PROTRANS Domino System
HLA – disease associations
Celiac Disease

REF 201 093

Instruction

Protrans SSP Cycler Program			
Initial denaturation	94℃	2 min	Hold
Denaturation	94℃	15 sec	10 cycles
Annealing and Extension	65℃	60 sec	
Denaturation	94℃	15 sec	20 cycles
Annealing	61℃	50 sec	
Extension	72℃	30 sec	
Hold	4℃	15 min	Hold
Ramp rate 1℃/sec			

1. Take the Protrans PCR workstation out of the freezer (-20℃).
   Take the Protrans **Buffer R (yellow cap)** and **Protrans Buffer Y** out of the freezer (-20℃).
   Place the **Protrans Buffer R** in the PCR workstation.
   Thaw Protrans **Buffer Y** and place it in the PCR workstation.
2. Take the Protrans **Celiac Disease Domino-Strip** out of the refrigerator (2-8℃) and place it in the PCR works tation.
3. Preparation of the **Pre Master Mix**.
   For each DNA sample pipette in a 1.5ml reaction tube:

Protrans Domino-Strip Celiac Disease	Buffer R	Buffer Y	Taq Polymerase 5U/µl
	70 µl	**140 µl**	**1.6 µl**

4. Vortex **Pre Master Mix** very thoroughly and spin the tube shortly.
5. Mark the Protrans Celiac Disease Domino-Strip.
6. **Negative control**
   Dispense **10µl** of the **Pre Master Mix** without the DNA in **position 24**.

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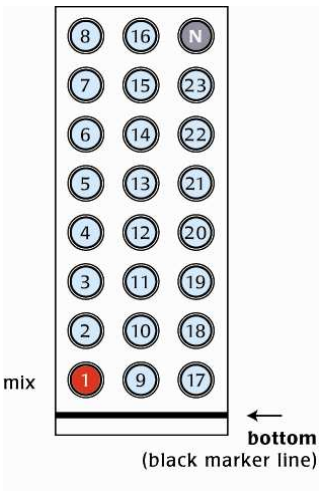
7. Add **50 µl of the DNA sample (50-100ng/µl)** to the **Pre Master Mix**.
8. Vortex **Master Mix** including the individual DNA very thoroughly and spin the tube shortly.
9. **Dispense of each Master Mix** with a stepper:
   10µl in **all 23 positions** of the Protrans **Celiac Disease Domino-Strip**.
10. Shake down the red Master Mix drops in the Protrans Celiac Disease Domino-Strip.
11. Close the Protrans Celiac Disease Domino-Strip with the cap strips and seal it very carefully by pressing the caps into the wells.
12. Place Protrans Celiac Disease Domino-Strip directly in the thermocycler, final volume 10µl and start the amplification immediately or store the covered Domino-Strip at 2-8℃ and start the amplification within 2 hours.



For the complete USER MANUAL PROTRANS SSP please refer to www.protrans.info

Domino-Strips

All primermixes are prepipetted in the right order on 24-well Domino-Strips.
PROTRANS Buffer R and Buffer Y are required for the preparation of the mastermix.
All primermixes have been named numerically.

Primermix 1 is located at the buttom left corner of the Protrans 24-well Domino-Strip.
The buttom is indicated by a **black marker line**.

Resolution

With the Protrans Domino System Celiac Disease the relevant alleles occurring in Celiac Disease can be assigned.

Working procedure

The amplification reaction can be started just after adding the Mastermix to the wells of the Domino-Strip (see Instruction).
The typing result can be obtained after electrophoresis of the amplification products (PROTRANS Electrophoresis Equipment).

REF	Article	Position of the Primermixes	Wells per typing	Resolution of relevant alleles
201 093	Protrans HLA Disease association Celiac Disease	See instruction	23	DQB1*02:01, *02:02, *03:01, *03:02 DQA1*02:01, *03:01,*05:01, *05:05 DRB1*03:01, *04, *07, *11, *12

Interpretation

The PROTRANS Reaction Protocol and the PROTRANS Amplification-Table gives the exact position, designation, allele specificity and fragment size of each Primermix.
The internal control primer pairs generate a 440 bp amplification fragment.
The allele- or group specific amplification products are < 240 bp.

Storage / Durability

Upon receipt, keep the Domino-Strips cool at 4-8℃.
Keep the Buffer R, Y and the Taq Polymerase frozen at -20℃ until use.
For the stability of the reagents see expiration date.