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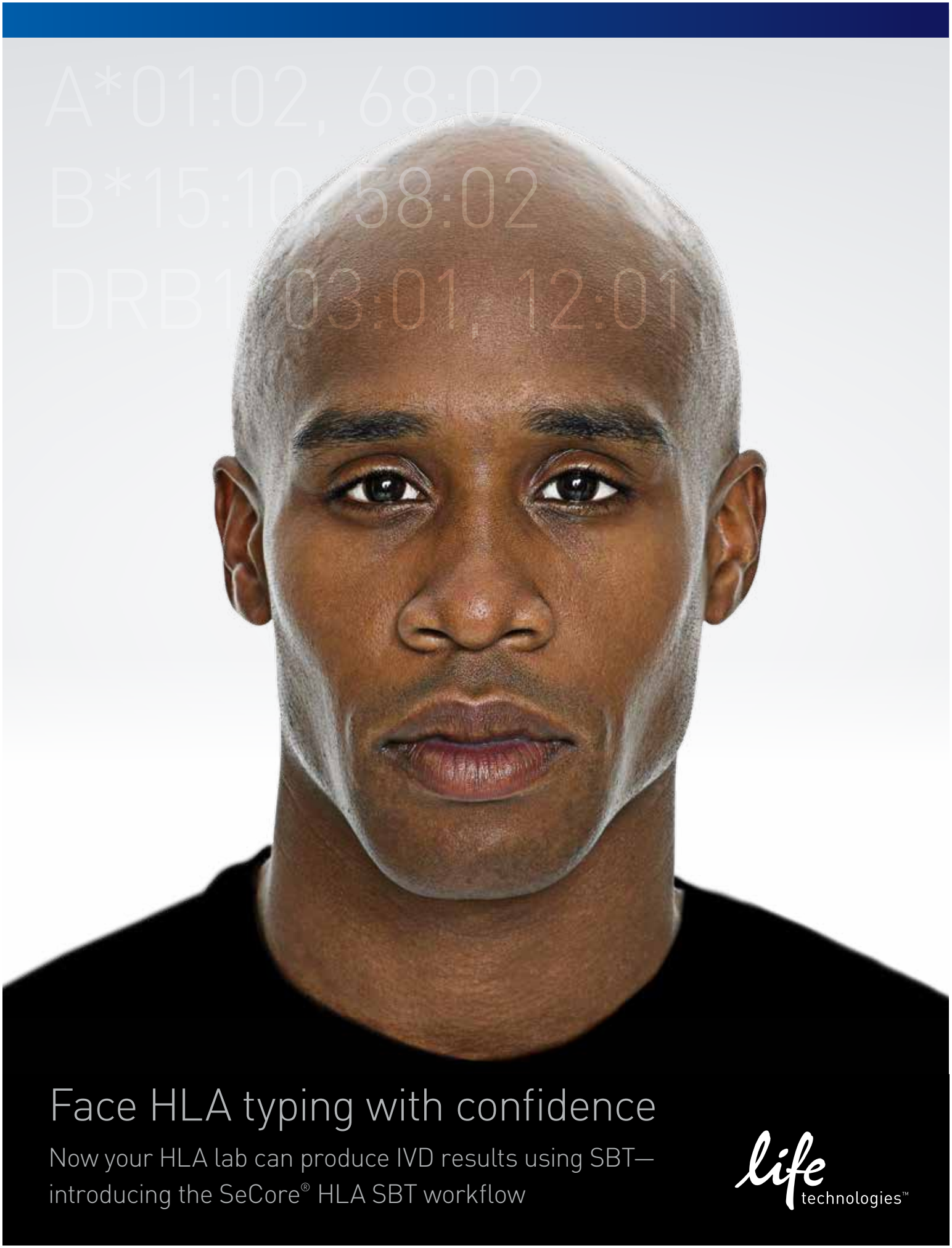


Face HLA typing with confidence

Now your HLA lab can produce IVD results using SBT—introducing the SeCore® HLA SBT workflow

life technologies™


A*26:02, 26:05
B*40:02, 51:01
DRB1*04:04, 14:05

A workflow that raises the bar

Clinical HLA labs can now access a sequence-based typing (SBT) workflow that produces a diagnostic result. We brought together individual components and designed them to work together as a unified workflow to produce diagnostic human leukocyte antigen (HLA) results utilizing sequenced-based typing. Through clinical trials, this workflow has demonstrated accuracy and repeatability. Life Technologies is proud to offer built-in quality because transplant patients depend on all of us—to help improve outcomes.

The Life Technologies HLA SBT typing workflow solution helps provide the answers you need today

Utilizing Sanger sequencing—the gold-standard technology for HLA typing—allows for the ability to accurately detect and identify an astounding level of allelic diversity, with an easy protocol that provides results. Only sequencing for HLA typing lets you store and retrieve nucleotide data for future database updates. That means you will be working with the latest information, a critical consideration for labs that perform HLA typing for diagnostic purposes. The complete solution for producing *in vitro* diagnostic (IVD) results for HLA typing includes the following Invitrogen™ and Applied Biosystems® components:

- SeCore® HLA SBT Kits
- Veriti™ Dx Thermal Cycler (0.2 mL 96-well)
- 3500 Dx CS2 Genetic Analyzer
- uTYPE® Dx HLA Analysis Software

Patients are depending on us

Delivering the most accurate results possible is our responsibility—together. HLA labs adopting this IVD HLA SBT workflow can have increased confidence in delivering accurate results to both transplant teams and patients.

Advantages of the IVD HLA SBT workflow from Life Technologies

Shorter time-to-result/ The sequencing experts at Life Technologies have precisely tuned the workflow instruments to produce optimal results in the shortest possible assay time. Adopting the workflow gives your lab the opportunity to deliver typing results within one workday (Table 1). Time-to-results can be critical, and laboratories that employ a faster workflow can provide patient typing results in shorter time frames.

An even easier-to-use workflow with reduced variability through optimized Dx instrumentation /Since instrument protocol adjustments are eliminated, variability among technicians can be reduced. Instrument settings are pre-set for optimal performance for HLA typing using SeCore® kits, giving you confidence that your team is working with technology that minimizes variation.

Accuracy utilizing the gold standard for HLA typing /Sanger sequencing has the significant advantage of producing the sequenced DNA in contiguous form; therefore, stored data can be utilized at any time for analysis against the most recent IMGT database. Adopting a workflow with proven accuracy, repeatability, and conformance will give your lab confidence about the results they provide to transplant teams and patients.

Flexibility /Our HLA scientific experts can review your lab's historical data to help you predict the group-specific sequencing primers (GSSPs) needed to achieve greater resolution. uTYPE® Dx software offers flexibility because users can analyze and interpret the standard and GSSP-sequenced data either separately or simultaneously. This "frontloading" of GSSPs enables final typing results to be produced in a shorter time frame.

Simplified analysis with powerful software /uTYPE® Dx software guides you through the sample analysis process, which helps make you and your staff comfortable with the technology from the start. Built-in features that accurately filter data are powered by proprietary algorithms, and easy-to-use tools enable you to get the final typing results faster.

Expert implementation /Our highly trained and skilled personnel provide the installation and training needed for process verification. Our knowledgeable professionals can recommend a laboratory workflow layout so your lab can get up and running quickly and operating at optimal efficiency.

Confidence and trust /SeCore® kits and uTYPE® Dx software are developed and manufactured in the USA and in a manufacturing facility that is ISO 13485-certified. Our supply chain has been carefully and purposely built with internal sourcing and USA suppliers—the result is kits you can trust to consistently perform with the quality you expect. Multiple North American stocking locations offer short shipping distances, ample stock, and quick turnaround times.

Figure 1. The Life Technologies HLA SBT workflow produces high-resolution IVD results. (Requires POP-6™ polymer for improved short-fragment resolution, 500 bp read length, and a run time of about 1 hour.)

Table 1. Estimated typical workflow time required for the Life Technologies HLA SBT workflow. The estimates are based on the time required to type five samples for A, B, C, DRB1, DQB1, and DPB1, starting with purified DNA.

	Hands-on time	Hands-off time
PCR	12 min	1.5 hr
PCR purification	5 min	40 min
Sequencing reaction	22 min	1 hr
Sequencing reaction purification	18 min	40 min
Capillary electrophoresis (3500xL Dx Genetic Analyzer, RapidSeq POP-6™ polymer)	31 min	8 hr
Typing analysis (using uTYPE® Dx software)	30 min	0 min
	Total: 118 min	**Total: 11.83 hr**
Results classification	**High-resolution result, IVD**	

SeCore® HLA SBT kits streamline Class I and Class II typing

SeCore® HLA SBT kits include all the necessary reagents and protocols. Complete kits give your lab an advantage of operating with a more efficient workflow that provides high-resolution typing results (sidebar and Table 2).

Why waste precious time ordering and tracking additional materials, performing supplemental pipetting steps, and carrying out additional quality control procedures? BigDye® chemistry is used in SeCore® kits so you achieve long, high-quality reads and accurate results, and base assignments (particularly in heterozygote detection), strong signal, excellent peak uniformity, and low background.

Titration of the final primer cocktail formulation is carefully performed, and the results help ensure an optimal allele balance. Rigorous quality control procedures include thorough testing of a range of DNA samples.

Every SeCore® kit uses the same standardized amplification program as well as the same sequencing program. A complete sample data set can be obtained without the need for locus-specific handling.

SeCore® kits offer:

- BigDye® chemistry for high-quality, long reads, accurate results and base assignments (particularly in heterozygote detection), strong signals, excellent peak uniformity, and low background
- Optimized primer cocktails for superior allele balance
- Improved laboratory efficiency—one protocol for all loci
- A streamlined sequencing workflow for reduced sample-handling errors and improved technician efficiency
- Highest resolution through an impressive stock of GSSPs that are current and comprehensive

Table 2. The targeted exons for Class I and II SeCore® sequencing kits.

Sequencing kit*	Exon target	Quantity
Class I		
SeCore® Locus A	Bidirectional sequencing of exons 2, 3, and 4 (amplicon includes exons 1–5)	25/500 tests
SeCore® Locus B	Bidirectional sequencing of exons 2, 3, and 4	25/500 tests
SeCore® Locus C	Bidirectional sequencing of exons 2, 3, and 4 (amplicon includes exons 1–6)	25/500 tests
Class II		
SeCore® Locus DRB1	Bidirectional sequencing of exon 2 and codon 86	25/500 tests
SeCore® Locus DRB1	Bidirectional sequencing of exons 2 and 3 and codon 86	25/500 tests
SeCore® Locus DRB1 group	Bidirectional sequencing of exon 2 and codon 86	25 tests
SeCore® Locus DQB1	Bidirectional sequencing of exons 2 and 3	25/500 tests
SeCore® Locus DPB1	Bidirectional sequencing of exons 2, 3, and 4, and codons 8 and 85	25/500 tests

*Each SeCore® kit includes amplification mix, FastStart® *Taq* polymerase, purification cocktail, sequencing mixes (including BigDye® 1.1 Terminators), and precipitation buffer.

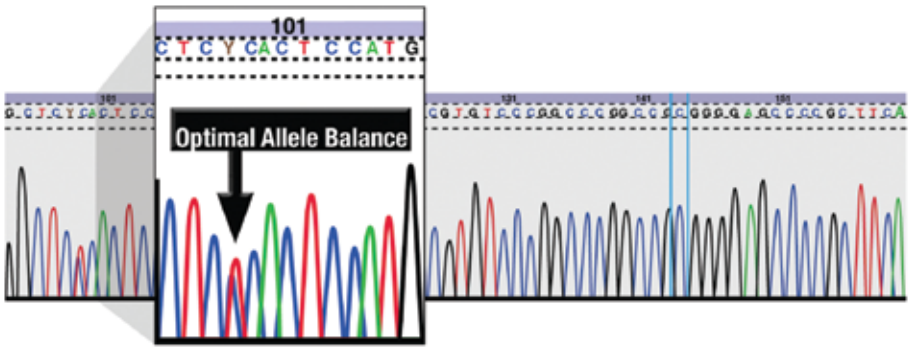


Figure 2. The right primers at the right concentrations and BigDye® chemistry help ensure that you get superior signal and the best peak spacing, peak height, and peak balancing for reduced allele dropout and overall improved data quality.


A*01:01, 03:01
B*39:20, 55:01
DRB1*08:04, 14:54

Meeting diagnostic lab challenges

Life Technologies is committed to simplifying the complexity in HLA typing analysis, and we designed uTYPE® Dx HLA Analysis Software to do just that. Incorporating sophisticated algorithms, uTYPE® Dx software translates sequencing data from the Applied Biosystems® 3500 Dx CS2 Genetic Analyzer into an intuitively guided analysis.

uTYPE® Dx Analysis Software features designed with HLA labs in mind

uTYPE® Dx Analysis Software highlights

To help ensure control of the sequencing data transfer process, uTYPE® Dx Analysis Software only accepts .ab1 files from the 3500 Dx Genetic Analyzer CS2 instrument. Control is also maintained in the update process since only uTYPE® Dx allele library updates are accepted. These built-in controls give you greater assurance of maintaining a protocol that achieves diagnostic results.

- Allele library updates are maintained on a quarterly basis, so you are working with the most recent information.
- The IMGT alignment database is integrated in the software and can be viewed simultaneously with sample data. This convenient feature means there is no need to compare sample data to printed charts or use multiple screens.
- The National Marrow Donor Program (NMDP) codes can be easily imported and used with the software.
- Sample database files are simply organized, and search features make locating sample data easy .
- All windows on the main screen are re-sizable to maximize and customize the user-interface experience.

Data analysis tools

- Color coding helps visually compare allele sequences that simplify the identification of mismatched positions (Figure 3).
- A layered review system provides the ability to track analysis by technician and supervisor levels to meet clinical lab requirements.
- A flexible search-and-load feature organizes the sample list for a simplified and uncluttered view.

Resolution strategy recommendations

- Single aligned electropherogram display including GSSP sequence and standard sequence data.
- Proprietary algorithms recommend the fewest GSSPs necessary.
- View GSSP alignment and ambiguity resolving positions.
- Search capability for resolving GSSPs of any given theoretical ambiguity.
- Interpret sample data from both locus-specific kits and GSSPs simultaneously.

Graphical data viewing

- The peak detail view provides a flexible display to locate and view each data point along the electropherogram.
- A magnifier view that works as a zoom for viewing any portion of the screen at close range.

Streamlined data analysis

- The marker position bar is color-coded to visually highlight base points along the electropherogram for quick identification of the key positions.
- Built-in allele sorting algorithms for conveniently reviewing rare, common, null, alternatively expressed, and intronic ambiguities.

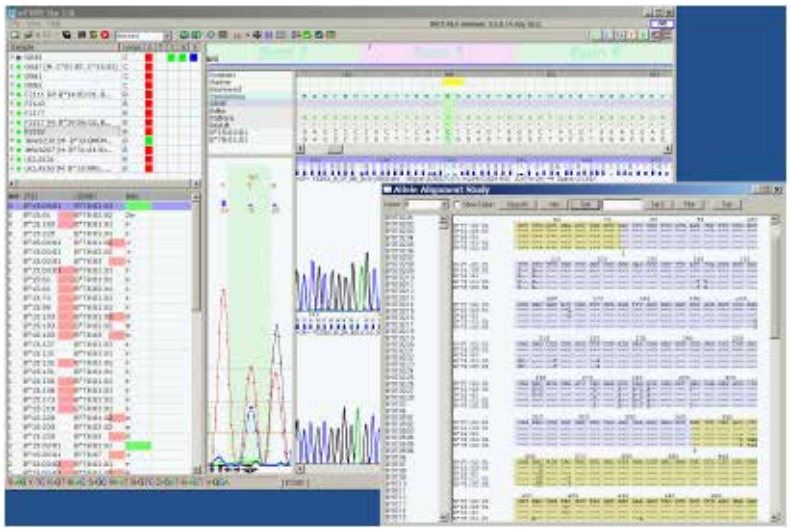
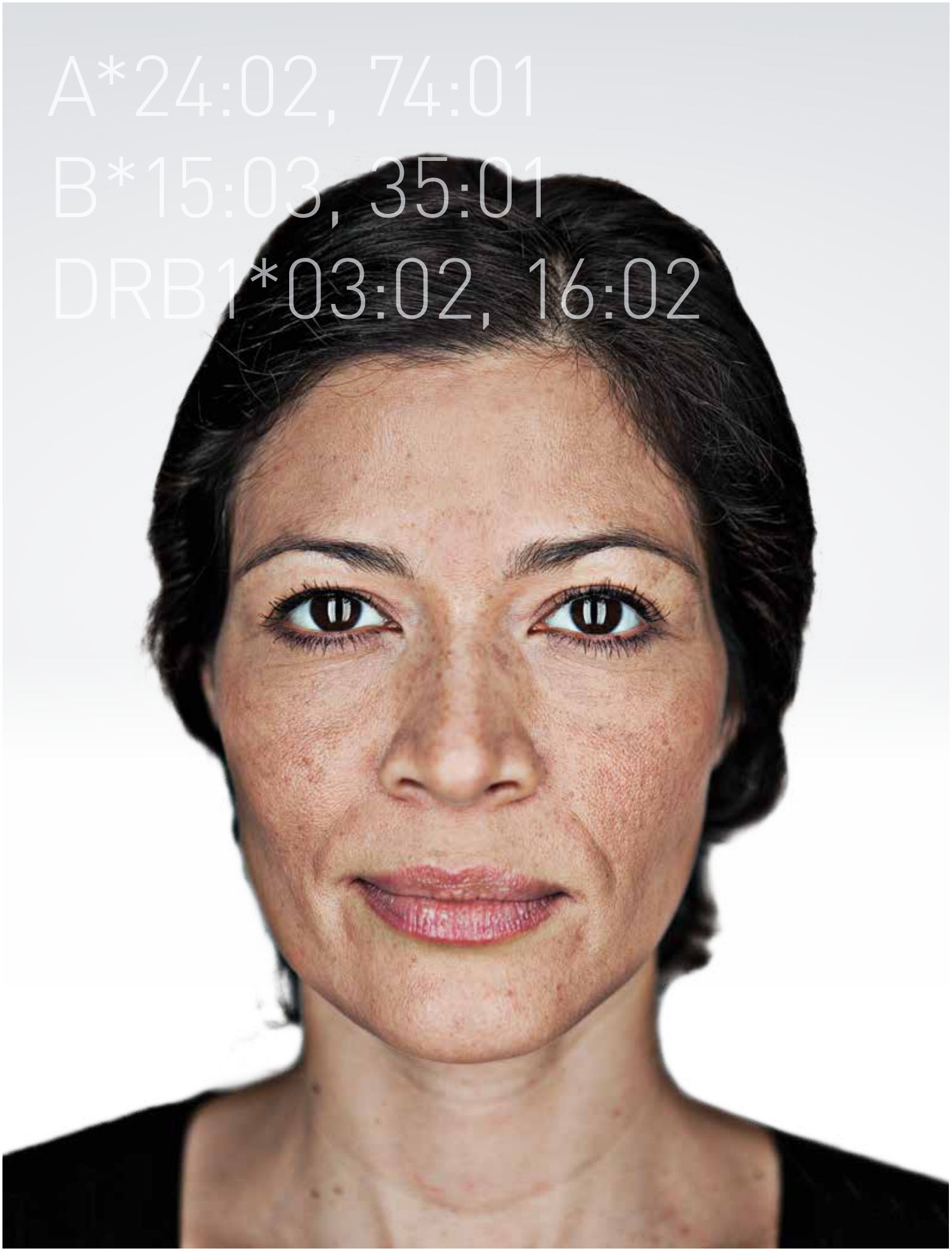


Figure 3. uTYPE® Dx software shows the marked positions in color, with peak detail and allele alignment all in one window.


A*24:02, 74:01
B*15:03, 35:01
DRB1*03:02, 16:02

The proof is in the performance

Each of three sites tested 100 samples for each of the following genes: HLA-A, -B, -C, -DRB, -DQB1, and -DPB1. The study device was the Life Technologies SBT workflow (Veriti™ Dx Thermal Cycler, 3500 Dx Series Genetic Analyzer, SeCore® sequencing kits, and uTYPE® Dx software).

External comparative study

For each SeCore® kit, the concordance rate was calculated and the corresponding exact two-sided 90% confidence interval was calculated by the Clopper-Pearson method. The lower confidence limit was compared to the benchmark acceptance criterion concordance rate of 0.95. For all kits, the lower confidence limit is higher than 0.95, demonstrating that the concordance rate is at least 0.95.

Primary analysis of concordance rate (Clopper-Pearson CI)			
SeCore® kit	**Concordance[1]**	**90% confidence interval[2]**	**Success[3]**
A Locus	100.0% (299/299)	(99.00%, 100.0%)	Yes
B Locus	99.7% (298/299)	(98.42%, 99.98%)	Yes
C Locus	99.7% (298/299)	(98.42%, 99.98%)	Yes
DRB1 Locus	100.0% (299/299)	(99.00%, 100.0%)	Yes
DR Group Kit (DRB1 Locus)	100.0% (299/299)	(99.00%, 100.0%)	Yes
DR Group Kit (DRB3, 4, & 5 Loci)	99.7% (298/299)	(98.42%, 99.98%)	Yes
DQB1 Locus	100.0% (299/299)	(99.00%, 100.0%)	Yes
DPB1 Locus	97.3% (291/299)	(95.22%, 98.66%)	Yes
All kits	99.5% (2381/2392)	(99.24%, 99.74%)	Yes

1. Concordance is reported in percentages (number concordant/number of samples).
2. Confidence interval calculated by the Clopper-Pearson method.
3. Success is determined by a lower bound on the 90% confidence interval greater than 0.95.

External reproducibility study

Study design

The reproducibility study was carried out at three external sites utilizing two operators per site. Each operator independently carried out assay setup through data analysis of 4 well-characterized samples set up in triplicate, run 6 times over 6 nonconsecutive days. Testing was performed with all SeCore® kits. For each SeCore® kit, the concordance rate was calculated and the corresponding exact two-sided 90% confidence interval was calculated by the Clopper-Pearson method. The lower confidence limit was compared to the benchmark concordance rate of 0.95. For all kits, the lower confidence limit is higher than 0.95, demonstrating that the concordance rate is at least 0.95.

Primary analysis of concordance rate (Clopper-Pearson CI)

SeCore® kit	Concordance[1]	90% Confidence interval[2]	Success[3]
A Locus	100.0% (432/432)	(99.15%, 100.0%)	Yes
B Locus	100.0% (432/432)	(99.15%, 100.0%)	Yes
C Locus	100.0% (432/432)	(99.15%, 100.0%)	Yes
DRB1 Locus	100.0% (432/432)	(99.15%, 100.0%)	Yes
DRB Group Kit	99.7% (298/299)	(98.42%, 99.98%)	Yes
DQB1 Locus	100.0% (432/432)	(99.15%, 100.0%)	Yes
DPB1 Locus	100.0% (432/432)	(99.15%, 100.0%)	Yes

1. Concordance is reported in percentages (number concordant/number of samples).
2. Confidence interval calculated by the Clopper-Pearson method.
3. Success is determined by a lower bound on the 90% confidence interval greater than 0.95.

In a study designed to test the performance of the SeCore® kits, a set of nine samples was assayed in parallel in three separate SBT runs (each of the three SBT runs was performed by a separate operator on a separate analysis system using different lots of all seven of the SeCore® kits (locus A, B, C, DRB1, DRB group, DQB1, and DPB1).

Clinical trial results

The results for the Life Technologies SBT workflow met the acceptance criteria of at least 0.95, both before and after adjusting for multiplicity, with 90% confidence for all Class I and Class II loci tested (A, B, C, DRB1, DRB3, DRB4, DRB5, DQB1, and DPB1). All samples tested in the comparative study demonstrated an overall concordance of 99.5% with a 90% confidence between the two methods. For all SeCore® kits, the lower 90% confidence limit is higher than 0.95, demonstrating that the 95% concordance primary endpoint of the clinical study was achieved. All clinical trial sites identified potential new alleles in HLA-B, -C, -DRB4, and -DPB1.

Internal validation results

Using an acceptance criterion of ≥300 RFU, the Class I analysis yielded an acceptable signal for the DNA concentration range of 0.5–100 ng/µL (Figure 4). Class II studies resulted in passed signal for the DNA concentration range of 0.2–100 ng/µL input sample DNA (Figure 5). Class I and Class II kits passed the criterion of ≤8% background for the range 0.2–100 ng/µL DNA (Figure 5). The OD and DNA concentration ranges verified in this study for the SeCore® kits were 1.7–1.9 and 15–30 ng/µL, respectively.

Performance data summary

Figure 4. Signals clearly exceed the acceptance criterion (≥300 RFU signal) over a wide range of DNA concentrations.

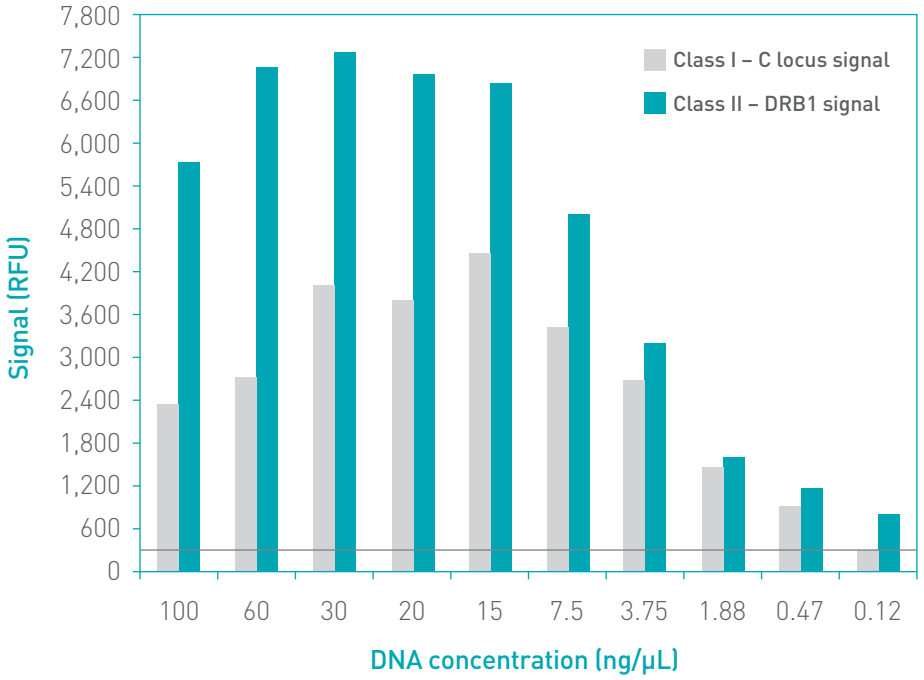
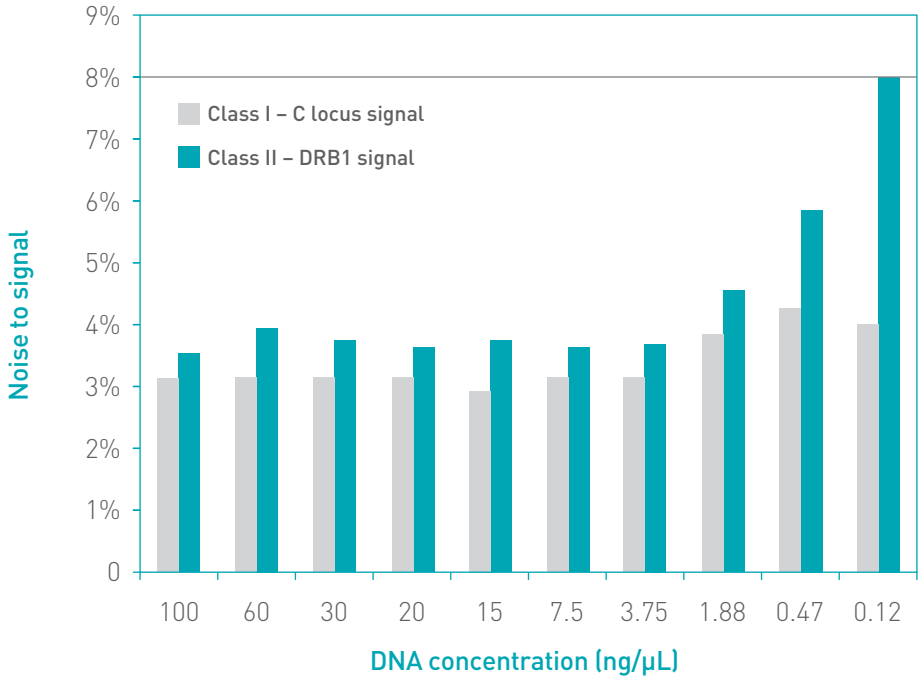


Figure 5. Superior data quality is achieved over a wide range of DNA concentrations, and noise-to-signal ratios are well below the acceptance criterion (≤8%).



Fast. Simple. Flexible. And designed to deliver a diagnostic result.

IVD results from a simple and short HLA SBT workflow Engineered and optimized to operate as a complete system, SeCore® HLA SBT Kits and uTYPE® Dx HLA Analysis Software together with the 3500 Dx CS2 Series Genetic Analyzer and Veriti™ Dx Thermal Cycler, provides IVD results with a shorter and easier workflow. Flexibility and cost-effectiveness are built into the workflow since the right GSSPs for your typing needs can be loaded up-front or after the first round of sequencing. Labs adopting this workflow can achieve diagnostic results with confidence because they know they are using an integrated system supported by clinical trial data attesting to accuracy and reproducibility. Together, we are doing everything we can to improve HLA typing and contribute to better outcomes for transplant patients.

The easiest-to-run, easiest-to-own sequencer to date

At the heart of the Life Technologies HLA typing SBT workflow, the Applied Biosystems® 3500 Dx Series CS2 delivers an IVD-labeled capillary electrophoresis solution, integrating a number of platform improvements designed to provide the highest level of performance from an Applied Biosystems® Genetic Analyzer to date. This integrated component set was designed to match the needs of molecular diagnostic laboratories. In total, you can get both superior data quality and an IVD result.

- Employs IVD-labeled consumables, reagents, and data collection software to support the demanding needs of process-controlled environments
- RFID-tagged, snap-in-and-run consumables pouches give you trackable and auditable reagent status and usage
- IVD-labeled instrument

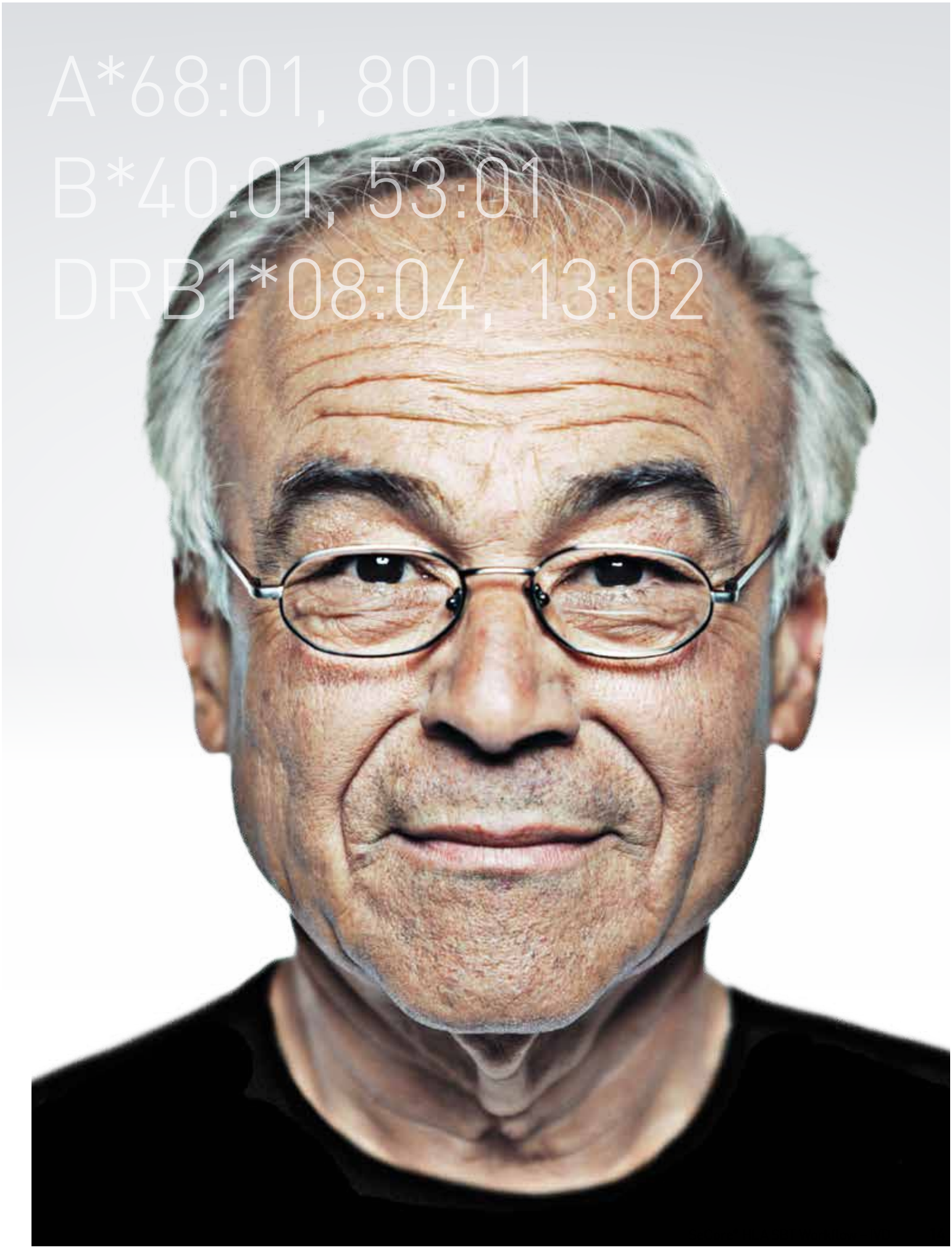
Fast, flexible PCR instrument

The Veriti™ Dx 96-Well Thermal Cycler delivers the reliability you need for SBT analysis.

- Features an easy-to-use graphical interface (6.5 inch VGA touch screen)
- Innovative VeriFlex™ Blocks provide convenient PCR optimization
- Standard and Fast-enabled cycling to address your current and future PCR needs
- Reduced PCR reaction time when using faster ramp rates
- IVD-labeled instrument

Veriti™ Dx Thermal Cycler

3500 Dx Genetic Analyzer CS2


A*68:01, 80:01
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