

# Olerup SSP®

## Olerup SSP® HLA-B\*13

**Prod. No:** 101.515-12/12u  
**Lot No:** 97F  
**Expiry Date:** 2011-July-01

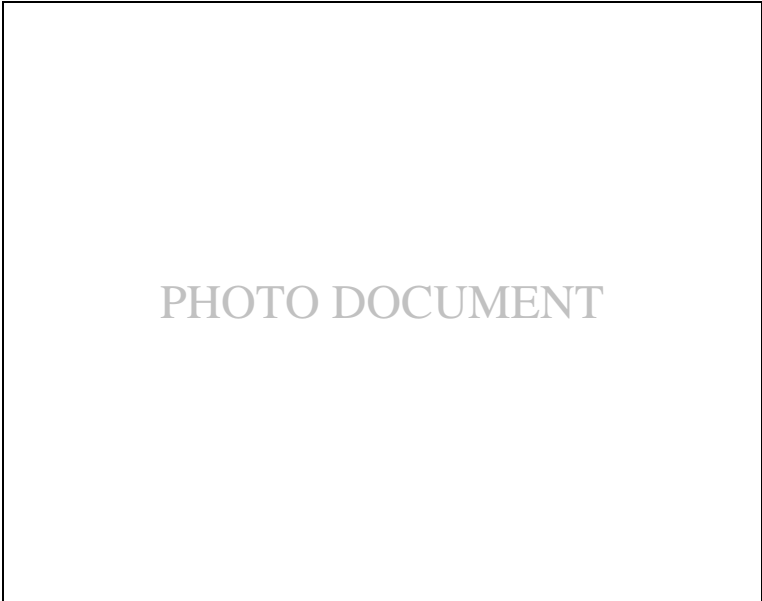
**Name:** \_\_\_\_\_ **Sample ID:** \_\_\_\_\_

**DNA Extract Date:** \_\_\_\_\_ **Conc.(ng/ul):** \_\_\_\_\_

**Test Date:** \_\_\_\_\_ **Review Date:** \_\_\_\_\_

**Tested By:** \_\_\_\_\_ **Reviewed By:** \_\_\_\_\_

**Interpretation:** \_\_\_\_\_



### Specificities and sizes of the PCR products of the 16 primer mixes used for HLA-B\*13 SSP typing.

Primer Mix	Size of spec. PCR product <sup>1</sup>	Size of control band <sup>2</sup>	Amplified HLA-B*13 alleles	Other amplified HLA Class I alleles <sup>3</sup>
1	140 bp	800 bp	1301-1304, 1306-1308Q, 1310-1324	4460
2 <sup>4,5</sup>	95 bp	800 bp	1301-130205, 1307N-1311, 1313-1321, 1323	4091
3	245 bp	1070 bp	1301, 1307N, 1313, 1317, 1320-1324	0712, 0714, 071801, 071802, 2719, 2730, 3515, 3533, 3588, 4004, 4030, 4034, 4059, 4064, 4068, 4416, 4421, 446401, 446402, 4817, 5713, <b>Cw*0334</b>
4	245 bp	1070 bp	130201- 130205, 1308Q, 1309, 1314- 1316, 1318, 1319	2714, 40060101-400602, 4044, 4053, 4070, 4075, 4083, 4086, 4093, 4095, 4096, 5110, 5116, 5131, 5134, 5208, 5509, 5522, 5524, 7301
5 <sup>4,8</sup>	125 bp, 150 bp, 180 bp	800 bp	1303, 1316, 1320, 1322, 1324	1542, 1573, 1586, 3560, 3917, 4071, 4410, 4415, 4418, 4501, 4504-4508, 4611, 4618, 490101-4903, 5001, 5002, 5004, 5115, 5162, 5401-5403, 5405N, 5407, 5408N, 5410, 5412, 5413, 5417, 550101-5503, 5505, 5507, 5509-5512, 5515, 5516, 5518, 5519, 5521, 5522, 5524-5526, 5529-5531, 5533, 5534, 5601, 5602, 5604, 5607, 5608, 5610, 5613, 5614, 5616, 5617, 5619N, 5620, 5623, 5624, 5901, 5904, 8201, 8202
6 <sup>4</sup>	105 bp	1070 bp	1304	1504, 1516, 1567, 1595, 9537, 9555, 3537, 390601, 390602, 3933, 3934, 4086, 4502, 4509, 4904, 4905, 511301, 511302, 5137, 5163, 5214, 5414, 5513, 5523, 5532, 5622, 5903, 7301
7 <sup>6,7</sup>	190 bp	1070 bp	1306	0734, 080101-0806, 0808N-0810, 0812, 0813, 0815-0820, 0822-0824, 0826, 0827, 0829-0836, 0838, 1542, 1583, 1586, 1826, 3560, 3587, 3712, 380101, 380201-3807, 3809-3819, 39010101-39010102L, 390103-390602, 3908-3914, 3916, 3918-3920, 3922-3924, 3926-3928, 3930, 3931, 3934-3937, 3939, 3941, 3942, 3944-3946, 3948, 4039, 4051, 4101-410302, 4105-4108, 4201, 4202, 4204-4208, 4210, 4618, 5121, 5136, 5401, 5402, 5404, 5405N, 5407, 5408N, 5410-5413, 5417, 550101-5505, 5507, 5510-5513, 5515-5521, 5525, 5526, 5528- 5534, 5610, 5612, 5623, 5820, 5901-5903, 670101-6702
8 <sup>6,7,9</sup>	185 bp, 225	1070 bp	1307N, 1318	1504, 9537, 3933, 5105, 5129, 5154, 5161, 5410, 5509, 5521

	bp			
<b>9</b>	230 bp	1070 bp	1308Q	
<b>10<sup>4,10</sup></b>	105 bp , 210 bp	<b>800 bp</b>	1309, 1321	070501-0706, 0732, 0734, 0740, 0753, 0769, 080102, 0806, 0820, 1553, 9550, 2707, 2708, 2711, 2712, 2718, 2720, 2724, 2726, 2727, 2733, 2740, 2742-2744, 350902, 3518, 3531, 3563, 3584, 3588, 3590, 400101-4016, 4018-4040, 4042-4096, 4101-4104, 4106-4108, 4409, 4431, 4446, 4501-4509, 4610, 4702, 480101, 480102, 480301-4807, 4809-4811, 4813, 4815, 4816, 4818, 4819, 5001, 5002, 5004, 510109, 5110, 512402, 5411, 5504, 5508, 7301, 8101-8104N
<b>11</b>	280 bp	<b>800 bp</b>	1304, 1310	0709, 0711, 0717, 0828, 0835, 0837, 15010101-1508, 151101-1516, 1518-1521, 1523-1529, 1531-1536, 1538-1540, 1543, 1544, 1546, 1547, 1549-1557, 1560-1562, 1564-1572, 1574-1576, 1578-1582, 1584, 1585, 1587-1589, 1591-1598, 9501-9529, 9531, 9532, 9534-9536, 9538-9549N, 9551-9560, 180101-1815, 1817N-1825, 1827-1833, 2741, 350101-350107, 3505, 3507-350803, 3510, 3511, 351401-3517, 3519-3521, 3523-3530, 3532, 3535, 3537, 3540N-3543, 3545-3554, 3557, 3558, 3561-3564, 3566-3569, 3571, 3572, 3576-3580, 3582, 3586, 3589-3594, 3597, 3599, 3907, 3943, 4003, 4020, 4038, 4052, 4059, 4060, 4209, 4417, 4443, 4509, 460101-4610, 4612-4617, 4802, 4814, 4904, 4905, 5137, 5145, 5163, 530101-5303, 5305, 5306, 5308-5318, 5406, 5409, 5414, 5514, 5523, 5532, 5603, 5609, 5618, 570101-570103, 5706, 5708, 5710, 5711, 5713-5716, 5718, 5719, 580101-5802, 5804-5807, 5809-5819, 5821
<b>12<sup>4</sup></b>	90 bp	1070 bp	1311	<b>Cw*0206</b>
<b>13<sup>4,11</sup></b>	90 bp, 180 bp	<b>800 bp</b>	1312, 1319	
<b>14<sup>4</sup></b>	130 bp	1070 bp	1313, 1321	4091
<b>15<sup>4,12</sup></b>	105 bp, 245 bp	<b>800 bp</b>	1314, 1322, 1323	1573, 1809, 2701, 3710, 380201-3804, 3808, 3815, 3818, 4047, 4048, 4096, 4415, 4455, 4902
<b>16<sup>4,13</sup></b>	90 bp, 205 bp	1070 bp	1315, 1317	3546, 5162

<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-B\*13 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 length 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-B\*13 subtyping.

In addition, wells number 2, 5, 10, 11, 13 and 15 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-B alleles some non-HLA-B\*13 alleles will be amplified by primer mixes 1, 3 to 8 and 10 to 12, 15 and 16. In addition, the Cw\*0206 allele will be amplified by primer mix 12 and the Cw\*0334 allele will be amplified by primer mix 3.

<sup>4</sup>Short specific PCR fragments are less intense and not as sharp as longer specific bands.

<sup>5</sup>Primer mix 2 may give rise to a weak band of about 400 bp. This band should be disregarded when interpreting HLA-B\*13 SSP subtypings.

<sup>6</sup>Primer mixes 5 may give a lower yield of specific PCR product than the other HLA-B\*13 primer mixes.

<sup>7</sup>Primer mixes 7 and 8 have a weak tendency of nonspecific amplifications.

<sup>8</sup>Primer mix 5: Specific PCR fragment of 125 bp in the B\*1322, 1324 and the B\*1586, 3560, 4508, 4618, 490102, 5401, 5402, 5405N, 5407, 5408N, 5410, 5412, 5413, 5417, 5502, 5507, 5510, 5512, 5516, 5518, 5519, 5522, 5526, 5530, 5534, 5610, 5619N and 5623 alleles. Specific PCR fragment of 150 bp in the B\*1316 and 1320 alleles. Specific PCR fragment of 125 and 150 bp in the B\*1542, 550101-550104, 5503, 5505, 5509, 5511, 5515, 5521, 5524, 5525, 5529, 5531 and 5533 alleles. Specific PCR fragments of 125 and 180 bp in the B\*1303, 4071, 4410, 4415, 4418, 4501, 4504-4507, 4611, 490102, 5115, 5403, 5601, 5602, 5604, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 5624, 5904,

8201 and 8202 alleles. Specific PCR fragments of 125, 150 and 180 bp in the B\* 1573, 490101, 4902, 4903, 5001, 5002, 5004 and 5162 alleles. All bands may not always be visible.

<sup>9</sup>Primer mix 8: Specific PCR fragment of 185 bp in the B\*1307N allele. Specific PCR fragment of 225 bp in the B\*1318 and the B\*1504, 9537, 3933, 5105, 5129, 5154, 5161, 5410, 5509 and 5521 alleles.

<sup>10</sup>Primer mix 10: Specific PCR fragment of 105 bp in the B\*1321 and the B\*070501-0706, 0732, 0734, 0740, 0753, 0769, 080102, 0806, 0820, 9550, 2707, 2711, 2720, 2724, 2727, 2743, 350902, 3518, 3531, 3588, 4010, 4012, 4013, 401403, 4019, 4021, 4047, 4058, 4076, 4088, 4096, 4431, 480101, 480102, 480301-4807, 4809-4811, 4813, 4815, 4816, 4818, 4819, 510109, 5110, 512402, 5411, 5504, 5508 and 8101-8104N alleles. Specific PCR fragment of 210 bp in the B\*1309 and the B\*1553, 2708, 2712, 2718, 2726, 2740, 2742, 2744, 3563, 3584, 3590, 4003, 4009, 4018, 4020, 4024, 4031-4033, 4042, 4044, 4052, 4059, 4060, 4071, 407201, 4077, 4087, 4089, 4095, 4101-4104, 4106, 4107, 4409, 4446, 4501-4509, 4610, 4702, 5001, 5002, 5004 and 7301 alleles. Specific PCR fragments of 105 and 210 bp in the B\*2733, 400101-400204, 4004-4008, 4011, 401401, 401402, 4015, 4016, 4022, 4023, 4025, 4027, 4029, 4030, 4034-4040, 4043, 4045, 4046, 4048, 4049-4051, 4053-4057, 4061-4070, 407202-4075, 4078-4086, 4088, 4090-4094 and 4108 alleles.

<sup>11</sup>Primer mix 13: Specific PCR fragment of 90 bp in the B\*1319 allele. Specific PCR fragment of 180 bp in the B\*1312 allele.

<sup>12</sup>Primer mix 15: Specific PCR fragment of 105 bp in the B\*1322 and the B\*1573 and 4048 alleles. Specific PCR fragment of 245 bp in the B\*1314 and 1323 and B\*1809, 2701, 3710, 380201-3804, 3808, 3815, 3818, 4047, 4096, 4415, 4455 and 4902 alleles.

<sup>13</sup>Primer mix 16: Specific PCR fragment of 90 bp in the B\*1317 and B\*3546 alleles. Specific PCR fragment of 205 bp in the B\*1315 and B\*5162 alleles.

'w', may be weakly amplified.

Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<b>HLA-B allele<sup>1</sup></b>																
<b>*1301</b>	1	2	3													
<b>*130201-130205</b>	1	2		4												
<b>*1303</b>	1				5											
<b>*1304</b>	1					6					11					
<b>*1306</b>	1						7									
<b>*1307N</b>	1	2	3					8								
<b>*1308Q</b>	1	2		4					9							
<b>*1309</b>		2		4						10						
<b>*1310</b>	1	2									11					
<b>*1311</b>	1	2										12				
<b>*1312</b>	1												13			
<b>*1313</b>	1	2	3											14		
<b>*1314</b>	1	2		4												15
<b>*1315</b>	1	2		4												16
<b>*1316</b>	1	2		4	5											
<b>*1317</b>	1	2	3													16
<b>*1318</b>	1	2		4				8								
<b>*1319</b>	1	2		4									13			
<b>*1320</b>	1	2	3		5											
<b>*1321</b>	1	2	3							10				14		
<b>*1322</b>	1		3		5											15
<b>*1323</b>	1	2	3													15
<b>*1324</b>	1		3		5											
<b>*070501-0706, 0732, 0740, 0753, 0769, 9550, 2707, 2708, 2711, 2712, 2718, 2720, 2724, 2726, 2727, 2733, 2740, 2742-2744, 350902, 3518, 3531, 3584, 400101-400204, 4005, 4007-4016, 4018, 4019, 4021-4029, 4031-4033, 4035-4037, 4040, 4042, 4043, 4045, 4046, 4049, 4050, 4054-4058, 4061-4063, 4065-4067, 4069, 407201-4074, 4076-4082, 4084, 4085, 4087-4090, 4092, 4094, 4104, 4409, 4431, 4446, 4503, 4702, 480101, 480102, 480301-4807, 4809-4811, 4813, 4815, 4816, 4818, 4819, 510109, 512402, 5508, 8101-8104N</b>										10						



*4071					5				10	12						
*4086, 7301				4		6			10							
*4091	2								10			14				
*4096				4					10				15			
*4410, 4418, 4611, 490101, 490102, 4903, 5115, 5403, 5601, 5607, 5608, 5613, 5614, 5616, 5617, 5619N, 5620, 5624, 5904, 8201, 8202					5											
*4415, 4902					5								15			
*4460	1															
*4501, 4504-4508, 5001, 5002, 5004					5				10							
*4502						6			10							
*4509						6			10	11						
*5105, 5129, 5154, 5161								8								
*511301, 511302, 5214, 5622						6										
*5162					5						12			16		
*5410, 5521					5	7	8									
*5509				4	5			8								
*5522, 5524				4	5											
*5532						6	7			11						
*5610					5	7					12					
Cw*0206											12					
Length of spec. PCR product(s) <sup>2</sup>	140	95	245	245	125	105	190	185	230	105	280	90	90	130	105	90
					150			225		210			180		245	205
					180											
Well No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Specific Reaction																
1st match: B*																
2nd match: B*																
Control Reaction																
Failed Controls																
False Positive																
False Negative																

<sup>1</sup>The sequence of the B\*1305 allele has been shown to be identical to B\*1304.

<sup>2</sup>Primer mix 5: Specific PCR fragment of 125 bp in the B\*1322, 1324 and the B\*1586, 3560, 4508, 4618, 490102, 5401, 5402, 5405N, 5407, 5408N, 5410, 5412, 5413, 5417, 5502, 5507, 5510, 5512, 5516, 5518, 5519, 5522, 5526, 5530, 5534, 5610, 5619N and 5623 alleles. Specific PCR fragment of 150 bp in the B\*1316 and 1320 alleles. Specific PCR fragment of 125 and 150 bp in the B\*1542, 550101-550104, 5503, 5505, 5509, 5511, 5515, 5521, 5524, 5525, 5529, 5531 and 5533 alleles. Specific PCR fragments of 125 and 180 bp in the B\*1303, 4071, 4410, 4415, 4418, 4501, 4504-4507, 4611, 490102, 5115, 5403, 5601, 5602, 5604, 5607, 5608, 5613, 5614, 5616, 5617, 5620, 5624, 5904, 8201 and 8202 alleles. Specific PCR fragments of 125, 150 and 180 bp in the B\* 1573, 490101, 4902, 4903, 5001, 5002, 5004 and 5162 alleles. All bands may not always be visible.

Primer mix 8: Specific PCR fragment of 185 bp in the B\*1307N allele. Specific PCR fragment of 225 bp in the B\*1318 and the B\*1504, 9537, 3933, 5105, 5129, 5154, 5161, 5410, 5509 and 5521 alleles.

Primer mix 10: Specific PCR fragment of 105 bp in the B\*1321 and the B\*070501-0706, 0732, 0734, 0740, 0753, 0769, 080102, 0806, 0820, 9550, 2707, 2711, 2720, 2724, 2727, 2743, 350902, 3518, 3531, 3588, 4010, 4012, 4013, 401403, 4019, 4021, 4047, 4058, 4076, 4088, 4096, 4431, 480101, 480102, 480301-4807, 4809-4811, 4813, 4815, 4816, 4818, 4819, 510109, 5110, 512402, 5411, 5504, 5508 and 8101-8104N alleles. Specific PCR fragment of 210 bp in the B\*1309 and the B\*1553, 2708, 2712, 2718, 2726, 2740, 2742, 2744, 3563, 3584, 3590, 4003, 4009, 4018, 4020, 4024, 4031-4033, 4042, 4044, 4052, 4059, 4060, 4071, 407201, 4077, 4087, 4089, 4095, 4101-4104, 4106, 4107, 4409, 4446, 4501-4509, 4610, 4702, 5001, 5002, 5004 and 7301 alleles. Specific PCR fragments of 105 and 210 bp in the B\*2733, 400101-400204, 4004-4008, 4011, 401401, 401402, 4015, 4016, 4022, 4023, 4025, 4027, 4029, 4030, 4034-4040, 4043, 4045, 4046, 4048, 4049-4051, 4053-4057, 4061-4070, 407202-4075, 4078-4086, 4088, 4090-4094 and 4108 alleles.

Primer mix 13: Specific PCR fragment of 90 bp in the B\*1319 allele. Specific PCR fragment of 180 bp in the B\*1312 allele.

Primer mix 15: Specific PCR fragment of 105 bp in the B\*1322 and the B\*1573 and 4048 alleles. Specific PCR fragment of 245 bp in the B\*1314 and 1323 and B\*1809, 2701, 3710, 380201-3804, 3808, 3815, 3818, 4047, 4096, 4415, 4455 and 4902 alleles.

Primer mix 16: Specific PCR fragment of 90 bp in the B\*1317 and B\*3546 alleles. Specific PCR fragment of 205 bp in the B\*1315 and B\*5162 alleles.

<sup>3</sup>w, may be weakly amplified.