SBT RESOLVER DRBI

Technical Note SBT Resolver™ DRB1 Update

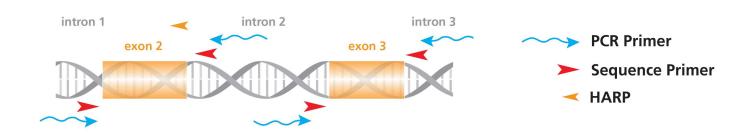
Product Codes: LG-PD5.2-7(20); LG-PD5.2-7(50)

UPDATE DESCRIPTION

As part of our commitment to the improvement of our SBT ResolverTM product line, we are pleased to announce the release of SBT ResolverTM DRB1 LG-PD5.2-7 (527). This updated kit now includes an exon 3 amplicon that is sequenced bi-directionally and includes ALL DRB1 allele groups.

This new 527 kit will replace HH-PD5.2-5 (525), which was the first commercially available, short amplicon, multiplex assay to include exon 3. 525 included an amplicon and sequencing strategy for a subset of the DRB1 alleles in exon 3. Importantly DRB1*14:54 could be unambiguously reported.

Due to the updates and required transition from DRB1 kit 525 to kit 527, there are some changes for the end user that are highlighted in this document.



APPLICATIONS

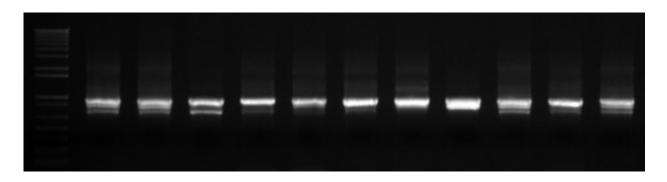
The 527 kit includes sequencing primers for bi-directional sequencing of exon 3. Exon 3 can be sequenced at the point of initial SBT or at the time of ambiguity resolution following primary typing.



IMPACT OF UPDATE

There are no changes to the PCR or sequencing protocols.

Modifications to the exon 3 amplicon from 525 to 527 have resulted in a difference in the size of the fragments. As a result the gel electrophoresis pattern has changed.



527 is designed to enable complete resolution of allele ambiguities characterised by polymorphisms in exon 3.

G Group Alleles Resolved in Exon 3

G Group	Alleles	Resolved with 525	Resolved with 527
DRB1*01:01:01G	01:01:01/01:50	No	Yes
DRB1*04:06:01G	04:06:01/04:06:02	No	Yes
DRB1*07:01:01G	07:01:01+/07:34	No	Yes
DRB1*11:06:01G	11:06:01/11:129	No	Yes
DRB1*15:01:01G	15:01:01+/15:01:17	No	Yes
DRB1*03:01:01G	03:01:01+/03:01:08	Yes	Yes
DRB1*11:01:01G	11:01:01/11:01:08/11:97	Yes	Yes
DRB1*11:13:01G	11:13:01/11:13:02	Yes	Yes
DRB1*13:01:01G	13:01:01/13:117	Yes	Yes
DRB1*14:01:01G	14:01:01/14:54:01	Yes	Yes
DRB1*12:01:01G	12:01:01/12:06/12:10/12:17	12:10 remains unresolved	12:10 remains unresolved

⁺ Alleles characterised by polymorphisms in non-coding regions have been condensed to 3 fields. Information in the table derived from IMGT database; Release 3.17.0.1, Date 21/08/2014.



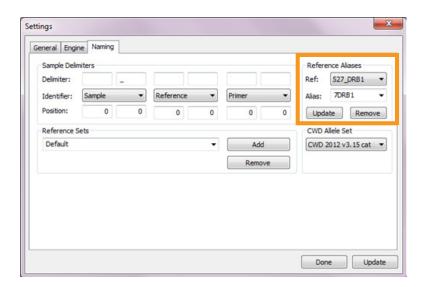
ANALYSIS IN ASSIGN™

The 527 assay requires a unique reference. DRB1 references for 527 will be named 527_DRB1. DNA sequence files produced using the 527 assay will need to have a unique alias that points to the 527_DRB1 reference.

Example:

EPG file name:

20140910_7DRB1.2F_A01.ab1



In the example shown, a unique alias (7DRB1) is required within the sequence filename to ensure that DRB1 527 sequence data is analysed against the 527_DRB1 reference.

NOTE: 527_DRB1 is used as the default alias. A user defined alias, such as the one illustrated in the example above, may be applied. However, it is recommended that any changes to an existing alias happen at the beginning of the alias name and not the end i.e. 7DRB is preferred to DRB7.

Sequence analysis has also been simplified as the exon 2 and 3 data from 527 is analysed in the same analysis layer, as opposed to 525 which required the data from each exon to be analysed in a separate layer as a result of exon 3 covering a subset of alleles.

Analysis of 525:

R880013054 DRB1	-FullX2					
Start: 14 (101)	cDNA 14, Exons 101	, Exo	n 2 1			
Stop: 283 (370) cDNA 283, Exons 370, Exon 2 270						
Allele 1	Allele 2	MM 0	MM 1	MM	2 Differences	
DRB1*01:01:01	DRB1*15:01:01:01	0			0	
DRB1*01:01:01	DRB1*15:01:01:02	0			0	
DRB1*01:01:01	DRB1*15:01:01:03	0	0		0	
DRB1*01:01:01	DRB1*15:01:01:04	0			0	
DRB1*01:01:01	DRB1*15:01:17	0			0 Exon 3	
DRB1*01:50	DRB1*15:01:01:01	0	C		0 Exon 3	
DRB1*01:50	DRB1*15:01:01:02	0	0		0 Exon 3	
DRB1*01:50	DRB1*15:01:01:03	0	C		0 Exon 3	
DRB1*01:50	DRB1*15:01:01:04	0			0 Exon 3	
DRB1*01:50	DRB1*15:01:17	0	C		0 Exon 3	
DRB1*01:18	DRB1*15:07:01	0			1 Exon 2	
DRB1*01:24:01	DRB1*15:05	0	0		1 Exon 2	
DRB1*01:01:18	DRB1*15:01:04	0	0		2 Exon 2	
DRB1*01:20	DRB1*15:02:01	0			2 Exon 2	
DRB1*01:15	DRB1*15:66	0	0		3 Exon 2	

Analysis of 527:

R880013054 527	DRB1			
Start: 14 (101)	cDNA 14, Exons 101	, Exo	n 2 1	
Stop: 565 (652	cDNA 565, Exons 6	552, E	xon 3	282
Allele 1	Allele 2	MM 0	MM 1	Differences
DRB1*01:01:01	DRB1*15:01:01:01	0	0	
DRB1*01:01:01	DRB1*15:01:01:02	0	0	
DRB1*01:01:01	DRB1*15:01:01:03	0	0	
DRB1*01:01:01	DRB1*15:01:01:04	0	0	
DRB1*01:18	DRB1*15:07:01	0	1	Exon 2
DRB1*01:24:01	DRB1*15:05	0	1	Exon 2
DRB1*01:01:18	DRB1*15:01:04	0	2	Exon 2
DRB1*01:20	DRB1*15:02:01	0	2	Exon 2
DRB1*01:15	DRB1*15:66	0	3	Exon 2



If you have any questions in regard to our new product, please contact your local Olerup representative or contact Conexio Genomics at support@conexio-genomics.com



Conexio Genomics Pty Ltd 2/49 Buckingham Dr Wangara 6065 Western Australia Australia



Qarad bvba Cipalstraat 3 B-2440 Geel Belgium



Designed, developed and manufactured by Conexio