Process

Preparation of the PCR for HLA B-5 and B-51

1. Add 6 µl B-5, B-51 Master-Mix to the tube.
2. Add 2.7 µl of B-5 Primer Mix or 2.7 µl of B-51 Primer Mix to tube.
3. Add 100-500 ng of your sample to each tube. Then add nuclease free water to 12 µl, then pipette up and down (Table 1 and 2).
   Note: Do not use lower DNA concentrations.
4. Spin down the solution and close the tube lids or seal the plate properly with the PCR adhesive foil.
5. Put the Tubes/plate into the thermal cycler and start the program (see Table 3).

Table 1. Prepare PCR Reactions for HLA B-5

Component	Volume (1 Reaction)
B-5 Master Mix, 2X	6 µl
B-5 Primer Mix	2.7 µl
Sample	100-500 ng
ddH2O	To 12 µl
Final Volume	12 µl

Table 2. Prepare PCR Reactions for HLA B-51

Component	Volume (1 Reaction)
B51 Master-Mix	6 µl
B-51 Primer Mix	2.7 µl
Sample	100-500 ng
ddH2O	To 12 µl
Final Volume	12 µl

Table 3. PCR Program for HLA B-5 and HLA B-51

Cycles	Step Name	Temperature	Duration
--	**Initial Incubation**	25°C	3 min
--	**Initial Denaturation**	96°C	2 min
10 Cycles	**Denaturation**	94°C	10 sec
	Annealing	65°C	1 min
20 Cycles	**Denaturation**	94°C	10 sec
	Annealing	61°C	50 sec
	Extension	72°C	30 sec
--	**Final Extension**	72°C	5 min
--	**Store**	4°C	----

Agarose Gel Electrophoresis

The PCR products are identified using agarose gel electrophoresis followed by detection of the DNA bands in UV light.

Preparing 2% (W/V) Agarose Gel

- Dissolve 2.0 grams of electrophoresis grade agarose powder in 100 ml of 1X TAE buffer (TAE, 10X, Filtered, Sterile, Molecular Cat NO: BU983104).
- Melt the agarose powder completely by boiling in a microwave oven or on a heating apparatus. Stir constantly. If evaporation occurs, replenish with ddH2O.

Casting Gel

- Cool the heated agarose gel to ~60 ºC.
- Add at least 2 µl of safe stain to the heated agarose. Stir until it is thoroughly incorporated.
- On a balanced surface, set up a gel plate with combs.

- Cast a 5mm thick gel on the plate.
- Allow the gel to settle.

Gel Electrophoresis

1. Submerge the gel in 1X TAE buffer in a gel box.
2. Gently remove the tube lids without splashing the PCR products.
3. Load a 5 to 8 µl of PCR product into each well on the gel.

   Note: The PCR products of HLA B-5 and B-51 are stained with cresol red. There is no need to load additional "loading buffer" or dye.

   Optional: Reserve the first well on each row for molecular weight standards, in increments of 100bp from 50 to 1000bp.

4. Connect the electric leads and turn on the power supply (115V AC). Electrophoresis for ~ twenty (25) minutes, or until two thirds (2/3) of the lane.
5. Turn off the power supply, and remove the gel from the gel box.
6. Transfer the gel tray with the gel to a UV transilluminator.
7. Photograph the gel with or without the gel tray.
8. Mark the photograph according the rules of the laboratory.

Results

Evaluation

PCR products specific for HLA and belonging to the internal control become visible in the gel under UV light. HLA B-5 and B-51 primer mix contains control primers which amplify a 796 bp fragment of human growth hormone (HGH) (In low concentrations of sample you might observe a non-specific band in 1250 bp, this band has no effect on your diagnosis and you can simply ignore it). The concentration of these primer pairs is lower than the allele specific primer pairs and their purpose is to provide an internal control of successful PCR amplification. This amplification generally always occurs, both in presence or in absence of an allele or group-specific PCR fragment. The control band can therefore generally be seen in all PCR reactions. From time to time, the control band can appear

weak or is completely missing in the presence of an allele-specific HLA PCR product. This is not a limitation of the method, as the specific band provides a check on the success of the PCR amplification. The composition of the primers permits positive identification of the HLA characteristics. The interpretation is based on whether a specific band is present on the gel or not. The size of the amplified DNA fragments does not need to be taken into consideration when evaluating the test; nevertheless, it might be helpful for the test interpretation. For evaluation, the pattern of the specific bands is transferred to the result sheet supplied and the typing result read off with the aid of the reaction pattern.

Gel Interpretation

Reaction	Positive	Negative	No amplification	Size (bp)
Gel lane				
Control band			None	796
Specific band		None	None	451
Primer dimers				≤100

Figure 1: schematic diagram of possible results of B-51 typing at the gel analysis

Note: In low concentrations of sample, you might observe a non-specific band in 1250 bp, this band has no effect on your diagnosis and you can ignore it.

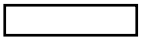
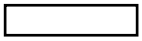
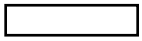
Reaction	Positive	Negative	No amplification	Size (bp)
Gel lane				
Control band			None	796
Specific band		None	None	401
Primer dimers				≤100

Figure 2: schematic diagram of possible results of B-5 typing at the gel analysis

Note: In low concentrations of sample, you might observe a non-specific band in 1250 bp, this band has no effect on your diagnosis and you can ignore it.

For the interpretation of the results, it is important if a band is present in the gel or not. The composition of those positive bands identifies the HLA characteristics. The size of the fragments (using a molecular weight standard) can help to interpret the results, but it is not essential for the evaluation of the test. This way it is ensured that the results are not based upon false positive bands caused by unspecific reactions or specific primers carried from one well to another.