

Lot No.: **34G**

Lot-specific information

## **Olerup SSP<sup>®</sup> HLA-A\*66**

Product number: 101.427-06u – without *Taq* polymerase  
Lot number: 34G  
Expiry date: 2011-October-01  
Number of tests: 6  
Number of wells per test: 12  
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. 34G.**

### **CHANGES COMPARED TO THE PREVIOUS *OLERUP SSP*<sup>®</sup> HLA-A\*66 LOT**

The HLA-A\*66 specificity and interpretation tables have been updated for the HLA-A alleles described since the previous *Olerup SSP*<sup>®</sup> HLA-A\*66 lot was made (Lot No. 28F).

Four wells have been added to the HLA-A\*66 kit,  
wells **9 to 12**.

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

Well	5'-primer	3'-primer	rationale
1	-	Added	Primer added for the A*6610 allele.
4	Moved	Moved	Primer pair moved to well 9.
9	Added	Added	Primer pair from well 4 for improved resolution of the A*6606 allele.
10	New	New	New primer pair for the A*6609 allele.
11	New	New	New primer pair for the A*6610 allele.
12	New	New	New primer pair for the A*6608 allele.

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## PRODUCT DESCRIPTION

### HLA-A\*66 SSP subtyping

#### CONTENT

The primer set contains 5'- and 3'-primers for identifying the HLA-A\*6601 to A\*6610 alleles.

#### PLATE LAYOUT

Each test consists of 12 PCR reactions in a 16 well cut PCR plate.

1	2	3	4	5	6	7	8
9	10	11	12	empty	empty	empty	empty

The 16 well cut PCR plate is marked with 'HLA-A\*66' in silver/gray ink.

Well No. 1 is marked with the Lot No. '34G'.

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 heat-sealed with a PCR-compatible foil.

**Please note:** When removing each 16 well PCR plate, make sure that the remaining plates stay sealed. Use a scalpel or a similar instrument to carefully cut the foil between the plates.

#### INTERPRETATION

The interpretation of HLA-A\*66 SSP subtypings will be influenced by the A\*01, several A\*02 alleles, the A\*0343, the A\*11, two A\*24 alleles, the A\*25, the A\*26, two A\*31 allele, two A\*33 alleles, the A\*34, the A\*36, the A\*4301 allele, most A\*68 alleles, the A\*6901, the A\*7410 and the A\*8001 alleles when present on the other haplotype.

#### UNIQUELY IDENTIFIED ALLELES

All the HLA-A\*66 alleles, i.e. **A\*6601 to A\*6610 alleles**, recognized by the HLA Nomenclature Committee in July 2009<sup>1</sup> will give rise to unique amplification patterns by the primers in the HLA-A\*66 subtyping kit.

<sup>1</sup>HLA-A alleles listed on the IMGT/HLA web page 2009-July-17, release 2.26.0, [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla).

#### RESOLUTION IN HOMO- AND HETEROZYGOTES

The 10 HLA-A\*66 alleles can be combined in 55 homozygous and heterozygous combinations. Fifteen of these genotypes do not give rise to unique amplification patterns. (The different lengths of the specific PCR products in well 5 were not considered in these calculations.)

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+++--+--+	-----	6601, 6607 = 6604, 6605 = 6604, 6607 =
		6605, 6607 = 6607, 6607
+++--+---	-----	6601, 6604 = 6604, 6604
+++--+---	+---	6601, 6606 = 6606, 6606
+++--+---	-+---	6601, 6609 = 6609, 6609
+++--+---	---+	6601, 6610 = 6610, 6610
+++--+---	----+	6601, 6608 = 6608, 6608

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## SPECIFICITY TABLE

### HLA-A\*66 SSP subtyping

Specificities and sizes of the PCR products of the 12 primer mixes used for HLA-A\*66 SSP subtyping

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-A*66 alleles	Other amplified HLA-A alleles <sup>3</sup>
<b>1<sup>5</sup></b>	175 bp	<b>800 bp</b>	*6601, 6604-6610	*9235, 250101-2505, 2507, 2508, 260101-2603, 2605-2608, 2610-2633, 2635-2638, 2640, 4301
<b>2<sup>4</sup></b>	100 bp	1070 bp	*6601, 6604, 6606-6610	*0113, 0117, 9203, 110101-1111, 1113-1116, 1120-1127, 1129-1139, 1141-1144, 2502, 2613, 2619, 2633, 340101-3406, 3408
<b>3</b>	425 bp	1070 bp	*6601, 6602, 6604, 6606-6610	*0234-023503, 025601, 025602, 0262, 0278, 9203, 2419, 2613, 2619, 340101-3408, 680101-680202, 6806-6814, 6816-6819, 6821-6830, 6832-6835, 6837-6848, 6901
<b>4</b>	175 bp	1070 bp	*6602, 6603	
<b>5<sup>4,6</sup></b>	70 bp, 100 bp	<b>800 bp</b>	*6604, 6607	*0255, 2603, 2606, 2621, 2630, 3324, 6805, 6815, 6820
<b>6<sup>4</sup></b>	80 bp	<b>800 bp</b>	*6601, 6604-6610	*0113, 0128, 110101-1111, 1113-1116, 1119-1127, 1129-1139, 1141-1144, 2419, 2444, 2603, 2606, 2621, 3103, 340101-3408
<b>7</b>	560 bp	1070 bp	*6603	*0216, 9231
<b>8<sup>4</sup></b>	95 bp	1070 bp	*6605, 6607	*01010101-0104N, 0106, 0108-0112, 0114-0116N, 0118N-0135, 0137, 0341, 1117, 1119, 1140, 2444 <sup>w</sup> , 250101, 250102, 2503-2508, 260101-2612, 2614-2618, 2620-2629, 2631, 2632, 2634-2638, 2640, 3103, 3313, 3601-3604, 4301, 7410, 8001
<b>9</b>	155 bp	1070 bp	*6606	*3103, 3104, 3402-3404, 3406-3408
<b>10</b>	205 bp	1070 bp	*6609	*020301, 020302, 0225, 0238, 9217, 9248, 9271, 2622

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<b>11</b>	190 bp	1070 bp	*6610	*2629
<b>12</b>	220 bp	1070 bp	*6608	*340101 <sup>?</sup> , 340102 <sup>?</sup> , 3405 <sup>?</sup>

<sup>1</sup>Alleles 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 HLA-A\*66 SSP typings.

When the primers in a primer mix can give rise to specific PCR products of more than one length this is indicated if the size difference is 20 base pairs or more. Size differences shorter than 20 base pairs are not given. For high resolution SSP kits the respective lengths of the specific PCR product(s) of the alleles amplified by these primer mixes are given.

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.

<sup>2</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-A\*66 subtyping.

In addition, wells number 5 and 6 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

In the presence of a specific amplification the intensity of the control band often decreases.

<sup>3</sup>Due to the sharing of sequence motifs between HLA-A alleles non-HLA-A\*66 alleles will be amplified by primer mixes 1 to 3 and 5 to 12.

<sup>4</sup>Specific PCR fragments shorter than 125 base pairs have a lower intensity than longer PCR bands.

<sup>5</sup>Primer mix 1 may give rise to a PCR fragment approx. 500 bp in size. This band should be disregarded in the interpretation of HLA-A\*66 subtypings.

<sup>6</sup>Primer mix 5: Specific PCR fragment of 70 bp in the A\*0255, 2603, 2606, 2621, 2630, 3324, 6805, 6815 and 6820 alleles. Specific PCR fragment of 100 bp in the A\*6604 and A\*6607 alleles. 'w', might be weakly amplified.

'?', nucleotide sequence of the primer matching sequence is not known.

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INTERPRETATION TABLE								
HLA-A*66 SSP subtyping								
Amplification patterns of the HLA-A*6601 to 6610 alleles								
	Well <sup>4</sup>							
	1	2	3	4	5	6	7	8
Length of spec.	175	100	425	175	70	80	560	95
PCR product(s)					100			
Length of int.	800	1070	1070	1070	800	800	1070	1070
pos. control <sup>1</sup>								
5'-primer(s) <sup>2</sup>	418	282	28	423	261	302	282	282
	5' -AgA 3'5' -CAg 3'5' -TCg 3'5' -gCT 3'5'				-AAC 3'5' -ggA 3'5' -CAC 3'5'		-CAC 3'	
	423				517			517
	5' -gCT 3'				5' -AgA 3'		5' -AgA 3'	
3'-primer(s) <sup>3</sup>	559	341	282	559	292	341	559	341
	5' -CCg 3'5' -CgT 3'5' -gAC 3'5' -CTC 3'5'				-gTg 3'5' -CgT 3'5' -CTC 3'5'		-CgT 3'	
	559				566			566
	5' -CCg 3'				5' -CCg 3'		5' -CCg 3'	
					583			
					5' -gTg 3'			
Well No.	1	2	3	4	5	6	7	8
HLA-A allele								
*6601	1	2	3			6		
*6602			3	4				
*6603				4			7	
*6604	1	2	3		5	6		
*6605	1					6		8
*6606	1	2	3			6		
*6607	1	2	3		5	6		8
*6608	1	2	3			6		
*6609	1	2	3			6		
*6610	1	2	3			6		
*01010101-0104N, 0106, 0108-0112, 0114-0116N, 0118N-0127N, 0129-0135, 0137, 0341, 1117, 1140, 2506, 2604, 2609, 2634, 3313, 3601-3604, 7410, 8001								8
*0113, 110101-1111, 1113-1116, 1120-1127, 1129-1139, 1141-1144		2				6		
*0117		2						
Well No.	1	2	3	4	5	6	7	8

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INTERPRETATION TABLE				
HLA-A*66 SSP subtyping				
Amplification patterns of the HLA-A*66 alleles				
Well <sup>4</sup>				
9	10	11	12	
155	205	190	220	Length of spec. PCR product(s)
1070	1070	1070	1070	Length of int. pos. control <sup>1</sup>
423	355	423	652	5'-primer(s) <sup>2</sup>
5' -gCT 3' 5' -CCg 3' 5' -gCT 3' 5' -CTg 3'				
539	517	570	829	3'-primer(s) <sup>3</sup>
5' -TCA 3' 5' -CgT 3' 5' -CCg 3' 5' -CTC 3'				
9	10	11	12	Well No.
				HLA-A allele
				*6601
				*6602
				*6603
				*6604
				*6605
9				*6606
				*6607
			12	*6608
	10			*6609
		11		*6610
				*01010101-0104N, 0106, 0108-0112, 0114-0116N, 0118N-0127N, 0129-0135, 0137, 0341, 1117, 1140, 2506, 2604, 2609, 2634, 3313, 3601-3604, 7410, 8001
				*0113, 110101-1111, 1113-1116, 1120-1127, 1129-1139, 1141-1144
				*0117
9	10	11	12	Well No.

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Length of spec.	175	100	425	175	70	80	560	95
PCR product(s)					100			
Well No.	1	2	3	4	5	6	7	8
*0128, 1119						6		8
*020301, 020302, 0225, 0238, 9217, 9248, 9271								
*0216, 9231							7	
*0234-023503, 025601, 025602, 0262, 0278, 9203, 680101-680202, 6806-6814, 6816-6819, 6821-6830, 6832-6835, 6837-6848, 6901			3					
*0255, 3324, 6805, 6815, 6820					5			
*9235	1							
*2419			3			6		
*2444						6		w
*250101, 250102, 2503-2505, 2507, 2508, 260101-2602, 2605, 260701-2608, 2610-2612, 2614-2618, 2620, 2623-2628, 2631, 2632, 2635-2638, 2640, 4301	1							8
*2502, 2633	1	2						
*2603, 2606, 2621	1				5	6		8
*2613, 2619	1	2	3					
*2622	1							8
*2629	1							8
*2630	1				5			
*3103						6		8
*3104								
*340101, 340102, 3405		2	3			6		
*3402-3404, 3406, 3408		2	3			6		
*3407			3			6		
HLA-A allele								
Well No.	1	2	3	4	5	6	7	8



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155	205	190	220	Length of spec. PCR product(s)
9	10	11	12	Well No.
				*0128, 1119
	10			*020301, 020302, 0225, 0238, 9217, 9248, 9271
				*0216, 9231
				*0234-023503, 025601, 025602, 0262, 0278, 9203, 680101-680202, 6806-6814, 6816-6819, 6821-6830, 6832- 6835, 6837-6848, 6901
				*0255, 3324, 6805, 6815, 6820
				*9235
				*2419
				*2444
				*250101, 250102, 2503-2505, 2507, 2508, 260101-2602, 2605, 260701-2608, 2610- 2612, 2614-2618, 2620, 2623- 2628, 2631, 2632, 2635-2638, 2640, 4301
				*2502, 2633
				*2603, 2606, 2621
				*2613, 2619
	10			*2622
		11		*2629
				*2630
9				*3103
9				*3104
			?	*340101, 340102, 3405
9				*3402-3404, 3406, 3408
9				*3407
				HLA-A allele
9	10	11	12	Well No.

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<sup>1</sup>The internal positive control primer pairs amplify segments of the human growth hormone gene. The two different control primer pairs give rise to either an internal positive control band of 1070 base pairs, for most wells, or a band of 800 base pairs, for some wells.

Well number 1 contains the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to help in the correct orientation of the HLA-A\*66 subtyping. .

In addition, wells number 5 and 6 contain the primer pair giving rise to the shorter, 800 bp, internal positive control band in order to allow kit identification.

<sup>2</sup>The nucleotide position, in the 1<sup>st</sup>, 2<sup>nd</sup> or 3<sup>rd</sup> exons, matching the specificity-determining 3'-end of the primer is given. Nucleotide numbering as on the [www.ebi.ac.uk/imgt/hla](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>3</sup>The nucleotide position, in the 2<sup>nd</sup> or 3<sup>rd</sup> exons, 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](http://www.ebi.ac.uk/imgt/hla) web site. The sequence of the 3 terminal nucleotides of the primer is given.

<sup>4</sup>Primer mix 5: Specific PCR fragment of 70 bp in the A\*0255, 2603, 2606, 2621, 2630, 3324, 6805, 6815 and 6820 alleles. Specific PCR fragment of 100 bp in the A\*6604 and A\*6607 alleles. 'w', might be weakly amplified.

'?', nucleotide sequence of the primer matching sequence is not known.

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CELL LINE VALIDATION SHEET																
HLA-A*66 SSP subtyping kit																
					Well											
					1	2	3	4	5	6	7	8	9	10	11	12
				Lot No.:	200964201	200853102	200853103	200964204	200853105	200853106	200853107	200853108	200964209	200964210	200964211	200964212
	IHWC cell line	A*	A*													
1	9001 SA	*2402			-	-	-	-	-	-	-	-	-	-	-	-
2	9280 LK707	*0201			-	-	-	-	-	-	-	-	-	-	-	-
3	9011 E4181324	*0101			-	-	-	-	-	-	-	+	-	-	-	-
4	9275 GU373	*3001			-	-	-	-	-	-	-	-	-	-	-	-
5	9009 KAS011	*0101			-	-	-	-	-	-	-	+	-	-	-	-
6	9353 SM	*0201	*2603		+	-	-	-	+	+	-	+	-	-	-	-
7	9020 QBL	*2601			+	-	-	-	-	-	-	+	-	-	-	-
8	9025 DEU	*3101			-	-	-	-	-	-	-	-	-	-	-	-
9	9026 YAR	*2601			+	-	-	-	-	-	-	+	-	-	-	-
10	9107 LKT3	*2402			-	-	-	-	-	-	-	-	-	-	-	-
11	9051 PITOUT	*2902			-	-	-	-	-	-	-	-	-	-	-	-
12	9052 DBB	*0201			-	-	-	-	-	-	-	-	-	-	-	-
13	9004 JESTHOM	*0201			-	-	-	-	-	-	-	-	-	-	-	-
14	9071 OLGA	*3101			-	-	-	-	-	-	-	-	-	-	-	-
15	9075 DKB	*2402			-	-	-	-	-	-	-	-	-	-	-	-
16	9037 SWEIG007	*2902			-	-	-	-	-	-	-	-	-	-	-	-
17	9282 CTM3953540	*0301	*8001		-	-	-	-	-	-	-	+	-	-	-	-
18	9257 32367	*3303	*7401		-	-	-	-	-	-	-	-	-	-	-	-
19	9038 BM16	*0201			-	-	-	-	-	-	-	-	-	-	-	-
20	9059 SLE005	*0201			-	-	-	-	-	-	-	-	-	-	-	-
21	9064 AMALA	*0217			-	-	-	-	-	-	-	-	-	-	-	-
22	9056 KOSE	*0201			-	-	-	-	-	-	-	-	-	-	-	-
23	9124 IHL	*0201	*3401		-	+	+	-	-	+	-	-	-	-	-	+
24	9035 JBUSH	*3201			-	-	-	-	-	-	-	-	-	-	-	-
25	9049 IBW9	*3301			-	-	-	-	-	-	-	-	-	-	-	-
26	9285 WT49	*0205			-	-	-	-	-	-	-	-	-	-	-	-
27	9191 CH1007	*2410	*2901		-	-	-	-	-	-	-	-	-	-	-	-
28	9320 BEL5GB	*0201	*2902		-	-	-	-	-	-	-	-	-	-	-	-
29	9050 MOU	*2902			-	-	-	-	-	-	-	-	-	-	-	-
30	9021 RSH	*3001	*6802		-	-	+	-	-	-	-	-	-	-	-	-
31	9019 DUCAF	*3002			-	-	-	-	-	-	-	-	-	-	-	-
32	9297 HAG	*0201			-	-	-	-	-	-	-	-	-	-	-	-
33	9098 MT14B	*3101			-	-	-	-	-	-	-	-	-	-	-	-
34	9104 DHIF	*3101			-	-	-	-	-	-	-	-	-	-	-	-
35	9302 SSTO	*3201			-	-	-	-	-	-	-	-	-	-	-	-
36	9024 KT17	*0206	*1101		-	+	-	-	-	+	-	-	-	-	-	-
37	9065 HHKB	*0301			-	-	-	-	-	-	-	-	-	-	-	-
38	9099 LZL	*0217			-	-	-	-	-	-	-	-	-	-	-	-
39	9315 CML	*0101	*0301		-	-	-	-	-	-	-	+	-	-	-	-
40	9134 WHONP199	*0207	*3001		-	-	-	-	-	-	-	-	-	-	-	-
41	9055 H0301	*0301			-	-	-	-	-	-	-	-	-	-	-	-
42	9066 TAB089	*0207			-	-	-	-	-	-	-	-	-	-	-	-
43	9076 T7526	*0207			-	-	-	-	-	-	-	-	-	-	-	-
44	9057 TEM	*6601			+	+	+	-	-	+	-	-	-	-	-	-
45	9239 SHJO	*2301	*2402		-	-	-	-	-	-	-	-	-	-	-	-
46	9013 SCHU	*0301			-	-	-	-	-	-	-	-	-	-	-	-
47	9045 TUBO	*0216	*0301		-	-	-	-	-	-	-	+	-	-	-	-
48	9303 TER-ND	*0201	*1101		-	+	-	-	-	+	-	-	-	-	-	-

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## CERTIFICATE OF ANALYSIS

### **Olerup SSP<sup>®</sup> HLA-A\*66 SSP**

**Product number:** 101.427-06u – without *Taq* polymerase  
**Lot number:** 34G  
**Expiry date:** 2011-October-01  
**Number of tests:** 6  
**Number of wells per test:** 12

#### **Well specifications:**

Well No.	Production No.	Well No.	Production No.
1	2009-642-01	9	2009-642-09
2	2008-531-02	10	2009-642-10
3	2008-531-03	11	2009-642-11
4	2009-642-04	12	2009-642-12
5	2008-531-05		
6	2008-531-06		
7	2008-531-07		
8	2008-531-08		

The specificity of each primer solution of the kit has been tested against 48 well characterized IHWC cell line DNAs.

No DNAs carrying the alleles to be amplified by primer solution 11 and 12 were available. The specificities of the primers in primer solution 11 were tested by separately adding one additional 5'-primer, respectively one additional 3'-primer. In primer solution 12 it was only possible to test the 5'-primer, the 3'-primer was not possible to test. The specificities of additional primers in primer solution 5 and 8 were tested by separately adding one additional 5'-primer, and/or one additional 3'-primer. One of the 3'-primers in primer solutions 5 and 8 was not possible to test.

**Results:** No false positive or false negative amplifications were obtained.

**Date of approval:** 2009-October-23

**Approved by:**

**Quality Control, Supervisor**

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## Declaration of Conformity

**Product name:** *Olerup* SSP® HLA-A\*66  
**Product number:** 101.427-06u  
**Lot number:** 34G

**Intended use:** HLA-A\*66 high resolution histocompatibility testing

**Manufacturer:** *Olerup* SSP AB  
Hasselstigen 1  
SE-133 33 Saltsjöbaden, Sweden  
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We, *Olerup* SSP AB, hereby declare that this product, to which this Declaration of Conformity relates is in conformity with the following Standard(s) and other normative document(s) ISO 9001:2008 and ISO 13485:2003, following the provisions of the 98/79/EC Directive on *in vitro* diagnostic medical devices, Annex II List B, conformity assessed using Annex IV, as transposed into the national laws of the Member States of the European Union.

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Saltsjöbaden, Sweden  
2009-October-23

Olle Olerup  
Managing Director

Lot No.: **34G**

Lot-specific information

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