Design Considerations in High-Throughput Automation for Biotechnology

Aura-Maria Cardona and Zvi S. Roth
Department of Electrical Engineering
Florida Atlantic University
777 Glades Road
Boca Raton, FL 33431
acardon5@fau.edu, rothz@fau.edu

ABSTRACT
This paper examines automation design issues related to a simple Biotechnology protocol case study. The example chosen is the preparation of RNA from tissue-cultured cells. The starting point in automation design is to first set the process as a manual labor production line and identify processes that can be automated. The paper explores the vast range of possible solutions, some may be robotic-based, and optimality issues that arise when designing a flexibly automated high-throughput systems for a given family of Biotechnology protocols. The paper examines the potential application of Group Technology to Biotechnology applications. It also explores a specific Industrial Engineering program called Arena as a potential computer-aided tool to address the problem of optimal automation design considering cost, foot print and throughput constraints.

Keywords
High-Throughput protocols, Automation, Biotechnology, Group Technology, Arena software.

1. INTRODUCTION
Much industrial experience gained over the years in automation design for automotive, consumer electronics and many other applications has formed the basis of Industrial Engineering now viewed as a mature field of engineering [4]. The design of any automation system strives to minimize capital and operational costs as well as floor space and maximize throughput, yield and product variety. The latter is referred to as Flexible Automation. This multi-objective optimization problem is large-scale thus often requiring computer-aided design. The optimization often starts with a construction of an ad-hoc "feasible solution" that meets a given set of cost, space and throughput constraints and proceeds with incremental design modifications aimed at yielding incremental performance improvements.

Biotechnology instrumentation has attracted significant attention in the past few years. Following the completion of genome sequencing of many organisms, and in order to decipher the function of genes and proteins, demand for high-throughput experimentation and protocols became incredibly large.

Automation plays an increasingly important role in Life Sciences and especially in Biotechnology. With advances in automation, the human genome and other genomes have been sequenced. Modern Molecular Biology and Biotechnology have contributed to new assays that, when automated, provide more accurate and rapid large amounts of information. Similarly, the pharmaceutical industry is heavily dependent on automation, especially as it shifts from products that treat diseases, to analytical methods that detect and classify diseases. Automation for the Life Sciences is therefore broad and includes fluid handling and assay processing, high-throughput screening and drug discovery, high-throughout production and analysis of protein and DNA microarrays, devices for analyzing living cells, lab-on-a-chip analysis tools, and numerous detection methods.

Much on-going basic research evolves around specific subsets of such high-throughput automation systems. This includes study and development of automated lab-on-a-chip, automated systems for liquid handling, fermentation reaction and process automation, genomics and proteomics software automation, DNA and protein micro-array fabrication automation, pharmaceutical fabrication and drug screening automation, detection technologies that enable automation for biological processes, automated systems for DNA, proteins, and cell manipulation and analysis, automated scanning problem microscopy-based systems for bio-applications, liquid chromatography (LC) and mass spectrometry (MS) bioinstrumentation automation, and system integration including interconnects and interfaces among automated modules.

Our chief concern in this paper is Industrial Engineering considerations in the design of the full automation system for such applications, as is done in [9], where Nam Suh's Axiomatic Theory [13] is applied to high-throughput automation for both, "upstream" (sample preparation) and "downstream" (sample analysis) protocols. It was found that due to the intrinsic constraints to both common architecture approaches found in laboratory automation, that is robotic-based and track-based, the possibly best solution is to develop a system that uses both architecture approaches for a flexible laboratory system.

We decided to narrow down the scope of the paper to the upstream (sample preparation) protocols taking part in Biotechnology. Questions that arise, motivated by [9], are: How can we fully automate high-throughput protocols for Biotechnology? What are the constraints and the goals? Where are the challenges and bottlenecks leading to protocols that must be improved or overcome with automation?

Section 2 describes some of the relevant literature sources. Section 3 defines the problem of "Design Considerations in High-Throughput Automation for Biotechnology" and related research issues. Section 4 contains a detailed case study example for a manual-labor protocol preparation line. It hypothesizes how each station is to be automated. Section 5 focuses on Group Technology issues in Biotechnology. Section 6 introduces the Arena software and its potential use for automation design for Biotechnology applications. The last section discusses open issues and possible future work.

2. LITERATURE REVIEW

Najmabadi et al [9-10] provide a system level study of high-throughput automation for both upstream and downstream protocols in Biotechnology. These works apply Axiomatic Design Theory [13] to the design and evaluation of candidate architectures for Biotechnology laboratory systems. Such design theory directly addresses laboratory automation hardware flexibility. Two architectures presented in [9] are that of Total Modular Laboratory Automation (TMLA) and that of Distributed Operations Laboratory Automation (DOLA). Both describe a Tower-based Architecture (see Figure 1).

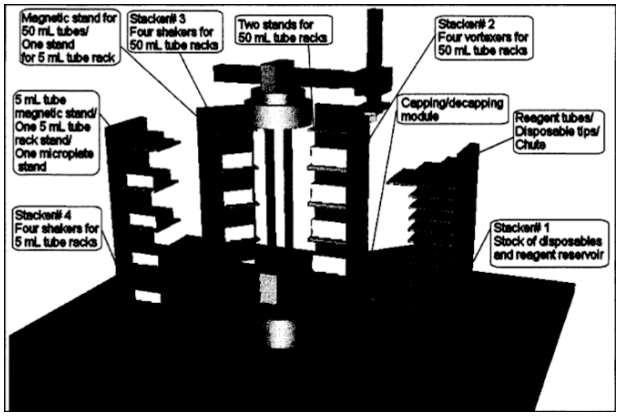

Figure 1. Tower-based automated work-cell conceptual design for magnetic protein complex purification protocol [9].

According to [9] the design of Biotechnology automation systems evolves around automation of generic operations and processes termed as Laboratory Unit Operations (LUO's). Regardless of the specific applications or protocols these LUO's are the building blocks for most, if not all, known laboratory protocols. The next sections demonstrate some of these LUO's.

The University of Washington ACAPELLA system [8] takes DNA (or other biochemical samples) and automatically processes it as specified in a user-defined protocol. Available processing techniques include aspiration, dispensing, mixing, thermal cycling, and imaging. The instrumentation of ACAPELLA includes a rotary table, glass capillaries, low-volume disposable containers, and piezoelectric dispensers for precision delivery of reagents (see Figure 2). The system successfully demonstrates high-throughput preparation of restricted enzyme digestion, polymerase chain reaction (PCR), and sequencing reaction for genome analysis.

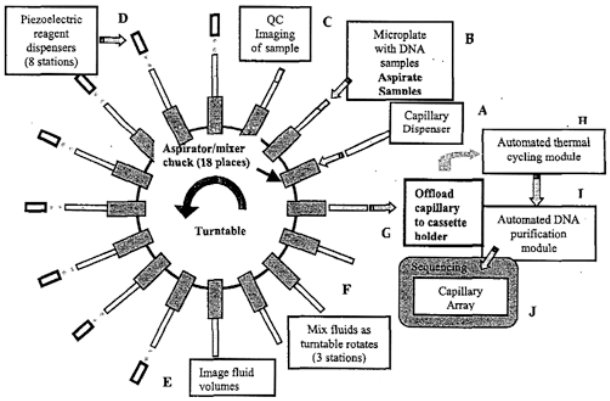

Figure 2. Functional schematic of fluid sample handling with the ACAPELLA-5K automated system [8].

The design team of ACAPELLA-5K developed a real-time PCR test-bed for the analysis of up to 48 1µl to 2µl reactions in glass capillaries. This PCR test-bed is suitable for high-throughput experimentation when used as a downstream module for ACAPELLA-5K. The test-bed features a laser-induced fluorescence scanner with high-sensitivity photomultiplier tubes for the detection of three spectral wavelengths. The scanner is used to monitor the reactions taking place inside the test-bed, in order to detect the copied genes.

The Translational Research Institute at the Scripps Research Institute – Florida [6] enables drug-target lead identification via ultra-high-throughput (see figure 3) screening technology. Using state-of-the-art instrumentation Scripps scientists develop and execute biochemical and cell-based high-throughput screening assays in a miniaturized microliter plate format.

Figure 3. The Scripps Research uHTS platform. a) industrial anthropomorphic robotic arm, b) pin tool, c) liquid handlers, d) incubators, e) multimode plate reader, and f) kinetic imaging plate reader [6].

Other related works in Biotechnology automation include the monitoring, sensing, and control of the processes at the University of Washington [11] and University of Munich [3]; and other upstream operations such as new liquid dispensing systems at the Robotic Institute of HIT [7], and dispensing for DNA microarrays at the New Jersey Institute of technology [2].

3. PROBLEM DESCRIPTION

In any automation design, regardless of application and discipline, features such as desired throughput, size of parts, tolerances, precision, timing and procedures involved, among many others, have to be taken into consideration. It is obvious that automated solutions for automotive production line, electronic assembly, integrated circuit production, and high-

throughput Biotechnology protocols are very different. The environmental conditions at which the processes involved have to take place (temperature, humidity, cleanliness, etc), the sizes and amounts of the parts and samples, and the reaction times for some processes may dictate different robot end effectors (for instance) for different applications.

Let us take for example the fabrication of integrated circuits (IC). IC fabrication has to be done in clean rooms, the equipment employed is very specialized, has tight precision requirements, and most of the steps are intrinsic to IC fabrication (meaning that these are only used for IC fabrication). Some of the key characteristics of Biotechnology applications may include the very small sample size, and special the extra special care needed sometimes in order to manipulate the samples.

The most common and basic protocols used in Biotechnology are: DNA isolation, construction of recombinant plasmid, PCR, transcription of genomic DNA and analysis of the resulting mRNA, transformation and gene expression, and analysis of DNA and RNA. These protocols have common steps that can be automated such as the addition of a given reagent to a sample. Some challenging steps for automation and bottlenecks may include the loading of specific equipment such as centrifuges, and the time taken for some of such processes to complete.

By increasing the throughput in Biotechnology, one is able to produce more samples in a shorter amount of time and perform more genomic and proteomic experiments. Reagents are expensive and often large amounts of viable samples may not be available. Therefore one of the goals of Biotechnology automation is to reduce the amount and size of the reactions. Along with sample volume reduction several benefits such as improved repeatability, quality and efficiency become possible as well.

Automation design can be cast as a multi-objective multi-constraint optimization problem. The objectives are to increase throughput, reduce size of samples (which will decrease cost), and try to have a flexible system that could perform more than one type of experiment and be easily reconfigurable. There are typically multiple constraints such as limited footprint, a limited budget, and the protocol-specific constraints (i.e. timing, reaction conditions and environmental condition constraints.)

Twelve common generic operations and processes (termed LUO's) are identified in [9]:

1. Manipulation: Physical handling and transportation of lab ware.
2. Liquid handling: Such as that of assay reagents, samples and buffers.
3. Separation or Purification: separation of specific molecules or particles from a solution.
4. Conditioning: Modifying and controlling the sample environment.
5. Washing/Drying: washing and drying of reusable lab ware
6. Agitation: Blending reagents together or with other molecules or particles.
7. Homogenization: Reducing sample particles size and creating a uniform sample.
8. Breaking/Fragmentation: Breaking cells or dividing large molecules.
9. Weighting: quantitative measurement of sample mass.
10. Measurement and direct detection of specific physical properties.
11. Analysis and data extraction
12. Documentation.

It is argued in [9] that typically there is not much to optimize for the last 10 LUO's. Each may require specific specialized equipment, and what may be available does not leave much choice. It is further argued that the first two LUO's tend to distinct one automated Biotechnology application from another. Liquid handling and manipulation do dominate the automation design process.

4. CASE STUDY: PREPARATION OF RNA FROM TISSUE CULTURE CELLS

Let us present a typical Biotechnology process in full detail. The process consists of a group of experiments developed for the Florida Atlantic University BSC4403 Biotechnology Lab 1 course. These manual "wet lab" experiments cover about one third of a semester. The overall goal of this group of experiments is the preparation of RNA from tissue-cultured cells (see Figure 4). The set of experiments are first laid out conceptually as a manual-labor production line. This production line presentation, with its sequential stages, serves as a basis for further deductive process as to how each station may be automated. The process of conversion to automation involves the identification of sensors and actuators and other equipment needed for the various process steps.

The goal of the process is the Isolation of RNA from Drosophila Schneider 2 cells that were transfected with a plasmid expressing dact mRNA (RE37047) [1]. The manual production requires QIAshredder columns, RNeasy columns, Lysis buffer RLT, B-mercaptoethnol, 70% ethanol, RW1 buffer, Wash buffer RPE, and RNase free water, all shall be explained next.

The experiment utilizes an RNeasy Mini - QIAshredder Kit, available from QIAGEN Sample and Assay Technologies (see figure 5). The kit contains QIAshredder mini spin columns, a RNeasy mini spin column, and all the needed reagents for the protocol including the buffers and the RNase free water. Generally speaking a spin column acts as a filter which, depending on its binding chemical properties, filters out from

the solution poured into it certain specific molecules and retains other specific molecules.

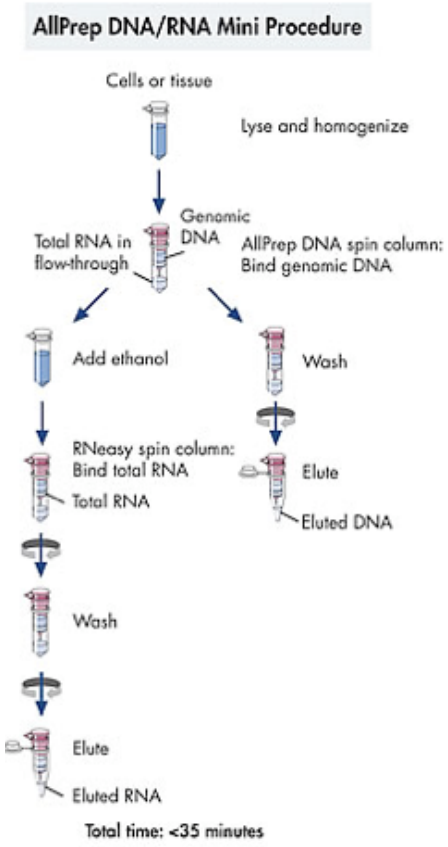

Figure 4. Preparation of RNA from Tissue Culture cells [14]

The process starts by taking frozen Drosophila cells and adding to it an RLT buffer. The buffer helps in the next step in which the cells are lysed by vortexing the sample. That is, the cells will be broken down by causing damage to its plasma membrane as happens by rotating rapidly the sample. The cells lysate is then transferred into a QIAshredder mini spin column. The QIAshredder is a cell-lysate homogenizer; that is, it breaks down fat into such small particles that stay suspended in liquid, rather than rise to the top of the column. It is used in nucleic acid mini-prep, a procedure to extract plasmid DNA from bacteria. The homogenized lysed cells are then centrifuged for two minutes at 14000 rpm in a 4°C incubator. A centrifuge works using the Sedimentation Principle, where the centripetal acceleration separates substances of greater and lesser density. Even though the RNA and the plasmid DNA are chemically similar and both are negatively charged, one is able to separate them because the plasmid DNA is more negatively charged than RNA. Plasmid DNA particles are bigger than the RNA particles and stick to the recedue of the column that is positively charged. Afterwards, one has to add a 70% ethanol to the lysate in the collection tube in order to make the environment hydrophobic for precipitation; that is, the formation of a solid in a solution during a chemical reaction. The solution is then transferred to an RNeasy column to which the RNA particles stick. It is then centrifuged again. At this point, the RNA particles are attached to the recedue, but so do other undesired compounds (such as genomic DNA). At that point there is a need to add buffer RW1 to a spin column and centrifuge again. This makes the column less attractive to the unwanted particles. The next two steps involve again centrifuging this time around with a wash buffer RPE added to get rid of all unwanted particles. Finally, all RNA particles are eluted or washed out by adding RNase free water to the column and performing one more round of centrifuging. RNase free water is used because any presence of the RNase enzyme causes the obtained RNA product to be rapidly cut, which is of course undesired. At the end the RNA particles in RNase free water accumulate in a collection tube.

Figure 5. Equipment used for preparation of RNA from tissue culture cells: A) centrifuge, B) collecting tubes, and C) RNeasy spin column [14]

As all columns and buffers are part of a commercial kit provided by QIAGEN the lab quantities, timings, and speeds are all specified by the kit manufacturer. The RNeasy Mini - QIAshredder Kit is intended for simple and rapid homogenization of cell and tissue lysates and purification of up to 100 µg of total RNA. It reduces loss of sample material, eliminates cross-contamination between samples, and it gives high-quality total RNA in about 20 minutes and ready-to-use RNA for any downstream application. The quantitative specifications for the columns are:

QIAshredder

- Format: Mini spin columns
- Sample source: Animal or human cell or tissue lysates, plant cell or tissue lysates, white blood cell lysates
- Sample size: Up to 700µl cell lysate

- Homogenization time: 2 minutes

RNeasy Mini Kit

- Format: Mini spin columns with 1.5ml and 2ml collection tubes
- Sample size
  - Animal cells: $10 – 1\times10^7$ cell/ml
  - Tissue: 0.5 – 30mg
  - Bacteria: $< 1\times10^9$ cell/ml
  - Yeast cells: $< 5\times10^7$ cell/ml
- Preparation time: 20 minutes
- Binding capacity: Up to 100 µg RNA
- Elution volume: 30–100 µl

The sample size for the columns is given in terms of the concentration for the different samples.

Below is a summary of the steps of the experiment. This experiment is designed to be executed by a single operator that retrieves from a storage a tube filled with frozen cells and work through all the process steps to obtain at the end the RNA suspended in 50µl of RNase free water. An expert operator can execute the protocol in approximately 20 minutes.

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 spin column placed in a 2ml collection tube.
4. Centrifuging of the tube for 2 minutes at 14,000 rpm in a 4°C incubator.
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. Into or onto??
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. Is it onto or into?
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 at -20°C in a freezer.

In order to conceptualize the above experiment protocol as a manual-labor production line, the steps mentioned above have to be broken down into smaller more basic tasks.

1. Picking up a tube filled with frozen cells.
2. Adding a specific amount of RLT buffer
3. Vortexing the tube
4. Transferring the lysate into a QIAshredder spin column
5. Coupling the QIAshredder spin column into a collection tube of a specific size
6. Centrifuging for a given amount of time at a given speed and at a given ambient temperature
7. Adding a specific quantity of 70% ethanol to the lysate in the collection tube.
8. Discharging the QIAshredder spin column
9. Transferring the combined solution, including any precipitate that may have formed, to a RNeasy mini column
10. Coupling the RNeasy spin column into a collection tube of a specific size
11. Centrifuging for a given amount of time at a given speed and at a given ambient temperature
12. Discarding of the flow-through and collection tube
13. Attaching a new collection tube to the RNeasy mini column.
14. Adding a specific quantity of buffer RW1 to the RNeasy mini column
15. Centrifuging for a given amount of time at a given speed and at a given ambient temperature
16. Discarding of the flow-through and the collection tube.
17. Attaching a new collection tube to the RNeasy mini column.
18. Adding a specific quantity of the wash buffer RPE onto the RNeasy mini column.
19. Centrifuging for a given amount of time at a given speed and at a given ambient temperature
20. Discarding the follow-through and collection tube
21. Attaching a new collection tube to the RNeasy mini column.
22. Adding a specific quantity of the wash buffer RPE onto the column.
23. Centrifuging for a given amount of time at a given speed and at a given ambient temperature

24. Discarding the follow-through and collection tube
25. Adding a specific quantity of RNase free water
26. Attaching a new collection tube to the RNeasy mini column.
27. Eluting the RNA from the column by centrifugation for a given amount of time at a given speed and at a given ambient temperature
28. Storing the collecting tube at -20°C in a freezer.

The 28 manual-tasks described above are first set up as a manual-labor production line. These tasks can be arranged in 17 stations where a single human operator tends each station. It appears that only one storage unit (a freezer) is needed. This storage place holds the tubes with frozen cells and this is where we could store the final tube that contains the extracted RNA. It is assumed that there is a rack located between stations where the tube prepared by one-operator is left to the operator of the next stage. The resulting production line stations are (see Figure 6):

1. Picking up sample from storage (Task 1)
2. Adding RLT Buffer (Task 2)
3. Vortex (Task 3)
4. Transferring into spin mini-column (Tasks 4 and 5)

   Station includes the storage of new spin mini-columns.
5. Centrifuging (Task 6)
6. Adding ethanol (Tasks 7 and 8)

   Station includes the storage of spin mini-columns
7. Transferring into mini-column (Tasks 9 and 10)

   Station includes storage of the mini-columns and collection tubes.
8. Centrifuging (Task 11)
9. Discarding flow-through, attaching collecting tube, and adding RW1 (Tasks 12, 13, and 14)

   Station includes storage for the new collection tubes and disposal place for the used collection tubes.
10. Centrifuging (Task 15)
11. Discarding flow-through, attaching collection tube, and adding RPE (Tasks 16, 17, and 18)

    Station includes storage for the new collection tubes and disposal place for the used collection tubes.
12. Centrifuging (Task 19)
13. Discarding flow-through, attaching collection tube, and adding RPE (Tasks 20, 21, and 22)

    Station includes storage for the new collection tubes and disposal place for the used collection tubes.
14. Centrifuging (Task 23)
15. Discarding flow-through, attaching collection tube, and adding 50µl of RNase free water (Tasks 24, 25, and 26)

    Station includes storage for the new collection tubes and disposal place for the used collection tubes.
16. Centrifuging (Task 27)
17. Storing of the collecting tube (Task 28)

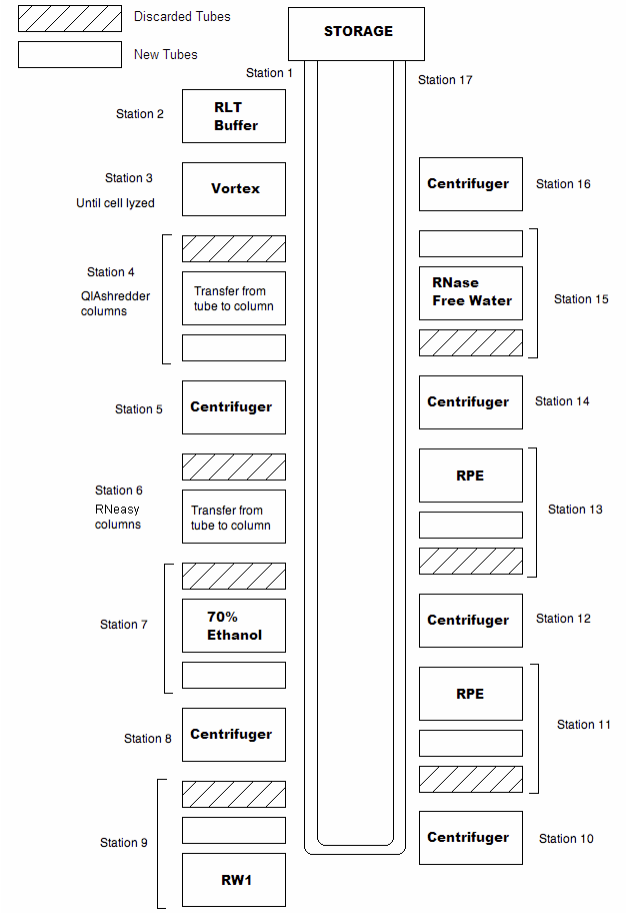

Figure 6. Conceptual design for the preparation of RNA from tissue cultured cells production line.

Initially the manual production line is thought of as a set of 17 stages each with one human operator in charge. That is the human operators perform simple tasks such as pipette reagents, discarding used ware, preparing the samples to be loaded, and loading and unloading the centrifuge. A question that may be asked is whether each operator can tend more than one station. It can be done, but in order to keep production going with the same throughput, all timings and stations have to be well synchronized and one would have to include labeling steps in each station. For the time being we opt to avoid including such extra steps.

Once the manual production line is conceptually set, the operators are then removed replaced by automation equipment. A conveyor replaces the handing of the samples from one operator to the next. All equipment is set in close proximity and

with access to and from a conveyor. The equipment needed for each station is listed next:

Station 1: Feeder from the storage into the conveyor.

Station 2: De-caper, caper, and dispenser, storage RLT buffer

Station 3: Vortex

Station 4: De-caper, caper, liquid transfer equipment (pipetting)

Station 5: Centrifuge

Station 6: De-caper, dispenser, storage for ethanol

Station 7: Liquid handling equipment, caper

Station 8: Centrifuge

Station9: De-caper, caper, dispenser, storage RW1

Station 10: Centrifuge

Station 11: De-caper, caper, dispenser, storage RPE

Station 12: Centrifuge

Station 13: De-caper, caper, dispenser, storage RPR

Station 14: Centrifuge

Station 15: De-caper, caper, dispenser, storage RNase free water

Station 16: Centrifuge

Station 17: Feeder into storage

Each station has in addition interfaces to and from the conveyor belts to transport the columns and tubes from station to station. In other words, the first automation strategy to be explored is that that involves no robotic devices at all. In this paper case study one can discard this first possibility from the start as it is not feasible. The main reason is that centrifuges cannot be loaded and unloaded by means of feeders to and from conveyors, at least not the commercial centrifuges. These need to be loaded and unloaded by means of a pick and place mechanism as it has to be done from the top. This immediately introduces the need for a robotic arm (or other pick and place device) that could perform this task of loading and unloading of the centrifuges. As seen in the description of the protocol, this case study procedure utilizes no less than six centrifuging steps. Questions that need to be raised are: Should the automation designer attach a robot for each centrifuge station? Do we need to have six distinct centrifuges at all? The latter question can be answered right away: reduction in the number of centrifuges indicates that the centrifuges become involved in more than one step. This means that some labeling steps have to be included. Once again, for the time being we are going to avoid adding extra steps to the protocol and we shall leave labeling steps as an open issue.

Let us return to the first question about one robot for each centrifuge. There are of course six possible options depending on how many robots are to be employed. This is where computational tools come into play. Utilizing six robotic devices does increase significantly the overall system's footprint due to each robot's workspace and it increases the total capital cost as a robot is surely one of the more costly pieces of hardware in the system; However it could theoretically yield the best throughput as each of the robots is always ready to tend its respective centrifuge. At the other extreme, using only one robotic device increases footprint and cost six-fold less, but it introduces a timing complexity that might compromise the system's throughput. Some tasks are critical and need to be performed as soon as samples become ready. Consider for instance the task of Station 14: As soon as the RNA is found in the column the RNase free water must be added in order to get the largest amount of RNA. Clearly, one of the solution outcomes of this multi-objective automation design problem (which involves equipment cost, floor footprint and throughput) should be a recommendation as to how many robots need to be taken.

On the other hand, centrifuging cannot be performed with only one tube or column in; centrifuges must be geometrically balanced in order to work properly. That means that depending on the number of available slots that a certain centrifuge may have, a certain number of samples could potentially be loaded. If for example the centrifuge has six slots, it could only simultaneously process two, three, four, or six samples symmetrically arranged. Bigger centrifuges have more potential combinations. The important consideration that has to be kept in mind is that more than one sample has to be loaded prior to each centrifuging action, and how many is one of the design parameters. This is a timing calculation and synchronization problem that has to be answered as part of automation design. Some of the centrifuging steps take long time, significantly more than other steps. One begins to appreciate the complexity of the automation design problem.

5. GROUP TECHNOLOGY APPLIED TO BIOTECHNOLOGY

Group Technology (GT) is a methodology to the production of parts in medium quantities [4], [12]. GT is based on the notion that although parts may be different, they also possess similarities. These similarities can be associated with similar production process steps or tooling needed to manufacture these parts. GT has become a standard methodology in mechanical and electronic assembly automation design however it has not been employed much in designs of high-throughput automation for Biotechnology.

In GT similar parts are identified and grouped together in order to take advantages of their similarities for design and production. Each production cell is designed to produce one part family following the principle of specialization operation, which involves the design of special production equipment to produce the part family.

Parts are classified into families, which have similarities in processing steps used in their manufacture. There are three common classification schemes: Systems based on part design attributes, systems based on part manufacturing attributes, and systems based on both. For example, in mechanical assembly common part design attributes taken into account for part classification are: major dimensions, basic external shape, basic internal shape, and material type. Common part manufacturing attributes that are taken into account for part classification are: major process, operation sequence, machine tools, cutting tools, and tolerances.

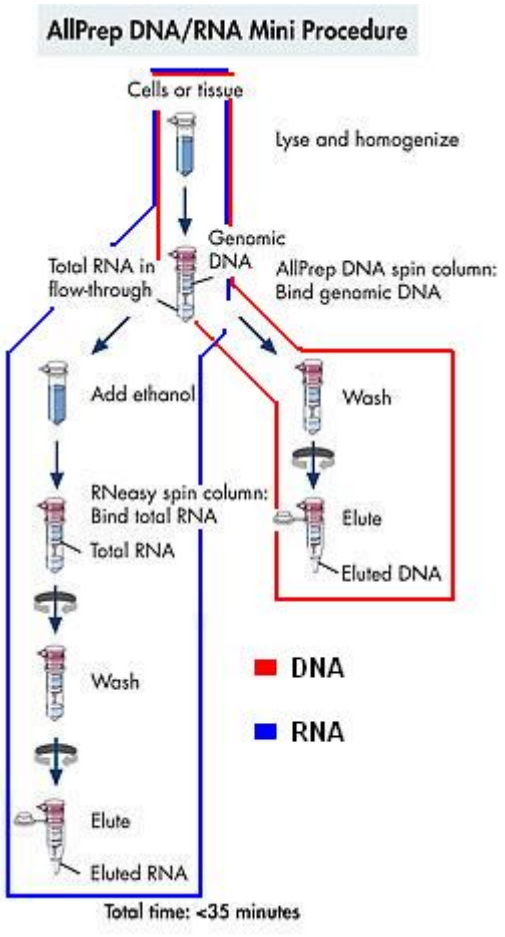

Figure 7. AllPrep DNA/RNA Mini Procedure [14]

If we go back to the example of preparation of RNA from tissue-cultured cells, one can demonstrate an example for GT production based on part manufacturing attributes. Say that in addition to RNA production from culture-cells one wishes to extract the RNA from bacteria or yeast cells. Extraction of RNA regardless of the kind of desired product – animal cells, tissue, bacteria, or yeast cells – use the same protocol (described in detail in section 4). One would use the same protocol, with the same columns, and same reagents; in other words the major process is the same for different "parts", better called in Biotechnology applications "samples". The operation sequences are the same for different samples, and the equipment used is the one used before: the same kind of centrifuges, vortex devices, and dispensers can be used for all kinds of samples. Evidently the use of GT is very beneficial in this case as although one uses different material types, different samples, and getting different products (that is different kinds of RNA), it is possible to group all of these "parts" into the same family because these have the same manufacturing attributes and share the same protocol.

GT based on part design attributes approach can also be applied in Biotechnology. If we study figure 7 carefully it is seen that there are two distinct protocols: the left side branch is the preparation of RNA form tissue-culture cells, and the right side branch is the preparation of DNA from the same tissue-culture cells. The protocol used is very similar to the one described in the case study section. For better understanding of the problem let us write the steps below for each of these two processes:

Preparation of RNA from tissue culture cells using AllPrep DNA/RNA Mini Procedure:

1. Collection of tube with cells or tissue lyse and homogenized
2. Transferring of the lysate into an AllPrep DNA/RNA spin column placed in a 2ml collection tube.
3. Centrifuging of the tube for 2 minutes at 14,000 rpm in the 4°C incubator.
4. Addition of 350 µl of 70% ethanol to the lysate in the collection tube.
5. Transferring of the combined 700µl including any precipitate that may have formed to an RNeasy mini column in a 2ml collection tube.
6. Centrifuging of the tube for 15 seconds at 14,000 rpm.
7. Discarding the flow-through and reattach the collection tube to the mini column.
8. Addition of 700µl of buffer RW1
9. Centrifuging of the tube for 15 seconds at 14,000 rpm.
10. Discarding the flow-through and the collection tube.
11. Transferring of the RNeasy mini column to a new 2ml collection tube.
12. Addition of 500µl of the wash buffer onto the column.
13. Centrifuging of the tube for 15 seconds at 14,000 rpm.
14. Discarding follow-through and reattach the collection tube to the mini column.
15. Addition of 50µl of RNase free water and attach a new collection tube.
16. Elution of the RNA from the column by centrifugation for 2 minutes at 14,000 rpm.
17. Storage of the collecting tube at -20°C in the freezer

Preparation of DNA from tissue culture cells using AllPrep DNA/RNA Mini Procedure:

1. Collection of tube with cells or tissue lyse and homogenized
2. Transferring of the lysate into an AllPrep DNA/RNA spin column placed in a 2ml collection tube.
3. Centrifuging of the tube for 2 minutes at 14,000 rpm in the 4°C incubator.
4. Discarding the flow-through and the collection tube.
5. Transferring of the AllPrep DNA/RNA mini column to a new 2ml collection tube.
6. Addition of 500µl of the wash buffer onto the column.
7. Centrifuging of the tube for 15 seconds at 14,000 rpm.
8. Discarding follow-through and reattach the collection tube to the mini column.
9. Addition of 50µl of elution buffer.

10. Elution of the DNA from the column by centrifugation for 2 minutes at 14,000 rpm.

11. Storage of the collecting tube at -20°C in the freezer

Note that in these two protocols we use an AllPrep DNA/RNA mini column. The AllPrep column has chemical properties that make the genomic DNA bind to the residue in the column. This is very convenient because one can elute the DNA directly from the AllPrep column, and one can obtain the RNA from the flow through of the AllPrep column and follow the same protocol from there on as described in section 4 and in the steps presented for preparation of RNA in this section.

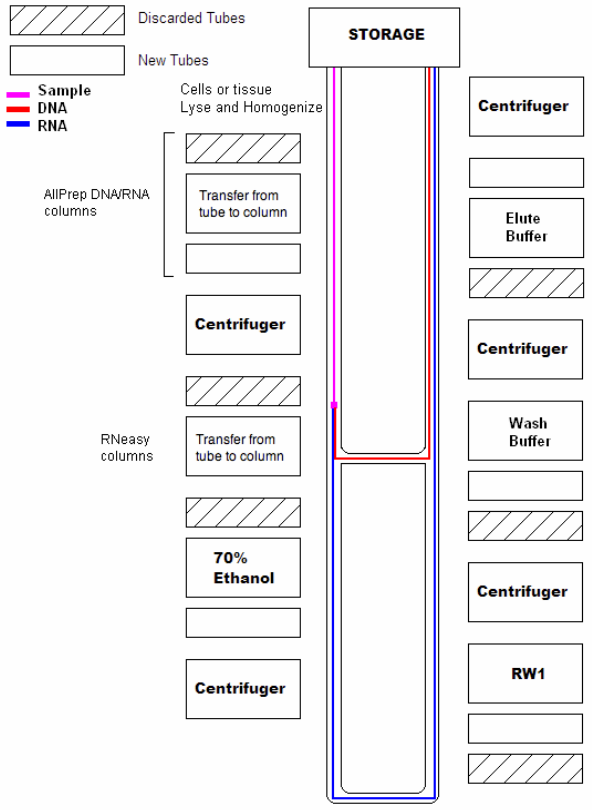

Figure 8. Conceptual design for the preparation of DNA and RNA from tissue cultured cells production line

Writing these two protocols side by side, it can be observed that the protocols are almost the same, but the preparation of DNA has fewer steps than the preparation of RNA. In this case one can apply GT production based on part design attributes. Although the process is not exactly the same, we do have the same material type because the same type of samples (RNA and DNA are being produced from the same kind of cell, tissue, or bacteria) are used, which means that the basic external and internal shapes are the same. In other words one is able to classify these parts as one family. The modified conceptual design for the preparation of RNA and DNA from tissue culture cells applying Group Technology is shown in figure 8.

There is an important concept in GT known as Composite Part. Composite part is a hypothetical part that includes all design and manufacturing attributes of the family. It is the correlation between part design features and manufacturing operations that produce those features. In the classical sense the machine cell design includes all machines required to make the composite parts and it should allow size variation.

The benefits that come with the use of GT are many to name a few: It promotes standardization of tooling and steps; the material handling is reduced; it simplifies the production scheduling; manufacturing lead time is reduced; work in process is reduced; process planning is simplified; and this will all result in higher quality work. Group technology also comes with disadvantages. The main two are: the production machines have to be rearranged, and identification of a part family could be complicated.

6. ARENA SIMULATION SOFTWARE

We are still in search of possible evaluation software to assist in optimizing automation design for Biotechnology. One candidate is a program called Arena. It is a simulation tool designed to model various automation processes. Here is a brief example that that illustrates the potential use of Arena as a computational tool to achieve our goal.

Arena simulation software was developed for modeling and analyzing business, service, or manufacturing processes or flows. It is most effective when analyzing complex, medium to large-scale projects highly sensitive to changes in the supply chain, manufacturing, processes, logistics, distribution, warehousing, and service systems [15].

In addition, Arena is used to create customized simulation modeling products; that is, templates focused on specific applications or industries. With Arena, customers develop custom templates that consist of "libraries" of modeling objects that make it significantly easier and faster to develop models that require repeat logic.

Examples of applications include:

- Analyzing business processes typically related to customer or paperwork handling, front and back office procedures in insurance, finance, or banking industries
- Detailed analysis of complex manufacturing processes that include material-handling intensive operations
- Modeling complex customer-handling activities such as passenger movement and baggage handling in airports, customer service in entertainment parks and other service systems
- Improving local and global supply chain processes
- Detailed analysis of warehousing, logistics, or transportation, military and mining applications
- Creating a custom Arena-based template for modeling logistics network of a global travel agency
- Creating a custom Arena-based template for modeling manufacturing flow of a major automotive manufacturer

Arena has the capability of executing detailed analysis of complex manufacturing processes. Arena is able to calculate all timings, queues, and time use for the different resources in the model, all costs associated with the entities, and can associate

statistical values to each element of the model in order to make a better simulation (that might include realistic sub-system failure probabilities accounting for less than perfect yields). The different elements shall be explained as we describe the example used to evaluate the effectiveness of Arena. It is demonstrated how to define entities, processes, resources, and queues to model two simple steps taken from this paper's case study.

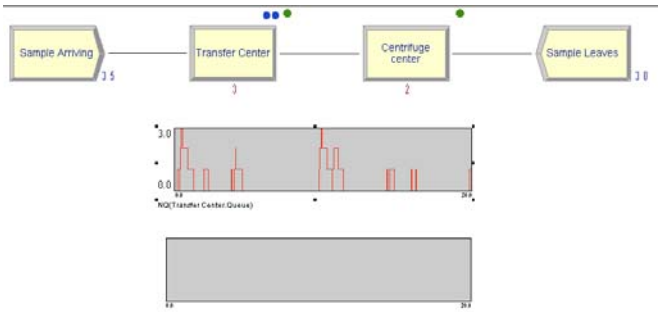

Figure 9. Arena model for steps 3 and 4: transferring of the lysate into a QIAshredder spin column placed in a 2ml collection tube, and centrifuging of the tube for 2 minutes at 14,000 rpm in the 4°C incubator.

Arena is used to model steps 3 and 4 of the case study presented in the previous section, that is, transferring of the lysate into a QIAshredder spin column placed in a 2ml collection tube, and centrifuging of the tube for 2 minutes at 14,000 rpm in the 4° C incubator (see figure 9). The simulation is set as a linear production line that starts with a block that represents the sample arriving at the system. This system consists of an entity (sample) that arrives at the system, passes through a Transfer Center (process), where it uses the transferring sample equipment (dispenser), then it goes to the Centrifuge Center (process), where it uses the centrifuge resource, and finally, the sample leaves the system. We define queues associated with both of our processes that appear graphically underneath the blocks' systems.

The first element that has to be defined is the entity to be used. The entities are the parts that are used and transformed throughout the processes. The entities in this case are the samples. The entities could have associate holding cost per hour, initial VA cost, that is, value added cost incurred by the entities throughout the system, initial NVA cost, that is, non-value added cost incurred by the entities throughout the system, initial waiting cost, initial transfer cost, and initial other cost. This information is going to be presented and used to calculate the total and average costs for the entities in the Category Overview Report in the Key Performance Indicator.

The second element that is added into the system is the block that represents the sample arriving at the system. A Create block describes the sample arriving at the system. The Create block allows us to specify what type of entity enters the system, which in our case is a sample, time between the arrivals of each sample, distribution for this arrival times, number of entities that arrive at a time, and maximum samples that arrive over the simulation period.

The Processes that are added to the system are the Transfer Center and the Centrifuge Center. To any process one can specify the action it executes (seize, delay, and release), the priority of the process, resources used in the process, and the type of delay. The actions for the Transfer Center are seize, delay, and release, the resource used is a dispenser, and it has a triangular probability density function for the delay with an average value of 20 seconds. The actions for the Centrifuge Center are seize, delay and release, the resource used is a centrifuge, and it has a constant delay of two minutes.

The two resources used in this specific example are the dispenser and the centrifuge. In this case we can set the type of capacity, the value of the capacity, and the time busy and idle per hour if needed. The dispenser and the centrifuge have fix capacity and the capacity for the dispenser is one sample at the time, and for the centrifuge are six samples at the time. We assume that a six slots centrifuge is used, and that one needs to wait to have six samples placed inside the centrifuge before running it. The processes also have queues associated with them. One can set the type of queue such as first in first out.

At the end of the run of the simulation the user gets a Category Overview Report. It has some Key Performance Indicators. The report is divided into the different categories of the system, in this case entity, process, queue, and resource. Te report gives information about the entity such as time: VA time, NVA time, wait time, transfer time, other time, and total time, and Number In: number of entities that entered the system for each entity type, Number Out: number of entities that left the system for each entity type, and WIP: work in process for each entity type. In the process section one finds information such as time per entry, accumulated time, and number in and out for each of the processes. In the queue section we find information about the wait time and the number of samples waiting for each of the processes. Finally, the resource section gives us usage information, such as instantaneous utilization, number busy, number scheduled, scheduled utilization, and total number seized for each of the resources.

Arena can generate all the time information and cost information related to the entities, processes, resources and the system in general. Arena has the capability of drawing the layout of the systems giving the user the total footprint size. It does not have any optimization functions, but it is a strong computational tool that can potentially produce optimization results if used repeatedly for different hardware selection and floor arrangement conditions.

7. OPEN ISSUES AND FUTURE WORK

There are still many questions that have to be answered. The first deals with the relationship between upstream and downstream protocols. Can we completely separate the upstream protocols from the downstream protocols? Or does one need to have some information about what the samples are needed for downstream in order to produce them more optimally upstream?

The second question is related to storage capacity of Biotechnology products. Is the maximizing of product output quantity per given time period always the desired goal for every single protocol and study? Do we always strive to increase throughput and assume that we can store the excess samples?

Some applications that can be envisioned may involve only fresh output samples. Is it viable in a given automation line to store samples and for how long before the samples die?

One has to always keep in mind when coming up with the "best solution" that we deal with live samples and chemicals. Some of these might need special manipulation equipment depending on temperature, humidity, and stability, among other important specific conditions, and some of these may need specific treatment and may have different lifetimes.

Future works that may involve feedback monitoring of the protocols are still an open issue. For example, in the case study of this paper every stage in the automation system is done in open loop. Present day Biotechnology automation systems are fully pre-programmed to perform automatically laboratory protocols. It would be very helpful to implement control concepts when setting up the experiments, and use the results of previous experiments to reconfigure future experiments in real time.

Another possible direction for a future study is to look into the automation of existing and new equipment for the remaining laboratory unit operation (LUO's). This is an area that has on going research in every aspect and new equipment is constantly being developed. The tendency these days inclines towards real-time processes, and in order to achieve this monitoring devices and sensors are being included in different operations.

Finally, there are still gaps in the different protocols that have not yet been automated and these gaps create bottlenecks. Coupling of different customized and generic equipment is desired.

8. ACKNOWLEDGEMENTS

We are grateful to Dr. Andrew Goldenberg (University of Toronto) who introduced us to this research problem. Thanks also to several Florida Atlantic University faculty - Dr. Oren Masory, for suggesting the Group Technology approach to this research, Dr. Chingping (Jim) Han, for introducing us to the Arena software, Dr. Salvatore Morgera for providing partial financial support through the FAU Bioengineering program, and to Dr. Maria Larrondo-Petrie for providing financial support through the LACCEI program. Finally we acknowledge the grant from T-VEC Technologies (Boca Raton, FL) that made this project possible.

9. REFERENCES

[1] *Biotechnology Laboratory I Manual*. Biotechnology Laboratory I, Biological Science, Florida Atlantic University, Fall 2006.

[2] T. N. Chang, S. Parthasarathy, T. Wang, K. Gandhi, and P. Soteropoulos. *Automated Liquid Dispensing Pin for DNA Microarray Applications.* IEEE Transactions on Automation Science and Engineering, Vol. 3, No. 2, April 2006, 187-191.

[3] T. Geisler, J. Ressler, H. Harz, B. Wolf, and R. Uhl. *Automated Multiparametric Platform for High-Content and High-Throughput Analytical Screening on Living Cells.* IEEE Transactions on Automation Science and Engineering, Vol. 3, No. 2, April 2006, 169-176.

[4] Groover, M. *Fundamentals of Modern Manufacturing Materials, Processes, and Systems*. Prentice Hall, Upper Saddle River, NJ, 1996.

[5] Hardin, C. Edwards, J., Riell, A. Presutti, D., Miller, W., and Robertson, D. Cloning. *Gene Expression, and Protein Purification Experimental Procedures and Process Rationale*. Oxford University Press, New York, NY, 2001.

[6] P. Hodder, P. Baillargeon, P. Chase, L. DeLuca, F. Madoux, B. Mercer, D. Minond, S. Saldanha, L. Scampavia, T. Spicer, P. Subramaniam, L. Sullivan. *Probe and Drug Discovery: The Lead Identification Department*. Translational Research Institute, Scientific Report 2007.

[7] Y. Liu, L. Chen, and L. Sun. *Automated Precise Liquid Dispensing System for Protein Crystallization*. Proceeding of the 2007 IEEE International Conference on Mechatronics and Automation, August 5 – 8, 2007, Harbin, China, 3616 – 3621.

[8] Meldrum, D., Fisher, C., Moore, M., Saini, M., Holl, M., Pence, W., Moody, S., Cunningham, D., and Wiktor, P. *ACAPELLA-5K, A High-Throughput Automated Genome and Chemical Analysis System.* Proceedings of the 2003 IEEE Intl. Conference on Intelligent Robots and Systems, Las Vegas, Nevada 2003, 2321-2328.

[9] Najmabadi, Peyman. *New Laboratory Automation System Architectures for Biotechnology Applications.* Ph.D. Thesis, University of Toronto, Ottawa, Canada 2006.

[10] P. Najmabadi, A. A. Goldenberg and A. Emili. *Hardware Flexibility of Laboratory Automation Systems: Analysis and New Flexible Automation Architectures*. Journal of the Association for Laboratory Automation, Volume 11, Issue 4, August 2006, Pages 203-216

[11] P. N. Ngatchou, M. R. Holl, C. H. Fisher, M. S. Saini, J. Dong, T. T. H. Ren, W. H. Pence, D. L. Cunningham, S. E. Moody, D. A. Donaldson, and D. R. Meldrum. *A Real-Time PCR Analyzer Compatible With High-Throughput Automated Processing of 2-µl Reactions in Glass Capillaries*. IEEE Transactions on Automation Science and Engineering, Vol. 3, No. 2, April 2006, 141-151.

[12] Singh N. *Systems Approach to Computer-Integrated Design and Manufacturing*. John Wiley & Sons, Inc., New York, NY, 1996.

[13] Suh, N. *Axiomatic Design Advances and Applications*. Oxford University Press, New York, NY, 2001.

[14] QIAGEN – Sample and Assay Technologies www.quigen.com

[15] Kelton, W., Sadowski, R., Sadowski, D. *Simulation with Arena*. McGraw Hill, New York, NY, 2002.