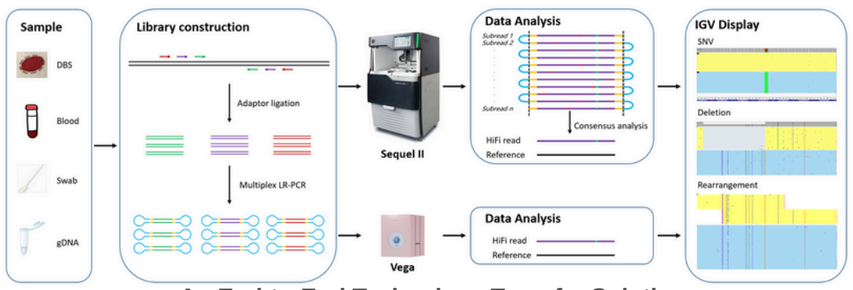
Thalassemia is a group of inherited hemolytic disorders characterized by reduced or absent production of globin chains, involving a spectrum of globin gene defects. This genetic complexity significantly limits the detection coverage of routine molecular assays, creating blind spots in comprehensive variant profiling. These limitations can lead to increased turnaround times, higher costs, and added burdens in clinical aspects.

Now, with advances in long-read sequencing (LRS) technologies, it is possible to offer an all-in-one assay capable of detecting a broad spectrum of variants in a cost-effective manner — Comprehensive Analysis of Thalassemia Alleles (CATSA).

CATSA - LRS Application Built for Commercial Scale

Developed by Berry Genomics, CATSA is a targeted LRS panel specifically designed to streamline the detection of thalassemia variants into a single, standardized assay. Optimized for large-scale applications, it includes a set of reagents and integrated software, enabling a seamless workflow from sample management to variant reporting.

IVD certificated solution
350,000 cases I 50+ publications



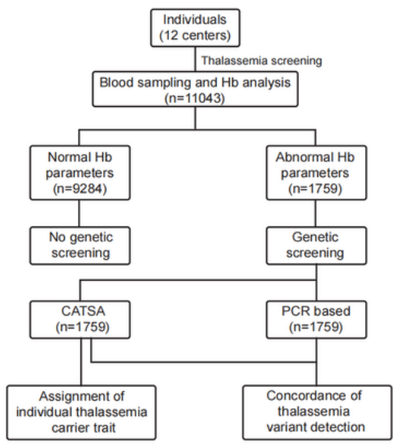
An End-to-End Technology Transfer Solution

Prospective Multi-Center Study

A total of 1,759 blood samples with abnormal hemoglobin parameters were collected across 10 centers to compare CATSA with a standard PCR panel. All CATSA-detected pathogenic variants were further confirmed.

Highlighted Findings:

- CATSA detected pathogenic thalassemia variants in 1,159 samples (65.9%), identifying 1,317 variants
- CATSA demonstrated 100% accuracy in detection
- PCR assays produced 2 false negatives and 7 false positives within its panel
- *HBA1*/*HBA2* structural variants in 10 samples were missed or misclassified as $-\alpha^{3.7}$ by PCR assays



Study design

Information derived from reference [2]

Streamline detection with CASTA

CATSA
All-in-one assay

α-thalassemia
- 30 Deletions
- 4 Homologous recombination
- 80 *HBA1* gene mutations
- 139 *HBA2* gene mutations

β-thalassemia
- 28 Deletions
- 340 *HBB* gene mutations

Hemoglobinopathy
- 324 *HBA1* gene mutations
- 386 *HBA2* gene mutations
- 694 *HBB* gene mutations

Others
- 160 *HBD* gene mutations
- *HBA*/*HBB* gene low frequency mutations, variant of uncertain significance (VUS) associated with thalassemia

Complete blood count + Hemoglobin electrophoresis

Some carriers (e.g., α^+/β^+-thalassemia, α-triplications) may be missed

Sequential hematological screening MCV + MCH and HbA2		
	Sensitivity	Specificity
α-thalassemia	16.53%	97.24%
β-thalassemia	73.74%	96.10%

Compare with NGS panel in China Dai nationality. Information derived from reference [1]

PCR-based assays

Missing carriers with rare deletions, homologous recombination, and uncommon SNVs/InDels

Routine PCR panel in China for common variants	
HBA1/ HBA2	3 Deletions: $--^{SEA}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$ 3 SNVs: Hb Westmead, Hb Quong Sze, Hb Constant Spring
HBB	17 SNVs/InDels: -32, -30, -29, -28, Initiation codon, Cap+1, Codon 17, Codon 14/15, HbE, Codon 27/28, Codon 31, Codon 43, Codon 41/42, Codon 71/72, IVS-I-1, IVS-I-5, IVS-II-654

Next-generation sequencing (NGS)

The limitations of short-read sequencing may lead to inaccurate detection of large deletions and recombination, and often fail to resolve the *cis*/*trans* configuration of variants.

Comparison Between CATSA and NGS

NGS and CATSA were simultaneously performed for 1,122 individuals in Hainan Province. All discordant variants were subsequently confirmed.

Highlighted Findings:

- 1,105 samples (98.48%) showed concordant results between CATSA and NGS, while 17 samples (1.52%) had discordant results
- CATSA improved the positive detection rate by 2.28%

NGS		CATSA	
HBA Variants	*HBB* Variants	*HBA* Variants	*HBB* Variants
$-\alpha^{3.7}/+$	ND	HKαα/αα	ND
ND	ND	HBA2:c.369C>G Hete	ND
ND	ND	$--^{SEA}/\alpha\alpha$	ND
ND	c.126_129del CTTT Homo	ND	c.126_129del CTTT Hete
ND	c.126_129del CTTT Hete	$\alpha\alpha\alpha^{anti4.2}/\alpha\alpha$	c.126_129del CTTT Hete
$--^{SEA}$/HKαα (?)	ND	$--^{SEA}$/HKαα	ND
ND	c.341T>A Hete	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	c.341T>A Hete
αFusion/αFusion	ND	αFusion/αα	ND
ND	ND	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	ND
HBA2:c.*98T>C Hete	ND	$\alpha\alpha\alpha^{anti4.2}$/HBA2:c.*98T>C	ND
ND	ND	$\alpha\alpha\alpha^{anti4.2}/\alpha\alpha$	ND
ND	ND	$\alpha\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	ND
ND	ND	$\alpha\alpha\alpha^{anti3.7}/\alpha\alpha$	ND

Discordant Results Between CATSA and NGS

Information derived from reference [3]

A total of 2,926 participants were retrospectively enrolled in Shanghai for carrier screening of 5 diseases using both NGS and LRS panels, with CATSA applied for thalassemia.

Highlighted Findings:

- LRS detected 16 α^0 variants, 47 α^+ variants, and 50 α-triplications /quadruplications
- NGS failed to identify all α-triplications and misclassified 10 HKαα variants as $-\alpha^{3.7}$, resulting in an error rate of 13.7% (10/73) among α-thalassemia positive samples

Gene	Variant	Carriers of the variant		Carriers of the gene	
		LRS	NGS	LRS	NGS
HBA1, HBA2	*HBA2*:c.342delC (α^+)	1	1	63	63
	HBA2:c.369C>G (α^+)	1	1		
	HBA2:c.427T>C (α^+)	1	1		
	$--^{SEA}$ (α^0)	15	15		
	$--^{THAI}$ (α^0)	1	1		
	$-\alpha^{27.6}$ (α^+)	1	1		
	$-\alpha^{3.7}$ (α^+)	37	37		
	$-\alpha^{4.2}$ (α^+)	6	6		
	False positive $-\alpha^{3.7a}$	0	10[b]	0	10[b]
	$\alpha\alpha\alpha^{anti3.7}$	29	0	50	0
	$\alpha\alpha\alpha^{anti4.2}$	20	0		
	$\alpha\alpha\alpha\alpha^{anti4.2}$	1	0		
HBB	c.−78A>G	2	2	19	19
	c.17_18delCT	1	1		
	c.52A>T	2	2		
	c.79G>A	2	2		
	c.92+5G>C	1	1		
	c.126_129delCTTT	5	5		
	c.316-197C>T	6	6		

Thalassemia carriers identified by LRS and NGS panels

Information derived from reference [4]

Potential Impacts on Prenatal Aspects

A total of 278 amniotic fluid samples from at-risk pregnancies were collected across 9 hospitals. PCR-based methods were used for prenatal diagnosis, while CATSA was performed retrospectively and blindly on all samples. All inconsistent results underwent variant confirmation.

Highlighted Findings:

- CATSA corrected the results in 15 samples (5.4%) with 100% accuracy: 4 within the PCR panel, 4 outside the panel, and 7 classified as variants of uncertain significance
- 8 of them had revised phenotype prediction, including 2 reclassified from β-thalassemia trait to intermedia, and 1 from β-thalassemia major to trait

Information derived from reference [5]

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References: [1] He J, Song W, Yang J, et al. Next-generation sequencing improves thalassemia carrier screening among premarital adults in a high prevalence population: the Dai nationality, China. *Genet Med*. 2017;19(9):1022-1031. [2] Liang Q, Gu W, Chen P, et al. A More Universal Approach to Comprehensive Analysis of Thalassemia Alleles (CATSA). *J Mol Diagn*. 2021;23(9):1195-1204. [3] Huang R, Liu Y, Xu J, et al. Back-to-Back Comparison of Third-Generation Sequencing and Next-Generation Sequencing in Carrier Screening of Thalassemia. *Arch Pathol Lab Med*. 2024;148(7):797-804. [4] Li S, Hua R, Han X, et al. Targeted long-read sequencing facilitates effective carrier screening for complex monogenic diseases including spinal muscular atrophy, α-/β-thalassemia, 21-hydroxylase deficiency, and fragile-X syndrome. *J Transl Med*. 2025;23(1):307. [5] Liang Q, He J, Li Q, et al. Evaluating the Clinical Utility of a Long-Read Sequencing-Based Approach in Prenatal Diagnosis of Thalassemia. *Clin Chem*. 2023;69(3):239-250.