Real-time Reporter Gene Assay

Progress of real-time reporter assay using bioluminescence protein gene.

1. Typical reporter protein in Japan.
2. Real-time reporter gene assay by photon counting system.
3. Real-time reporter gene assay by imaging system.

Feature of Bioluminescence Reporter Assay

High Quantum Yield	**QY=0.88 (Firefly) QY=0.28 (VL)**
High Selectivity	**Low Backgrand Noise High S/N**
Low Toxicity of Substrate	**Suitable for a continuous real-time reporter assay**

Photo: Dr. Osamu Shimomura

Aequorea aequorea

GFP

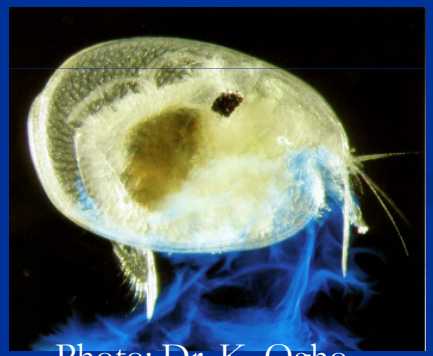


Photo: Dr. K. Ogho

Valugula hilugendorfii

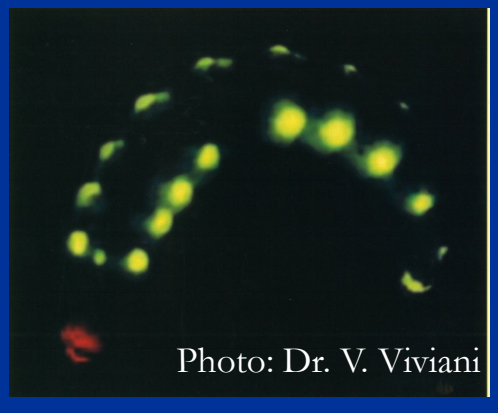
Photo: Dr. K. Niwa

Firefly

Luciola lateralis

Bioluminescence Reporter Gene

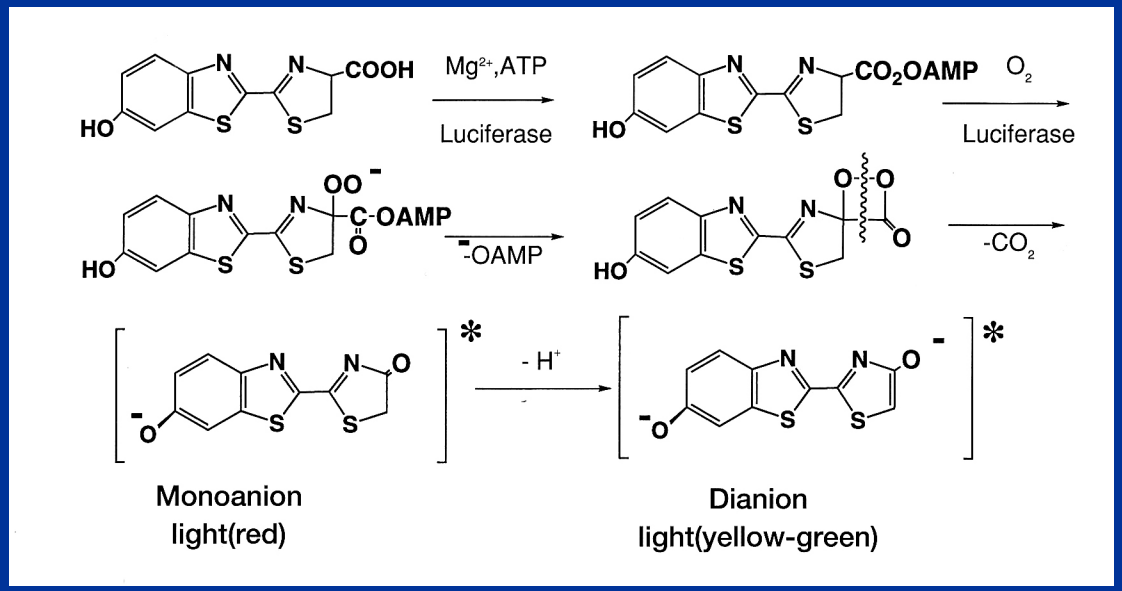
Bioluminescence Protein		Substrate	Max. Wavelength	
Luciferase				
	Firefly (*Photinus pyralis*)	D-Luciferin	560～620nm	The luminescence color is changed by the pH.
	Railroad worm	D-Luciferin	620nm	The luminescence color is constant .
	Brazilian Click Beetle (*Phrophourus plagiopthalamus*)	D-Luciferin	540nm	The luminescence color is constant .
	Firefly (*Rhagophthalmus ohbai*)	D-Luciferin	560, 580nm	The luminescence color is constant .
	Renilla	Coelenterazine	470nm	
Secreted Luciferase				
	Ostracod(*Cypridina Noctiluca*)	Cypridina Luciferin	462nm	
	Copepod(*Metridia longa*)	Coelenterazine	470nm	

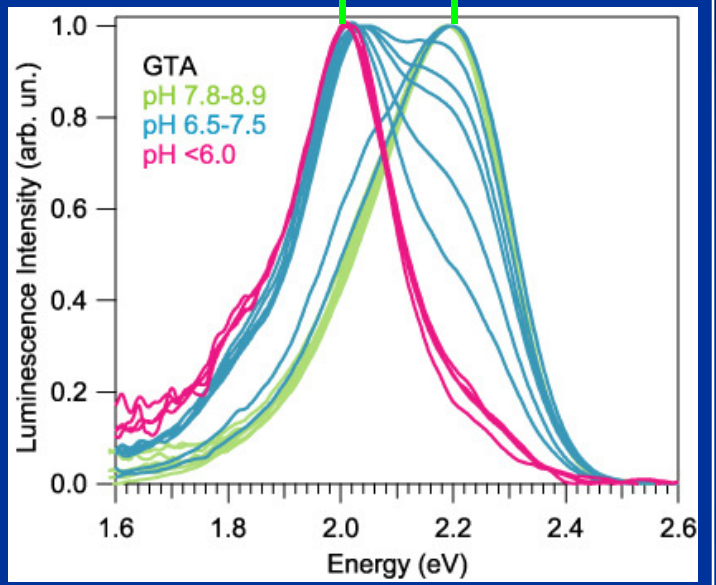


Railroad worm

Oxidation of Luciferin & Coelenterazine

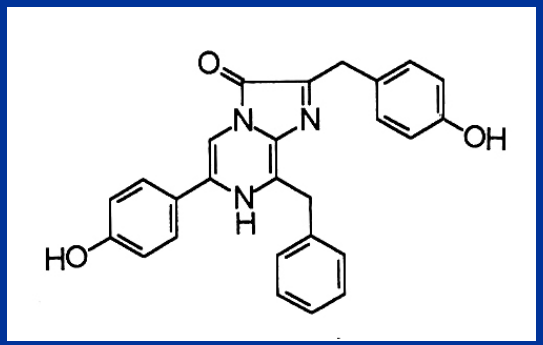
Luciferin





Provided by Dr.H.Akiyama

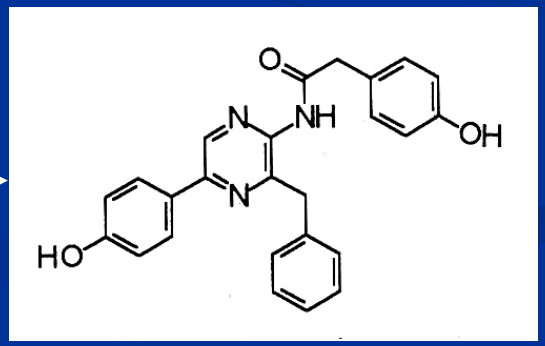
Coelenterazine



O_2

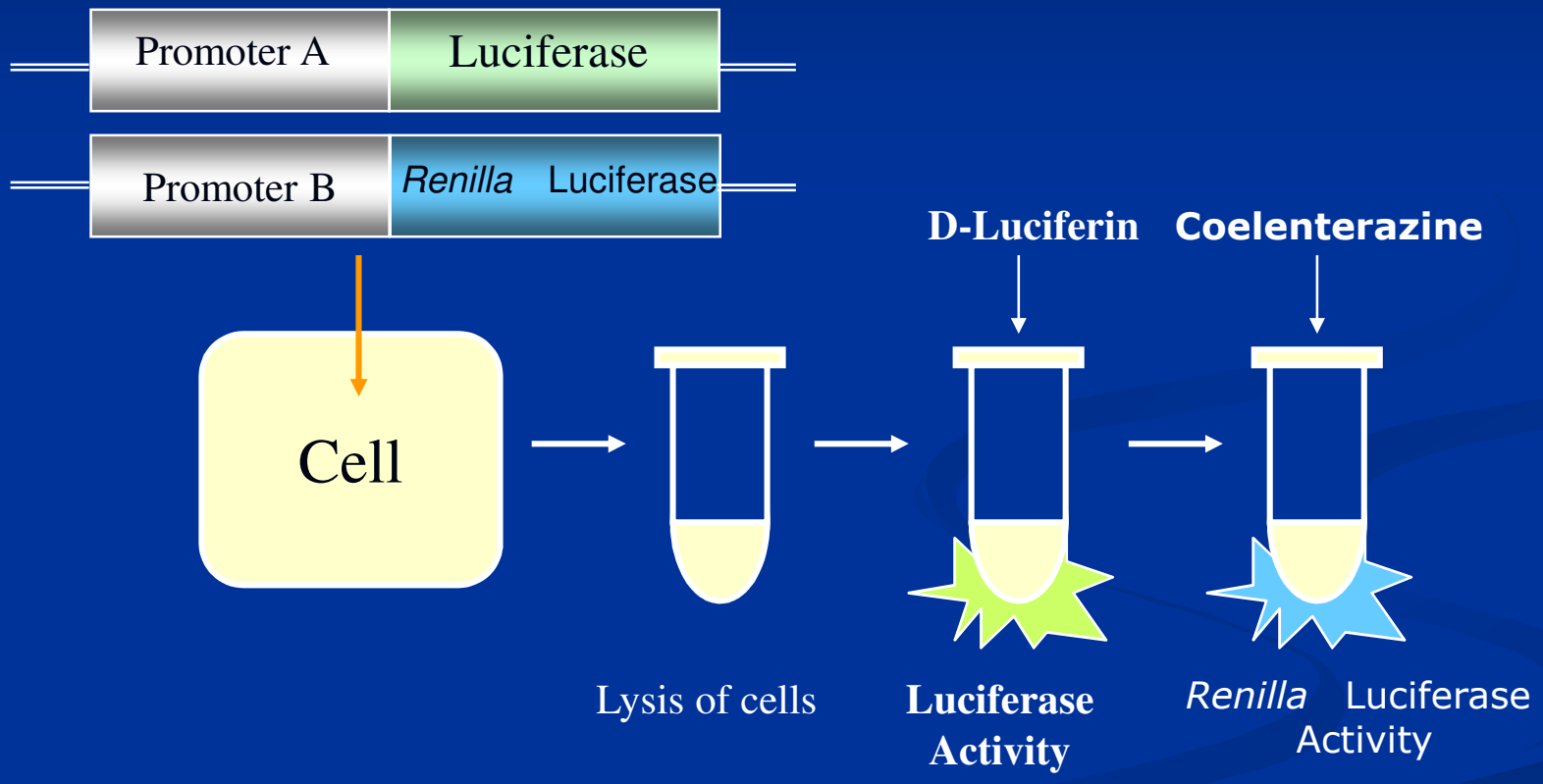
Renilla Luciferase

Coelenteramide

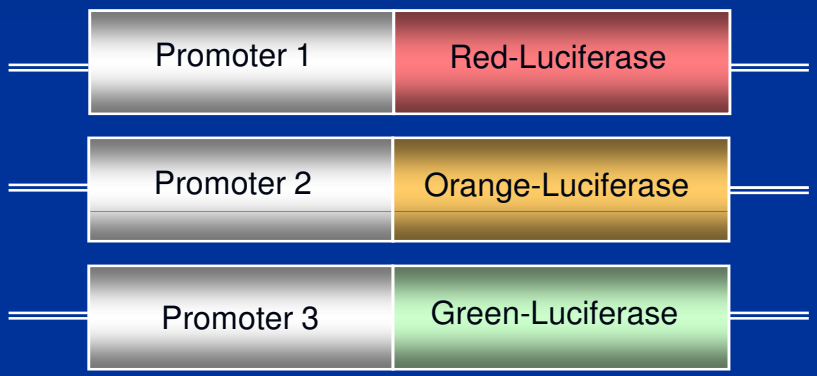


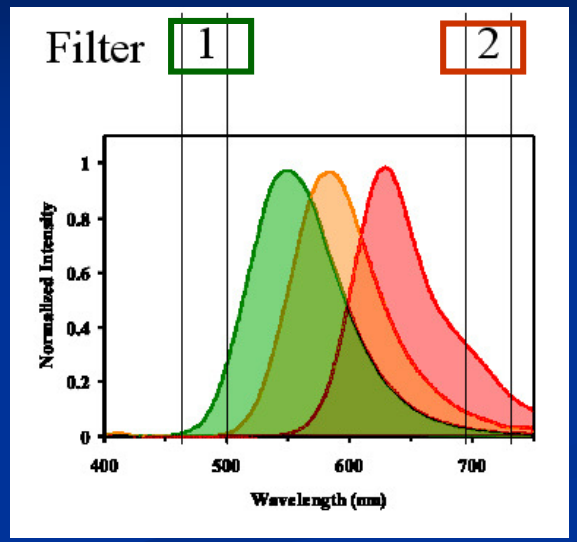
$+CO_2 + h\nu$

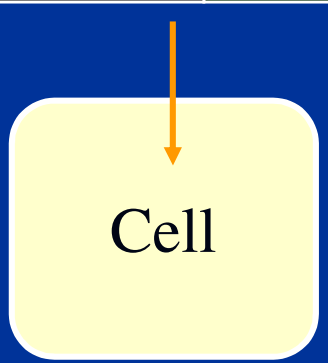
Dual Reporter Assay



Multi-Color Reporter Assay



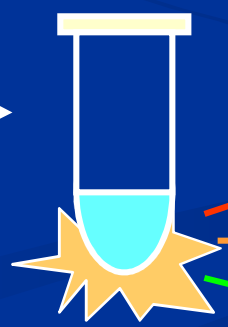




Culture

Lysis of Cells

D-Luciferin



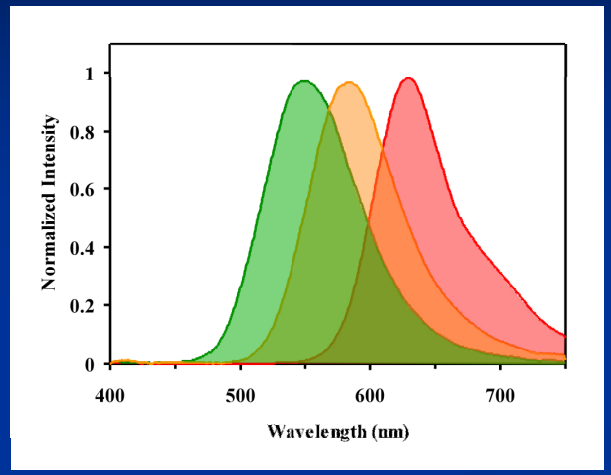
measurement of Luminescence Intensity

Red

Orange

Green

Advanced Method of Quantitative Color Detection

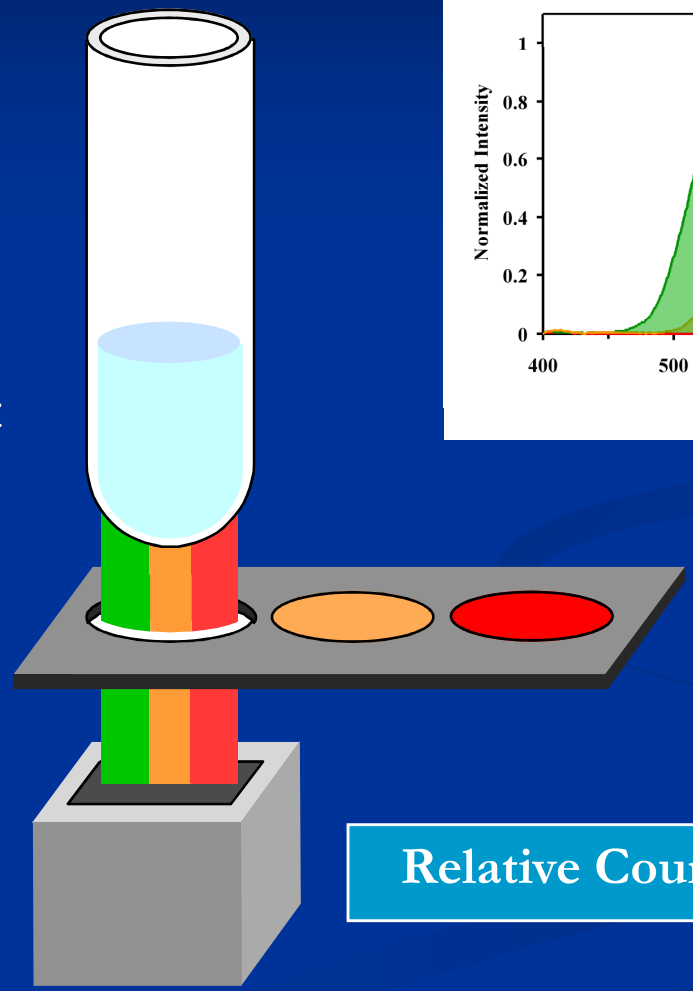


Sample

Optical filter unit

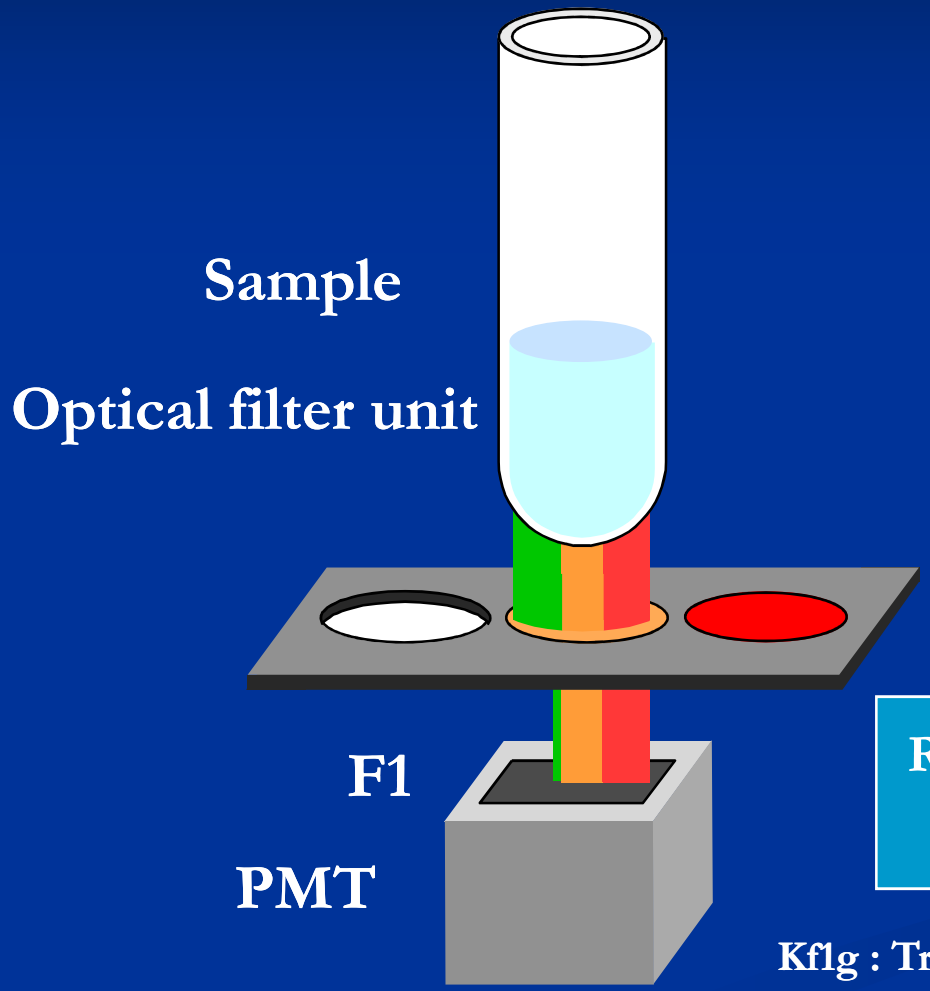
F0

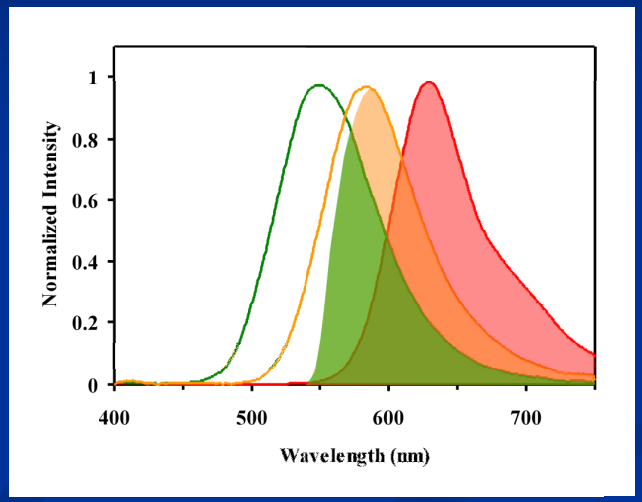
PMT



Relative Counts (F0) = G+O+R

Advanced Method of Quantitative Color Detection

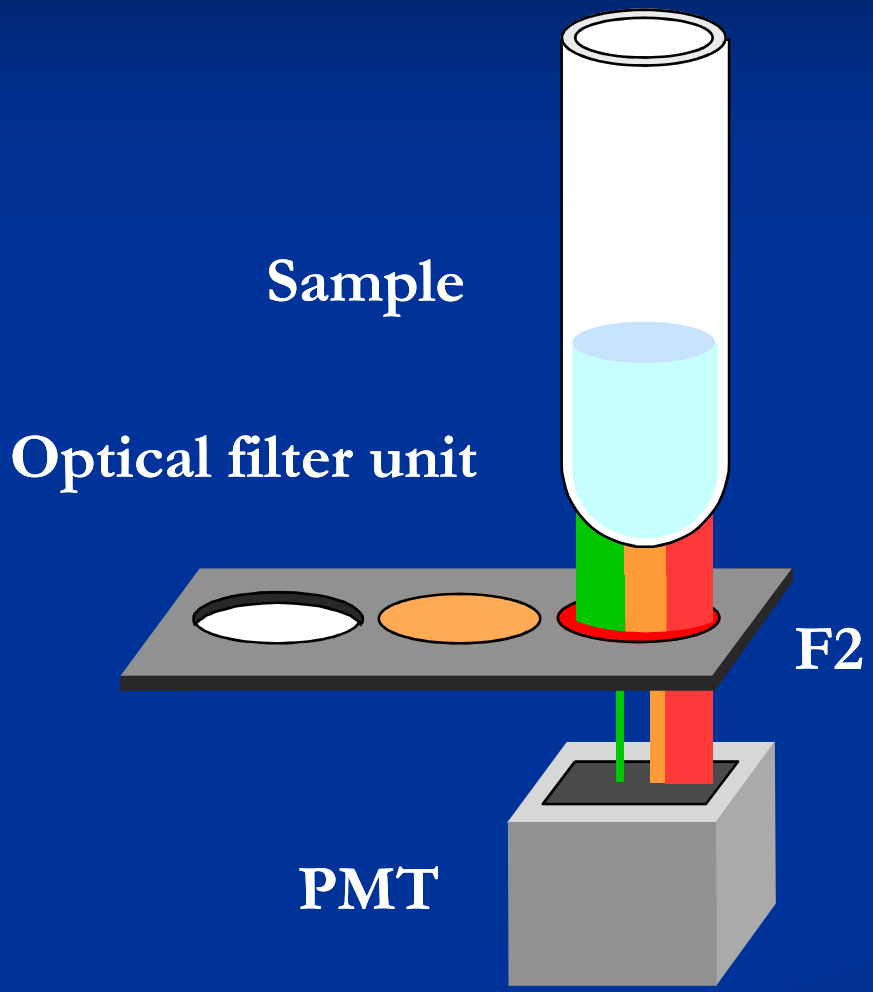


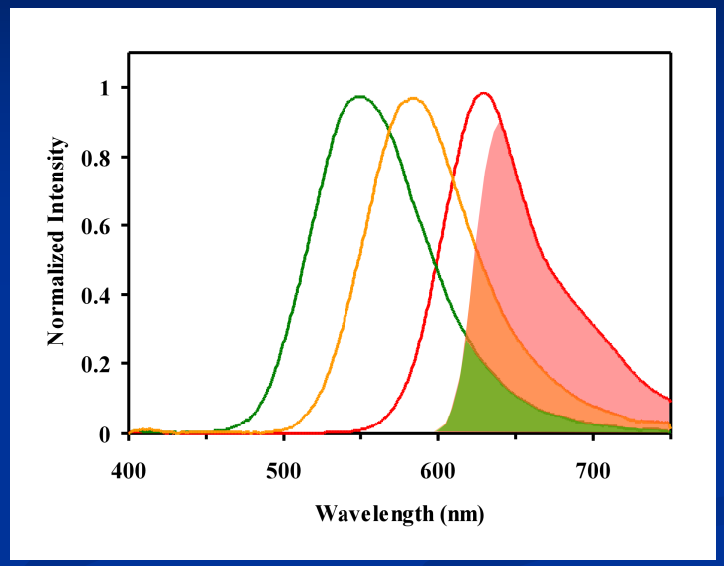


Relative Counts (F1) =
$Kf\ 1g \times G + Kf\ 1o \times O + Kf\ 1r \times R$

Kf1g : Transmittance of green luminescence of f1 filter

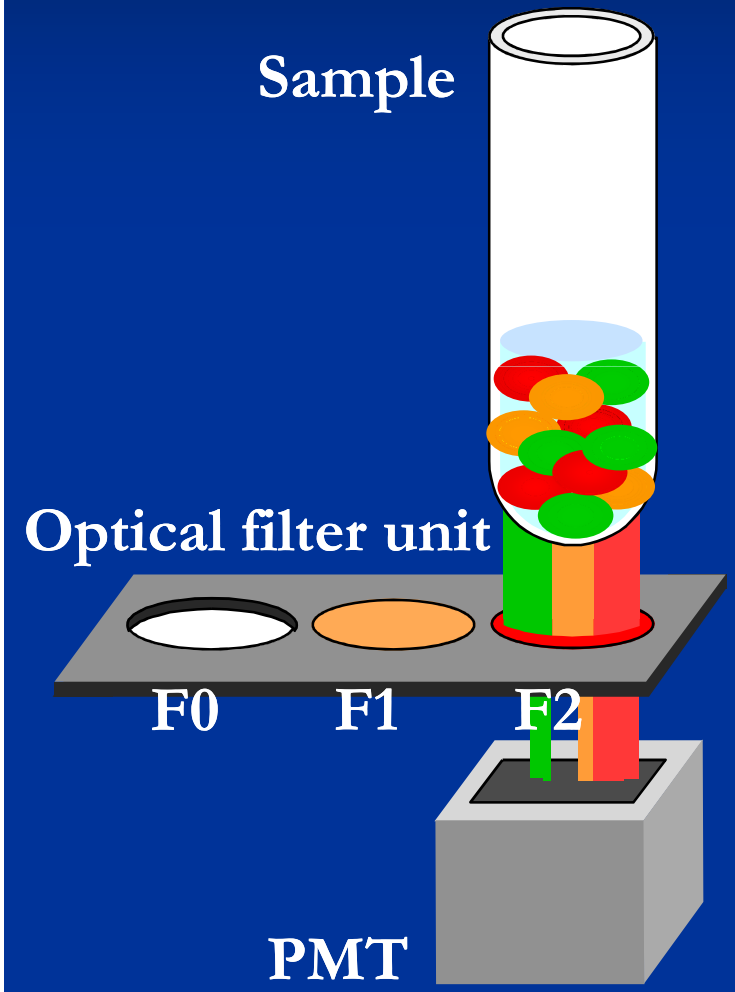
Advanced Method of Quantitative Color Detection

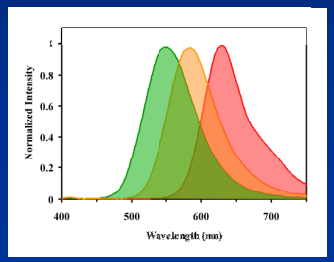


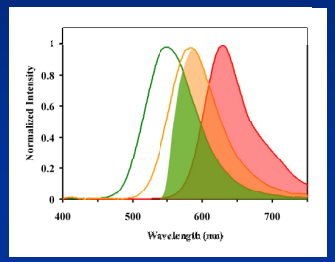


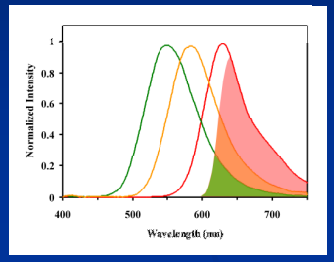
Relative Counts (F2) =
$Kf_{2g} \times G + Kf_{2o} \times O + Kf_{2r} \times R$

Advanced Method of Quantitative Color Detection









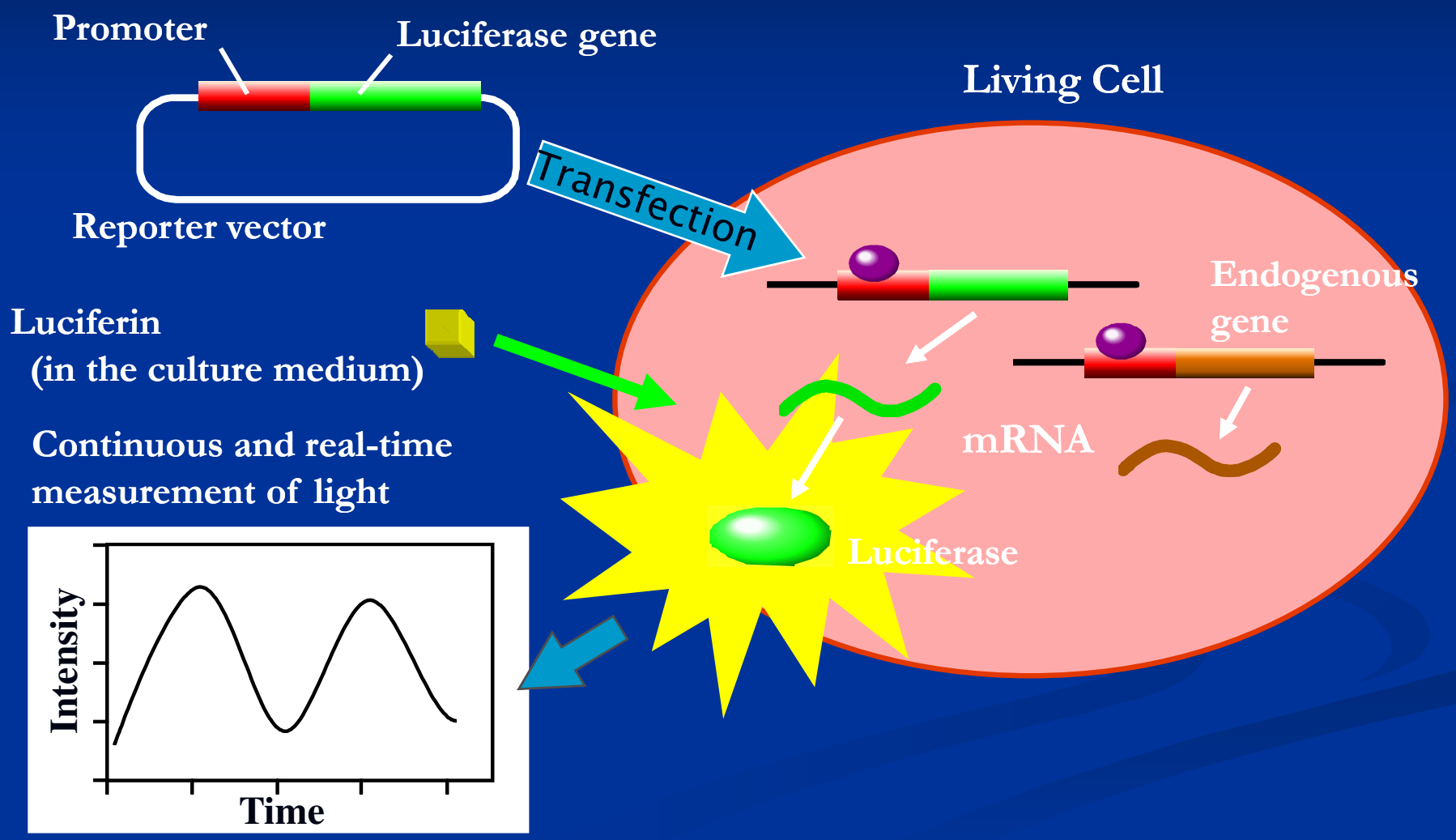
$$\begin{pmatrix} F0 \\ F1 \\ F2 \end{pmatrix} = \begin{pmatrix} 1 & 1 & 1 \\ \kappa_{f1g} & \kappa_{f1o} & \kappa_{f1r} \\ \kappa_{f2g} & \kappa_{f2o} & \kappa_{f2r} \end{pmatrix} \begin{pmatrix} G \\ O \\ R \end{pmatrix}$$

F : Measurement value

κ : Transmittance of each color light of each optical filter

G, O, R : Each color light

Real-Time Reporter Assay (Live Cell Reporter Assay)

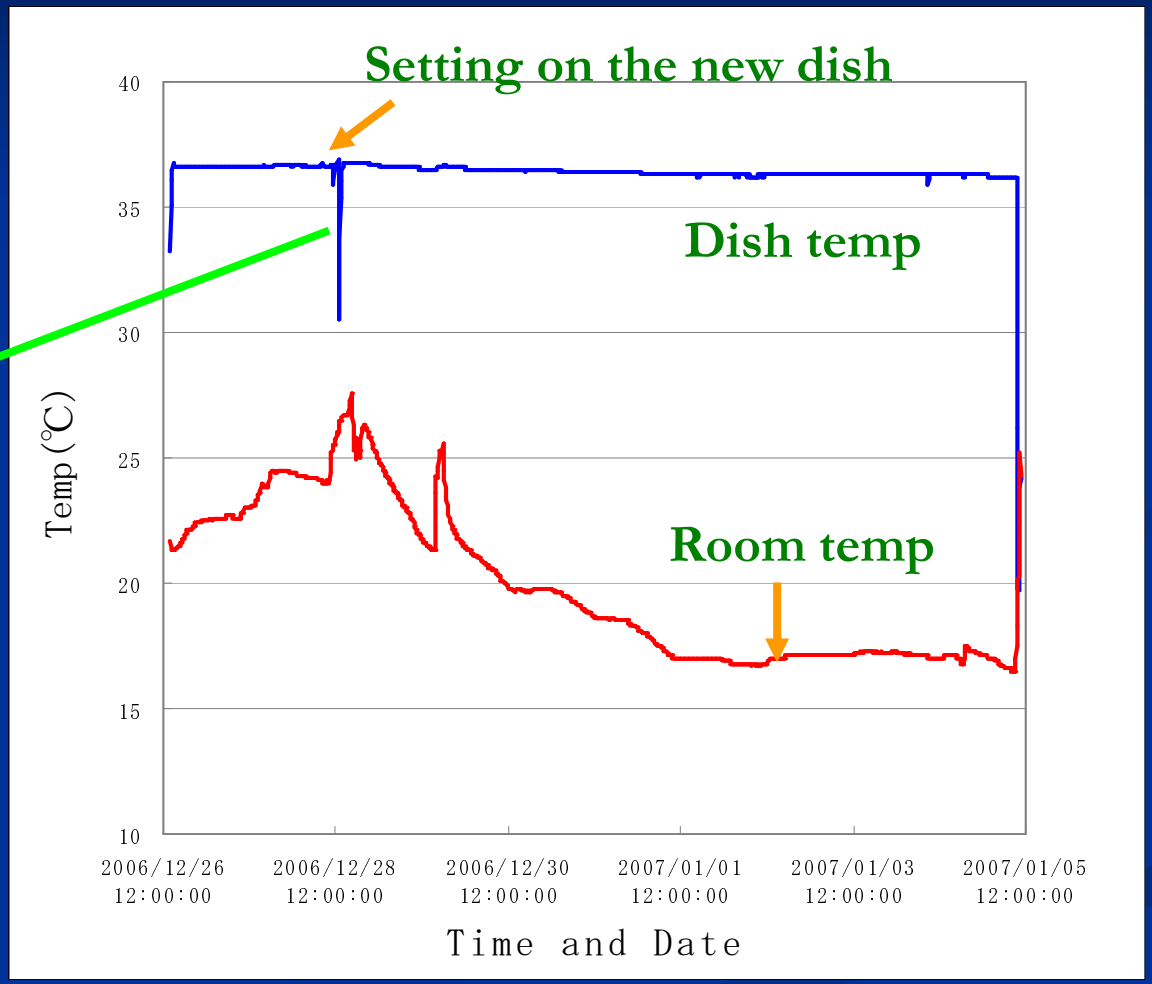


Luminometer
for real-time reporter assay in living cell

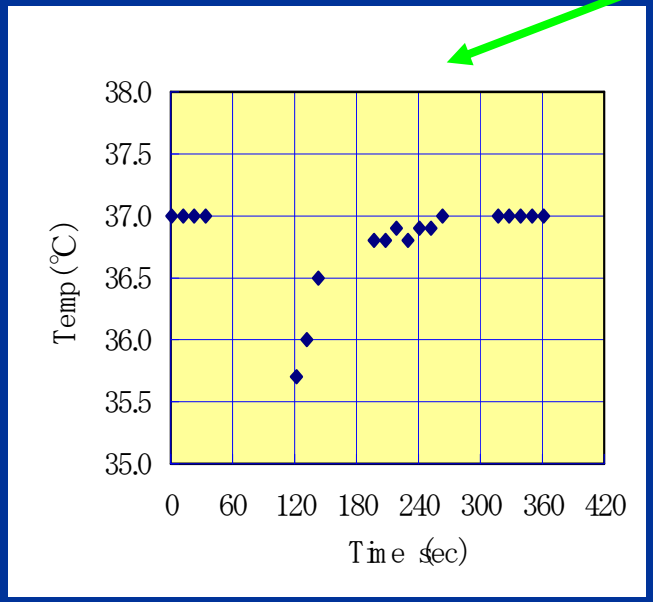
Kronos (AB-2500)

Temperature Stability

Sensor in dish

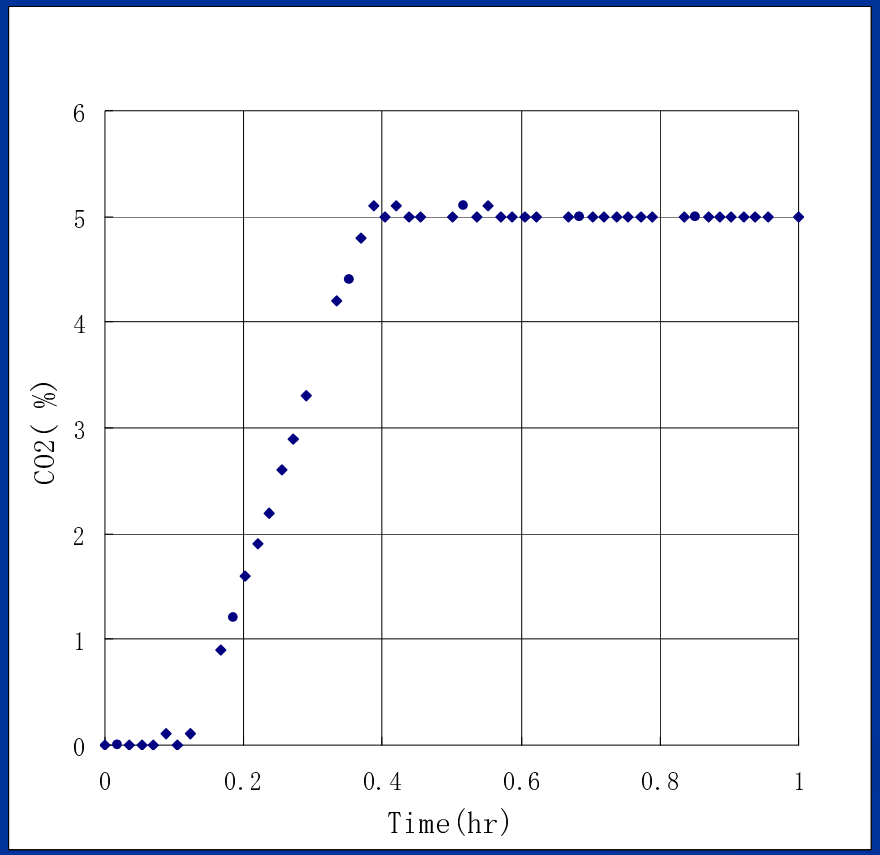


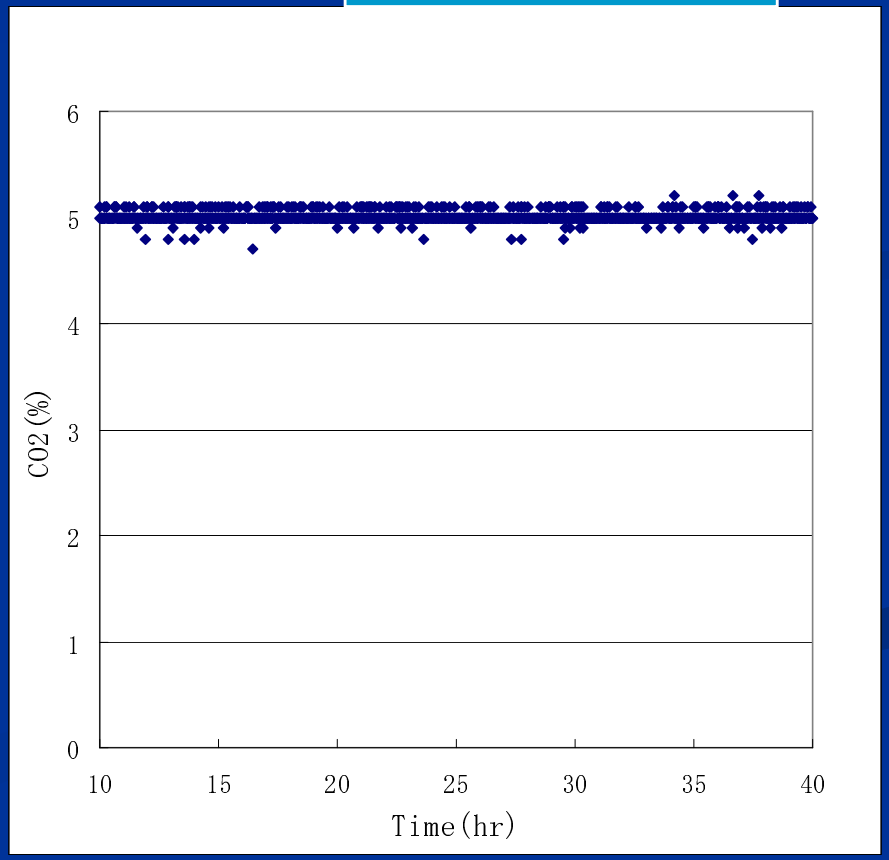
Sensor in kronos



Preset Temperature : 37℃

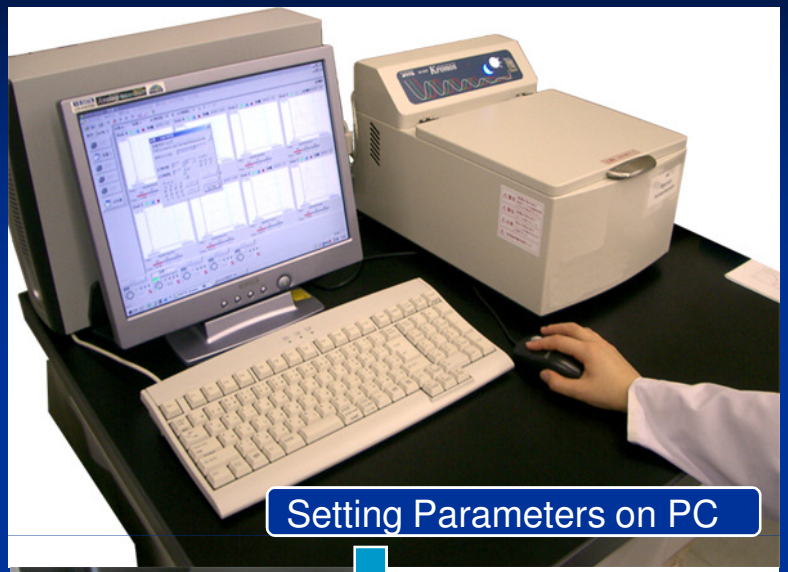
CO_2 Concentration





Procedure

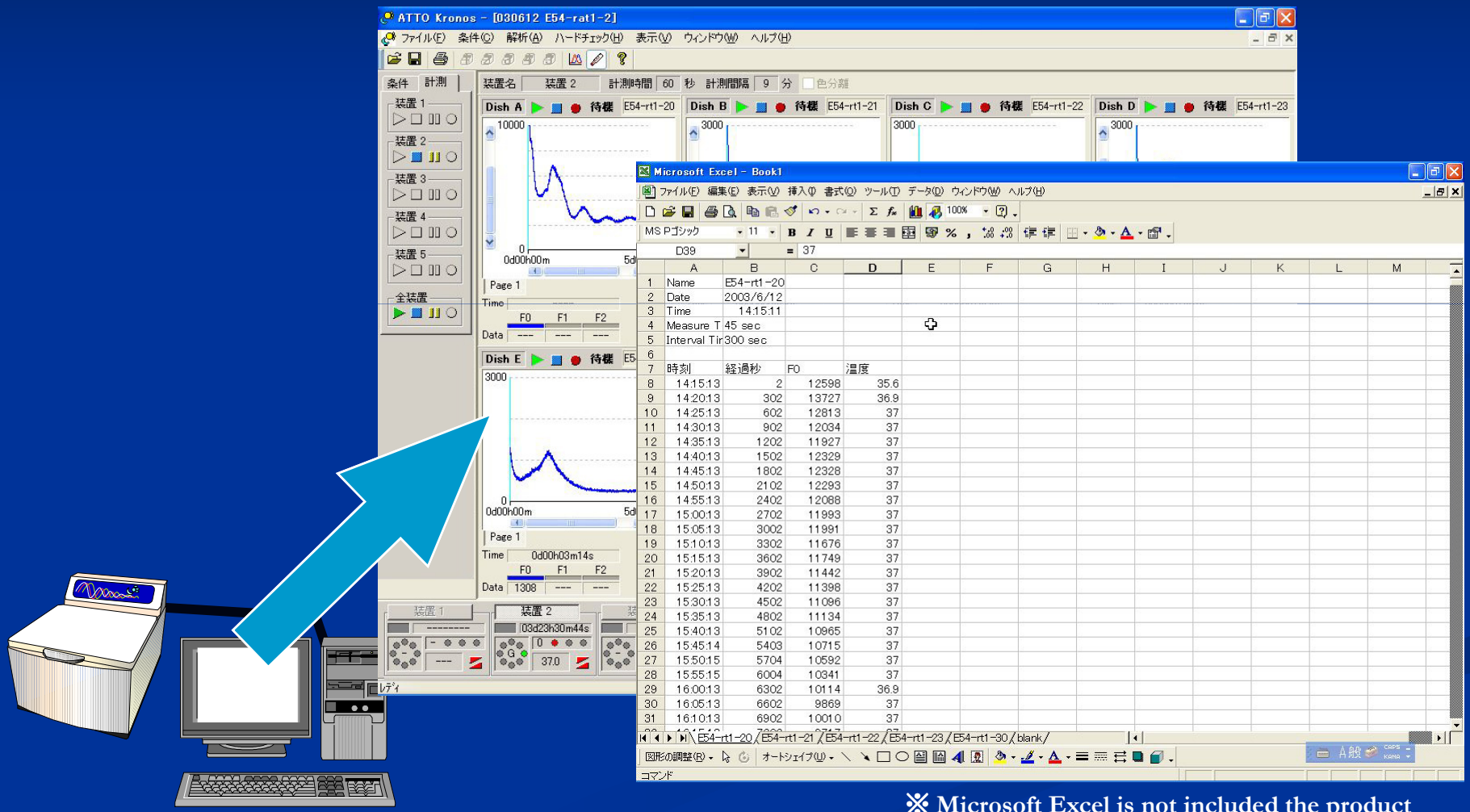

Addition of Luciferin
Culture Cell
(Reporter gene transfected)


Setting Parameters on PC


Setting Dishes in Kronos

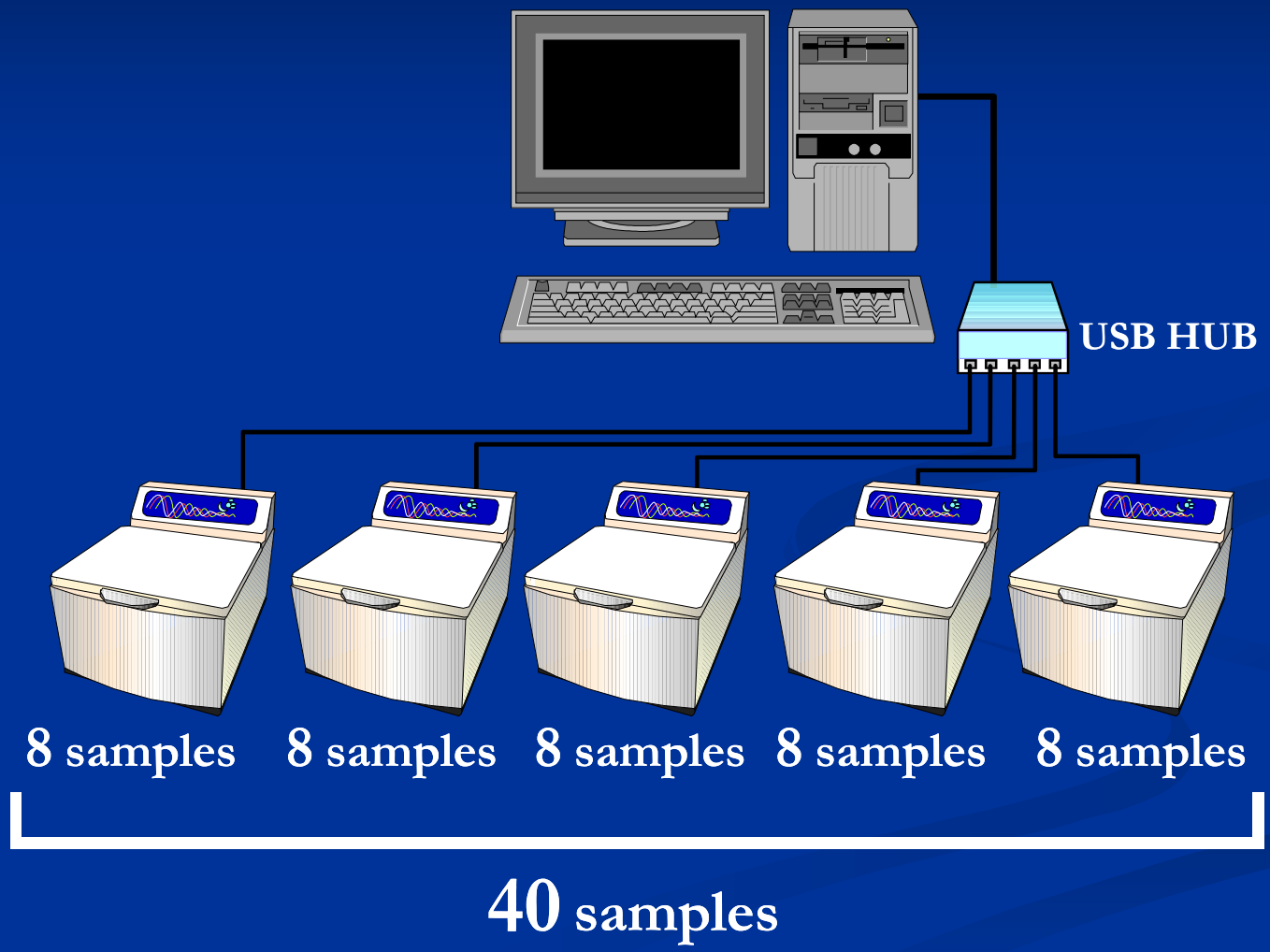

Click Mouse to Start

Output Data on PC



※ Microsoft Excel is not included the product

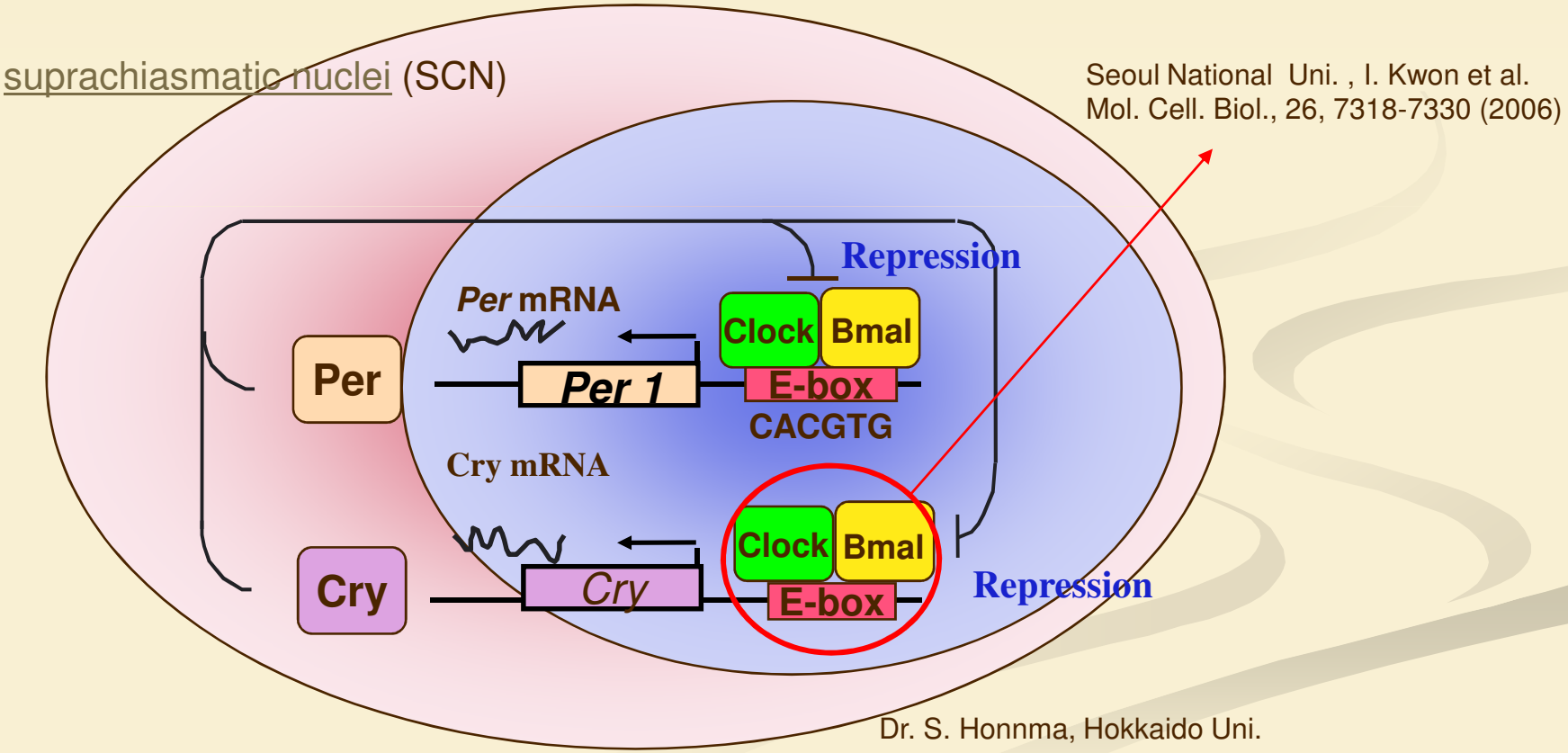
1 PC Can Control
5 Sets of Kronos



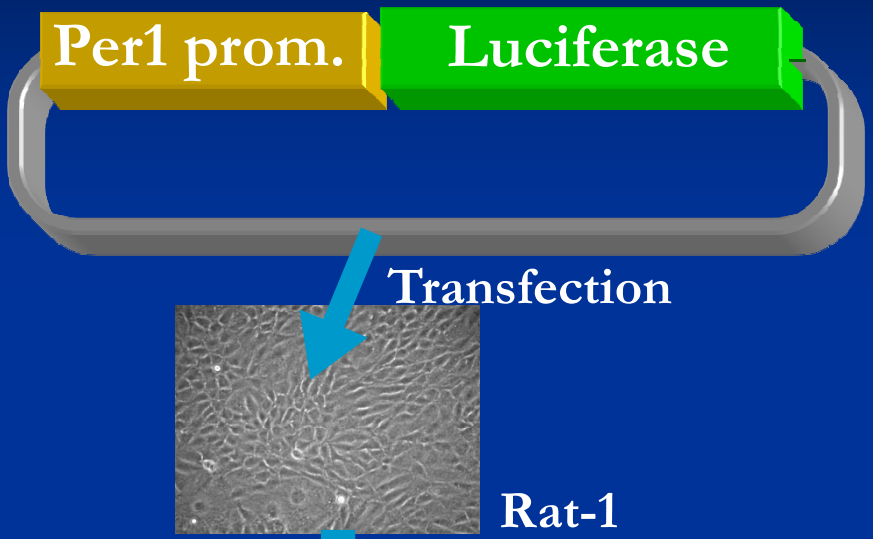
Feedback loop for circadian rhythms

Positive elements: *Clock, Bmal1*

Negative elements: *Per1, Per2, Cry1,Cry2*



Monitoring Circadian Rhythm by Kronos



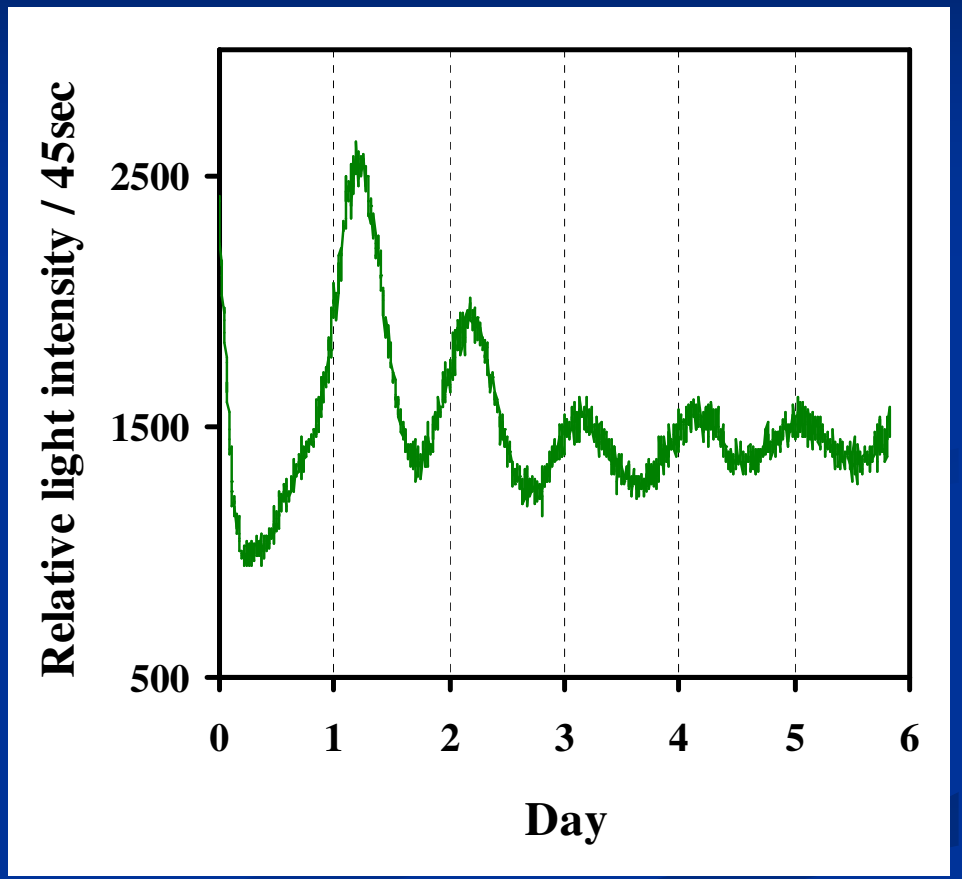
Transfection

Rat-1
fibroblast cell

Stimulation(by Dex)

Medium change and
Addition of Luciferin

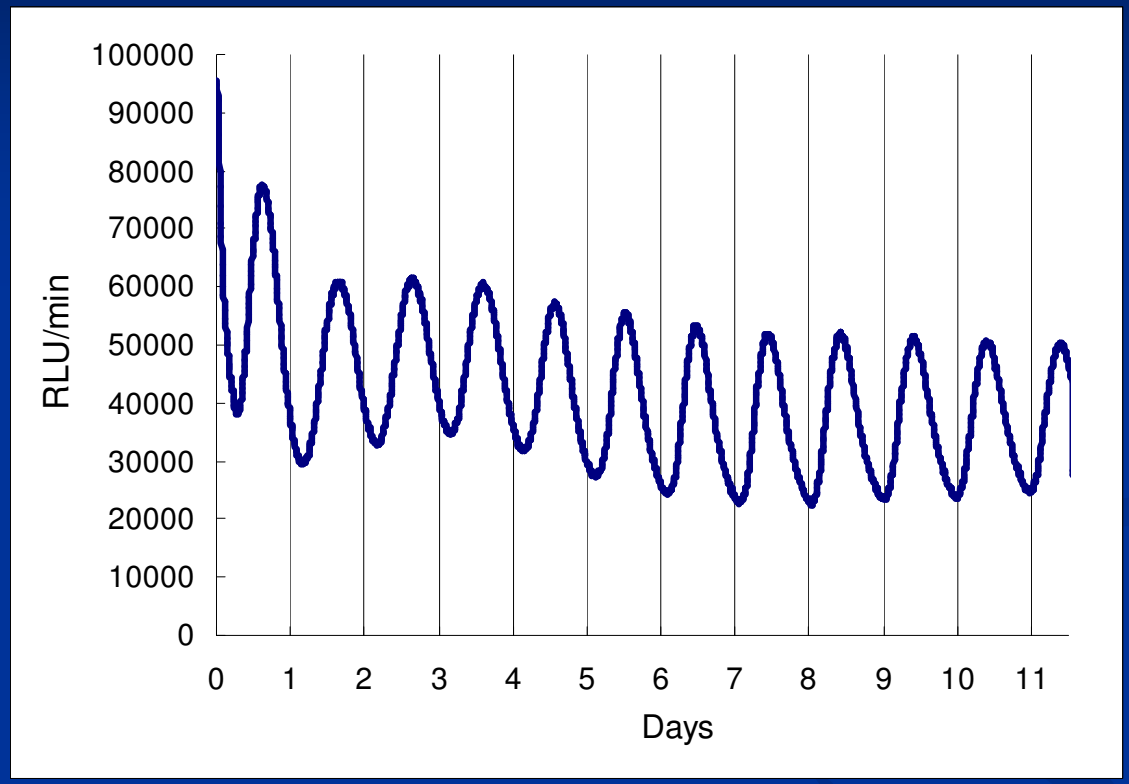
Measurement by Kronos



D r . Y.Nakajima,
National Institute of Advanced Industrial Science and Technology (AIST)

Self-sustained circadian rhythms in SCN

SCN section of mouse transgenic for *Per1*-Luciferase (300μm)

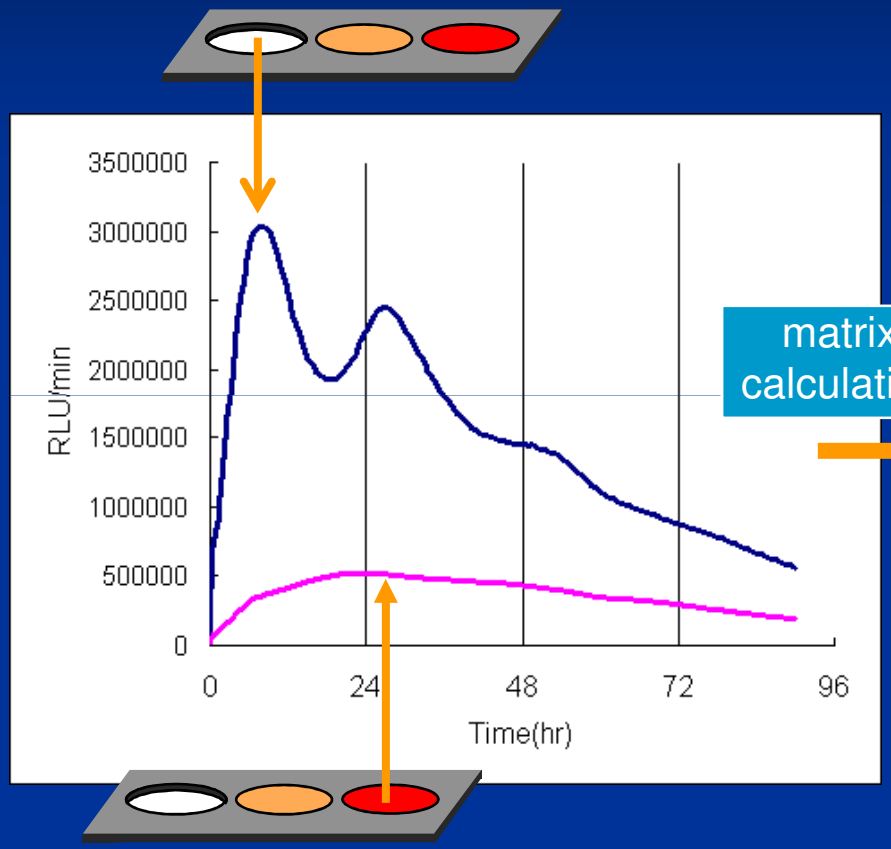


Dr.Ken-ichi Honma , Hokkaido Uni.

Reporter: Per1 Promoter-FLuc
Medium : DMEM, 10mM HEPES, 0.1mM D-Luciferin K

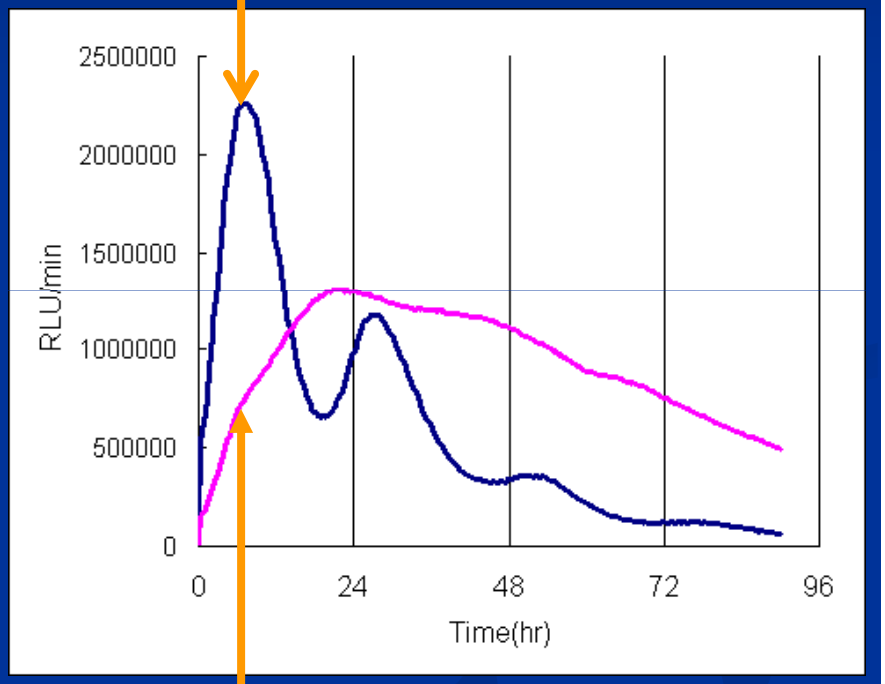
Real-Time Dual Color Reporter Assay

Relative Counts of Per2-Gluc and SV40-Rluc



matrix calculation

Relative Counts of Per2-Gluc



Relative Counts of SV40-Rluc

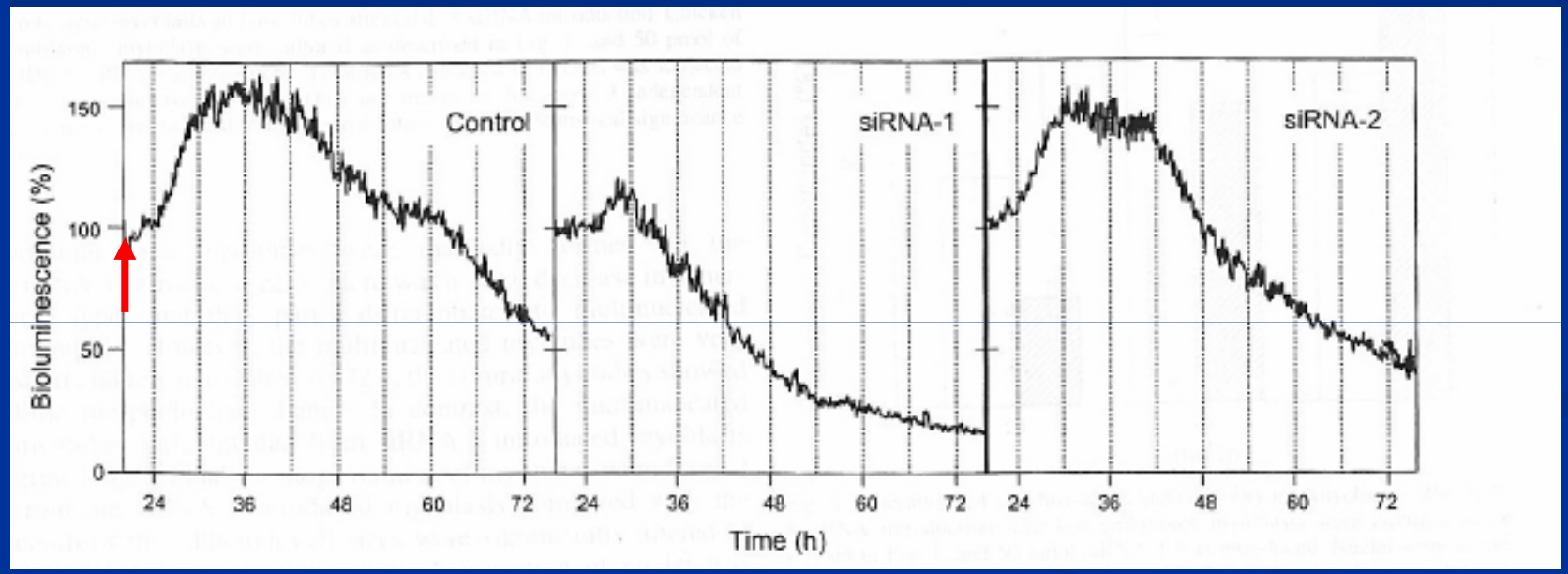
Filtered Relative Counts of Per2-Gluc and SV40-Rluc

Dr. Y. Nakajima, AIST

Cell :NIH3T3
Reporter: Rer2 Promoter-Gluc , SV40-RLuc
Medium : DMEM, 10%FBS
Transfection: Rer2 Promoter-Gluc 1μg , SV40-Rluc 0.2μg

Small interference RNA

Control GDF-8-siRNA siRNA(negative contorol)



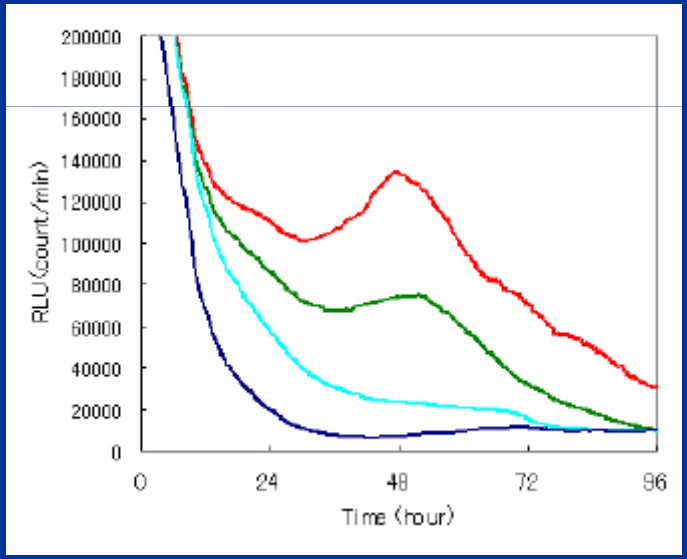
Growth and Differentiation Factor 8 (GDF-8) : TGF-β superfamily

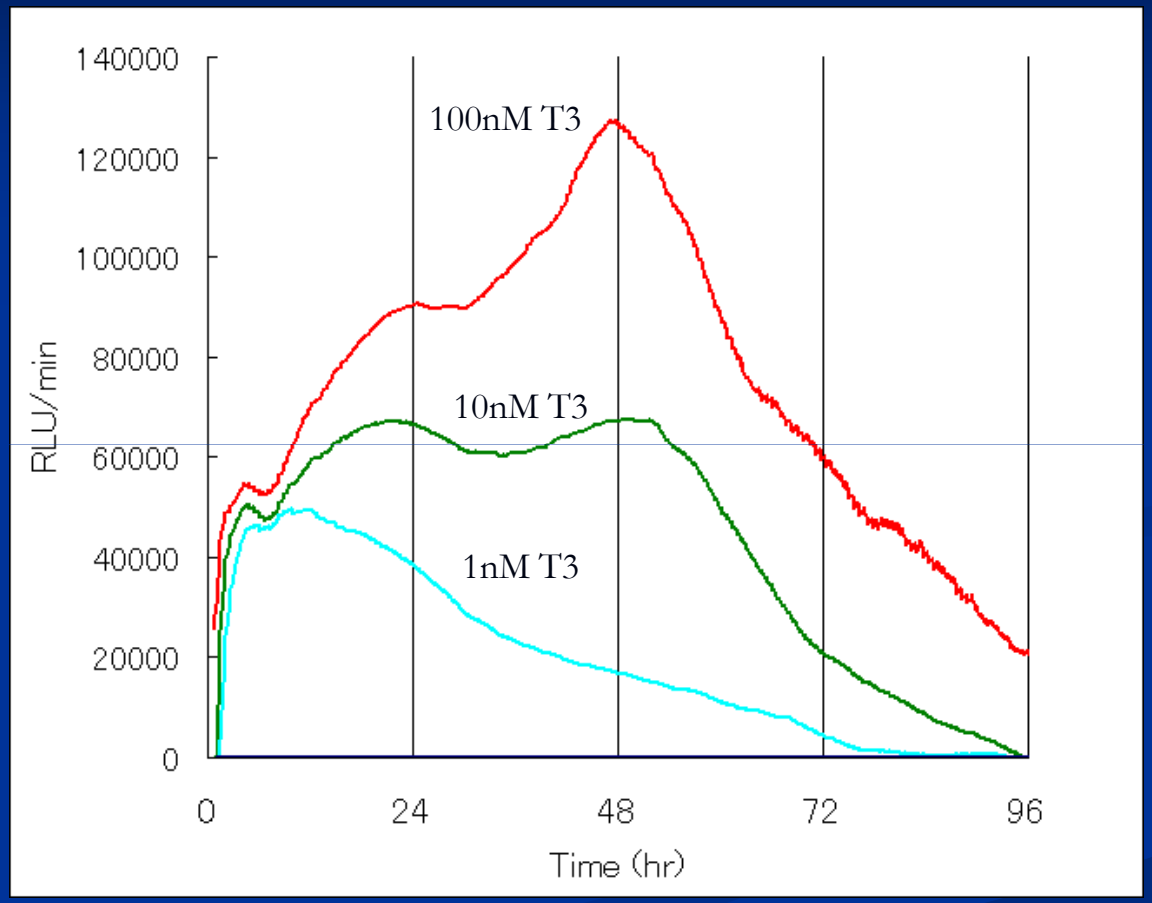
Cell :chicken embryonic myoblasts
Reporter: GDF- 8 (Myostatin) Promoter-pGL3 luc
Medium : Opti-MEM, 7.5%knockout serum replacement
Transfection:50pmol GDF-8-siRNA, Lipofectamine2000
Normalization time:20hr

Dr. M. Hattori , Kyusyu Uni.

Fujimori Sato et. al., *Am J Physiol Cell Physiol* , 291, C538-C545(2006)

The promoter activity of growth hormone by Thyroid hormone(T3)



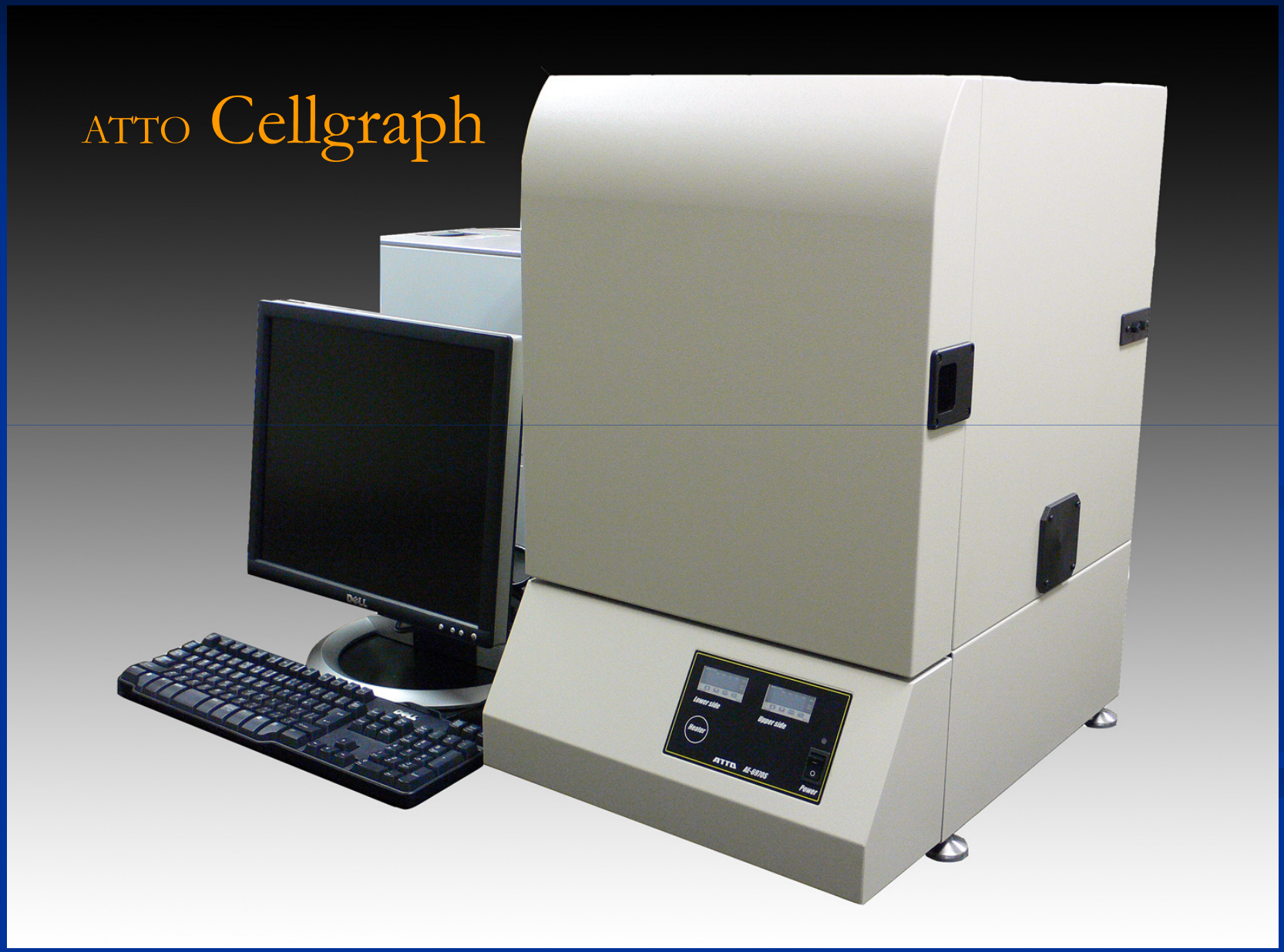


T. Enomoto, ATTO Corp.

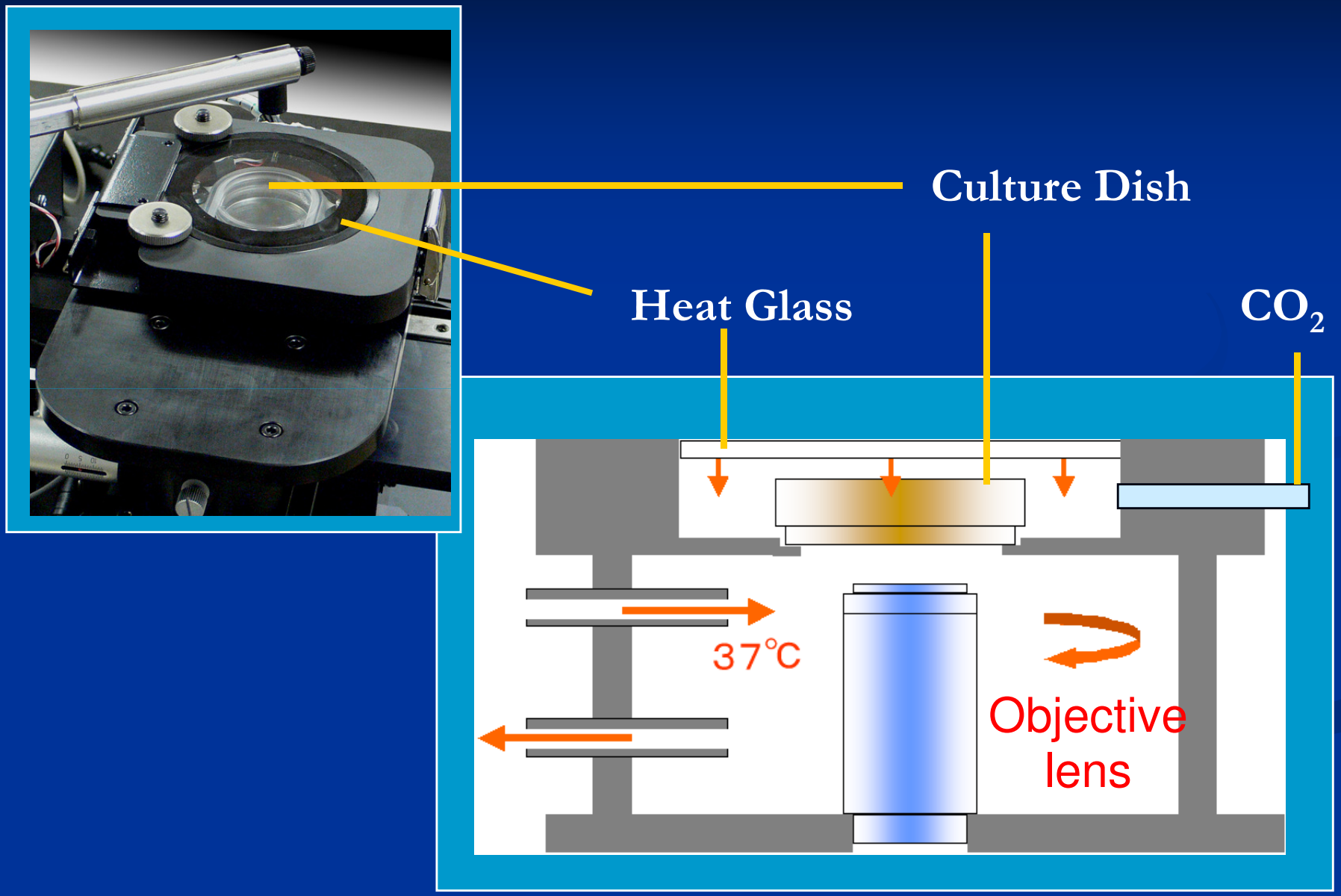
Cell :GH3 (rat pituitary adenoma cell)
Reporter:GH Promoter-Fluc
Medium : OPTI-MEM

Ref: Y.Tanahashi et.al. Anal. Biochem. 289, 260-266 (2001)

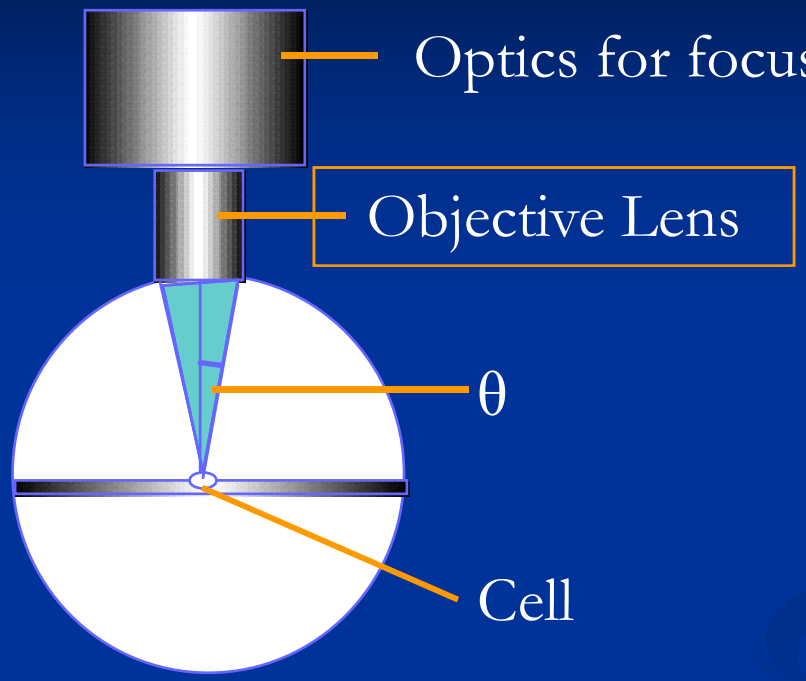
Real-time Imaging System in Living Cell



Incubation system



Collective efficiency and transmission of opitcal system



NA	n (%)
0.1	0.3
0.15	0.6
0.2	1
0.25	1.6
0.3	2.3
0.35	3.2
0.4	4.2
0.45	5.4
0.5	6.7
0.55	8.3
0.6	10
0.65	12
0.7	15
0.75	17
0.8	20
1	50

$$\eta = \frac{1-\sqrt{1-\sin^2\theta}}{2} = \frac{1-\sqrt{1-NA^2}}{2}$$

η : Collective Efficiency

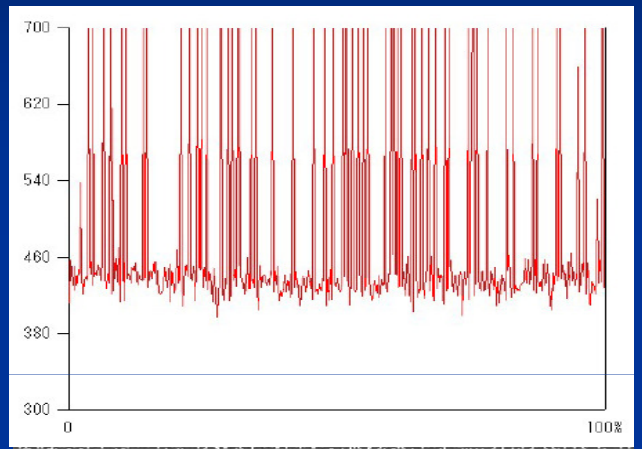
NA : numerical aperture (NA=nsinθ)

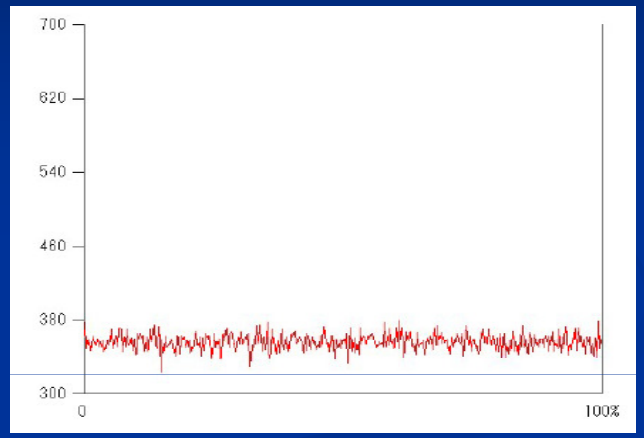
n : refractive index

High Sensitive & Low Noise Cooled CCD

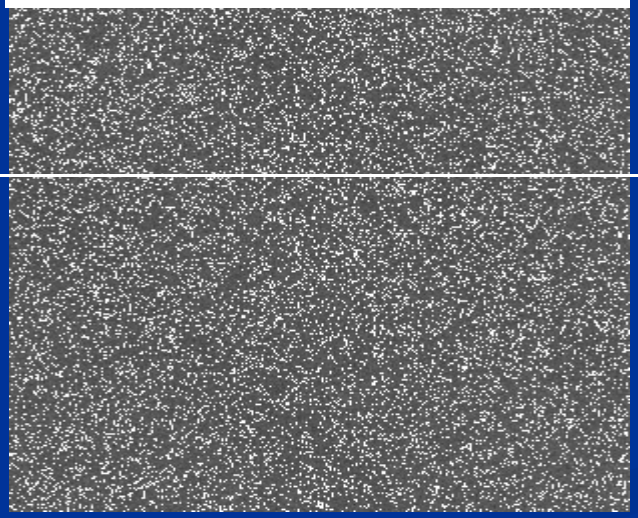
Exposure time : 10min

Image brightness on the drawn line





Dark Image

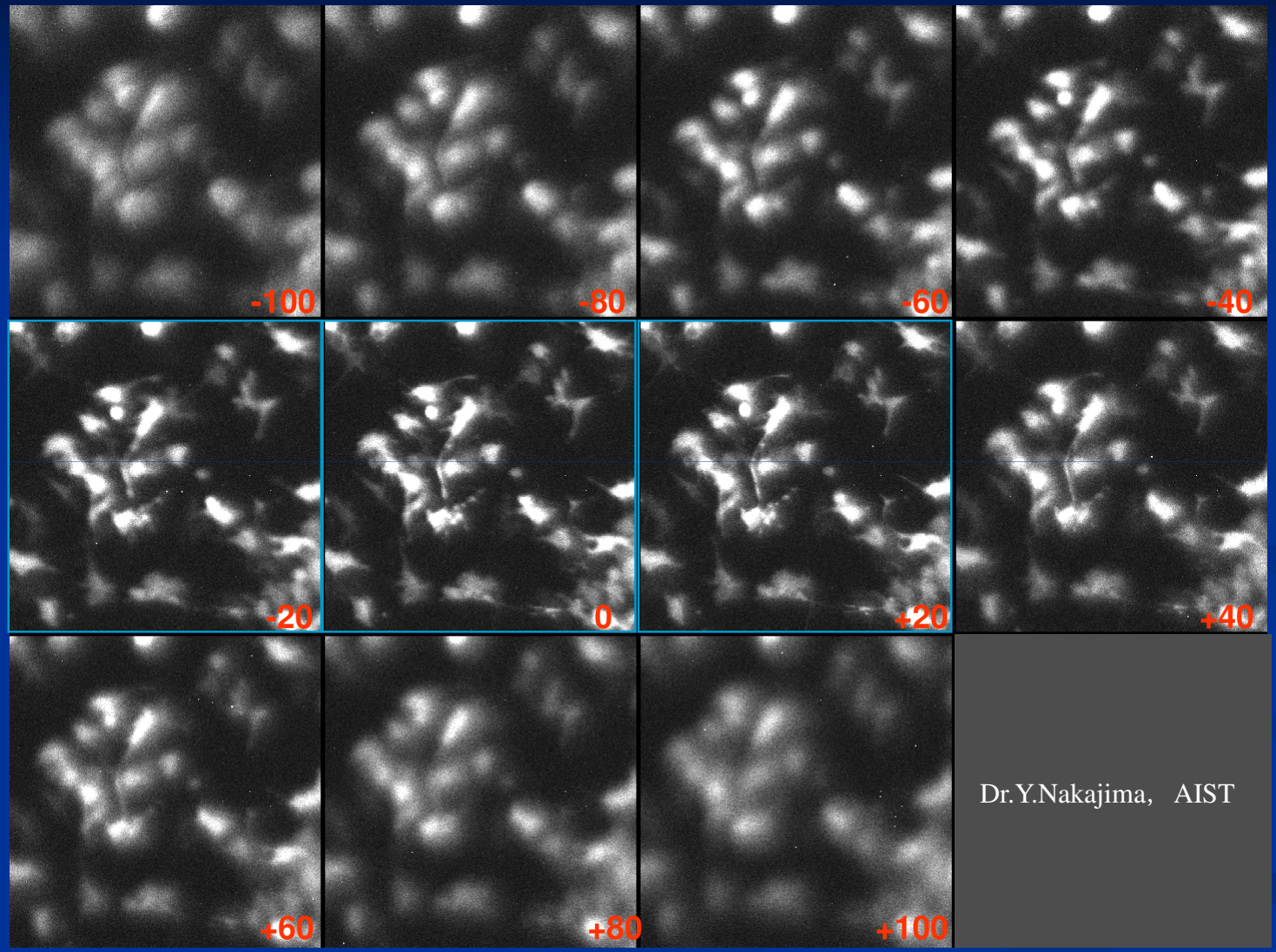


Cooled CCD Camera

Cellgraph

Focus adjustment

PTGR-Cytosol, (x20 lens)



SV40-PTGRm-Cytosol in NIH3T3-3-4 cells
200 mM LH2K in 10% FBS, 25 mM Hepes

X20 lens
3 min exposure

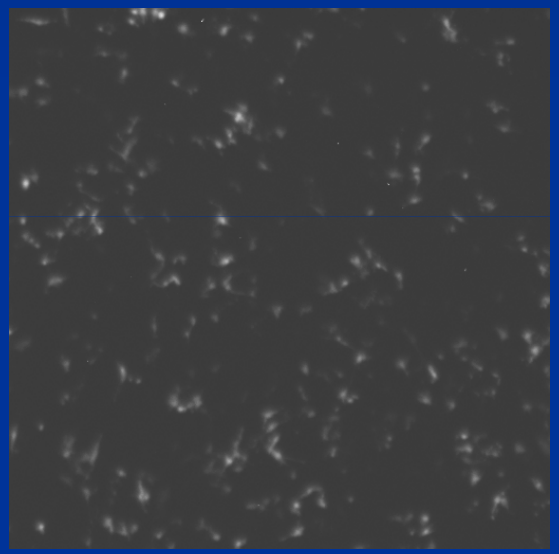
20 mmStep
Kronos: 1 x 10^8 (No.1)

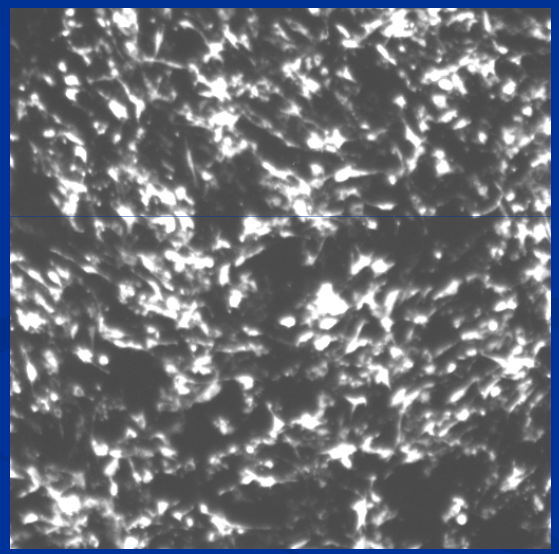
The best Luciferase(Eluc) for cell imaging

Bioluminescence imaging of ELuc and FLuc in NIH3T3 cells

Fluc(Conventional) **Eluc(New)**

x5.6 lens
3 min exposure





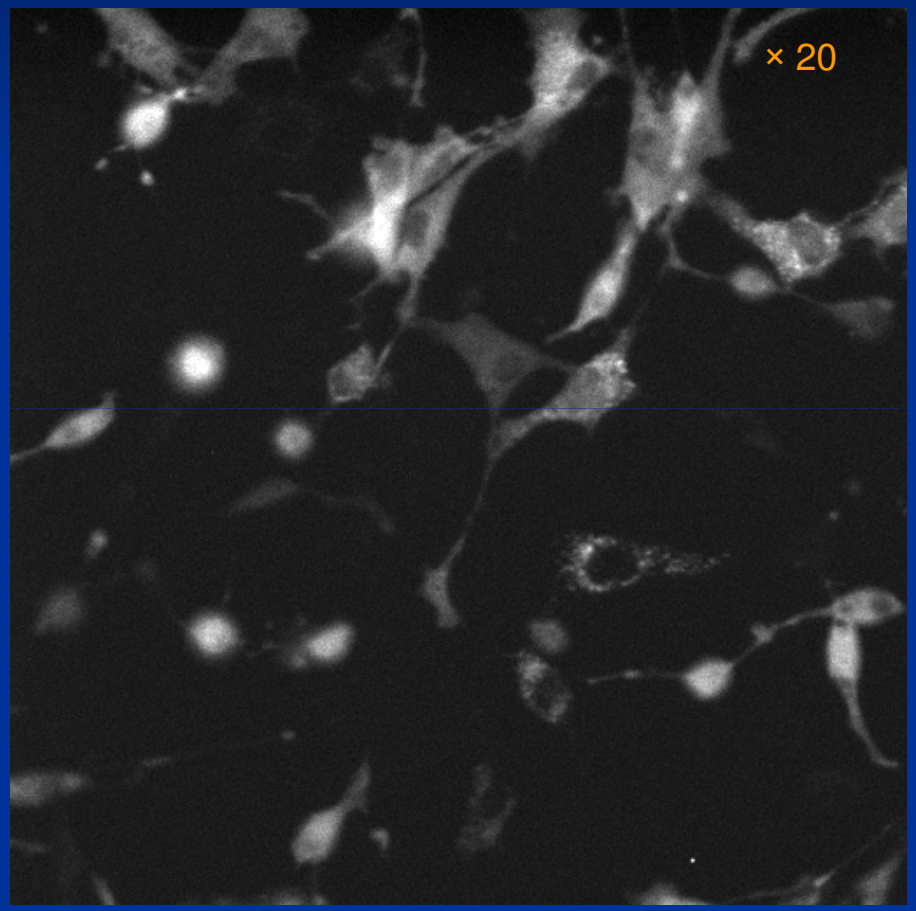
High Quantum Yield in Cell

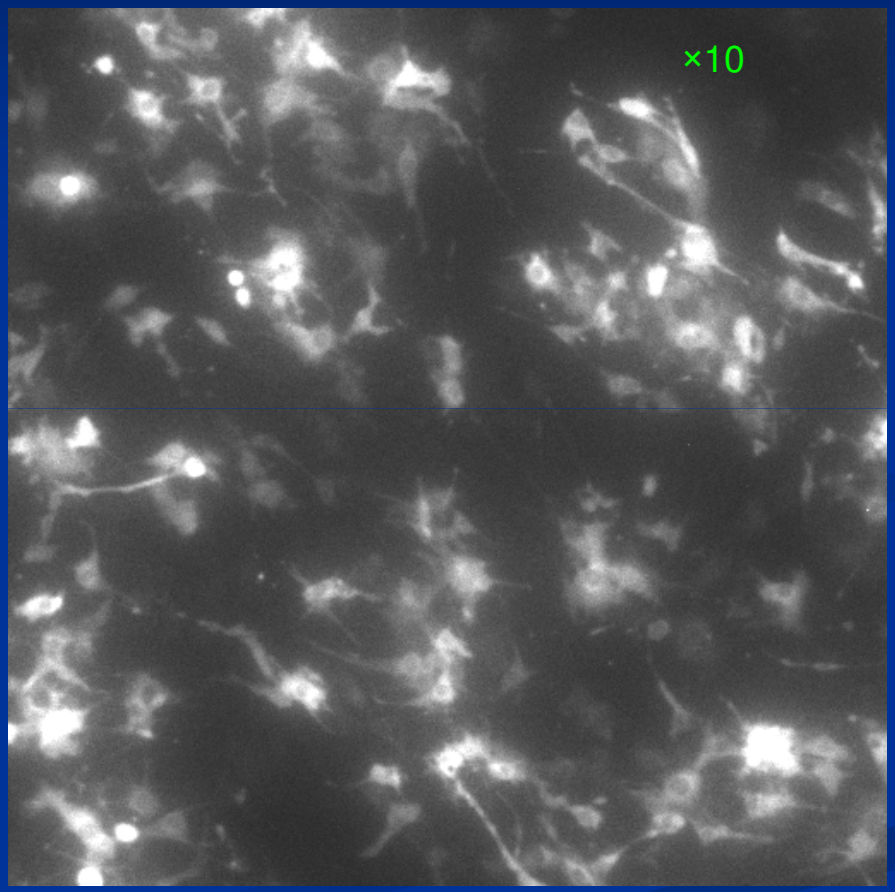
Dr.Y.Nakajima, AIST

SV40-PTGRm-Cytosol in NIH3T3-3-4 cells
200 mM Luciferin K in 10% FBS, 25 mM Hepes
Medium: DMEM+10%FBS , 200μM D-Luciferin

Bioluminescence image of NIH3T3

Transfection of the CMV– Eluc reporter construct into NIH3T3 cells.



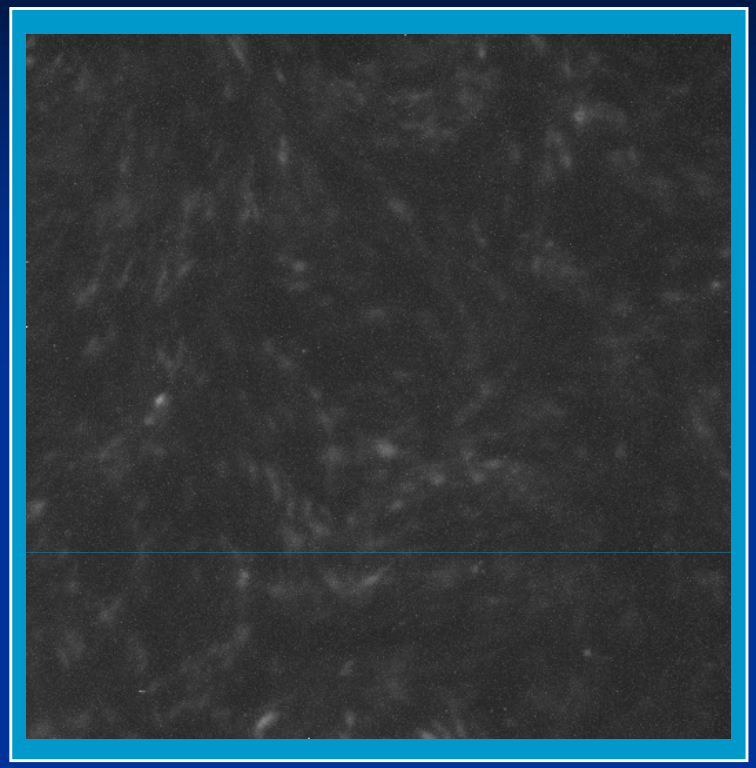


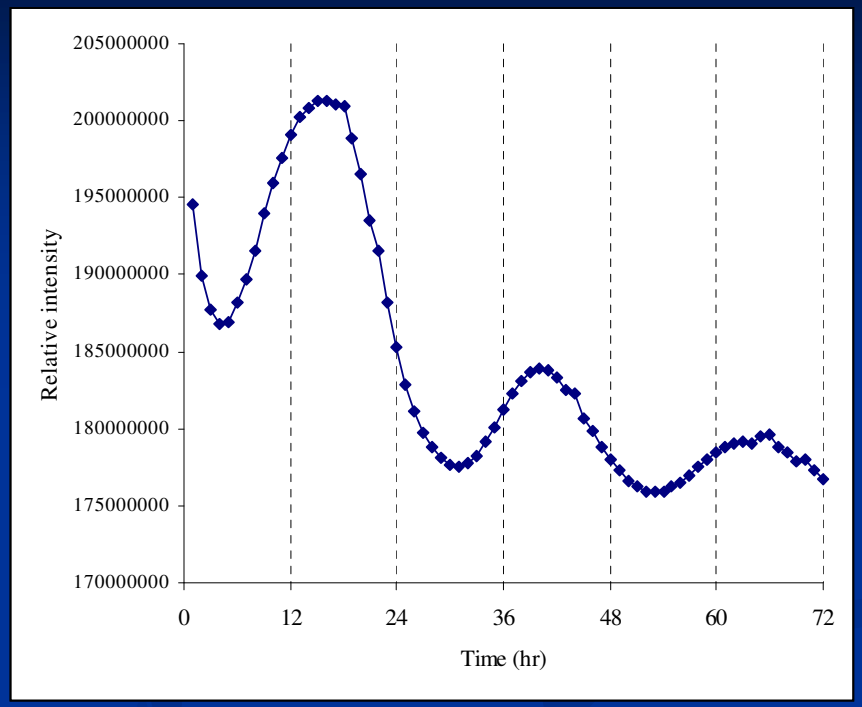
CMV promoter – Eluc(2 μ g)
Transfection : lipofection
Medium: DMEM+10%FBS , 200μM D-Luciferin

Magnification of objective lens :×20, ×10
Exposure time : **3min(×10)**

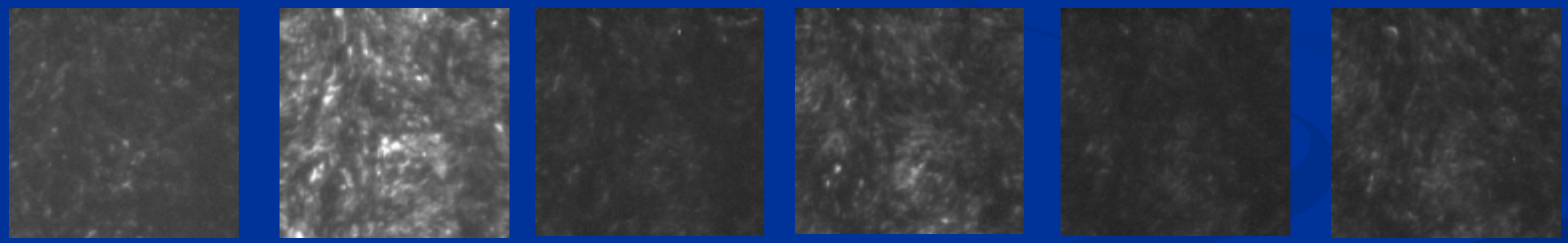
Dr.Y.Nakajima, AIST

The movie of *Bmal1* expression





Dr. Ken-ichi Honnma ,Hokkaido Uni.

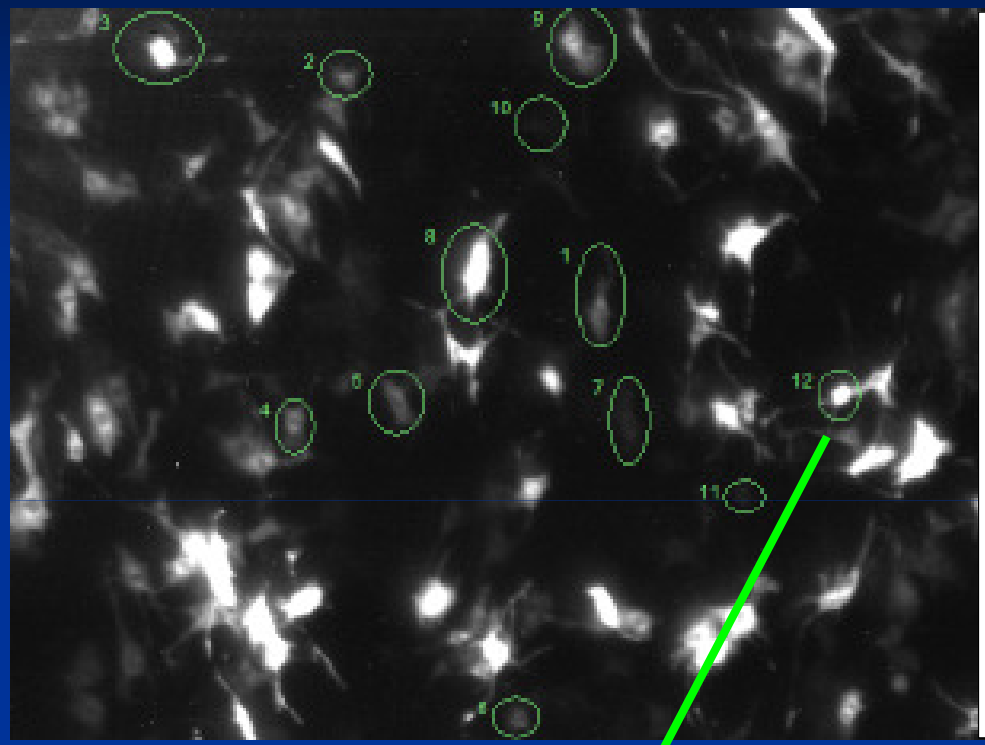


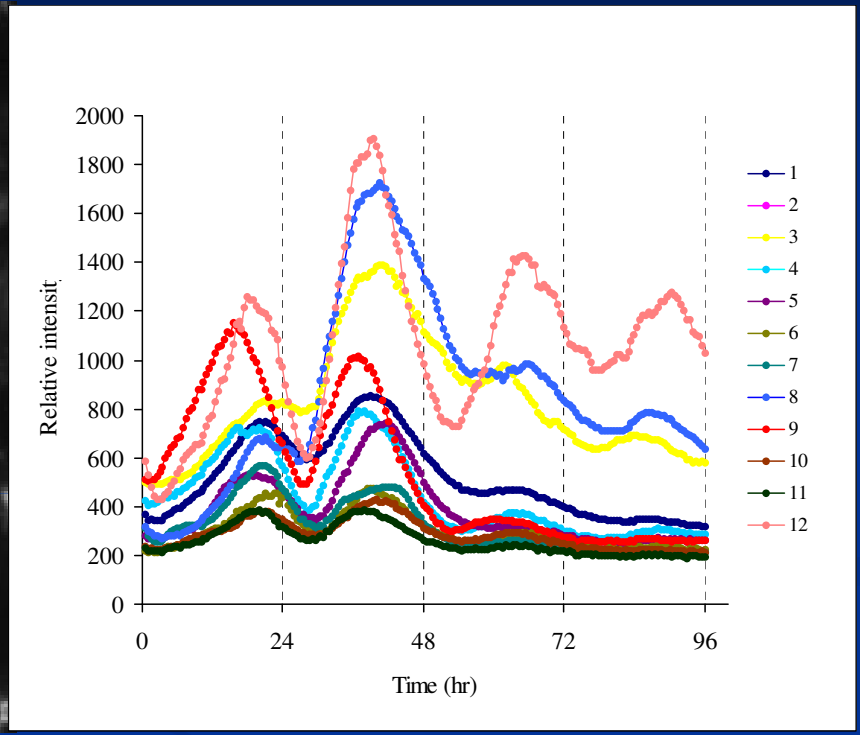
4hr 16hr 28hr 40hr 52hr 64hr

Cell : Rat1
Reporter: Bmal1 Promoter-Fluc
Medium : DMEM, 10%FBS, 25mM HEPES, 0.2mM D-Luciferin

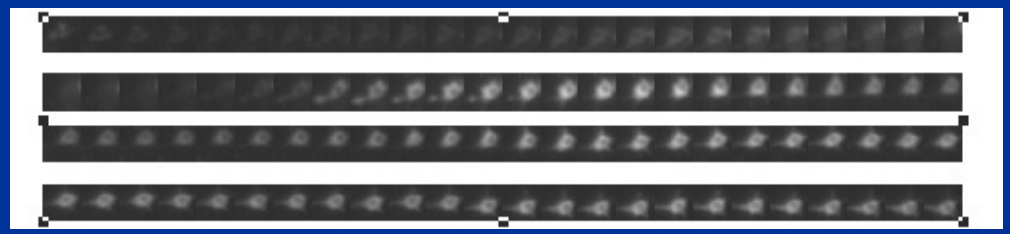
Magnification of objective lens :×5.2
Exposure time : 20min

Image Analysis





1hr



96 hr

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Cell : NIH3T3
Reporter: Bmal1 Promoter-Fluc
Transfection: 2μg
Medium : DMEM, 10%FBS, 25mM HEPES, 0.2mM D-Luciferin
Magnification of objective lens :×5.6
Exposure time : 20min

Human genome sequence

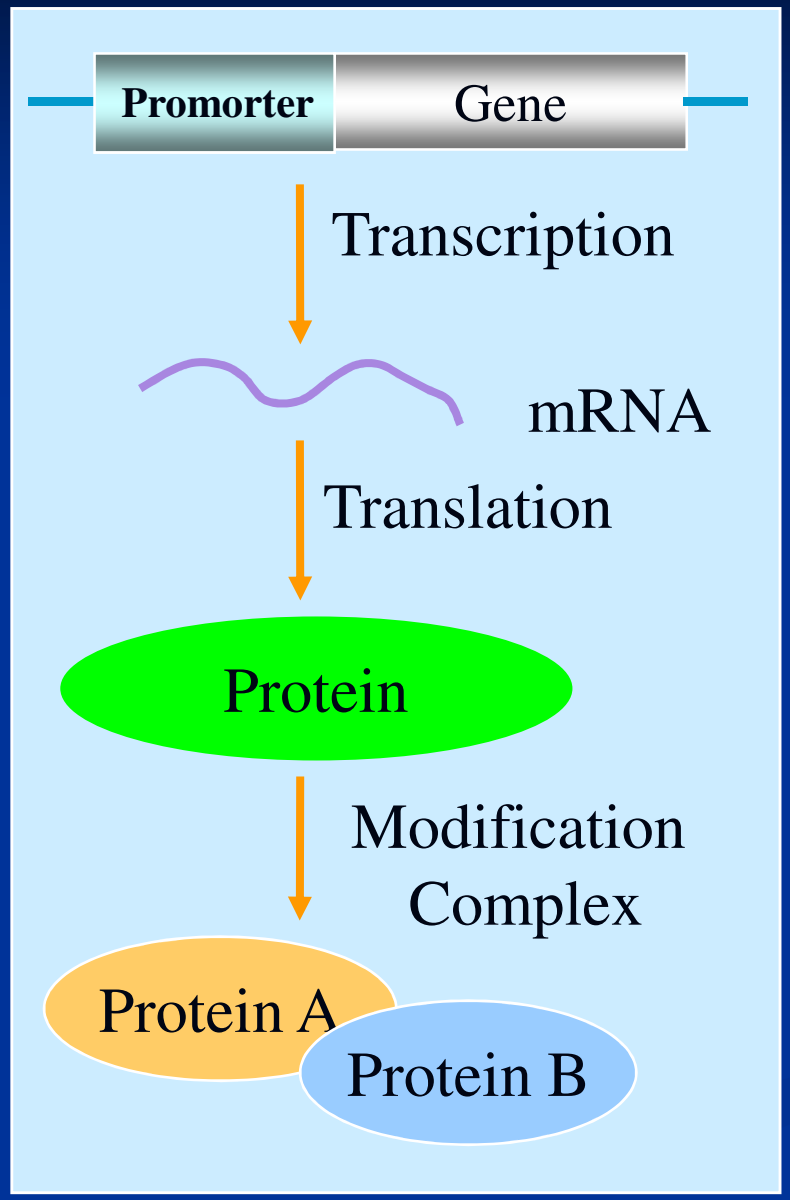
articles

The DNA sequence of human chromosome 21

The chromosome 21 mapping and sequencing consortium
M. Hattori*, A. Fujiyama*, T. D. Taylor*, H. Watanabe*, T. Yada*, H.-S. Park*, A. Toyoda*, K. Ishii*, Y. Totoki*, D.-K. Choi*, E. Soeda†, M. Ohki‡, T. Takagi§, Y. Sakaki*§; S. Taudien‖, K. Blechschmidt‖, A. Polley‖, U. Menzel‖, J. Delabar¶, K. Kumpf‖, R. Lehmann‖, D. Patterson#, K. Reichwald‖, A. Rump‖, M. Schillhabel‖, A. Schudy‖, W. Zimmermann‖, A. Rosenthal‖; J. Kudoh☆, K. Shibuya☆, K. Kawasaki☆, S. Asakawa☆, A. Shintani☆, T. Sasaki☆, K. Nagamine☆, S. Mitsuyama☆, S. E. Antonarakis**, S. Minoshima☆, N. Shimizu☆, G. Nordsiek††, K. Hornischer††, P. Brandt††, M. Scharfe††, O. Schön††, A. Desario‡‡, J. Reichelt††, G. Kauer††, H. Blöcker††; J. Ramser§§, A. Beck§§, S. Klages§§, S. Hennig§§, L. Riesselmann§§, E. Dagand§§, T. Haaf§§, S. Wehrmeyer§§, K. Borzym§§, K. Gardiner#, D. Nizetic‖‖, F. Francis§§, H. Lehrach§§, R. Reinhardt§§ & M.-L. Yaspo§§

ATTO Gel Documentation Analysis System

Analysis Tool

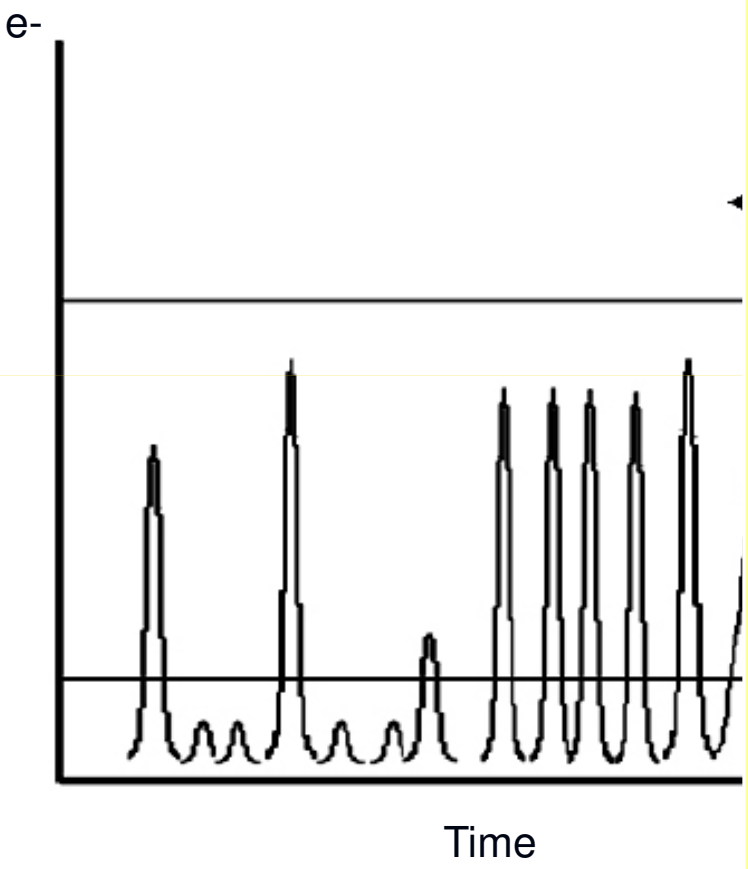


Reporter Gene Assay

DNA Chip
Real-time PCR
Northern Blotting

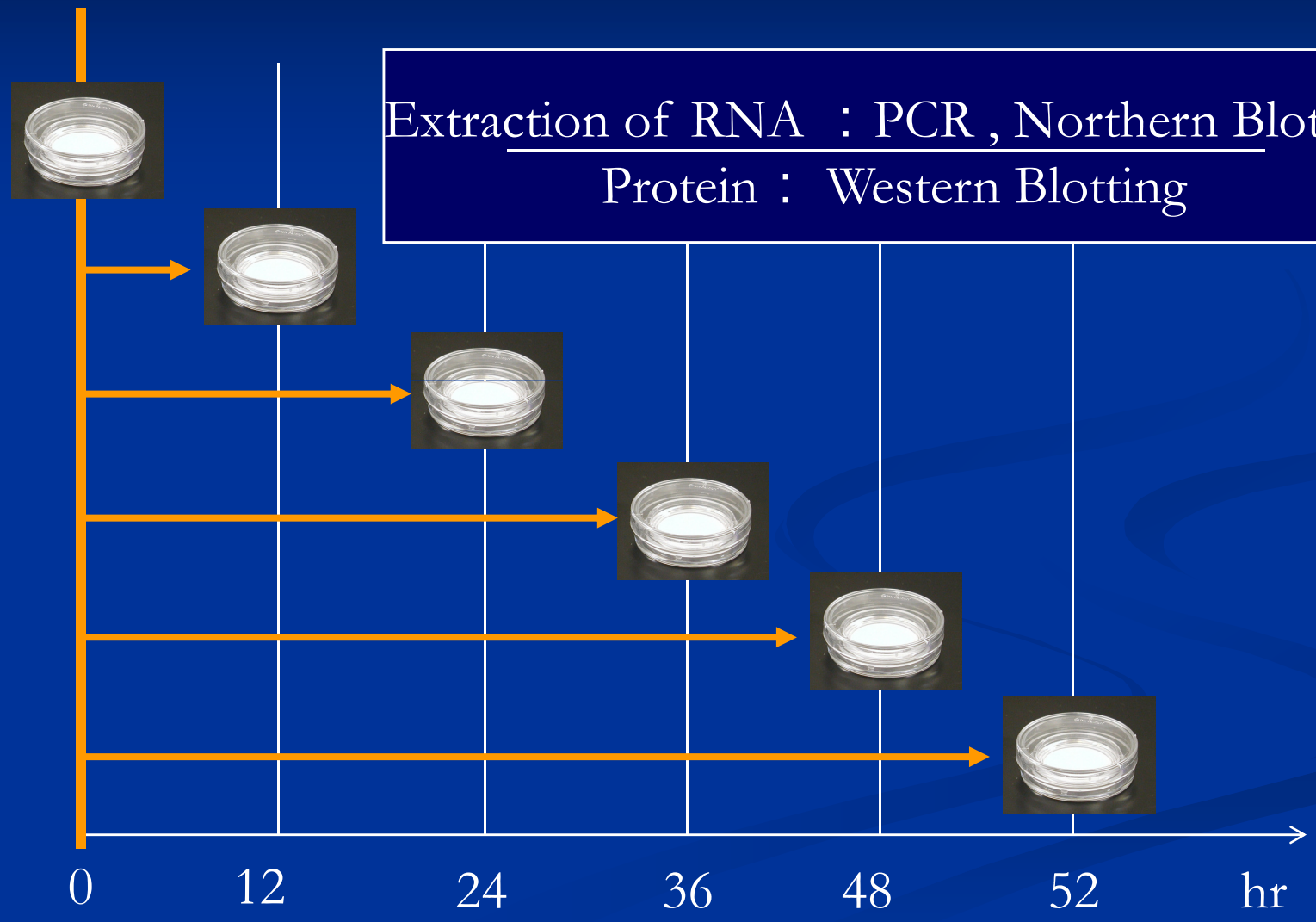
HPLC
2D-Electrophoresis
MS
Western Blotting

Photon Counting



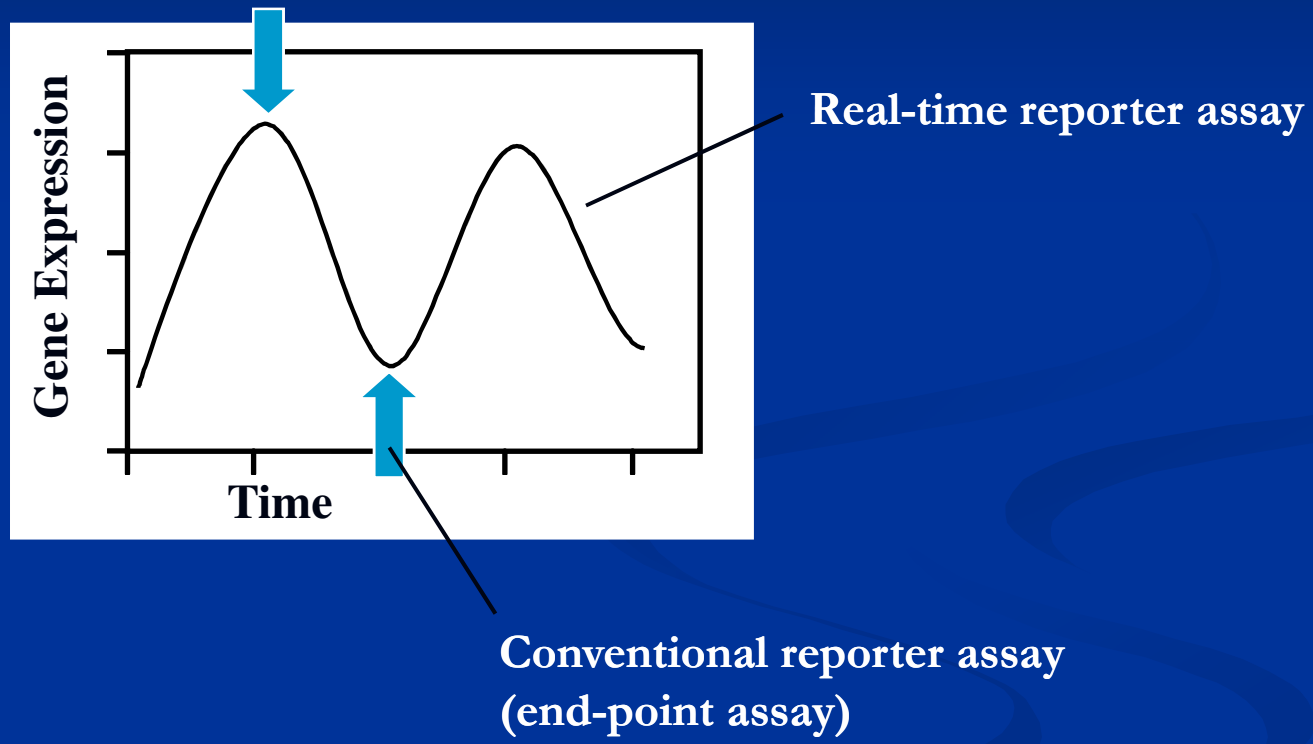
PMT

Quantitative Analysis of mRNA and Protein

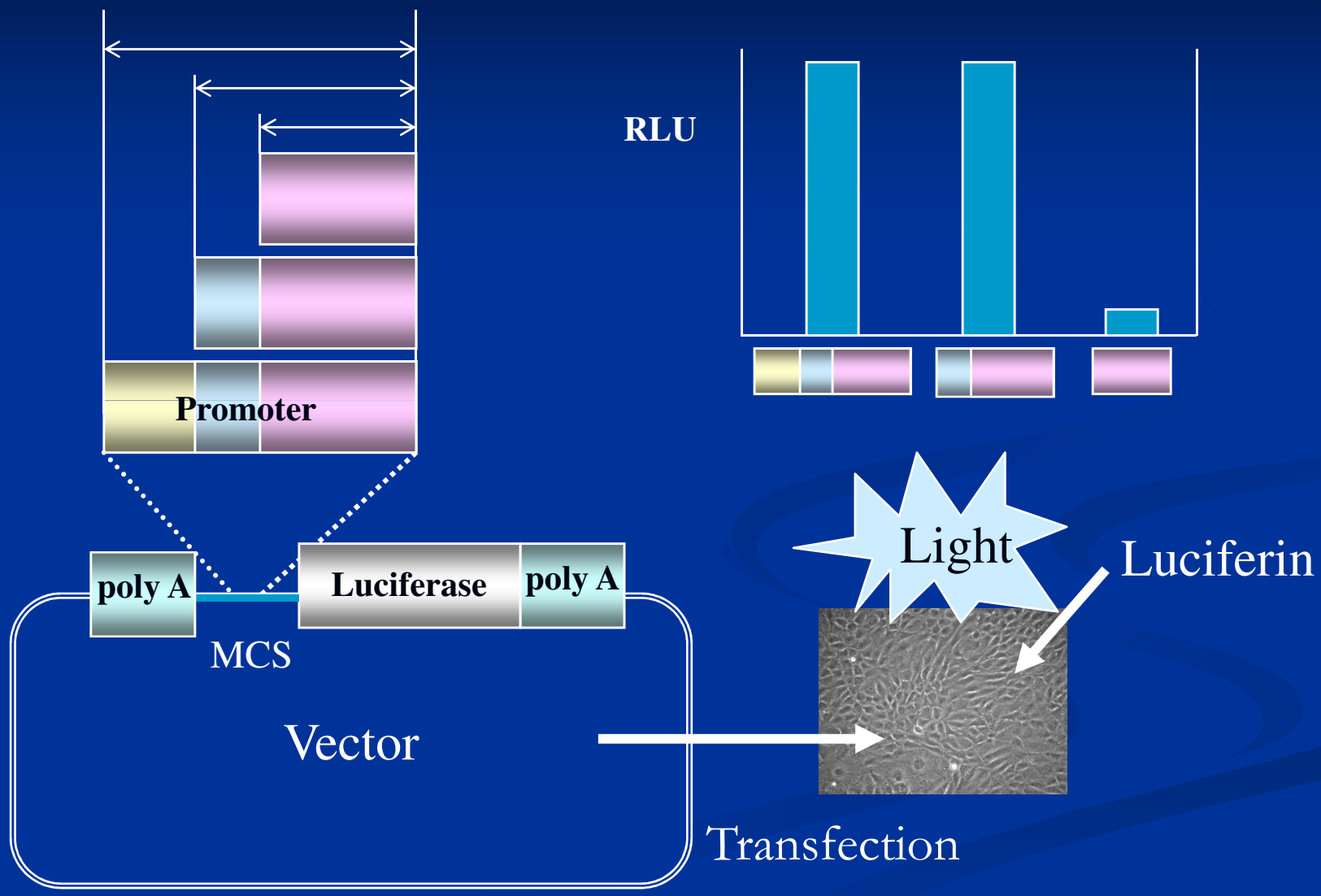


Extraction of RNA ：PCR , Northern Blotting
Protein： Western Blotting

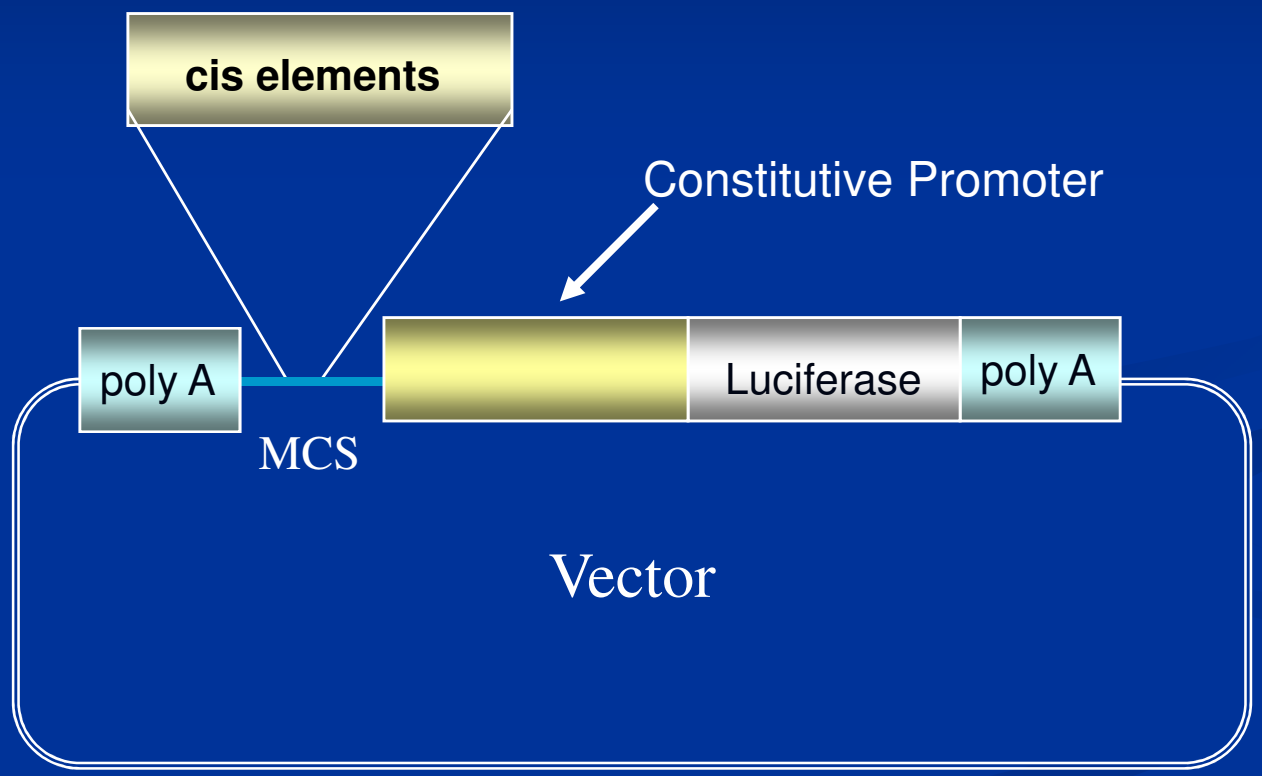
Monitoring of Real-time Changes



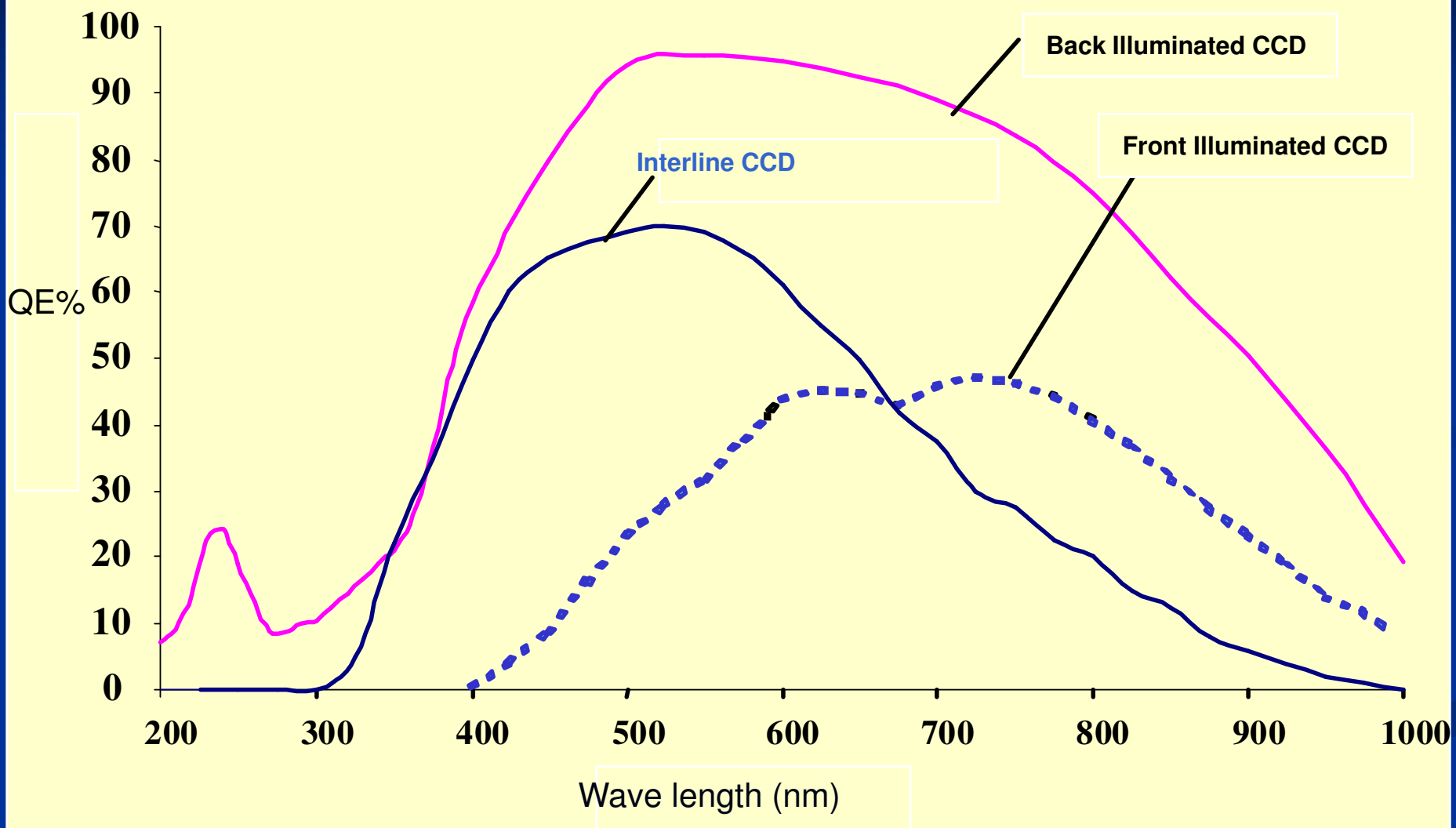
Promoter Analysis



Element Analysis



Quantum Efficiency of CCD



Analysis of Gene Expression by Luciferase Reporter Assay