PCR SET-UP ROOM – LAB 3
PROCEDURES

Contamination can occur from several sources: *i*) previous amplification and purification of plasmid clones; *ii*) repeated isolation of genomic nucleic acids; *iii*) previously amplified molecules ("amplicons"). It is the latter that is the primary source of PCR contamination - aerosoled PCR products from the post-PCR phase (after you have amplified the DNA in the PCR machine). This is especially a problem when you are working on repeated analysis of selected templates, different individuals of the same species or closely related species, or degraded DNA (template from scat, museum specimens, bone, etc). Often contamination of these sources cannot be observed and can call results into question.

Research based on PCR can be divided into the following activities: DNA extraction/purification, PCR reaction assembly, PCR execution, and post-PCR analysis. These activities can be collected into two major categories, pre-PCR activities (sample preparation and PCR preparation) and post-PCR activities (PCR execution and analysis). The crucial parts of contamination control include space and time separation of pre- and post-PCR activities, use of physical aids, ultraviolet light, aliquoted PCR reagents, dedicated supplies, and numerous positive and negative (blank) controls. To help prevent contamination, we have set up a pre-PCR room and further segregated this room by activities. Benches and particular areas have been designated for specific procedures and other activities should not take place at these locations.

--Bench 1 (behind the door) - DNA extraction from bone
--Bench 2 – DNA extraction from other types of museum specimens (feathers, toepads, baleen, museum skins)
--Bench 3 – DNA extraction from scat
--Bench 4 & 5 (separated in space from other extraction benches) – DNA extraction from modern tissues

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--Bench 6 & 7 – PCR template addition (genomic DNA, bone extract, whatever your extracted DNA is)
--PCR hoods – There are two PCR hoods at the back of the room. This is where the PCR master-mix is prepared then the tubes are taken to the template addition bench to add your extracted DNA.

Pipettmen and racks have been dedicated to particular benches for a specific use. The supplies and pipettmen inside the PCR hoods do not leave the hoods nor can the template addition pipettmen be used for other uses.

There are certain procedures that must be followed to prevent contamination not only of your research materials but also of others.

* Do not wear gloves into lab 3! This is one of the fastest ways to introduce PCR products and contaminate someone's research. **Always** put on a new pair of gloves when you enter lab 3 (even if you were in the other lab only briefly).

* When you enter lab 3 you have the option of putting on a lab coat dedicated for this room (rack by door) but do <u>not</u> wear a lab coat worn in the other labs into lab 3.

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* Samples housed in the other labs cannot be brought into lab 3.
[Note: In the initial set-up of lab 3 we are wiping down samples boxes and tubes with DNAaway as we bring them into lab 3 for permanent storage. Once the initial set-up period is over, we can no longer transfer materials or samples from the other labs, as this could introduce the PCR products that we are trying to avoid.]

* Supplies, equipment and reagents cannot be brought into lab 3 from the other labs. All supplies must flow in one direction; therefore they must come from the supply closet or a newly received reagent order.

* After assembling your PCR reaction and adding DNA template you should seal your plate – or close the lid to the tubes - and carry them into the other lab for cycling without a rack. It is Very Important that you DO NOT TAKE RACKS from lab 3 to lab 2 or visa versa.

* If you are sub-sectioning samples (scat, tissue or whatever) that are housed outside the molecular lab, the sub-sectioning needs to be done in lab 3 and then your sectioned working sample needs to be maintained in lab 3.

* Use pipettmen and racks designated for their specified use and bench/area.

* Use barrier tips when setting up PCR reactions and for the addition of template. [This also includes addition of amplified DNA in the hood in lab 1].

* In the freezer are three “ice boxes” that tubes can be put into to be kept cold. If you absolutely must have ice in lab 3, there is a dedicated ice bucket for lab 3. This bucket cannot be brought into any of the other labs; bring the ice scoop to the bucket in the hallway.

* Make sure to turn the UV light on when you are finished using the hood. [UV will only eliminate DNA from the outer surface of an object – it will not penetrate the surface of any liquid, the wall of a tube, or the cover of a box/container.]

* We need to regularly use DNAaway on the pipettmen and racks at the template addition benches and the extraction benches.

* **Amplified DNA must NEVER be brought into lab 3!!** If you need to use amplified DNA as your template, prepare your master-mix in lab 3 PCR hoods then take the tubes with master-mix into lab 1. There is a hood dedicated to the addition of amplified DNA at the front of lab 1.

* **Only use the door leading to lab 2** (next to the PCR hoods) **in case of AN EMERGENCY!!!** If you open this door, you can contaminate the room with amplified DNA. (Remember that there are PCR machines and gel rigs right next to this door – each time you open a PCR tube it aerosols amplified DNA into the air.).

* Carboys for de-ionized water and buckets for the Biomek robot need to be cleaned (in the hallway) with DNAaway before being taken into lab 3 to fill. Be very careful to wash all the external surfaces without getting DNAaway in the nozel or screw-top lid. Use the biomek trolley to bring the input bucket as far as the external door, then carry the bucket in and fill it- the trolley and the output bucket should NOT enter lab 3! (Empty the output bucket in lab 1 or 2.)

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Please be considerate of the research of others as you want them to be considerate of yours. No one wants contamination or to have their results questioned because of this issue.