

FY'00 Shared Biomedical Research Equipment Proposal for the Purchase of a Qualitative and Quantitative PCR LightCycler System.

Submitted by Kenneth R. Peterson, Ph.D. and Glen K. Andrews, Ph.D.
Department of Biochemistry and Molecular Biology

1. Description of equipment and price.

We propose to purchase a LightCycler system from Roche Molecular Biochemicals. The price quoted from Roche is \$55,000, which includes the LightCycler instrument and a desktop computer/printer. In addition, we will purchase a one-year extended warranty beyond the initial warranty period (\$3,600), an additional copy of the LightCycler software (\$300), a carousel for multiple samples (\$1,000), and a cooling block and adapter (\$360). Shipping and handling costs will be \$150, bringing the **total amount to \$60,410**. Investigators, centers and departments using the equipment will **contribute a total of \$11,750.00**. Therefore, the total amount requested from the **School of Medicine is \$48,660**.

2. Statement of equipment need.

Studies of gene expression traditionally relied upon RNase protection, S1 nuclease protection, Northern blot hybridization or quantitative RT-PCR. The first three are time-consuming, technically demanding and require the use of radiolabeled nucleic acid probes; the latter requires intense empirical standardization to obtain valid data. All of these approaches require gel electrophoresis and gel staining or autoradiography. New technology has been developed that allows: 1) precise quantitation of mRNA species by coupling PCR with fluorescent probes, 2) elimination of gel techniques, and 3) completion in minutes as opposed to days. In addition to simplicity and speed, advantages include safety (no radionuclides required), and simultaneous analysis of multiple samples. Essentially, the LightCycler combines fluorescent technologies with ultra-rapid thermal cycling that allows complete amplification and analysis of up to 32 samples in less than 20 minutes. Thus, many investigators can obtain data in a single day. Amplification and detection are performed in the same tube so that results can be monitored on-line and in real-time. Multiple sequences can be studied concurrently within the same reaction. The sensitivity of the instrument allows detection of 100 mRNA copies in 10 pg of total RNA or a single-copy gene in 3 pg of human genomic DNA. In addition to measurement of gene expression levels, the LightCycler accommodates the following types of analyses.

- 1) Study target sequences by looking at the melting behavior of the amplified product.
- 2) Differentiate between specific and nonspecific fragments.
- 3) Identify different genotypes of a target molecule without sequencing.
- 4) Detect point mutations.

This proposal has identified 12 active users from four basic science departments, and has obtained commitment for support from a center (Reproductive) and from the Department of Biochemistry and Molecular Biology. This equipment will directly support the efforts of 22 NIH funded grants, including program project and center grants, as well as several other grants and 3 pending NIH grants.

The LightCycler will be available to the aforementioned investigators as well as other faculty of the University of Kansas Medical Center. We anticipate that participants of this proposal will use this instrument 80-90% of the time, on a first-come, first-serve basis. This will apply to users not a part of this proposal as well.

3. This is a new piece of equipment for the University of Kansas Medical Center.
4. Drs. Kenneth R. Peterson and Glen K. Andrews of the Department of Biochemistry and Molecular Biology will be responsible for the LightCycler system, including maintenance. It will be housed in Wahl Hall East, room 4005.
5. Names of investigators and brief summaries of the research programs that will share and benefit from the equipment.

Dr. Glen K. Andrews Biochemistry and Molecular Biology

Our research focuses on the mechanisms of regulation of metallothionein gene expression. The molecular mechanisms of metal induction of these genes, as well as the mechanisms regulating the cell-specific expression of these genes during development are being studied. We are also developing a new area of study involving expression and regulation of zinc transporter genes during pregnancy. The LightCycler will greatly facilitate quantitative measurements of mRNA levels in small samples.

NIH grants to be supported by this equipment include:

Environmental toxicology using transgenic mouse models: ES 05704
Metallothioneins during reproduction and development: CA 61262

Dr. Kenneth Peterson Biochemistry and Molecular Biology

My research focuses on understanding genetic regulatory mechanisms with emphasis on the delineation of the function of locus control regions (LCRs). The human γ -globin locus serves as the primary model system and the effects of mutations on LCR function are being analyzed in the context of the intact γ -globin locus throughout development using YACS. Functional analysis of gene expression is performed by RNase protection currently. The LightCycler instrument technology will greatly facilitate my analyses of globin gene expression by eliminating radiolabeled probes, gel technology and autoradiography. In addition, my studies can be completed in hours, instead of days.

NIH grants supported by this equipment include:

Role of the LCR in human γ -globin gene regulation: DK 53510
Kansas Interdisciplinary Center for PKD Research; Project 6, "Test of the two-hit hypothesis in a PKD mouse model" DK 57301

Dr. S.K. Dey Molecular and Integrated Physiology

The research focus of our ongoing program is to identify genes that are critical for uterine receptivity for implantation and genes that are involved for embryo-uterine attachment reaction to initiate the process of implantation.

NIH grants supported by this equipment include:

Aspects of blastocyst implantation: HD 12304
Aspects of uterine receptivity for implantation: HD 29968

Dr. Michael P. Sarras Jr. Anatomy and Cell Biology

Our laboratory has utilized the invertebrate system Hydra and the vertebrate system zebrafish to analyze the expression of ECM and MMPs genes during development.

NIH grants supported by this equipment include:

An in vivo model for glucose-mediated basement thickening: DK 47840

Metalloproteinases in Connective Tissue Matrix Breakdown: AR 39189

**Dr. Joan S. Hunt Department of Anatomy and Cell Biology/Pathology and
Laboratory Medicine**

Two major projects in our laboratory utilize PCR/RT-PCR to assess gene expression and regulation. The first is HD24212, where we are evaluating one of the tumor necrosis superfamily genes, TRAIL, in human placentas. Real time PCR would be of major assistance as we assess the impact of endogenous and pathological biological molecules that modulate expression of this gene. At present we read against housekeeping genes, but essentially all of these are profoundly or subtly affected by our cytokine and hormone modulators. In a second project, we are investigating genomic elements that include regulatory sequences, and also isoform-specific messages in human placental cells that encode membrane-bound and soluble isoforms of HLA-G, a novel transplantation antigen in reproduction. Again, Real time PCR would permit us to perform fine analyses of levels of gene expression under various conditions.

NIH grants supported by this equipment include:

Class I MHC Gene Expression by Human Trophoblast Cells: HD 26429

Role of Tumor Necrosis Factor-alpha in Development: HD 29156

Decidual Cell/Placental Interactions: HD 24212

Dr. Hiroaki Serizawa Biochemistry and Molecular Biology

Transcription factor TFIIF by RNA polymerase II (RNA pol II) is a multi-subunit enzyme possessing cyclin-dependent kinase-activating kinase (CAK), DNA repair activity, and is essential for transcription. TFIIF subunits, CDK7 and cyclinH, are capable of phosphorylating the carboxyl-terminal domain (CTD) of the largest subunit of RNA pol II, an important event for transcriptional regulation. Recently, it has been suggested that CTD phosphorylation by CDK7 plays an important role in cardiac muscle hypertrophy. Cardiac hypertrophy is accompanied with enhanced activity of RNA pol II, and the enhancement is thought to be regulated via CTD phosphorylation by CDK7, TFIIF kinase subunit. The primary goal of the research program is to determine the biochemical activities and subunits of TFIIF-p16. Investigations proposed in the research program will provide important information on understanding biochemical functions of p16INK4A and TFIIF in transcription. These investigations are important for elucidating molecular mechanism of cardiac muscle hypertrophy.

A grant supported by this equipment is:

American Heart Association, Heartland Affiliate: G 5380005

Dr. Robert C. De Lisle Anatomy and Cell Biology

My lab works on exocrine cell biology in normal and diseased states. The project will benefit from this equipment has as its long-term objective to understand how loss of functional CFTR (cystic fibrosis transmembrane regulator), which is the genetic defect causing cystic fibrosis, affects expression and posttranslational processing of sulfated glycoconjugates. Accumulation of abnormally processed sulfated glycoconjugates are believed to contribute to

development of pathologies of tissues affected in cystic fibrosis (exocrine pancreas, intestines, and lungs). Muclin, a 300 kDa sulfated mucin-type glycoprotein, is being used as a model sulfated glycoprotein, to investigate the relationship between loss of CFTR and overexpression of Muclin. Muclin is overexpressed in pancreas and intestines of CF mice. We plan to measure the mRNA levels of various cytokines and other cell signaling molecules which are expected to be expressed in the CF intestine and are candidates for regulation of Muclin expression by epithelial cells. The Roche Light Cycler will make these experiments feasible.

NIH and other grants supported by this equipment include:

Pathogenesis of cystic fibrosis in the GI system: DK 5679

Sulphated gp300 in the GI system of CF and normal mice: DELISL96PO; Cystic Fibrosis Foundation (G 5258086).

Dr. Paul Terranova Molecular and Integrative Physiology

The objective of this project is to delineate the mechanisms by which tumor necrosis factor alpha (TNF) and its receptor system inhibit gonadotropin-stimulated ovarian granulosa cell estradiol secretion using a mouse model. The site of action of TNF in human and mouse granulosa cells appears to be at post cAMP sites, whereas in the rat model TNF inhibits at sites prior to cAMP, as well as at post cAMP sites. The real time PCR machine would be used to determine aromatase expression in human granulosa cells. Since the amount of RNA is limited in the human model this machine will be especially helpful in quantitative aspects.

NIH grants supported by this equipment include:

Ovarian Tumor Necrosis Factor: CA 50616

Center for Reproductive Sciences: P30 HD 33994

(I am the Director of the Center)

Dr. Alan R. Godwin Molecular and Integrative Physiology

Our research focuses on understanding the role of two murine *Hox genes*: *Hoxc12* and *Hoxc13*. Mice carrying targeted gene disruptions in *Hoxc13* have brittle hair, abnormal nails, and broken filiform papillae on the surface of the tongue. All of these defects are consistent with structural problems in the respective body region. Further, *Hoxc13* as well as the hair keratins are expressed in each of these body regions. Therefore, *Hoxc13* has excellent candidates for the downstream genes that it regulates: the hair keratin and hair keratin associated genes. In addition, we have generated mice carrying *Hoxc13-GFP* (green fluorescent protein) gene fusions allowing isolation of cells expressing GFP from homozygotes and heterozygotes. Comparison of the mRNAs expressed in these two cell populations will identify additional genes controlled by *Hoxc13*. A method of further verification that *Hoxc13* actually controls these genes is needed. In addition, quantitation of the expression level differences between heterozygotes and homozygotes is needed. The sensitivity and speed of analysis using the PCR LightCycler System will achieve both these later two considerations for us. In addition, the ability to simultaneously analyze multiple genes will greatly simplify our studies and greatly decrease the amount of time needed to analyze the expression of multiple genes.

Grants supported by this equipment include:

Institutional start-up funds

Pending grants:

Hoxc13 and hair follicle morphogenesis: RO1.

Dr. James P. Calvet **Biochemistry and Molecular Biology**

Most cases of human polycystic kidney disease are caused by mutations in two genes, *PKD1* and *PKD2*. The *PKD1* protein, polycystin-1, is a membrane-associated glycoprotein. We hypothesize that polycystin-1 functions as a G-protein coupled receptor. To test this idea, we are performing the following experiments. 1) Amino acid residues required for the *in vitro* binding interaction between polycystin-1 and heterotrimeric G-proteins will be determined. 2) The potential for polycystin-1 to engage in heterotrimeric G-protein coupled signal transduction will be analyzed. 3) The potential for polycystin-1 to interact with regulators of heterotrimeric G-protein coupled receptors will be assessed. 4) The importance of the conserved G-protein activation domain will be tested in transgenic mice. The LightCycler will help us with the studies described in the last of these experiments. It will allow us to accurately genotype our "knock-in" mice as well as perform necessary *PKD1* gene expression studies using RT-PCR.

NIH grants supported by this equipment include:

Mouse PKD1 protein and its biochemical interactions: DK 51047

Molecular mechanisms of progressive renal disorders; Project 2, "Molecular Mechanisms of Polycystin Function:" P01 DK 53763

Kansas interdisciplinary center for PKD research; Project 3, "Polycystin G-protein Signal Transduction:" P50 DK 57301

Dr. R. Padmanabhan **Biochemistry and Molecular Biology**

Dengue virus project: Overall goal of this project is to understand the function of dengue virus proteins, NS3 and NS5, in the biological processes such as polyprotein processing and viral RNA replication in the virus life cycle. It is important to be able to quantitate the viral RNA synthesized in wild type and mutant infected cells by PCR. Several site-specific mutants of the viral RNA genome will be constructed in the cDNA constructs and transcribed *in vitro*. The RNAs will be transfected into mammalian cells and their replication potential will be assessed. A highly sensitive instrumentation for quantitation will be extremely useful for this project.

Hepatitis C virus: Overall goal of this project is to analyze the role of phosphorylation in the function of NS5A protein coded by the virus. NS5A protein interacts with and is phosphorylated by cellular kinase(s). The specific aims are focused on identification of these cellular kinase(s) and map the sites of phosphorylation. The function of this protein modulation of cell cycle signaling is under investigation. Results of our work indicate that NS5A expression retards cell growth as revealed by analysis of growth properties, efficiency of colony formation and increased expression of p21cip1/waf 1 both at mRNA and protein levels. To pursue these observations at molecular details, we need a very sensitive instrument such as the Real Time PCR machine to measure the levels of mRNAs encoding cell cycle regulatory proteins.

NIH grants supported by this equipment include:

Functional Analysis of dengue viral: AI 32078

Virus/Host Interactions Modulated by Hepatitis: AI 44036

Dr. Leslie L. Heckert **Molecular and Integrative Physiology**

The research in my laboratory focuses on understanding the transcriptional and cell-signaling processes that are important for gonadal function and development. We are currently studying the genes that encode the follicle-stimulating hormone receptor (FSHR), a protein expressed only in somatic cells of the gonads, and steroidogenic factor 1 (SF-1), an orphan nuclear receptor required for gonad and adrenal formation. Aspects of these projects require

quantification of mRNA levels for various genes during development of the gonads. Because of the limiting amount of starting material, these studies will require a very sensitive and accurate method for quantification of mRNA. The Roche Light Cycler will provide the needed sensitivity and quantitative accuracy. In addition, the method of real time PCR will allow us to measure many different messages in significantly less time than more conventional techniques.

NIH grants supported by this equipment include:

Gonadal roles by a new transgenic approach: HD 35871

Transcriptional regulation of the FSH receptor: HD 35217

Identification of bHLH proteins in Sertoli cells: P30 HD 33994

Pending grants:

Regulation of SF-1 expression in the gonads: HD38498

Identification of HLH proteins in the testis: HD 38923

6. Outline of commitments for sharing the cost of purchase.

<u>Name</u>	<u>Department</u>	<u>Amount committed</u>
Dr. G. K. Andrews	Biochemistry and Molecular Biology	\$1,000
Dr. Kenneth Peterson	Biochemistry and Molecular Biology	1,000
Dr. S.K. Dey	Molecular and Integrated Physiology	500
Dr. Michael P. Sarras Jr.	Anatomy and Cell Biology	500
Dr. Joan S. Hunt	Anatomy and Cell Biology/Pathology and Laboratory Medicine	1,000
Dr. Hiroaki Serizawa	Biochemistry and Molecular Biology	1,000
Dr. Robert C. De Lisle	Anatomy and Cell Biology	750
Dr. Paul Terranova	Molecular and Integrated Physiology	500
Dr. Alan R. Godwin	Molecular and Integrative Physiology	500
Dr. James P. Calvet	Biochemistry and Molecular Biology	1,000
Dr. R. Padmanabhan	Biochemistry and Molecular Biology	1,000
Dr. Leslie L. Heckert	Molecular and Integrative Physiology	500
<u>Center for Reproductive Sciences</u>		500
<u>Department of Biochemistry and Molecular Biology</u>		<u>2,000</u>
Total cost share:		\$11,750.00
Requested from the School of Medicine:		\$48,660.00

01 OCTOBER 1999

Dr. Ken Peterson / Customer number 55059272
4034 Wahl Hall East
KU Med Center / 3901 Rainbow Blvd.
Kansas City, KS 66160
Phone 913-588-6907
Fax 913.588.7440

Dear Dr. Peterson,

Roche Molecular Biochemicals (RMB) is pleased to offer the following proposal for the LightCycler™:

Pricing Information:

Effective dates of proposal	*Description	RMB Product Number	Price
01 Oct 1999 through 31 Dec 1999	LightCycler™ Instrument		\$55,000
	(with desktop computer)	2043 912	
	(with laptop computer)	2011 468	

*System components and specifications - Exhibit A (attached)

LightCycler™ reagents and accessories - Exhibit B (attached)

LightCycler™ unique specifications and features - Exhibit C (attached).

Instrument Warranty:

- Complete coverage for parts and labor for one year.
- Technical assistance hotline for hardware, software and application chemistry
- 48 hour response time for on-site repair/replacement

Available Extended Warranty Service Agreement:

For coverage beyond the initial warranty period:

Coverage Period	RMB Product Number	Cost per instrument
One year extended coverage	101607	\$3,600.00
Two year extended coverage	101611	6,100.00
Three year extended coverage	101615	8,800.00

Five year extended coverage	101625	13,750.00
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KUMC / DR. KEN PETERSON

LightCycler™ Proposal for Sale

Additional Copies of LightCycler Software (one copy provided with each LightCycler™ purchased):

Additional Software	RMB Product Number	Price Per Copy
Additional Copy	1909304	\$ 300

Delivery and installation:

- SHIPPING CHARGES ADDITIONAL (see below)
- RMB installs instrument and provides on-site training for hardware and software

Estimated freight charges quoted are approximate and subject to change:

SHIPPING CHARGES	Carrier	Estimated Cost
Based upon estimated 150lbs. <ul style="list-style-type: none"> • <i>LightCycler™ – 35 lbs..</i> • <i>Miscellaneous Items (1 box of capillaries, etc...) – 20 lbs.</i> • <i>Desk Top Computer and printer – 60 lbs.</i> 	Fed Ex	\$150.00
Based upon estimated 150lbs. <ul style="list-style-type: none"> • <i>LightCycler™ – 35 lbs..</i> • <i>Miscellaneous Items (1 box of capillaries, etc...) – 20 lbs.</i> • <i>Lap Top Computer and printer – 10 lbs.</i> 	Fed Ex	\$120.00

Ordering:

To place your order, please contact me at 1-800-845-7355, mailbox number 8052 (voicemail). Please refer any questions or requests for additional information to me as well.

LIGHT CYCLER ASPECTS YOU WERE INTERESTED IN

- ACCESSORIES for the Light Cycler:

Software	\$ 300
Carrousel	\$ 1000
Cooling Block & Adapter	\$ 360

- "ON-LINE" vs. "REAL TIME"

"Real Time" means that data is collected for each sample in each cycle. "On Line" means that the "real time" data is displayed after each cycle. THE LIGHT CYCLER OFFERS "REAL TIME" DATA in addition to "On Line" data. The 7700 does not offer "On Line" data. "On Line" data allows you to stop if nothing is seen in the PCR, and allows you to continue an additional 10, 20, 30.... cycles if you desire.

- SPEED: FLEXIBILITY AND THROUGHPUT

The Light Cycler does 30 PCR cycles in less than 30 minutes. This allows greater THROUGHPUT in an 8 hour day (512 vs. 384 samples). This also allows greater FLEXIBILITY in that 16 researchers can use the unit in an 8 hour day, either using 16 separate runs, or 16 runs with different parameters.

Another aspect of flexibility is the fact that the LIGHT CYCLER CAN USE TAQMAN PROBES, but the 7700 cannot use Light Cycler probes.

- COSTS: SHORT TERM vs. LONG TERM

The short term costs would include the PURCHASE PRICE of \$55,000 instead of \$95,000. The long term costs would include MAINTENANCE COSTS (replacement of lasers vs. LEDs), and per-reaction costs. We estimate that our PER-REACTION COSTS are \$2.35; for the 7700, it is \$2.85.

- QUANTITATION

The Light Cycler can quantitate 3 PG OF HUMAN GENOMIC DNA, which is the equivalent of one genome (see page 11 of the large brochure). Also, the Light Cycler can DIFFERENTIATE BETWEEN 1 AND 10 COPIES in this range. The 7700 cannot differentiate at this low range; they are limited to differentiate in the range of 5,000 and 20,000 copies.

Please note also that shipping and handling is not included in the price of the Light Cycler. You may also be interested in purchasing an additional **Instrument Warranty**.

Sincerely,

John Reilly