



**PCR Protocol**

HLA-Locus: \_\_\_\_\_

Date: \_\_\_\_\_

**Samples and negative control (HPLC-water):**

	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						

	7	8	9	10	11	12
A						
B						
C						
D						
E						
F						
G						
H						

PCR	1x [µl]	96x [µl]	Calculation n number of samples	____x
Ready to Use PCR Mix	17.85	1885	$n \cdot 17.85 + (0.1 \cdot (n \cdot 17.85))$	
Taq DNA Polymerase (5U/µl)	0.15	16	$n \cdot 0.15 + (0.1 \cdot (n \cdot 0.15))$	
total	18	1901		
		18µl/well		18µl/well
DNA sample / well	2	2µl/well		2 µl/well

Thermocycler program for initial PCR:

1 Cycle: 96°C: 120 sec	5 Cycles: 96°C: 30 sec. 65°C: 30 sec. 72°C: 120 sec.	35 Cycles: 96°C: 30 sec. 62°C: 30 sec. 72°C: 120 sec.	10°C ∞
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Thermocycler used:

**Performed by**

\_\_\_\_\_ Date and signature:

**Positive results according to the gel picture:**

**(Mark positive results [√])**

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												



Gel picture:

Evaluated by

\_\_\_\_\_

Date and signature:

Purification of initial PCR:

Purification	1x [ $\mu$ l]	96x [ $\mu$ l]	Calculation n number of samples	____x
ExoSAP-IT <sup>®</sup>	2	192		
		2 $\mu$ l / well		2 $\mu$ l / well

Thermocycler incubation program for ExoSAP-IT<sup>®</sup>:

Incubation:	Enzyme inactivation	
1 Cycle: 37°C: 15 min	1 Cycle: 85°C: 15 min.	10°C $\infty$

Performed by

\_\_\_\_\_

Date and signature:

Dilution of the Samples:

Dilution:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Performed by

\_\_\_\_\_

Date and signature:



Appendix 8.1.2: Cycle Sequencing Protocol

**Cycle Sequencing Protocol**

**HLA- Locus:** \_\_\_\_\_

**Prepare separately for each Ready to Use Sequencing Mix:** \_\_\_\_\_  
 Name of Ready to Use Sequencing Mix

Cycle sequencing	1x [ $\mu$ l]	96x [ $\mu$ l]	Calculation n number of samples	____x
Ready to use Sequencing Mix	7.75	815	$n*7.75\mu\text{l} + (0.1*n*7.75\mu\text{l})$	
BigDye™ Terminator Mix Version 3.1	0.25	26.4	$n*0.25\mu\text{l} + (0.1*n*0.25\mu\text{l})$	
total	8	841.6		
		8 $\mu$ l/well		8 $\mu$ l/well
DNA sample / well	2	2 $\mu$ l/well		2 $\mu$ l/well

Thermocycler Cycle Sequencing Program:

1 Cycle: 96°C: 1 min.	40 Cycles: 96°C: 10 Sec. 60°C: 2 min.	10°C: $\infty$
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**Thermocycler:**

**Samples:**

Samples are prepared according to the standard protocol:  
 Ready to Use Sequencing Mix 7.75 $\mu$ l + 0.25 $\mu$ l BigDye Terminator V. 3.1 + 2 $\mu$ l purified sample

No.	Serial Number:	Internal Number:	Sequencing Primer	Sample (2 $\mu$ l)	BigDye (0.25 $\mu$ l)	Sequencing Mix (7.75 $\mu$ l)	Exon
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
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25							
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27							
28							
29							
30							
31							
32							
33							
34							
35							



Appendix 8.1.2: Cycle Sequencing Protocol

No.	Serial Number:	Internal Number:	Sequencing Primer	Sample (2µl)	BigDye (0.25µl)	Sequencing Mix (7.75µl)	Exon
36							
37							
38							
39							
40							
41							
42							
43							
44							
45							
46							
47							
48							
49							
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86							
87							
88							
89							
90							
91							
92							
93							
94							
95							
96							

Performed by \_\_\_\_\_

\_\_\_\_\_ Date and signature



**Purification of samples:**

**Method:**

\_\_\_\_\_ Name of the method

**Final volume / sequencing reaction:**

\_\_\_\_\_

**Performed by**

\_\_\_\_\_ Date and signature

**Sequencing Automat:**

**No:** \_\_\_\_\_

**Loaded by**

\_\_\_\_\_ Date and signature

**Remarks:**