

Title: Non-Stop Lab Week: a real laboratory experience for Life Sciences postgraduate courses

Running title: Non-Stop Lab Week: a real laboratory experience

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Abstract

At the Portuguese universities, practical classes of Life Sciences are usually professor centered 2-hour classes. This approach results in students underprepared for a real work environment in a research/clinical laboratory. To provide students with a real life laboratory environment, the Non-Stop Lab Week was created in the Molecular Biomedicine Master program at the University of Aveiro, Portugal. The unique feature of the Non-stop Lab Week is its intensity: during a one-week period, students perform a subcloning and a protein expression project in an environment that mimics a real laboratory. Students work autonomously and progression of work depends on achieving the daily goals. Through 3 curricular years, most students considered the intensity of the Non-stop Lab Week a very good experience and fundamental for their future. Moreover, after some experience in a real laboratory, students state that both the techniques and the environment created in the Non-stop Lab Week were similar to what they experience in their current work situation. The Non-stop Lab week fulfills a gap in postgraduate students learning, particularly in practical skills and scientific thinking. Furthermore, the Non-stop Lab Week experience provides skills to the students that are crucial to their future research area.

Key Words

Practical classes; postgraduate students; practical skills, student-centered teaching.

Introduction

Teaching laboratory skills at the University level is traditionally dependent on theoretical classes and/or professor-centered practical classes. This approach tends to give rise to graduate students without adequate laboratory skills to be successful in a laboratory real environment. It is common knowledge that good laboratory classes are valuable for students' research work [13]. The traditional method of practical classes restricts the "hands-on" experience to a few students since they are performed in students groups and only part really make the practical experience [4]. This results in a gap in students' learning. Laboratory experience gives the students an opportunity to practice theoretical concepts learned in lecture-based classes (for example, safety procedures in the laboratory), experience a working laboratory environment and acquire basic laboratory skills through repetition [5]. Moreover, it facilitates the development of scientific thinking, exposes the students to scientific discovery and encourages them to follow a scientific research career [6]. Several approaches can be undertaken to successfully provide a laboratory experience to undergraduate and graduate students: from a "cookbook" 2-hour class to a several week project based [6; 7]. To fulfill the needs of laboratory experience in the Molecular Biomedicine Master program at the University of Aveiro, Portugal, since 2012, a one-week intensive laboratory module was created: the Non-stop Lab Week (NSLW). This practical course is characterized by its intensity: throughout one week students spend their days at the laboratory. During the week, students must subclone a protein coding DNA sequence (CDS) into an expression vector, express in bacteria the respective protein and, finally, detect it by immunoblot and gel staining

techniques. These procedures are common practice in a molecular biology and biochemistry research laboratory. This approach mimics the real laboratory environment, since students experience a day-to-day laboratory atmosphere, focus only in the project at hands and must overcome obstacles independently. The present article reports the results obtained from 3 consecutive years of NSLW.

Organization of the Non-Stop Lab Week module

Students and professors background

From 3 distinct curricular years, 54 students enrolled in the NSLW module. Since the NSLW is a module of the Biomolecular Laboratories 2 curricular unit, in the Molecular Biomedicine Master, the student's background is diverse. Most students had a Biomedical Sciences bachelor (61%) obtained at the University of Aveiro. The other 39% of students had bachelors in diverse areas of the Life Science, specifically, Biology, Biotechnology, Genetics, Pharmacy, etc. These bachelors were obtained in several Portuguese Universities. Two of the students already had a Master degree. It is noteworthy to mention that students from the Biomedical Sciences bachelor were familiar with a student centered learning approach, since the bachelor is based on Problem Based Learning (PBL) [8]. All students had laboratory experience from previous courses. However, the experience was diverse from student to student and was based in traditional professor-centered learning.

The coordination of the NSLW relied on two or three professors per year: an assistant professor, a postdoc and two PhD fellows. All professors had extensive laboratory experience, particularly in the tasks developed throughout the NSLW. More important, throughout the NSLW, the professors were 100% available and

present at the laboratories to assist the students in any problem, as well as, to observe and assess every student progress.

NSLW design

One week prior to the beginning of the NSLW, students had access to the NSLW guidebook. This document contained all the information necessary to achieve a successful week: class schedules; laboratory localization; week goals; students' assessment criteria and standard protocols for the techniques used throughout the week (supplementary data 1, SD1 – note that the original guidebook is in Portuguese). Two days prior to the beginning of the NSLW, students had the opportunity to discuss the contents of the guidebook in a 2-hour class with the laboratory professors. In this class it was expected that students ask all questions regarding all aspects of the NSLW. They were, also, informed that during the laboratory week, although professors were always present, they were supposed to be observing and only intervene when a problem appeared. Students should be able to solve problems independently. Nevertheless, students were always supervised in the case they could not solve any issue autonomously.

During the NSLW, the techniques performed were simple and did not demand expensive material/equipment. However, since most the protocols were performed individually, the financial cost increased when compared to student group practical classes.

The four days that composed the NSLW, began at 8.00 am and finished when the day goals were achieved. Since the progression of the work depended on the achievement of established daily goals, if for some unpredictable reason, goals were

not achieved professors guarantee the continuity of the work. Due to material limitations, students need to coordinate the use of the equipment (e.g. centrifuges).

The NSLW project was characterized by the sub-cloning of SARP2 (several ankyrin repeat protein 2- Gene Bank code: EF041819 [9]), in a bacterial expression vector (pET 28). After expression and extraction of an enriched bacterial extract of SARP2, immunoblotting and gel staining allowed detection of SARP2. A more detailed description of daily tasks is listed in table 1. All the necessary materials and reagents to perform the tasks were provided and the laboratory was fully equipped with all the necessary equipment.

Student's assessment was based on laboratory attendance, on theoretical knowledge, practical achievement and a final report (0-20 scale). The final report was written individually as a research article: with an abstract, introduction, methods, results and discussion sections. Laboratory reports were due one week after the NSLW. Reports allowed to realize students' learning by assessing how previous knowledge was introduced, methods were summarized and how results were described and discussed. Regarding results discussion, students were not penalized if results were different from the expected. Table 1 describes the NSLW organization and the criteria used for students' assessment, as well as, the correspondent percentage of the final grade. During the NSLW, professors had a record sheet for every student (supplementary data 2 - SD2), which allowed assessing them on the spot.

(table 1)

Evaluation of the NSLW by the students

At the end of the NSLW, students were asked to fulfill an anonymous Survey 1 (Supplementary data 3, SD3 – note that the original was written in Portuguese) to evaluate student's satisfaction about several aspects of the NSLW, specifically, organization, professors, work and global assessment. Also, students were asked to point out two positive aspects and two negative aspects. Moreover, a Survey 2 (Supplementary data 4, SD4 – note that the original was written in Portuguese) was presented to the students to evaluate the impact of the knowledge acquired during the NSLW on their current working situation. This survey was presented to the students 6 months after the NSLW.

Results

The NSLW was created and designed to provide students a real life laboratory experience and to fill a gap in life science students: hands-on experience in basic but relevant techniques. Without doubt a practical component on life science students training is crucial for a future in a laboratory environment. The student's satisfaction and the contribution of the NSLW to their future was assessed. To the teaching staff, more fundamental than the student's grades was to understand if the NSLW prepares future researchers. Therefore the results presented in this article focus on students' opinion, satisfaction and the importance of the NSLW on their future. Please note that answering the surveys was a volunteer and anonymous action.

Non stop lab week - Students performance and satisfaction

In general, all students were able to fulfill daily tasks. In 3 scholar years and 54 students, only one student performed poorly. The average grade of the NSLW module was 15.8 (from a 0 to 20 scale).

At the end of the NSLW (SD3), when students were asked about the importance of the NSLW for their education, three quarters believed that the NSLW was very good or excellent for their education (Fig.1). Regarding the relevance of the knowledge acquired to their education, half of the students considered that it was very good for their future and 38.9% considered it was excellent. More than 80% of the students stated that the NSLW was up to their expectations and the majority of the students evaluated the NSLW as a very good or excellent experience. When asked to describe two positive aspects on the NSLW, most students pointed-out the potential relevance of the techniques to their future career. Moreover, some students' stated that the intensity of the NSLW was a positive aspect, since it mimics the day-to-day life in a laboratory. Regarding negative aspects of the NSLW, the students' opinion was unanimous: lack of material/reagents and laboratory conditions. (figure 1)

Six months after the NSLW, the student's opinion about the relevance of the NSLW to their current professional/academic situation was assessed (SD4). First, to evaluate if the NSLW resembles a real life situation, we questioned the students if they performed (in their current activity situation) any of the techniques executed during the NSLW. Around 54% of the students were affirmative in at least one of the techniques of the NSLW. These techniques varied from immunoblot, sub-cloning and protein expression. Most students (>90%) confirmed that the practical knowledge acquired on the NSLW was relevant or very relevant to their current activity. Only to 8.3% of the student the NSLW had small relevance to their future (theoretical

knowledge relevance obtained very similar results). Since, the main goal of the NSLW was to create a “work environment” reality, students were questioned about the similarity between the NSLW and their current activity. Students agree that the environment created through the NSLW is similar or very similar to their current reality. Finally, 33.3 % of the students stated that the relevance of the NSLW to their performance as scientists was high and 100% think that a similar NSLW activity must be part of an undergraduate/master degree. Figure 2 represents the results obtained.

Discussion

Most practical laboratory classes, in Portuguese undergraduate and graduate courses, are designed to improve student’s practical skills. Also, the vast majority of practical laboratory classes are dispersed throughout a semester period and are mostly unrelated to each other [10]. Consequently, the knowledge acquired by the students is fragmented. The design of a practical laboratory course influences greatly the knowledge and skills acquired by students. A traditional “cookbook” laboratory manual increases the technical skills and reinforces theoretical knowledge. However, a project-based laboratory course promotes scientific thinking [11; 12]. Since we believe that both approaches have qualities and can reflect a real laboratory situation, we created the NSLW.

The NSLW is a halfway experience between a “cookbook” experience and a complete laboratory project. At the beginning of the NSLW, the main goal is presented to the students, as well as, the techniques necessary to achieve it. Thereafter, students have 4 days to complete the project. Throughout the 4 days,

the students focus only on the NSLW. Students must perform the experiments, coordinate to use the equipment, prepare reagents for everyone (e.g. SDS-PAGE gel, DNA agarose gel), organize their time, discuss their results and write an article type report. Moreover, there was no strict schedule on the laboratory – the class began at 8 am and only finished when the work for the day was complete; meal breaks were scheduled by the students without any restrictions. We believe this approach mimics a real laboratory situation, promotes student independence, problem solving skills and responsibility for their own work.

Besides mimicking a real laboratory environment, the NSLW is essential for student's future laboratory work. To assess the student's satisfaction, the students answered two surveys, voluntarily. Taking into account the student's answers and the comments, the NSLW was a very positive experience. Students believe that the knowledge acquired throughout the NSLW was essential to their future work, which was then confirmed when asked if in their current work situation the knowledge acquired was relevant. Moreover, half of the students repeated one or more techniques performed in the NSLW. The knowledge (practical and theoretical) acquired in the NSLW was directly applied on a real world situation, reinforcing the already documented benefits of practical courses on academic and professional success [13].

The original feature of the NSLW, was its intensity. To the satisfaction of the teaching staff, some students stated that the intensity of the NSLW was a positive aspect, since it gave them a sense of a real life situation on a laboratory environment. This approach on practical laboratory classes must be implemented in Master degrees.

Typically, at the end of the Master degree students develop a small project. We believe that a previous experience, such as the NSLW, can be valuable for student's success. Again, this is supported by the student's opinion. According to the students, the environment created on the NSLW is similar to what they encountered on their work situation. Moreover, most students believed that the NSLW contributed to their performance as a scientist, which reinforces the similarity between the NSLW approach and the real life situation.

Regardless of the teaching staff efforts, the lack of materials and the laboratory conditions was the most criticized aspect. In each scholar year, the number of students was around 20. The material available did not allow each student to have its set of equipment (for example a set of micropipettes). To overcome this issue, a new laboratory was used in the year 2014 since the Health Sciences department had brand new facilities fully equipped.

In conclusion, the NSLW appears to be a step forward as a new approach on laboratory courses. It allows students to practice theoretical knowledge previously acquired and, more important, experience a laboratory work environment.

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Table 1. Non-Stop Lab Week organization. Tasks, students' assessment and correspondent percentage of the final grade of the NSLW.

Days	Task	Criteria	% of final

			grade
All week and			
preparation	Attend the classes	Presence	10%
class			
All week and		Works independently;	
preparation	Preparation and theoretical knowledge	Finds solutions to any problem;	10%
class		Prepares the experiments previously	
Digest the cDNA of the protein of interest and the bacterial expression vector;		Presence of correct size cDNA bands	5%
Confirm the digestion by agarose gel			
1st day	Purify the cDNA fragments; Quantify the cDNA fragments by spectrophotometry	Obtain sufficient cDNA to perform a ligation reaction	5%
Clone the cDNA into the bacterial expression vector;		Presence of colonies	5%
Transform the plasmid into competent cells			
Extract the plasmid by miniprep		Presence of cDNA	5%
2nd day	Confirm the cloning by digestion of the plasmid;	Presence of correct size cDNA bands	5%
Analyze the cDNA by agarose gel			
Express the protein of interest			
3rd day	in bacteria;	Obtain a protein extract	5%
Prepare a protein extract from			

	bacteria	
	Prepare an SDS –PAGE system;	Prepare stacking and resolving gels,
	Run an SDS-PAGE; Perform an electroblotting	prepare and load protein samples, transfer protein samples to a nitrocellulose membrane
4th day	Coomassie blue gel staining	Presence of correct size protein band 5%
	Immunoblotting	Presence of correct size protein band 5%
		Well defined goals
		Concise and careful writing
		Appropriate tables and figures
		Discussion of obtained results
One week later	Final Report	Temporal perception of the experiments 35%
		Conclusions
		References

Legends

Table 1. Non-Stop Lab Week organization. Tasks, students' assessment and correspondent percentage of the final grade of the NSLW.

Figure 1. Students' answers to Survey 1. Students answers to question 7, 14, 16 and 21 of survey 1.

Figure 2. Students' answers to Survey 2 (SD4). Students answers to question 2, 3, 5, 6 and 7 of survey 2.

SD1. Non-stop Lab Week guidebook. The guidebook is provided to the students a week before the non-stop lab week. It contains all the necessary information for a successful non-stop lab week.

SD2. Record sheet. Sheet used by the professors to score the practical and theoretical knowledge of students.

SD3. Survey 1. Survey presented to students to evaluate their satisfaction on several aspects of the Non-stop Lab Week

SD4. Survey 2. Survey presented to students, 6 months after the Non-stop Lab Week, to evaluate the impact of the Non-stop Lab Week in their work experience.

Table 1. Non-Stop Lab Week organization. Tasks, students' assessment and correspondent percentage of the final grade of the NSLW.

Days	Task	Criteria	% of final grade
All week and preparation class	Attend the classes	Presence	10%
All week and preparation class	Preparation and theoretical knowledge	Works independently; Finds solutions to any problem; Prepares the experiments previously	10%
	Digest the cDNA of the protein of interest and the bacterial expression vector; Confirm the digestion by agarose gel	Presence of correct size cDNA bands	5%
1 st day	Purify the cDNA fragments; Quantify the cDNA fragments by spectrophotometry	Obtain sufficient cDNA to perform a ligation reaction	5%
	Clone the cDNA into the bacterial expression vector; Transform the plasmid into competent cells	Presence of colonies	5%
	Extract the plasmid by miniprep	Presence of cDNA	5%
2 nd day	Confirm the cloning by digestion of the plasmid; Analyze the cDNA by agarose gel	Presence of correct size cDNA bands	5%
	Express the protein of interest in bacteria; Prepare a protein extract from bacteria	Obtain a protein extract	5%
3 rd day	Prepare an SDS –PAGE system; Run an SDS-PAGE; Perform an electroblotting	Prepare stacking and resolving gels, prepare and load protein samples, transfer protein samples to a nitrocellulose membrane	5%
4 th day	Coomassie blue gel staining	Presence of correct size protein band	5%
	Immunoblotting	Presence of correct size protein band	5%
		Well defined goals Concise and careful writing Appropriate tables and figures Discussion of obtained results Temporal perception of the experiments Conclusions References	35%
One week later	Final Report		

Non-Stop Lab Week

Molecular Biomedicine 2, Master in Molecular Biomedicine

1st year / 2nd semester



Professors: Margarida Fardilha and lab tutor

Timetable

Day	Content	Responsible	H	Room
March 27	Non-Stop Lab Week Preparation	Margarida Fardilha Maria João Freitas	9h-11h	30B.1.41
March 31 -3 April	Non-Stop Lab Week	Margarida Fardilha Maria João Freitas	9h-19h	30B.1.41
April 11	Reports due date	Margarida Fardilha Maria João Freitas		30B.1.41

Evaluation

Task	%	Reviews
Assiduity	10	Attendance
Preparation of the Non-Stop Lab Week	10	Knowledge of the contents of the protocols including techniques and questions raised
Non-Stop Lab Week	45	See table 1, page 4
Report	35	

Competences

1. To develop interpersonal communication skills
2. To develop organizational skills
3. To understand and perform molecular biology protocols
4. To work with equipment used in molecular biology laboratories
5. To deal with unexpected problems and obtain alternative solutions to overcome them in time
6. To develop skills of interpersonal relationships in tense situations

Design of the Non-Stop Lab week

Table 1. Design and assessment of students during the Non-stop lab week.

Goals	Task	% grade	% grade
1	DIGEST SARP2 cDNA FROM pBLUESCRIPT VECTOR AND pET VECTOR WITH RESTRICTION ENZYMES	15	Bands in the correct size - 5%
	PREPARE AGAROSE GEL FOR DNA EXTRACTION		Sufficient amount of DNA - 5%
	PURIFY THE SARP2 AND pET FRAGMENTS		
	QUANTIFY SPECTROPHOTOMETRICALLY THE FRAGMENTS PURIFIED (SARP2 AND pET)		Colonies - 5%
2	LIGATE SARP2 TO THE pET VECTOR	10	
	TRANSFORM THE LIGATION PRODUCT INTO E. coli XL-1 BLUE		
	EXTRACT THE DNA FROM BACTERIA - USE THE ALKALYNE LYSIS METHOD		DNA - 5%
3	DIGEST THE DNA WITH RESTRICTION ENZYMES	10	Positives - 5%
	PREPARE AN 1% AGAROSE GEL TO ANALYZE THE EXTRACTED DNA		
	PREPARE BACTERIAL EXTRACT		Extract - 5%
4	PREPARE SDS-PAGE GEL	10	Gel loading - 5%
	TRANSFER THE PROTEINS FROM THE GEL TO A NITROCELLULOSE MEMBRANE		
	STAIN THE SDS-PAGE GEL WITH COOMASSIE BLUE		Band at the correct size - 5%
	PERFORM AN IMMUNOBLOTTING		Band at the correct size - 5%

Non-Stop Lab Week

- Day 1 -

Note: All the reagents and solutions are indicated in Reagents and solutions (table 6)

DIGEST THE cDNA SARP2 (pBLUESCRIPT) AND THE pET VECTOR WITH RESTRICTION ENZYMES

Restriction enzymes:

- NEVER vortex
- ALWAYS keep at -20°C

Table 2. Restriction Mix

Total volume	20
H ₂ O	16,8 – x
DNA	x
Buffer 10x	2
BSA 100x if necessary	0,2
Enz. Restriction 1	0,5
Enz. Restriction 2	0,5

1. Prepare the mix by adding H₂O, DNA, buffer, BSA, restriction enzymes (chosen in the preparation class).
2. Mix by tapping, spin-down and put the tubes in the water bath at 37°C for 2h.

NOTES:

PREPARE AN AGAROSE GEL FOR DNA EXTRACTION

Note: GreenSafe is fluorescent dye used to stain nucleic acids. It is a safe alternative to ethidium bromide. The **Transiluminator** is a unit of ultraviolet (UV) light used to visualize DNA stained with GreenSafe. Always use material to protect the eyes when watching a DNA through a transilluminator, to prevent eye damage due to ultraviolet rays.

1. Mount the gel apparatus (see Figure 1).
2. Prepare a 1% agarose gel in 120mL of TAE (1X) by boiling to dissolve the agarose.
3. Cool the agarose at Room Temperature (RT) until temperature is approximately 60°C and add 3µl GreenSafe.
4. Pour the solution on the mold and remove any air bubbles (see Figure 1).



Figure 1. DNA gel electrophoresis. Images illustrating how to cast an agarose gel, load a sample into a well and a complete DNA electrophoresis apparatus

- 5 Allow the gel to set.
- 6 Prepare the samples to load into the gel: in each DNA sample add 4µl of loading buffer (6x) Place the gel in the electrophoresis apparatus, fill the tub with TAE (1X) to cover the gel and take the comb out.
- 7 Apply the samples onto the gel. In one well load the molecular weight marker (5µl).
- 8 Run at 80 Volts. Stop only when the migration front reaches half the gel. DNA migrates to the positive electrode (usually red).
- 9 Analyze the gel in the UV transilluminator and photograph the gel.
- 10 Determine the size of the DNA band and extract the fragments corresponding to SARP2 and pET.

PURIFY THE DNA FRAGMENTS CORRESPONDING TO SARP2 AND pET

- 1 Weigh the extracted DNA gel bands (pre weigh the microtube).
- 2 Add 3 volumes of buffer QG to 1 volume of the extracted DNA gel fragment (100mg = 100 µl)

3 Incubate at 50 °C for 10min (or until the fragment is completely dissolved). To help dissolve, vortex the tube every 2 or 3min during incubation.

Note: It is essential that the agarose is completely dissolved. Verify if the color of the mixture is yellow (similar to the color of the Buffer QG prior to dissolve).

4 Load the DNA mixture to a QIAquick column and centrifuge for 1min at top speed.

5 Discard the liquid and load 750µl of PE buffer into the the column.

6 Centrifuge for 1min at top speed.

7 Discard the liquid and elute the DNA with 50µl nuclease free water.

8 Centrifuge for 1min at top speed.

NOTES:

QUANTIFY THE DNA CONCENTRATION OF SARP2 AND PET FRAGMENTS (SPECTROPHOTOMETRICALLY)

- 1 To 5 μ L of sample, add 1mL of water (vortex).
- 2 Measure absorbance at 260nm and 280nm.

Note: It is considered that 1 unit of double stranded DNA (at 260nm) corresponds to 50 mg/mL in water.

Optical density must be between 0.1 and 1.0. It is considered that pure DNA has a ratio of $A_{260}/A_{280} >$ or equal to 1.8.

Ratio < 1.8 indicates that the preparation is contaminated with protein and alcohols.

Ratio > 2 indicates RNA contamination.

NOTES:

LIGATE SARP2 cDNA WITH DIGESTED pET VECTOR

1. Determine the SARP2 amount to be used:

1.1. (Vector amount/ Vector size (bp)) x SARP2 size = SARP2 amount (ratio 1:1)

1.2. Use 40ng of vector and SARP2 cDNA in a ratio of 1:3 and 1:5

Vector pET28a - 5369bp

SARP2 – ~1600bp

Table 3. Ligation conditions

	Vector (ng)	SARP2 (μ l)	Buffer 10x (μ l)	Ligase (μ l)	H ₂ O (μ l)	Total (μ l)
NC	40	-	2	1		20
1:3	40		2	1		20

Legend: NC – negative control

2. Incubate 2h at RT.

NOTES:

TRANSFORM THE LIGATION RESULT (SARP2-pET) INTO E.COLI XL-1 BLUE

Note: work in sterile conditions.

- 1 Keeping the tubes on ice, to 50µl of competent cells (stored at -80°C) add 5µl of ligation mixture.
Mix gently
- 2 Incubate 15min on ice.
- 3 Place tubes in the water bath at 42°C for 90sec (heat chock).
- 4 Incubate 5min on ice.
- 5 To each tube, add 950µl of SOC medium.
- 6 Incubate 45min at 37°C with shaking (~ 180 rpm).
- 7 Centrifuge 90sec at top speed. Discard the supernatant and resuspend the pellet in the remaining SOC (~ 100µl).
- 8 Plate the cells onto LB/kanamycin plates (use glass beads).
- 9 Incubate at 37°C over night (ON).

Table 4. Transformation conditions, expected results and obtained result

	Condition	Expected result (colonies)	Result
1	Competent cells	0	
2	pET uncut (5ng)	Many	
3	pET cut (5ng)	0	
4	Ratio 1:3	Some	
5	Ratio 1:5	Some	

NOTES:

EXTRACT PLASMID DNA FROM BACTERIA- ALKALINE LYSIS METHOD

- 1 Inoculate an isolated colony into a test tube containing 3mL of LB/kanamycin.
- 2 Incubate at 37°C, ON, with shaking (~180rpm).

- Day 2 -

- 3 Centrifuge for 90 sec, top speed, 1.5mL of the culture. Remove the supernatant.
- 4 Dilute the cells in 100µl of solution I (4°C). Vortex vigorously.
- 5 Add 200µl of solution II and mix gently by inverting the tube 5x. Place the tube on ice.
- 6 Add 150µl of solution III (4°C) and invert the tube 10x. Keep on ice for 5min.
- 7 Centrifuge 10min at top speed, at 4°C. Remove, carefully, the supernatant to a new microtube. Discard the rest.
- 8 Add 2 volumes (~ 900µl) of 100% EtOH (-20 °C). Vortex thoroughly and incubate 2h at -20C.
- 9 Centrifuge 5min at top speed, at 4°C. Discard the supernatant.
- 10 Add 750 µl of 70% EtOH (-20 °C).
- 11 Centrifuge 5min, at top speed, at 4°C.
- 12 Remove the supernatant and dry the pellet (37°C, 30-60min).
- 13 Ressuspend the pellet in 50 µl RNase water (20 µg/mL).
- 14 Store the DNA at -20°C.

NOTES:

DIGESTSARP2-PET TO CONFIRM CLONING

Table 5. Restriction Mix

	to 5 microtubes (prepare the mix to 5.5 microtubes)
Total volume	55µl
H ₂ O	18.45µl
Buffer10x	5.5µl
BSA 100x if necessary	0.55µl
Enz. Restriction 1	1.5µl
Enz. Restriction 2	1.5µl

- 1 Place 5µl of DNA into a microtube.
- 2 Prepare the cutting mix by adding H₂O, buffer, BSA, Enzymes.
- 3 Mix gently and make a "spin down".
- 4 Add 5µl of cutting mix.
- 5 Mix, by tapping, a spin-down and place the tubes at 37°C for 2h.

NOTES:

PREPARE AN 1% AGAROSE GEL TO ANALYZE THE DNA AND PREPARE A BACTERIAL PROTEIN EXTRACT

Note: The positive clones will present two bands: 5369bp (pET vector), and the band corresponding to SARP2 (~ 1600bp)

- 1 Select one positive clone and transform into Rosetta bacteria (previously done by one of the professors).
- 2 Inoculate an isolated colony into 3mL of LB/kanamycin.
- 3 Incubate at 37°C, ON, (~180rpm).

- Day 3 -

- 4 Inoculate 12 mL of LB / kanamycin with 25 μ l of the pre-culture.
- 5 Incubate at 37°C until A_{600} is 0.5-0.6.
- 6 Add IPTG (Isopropyl β -D-1-thiogalactopyranoside) to a final concentration of 1mM.
- 7 Incubate at 37 °C for 3 h.
- 8 Transfer the culture into a 15mL Falcon and centrifuge at 4000rpm for 10min.
- 9 Discard the supernatant and add 200 μ l of 1% SDS.
- 10 Sonicate in cycles of 15sec 3x.
- 11 Remove 10 μ l and add $\frac{1}{4}$ LB.
- 12 Boil for 5min.

NOTES:

PREPARE THE SDS-PAGE GELS

- 1 Set up the vertical electrophoresis apparatus (consult the [Min-Protean Tetra cell Instruction Manual](#)).

The desired thicknesses of the gel (1.5cm) must be considered when choosing the appropriate spacers.

Note: The glass should be cleaned thoroughly, especially the side that contact the gel.

- 2 Prepare the lower gel (10mL). Final % of acrylamide must be 12%.

Lower gel:

H ₂ O	3.45mL
Acrylamide	4.0mL
4x LGB	2.5mL
10% APS	50µl
TEMED	5µl

- 3 Pour the lower gel between the glasses until two centimeters from the top.
- 4 Carefully, apply a small amount of isopropanol on top of the gel.
- 5 Let polymerize for 30-60min.
- 6 After the gel polymerizes, pour out the isopropanol and prepare the upper gel (5mL).

Upper gel:

H ₂ O	3.3mL
Acrylamide	0.6mL
5x UGB	1.0mL
10% SDS	50µl
10% APS	50µl
TEMED	5µl

- 7 Pour the upper gel between the glasses until the top.
- 8 Carefully place the comb, previously selected and clean.
- 9 Let polymerize for 30min.
- 10 Set up the electrophoresis system.
- 11 Fill the tank with 1x Running buffer.
- 12 Transfer the vertical device to the tank and carefully remove the combs.

- 13 Load the previously prepared samples into the gels (Each student must apply a sample in 2 different gels – to stain with Coomassie blue and to do an immunoblot) (figure 2). Free wells must be load with loading buffer 1x.

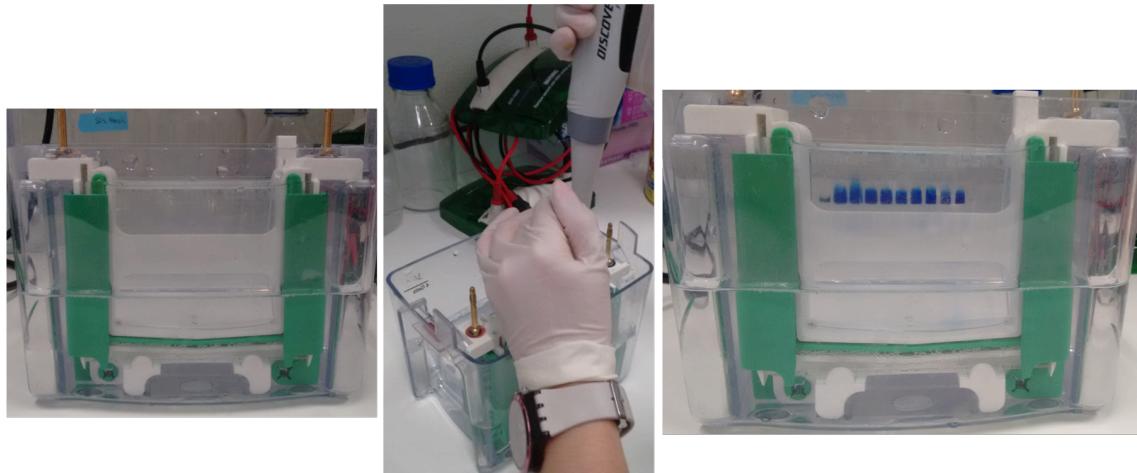


Figure 2. Protein electrophoresis. Images illustrating how to load a protein gel a complete electrophoresis apparatus.

- 14 Place the electrophoresis tank covered and connect the electrodes to the power source.
- 15 Run the gel at 100 Volts.
- 16 After the migration front reaches the bottom of the gel, turn off the power source and remove the tank cover.
- 17 Remove the vertical device and pour out the running buffer.

TRANSFER THE PROTEINS INTO A NITROCELULOSE MEMBRANE

Note: Only 1 of the gels will be transferred to the membrane..

- 1 Cut one nitrocellulose membrane for each gel;
- 2 Carefully wet the membranes in transfer buffer.
- 3 Assemble the transfer sandwich. The assembly must be done in transfer buffer and all components must be wet with transfer buffer.
- 4 Assemble the components according to Figure 3.

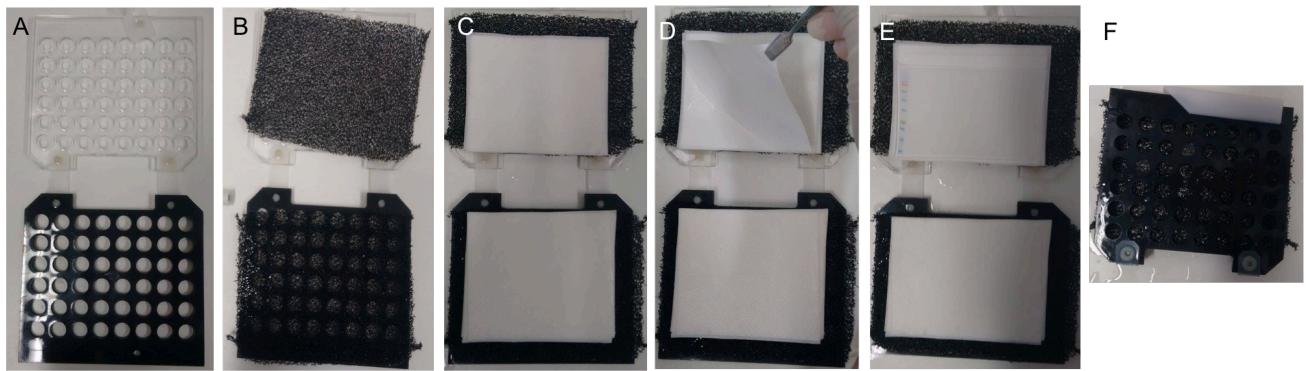


Figure 3. Steps (A-F) for arrangement of an electroblotting sandwich. Please note that each layer is being added carefully to minimize the air bubbles.

- 5 Insert the cartridge into the transfer unit.
- 6 Transfer at a constant current of 100mA for 2h;
- 7 Turn off the power source and take out the nitrocellulose membrane.
- 8 Dry the nitrocellulose membrane and set aside.

STAIN THE GEL WITH COOMASSIE BLUE

- 1 Place the polyacrylamide gel into the fixative solution for 30min with shaking.
- 2 Wash the gel in H₂O.
- 3 Add the staining solution and incubate 30min. with shaking.
- 4 Wash the gel in H₂O.
- 5 Add the destaining solution and incubate 3min.
- 6 Add new destaining solution and incubate ON.

- Day 4 -

- 7 Examine the band pattern.

PERFORM THE IMMUNOBLOTTING

- 1 Incubate the membrane in 1xTBS 5min.
- 2 Block the membrane in 5% milk/TBST 1x 1h.
- 3 Wash the membrane in 1xTBST.
- 4 Incubate the membrane with the primary antibody (anti -Histidines), 2h with shaking. The antibody must be diluted in 3% milk/1x TBST.
- 5 Wash 3x 10min in 1xTBST.
- 6 Incubate the membrane with secondary antibody (anti-mouse) (Diluted in 3% milk/1xTBST), 1h with shaking.
- 7 Wash 3x 10min with 1x TBST.
- 8 Analyze the band pattern using Odyssey Infrared Imaging System¹

NOTES:

¹Odyssey Infrared Imaging detects fluorophores attached to antibodies

Reagents and Solutions

Table 6: Reagents' and Solutions.

LB medium Deionized H ₂ O until 950mL Tryptone, 10g Yeast extract, 5g NaCl 10g	To prepare LB (Luria Bertani) stir until all solutes dissolved. Adjust pH to 7.0 with 5 N NaOH (approx. 0.2ml). Adjust the volume of solution to 1L with deionized H ₂ O. Sterilize by autoclaving 20 min.
Plates LB / Ampicillin Reagents used to prepare LB medium, Agar 15g/L Ampicillin 50µg/mL	After sterilization, slowly stir the melted agar. Be careful, the liquid may overheat and burn. Allow to cool to 50-60 ° C before adding the antibiotics. Avoid making bubbles. Pour de liquid into petri dishes (30-35mL each) When the medium solidify, invert the plates and kept at 4°C until use.
SOB Deionized H ₂ O until 950mL Tryptone, 20 g Yeast extract, 5 g NaCl, 0.5 g	Stir until all dissolved solutes. Add 10mL of 250 mM KCl (This solution is prepared by dissolving 1.86 g KCl in 100mL of deionized H ₂ O). Adjust the pH of the medium to 7.0 with 5 N NaOH (approx. 0.2mL). Adjust the solution volume to 1 L with deionized H ₂ O. Sterilize by autoclaving 20min. Prior to use add 5mL of sterile 2M MgCl ₂ solution (this solution is made by dissolving 19 g MgCl ₂ in 90mL of deionized H ₂ O, the solution volume is adjusted to 100mL with deionized H ₂ O and It is sterilized by autoclaving 20min).
SOC Deionized H ₂ O until 950mL Tryptone, 20g Yeast extract, 5g NaCl, 0.5g Glucose 1M	SOC is identical to SOB but it contains glucose. After the SOB was autoclaved allowed to cool to 60°C or less, and adding 20mL of sterile 1M glucose (This solution is prepared by dissolving 18 g of glucose in 90mL deionized H ₂ O. Adjusts the solution volume to 1L with deionized H ₂ O and sterilized by passing through a 0.22µm filter.
Solution I Glucose, 50mM Tris-Cl (pH 8.0), 25mM EDTA (pH 8.0), 10mM	Prepare the solution 1 from stock solutions of each of the components and sterilizing by autoclaving 15min.
Solution II	NaOH 0.2 N (diluted fresh from a stock 10 N) SDS, 1% (w / v) Prepare de solution just before use. Use it a RT.
Solution III	Potassium acetate, 5 M, 60mL Glacial acetic acid, 11.5mL H ₂ O, 28.5mL The resulting solution is 3M with respect to potassium and 5M with respect to acetate. Store the solution at 4°C and put it on ice before use.
EDTA 0.5M (pH 8.0)	Add 186.1g of EDTA • 2H ₂ O and 800mL H ₂ O. Stir vigorously using a magnetic stirrer. Adjust pH to 8.0 with NaOH (approx. 20 g NaOH). Aliquot and sterilize by autoclaving. The disodium salt EDTA cannot dissolve until the pH of the solution is adjusted to approximately 8.0.
NaOH	Preparation 10 N NaOH involves a highly exothermic reaction, which may lead to breakage of glass beakers. Therefore, prepare, this solution with extreme caution in graduated plastic cups. To 800mL of H ₂ O, and add slowly 400g of NaOH tablets, stirring constantly. As an added precaution, place the cup on ice. When it is fully dissolved adjust the volume to 1 liter with H ₂ O. Store this solution in a plastic bottle at room temperature. It is not necessary to sterilize.
SDS	To prepare a 20% solution (w/v), dissolve 200g SDS electrophoresis grade in

	900mL H ₂ O. Heat to 68 ° C and stir using a magnetic stirrer. If necessary adjust the pH to 7.2 by adding some drops of concentrated HCl. Adjust the volume to 1 liter with H ₂ O. Store at RT. Sterilizing is not necessary.								
Tris-HCl (1M)	Dissolve 121.1 g Tris base in 800mL of H ₂ O. Adjust the pH to the desired value by adding concentrated HCl: <table style="margin-left: 40px;"> <tr><td>pH</td><td>HCl</td></tr> <tr><td>7.4</td><td>70mL</td></tr> <tr><td>7.6</td><td>60mL</td></tr> <tr><td>8.0</td><td>42mL</td></tr> </table> Allow the solution to cool to RT before final pH adjustment. Adjust the volume of the solution to 1 liter with H ₂ O. Aliquot and sterilize by autoclaving. Tris pH solutions is temperature-dependent and decreases about 0.03 pH units for each 1°C increase in temperature. For example, a 0.05M solution has pH values of 9.5, 8.9 and 8.6 at 5 ° C, 25 ° C and 37 ° C respectively.	pH	HCl	7.4	70mL	7.6	60mL	8.0	42mL
pH	HCl								
7.4	70mL								
7.6	60mL								
8.0	42mL								
Loading buffer agarose gel (6x)	Bromophenol blue, 0:25% (w/v) Glycerol, H ₂ O, 30% (v/v) Store this solution at 4°C.								
TAE (50X)	Prepare a 50x stock solution in 1L of H ₂ O: Tris base, 242g Glacial acetic acid, 57.1mL 0.5 M EDTA solution (pH 8.0), 100mL A working solution (1x) and 40 mM Tris-acetate and 1 mM EDTA.								
Stock solution of 1M IPTG	Dissolve 2 g of IPTG in deionized water, and make up to 10mL. Aliquot (1mL) and storing this solution at -20°C.								
Acrylamide solution (30% acrylamide / 0.8% bisacrylamide)	Acrylamide 29.2g Bisacrylamide 0.8g Dissolve in deionized water and make up to 100mL. Filter the solution with a diameter of 0.2μm filter and storing the solution at 4°C.								
Solution 4x LGB ("Lower Gel Buffer") [1.5M Tris-HCl; 0.014M SDS]	Tris 181.65g 4g SDS Dissolved in deionized water, adjust the pH to 8.9 with HCl, and adjust the volume to 1L.								
Solution 5x UGB (Upper Buffer Gel) [0.63M Tris-HCl]	Tris 75.7g Dissolved in deionized water, adjust the pH to 6.8 with HCl, and adjust the volume to 1L.								
10% APS solution (ammonium persulfate)	(Note: This solution must be prepared at the time of use) Dissolve 0.5g of APS in 5mL of deionized water. Store the solution at 4°C.								
Solution 4x loading buffer (or LB, "Loading Buffer")	Tris stock solution (1M, pH 6.8) 2.5mL (final concentration: 250mM) SDS 0.8g (8%) 4mL glycerol (40%) b - Mercaptoethanol 2mL (20%) 1mg bromophenol blue (0.01%)								
Solution 10xRunning	30.3g Tris base (250mM) Glycine 144.2 g (2.5M) 10g SDS (1%) Dissolved in deionized water, adjust the pH to 8.3 and adjust the volume to 1L.								
Gel staining solution - Coomassie blue	Coomassie blue R-250 2.0g (0.2%) Methanol 500mL (50%) 100mL acetic acid (10%) Dissolved in deionized water and adjust the volume to 1L.								

Gel destaining solution	Methanol 500mL (25%) 100mL acetic acid (5%) Dissolved in deionized water and adjust the volume to 2L.
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Day	Activity	Attendance	Theoretical Knowledge/ Preparation	DNA bands	DNA quantity	Bacterial colonies	DNA concentration	Positive	Protein extract	Ge loading	Bands in Comassie	Bands in Immunoblot	Report
		0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.35
27/Mar	Presentation/ Goals												
31/Mar	Lab Week												
01/Abr													
02/Abr													
03/Abr													
11/Abr	Report												

Student _____

Grade _____ 0 to 20

Students Survey 1

Molecular Biomedicine Master Degree

Biomolecular Laboratories 2 – Non-stop lab week

State your opinion/evaluation on the topics below. Please use the following scale

1- Unsatisfying, 2- Fair, 3- Good, 4- Very Good, 5- Excellent

Non-stop lab week (NSLW) organization

1. The organization
 2. The syllabus
 3. The time dedicated to the NSLW
 4. The grade % in each training component
 5. Protocol provided
 6. General conditions (material, reagents, etc.)
 7. Contribution for your education
 8. Relevance for your education

Mentors/Teachers

9. The motivation
 10. The availability to answer questions
 11. Global performance of Margarida Fardilha
 12. Global Performance of Professor 1
 - 13 Global Performance of Professor 1

1	2	3	4	5

Students

14. The weekly work required
 15. Relevance of the practical and theoretical knowledge acquired for your education
 16. Important information was provided in time
 17. The NSLW fulfilled your expectations
 18. The learning goals were clearly provided

Evaluation

19. The evaluated topics correspond to the previously stated goals?
 20. The tools used to evaluated the knowledge acquired
 21. Opportunities to ask about how the evaluation was performed

1	2	3	4	5

22. NSLW global assessment

1	2	3	4	5

Please, provide two positive aspects about NSLW

Please, provide two aspects that need to be improved in NSLW

Additional commentary

Students Survey 2

Molecular Biomedicine Master Degree

Non-stop lab week

The following survey intends to access the relevance of the theoretical and practical knowledge acquired on the non-stop lab week (NSLW) on a real life work situation. Please, state your opinion on the topics below.

1. Which of the following activities did you pursue after the NSLW.

	Research
	Diagnostic and Clinic
	Academic degree (PhD, master, etc.)
	Other

If you answer "Other", please specify _____

2. After the NSLW did you carried out some of the techniques performed on the NSLW?

	Yes
	No
	Cannot remember

If you answer "Yes", please specify_____

3. The practical skills and knowledge acquired throughout the NSLW were _____ to my work.

	Not relevant
	Small relevance
	Relevant
	Very relevant

4. The theoretical knowledge acquired through the NSLW were _____ to my work.

	Not relevant
	Small relevance
	Relevant
	Very relevant

5. Regarding your current work experience, how similar is a working day in a research laboratory and the day on NSLW?

	Different
	Somewhat similar
	Similar
	Very similar

6. In your opinion, what was the NSLW contribution for your current work situation?

	None
	Reduced
	Fair
	High

7. The NSLW type activity is beneficial for students in an undergraduate/master degree?

	Yes
	No

Additional commentary

Thank you