LABORATORY 013 GUIDELINES

User responsibilities:

- Leave your workspace clean and replace filter paper if dirty. Please ask for more in the upstairs shop if there is none left in the lab.
- Refill and replace the common pipette tip boxes.
- Leave the box cutter and the sealing tape where they belong after use. This also applies to other tools like pens, calculator, parafilm, scissors, etc.
- Empty waste deposits every time you finish your experiments.
- **DO NOT USE OR TOUCH** any *screening* (or *deepwell*) which is meant for robot use only. Please contact the person responsible for the lab in special situations.

Materials

- The equipment in laboratory 013 is restricted to this lab only, and must remain unshared between other laboratories.
- Thoroughly rinse with water any glassware used and leave it to air dry in the dish drainer. When full, put the equipment back into their corresponding cabinets.
- There should always be a spare bag for every supply (*Eppendorf*, tips, falcons…). If you need to open this extra bag, you must ask for a new one in the upstairs shop.
- Clean the scales, stirrers, spatulas and stir bars after use.

pH meter

- Calibrate if necessary.
- Clean the electrode when you finish measuring. Refill electrolyte solution if empty.
- Turn it off after use.

Crystallization solutions

- Commercial *screening solutions* can be found in the first floor's cold room. Boxes should be returned after use.
- When commercial solutions are sufficiently empty so that *deepwells* cannot be refilled using the robot, they should be placed inside the boxes in laboratory 013, which are set aside for hand use. In order not to accumulate more boxes, users should try to complete the set of solutions per screen. The date in which they are available for hand use must be written on the falcon tubes.
- If there is a contaminated solution, it must be thrown away immediately to prevent its use by others.

Innovadine Crystallization Robot

- Please sign up on the sheet beside the robot and include any problems (if any).
- The cutter and sealing tape for the robot's plates must remain beside it.
- The detergent available for cleaning is reusable and should not be thrown away. Make more if necessary.
- Check water levels when you finish your experiments. You must refill and degas the water tank if it has reached the maximum level indicated on the deposit, so that it is ready for the next user.
- Change the helium tank when empty. Instructions are placed next to the robot. If you need any help, ask the people responsible for the 013 laboratory.
- When you finish using the robot, do not forget to shut off the helium tank (pull down the red lever on the side of the tank).

Gilson Robot

- Sign up in the note book if you are using the robot. Include any problems (if any).

- Make sure there is plenty of milliQ water and ethanol. You must sonicate both solutions when refilling in order to ensure the correct functioning of the robot.

Synchrotron *Loops* and Crystal Freezing Equipment

- There are templates that are to be filled out with the samples taken on each synchrotron trip. Make photocopies whenever there are only two left (around 40 copies will do).
- Make sure you clean the loops correctly. Ask for help if you are not sure about the protocol.
- When you finish freezing your crystals, return the equipment back to the cabinet when dry (dewars and styrofoam containers).
- The glue used for loop mounting is kept in room 107.
- Put back and organize loops and synchrotron equipment after use.

Below is a guide on how to plan a crystallization experiment in order to optimize the assays as well as minimize costs and equipment usage.

1. All crystallization experiments must be approved by the corresponding supervisor to avoid redundant or wasteful assays.
2. Before using commercial crystallization screens, optimal protein concentration must be evaluated by a “Pre-Crystallization Test” (PCT kit), instead of a “trial-and-error” approach. PCT should be used in 48-well sitting drop plates with sealing tape. Sealing the whole plate is recommended and wells should be uncovered and reused in future assays to avoid using new plates every time.

   The 48-well sitting drop plates allow 10 μl drops (5+5) and 100-150 μl in the reservoirs.
3. Robot plates “Innovadine SD2” have two independent wells. These should be used every time if possible, i.e. two mutants, two proteins, ligands…

4. The 48-well sitting drop plates must be used for the scaling up of crystallization conditions. *Limbro*-style sitting and hanging drop plates can only be used if authorized by the corresponding supervisor due to their high cost.
5. Diffraction experiments using the in-house diffractometer are recommended to evaluate cryoprotectants and to test crystals before a synchrotron trip. In this way, we can avoid taking salt crystals and we can optimize data collection.

PLEASE, IF YOU HAVE ANY QUESTIONS, ASK THE PEOPLE RESPONSIBLE FOR LABORATORY 013.

THERE ARE MANY OF US USING THIS LAB. WE MUST ALL BE CAREFUL WITH THE EQUIPMENT AND RESPECT OUR WORKPLACE SO WE ALL CAN BE MORE PRODUCTIVE.

THANK YOU.