Amplification test set-up

(Antibiotic resistance sequencing, GenoType® HAIN)

All molecular amplification procedures require the same constraints i.e. prevention of contamination, mainly due to the amplified DNA. The prevention principles must be rigorously implemented in order to limit contamination of the test results and therefore, make sure that those results can be safely used for sample-based diagnostic purposes.

In order to prevent amplicon-contamination,

- Using 3 separate rooms (named white (1), grey (2) and black (3)) is compulsory
- The technician must always use the 3 rooms in the following order: 1, 2 then 3, in a one-day period.
- The rooms must contain the necessary equipment.
- ***Equipment is to stay in this same dedicated room*** and shall never be transferred from room 3 to room 2, from room 2 to room 1, or from room 3 to room 1.
- The thermal cycler cannot be placed in either room 1 or 2.

1
WHITE area
DNA free

The DNA-free clean "white" room

It is used to prepare the PCR mix.

Rules

- The technician wears a disposable lab coat, nurses cap, shoe covers and gloves
- The pipettes, racks, and all equipment are new and are to stay in this room and be solely dedicated to PCR mix preparation.
- The mix is prepared and distributed in the tubes by a technician not having been in contact with amplified DNA on that same day. After s/he has finished working in the DNA-free clean "white" room, he cleans the workplace and does not come back on that day.
- Autoclave and calibrate pipettes regularly

Equipment and reagents

- Disposable lab coat, nurses cap, shoe covers and gloves
- PCR cabinet (protection of the product thanks to a vertical laminar flow) or, if not available, a clean work area dedicated to PCR mix.

- New 1000, 200 and 20 µL pipettes
- Disposable sterile pipette tips with filter
- Absorbent paper (if no PCR safety cabinet available)
- 200 µL PCR micro tubes (+ 96 wells microtiter plates or strips of 8 PCR tubes)
- Eppendorf tubes for mix preparation
- Eppendorf racks and disposable micro tube racks
- Freshly diluted 1% bleach
- 70% alcohol
- Molecular grade water (aliquoted)
- Refrigerator and -20°C freezer for PCR reagents conservation

Before using the work area

- Put on gloves and lab coat
- Decontaminate work area with freshly diluted 1% bleach (20 min), followed by 70% alcohol
- Cover the work area with clean paper towels
- Always use a new aliquot of molecular grade water

After each use

- Clean pipettes, racks, instruments (and the BSC) with freshly diluted 1% bleach (20 min), followed by 70% alcohol (and UV light)
  Note : containers containing bleach CANNOT be autoclaved!
- Use area-dedicated spray flasks or beakers (separate beakers for surface cleaning and instruments)

2
GREY area
DNA preparation

The "grey" room dedicated to non-amplified DNA handling

It is used for the introduction of DNA in the PCR mix.

Rules

- The technician wears a clean lab coat
- The pipettes, racks, and all equipment are new and are to stay in this room and be solely dedicated to non-amplified DNA handling.
- The reaction tubes prepared in the white room enter the gray room on a disposable rack that will never re-enter the white room.
- A technician not having been in contact with amplified DNA on that same day will be the one to handle the non-amplified DNA in this room.

Equipment

- Clean working surface or laminar flow safety cabinet protecting both the product and the technician
- 20 µL pipette

- Disposable sterile pipette tips with filter
- Absorbent paper (if no safety cabinet available)
- Eppendorf racks and disposable micro tube rack
- Container for infectious clinical waste

3
« black » area
Amplified DNA

The "black" room for amplified DNA handling

It is used for the detection of amplified DNA

Rules

- The technician wears black room dedicated lab coat and gloves
- Pipettes, racks and all equipment are to stay in this room and be solely dedicated to amplified DNA handling.
- After amplification, the tube enters the black room on a disposable rack that is to never re-enter the white and grey rooms.
- The technician handling amplified DNA can not return to the white and grey rooms on the same day in order to prevent contamination by introducing already amplified DNA into a new amplification process.

Equipment

- Clean working surface
- Gloves
- 1000, 200 and 20 µL pipettes
- Disposable sterile pipette tips with filter
- Absorbent paper
- Micro tubes racks
- Plastic tweezers to handle the test strips
- Container for infectious clinical waste
- Refrigerator for GenoType kits and amplified DNA conservation
- Twincubator, electrophoresis material

Optimal PCR laboratory layout

WHITE ROOM

GREY ROOM BLACK ROOM