

101.122-24/06 – including *Taq* pol., IFU-01
101.122-24u/06u – without *Taq* pol., IFU-02

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“Instructions for Use” (IFU)

Lot No.: **9F6**

Lot-specific information
Olerup SSP® DRB4

Product number:	101.122-24/06 – including <i>Taq</i> pol. 101.122-24/06u – without <i>Taq</i> pol.
Lot number:	9F6
Expiry date:	2020-07-01
Number of tests:	24 tests – Product No. 101.122-24 6 tests – Product No. 101.122-06
Number of wells per test:	31+1
Storage - pre-aliquoted primers:	dark at -20°C
- PCR Master Mix:	-20°C
- Adhesive PCR seals	RT
- Product Insert	RT

This Product Description is only valid for Lot No. 9F6.

Complete product documentation consists of generic Instructions for Use (IFU), lot specific Product Insert, Worksheet and Certificate.

**CHANGES COMPARED TO THE PREVIOUS OLERUP SSP®
DRB4 Lot (8E8)**

The DRB4 kit is updated to enable separation of:

- Confirmed DRB4 alleles as listed in the IMGT/HLA database¹
- Polymorphisms in exons outside of the region encoding the peptide binding domain
- Null and Alternatively expressed alleles

One well has been added to DRB4, well **32**.

The format of the Worksheet has been changed.

¹As described in section Uniquely Identified Alleles.

The DRB4 primer set, specificity and interpretation tables have been updated for the DRB alleles described since the previous *Olerup SSP®* DRB4 lot was made (**Lot No. 8E8**). The kit design is based on IMGT/HLA database 3.29.0.

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The primers of the wells detailed below have been exchanged, added or modified compared to the previous lot.

Well	5'-primer	3'-primer	rationale
13	-	Added	3'-primer added for the DRB4*01:45 allele.
24	-	Added	3'-primer added for the DRB4*01:54N allele.
25	-	Added	3'-primer added for the DRB4*01:56N allele.
26	-	Added	3'-primer added for the DRB4*01:56N allele.
28	-	Added	3'-primer added for the DRB4*01:45 allele.
31	Added	Added	Negative control moved to well 32, primer pair added for the DRB4*01:53 allele.

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Well **32** contains Negative Control primer pairs, that will amplify more than 95% of the *Olerup SSP®* HLA Class I, DRB, DQB1, DPB1 and DQA1 amplicons as well as all the amplicons generated by the control primer pairs matching the human growth hormone gene.

HLA-specific PCR product sizes range from 75 to 200 base pairs.
The PCR product generated by the positive control primer pair is 430 base pairs.

Length of PCR product	105	200	105	80	75	80	85
5'-primer¹	164	340	440	45	45	43	36
	5'-CAC ^{3'}	5'-Agg ^{3'}	5'-TTA ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-Tgg ^{3'}	5'-TAC ^{3'}
							36
							5'-TAT ^{3'}
3'-primer²	231	2nd I	507	59	58	57	47
	5'-TgC ^{3'}	5'-AAA ^{3'}	5'-TTg ^{3'}	5'-CTC ^{3'}	5'-ggC ^{3'}	5'-CTC ^{3'}	5'-ACA ^{3'}
							48
							5'-gCA ^{3'}
							48
							5'-gCC ^{3'}
							52
							5'-TgT ^{3'}
A*	+	+	+				
B*	+	+	+				
C*	+	+	+				
DRB1				+	+		
DRB3				+	+		
DRB5				+			
DQB1					+		
DPB1						+	
DQA1							+

¹The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon, matching the specificity-determining 3'-end of the primer is given. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

²The nucleotide position for HLA class I genes and the codon for HLA class II genes, in the 2nd or 3rd exon or the 2nd intron, matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide and codon numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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Lot-specific information

PRODUCT DESCRIPTION

DRB4 SSP subtyping

CONTENT

The primer set contains 5'- and 3'-primers for identifying the DRB4*01:01:01:01 to DRB4*03:01N alleles.

PLATE LAYOUT

Each test consists of 32 PCR reactions in a 32 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16
17	18	19	20	21	22	23	24
25	26	27	28	29	30	31	NC

The 32 well cut PCR plate is marked with 'DRB4' in silver/gray ink.

Well No. 1 is marked with the Lot No. '9F6'.

Wells 1 to 31 – DRB4 high resolution primers.

Well 32 – Negative Control (NC).

A faint row of numbers is seen between wells 1 and 2 or wells 7 and 8 of the PCR trays. These stem from the manufacture of the trays, and should be disregarded.

The PCR plates are covered with a PCR-compatible foil.

Please note: When removing each 32 well PCR plate, make sure that the remaining plates stay covered. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

INTERPRETATION

Due to the sharing of sequence motifs between DRB4 alleles, non-DRB4 alleles will be amplified by some primer mixes. For further details see Specificity Table.

The DRB1*15:01:01:01-15:01:34, 15:02:01:01-15:02:17, 15:03:01:01-15:147 and the DRB1*16:01:01-16:05:02, 16:07-16:47 alleles might be faintly amplified by primer mix 7.

UNIQUELY IDENTIFIED ALLELES

All the DRB4 alleles, i.e. **DRB4*01:01:01:01 to DRB4*03:01N**, recognized by the HLA Nomenclature Committee in August 2017^{1,2} will be amplified by the primers in the DRB4 subtyping kit.

The DRB4 kit enables separation of the confirmed DRB4 alleles as listed in the IMGT/HLA database. An HLA allele is listed as confirmed by IMGT/HLA if it has been sequenced by more than a single laboratory or from multiple sources. Current allele confirmation status for DRB4 alleles is listed below.

The DRB4 kit also enables identification of polymorphisms in exons outside of the region encoding the peptide binding domain and of null and alternatively expressed alleles.

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The following DRB4 alleles can be distinguished by the different sizes of the HLA-specific PCR product:

Alleles	Primer mix
DRB4*01:20, 01:36	21
DRB4*01:24, 01:37	23
DRB4*01:32, 01:43, 01:54N	24
DRB4*01:38N, 01:40	22

¹DRB4 alleles listed on the IMGT/HLA web page 2017-August-10, release 3.29.0, www.ebi.ac.uk/imgt/hla.

²Alleles that have been deleted from or renamed in the official WHO HLA Nomenclature up to and including the last IMGT/HLA database release can be retrieved from web page <http://hla.alleles.org/alleles/deleted.html>.

ALLELE CONFIRMATION STATUS

Allele	Status ¹	Allele	Status ¹	Allele	Status ¹	Allele	Status ¹
DRB4*01:01:01:01	Confirmed	DRB4*01:13	Confirmed	DRB4*01:33	Unconfirmed	DRB4*01:53	Confirmed
DRB4*01:01:02	Confirmed	DRB4*01:14	Confirmed	DRB4*01:34	Unconfirmed	DRB4*01:54N	Unconfirmed
DRB4*01:02	Confirmed	DRB4*01:15	Unconfirmed	DRB4*01:35	Unconfirmed	DRB4*01:55	Unconfirmed
DRB4*01:03:01:01	Confirmed	DRB4*01:16N	Confirmed	DRB4*01:36	Confirmed	DRB4*01:56N	Unconfirmed
DRB4*01:03:01:02N	Confirmed	DRB4*01:17	Confirmed	DRB4*01:37	Unconfirmed	DRB4*02:01N	Unconfirmed
DRB4*01:03:01:03	Unconfirmed	DRB4*01:18	Unconfirmed	DRB4*01:38N	Unconfirmed	DRB4*03:01N	Unconfirmed
DRB4*01:03:02	Unconfirmed	DRB4*01:19	Unconfirmed	DRB4*01:39	Confirmed		
DRB4*01:03:03	Confirmed	DRB4*01:20	Unconfirmed	DRB4*01:40	Confirmed		
DRB4*01:03:04	Unconfirmed	DRB4*01:21	Unconfirmed	DRB4*01:41	Confirmed		
DRB4*01:03:05	Unconfirmed	DRB4*01:22	Confirmed	DRB4*01:42	Unconfirmed		
DRB4*01:04	Confirmed	DRB4*01:23	Unconfirmed	DRB4*01:43	Unconfirmed		
DRB4*01:05	Unconfirmed	DRB4*01:24	Unconfirmed	DRB4*01:44	Confirmed		
DRB4*01:06	Unconfirmed	DRB4*01:25	Unconfirmed	DRB4*01:45	Confirmed		
DRB4*01:07:01	Confirmed	DRB4*01:26	Unconfirmed	DRB4*01:46	Unconfirmed		
DRB4*01:07:02	Confirmed	DRB4*01:27	Unconfirmed	DRB4*01:47	Unconfirmed		
DRB4*01:08	Unconfirmed	DRB4*01:28	Confirmed	DRB4*01:48	Unconfirmed		
DRB4*01:09	Unconfirmed	DRB4*01:29	Unconfirmed	DRB4*01:49	Unconfirmed		
DRB4*01:10	Unconfirmed	DRB4*01:30	Unconfirmed	DRB4*01:50	Unconfirmed		
DRB4*01:11	Confirmed	DRB4*01:31	Unconfirmed	DRB4*01:51	Unconfirmed		
DRB4*01:12	Unconfirmed	DRB4*01:32	Unconfirmed	DRB4*01:52	Unconfirmed		

¹Allele status “confirmed” or “unconfirmed” as listed on the IMGT/HLA web page 2017-August-10, release 3.29.0, www.ebi.ac.uk/imgt/hla.

RESOLUTION IN HOMO- AND HETEROZYGOTES

Results file with resolution in DRB4 homo- and heterozygotes is available upon request.

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SPECIFICITY TABLE

DRB4 SSP subtyping

Specificities and sizes of the PCR products of the 31+1 primer mixes used for
DRB4 SSP subtyping

Primer Mix	Size of spec. PCR product ¹	Size of control band ²	Amplified DRB4 ³ alleles	Other amplified DRB alleles
1	185 bp	515 bp	*01:01:01:01-01:01:02, 01:03:01:01-01:03:05, 01:05-01:26, 01:28-01:56N	
2⁷	140 bp	430 bp	*01:02	
3	130 bp	430 bp	*01:01:01:01, 01:01:02 [?] , 01:04 [?] -01:05 [?] , 01:06, 01:07:01 [?] -01:08 [?] , 01:11 [?] -01:56N [?] , 02:01N, 03:01N	DRB1*10:19, DRB1*15:141
4	245 bp	515 bp	*01:01:01:01-01:03:01:01, 01:03:01:03-01:04, 01:05 [?] , 01:06-01:56N, 02:01N	
5⁶	155 bp	430 bp	*01:03:01:02N	
6⁴	115 bp 200 bp	430 bp	*01:12 *01:04, 01:14	
7^{5,6}	155 bp	430 bp	*01:02-01:03:05, 01:04 [?] -01:05 [?] , 01:07:01 [?] -01:08 [?] , 01:09-01:10, 01:11 [?] -01:56N [?]	DRB1*15:01:01:01^w-15:01:30^w, DRB1*15:01:32^w-15:02:17^w, DRB1*15:03:01:01^w-15:137N^w, DRB1*15:140^w, DRB1*15:142^w-15:147^w, DRB1*16:01:01^w-16:05:02^w, DRB1*16:07^w-DRB1*16:41N^w, DRB1*16:45^w-16:47^w
8⁸	290 bp	515 bp	*01:01:01:01, 01:01:02 [?] , 01:04 [?] -01:56N [?] , 02:01N [?] , 03:01N	
9⁷	155 bp	515 bp	*01:05	
10⁴	85 bp	515 bp	*02:01N	
11⁴	110 bp	430 bp	*01:06	
12	210 bp	430 bp	*01:07:01-01:07:02, 01:14	
13⁴	110 bp 140 bp 215 bp	430 bp	*01:12, 01:39 *01:45 *01:08	
14	220 bp	430 bp	*01:09	
15	190 bp 245 bp	430 bp	*01:10, 01:30 *01:02 [?] , 01:21	
16⁴	125 bp 265 bp	430 bp	*01:13 *01:02 [?] , 01:17	
17	255 bp	430 bp	*01:02 [?] , 01:17, 01:21-01:22, 01:26	
18	200 bp	430 bp	*01:29-01:31	
19	210 bp	430 bp	*01:27-01:29	

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20	225 bp	430 bp	*01:23, 01:27	DRB1*04:109, DRB1*12:65, DRB1*15:42
21⁴	250 bp 105 bp 225 bp	430 bp	*01:02 ² , 01:22 *01:20 *01:05 ² , 01:07:01 ² , 01:36, 01:42	
22⁴	100 bp 230 bp	430 bp	*01:33, 01:40 *01:38N	
23⁴	105 bp 240 bp	430 bp	*01:24 *01:37	
24⁴	75 bp 175 bp 240 bp	430 bp	*01:32 *01:35, 01:43 *01:54N	
25⁴	75 bp 160 bp	430 bp	*01:44 *01:15, 01:35, 01:56N	
26	135 bp 165 bp	430 bp	*01:25 *01:18, 01:34, 01:56N	
27	160 bp 220 bp	430 bp	*01:19, 01:34 *01:42	
28	140 bp	430 bp	*01:16N, 01:25, 01:45	
29	140 bp	430 bp	*01:11	
30	235 bp	430 bp	*01:41	
31⁴	125 bp	430 bp	*01:53	
32⁹	-	-	Negative Control	

¹Alleles are assigned by the presence of specific PCR product(s). However, the sizes of the specific PCR products may be helpful in the interpretation of DRB4 SSP typings. When the primers in a primer mix can give rise to HLA-specific PCR products of more than one length this is indicated if the size difference is more than 20 base pairs. Size differences of 20 base pairs or less are not given. For high resolution SSP kits, the alleles listed are specified according to amplicon length. Nonspecific amplifications, i.e. a ladder or a smear of bands, may sometimes be seen. GC-rich primers have a higher tendency of giving rise to nonspecific amplifications than other primers. PCR fragments longer than the control bands may sometimes be observed. Such bands should be disregarded and do not influence the interpretation of the SSP typings. PCR fragments migrating faster than the control bands, but slower than a 400 bp fragment may be seen in some gel read-outs. Such bands can be disregarded and do not influence the interpretation of the SSP typings. Some primers may give rise to primer oligomer artifacts. Sometimes this phenomenon is an inherent feature of the primer pair(s) of a primer mix. More often it is due to other factors such as too low amount of DNA in the PCR reactions, taking too long time in setting up the PCR reactions, working at elevated room temperature or using thermal cyclers that are not pre-heated.

²The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases. In the presence of a specific amplification the intensity of the control band often decreases.

³For several DRB1 alleles 1st and/or 3rd exon(s) and beyond, as well as intron nucleotide sequences, are not available. In these instances it is not known whether some of the primers of the SSP sets are completely matched with the target sequences or not. Assumption is made that unknown sequences in these regions are conserved within allelic groups. The complete 2nd exon nucleotide sequence of the DRB4*01:05 allele is not known. Thus, it is not known whether the DRB4*01:05 allele will be amplified by primer mix 4 or not.

⁴HLA-specific PCR products shorter than 125 base pairs have a lower intensity and are less sharp than longer PCR products.

⁵The DRB1*15:01:01-15:01:34, 15:02:01:01-15:02:17, 15:03:01:01-15:147 and the DRB1*16:01:01-16:05:02, 16:07-16:47 alleles might be faintly amplified by primer mix 7.

⁶Primer mixes 5 and 7 may have tendencies of unspecific amplifications.

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⁷Primer mixes 2 and 9 have a tendency to giving rise to primer oligomer formation.

⁸Primer mix 8 may give rise to a lower yield of HLA-specific PCR product than the other DRB4 primer mixes.

⁹Primer mix 32 contains a negative control, which will amplify more than 95% of HLA amplicons as well as the amplicons generated by the control primer pairs matching the human growth hormone gene. HLA-specific PCR product sizes range from 75 to 200 base pairs and the PCR product generated by the HGH positive control primer pair is 430 base pairs.

‘?’ , nucleotide sequence information not available for the primer matching sequence.

‘w’ , may be weakly amplified.

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PRIMER SPECIFICATION

Well No.	1	2	3	4	5	6	7	8	9	10	11	12
Length of spec. PCR product	185	140	130	245	155	115 200	155	290	155	85	110	210
Length of int. pos. control ¹	515	430	430	515	430	430	430	515	515	515	430	430
5'-primer(s) ²	28(170) 5'-gAT 3'	42(213) 5'-AgT 3'	105(401) 5'-AAA 3'	1 st I 5'-ggg 3'	5(101) 5'-CAA 3'	28(170) 5'-gAT 3'	96(375) 5'-CAA 3'	2 nd I 5'-TgA 3'	42(213) 5'-AgT 3'	28(170) 5'-gAT 3'	112(421) 5'-ACT 3'	28(170) 5'-gAT 3'
3'-primer(s) ³	76(314) 5'-TgT 3'	76(314) 5'-TgC 3'	135(490) 5'-gCT 3'	1 st I 5'-TgC 3'	42(213) 5'-TCA 3'	53(244) 5'-CAT 3'	135(490) 5'-gCC 3'	2 nd I 5'-TTC 3'	81(328) 5'-gTg 3'	42(213) 5'-TCA 3'	135(490) 5'-gCT 3'	83(335) 5'-CgC 3'
						77(317) 5'-AgT 3'						84(337) 5'-CCg 3'
						83(335) 5'-CgC 3'						84(337) 5'-CCT 3'
Well No.	1	2	3	4	5	6	7	8	9	10	11	12

Well No.	13	14	15	16	17	18	19	20	21	22	23	24
Length of spec. PCR product	110 140 215	220	190 245	125 265	255	200	210	225 250	105 225	100 230	105 240	75 175 240
Length of int. pos. control ¹	430	430	430	430	430	430	430	430	430	430	430	430
5'-primer(s) ²	28(170) 5'-gAT 3'	96(375) 5'-CAA 3'	17(138) 5'-TTg 3'	12(121) 5'-CTg 3'	12(121) 5'-CTg 3'	30(175) 5'-gAg 3'	28(170) 5'-gAg 3'	17(137) 5'-TTC 3'	28(170) 5'-gAT 3'	28(170) 5'-gAT 3'	28(170) 5'-gAT 3'	28(170) 5'-gAT 3'
			36(195) 5'-gAC 3'	58(259) 5'-ACC 3'	15(131) 5'-gTA 3'	36(195) 5'-gAC 3'	30(175) 5'-gAg 3'	23(155) 5'-gCA 3'				
			38(199) 5'-ACA 3'		17(137) 5'-TTC 3'	37(196) 5'-AgC 3'	30(175) 5'-gAC 3'	28(170) 5'-gAg 3'				
					17(138) 5'-TTg 3'							
3'-primer(s) ³	51(239) 5'-CCA 3'	156(555) 5'-gTA 3'	86(344) 5'-CCA 3'	86(344) 5'-CCA 3'	86(344) 5'-CCA 3'	86(344) 5'-CCA 3'	86(344) 5'-CCA 3'	86(344) 5'-CCA 3'	49(233) 5'-CCA 3'	46(223) 5'-CTT 3'	49(232) 5'-CgT 3'	39(203) 5'-AgT 3'
	53(244) 5'-CAT 3'								88(350) 5'-Agg 3'	48(230) 5'-CCC 3'	94(368) 5'-CTT 3'	71(299) 5'-gCT 3'
	61(270) 5'-TTg 3'								93(365) 5'-gCT 3'	92(361) 5'-CTA 3'		74(309) 5'-ACA 3'
	87(346) 5'-CTT 3'											94(367) 5'-TCA 3'
Well No.	13	14	15	16	17	18	19	20	21	22	23	24

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Well No.	25	26	27	28	29	30	31
Length of spec.	75	135	160	140	140	235	125
PCR product	160	165	220				
Length of int.	430	430	430	430	430	430	430
pos. control ¹							
5'-primer(s) ²	28(170)	28(170)	28(170)	28(170)	15(130)	21(149)	28(170)
	5'-gAT 3'	5'-gAT 3'	5'-gAT 3'	5'-gAT 3'	5'-AgC 3'	5'-gAT 3'	5'-gAT 3'
3'-primer(s) ³	39(202)	60(266)	68(290)	60(266)	48(230)	86(344)	56(253)
	5'-gCA 3'	5'-AgC 3'	5'-CCg 3'	5'-AgC 3'	5'-CCT 3'	5'-CCA 3'	5'-AgT 3'
	64(277)	64(277)	70(295)	60(267)			
	5'-CTA 3'	5'-CTA 3'	5'-CCC 3'	5'-CAC 3'			
	71(299)	68(290)	88(350)	61(270)			
	5'-gCT 3'	5'-CCg 3'	5'-Agg 3'	5'-TTg 3'			
	72(301)	70(295)					
	5'-CCA 3'	5'-CTg 3'					
Well No.	25	26	27	28	29	30	31

¹The internal positive control primer pairs amplify segments of the human growth hormone gene. The internal positive control bands are 430 or 515 base pairs respectively, well distribution as outlined in the table. Well number 1 contains the longer, 515 bp, internal positive control band. The well distribution of the internal controls can help in orientation of the kit on gel photo, as well as allow for kit identification. In the presence of a specific amplification the intensity of the control band often decreases.

²The nucleotide position matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

³The nucleotide position matching the specificity-determining 3'-end of the primer is given in the anti-sense direction. Nucleotide numbering as on the www.ebi.ac.uk/imgt/hla web site. The sequence of the 3 terminal nucleotides of the primer is given.

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[illegible]

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¹The provided cell line HLA specificities are retrieved from the <http://www.ihwg.org/hla> web site. The specificity of an individual cell line may thus be subject to change.

²The specificity of each primer solution in the kit has been tested against 48 well characterized cell line DNAs and where applicable, additional cell line DNAs.

*f The DRB1*15:01:01:01-15:01:34, 15:02:01:01-15:02:17, 15:03:01:01-15:147 and the DRB1*16:01:01-16:05:02, 16:07-16:47 alleles might be faintly amplified by primer mix 7.

No DNAs carrying the alleles to be amplified by primer solutions 6 and 9 to 31 were available. The specificities of the primers in primer solutions 6, 9, 22 and 24 to 26 were tested by separately adding one additional 5'-primer, respectively, one additional 3'-primer. In primer solutions 10, 11, 15 to 20, 29 and 30, it was only possible to test the 3'-primers, the 5'-primers were not possible to test. In primer solutions 12 to 14, 21, 23, 27, 28 and 31 it was only possible to test the 5'-primer, the 3'-primers were not possible to test. In primer solutions 6, 22, 24 to 26 two or three of the 3'-primers were not possible to test.

One additional 3'-primer in primer solution 1 was tested by adding one additional 5'-primer.

101.122-24/06 – including *Taq* pol., IFU-01
101.122-24u/06u – without *Taq* pol., IFU-02

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