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Automation and Integration of Polymerase Chain Reaction with Capillary Electrophoresis for High Throughput Genotyping and Disease Diagnosis

by

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TABLE OF CONTENTS

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ABSTRACT

Genotyping is to detect specific loci in the human genome. These loci provide important information for forensic testing, construction of genetic linkage maps, gene related disease diagnosis and pharmacogenetic research. Genotyping is becoming more and more popular after these loci can be easily amplified by polymerase chain reaction (PCR). Capillary electrophoresis has its unique advantages for DNA analysis due to its fast heat dissipation and ease of automation. Four projects are described in which genotyping is performed by capillary electrophoresis emphasizing different aspects.

First, We demonstrate a principle to determine the genotype based on capillary electrophoresis system. VNTR polymorphism in the human D1S80 locus was studied. A pooled allelic ladder was chosen as the absolute standard. Genotyping can be accomplished by co-injection of the PCR products of an individual genomic DNA with the D1S80 ladder and then being separated by CE. The increases of relative peak intensities of certain alleles in the mixture compared with those in allelic ladder indicated the genotype clearly.

Second, the separation of four short tandem repeat (STR) loci vWF, THO1, TPOX and CSF1PO (CTTv) by using poly(ethylene oxide) (PEO) was studied in achieving high resolution and preventing rehybridization of the DNA fragments. Separation under denaturing, non-denaturing conditions and at elevated temperature was discussed.

Third, a 250 μm i.d., 365 μm o.d. fused silica capillary was used as the microreactor for PCR. Four tandem repeat (STR) loci vWA, THO1, TPOX and CSF1PO, as well as HIV-1 gag fragment were amplified by the microreactor. The microreactor was integratred to the separation capillary to achieve on-line PCR, standard loading, DNA denaturation, injection, separation and detection.

Fourth, direct PCR from blood was studied to simplify the sample preparation for genotyping to minimum. An integrated and multiplexed on-line instrument starting from blood to final determination of genotype was built based on freeze/thaw controlled multiplexed microfluidics and capillary array electrophoresis to achieve the goal of high speed, high throughput and automation for genotyping.

CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

This dissertation begins with a general introduction of the background. Recent research and progress are also provided in the literature. The following chapters are presented as four complete scientific manuscripts with accompanying literature cited, tables, and figures. General conclusions summarize the work. Appendixes provide supporting materials. Finally, a list of cited references for the general introduction concludes this dissertation.

Background

Characterization, or "typing," of blood, serum, and other body fluids and tissues has been used for forensic and clinical purposes for more than 50 years.[1] In the last decade, methods have become available for deoxyribonucleic acid (DNA) typing, that is, for showing distinguishing differences in the genetic material itself. Developments in genetics and modern molecular biology have accumulated large amount of knowledge about human genome,[2,3] especially after the significant progress of Human Genome Projects.[4,5] This knowledge has provided a sufficient number of regions of DNA which can be characterized.

The human genome consists of DNA molecules in the form of a double helix in which the two strands of the DNA duplex are held together by weak hydrogen bonds (Figure 1). A sugar with an attached base and phosphate group constitutes the basic repeat

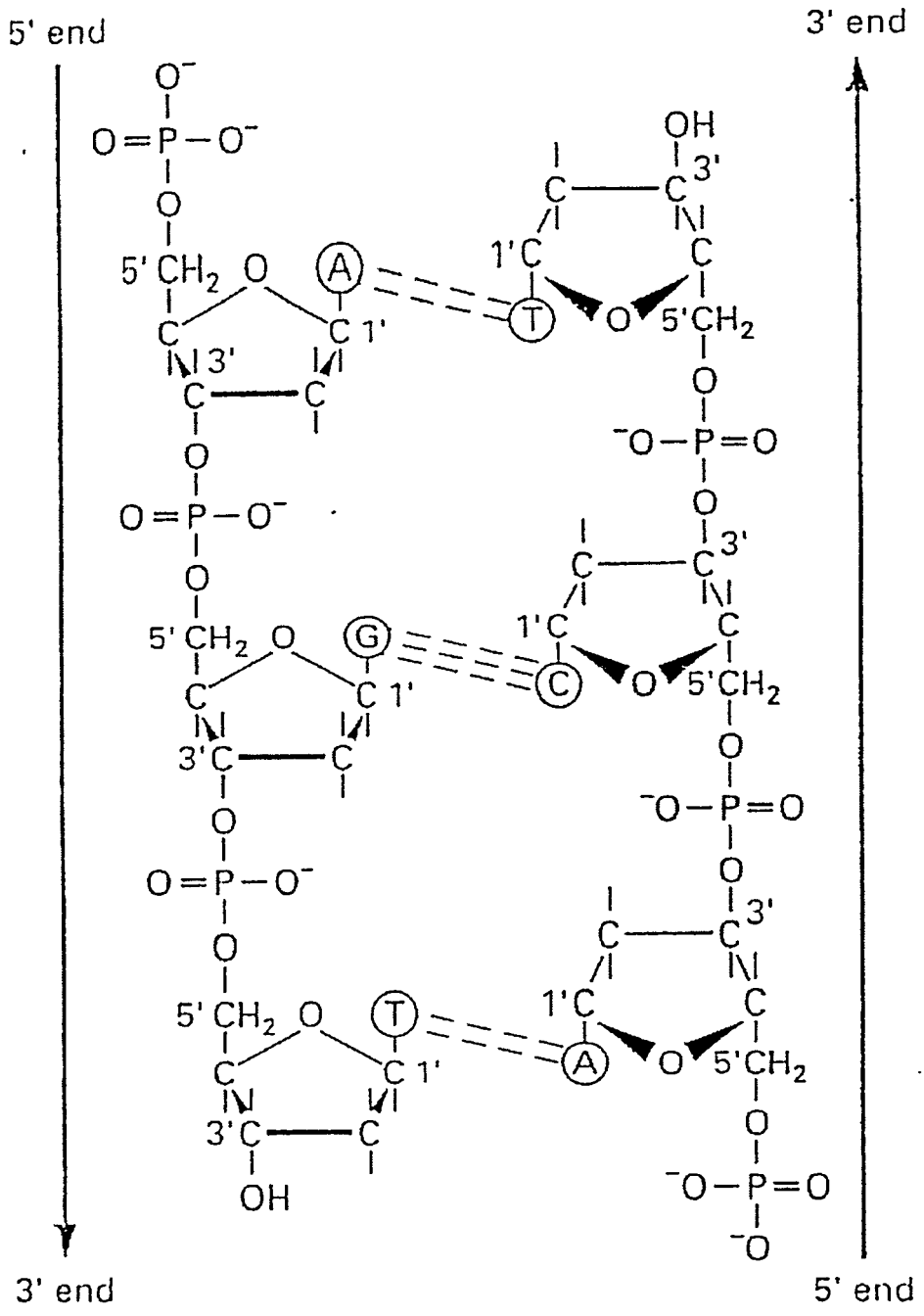


Figure 1 Structure of double-stranded DNA
A = adenine, C = cytosine, G = Guanine, T = thymine

unit of a DNA strand, a nucleotide. Genetic information is encoded by the sequence of bases in the DNA strands. The total number of nucleotides is about 3 billion in the human genome. Genes are segments of the DNA molecules. A human has 23 pairs of chromosomes. Pairs of DNA sequences, which are located at identical positions on each pair of chromosomes (except sex chromosomes X and Y), are referred to as alleles. An individual is said to be homozygous or heterozygous at a specific locus if the two alleles at that locus are, respectively, identical or different sequences. DNA technology has revealed variations in the genome. In noncoding regions of DNA, it is estimated that at least one nucleotide per 300-1,000, on the average, varies between two people.[6] Some regions of DNA contain repetitive units. In VNTRs (variable number of tandem repeats), the repetitive unit is longer than seven bases,[7,8] while STR (short tandem repeat) has a repeat unit shorter than seven bases.[9-11] The repeat unit may be consensus or complex.[13] These are specific DNA regions which will be studied in this dissertation, because they are highly polymorphic,[14] they are also related to certain diseases.[15] DNA typing in VNTR and STR regions is becoming a powerful tool in applications such as personal identification,[16-19] agriculture genetics, genetic linkage mapping,[20-21] gene related disease diagnosis and pharmacogenetics research.

The technologies included in DNA typing are restricted enzyme digestion, probe hybridization, electrophoresis and polymerase chain reaction (PCR). PCR is most robust and efficient. The introduction of PCR by Kary Mullis in 1985 has led the revolution of modern molecular biology. Its ease of amplification produces millions of copies of specific DNA fragments and has made detection based on radioactive substance obsolete. Laser induced fluorescence is one of the most important methods, which made automation

possible because the capability of on-line detection and data interpretation. Certain dye is covalently bonded to 5' end of a primer in PCR. In another case, intercalating dyes are used to enhance the fluorescence signal of DNA fragments. The variability of PCR itself (Table1) and the technical improvement of slab gel electrophoresis afford more options of analysis methods. Meanwhile the rapid progress of capillary electrophoresis (CE) and other micro devices such as chips provide a powerful tool to genetic analysis. DNA typing becomes faster, more sensitive by using these devices. The throughput has also improved.

Sample Preparation for DNA Typing

Polymerase chain reaction (PCR) for amplifying DNA

The PCR process (Figure 2) is simple; indeed, it is analogous to the process by which cells replicate their DNA.[22,23] Two short oligonucleotides are hybridized to the opposite strands of a target DNA segment in positions flanking the sequence region to be amplified. The two oligonucleotides serve as primers for an enzyme-mediated replication of the target sequence. The PCR amplification process itself consists of a three-step cycle:

1. The double-stranded template DNA is dissociated into single strands by incubation at high temperature, typically 94°C.
2. The temperature is lowered to allow the oligonucleotide primers to bind to their complementary sequences in the DNA that is to be amplified.
3. A DNA polymerase extends the primers from each of the two primer-binding sites across the region between them, with the target sequence as template.

The yield from 30 cycles amplification is generally about 10^6-10^7 copies of the target sequence. The efficiency can be improved by amplifying several different products in the same reaction mix; this is termed multiplex amplification.[24,25] PCR is the most important biotechnology throughout this study.

Table 1. Some PCR-Based Systems for the Detection of Genetic Variation

Sequence-based detection systems
Allele-specific oligonucleotide (ASO)[7]
Allele-specific priming of PCR[8]
Oligonucleotide-ligation assay (OLA)[9]
Restriction-site-specific cleavage (Amp-FLPs)[10]
Denaturing gradient gel electrophoresis[11]
Chemical cleavage of mismatched heteroduplxes[12]
Length-variation systems
Simple insertions and deletions
VNTR polymorphisms[13]
Analysis of nucleotide sequences

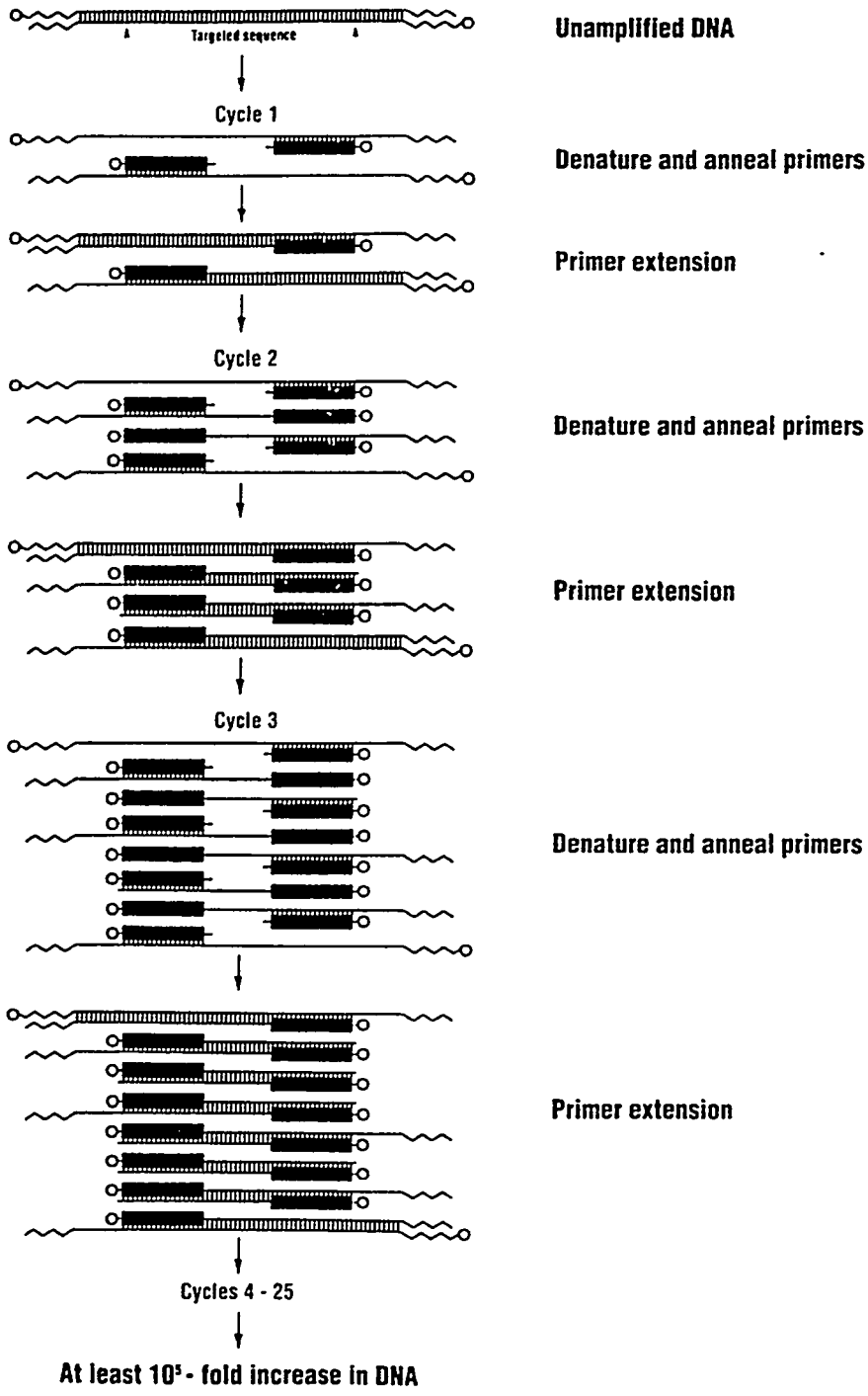


Figure 2 Principle of polymerase chain reaction

DNA extraction and purification

The extraction and purification of nucleic acid (DNA or RNA) from a biological material requires lysis of the cell membrane, inactivation of the cellular nucleases, and separation of the desired nucleic acid from the cellular debris. Cellular lysis can be accomplished with enzymatic treatment, detergents, or chaotropic agents. Traditionally, the subsequent extraction of the nucleic acid uses phenol-chloroform which preferentially partitions the nucleic acid into the aqueous phase and the other cellular components including protein, into organic phase or phase interface. Unfortunately, this process is very time consuming and costly.[26-32] There are many improved methods which no longer use organic extraction but still providing appropriate template for PCR. For example, using Chelex-100 resin can finish extraction in one vial within an hour.[33,34] However, centrifugation, incubation and liquid transferring are still involved.

Another category of methods of DNA extraction is the magnetic isolation of cells. Magnetic beads can quickly prepare high quality samples for downstream molecular biology applications. Magnetic beads are usually coated with antibodies against a specific cell type or bacteria, even virus. The cells or bacteria bound to the antibody coated magnetic beads are isolated and washed using a magnet. After isolation, microwave heating or boiling is generally sufficient for preparing template for PCR. Cells have been isolated by this method including human leukocytes [35,36] and hundreds other cells.

Advanced High-throughput Instruments for DNA Analysis

Slab gel electrophoresis has been used traditionally for DNA analysis such as sequencing, restricted fragment length polymorphism, mutation test and hybridization

methods. It involves manual gel pouring and sample loading. It is relatively slow because of the low heat dissipation so that only lower voltage can be applied compared to capillary electrophoresis. The speed of separation has been improved by using thin gel. Slab gel electrophoresis is still the dominating method right row. Commercially available instruments such as ABI sequencers and gene scanners are widely used among biochemistry lab. Although CE has the advantages of speed, resolution, ease of automation, only one capillary can be run and detected at a time, so that total throughput is no better than that with slower slab gels running multiple lanes. That is the reason for the introduction of capillary array electrophoresis and multi-channel chip devices for separation and detection.

Capillary array electrophoresis (CAE)

In the last few years, our group and several other groups have developed different setups and detection systems to accommodate parallel arrays in capillary electrophoresis. There are four major setups. First, Mathies's group developed the confocal fluorescence scanner equipped with a mechanical stage to translate the capillary array across the optical region at 1 Hz rate.[38-40] The small local illumination and detection volume requires only modest excitation power. However, this sweeping method may be limited to hundreds of capillaries. Recently, this group used a rotating objective instead of horizontal sweeping scanner, it led up to 1000 capillaries.[41] The detection components were photomultiple tubes. Second, Takahashi et al developed multiple sheath-flow gel capillary-array electrophoresis. To eliminate excitation light scattering at the capillary surfaces and to irradiate all the migration tracks simultaneously, the capillary tubes were removed from

the irradiated region. DNA fragments were eluted from the tips of gel capillaries and flowed into the lower open capillaries. CCD was employed as the fluorescence detector. The advantage was the fluorescence background was very small. Higher throughput can be easily achieved.[42] Our group developed several excitation and detection schemes. One arrangement involved a beam expander and a cylindrical lens to distribute the laser into a thin line that intersected the axes of the capillaries. A CCD camera was used to detect fluorescence at 45° relative to the excitation beam. Laser power was required to be stronger because of the Gaussion distribution of laser power along the thin line,[43] but the setup was robust. DNA sequencing data was obtained with good signal to noise ratio for all 96 capillaries. Also, statistics on genotype identification were presented regarding the entire array.[44] Another excitation mode is side-entry. By immersing the capillary wall in an index-matching fluid, scattering and refraction from the capillary walls are minimized and the laser beam is able to pass through all capillaries with only minor losses.[45] A similar excitation mode is used in Chapter 5.

Thus, slab gel preparation can be replaced by automated capillary filling using a low viscosity matrix,[46-61] refillable capillaries, and a pressurized matrix-filling system. Manual sample loading, another tedious, labor-intensive step, is replaced by simultaneous electrokinetic injection of up to 96 samples.[44] Thus, the increase in sample throughput obtained with CAE relative to that of slab gel systems results from the elimination of gel pouring and automation of sample loading as well as from reduced electrophoresis time.

Microchip devices for DNA analysis

Microchips have miraculous power to miniaturize. Silicon chips compressed computers from room sizing of vacuum tubes and relays to the size of a hardback book. Now microchips are poised to perform the same downsizing on analytical laboratories. Microchips are very hot research area for DNA analysis because they have the potentials to provide one magnitude higher speed and higher throughput than capillary electrophoresis.[62-63]

Fused silica wafer is widely used material for making chip device. Plastic chips are also reported. The channels and vials for electrophoresis are fabricated using photolithography and chemical etching. The channels are then derivatized to eliminate the electro-osmotic flow. Polymer solution is filled into channels for separation. Cross-channel geometry is usually adapted in aligning separation and injection channel. Laser induced fluorescence is the method for detection. The chip size is usually several inches wide and long. The channel width varies around 50 μm. Research has been conducted to improve the separation resolution of chips. These efforts include channel coating, better sieving matrixes, decreasing in the length of injection plug and longer separation channel.[64-65] DNA sequencing separation to ~433 bases in only 10 min using one-color detection system was reported.[65] However the resolution of chip is still much lower than capillary electrophoresis at present time, because the loss of resolution due to decreased separation length in chips can't be compensated by the ability of reduced diffusion time, very narrow injection plug or high voltage. However, chip electrophoresis is attractive to genotyping, restriction fragments, PCR products and short oligonucleotides analysis where less resolution is required than sequencing.[66-70] Short tandem repeat DNA samples were

separated with baseline resolution in 2 min in a microfabricated device of only 2.6 cm in length.[71]

It is the same reason as the introducing of capillary array electrophoresis that array on chip is developed to increase the throughput and make parallel analysis of multiple samples practical for chip.[65,72] High-speed DNA genotyping using array electrophoresis chips was reported to simultaneously separate and detect 12 samples.[65] Because the capillary-array-chip lane to lane distance can be further reduced, it should allow higher lane density than capillary array electrophoresis in the same dimension range. The consumption and cost of reagents can be decreased significantly because only nanoliter volumes is injected. However, there are still problems for chips such as resolution, sample loading, and compatibility of supporting equipment. A breakthrough is needed in these areas.

Miniaturization of PCR

The widespread application of the polymerase chain reaction (PCR) and allied nucleotide amplification techniques has resulted in the development of many devices or systems to provide the thermal cycling necessary for these procedures. These devices are based a variety of design principles for the heat transfer including water baths, air baths, dry blocks, etc. Widely different conditions are investigated to achieve amplification. However, the overall aim of these developments has been to reduce cost, time of reaction, and contamination, while at the same time achieving adequate amplification of the targeted nucleotide sequence for subsequent detection or analysis. Miniaturization seems inevitable, because extremely small reaction volume reduces reagent cost, and thermal

response is faster for smaller chamber or reactors so that faster reaction can be achieved. Furthermore, miniaturized PCR devices are more easily integrated with micro or semi-micro separation devices such as capillary array electrophoresis and microchip electrophoresis to achieve final integration and automation. This important issue is discussed in the following section.

The representative of miniaturization is PCR in a silicon microstructure.[73] The silicon wafer was about 400 μm thick. The micro channels and chambers were made by planar photolithography or reactive ion etching. The chips were sealed with thin glass by certain bonding technique. A series of studies have been conducted. First, the manipulation and flow of biological fluids in straight channel micromachined in silicon was studied. It was found different fluids were easily manipulated in the microchannel and a variety of fluids revealed non-Newtonian behavior.[74] By means of micromachined filters (5 μm) located in channels, blood cells and microparticles were effectively separated from nanoliter-sized samples.[75] Second, surface passivation of microfabricated silicon-glass chips was studied. The purpose was to find a PCR friendly surface. An oxidized silicon (SiO_2) surface gave consistent amplification.[76] Finally, several PCR systems such as bacteria DNA, genomic DNA amplification, hot start PCR with TaqStart antibody, as well as PCR directly from intact human lymphocytes cells were investigated by this silicon-glass chip.[77] The total reaction volume was less than 10 μl. The chip was held by certain heater-cooler components. Manz's group demonstrated another excellent device, which was the continuous-flow PCR on chip. Three well-defined zones were kept at 95°C, 77°C and 60°C on a chip. The sample was hydrostatically pumped through a single channel (40

μm deep, 90 μm wide) etched into the glass chip. The channel passing through the three temperature zones defined the thermal cycling process. The time delay for a sample to reach a new temperature depended only on the time needed to transport the sample into the temperature zone. The heating and cooling times were less than 100 ms. Multiple sample plugs could be injected into the channel, and they could be amplified simultaneously as they traveled by the three temperature zones.[78]

Other miniaturization includes using small diameter capillary as reactors. For examples, 250 μm i. d. fused silica capillary was used as the reactor for DNA sequencing.[79] PCR inside 20-75 μm i. d. fused silica capillary was also reported. The PCR reaction volume was only nanoliter scale in this report.[80] The miniaturization not only means the scaling down of reaction volume, but also every component in the instrument. Actually, the full potential of miniaturization won't realize its full potential until all the functions needed to carry out a PCR test including batteries to drive the system, reaction chambers and filters to prepare the sample, and detectors to read the results-can be miniaturized as well.

Automation and Integration

The concept of automation is not new in genome community. A lot of efforts have been done on automation, especially after the proposing of Human Genome Project. Under the pressure that millions of samples needed to be analyzed everyday in bio-labs, methods for automation were born. First, automated fluorescence detection replaced the old radio active material ^{32}P and silver staining, which require long time and many after electrophoresis operations. Second, high throughput, which is an important aspect of

automation, has been achieved from sample preparation to instrumentation. For genotyping purpose, PCR has been multiplexed allowing several loci to be amplified in a single reaction. Quadruplex,[81] octoplex[82] and heptaplex[83] conditions were already described. Multiplexing PCR reduces the labor for DNA extraction and PCR setting same fold as the number of loci involved. Thin slab gel electrophoresis, capillary array electrophoresis and other microfabricated devices make the parallel analysis of multiple samples possible. Third, databasing is automated. Software is designed to determine automatically the fragment sizes. Once alleles have been sized, they are automatically designated to compile results into formatted spreadsheet which can be directly utilized in further analysis. Also, many thermocyclers have been designed to do faster PCR with higher throughput. Fourth, robots have been used to do certain tasks, especially in liquid handling process. For example, robot can pipette, dispense and transfer 96 samples at the same time. However, these automation efforts emphasize more individual process than the integration of these processes to become one automated system, which allow samples flow in and results come out without any human interpretation. So without integration, there is still a step away from the ultimate total automation.

The attempts of integration have been demonstrated in three categories: the lab-on-chip,[84] robotics[85] and capillary microfluidics.[79,86] The automation of the front-end of genotyping which is the DNA extraction has been largely neglected so far. The whole genotyping process includes extraction of DNA, PCR followed by separation and detection. This dissertation emphasizes the integration of all necessary steps into one automated instrument based on the last category--capillary electrophoresis and microfluidics. Chapter 2 discusses a novel internal standard method for genotyping by CE.

Chapter 3 studies the separation of STR fragments by PEO matrix. The integration of PCR to CE in a single capillary format consists of Chapter 4. Finally, the multiplexed DNA typing system in Chapter 5 emphasizes the integration and automation.

GENERAL SUMMARY

Human genome is very complicated. Thorough understanding of 3 billion sequence of DNA has not been achieved. Also, a lot of genes have not been localized and their functions in human life require more exploration. One outstanding character of nucleic acids analysis is the presence of large amount of samples. In response, technologies developed for genome research and applications are getting faster, with higher throughput and lower reagents consumption. Capillary electrophoresis, especially capillary array electrophoresis is replacing traditional slab gel due to its speed, resolution and ease of automation. Microchip devices also show great potentials in terms of speed and throughput. However capillary electrophoresis is a relative mature technology already. Sample preparation, an important step which is usually labor intensive, has been largely neglected in DNA analysis. At present time, the integration of individual technologies becomes the most challenging and important issue in genome community. The trend for genome research is miniaturization, automation and integration.

A series of studies have been conducted in this dissertation for genotyping by capillary electrophoresis. First of all, a novel sizing principle (Chapter 2) for alleles made the identification of allele precisely. No migration time calibration, which is usually the problem in CE, was necessary. Secondly, separation methods developing of STR fragments (Chapter 3) showed the importance of DNA denaturation and elevated temperature. Thirdly, the successful integration of sample preparation to CE system (Chapter 4) showed the potential of multiplexing automation and integration. Fourthly,

the automation and integration of genotyping from blood to final results was demonstrated for the first time (Chapter 5).

The future efforts following this work will be the expanding of the system to larger array. Also, the reaction volume of the PCR can be further scaled down by using smaller diameter of capillaries for the PCR reactors. It will be very interesting to demonstrate more applications such as drug screening or peptide mapping on similar systems.

APPENDIX A. AUTOMATION CONTROL

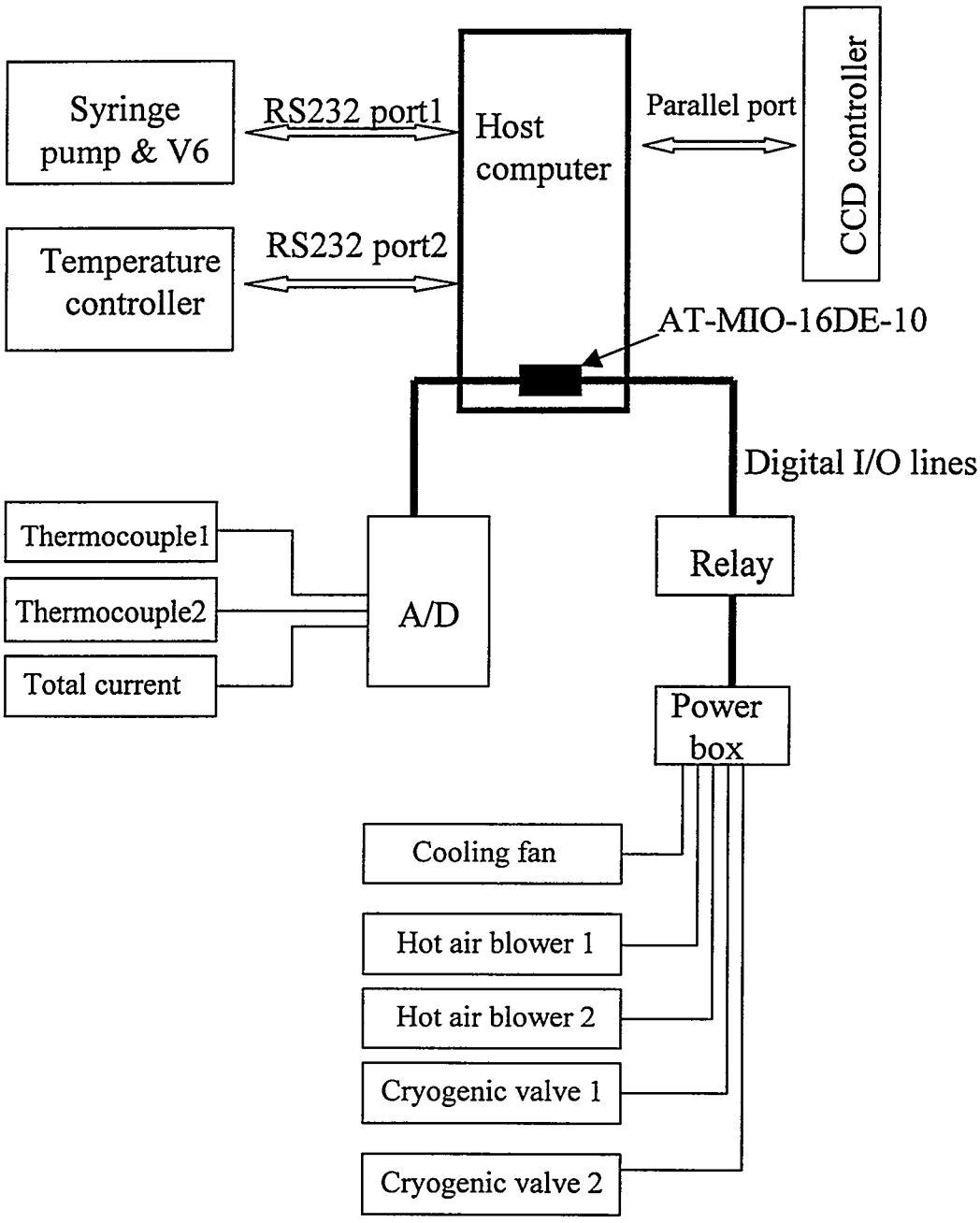


Figure A1 Control units for automation of multiplexed and integrated system

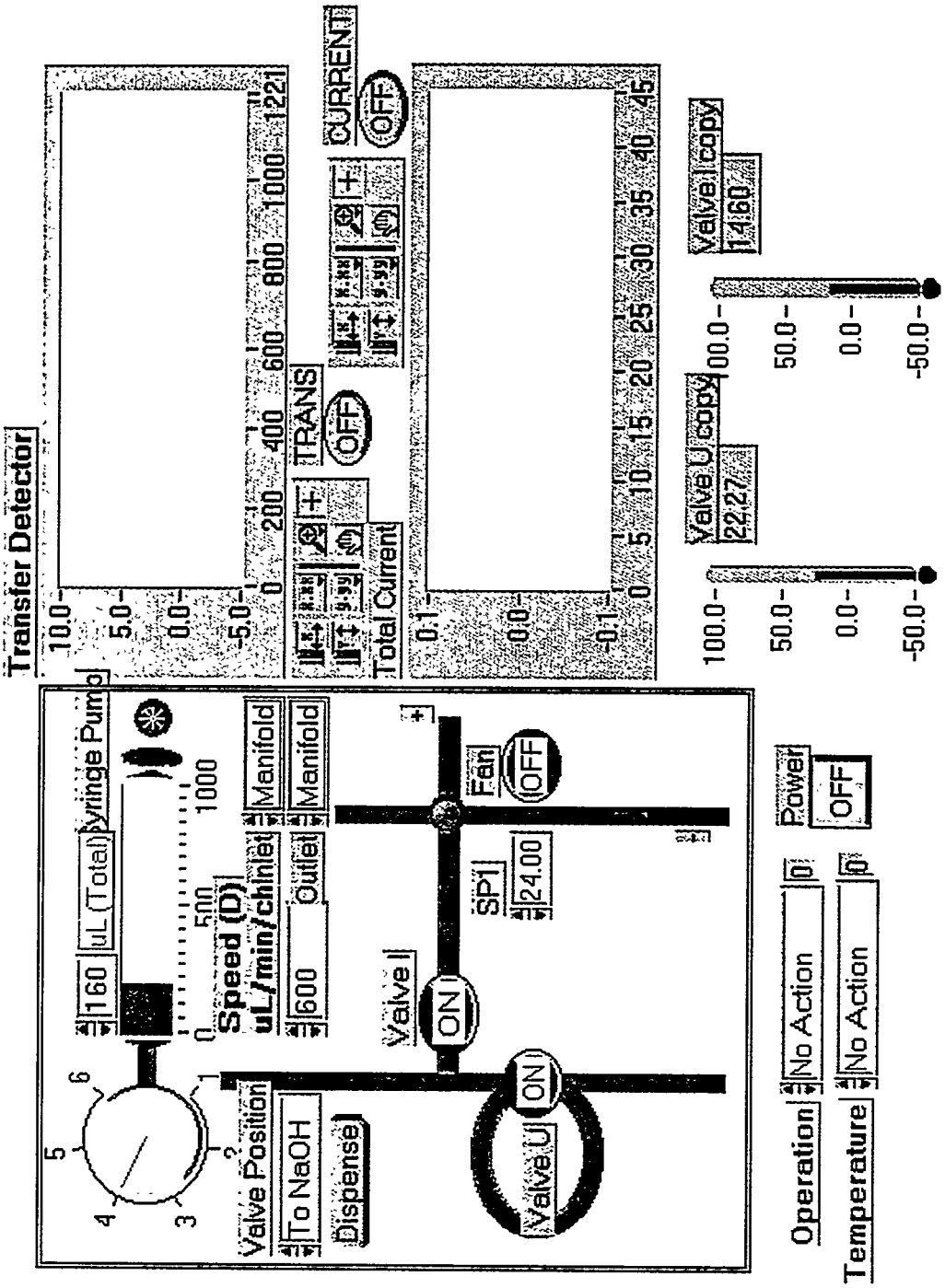


Figure A2 Front panel of instrumental control software

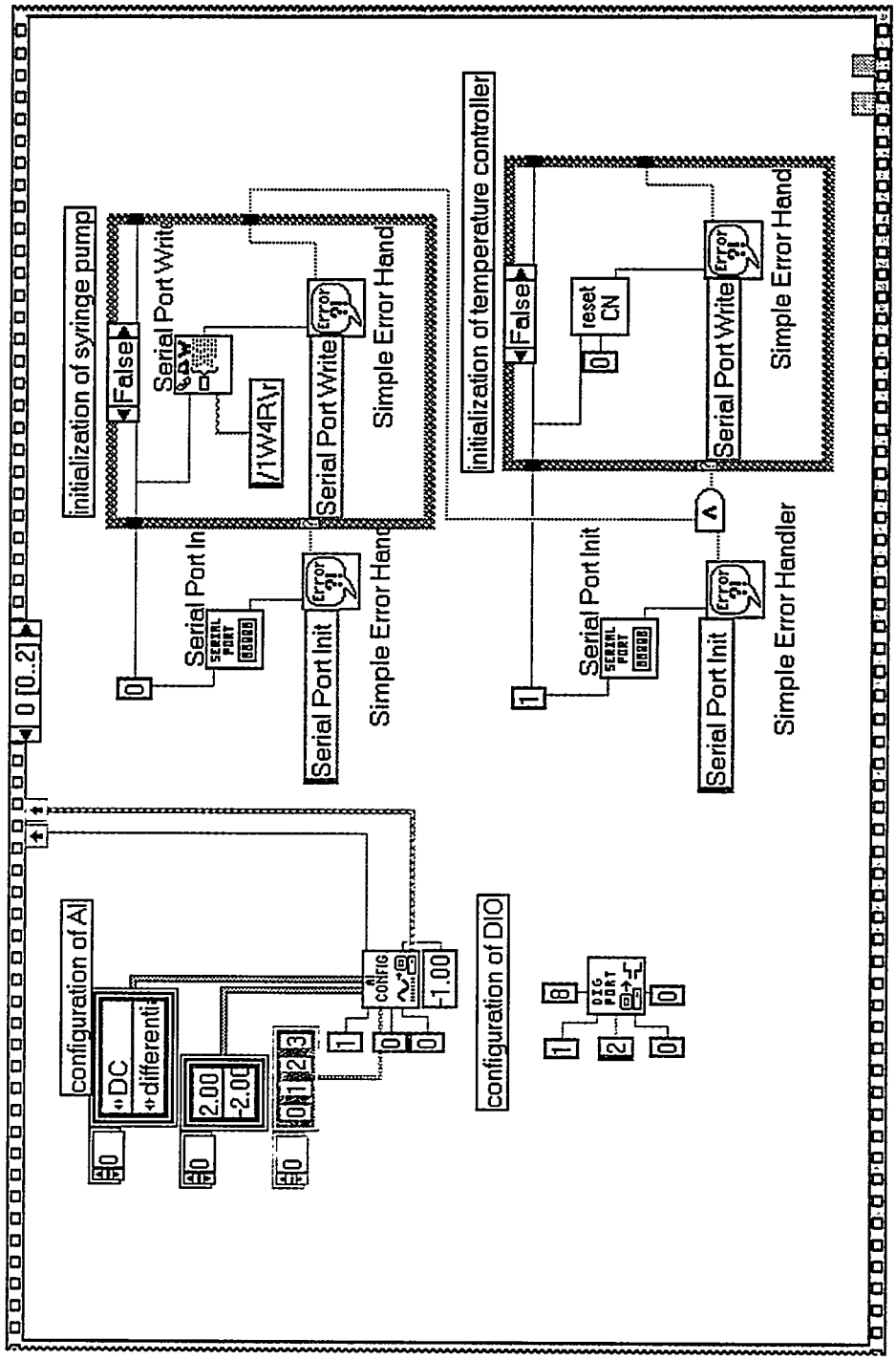


Figure A3 Block diagram of instrumental control software

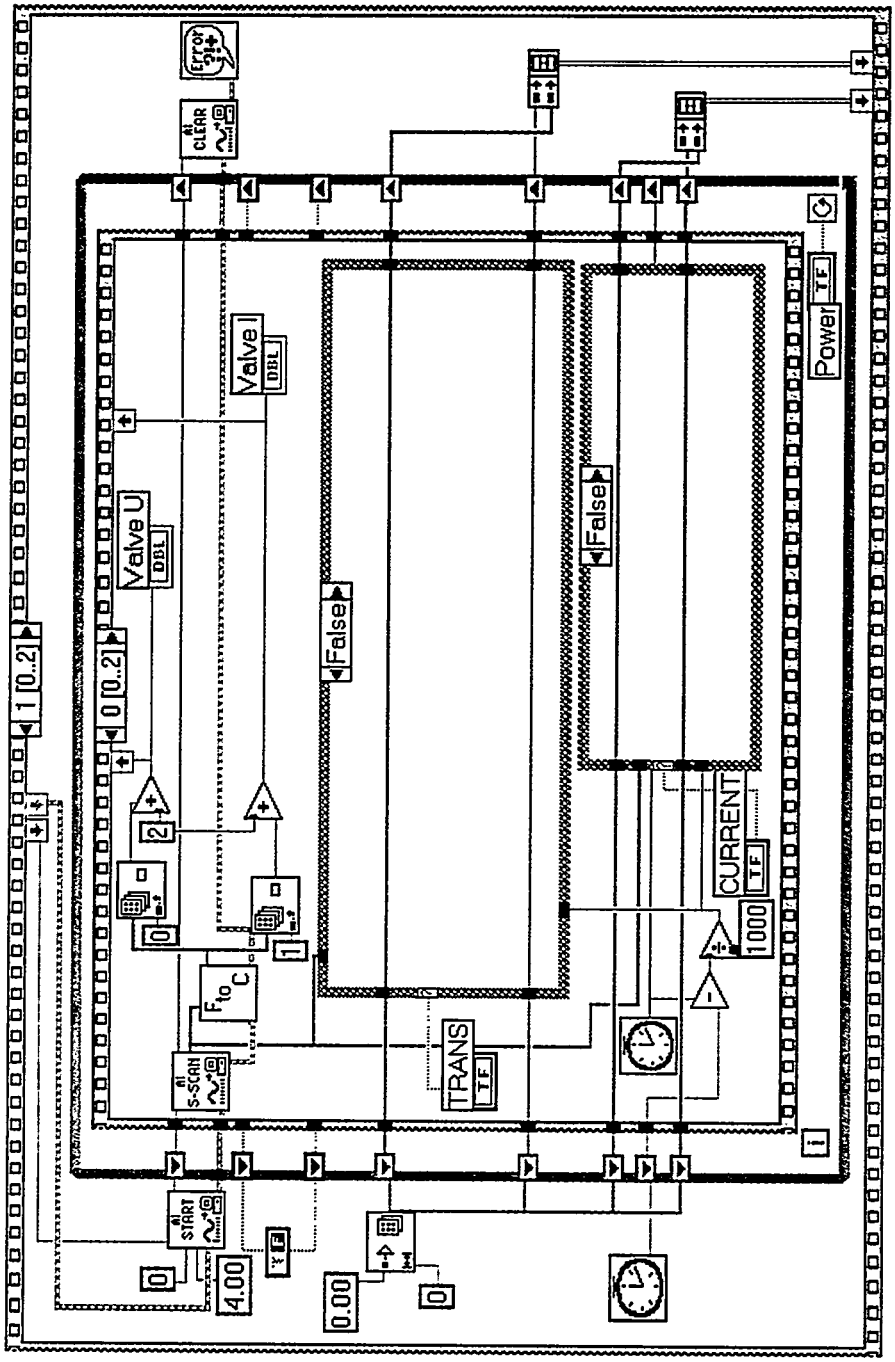
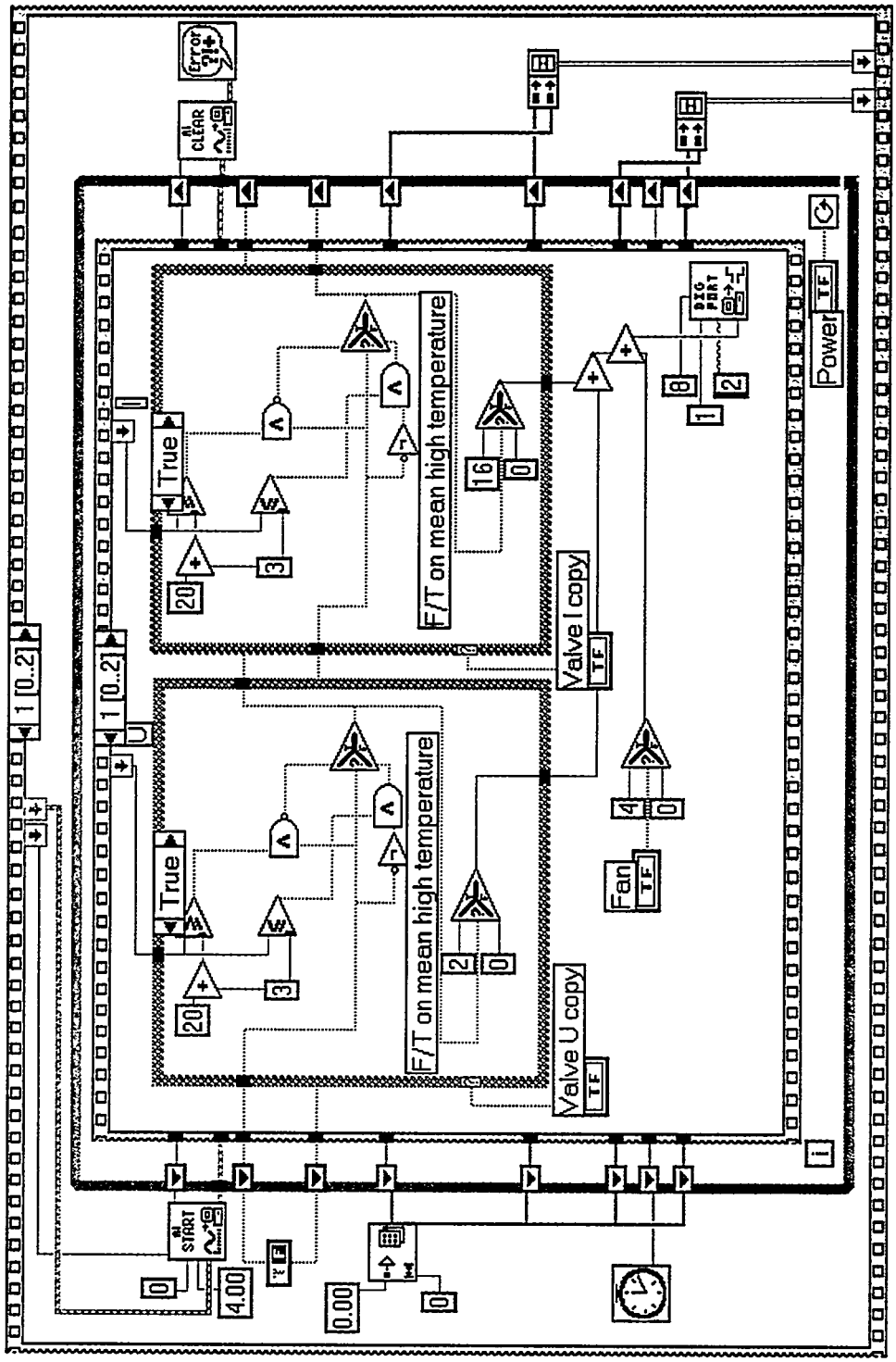


Figure A3 Block diagram of instrumental control software (continued)



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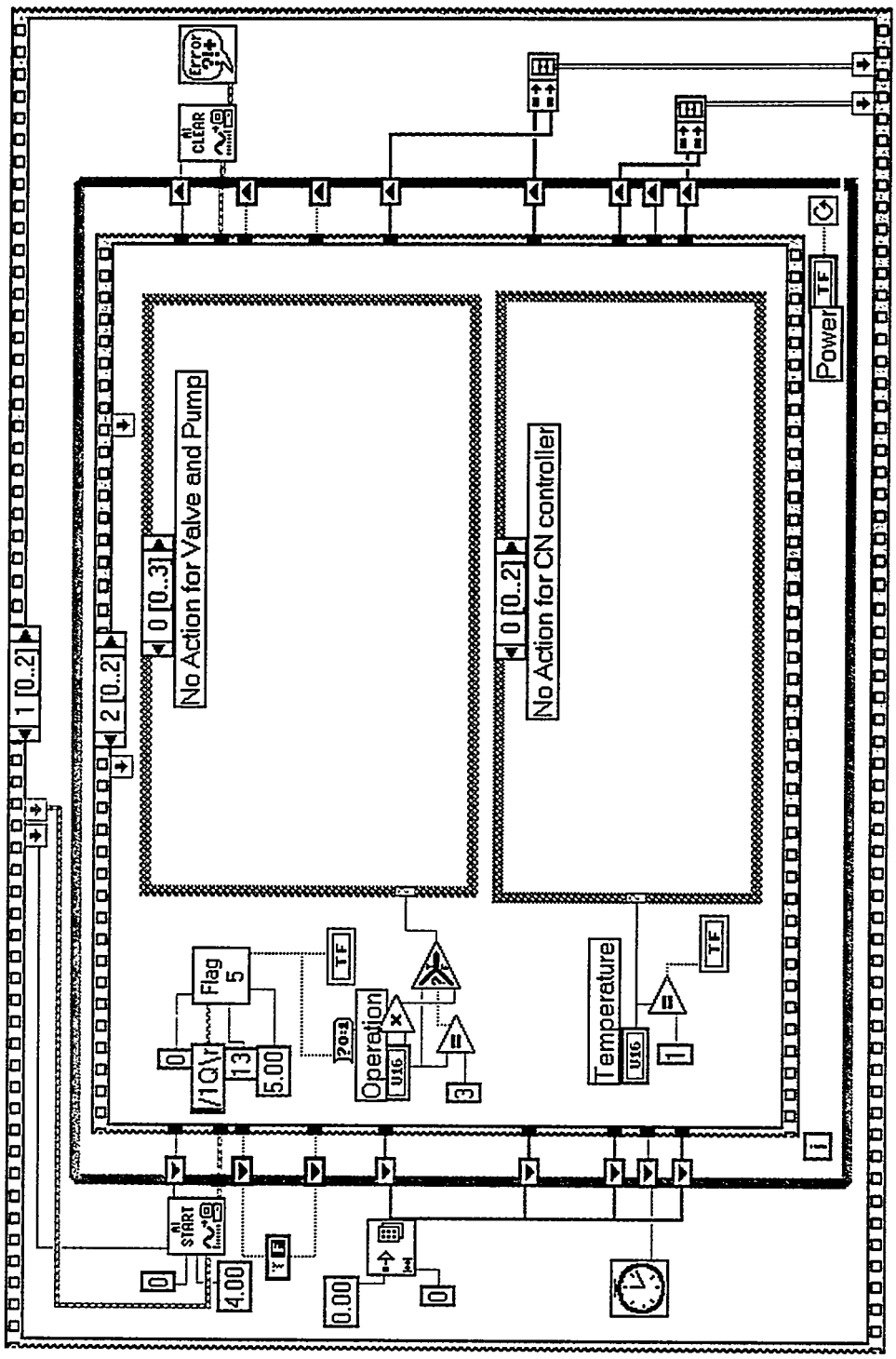

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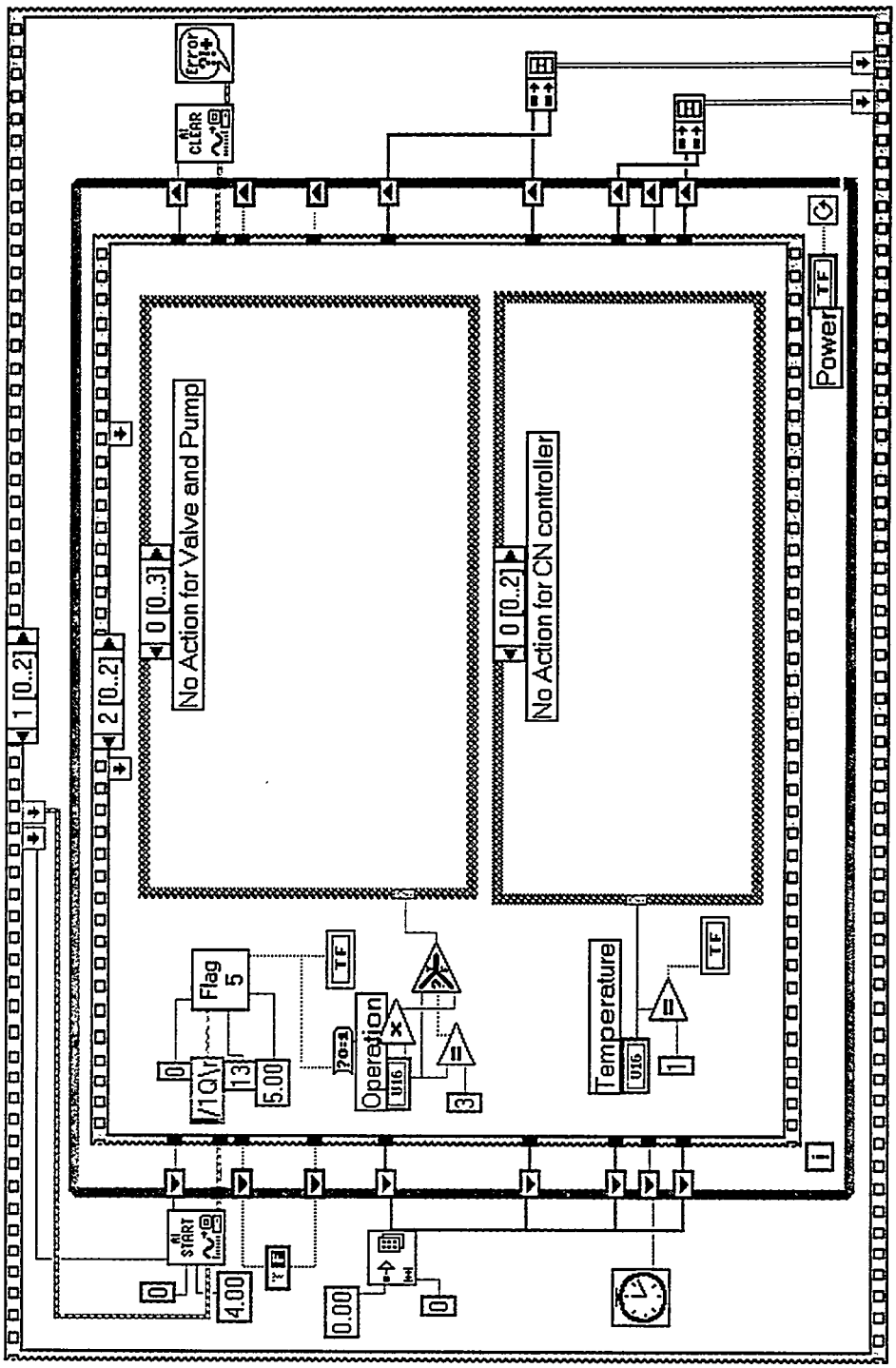

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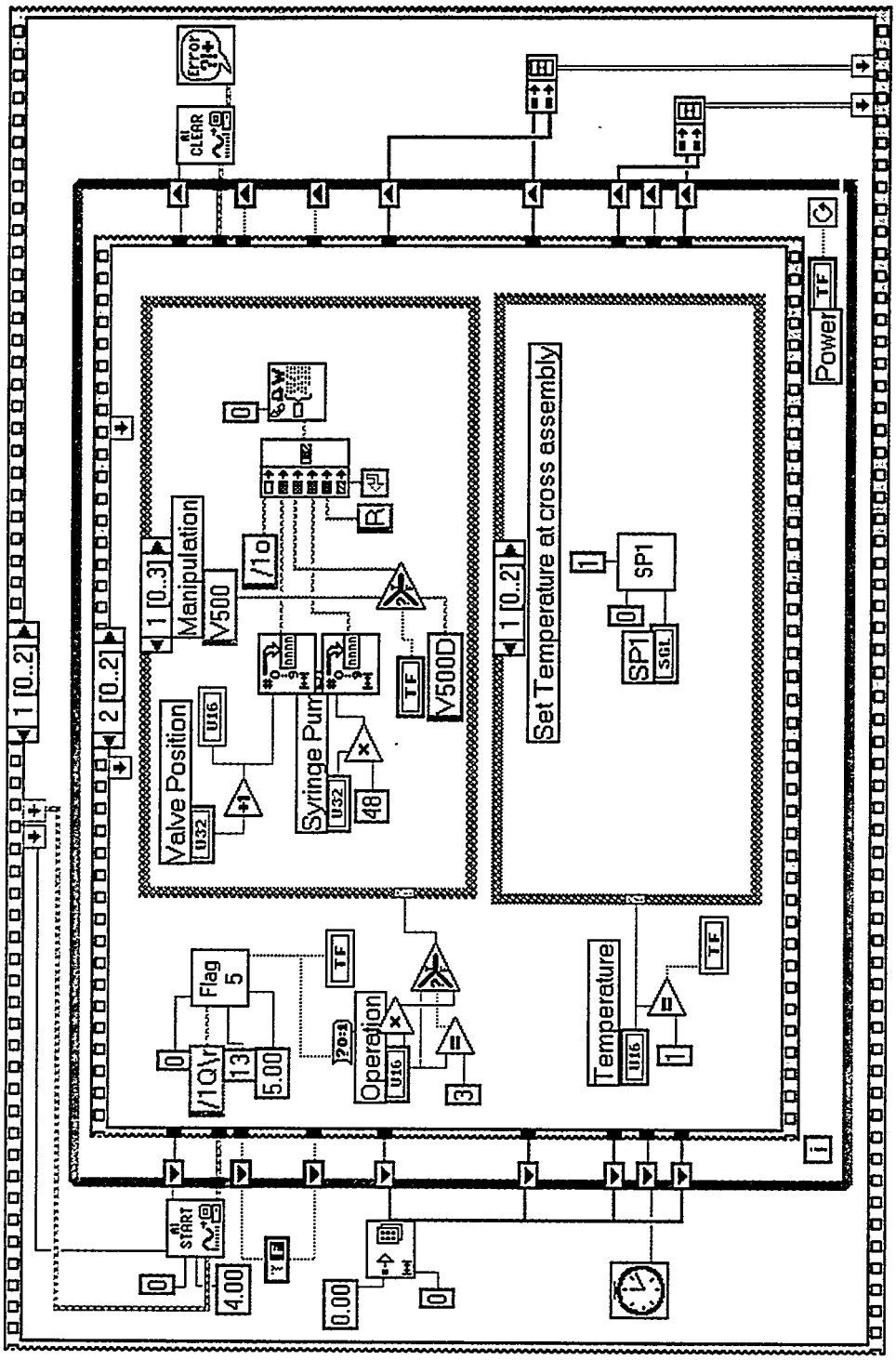

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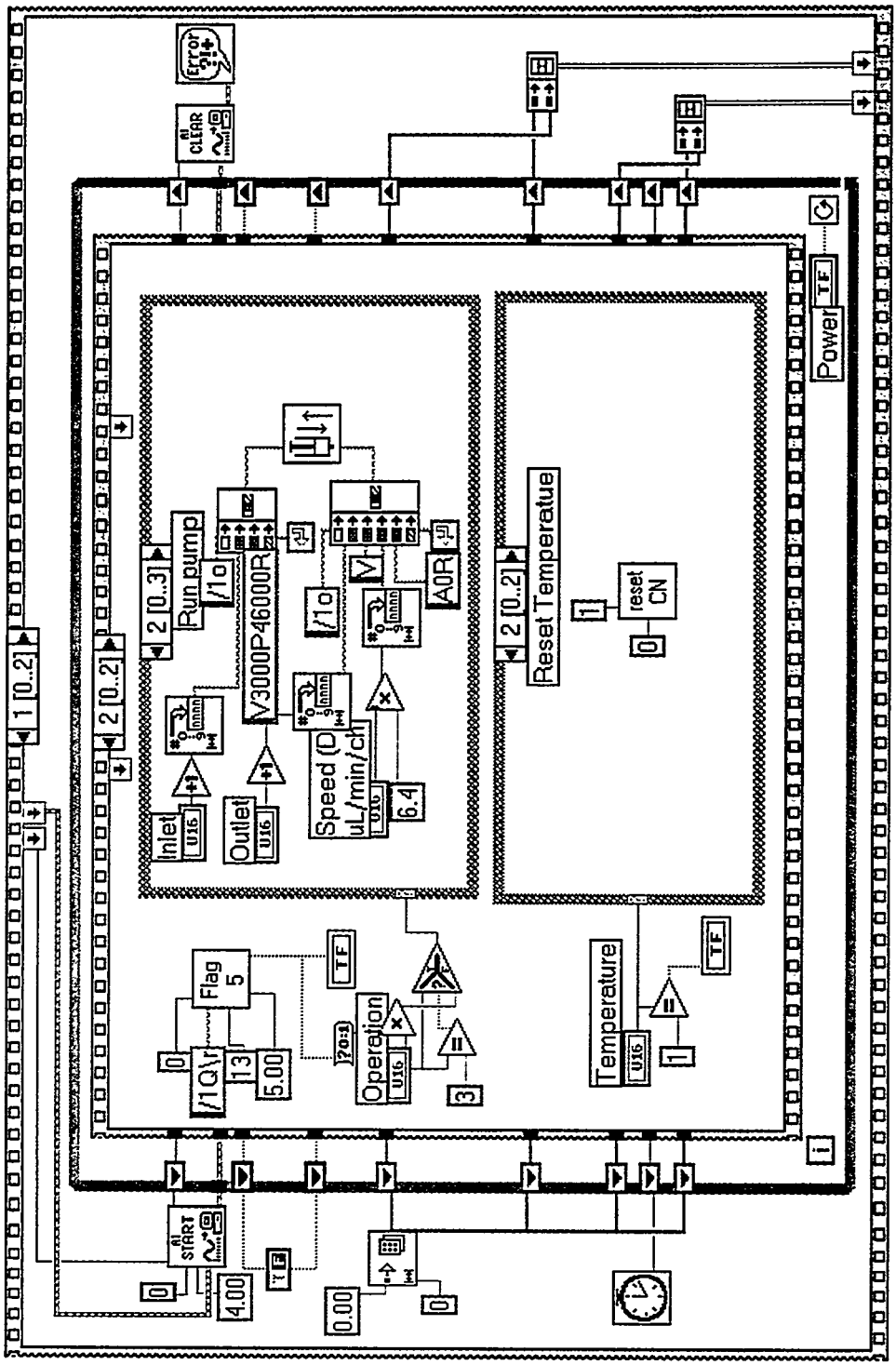

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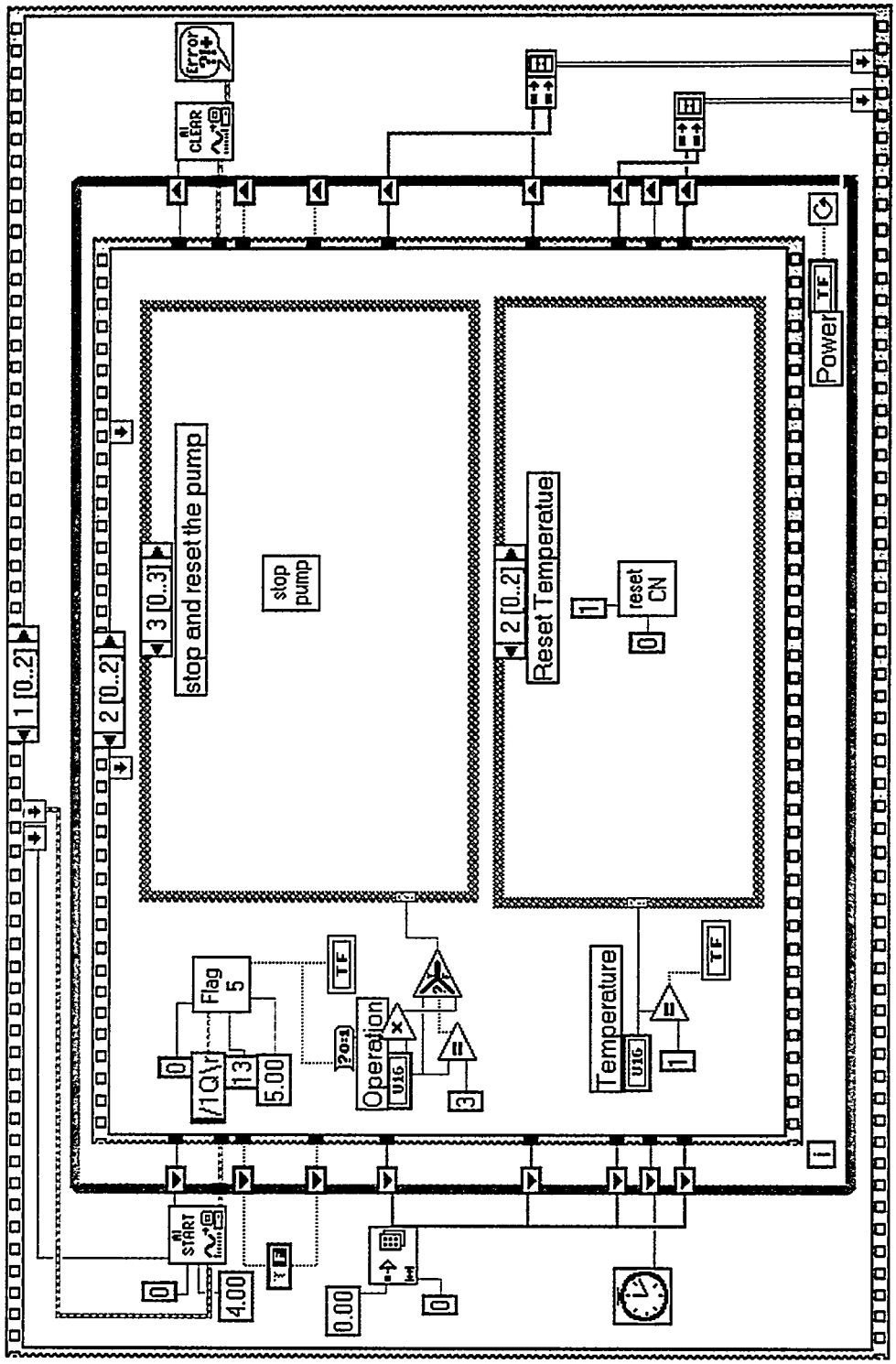

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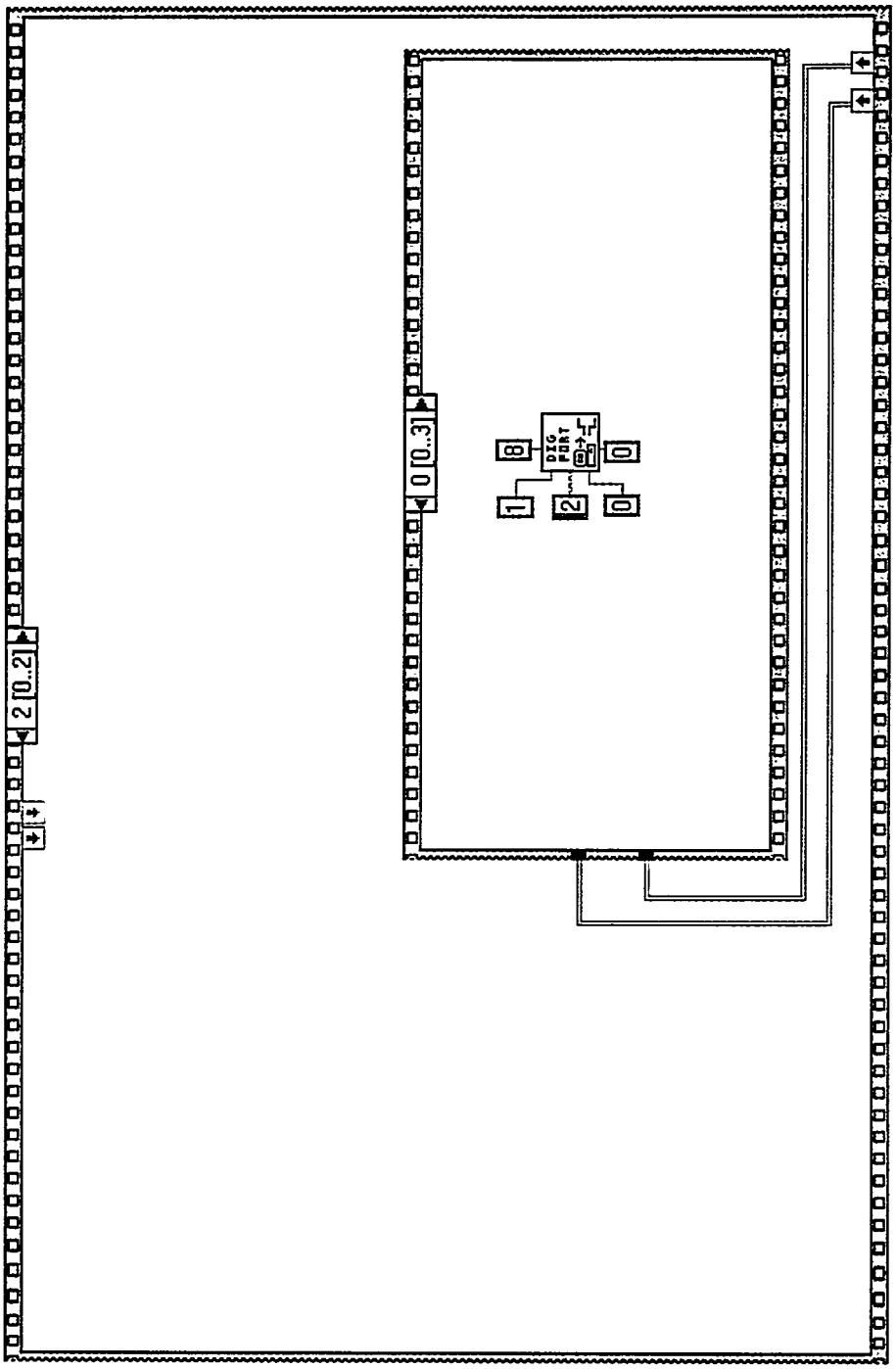

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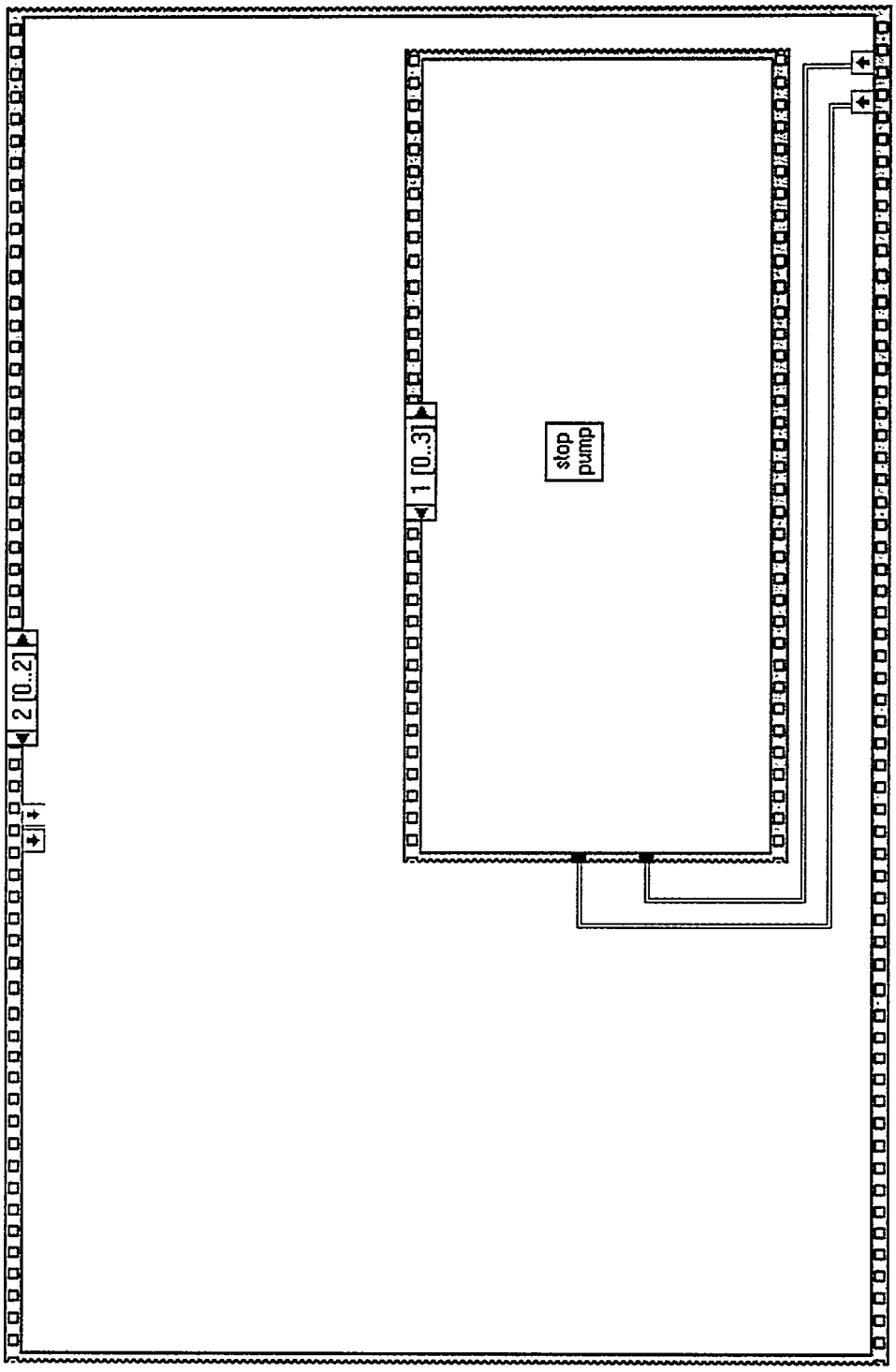

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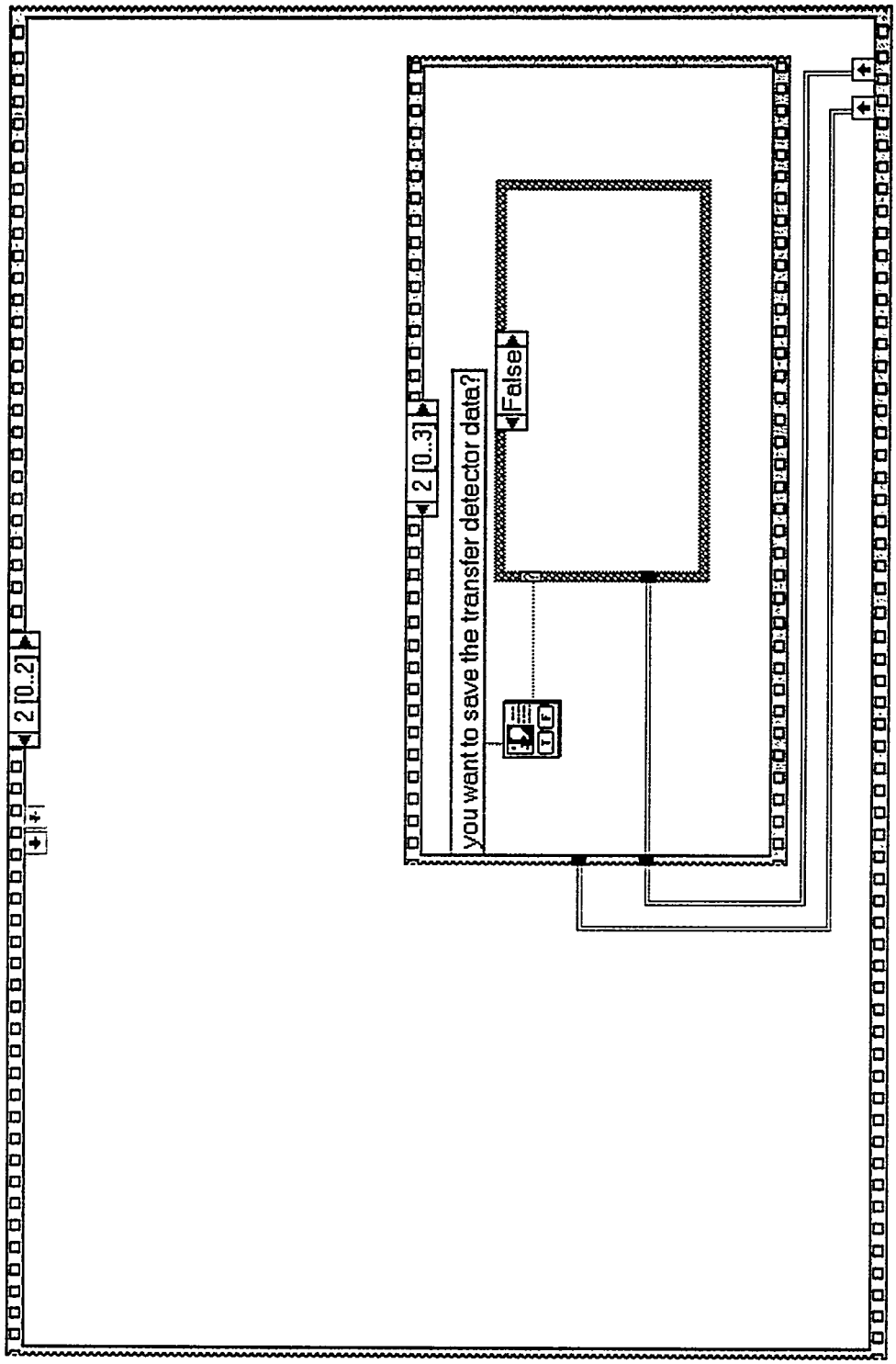

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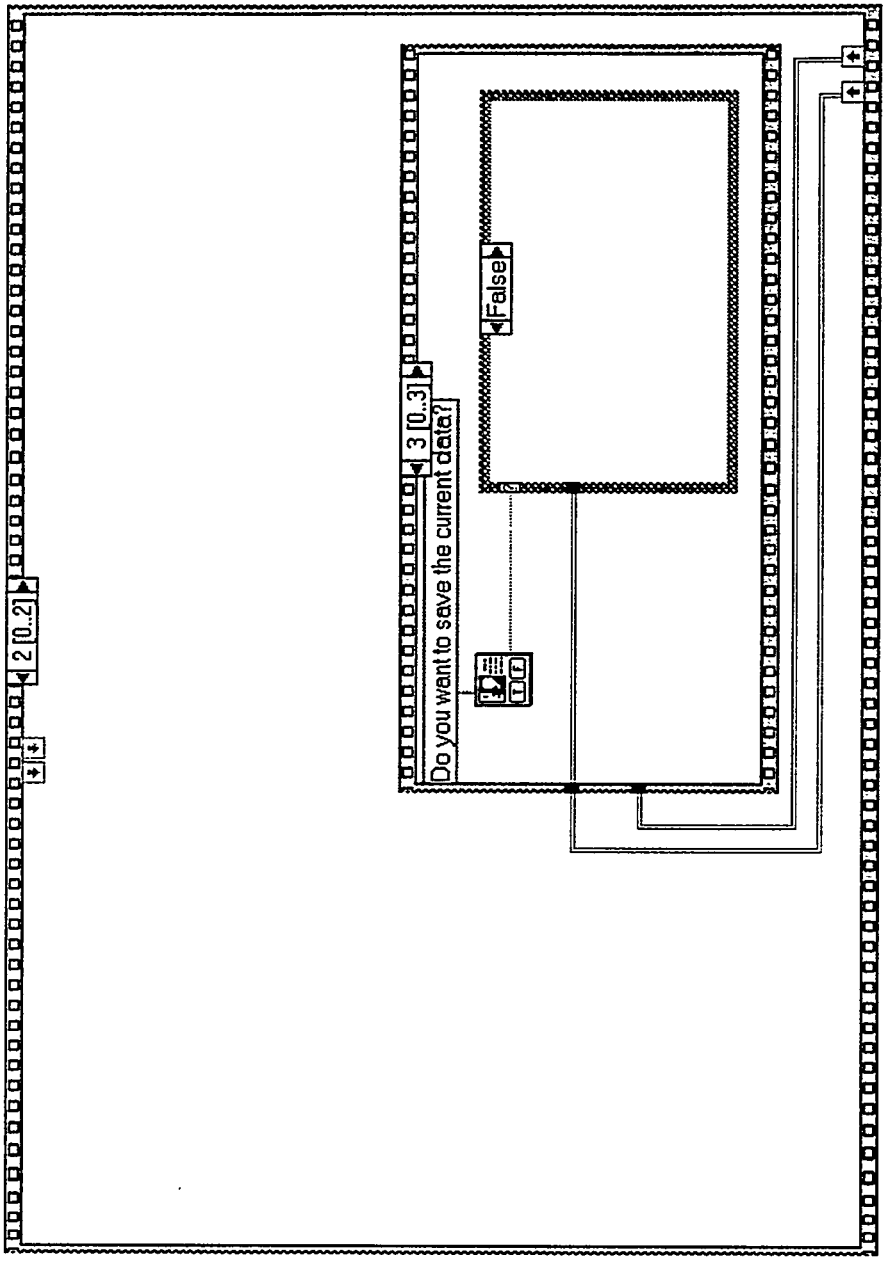


Figure A3 Block diagram of instrumental control software (continued)

APPENDIX B. TERMS AND ABBREVIATIONS

A/D	Analog and digital signal converter
Allele	One of several alternative forms of a gene or DNA sequence at a particular chromosomal position (locus). At each locus, an individual possesses two alleles. One inherited from father; one inherited from mother.
Allelic Ladder	Pooled alleles which have been observed among population.
BSA	Bovine serum albumin
CAE	Capillary array electrophoresis
CCD	Charge coupled devices
CE	Capillary electrophoresis
CTTv	Four short tandem repeat loci which are: vMA, THO1, TPOX and CSF1PO
DNA	Deoxyribonucleic acid (the basic constituent of the gene)
dNTPs	Deoxynucleotide triphosphate
EtBr	Ethidium bromide
Genotyping	A term for wide range of methods used for studing genetic variations
HPLC	High performance liquid chromatography
RSD	Relative standard deviation
HIV	Human immunodeficienty virus
LabVIEW	Laboratory virtual instrument engineering workbench
LIF	Laser induced fluorescence
MW	Molecular weight

PCR	Polymerase chain reaction
PEO	Poly (ethylene oxide)
PMT	Photomultiplier tube
STR	Short tandem repeat DNA. The repeat unit is shorter than seven bases in each locus. Examples are vWA, THO1, TPOX and CSF1PO loci.
SVR	Surface to volume ratio
1xTE	Buffer composed of 10 mM tris base and 1 mM EDTA, pH= ~8
1xTBE	Buffer composed with 89 mM tris base, 89 mM boric acid and 2 mM EDTA, pH= ~8.2
VNTR	Variable number of tandem repeat DNA. The length of repeat unit is between seven to hundreds. Example is D1S80 locus.

APPENDIX C. INSTRUMENTAL SURVEY

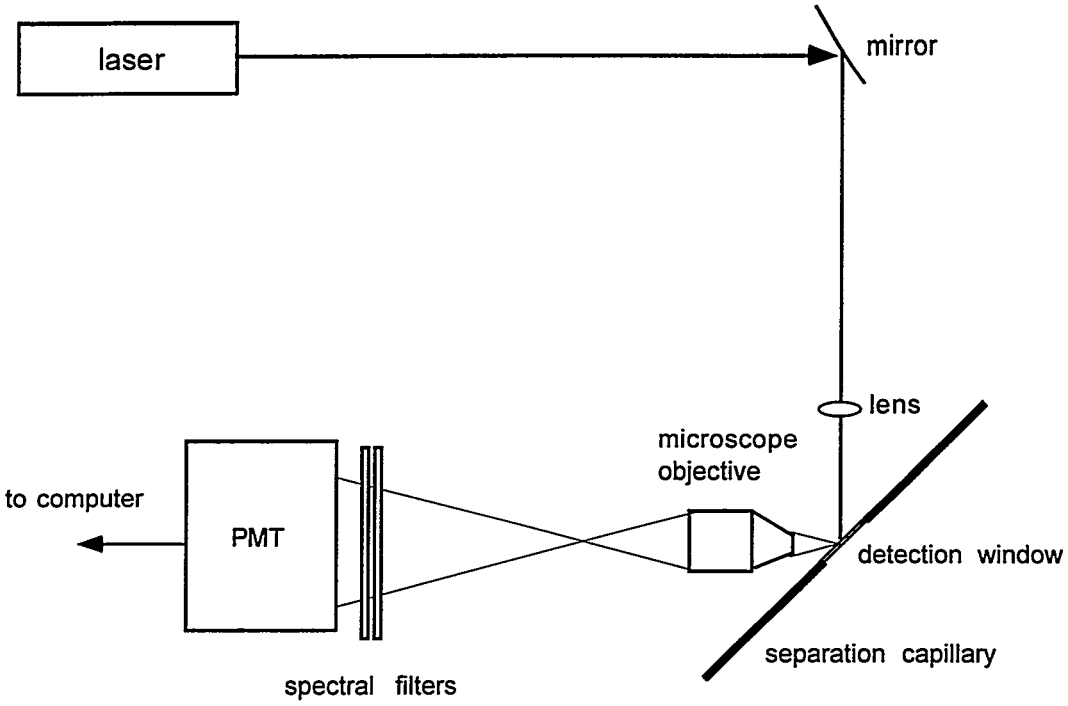
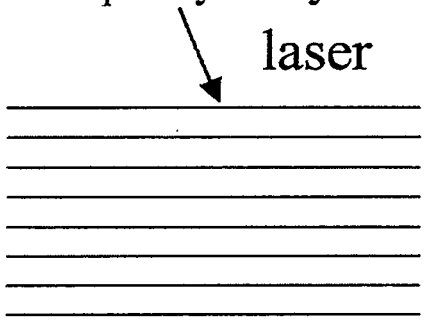


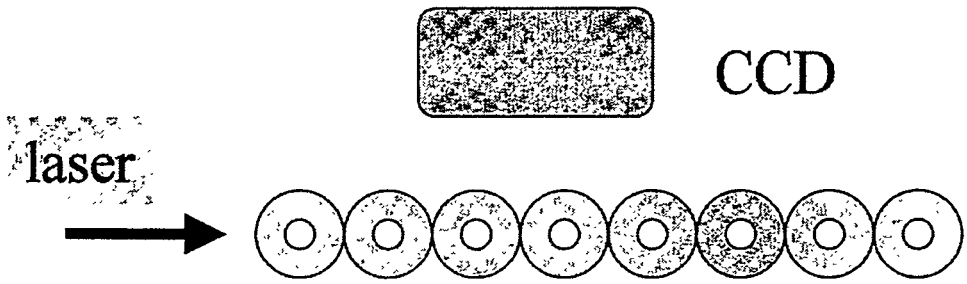
Figure C1 Schematic diagram of CE-LIF

Side Entrance Excitation

- Top View of Capillary Array:



- Cross-sectional View Along Laser Beam:



- Focusing and Image of the Background Fluorescence of the 8x capillary array :

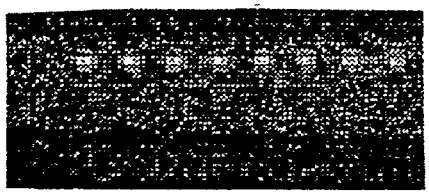


Figure C2 Laser excitation mode for capillary array electrophoresis used in this study

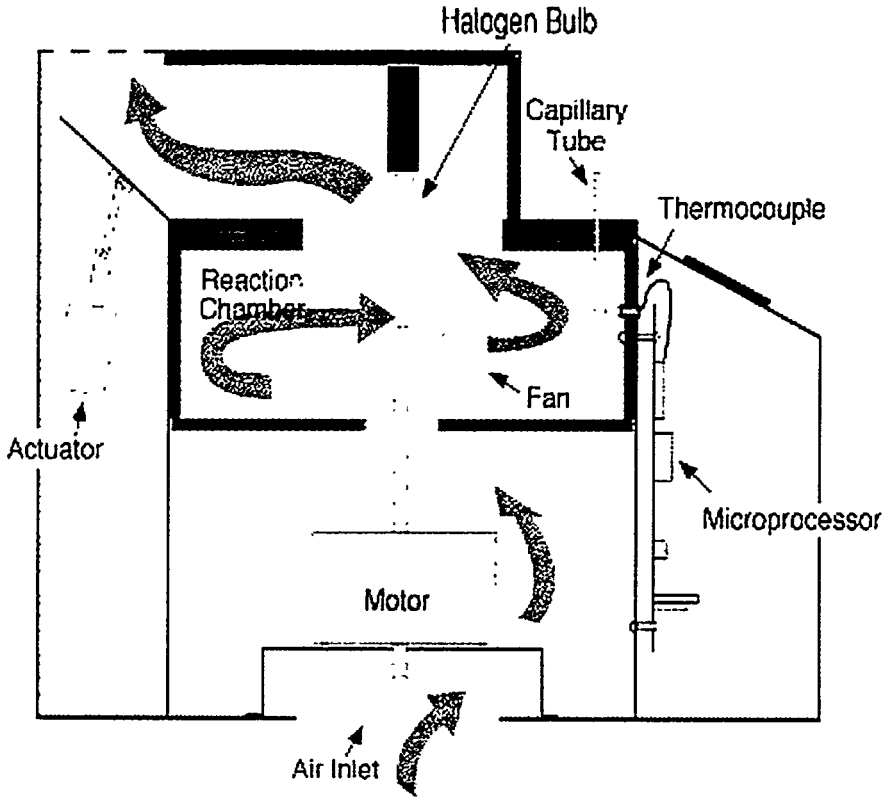


Figure C3 Interior view of the RapidCycler. Air flow through the machine is indicated by the arrows

Kloehn Company's syringe drive modules are mechanically designed for full optimization of the precision ground lead screw, a standard resolution 24,000:1 or optional 48,000:1 resolution is achieved, the industry's highest. Each Module contains dual stepper motor drive; enhancing the ability to create multi-position valving. Rotary valves available in 3, 4, 5, 6, and 8 way configuration. The modules feature easy change of a variety of syringe volumes, 25 µl through 50 ml. Each extended life syringe is constructed of borosilicate glass, Teflon and Kel-F.

Some of the features of 50300 are:

Non-volatile Program Storage, Store up to 10 user programs. Ten year program memory when power is removed. Run programs by name from a remote controller.

Extra User Outputs-Three user outputs for driving relay or other electronic devices under user program control.

User Digital Inputs-Inputs are relay or logic compatible. Inputs can be sampled under program control. Feedback reporting to controlling computer.

Analog Measurements-Digital volt meter allows user to measure external parameters; position, force, pressure, temperature, light intensity, and pH can be reported to a controlling computer.

Advanced Program Control-Control tatements allow program looping, decision and branching based on external conditions.

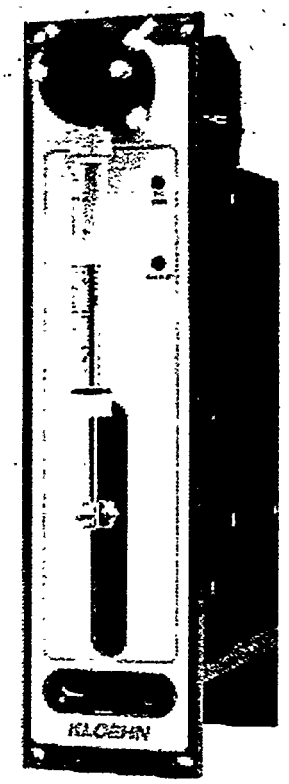
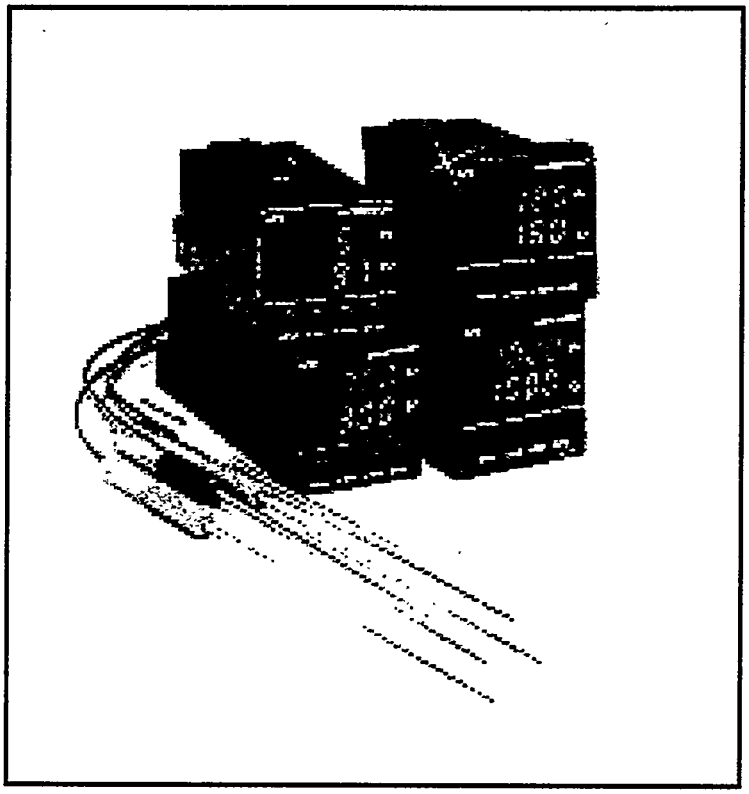


Figure C4 Sringe drives Model 50300 from Kloehn Ltd.

1/16 DIN MICROMEGA AUTOTUNE TEMPERATURE

& PROCESS CONTROLLERS



The CN77000 controllers offer unparalleled flexibility in process control. Each unit allows the user to select the input type, from 10 thermocouple types (J, K, T, E, R, S, B, C, N and J DIN), Pt RTDs (100, 200, 500 or 1000Ohms, with either 385 or 392 curve), or analog voltage or current inputs. The voltage/current inputs are fully scalable to engineering units, with selectable decimal point, perfect for use with pressure, flow or other process input.

•High ±0.5°C, 0.03% Rdg. Accuracy

•Universal Inputs Process Voltage, Current, RTD and Thermocouple

•Dual 4-Digit LED Display with Indicators for Output and Alarm Status

•Relay, ac SSR, dc Pulse, 0 to 10 V and 0 to 20 mA

•Output Types

•Ramp up to Setpoint Feature

•Universal Power Supply, 90-250 Vac or Vdc (Optional 10-34 Vac or Vdc)

•Dual Output and Dual Alarm Capability

•Optional RS-232, RS-485, Analog Output or Remote Setpoint Selection

•Optional Arbored Holesaw for Round Cutout Models

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