



## **GeneQuery™ HLA-A PCR+Sanger SBT Typing Kit (SBT-A)**

Catalog #GK891

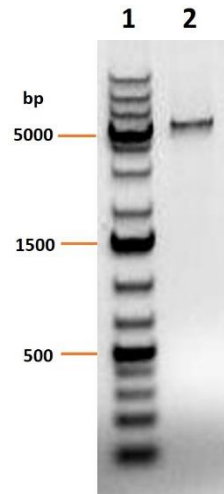
*40 samples*

### **Product Description**

The human leukocyte antigen (HLA) complex is a gene complex located on the short arm of human chromosome 6. The HLA complex is the human version of the major histocompatibility complex (MHC) which helps the immune system distinguish host cells from invaders. It is the most polymorphic locus in the human genome, and has been implicated in many pathologies including autoimmunity disorders and cancer.

GeneQuery™ HLA-A PCR+Sanger SBT Typing Kit (SBT-A) utilizes the Sequence-Based Typing (SBT) method, which is the gold standard for HLA typing. This method involves PCR amplification of HLA genes, followed by Sanger sequencing of the amplicons. The acquired sequences are aligned to an HLA allele database using the SOAPTyping software (BGI Genomics) to obtain the 4-digit genotypes of the samples. The two haplotypes from both parents can be typed simultaneously using this kit and the SOAPTyping software.

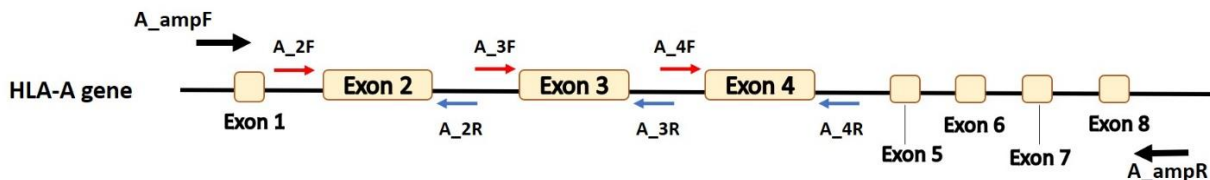
SBT-A kit contains a ScienCell proprietary HLA-A amplification primer set (A\_ampF primer mix and A\_ampR primer mix; Cat #GK891a) that can amplify the complete HLA gene sequence from the enhancer-promoter region to the 3' UTR region, and six sequencing primer mixes: A\_2F (Cat # GK891b), A\_2R (Cat # GK891c), A\_3F (Cat # GK891d), A\_3R (Cat # GK891e), A\_4F (Cat # GK891f), and A\_4R (Cat # GK891g) that can be used in the following Sanger sequencing of the PCR product. Please refer to Figure 2 for the binding location of the primers on the human genome. Each GeneQuery™ HLA typing kit contains a human Genomic DNA Reference (GDR) sample with known HLA alleles (Cat #GK891r). The GDR can be used to monitor the quality of the reagents and the instruments. The HLA-A alleles in GK891r are HLA-A\*02:01 and HLA-A\*25:01.



**Figure 1. Gel electrophoresis separation of PCR amplification products with human genomic DNA as the template using ScienCell GeneQuery™ HLA-A PCR+Sanger SBT Typing Kit.**

Lane 1, DNA ladder;

Lane 2, PCR product of HLA-A amplification PCR primer set (Cat #GK891a, size: ~5,500 bp)

**Figure 2.** Primer binding locations on the human genome.**Kit Components**

Cat #	Component	Quantity	Storage
GK891a	HLA-A amplification primer set (A_ampF and A_ampR)	400 µL	-20°C
GK891b	A_2F sequencing primer mix, 10 µM	200 µL	-20°C
GK891c	A_2R sequencing primer mix, 10 µM	200 µL	-20°C
GK891d	A_3F sequencing primer mix, 10 µM	200 µL	-20°C
GK891e	A_3R sequencing primer mix, 10 µM	200 µL	-20°C
GK891f	A_4F sequencing primer mix, 10 µM	200 µL	-20°C
GK891g	A_4R sequencing primer mix, 10 µM	200 µL	-20°C
GK891h	Nuclease-free H <sub>2</sub> O	4 mL	4°C
GK891r	Human genomic DNA reference (GDR), ~50 ng/µL (HLA-A*02:01 and HLA-A*25:01)	10 µL	-20°C
MB6038-1	2X EmeraldNStart HiFi Marathon PCR Master Mix, 1 mL	2 vials	-20°C

**Additional Materials Required (Materials Not Included in Kit)**

Component	Recommended
genomic DNA template	Customers' samples
PCR purification kit	SpeedDNA PCR Purification Kit (ScienCell, Cat #MB6948)
Sanger sequencing kit	
PCR plates or tubes	

**Quality Control**

The primers are validated by PCR, followed by gel electrophoresis and Sanger sequencing using GK891r as the template.

**Product Use**

SBT-A is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

**Shipping and Storage**

SBT-A is shipped on dry ice. Upon receipt, store the primers and EmeraldNStart HiFi Marathon PCR Master Mix at -20°C in a manual defrost freezer, and nuclease-free H<sub>2</sub>O at 4°C. Once the EmeraldNStart HiFi Marathon PCR Master Mix is thawed, store it at 4°C and do not refreeze. It is stable at 4°C for up to 6 months if stored properly.

## Procedures

**Important:** Only use nuclease-free reagents in PCR amplification.

1. Thaw the HLA-A amplification primer set (Cat #GK891a). Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.
2. For each genomic DNA sample, prepare one 100 µl PCR reaction solution as shown in Table 2. Aliquoting to multiple PCR tubes for the PCR reaction to keep the volume in each tube low is recommended.

**Table 2.**

Genomic DNA template	80 - 120 ng
HLA-A amplification primer set (Cat #GK891a)	10 µl
2X EmeraldNStart HiFi Marathon PCR Master Mix	50 µl
Nuclease-free H <sub>2</sub> O (Cat #GK891h)	variable
<b>Total volume</b>	<b>100 µl</b>

3. Seal the PCR reaction tubes. Centrifuge the tubes at 1,500x g for 15 seconds.
4. Refer to Table 3 for PCR program setup.

**Table 3.**

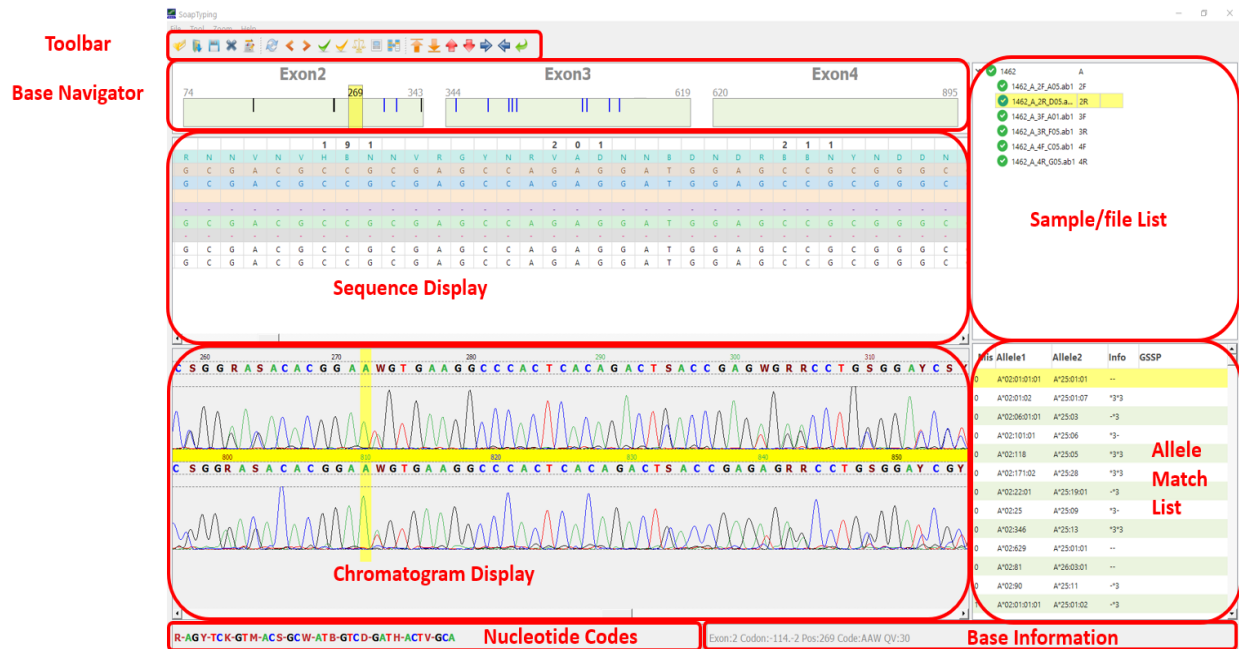
Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	30 sec	38
Annealing	64°C	30 sec	
Extension	72°C	4 min	
Final extension	72°C	10 min	

5. Refer to the Instructions for Use of the PCR purification kit to be used for the purification of the PCR products. Purified PCR products can be used for the Sanger sequencing.
6. Refer to the Instructions for Use of the Sanger sequencing kit to be used for the sequencing of the PCR products. Six sequencing reactions should be prepared with each of the 6 sequencing primers included in the kit.
7. Analyze the sequencing results with the SOAPTyping software (BGI Genomics) to obtain the 4-digit genotypes of the samples. The software can be downloaded at <https://github.com/BGI-flexlab/SOAPTyping/releases> (for Windows, Linux, and Mac) or <https://www.sciencellonline.com/products-services/genetics-genomics/soaptyping> (for Windows only). Please refer to <https://github.com/BGI-flexlab/SOAPTyping> for Instruction for Use of the SOAPTyping software. Appendix 1 provides a quick start guide for the SOAPTyping software. If allele-specific sequencing primers (ASSPs) are required to resolve the typing ambiguity, please refer to Appendix 2 for ASSPs designing guide.







## Appendix 1. Quick start guide for the SOAPTyping software

**Disclaimer:** SOAPTyping Software is a free and open-source software developed by BGI Genomics. ScienCell is NOT responsible for technical support of the software.

## 1. SOAPTyping

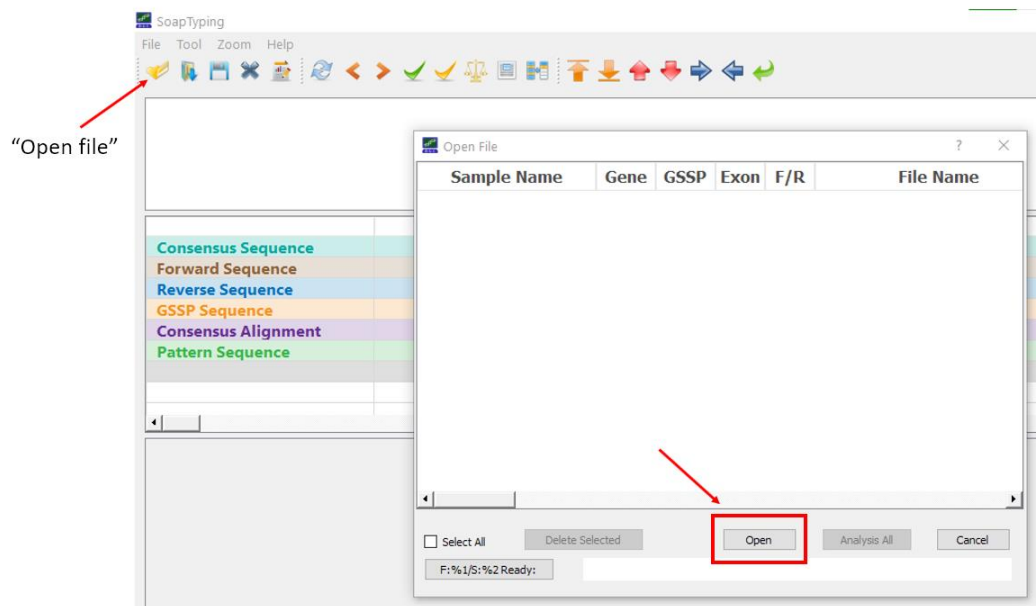


- Verify that the names of the .ab1 files to be analyzed are in the format of “Sample name”\_“HLA gene-of-interest”\_“Exon targeted and primer direction”\_“Additional info”.ab1. “HLA gene-of-interest” is “A” when using this kit for typing HLA-A gene. “Additional info” is a user-defined annotation to the file. The SOAPTyping software uses the underscore symbol “\_” to separate the sections. It is required to have 4 sections in the file name and no additional underscore symbol in the file names. For example,

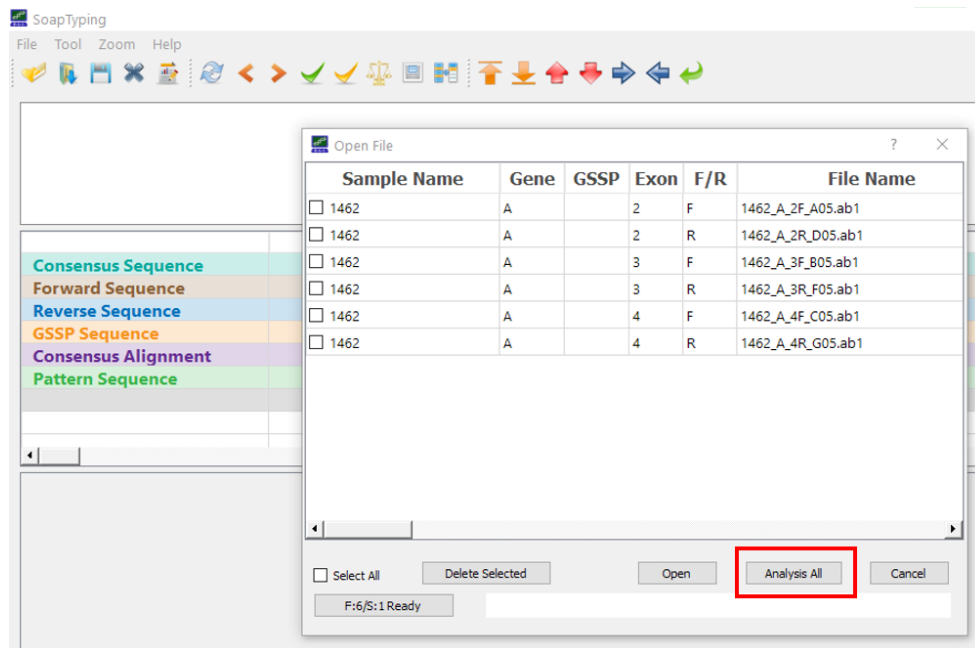
 1462\_A\_2F\_A05.ab1  
 1462\_A\_2R\_D05.ab1  
 1462\_A\_3F\_B05.ab1  
 1462\_A\_3R\_F05.ab1  
 1462\_A\_4F\_C05.ab1  
 1462\_A\_4R\_G05.ab1

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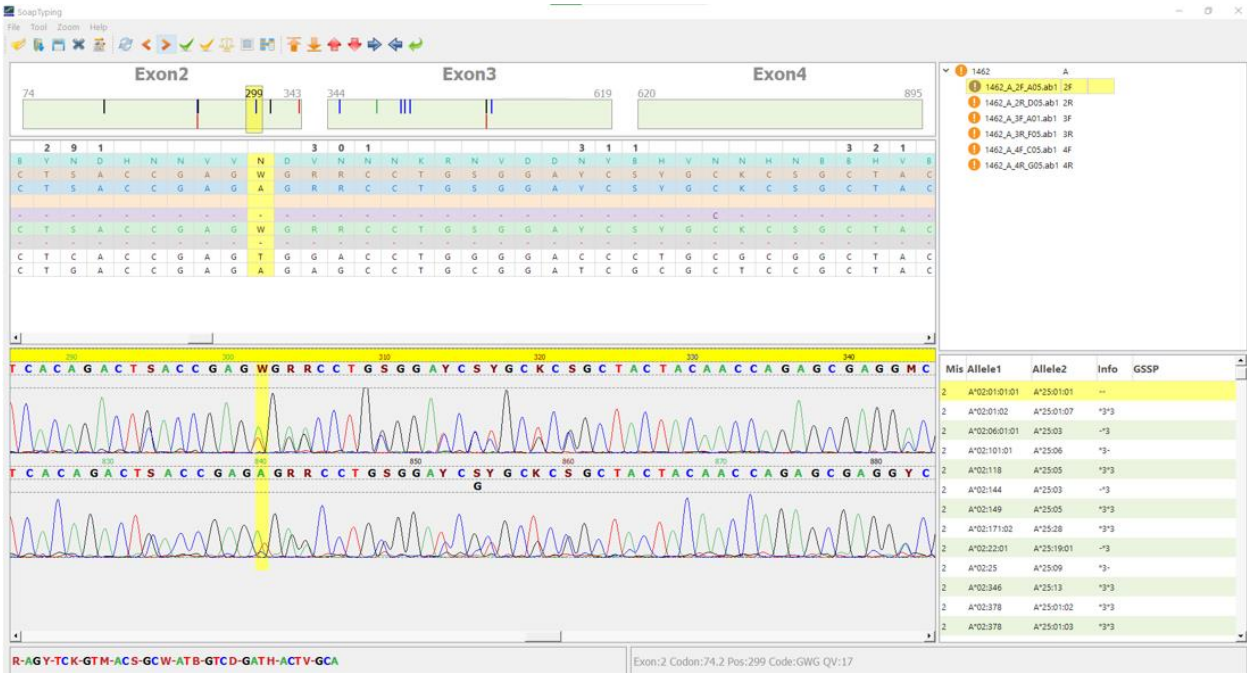
3. Run the SOAPTyping software, click “Open File”, then click “Open” button to select files for analysis.



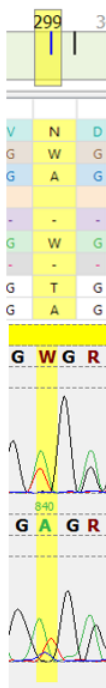
4. Select and open the files, then click “Analyze all” button to analyze the data. The “Gene”, “Exon”, and “F/R” sections should be filled automatically. If not, please check if the file names follow the format described in Step 2 of Appendix 1.



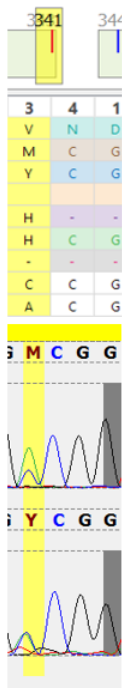
5. The results with ambiguities and mismatches to resolve will be listed.



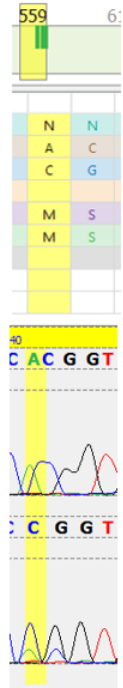
In the upper half of the Base Navigator panel, the ambiguities and mismatches are indicated by color-coded vertical lines. Blue lines indicate different but compatible forward and reverse sequences (e.g., W and A); Green lines indicate different and



The peak of T was NOT accounted for, therefore A should be W here



The peak of T should not be picked, therefore Y should be M here



This position should be an M. In forward sequencing, A and C were separated. In reverse sequencing the peak of A was NOT accounted for, causing a mismatch in forward and reverse sequences.

incompatible forward and reverse sequences (e.g. S and T); Red lines indicate differences between consensus sequence from database and pattern sequence of the sample; Black lines indicate manually changed and now compatible nucleotides.

The red lines in the lower half of the Base Navigator panel indicate mismatches between the pattern sequence and the sequence of the selected allele pair from the Allele Match List panel. These ambiguities and mismatches are usually caused by the interpretation of the Sanger sequencing results. All the ambiguities and mismatches in green and red must be manually corrected to get the final types approved.

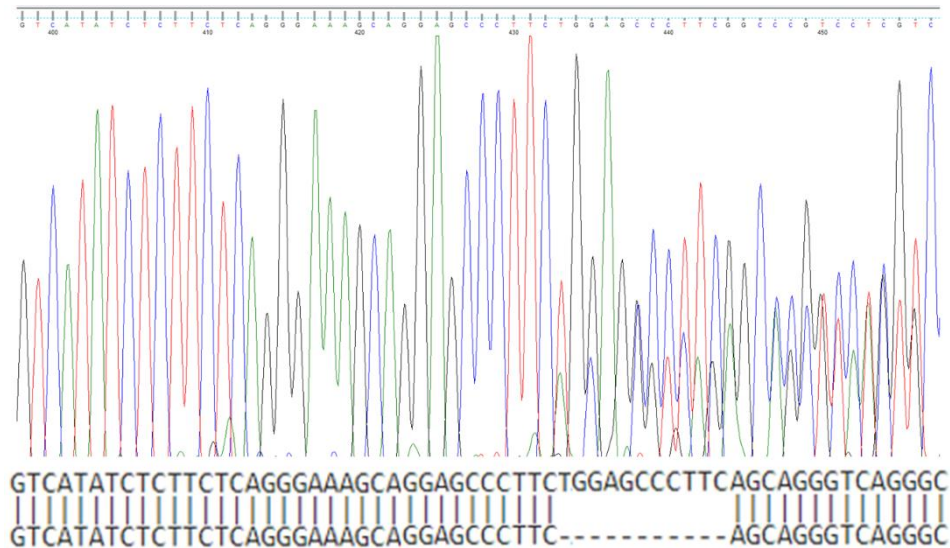




## Appendix 2. Allele-specific sequencing primers (ASSPs) designing guide

**Note:** ASSPs are different than the group-specific sequencing primers (GSSPs). Do NOT use the GSSP option in the SOAPTyping software.

When there is deletion/insertion polymorphism between the 2 alleles, the Sanger sequencing chromatograms will look like the figure shown below. It may cause difficulties in typing the alleles. In other cases, there may be multiple allele pairs matching the sequences analyzed, leading to typing ambiguities. Employment of allele-specific sequencing primers (ASSPs), therefore, may be necessary.



The ASSPs can be designed as described below.

1. At least 15 nucleotides long;
2. The  $T_m$  of ASSPs is 50-55°C;
3. The first different nucleotide is at the 3'-end of the primers.

An example of ASSPs based on the sequencing results in the figure above is given below.

GTCATATCTCTTCTCAGG GAAAGCAGGAGCCCTTCTGGAGCCCTTCAGCAGGGTCAGGGC  
 GTCATATCTCTTCTCAGG GAAAGCAGGAGCCCTTC-----AGCAGGGTCAGGGC

sequencing primer targeting allele 1:	GAAAGCAGGAGCCCTTCT
sequencing primer targeting allele 2:	GAAAGCAGGAGCCCTTCA

Sequence the purified PCR product from Procedure Step #5 using the designed ASSPs by Sanger sequencing. Only the targeted allele of each ASSP is sequenced. The other allele will not show in the sequencing results. Name the .ab1 files in the format described in Appendix 1 based on the exon targeted, and analyze the results using the SOAPTyping software. Alternatively, because only one allele is sequenced utilizing this method, the obtained sequence can be aligned to an HLA genomic sequence database for typing of the allele.