Modular Implementation of Laboratory Unit Operations (LUOs) for Automation of Biotechnology Protocols

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ABSTRACT
Protocols used in biotechnology consist of multiple steps that have to be strictly followed in order to obtain a desired chemical or biological outcome. Each step is an operation that has to be carried out in order to achieve a given reaction. For biotechnology, twelve generic operations considered the basis for all protocols, have been identified in the literature. These are called Laboratory Unit Operations (LUOs).

This paper examines automation design issues that are introduced to biotechnology protocols by the various LUOs. The paper first presents the biotechnology's LUOs classification found in literature and the laboratory equipment used for each operation. It also describes the possible challenges encountered in the process of integrating the equipment into an automated solution. Second, it discusses the integration aspects and possible scenarios for future automation implementation of equipment, and shows an implementation approach for the general and common cases among different LUOs. These common cases are translated into implementation modules, and these modules are simulated and validated using ARENA Simulation Software. Third, a methodology used to integrate the different ARENA modules for one complete Biotechnology protocol solution is shown.

Keywords
Biotechnology, Laboratory Unit Operations, Automation, and Simulation.

1. INTRODUCTION
Every protocol used in biotechnology consists of multiple steps that have to be strictly followed to obtain a desired chemical or biological outcome. Each step is an operation that has to be carried out systematically in order to achieve a given reaction. For biotechnology, twelve generic operations considered the basis for all protocols, have been identified in the literature. These are called Laboratory Unit Operations (LUOs) [4].

Automating a biotechnology protocol, as in automation done in any other industry, consists of automating the fundamental operations (LUOs) and integrating them into a production line (or cell). The equipment and technologies available for biotechnology can be classified into three groups:

Group 1 consists of all the manual equipment available for biotechnology, such as pipettes, syringes, and mortar and pestle.

Group 2 consists of the equipment that automates only one LUO at a time, such as a dispenser, centrifuge, robotic arm, and conveyors.

Group 3 consists of state of the art equipment that already automates and integrates more than one LUO, but is currently considered very expensive or is customized equipment.

This paper only considers automation for small-to-mid-size laboratories; this study contemplates only commercial off-the-shelf equipment that performs single operation (Group 2). Small-to-mid-size laboratories usually have available equipment that belongs in group 2.

The paper examines automation design issues that are introduced to biotechnology protocols by the various LUOs. First, it presents the biotechnology's LUOs classification found in literature and the laboratory equipment used for each operation. It also describes the possible challenges encountered in the process of integrating the equipment into an automated solution. Second, it discusses the integration aspects and possible scenarios for automation implementation of equipment, and shows an implementation approach for the general and common cases among different LUOs. These common cases are translated into implementation modules, and these modules are simulated and validated using ARENA Simulation Software [3], [7]. Third, a methodology used to integrate the different ARENA modules for one complete Biotechnology protocol solution is shown.

2. THE TWELVE LABORATORY UNIT OPERATIONS (LUO)
The concept of Unit Operations originates from Chemical Engineering where such are considered to be basic steps in any process. A subset of all Unit Operations constitutes the Lab Operations Units for Biotechnology (or in short LUO) as coined by [4]. A set consisting of twelve LUOs for

biotechnology was defined by modifying and completing the original classification reported by Zymark Corporation in 1988 to consider the specifications and requirements of current biotechnology laboratories. The original table found in the Laboratory Robotics Handbook by Zymark Corporation was based on specifications for chemistry laboratories. This section describes the twelve LUOs as applicable to automation design issues in biotechnology protocols.

The twelve LUOs described by Najmabadi are the following: manipulation (LUO 1), liquid handling (LUO 2), separation or purification (LUO 3), conditioning (LUO 4), washing-drying (LUO 5), agitation (LUO 6), homogenization (LUO 7), breaking-fragmentation (LUO 8), weighing (LUO 9), measurement and direct detection (LUO 10), analysis and data extraction (LUO 11), and documentation (LUO 12). These LUOs have been numbered for easier identification throughout the paper chapter. It is important to understand and keep in mind that not all twelve LUOs are necessarily used in every experiment and protocol; most of the protocols only use a small subset of the LUOs.

As mentioned earlier, the equipment that is in Group 2 is capable of performing only a single operation. The vital issues to examine whenever one automates any given LUO are the actual operation that takes place and the way it is done. The complete automated solution for any given LUO needs to take into account that for the operation to occur, the sample needs to be loaded into the equipment or transferred into the labware in which the operation takes place. Likewise, for the sample to be ready for the next operation, it needs to be unloaded from the equipment or transferred to another labware. For example, whenever separation (LUO 3) is performed by means of a centrifuge, the automated solution needs to involve the loading and unloading (manipulation) of the labware (LUO 1) in and out of the centrifuge. This example demonstrates that by mere inclusion of a centrifuge (LUO 3) into the production line, the automation task remains incomplete. Such a separation solution needs to include a centrifuge (LUO 3) that is integrated with manipulation operations (LUO 1) for it to be a valid automated solution for sample separation. This logic can be extended to all LUOs. Each of these represents different challenges and different sets of LUOs that need to be integrated in order to find truly functional solutions. The following sections explore all possible combinations among the different LUOs and present an automated solution for these sets of LUOs.

2.1 LUO 1: Manipulation

Manipulation in biotechnology LUO context is understood as the physical handling and transportation of labware, such as tubes, plates, caps, and arrays. This is better understood by dividing the manipulation operations into two categories: Category 1 includes the transportation of tubes, plates, and arrays between distinct locations (such as stationary equipment and stackers) and it is often realized by means of pick-and-place operations. Category 2 includes operations such as capping and uncapping of labware, lid openers, and dispensing of labware. Manipulation in Category 2 also refers to manipulation and deposition of different molecules; the operations included are, for example, cloning, colony picking and array deposition.

For Category 1, in a manual experiment, a human operator performs the transportation operations. The operator moves the labware to and from different equipment and loads and unloads the labware into and from the equipment. When extrapolating a manual protocol to an automated solution, the first issue that arises is how the samples are going to flow throughout the different protocol stations. This problem has been long solved. Basically, there are two main solutions, and depending on the applications, one or both of them can be present in a production line: laboratory robotic arms (cylindrical, articulated, or Cartesian) or conveyers. Nowadays there are many available robots and conveyers specialized for biotechnology applications.

The equipment used for category 2 operations are lid openers, cappers/decappers, microarreyers, and colony pickers among others. Nowadays, laboratories tend to rely on automated equipment to perform these operations. Depending on the size of the laboratory, this includes equipment that only performs a given operation whereas a human operator needs to load the labware to be manipulated, to more complex equipment that integrates more than one operation and that may or may not have integrated feeding mechanisms.

Assuming automation solutions for small-to-mid-size laboratories, we shall consider only commercial equipment that can perform single type of operation, assisted with a basic or even nonexistent feeder mechanism. The discussion of more complex manipulators support equipment is left as a future research direction.

There are two distinct ways to integrate other LUOs to the manipulation system: either by means of conveyors, operating jointly with feeder mechanisms such as dispensers; or by means of pick-and-place devices or robotic manipulators, needed for equipment such as centrifuges that have to be loaded from the top and have no feeder mechanisms, or for transferring samples if the operation such as filtration requires it. This will dictate the way in which the labware is transported to and from the equipment.

The manipulation operation describes the actual flow of the samples throughout the processing line. The assessment of what is needed is dictated by the requirements and constraints of the equipment used for the protocol. In other words, the integration between different elements in a process is defined by the operating principle of each of the

instruments; and depending on those requirements and constraints is determined the need for pick-and-place devices, robotic transporters, and/or conveyors [5].

2.2 LUO 2: Liquid Handling

The handling of all liquids such as assay reagents, reaction reagents, buffers, samples, and the solutions, involves operations such as pipetting, dispensing and aspiration. The liquid handling operations would be involved in the adding of specific given volumes at a very high precision (low tolerances), the transferring of the reaction solution from one labware (usually tube, capillary tube, or assay array) into another; and also in the mixing of some reactions if performed by systematically pipetting in and out of the solution until the reaction solution is at the desired mixing quality. As with addition and measuring of volumes, operations such as transferring or mixing have to be performed with extreme care so as to not lose any part of the solution in the process. At these small volume levels, every tiny drop or particle can make a difference. Other than the level of precision needed for these operations, the automation itself of this LUO is straightforward: pipette tips or syringes are attached to pick and place devices that actually move solutions from one position to another (in the case of transferring, dispensing, aspiration, or addition). A concern that will be addressed in future research directions is that of avoiding cross contamination between liquids [8].

An off-the-shelf dispenser that may or may not have feeder mechanisms is the automated equipment of choice generally used for liquid handling. The labware needed for the operation is placed underneath the actual dispenser's heads by means of feeder mechanism or pick-and-place devices. The dispenser may have movable heads equipped with pipette tips that perform dispensing and/or aspiration operations. The pipette tips are usually arranged in singles or in groups of 9 or 96 tips for conventional throughput operations, or in groups of 384 or 1536 for high throughput operations.

Whether or not the dispensing equipment has a feeder mechanism, dictates how it could be integrated into the production line. If the device is equipped with a feeder mechanism it can be easily integrated by means of conveyors, however if a feeder mechanism is not available, a pick-and-place device will be needed.

As explained before, depending on the type of dispenser available, the complete integration to an automated production line has to encompass not only the operation itself, but also what is needed in order to carry out such operation.

2.3 LUO 3: Separation and Purification

Separation and purification both involve the separation of specific molecules from a solution. It is called Separation whenever the operation objectives are the molecules that are separated from the solution. For example, after separation the resulting product of interest may be a genomic RNA. It is called Purification whenever the operation objective is the fluid that is cleared of the separated molecules.

There are variety of separation/purification methods such as filtration, centrifugation, magnetic separation, extraction, distillation, electrophoresis, and recrystallization. Each of these methods is based on different physical or chemical principles and proper instrumentation that has to be used in order to obtain the desired outcome.

These methods can be divided into three main groups. The methods used in the first group take place inside the labware containing the sample without having to add any extra reagent or element to the labware in order for the reaction to occur. This is the case of centrifugation or magnetic separation where the labware containing the sample is loaded into the equipment in order for the operation to take place. Methods in the second group involve the adding of elements into the sample's labware for the operation to take place. Recrystallization and magnetic beads are good examples for this group. For recrystallization, reagents and solvents are added in order for the operation and the reaction to take place. For magnetic beads, beads have to be added before the actual operation is carried out and for the operation to be capable of happening. The third group includes methods, such as filtration, distillation, or electrophoresis, where the sample has to be transferred and has to flow through a device in order for the operation to take place. Afterwards, collection of the sample is executed depending on the desire product and the steps that follow the operation. The collection process may also take more than one step.

These methods can eventually be broken down into different steps, and there might be a need to use more than one piece of equipment to carry out the complete operation. Some of the steps can be recognized as other basic operations. Each device used for the operation has different requirements, which dictate if it has to be integrated by means of a pick-and-place device or a conveyer mechanism.

2.4 LUO 4: Conditioning

As its name clearly states, these operations modify and control the samples environment in order for a specific given reaction to occur. This is a very critical set of operations since, for example, enzymes need a specific temperature range for the reaction to occur. If the ideal environmental conditions are not met, the reaction will not take place. Like enzymes, there are many similar examples in the biotechnology world.

There are two types of conditioning that must be carried out: the first one is the actual conditioning of the laboratory (room); and the second one is a conditioning that may or

may not be required for a specific step or steps in the protocol.

Biotechnology laboratories need to be enclosed within clean rooms; there is a constant monitoring of the room temperature, pressure and humidity. In this sense, laboratories are equipped with temperature, pressure, humidity and particle sensors. This first type of conditioning includes operations such as temperature control and atmosphere control. The integration of this type of conditioning LUO to an automated system is straightforward. The sensors are integrated and transfer the data directly to the laboratory management system. Since typically such environmental conditions have to be constant throughout the production line, there is no need to include or integrate this type of conditioning LUO to each step or another LUO for that matter.

The second type of conditioning is whenever specific steps of the protocol require special conditions. For example a given temperature needed for an enzyme to carry out a reaction or a centrifuge step that needs to be performed at a given temperature. For this second type of conditioning there are two possible scenarios for LUO integration. One occurs whenever a sample needs to be subjected to a different environment condition such as a temperature change for the enzymes to become active. In this case the LUO that needs to be integrated is the conditioning equipment by itself. Equipment used for this operations are hotplate heaters, incubators, refrigerators, ovens, freezers, vacuums fume hoods, and PCR machines. The second integration format for this type of conditioning operation is whenever another operation needs to be carried out at a different temperature, such as a centrifugation at a different temperature. In this case the integration of the conditioning LUO has to be thought out as integrated to the second LUO that actually does the operation. For example: a centrifuge that is placed inside a freezer or an incubator. Nowadays, some equipment such as centrifuges have also temperature control, and therefore the integration of such equipment will be just as the integration of any other centrifuge.

2.5 LUO 5: Washing/Drying

An important operation is that of washing and drying of reusable labware such as tubes, plates and caps, pipette tips, syringes, etc. Washing and drying is performed by means of operations such as washing, sonication and vacuum dryers.

This LUO is not part of any biotechnology protocol per se, but the operations have to be carried out if using reusable labware. Whenever using reusable labware the laboratory itself needs to be prepared to handle the washing and the drying operations keeping in mind that these are time consuming operations, and that the sufficient pieces of labware need to be available for the actual protocols At the same time, it is not integrated with any other operation and for its integrations the means of labware manipulation needs to be considered.

The washing/Drying LUO is a special integration case because it can be integrated as a separate cell on its own since it is not integrated into a protocol but into a production line; and it will be treated as such in the next section of this chapter.

2.6 LOU 6: Agitation

Agitation refers to the mixing of reagents together or with other molecules or particles. It is considered agitation whenever a reagent, molecule or particle is added into the solution at some point of the protocols and the new solution is mixed for further processing. Depending on the substance that is added to the solution and the protocol upstream agitation can be performed with different operations. Operations that carry out agitation are mixing, vortexing, shaking, and stirring.

For agitation, the off-the-shelf equipment available for the operations requires a pick-and-place device in order to load and unload the labware containing the samples into the equipment. Hence, a pick-and-pace device is needed to integrate the equipment that performs the operation into the production line. All these operations, mixing, vortexing, shaking, and stirring, are performed inside the labware in which these are contained so that its integration doesn't need a liquid handling operation.

Equipment used to perform the different operation methods for this LUO is: vortex, shaker, automated rocker, and automated stirrer.

2.7 LUO 7: Homogenization

Homogenization in biotechnology refers to making a solution homogeneous by reducing the samples particles sizes thus creating a uniform solution. It also refers to the cutting of the samples that has to take place in order to create a uniform solution. Like with the previous LUOs and especially LUO 3 (separation and purification), homogenization can be carried out by numerous operations, and these operations depend on the type of sample in-hand and the protocol that is being performed. This operation can be carried out by means of sonication, grinding, cutting samples from gels, and homogenization itself.

As with LOU 3, these homogenization methods can be broken down into different steps depending on the characteristics of the operations. For example: the integration of an operation that takes place inside the labware that contains the sample, such as sonication, is different than the integration of an operation that needs to be transferred to a different labware or equipment such as a homogenizer column or a grinder.

Again, the complete solution for the integration of the LUO in any case needs to include the manipulation of the

labware and the liquid handling of the sample and any other reagents needed to perform the operation.

2.8 LUO 8: Breaking/Fragmentation

Breaking and fragmentation refers to the breaking of cells and to the dividing of large molecules. The former is referred to as breaking and the latter as fragmentation. Operations such as lysis, mechanical or chemical cell disruption or fragmentation and sonication carry out breaking and fragmentation.

Since some of the operations used for breaking and fragmentation are also use for homogenization, the same integration challenges found for LUO 7 appear again for LUO 8. Even though LUO 7 and LUO 8 at first glance appear to be the same operation, since their goals are different, it is very important to distinguish between both LUOs. LUO 7 homogenization, aims to have a homogeneous solution by reducing the larger particles sizes; while LUO 8 breaking and fragmentation, only intend to break or fragment the sample, for example, lysis will break the cells' membranes allowing access to the RNA and DNA inside the cells.

Other operations used for this LUO present the same integration challenges such as loading and unloading the equipment, and the handling of reagent needed for, for example, chemical disruption.

2.9 LUO 9: Weighing

Weighing is defined as quantitative measurements of samples mass. This operation requires specialized equipment since the masses that are considered in biotechnology are very small (these could be as low as hundreds of picograms in terms of order of magnitude). This operation is performed by direct measurement.

In this case, the equipment is usually calibrated keeping in mind the labware used in the protocol in order to load the sample inside the instrument without having to transfer it from one place to another. The requirement that needs to be considered in this case is the interface of the equipment with the production line. There are some off-the-shelf scales available with feeder mechanisms that can be integrated by means of pick and place devices, but there are others that do not include a feeder mechanism making a pick-and-place device the only solution to load and unload the sample to and from the equipment.

2.10 LUO 10: Measurement and direct detection

Measurement and direct detection includes the direct measurement of physical properties such as pH, conductivity, absorbance and fluorescence. Some techniques to measure physical properties in biotechnology protocols include adding special dyes to the reaction. Then, by measuring the fluorescence and/or absorbance of the reaction and comparing it to a known desired outcome a physical property can be quantitatively measured. Other operations encompassed in direct measurements and detection are: pH measurements and conductivity measurements. There are two purposes for measuring physical properties of a reaction or sample. The first one would be to ensure and control that the protocol is carried out correctly. The second one, present in downstream protocols, is when the goal of the protocol is to measure the results of different reactions like when screening in drug discovery.

Whenever the purpose of measuring is to ensure that the protocol is being carried out correctly, measurements such as fluorescence, absorbance, and pH measurement involve the addition of reagents or manipulation of the sample that is not actually part of the protocol. For this reason these measurements are only performed to a sample of the samples, and these samples are kept for records or discarded, but do not continue in the process line since these have been tampered with. This is one of the reasons why in small-to-mid size biotechnology laboratories a close loop control is almost never performed: controlling the quality of the sample means discarding whatever has been tested and as already mentioned, one big issue with biotechnology automation is the limited quantity available of starting testing material (cells and tissues).

Whenever measuring the physical properties of the reaction is part of the protocol as in screening for drug discovery, the measurement is performed to all samples in order to process them. It is important to highlight that downstream protocols are beyond the scope of this paper. It is mentioned at this point to completely describe LUO 10, remembering that LUOs are the basic unit operations that describe any biotechnology protocol being an upstream protocol or a downstream protocol.

Going back to the problem in hand, integrating LUO 10 into the production line is significantly different from that of other LUOs. For once, after the operation takes place the sample is not put back into the production line, but instead it is stored in a different storage unit (or discarded). Also, it is not performed to all of the samples available.

2.11 LUO 11: Analysis and Data Extraction

Analysis and data extraction is defined as analysis of the outcome of the experiment using specific instrumentations. The operations included in LUO 11 are mass spectrometry, biochip analysis, and sequencer, among others. This LUO is more commonly used in sample processing protocols (downstream protocols). Operations that perform analysis and data extraction are carried out by specialized equipment that performs each of the operations, mass spectrometer, biochip screener, and sequencer. Labware need to be loaded and unloaded to and from the equipment by means of pick-and-place devices, or if the equipment has feeding mechanisms it can be integrated to a conveyor.

2.12 LUO 12: Documentation

Documentation in the context of biotechnology refers to creating the appropriate data documentation for retrieval and further analysis and reports. This operation is performed usually by database management software applications that can be commercial available solutions or customized for each laboratory. The integration of the documentation and data collection is available if management software is integrated to the equipment needed for the protocol. The completeness of the integration and the information collected is directly proportional to the integration of the management software to the equipment.

2.13 Summary

Table 1 summarizes the 12 LUOs, with the different operations and methods that carry out each, the equipment used in the methods, and the challenges encountered when integrating these LUOs into a production line.

Table 1. Summary of LUO methods and integration challenges.

LUO	Methods	Equipment	Challenges and highlights for integration
LUO 1	Manipulation of labware Capping/decapping, manipulation and deposition of different molecules	Pick-and-place devices, robotic manipulators and conveyors. Lid openers, cappers/decappers, microarreyers, and colony pickers among others	Manipulation of labware is the only operation that can stand by itself. This type of operation is carried out by different equipment where the sample needs to be loaded. Integration solution needs to include the loading and unloading of the labware to the equipment by means or a conveyor or a pick-and-place device depending on the equipments requirements.
LUO 2	Dispensing reagents, pipetting and aspiration samples.	Dispensers, pipettor and syringe.	Depending if the equipment use has feeding mechanisms or not, the complete integration of these operations involves a pick-and-place device or a conveyor.
LUO 3	Centrifugation and magnetic separation. Recrystallization and magnetic beads. Filtration, distillation, and electrophoresis.	Centrifuge and magnetic stand. Magnetic stand and different chemical compounds. Filtration systems.	Operations take place inside the labware the sample is in. The labware needs to be loaded into the equipment. For centrifugation because of the operation's principle, the centrifuge needs to be loaded by means of pick-and-place devices. These operations take place inside the labware containing the sample, but chemical reagents need to be added for the operation to take place. Afterwards the sample needs to be loaded into different equipment. These operations may consist in multiple steps and rounds. For these operations to take place, samples need to be transfer for it to flow through the actual device where the operation takes place (eg: a filter). It then has to be collected and transported to the next station.
LUO 4	Temperature control, atmosphere control, thermal cycling, and incubation.	Temperature, atmosphere, humidity sensor. Hotplate heater, incubator, refrigerator, oven, freezer, vacuum, fume hood, and PCR Machine.	One type of conditioning involves the conditioning of the laboratory room were the protocol takes place. The room usually has all necessary sensor integrated to the laboratory management software system to ensure proper conditions for the protocols to take place. A second type of conditioning is when the condition changes only for a given step in the protocol (eg: correct temperature for the enzymes to act). In this case the labware containing the sample needs to be manipulated inside the different equipment by means or feeding mechanisms or pick-and-place devices.
LUO 5	Washing and drying.	Washer, water bath, vacuum, autoclave.	Washing and drying of reusable labware operations are not part of a biotechnology protocol. They have to be taken into consideration because in order to carry out the actual protocol, labware needs to be available. Integration of these operations is done as a separate cell from the production line.

LUO 6	Mixing, vortexing, shaking, and stirring.	Vortex, shaker, stirrer, and rocker.	These operations are carried out inside the same tube they are in. Usually this equipment is not available with feeder mechanisms and has to be loaded and unloaded by means of pick-and-place actions.
LUO 7	Homogenization, gridding, cutting samples from gels. Sonication	Homogenization columns, blender, Mortar & pestle, chopper, razor blade. Sonicator	These operations are performed in more than one step that needs to be taken into consideration for integration. Liquid/sample handling operations need to be included to move the sample from the labware they are in, to where the operation actually takes place (eg: the sample needs to be move into a homogination column). These operations may require multiple manipulation steps as well. Depending if the equipment use has feeding mechanisms or not, the complete integration of these operations involves a pick-and-place device or a conveyor.
LUO 8	Lysis and chemical cell disruption. Mechanical cell disruption or fragmentation and sonication	Vortex, shaker. Mechanical disruptor, mortar & pestle, and sonicator	Liquid handling operations are needed for the addition of the reagents needed for the reaction to rake place. Loading of the labware into the equipment by means of pick-and-place action. Depending if the equipment use has feeding mechanisms or not, the complete integration of these operations involves a pick-and-place device or a conveyor.
LUO 9	Quantitative measurement of sample mass.	Balance and scale.	Depending if the balance used has or not feeding mechanisms, a pick-and-place device or a conveyor is needed.
LUO 10	Fluorescence and/or absorbance, pH measurements, and conductivity measurements.	UV lamp, UV cross linker, luminometer, fluorescent optical scanner, chemical reagents.	For upstream protocols: Measurements are not part of the protocol, and since different chemical reagents are added to observe the desired physical property, the samples that are tested do not continue into the production line (are either stored or discarded). Only a sample of the samples is tested. For downstream protocols: Depending if the equipment use has feeding mechanisms or not, the complete integration of these operations involves a pick-and-place device or a conveyor.
LUO 11	Mass spectrometry, biochip analysis, and sequencing, among others	Mass spectrometer, biochip screener, and sequencer, among others	Depending if the equipment use has feeding mechanisms or not, the complete integration of these operations involves a pick-and-place device or a conveyor.
LUO 12	Documentation and data collection	Data base management software	The integration will be successful as long as the equipment for the different protocol operations communicates between them.

3. COMMON CASES OF LUO INTEGRATION

Even though the twelve LUOs execute different operations, similarities as to how these carry out these operations can be established. In other words, the LUOs might use the same feeding mechanisms; carry out the operation within in the equipment or in separate containers, and so on. In this section, these similarities are taken into consideration and grouped together to find Common Integration Modules that represents and could incorporate into the production line different LUOs.

To integrate and automate any operation into a production line, issues regarding the operation principles of the LUOs have to be taken into consideration. It is vital to understand how the operation takes place, where the operation takes place, how many steps the operation has, the equipment used for the operation, and how the equipment is used for the operation works. The knowledge of the complete operation process provides the required information regarding the sequence of steps that need to be performed to carry out the given operation. Taking all the aforementioned considerations into account, a large amount of possible integrations of operations will arise and have to be considered.

LUOs can play three main roles within a protocol: LUOs that their main function is to move labware, compounds, reagents, and samples around the production line; LUOs that process the samples to obtain the required outcome; and LUOs that handle the data measurements and data collection of the protocols. For integration and automation purposes, LUOs that process the samples and collect data can be addressed using the same approach. In these operations samples need to be loaded or transferred to a different equipment or location. For these LUOs, the automation solution involves the arriving of the sample (loading of the sample or transferring sample) to the station, the operation itself, and the departing of the sample (unloading the sample or transferring sample) from the station. Manipulation operations that require specific equipment, such as a capper/decapper, lid openers, microarrayers, or colony picker, can also be automated using the same approach.

Automation of LUO1 (manipulation), which main function is to move around or transport labware throughout the production line, has long been solved by means of robotic manipulators, pick-and-place devices, or conveyors, and all their possible combinations. For this reason, this paper will not center on this matter, but instead will use these results to discuss the automation for the remaining LUOs. There are, also, two special cases for LOU automation, washing and drying and documentation, which will be discussed at the end of the section.

LUOs use more than one method to carry out the different operation (For example, there are more than one separation method.) as a result a generalization for each LOU is not possible. Instead, different LUO may have constraints and requirements common among them and can be group together to formulate a single automated solution for each group. Taking into consideration the 12 LUOs and the most common methods for each of them, implementation modules where created. Table 1 helps in identifying common constraints and requirements between the 12 different LUOs.

Each LUO is first broken down into the set of steps required to perform the given operation. Taking as an example LUO 3 and using filtration, the steps that have to be followed to perform separation are:

1. The sample arrives at the station.
2. The sample is taken out of the original labware and put into the container where the filter is present.
3. The sample goes through the filter and is collected in a new labware placed underneath the filter.
4. The new labware containing the filtered sample is taken to the following station or interface location (sample leaves the station).

As shown in the example above, the actual separation only takes place in step 3, but the sample has to go through two prior steps before separation actually takes place. These prior steps can also be expressed in term of the LUOs. The sample arrives to the station by means of labware manipulation (LUO 1), and is then transferred to a second container by means of liquid handling (LUO 2). At the end (step 4), the sample has to leave the station by means of labware manipulation (LUO 1). In summary, to automate a separation operation (LUO 3) using filtration method, the implementation module consists of the following set of operations: LUO 1, LUO 2, LUO 3, and LUO 1.

After the LUO is broken down into steps and the possible LUO implementation module is defined, the equipment needed for each of the steps is identified. As stated earlier, the manipulation of the labware throughout the production line is dictated by the requirements of the equipment itself. Going back to the example, the requirements of the actual filtering operation, dictates how the labware's manipulation and liquid handling operation is carried out. For filtration to take place a vacuum filter is used. A vacuum filter is a device that consists of a first chamber with a filter, where the sample is initially pour; a container or second chamber attached to the first one underneath the filter to collect the sample once it has flow through the filter; and a vacuum valve that is attached to the container to ensure that all the samples flow through the filter in a timely manner. A vacuum filter has no feeder mechanism to facilitate its integration into a production line. This device requires the sample to be transfer from its original labware to the device by means of a liquid handler, which can aspirate the sample from where it is originally contained and dispense it to the vacuum filter. This operation can be carried out by an automated dispenser. The dispenser also needs to be integrated into the production line. Dispensers with the capability to perform this task may or may not have feeder mechanisms; as a result, they can be integrated into the production line by means of a conveyor or pick-and-place device. Finally, the labware where the sample is collected has to be taken into the next station for further processing. As the vacuum filter has no feeder mechanisms of any kind, this operation is performed by a pick-and-place device that takes the sample to the next location. To sum up, the implementation module consists of a manipulator, a dispenser, a vacuum filter, and a pick-and-place device. Notice that because depending of the dispenser used, there are to possible solutions at this point: if the disperser is loaded by means of a pick-and-place device, or fed by means of a conveyor.

After the implementation module and the equipment needed for the automation of the desired LUO is identified, the manipulation requirements of the previous station and next station need to be addressed to fully integrate the desired operation into a production line. Considering the requirements of the previous station, there are two possible

scenarios. One scenario is when the previous station and the station that is being integrated have the same manipulation requirements to interact between them; in other words, that the sample leaves the previous station by means of a pick-and-place device and enters the station of interest by the same means, or that the sample leaves the previous station and enters the station of interest by means of a conveyor. This scenario is very straight forward and means that the sample can enter the station of interest coming directly from the previous station. The second scenario is when the previous station and the station of interest do not have the same manipulation requirements to interact between them; in other words, the sample leaves the previous station by means of a pick-and-place device and enters the station of interest by means of a conveyor, or vice versa. In this scenario, a sample exchange station to change the manipulation method needs to be included before the station of interest as an interface with the station. At this interface, a pick-and-place device drops the labware on a conveyor belt, or it picks up the labware from a conveyor to load the station of interest. The consideration of the requirements of the next station will throw the same conclusions.

In summary, the implementation module to automate the separation operation by means of filtration consists of a manipulator (LUO 1), a dispenser (LUO 2), a vacuum filter (LUO 3), and a pick-and-place device (LUO 1). In this example, since dispensers may or may not have feeder mechanisms, a specific dispenser may be chosen depending on the manipulation requirements of the previous station to avoid a sample exchange station. In the other hand, since after the operation has taken place, the labware needs to be manipulated by means of a pick-and-place device, the requirements of the next station dictates the need for an interface station or not. As illustrated in this example, even though the manipulation of the labware has more than one solution, it is considered to be the same implementation module since the group of LUOs needed is the same.

Following the aforementioned approach, basic implementation modules can be generalized. Table 1 shows that LUOs 1, 2, 6, 9, and 11, have only one implementation constraint and one implementation module can be use for all these operations. LUOs 3, 7, and 8, even though for some of the operation that carry outs these LUOs uses the same implementation solutions than the aforementioned LUOs, these three LUOs have more than one solution for other operations. And lastly, table 1 also shows that LUOs 4, 5, and 10, have special cases implementations. In summary, four common implementation modules and three special cases implementation modules are drawn to integrate any of the twelve biotechnology LUOs.

3.1 Implementation Module 1

The most common scenario found whenever aiming to integrate an LUO into a production line is that the operation is performed by specific equipment. In this case, the only issue left to consider for automation is how the sample is placed into the equipment before processing and how it is removed after it has finished. In general, the implementation module in this case includes LUO 1, followed by the equipment that carries out the operation for the given LUO, followed by LUO 1. Which form of LUO 1 is used depends on the requirements of the equipment itself and the stations that presides and follows the operation. The equipment is chosen among the off-the-shelf equipment available that can perform the given operation.

Some off-the-shelf equipment requires being loaded from the top because their operation principle doesn't allow them to have side openings, such as centrifuges or vortexes; others, simply have been designed and developed with no feeder or interface mechanisms with conveyors and also requires a pick-and-place device to put the sample in place for the operation to happen. In the other hand, some equipment may have a feeder mechanism that allows an easy interaction and integration with a conveyor belt, such as dispensers or incubators. Common equipment found in the literature for the different LUOs that could be integrated using Implementation Module 1 is shown below.

- LUO 1: capper/decapper, lid openers, and any other manipulation that requires a different equipment to handle labware.
- LOU 2: dispensers, pipetters, and syringes if the operation performed is addition of a reagent or compound to the labware that already holds the sample.
- LOU 3: centrifugation and separation via magnetic stands.
- LOU 4: hotplate heaters, incubators, refrigerators, ovens, vacuums, and PCR machines among others.
- LUO 6: shaker and vortex among others.
- LUO 7: sonicator.
- LUO 8: sonicator and vortex.
- LUO 9: balance.
- LUO 10: luminometer, optical scanner among others (not for upstream protocols)
- LUO 11: mass spectrometer and biochip scanner.

The following general set of steps can be concluded when breaking down the aforementioned LUOs' methods into the set of steps required for performing the given operation:

1. The labware arrives to the station and is placed or loaded inside the equipment.
2. The equipment performs the operation.
3. The labware is removed or unloaded from the equipment and moved to the next station.

For the LUOs that follow these set of steps, the actual operation is carried out in step two. The LUOs (methods) pertaining to this scenario have an implementation structure that follows the configuration LUO 1, LUO of interest, and LUO 1. This configuration can be evaluated using the Arena model shown in Figure 1.

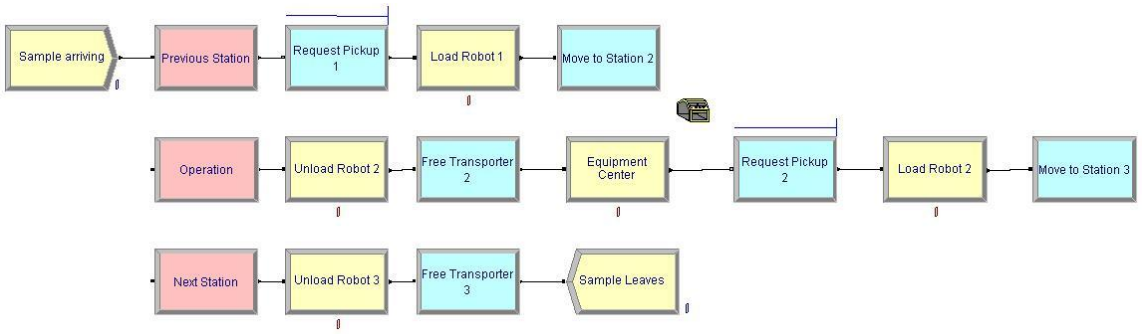

Figure 1. Implementation Module 1 (ARENA Model)

Figure 1 shows the Arena module used to evaluate Implementation Module 1. This module simulates a sample arriving from a previous station (LUO 1), the operation that takes place, and the sample leaving the station (LUO 1).

3.2 Implementation Module 2

A second possible solution scenario found when integrating an LUO to a production line is common to LUO 3 and 7. This module is used when the operation is not performed inside the labware containing the sample, but it needs to be transfer into a device for the operation to take place. The following operations can be integrated using this module:

- LUO 3: filtration
- LUO 7: homogenization

To perform these operations and integrate them into a production line, the following steps must be followed:

1. The sample arrives to the station.
2. For filtration, the sample is taken out of the original labware and into the container where the filter is present; for homogenization the sample if dispensed into a homogenization column.
3. The sample goes through the corresponding device and is collected in a new labware placed underneath filter or homogenization column.
4. The new labware containing the filtered sample is taken to the following station or interface location (sample leaves the station).

The common module used for both of this operations will include the following LUO sequence: LUO 1, LUO 2, LUO of interest (LUO 3 or LUO 7), and finally LUO 1. The Arena model use to evaluate and validate this implementation is shown in figure 2.

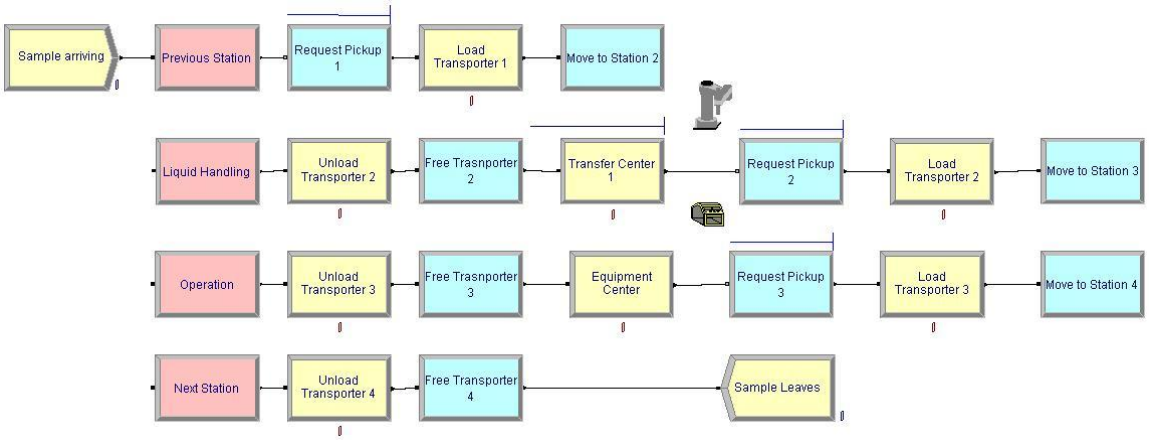

Figure 2. Implementation Module 2 (ARENA Model)

In this case, the actual operation takes place in step 3, but the manipulation of the labware and the transferring of the liquid sample are essential for the integration of the operation into a production line

3.3 Implementation Module 3

Other operations that carry out LUOs 3 and 8 need addition of reagents into the sample before the actual operation can take place. This is the case for the following operations:

- LUO 3: magnetic bead separation
- LUO 8: chemical disruption

These operations follow the following common steps:

1. The labware arrives to the station and is placed in the dispenser device.

2. In the case of magnetic separation, the bead is dispensed into the labware containing the sample; in the case of chemical disruption, the chemical disruptor is dispensed into the sample.

3. The labware with the sample is placed into the equipment that actually performs the operation, magnetic stand for LUO 3 and mechanical disruptor for LUO 8.

4. The equipment performs the operation

5. The labware is removed from the equipment and placed in a dispenser.

6. The sample of interest is transfer into a new tube (labware).

7. The labware is transported to the next station.

The sequence of LUO needed to integrate these operations into a production line is more complex as more steps are needed to carry them out. The sequence is: LUO 1, LUO 2, LUO 1, LUO of interest, LUO 1, LUO 2, and LUO 1. The Arena model used to evaluate this implementation module is shown in figure 3.

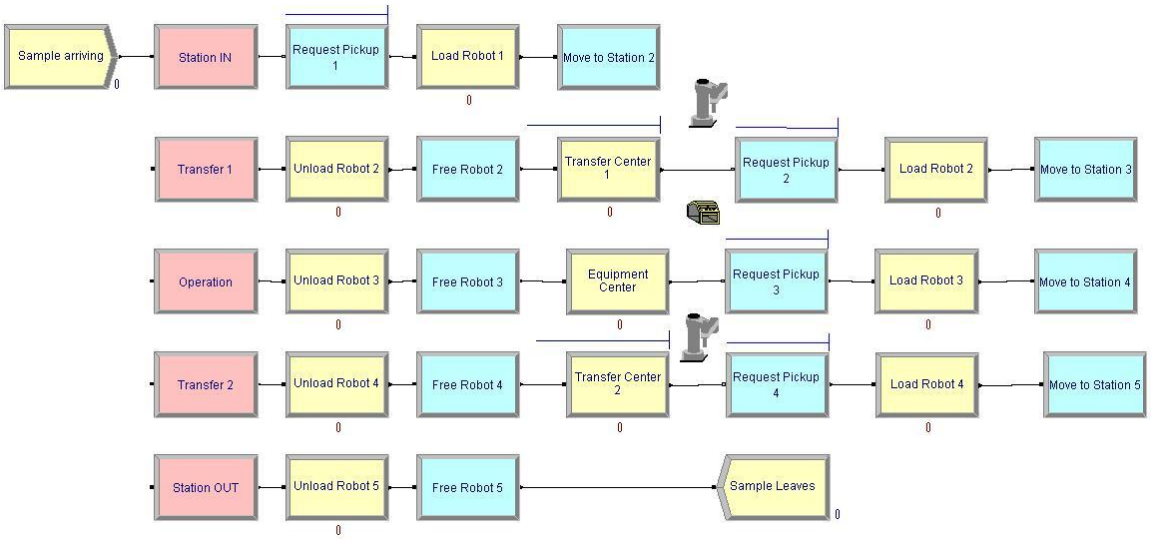

Figure 3. Implementation Module 3 (ARENA Model)

3.4 Implementation Module 4

Operations that are integrated using this last common module are:

- LUO 7: grinding, and cutting
- LUO 8: fragmentation

These operations follow the following common steps:

1. The labware arrives to the station.
2. The sample is transferred into a different labware that can adjust to the equipment.
3. The labware with the sample is placed into the equipment that actually performs the operation.
4. The equipment performs the operation
5. The labware is removed from the equipment.
6. The sample of interest is transfer into a new tube (labware).
7. The labware is transported to the next station.

The sequence of LUOs used in module 4 is the same as the ones used for module 3. Two different modules exist because even thought step 2 for both modules is LUO 2, in module 3 the operation needed is the dispensing of a reagent into the labware containing the sample; and in this case, the operation that is needed is the transferring of the sample from the current labware, to where the operation actually takes place. Figure 4 shows the Arena module that simulates and validates this solution.

The sequence of LUOs needed to perform this solution is: LUO 1, LUO 2, LUO 1, LUO of interest, LUO 1, LUO 2, and LUO 1.

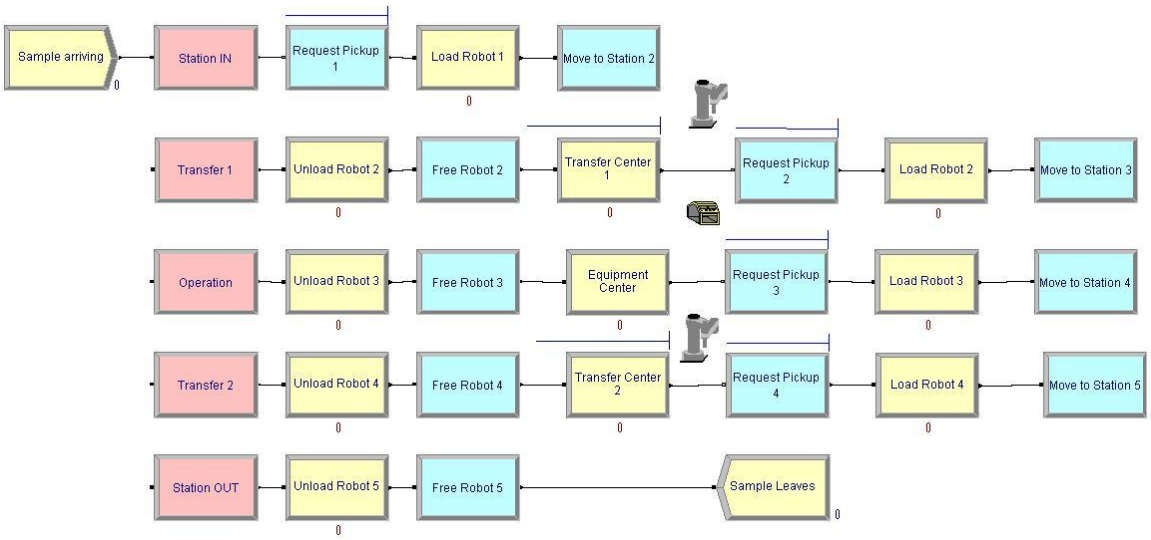

Figure 4. Implementation Module 4 (ARENA Model)

3.5 Implementation Module 5 (Special Case)

Implementation module 5 is a special case and is the solution to integrate LUO 5. As explained in the section before, washing and drying of reusable labware operations are not part of a biotechnology protocol per say. They have to be performed in order to have the labware available for the actual protocol. This is why this LUO is implemented as a separate cell from the actual production line. There is no interaction between this module and any other in the production line. Even though this is the case, to automate this operation the same process as before is followed.

LUO 5 has the following step sequence:

1. The labware arrives to the station and is placed inside the washing equipment.
2. The equipment performs the washing operation.
3. The labware is taken out of the washing equipment and into the dryer.
4. The equipment performs the drying operations.
5. The labware is removed from the equipment and taken to a storage unit.

The sequence of LUOs that is needed to perform this operation is: LUO 1, LUO 5, LUO 1, LUO 5, LUO 1.

The Arena module used for this implementation is shown in figure 5.

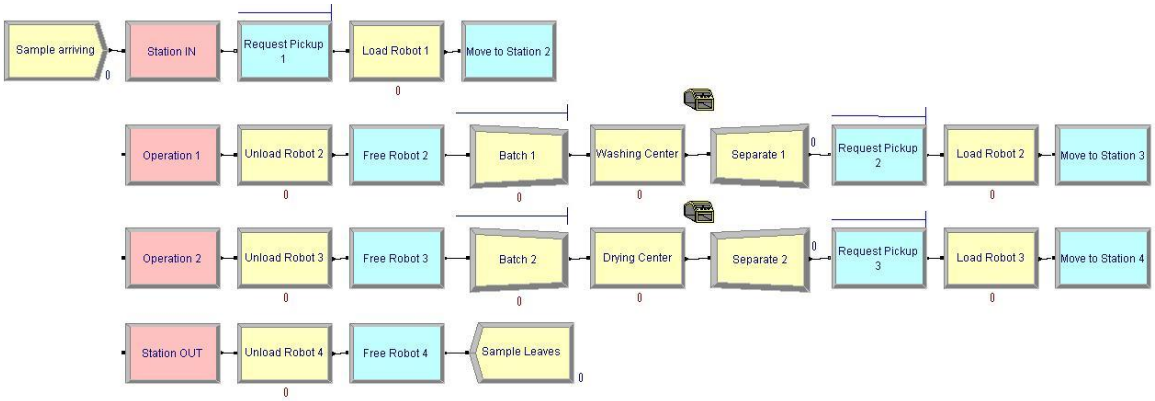

Figure 5. Implementation Module 5 (ARENA Model)

There is some of-the-shelf equipment that can perform both operations (washing and drying) inside the same piece of equipment. If the same equipment performs both the washing and the drying, the sequence of LUOs that is followed then is LUO 1, LUO 5, and LUO 1. This sequence can be modeled using Implementation Model 1. In this case it would have to be used as a standalone cell for the same reasons already discussed.

3.6 Implementation Module 6 (Special Case)

As explained in the previous sections, there are two types of conditioning (LUO 4). One is the actual conditioning of the laboratory, and the second one is the conditioning of a specific step in the protocol.

For the condition of the laboratory, heating units, air conditioning units, and humidifiers, among others are used. These are controlled by means of environmental sensors located around the lab such as thermometers, humidity sensors, pressure sensors, and so on. For this type of conditioning there is no Implementation Module required.

The conditioning of a specific step of the protocol can be just that the sample needs to be incubated, heated, frozen, or any other

condition change just to the sample. This is carried out with Implementation Module 1 as already explained because the sequence of steps for such operations is: LUO 1, LUO 4, and LUO 1.

There is a second possibility where an operation needs to be carried out at a different condition, for example, a centrifugation that needs to be carried out at lower than room-temperature temperature. In this case, there are extra steps needed to perform that same operation that includes the LUO of interest and LUO 4. Extra manipulation steps are required for this purpose. There various examples for this kind of conditioning and they cannot be all generalized in the same way. This is why when the designer encounters this kind of operation, the designer would have to develop the integration model following the steps described in this paper.

3.7 Implementation Module 7 (Special Case)

The last module is used to integrate LUO 10 in upstream protocols. This is a special case because as it was discussed in a previous section, these operations for upstream protocols are not performed to all of the samples and once the sample is taken from the production line is not put back into normal circulation.

The sequence of LUOs for this module is the same as for Implementation Module 1, but after the sample is processed it won't continue into the next station of the protocol, but it would go to a different storage unit or discarding station.

This operation follows the sequence:

1. One sample every given number of samples arrives to the station and is placed inside the equipment.
2. The equipment performs the pH measurement
3. The sample is removed from the equipment and stored or discarded.

Figure 6 shows the Arena module for validation of implementation 7. The sequence of LUOs for this operation is therefore LUO 1, LUO 10, and LUO 1.

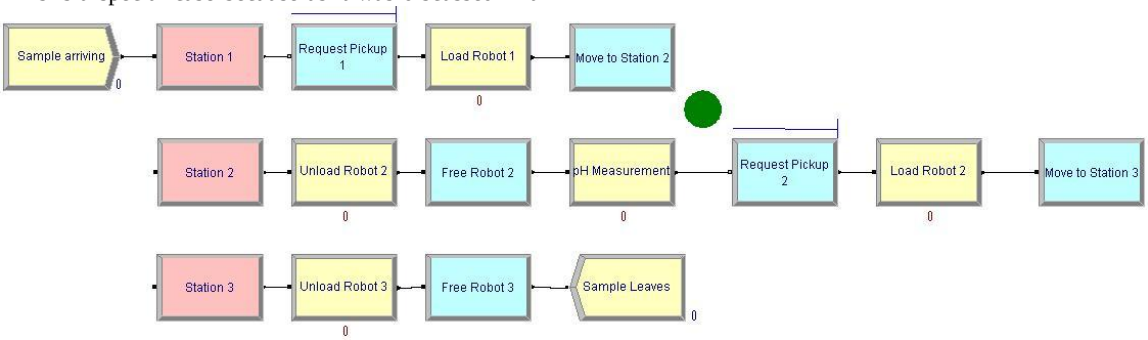

Figure 7. Implementation Module 6 (ARENA Model)

4. INTEGRATION OF IMPLEMENTATION MODULES

The idea behind the description of the Common Implementation Modules and the Special Case ones, is that having a Biotechnology protocol that needs automation, one can identify the LUOs needed for that protocol and using the Implementation Modules that already exist, use them as building blocks to simulate and validate the complete protocol that later could be use for automation.

To integrate these Implementation Modules into a production line and be able to simulate a complete Biotechnology protocol the following steps have to be followed:

1. Identify the LUOs used in the protocol
2. Identify the method used for each LUO.
3. Identify the Implementation Module that could be used to integrate the operation
4. Depending on the transportation and manipulation of the labware throughout the protocol, get rid of any redundant blocks to find a better solution.

4.1 Complete Example: Preparation of RNA from Tissue Culture Cells

Preparation of RNA from tissue-culture-cells protocol that was chosen as an example consists of 20 steps. As our interest in this paper is only to identify the LUOs of the protocol, there is no need to get into great detailed about the components, reagents or quantities used. The example is only been look at from an automation point of view [1].

The 20 steps for the preparation of RNA are [2]:

1. Retrieval of one tube full of frozen cells.
2. Addition of 350 µl of RLT buffer followed by gentle vortexing until cells are lyzed.
3. Transferring of the lysate into a QIAshredder [6] spin column placed in a 2ml collection tube.
4. Centrifuging of the tube for 2 minutes at 14,000 rpm
5. Addition of 350 µl of 70% ethanol to the lysate in the collection tube.
6. Transferring of the combined 700µl, including any precipitate that may have formed, to an RNeasy mini column in a 2ml collection tube.
7. Centrifuging of the tube for 15 seconds at 14,000 rpm.
8. Discarding of the flow-through and reattaching the collection tube to the mini column.
9. Addition of 700µl of buffer RW1
10. Centrifuging of the tube for 15 seconds at 14,000 rpm.
11. Discarding of the flow-through and the collection tube.
12. Transferring of the RNeasy mini column to a new 2ml collection tube.
13. Addition of 500µl of the wash buffer RPE onto the column.
14. Centrifuging of the tube for 15 seconds at 14,000 rpm.
15. Discarding of the follow-through and reattaching the collection tube to the mini column.

16. Addition of another 500µl of the wash buffer RPE onto the column.
17. Centrifuging of the tube for 2 minutes at 14,000.
18. Addition of 50µl of RNase free water and attaching a new collection tube.
19. Elution of the RNA from the column by centrifugation for 2 minutes at 14,000 rpm.
20. Storage of the collecting tube.

4.1.1 Identification of LUOs used, method used for each operation, and possible implementation module for each LUO

First, to identify each LUO, each step and operation needs to be understood. Then, the method used for each operation needs to be identify. The next step is to identify which Implementation Module (IM) could be used for each of the LUOs and steps present in the protocol. The LUOs, methods and IM for each step are:

- Step 1: LUO 1 – pick-and-place device
- Step 2: LUO 8 – Vortex, IM 3
- Step 3 and Step 4: LUO 7 – Homogenization column, IM 2
- Step 5: LUO 2 – liquid dispenser, IM 1
- Step 6: LUO 2 – pippetor, IM 1
- Step 7: LUO 3 – centrifuge, IM 1
- Step 8: LUO 1 – labware dispenser, IM 1
- Step 9: LUO 2 – liquid dispenser, IM 1
- Step 10: LUO 3 – centrifuge, IM 1
- Step 11: LUO 1 – labware discarder, IM 1
- Step 12: LUO 1 – pick-and-place device
- Step 13: LUO 2 – liquid dispenser, IM 1
- Step 14: LUO 3 – centrifuge, IM 1
- Step 15: LUO 1 – labware dispense, IM 1
- Step 16: LUO 2 – liquid dispenser, IM 1
- Step 17: LUO 3 – centrifuge, IM 1
- Step 18: LUO 2 – liquid dispenser, IM 1
- Step 19: LUO 3 – centrifuge, IM 1
- Step 20: LUO 1 – pick-and-place device

4.1.2 Remove redundancies

The remove redundancies each implementation module has to be written in the sequence to be implemented with its steps. The manipulation of labware between them is looked at and some of these steps will be removed. For example, looking into steps 1 to 3 in detailed we get as follows:

- Step 1:
    1. The labware is retrieved from a storage unit
- Step 2:
    1. Labware arrives to the station and is placed in the dispenser device.
    2. The chemical disruptor is dispensed into the sample.
    3. The labware with the sample is placed into the vortex.
    4. The equipment performs the operation
    5. The labware is removed from the equipment and placed in a dispenser.
    6. The sample of interest is transfer into a new labware.
    7. The labware is transported to the next station.
- Step 3:
    1. The sample arrives to the station.
    2. The sample if dispensed into a homogenization column.
    3. The sample goes through the homogenization column device and is collected in a new labware placed underneath the homogenization column.
    4. The new labware containing the sample is taken to the following station.

Inspecting the above example, the labware that is retrieved from the storage unit (step 1), can go straight to the dispenser device (step 2), that way only one manipulation operation is perform, hence to remove redundancy, only one pick-and-place device is needed between Station 1 and Station 2. Likewise, as the sample is transferred into a new labware and that labware goes to the next station, that same pick-and-place device leaves the sample into the dispenser unit. These three steps with 12 sub-steps can be reduced to 10 sub-steps:

1. The labware is retrieved from a storage unit and is placed in the dispenser device.
2. The chemical disruptor is dispensed into the sample.
3. The labware with the sample is placed into the vortex.
4. The equipment performs the operation
5. The labware is removed from the equipment and placed in a dispenser.
6. The sample of interest is transfer into a new labware.
7. The labware is transported to the next station.
8. The sample is transferred into a homogenization column.
9. The sample goes through the homogenization column device and is collected in a new labware placed underneath the homogenization column.
10. The new labware containing the sample is taken to the following station.

By doing this with the rest of the 20 steps and stations, the redundancy will be reduced as some manipulation equipment can be share between stations.

When building the ARENA model [3] with the different implementation Modules the process is the same. Each module is placed one underneath the other in the correct order and the redundant blocks can be erase to remove redundant equipment.

4.2 ARENA Model for Preparation of RNA

The complete ARENA model for this example is presented in figure 7.

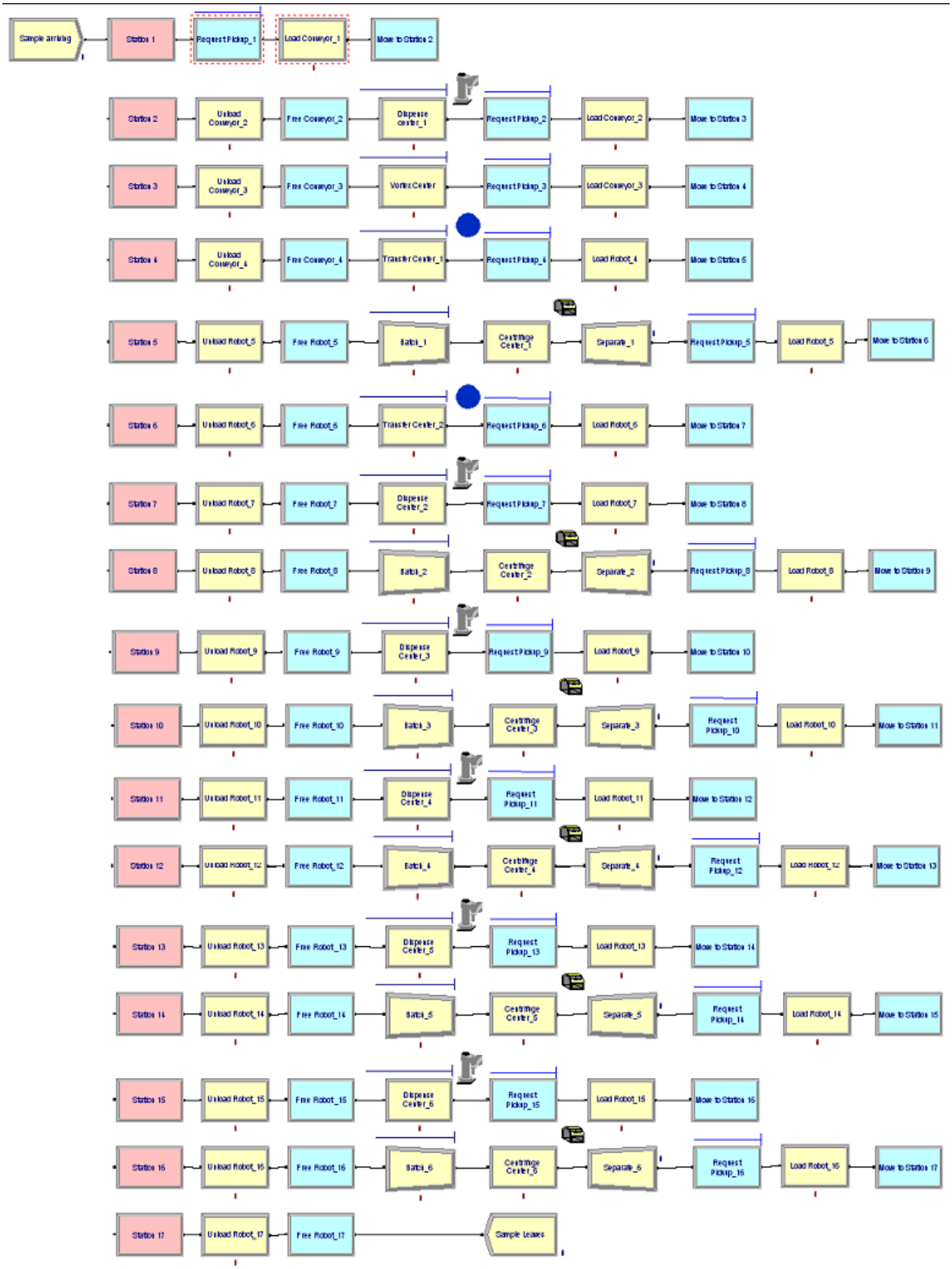

Figure 7. ARENA Model for Preparation of RNA

This simulation was carried out using the simplest possible manipulation method for each of the instruments used. This comprises conveyors for the operations that allow use of conveyors, and simple pick-and-place device for the instruments that absolutely demand it. Operations and equipment such as dispensers and automatic pipettes can be fed by means of conveyors and feeder mechanisms, while centrifuge and vortex definitely need pick-and-place devices. This configuration uses: one storage unit, six dispensers, one vortex, two automatic pipettes and column stands, and six centrifuges, a conveyor belt that goes around all 17 stations, and 7 pick-and-place devices that manipulates the labware from the feeders mechanisms in the conveyors to the vortex and centrifuges. Each pick-and-place device will serve one station and one instrument.

This model is simulated and validated using ARENA and its feasibility is demonstrated. The model for Preparation of RNA will be able to process 234 samples per 8 hours shift, taking on average 124 seconds per sample.

5. Future Directions

Customized equipment and equipment that can perform more than one operation at the time hasn't been addressed yet. Can Generic Implementation Modules be obtained to completely describe all equipment available in the market? Or do more general guidelines have to be drawn to be able to encompass a larger range of equipment? Also some automation operation hasn't been included into the twelve Biotechnology LUOs. It would be a good idea to include operations such as labeling steps that would help described better a complete automation for a Biotechnology protocol.

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