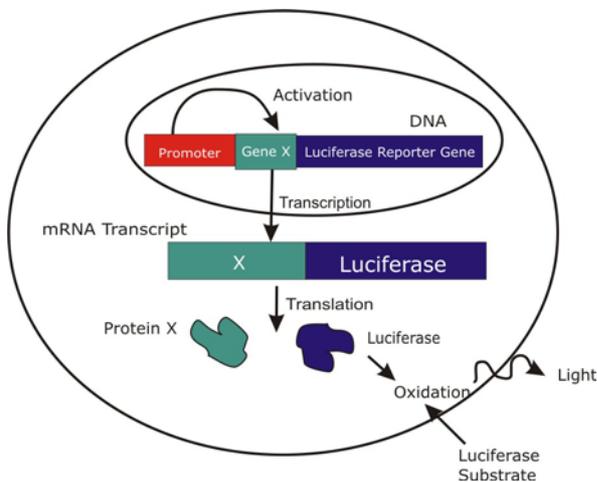


## Reporter Gene Assays

**Reporter genes** have become an invaluable tool in studies of **gene expression**. They are widely used in biomedical and pharmaceutical research and also in molecular biology and biochemistry. A gene consists of two functional parts: One is a **DNA-sequence** that gives the information about the protein that is produced (coding region). The other part is a specific DNA-sequence linked to the **coding region**; it regulates the transcription of the gene (promoter). The **promoter** is either activating or suppressing the expression of the gene.

The purpose of the reporter gene assay is to measure the regulatory potential of an unknown DNA-sequence. This can be done by linking a promoter sequence to an easily detectable reporter gene such as that encoding for the **firefly luciferase**.



Common reporter genes are  **$\beta$ -galactosidase**,  **$\beta$ -glucuronidase**, and **luciferase**. Various detection methods (see below) are used to measure expressed reporter gene protein. These include **luminescence**, **absorbance** and **fluorescence**.

Reporter	Detection Method		
	Luminescence	Fluorescence	Absorbance
Luciferase	+		
$\beta$ -Galactosidase (GUS)	+	+	+
$\beta$ -Glucuronidase (b-Gal)	+	+	
Secreted placental alkaline phosphatase (SEAP)	+		+
Green Fluorescent Protein (GFP)		+	

### Luciferase Reporter Gene Assay

The advantages of a luciferase assay are the high sensitivity, the absence of luciferase activity inside most of the cell types, the wide dynamic range, rapidity and low costs.

The most versatile and common reporter gene is the luciferase of the North American firefly *Photinus pyralis*. The protein requires no posttranslational modification for enzyme activity; it is not even toxic in high concentration (in vivo) and can be used in pro- and eukaryotic cells.

The firefly luciferase catalyzes the bioluminescent oxidation of the luciferin in the presence of ATP, Magnesium and Oxygen:



## Dual Luciferase™ Reporter Gene Assay

The Dual-Luciferase™ Reporter (DLR) Assay System contains two different luciferase reporter enzymes that are expressed simultaneously in each cell. The firefly luciferase and the *Renilla* (sea pansy) luciferase can discriminate between their respective bioluminescence substrates and do not cross-activate.



Validated DLReady™ the BERTHOLD instruments Mithras LB 940, Centro LB 960 and Lumat LB 9507 are ideally suited for this type of reporter gene assay. The instruments' software MikroWin contains predefined DLR parameter files ready to use.

## Chroma Glo™

The Chroma-Luc™ vectors encode luciferases that emit green and red luminescence, their peak luminescence wavelengths are separated by >75nm. These novel vectors are useful for dual measurements where closely similar reporter structures are preferred or where a single reagent addition is desired.

The Mithras LB 940 is one of the most sensitive and best suited instruments to perform this assay because of its unique optical luminescence path and fast filter change. The MikroWin software contains a predefined Chroma Glo™ parameter file, which is ready-to-use.

## Selection of Reporter Gene Assay Kits:

### Absorbance technology:

#### Kit:

- β-Galactosidase Assay Kit (Gene Therapy Systems Inc.)

#### BERTHOLD instruments:

- LB 911/912 Apollo Absorbance Readers
- LB 940 Mithras Multimode Reader

### Fluorescence technology:

#### Kit:

- BD Great EscAPe™ SEAP Fluorescence Detection Kit (BD Biosciences Clontech)
- BetaFluor β-Gal Assay Kit (Novagen)
- β-Glucuronidase Fluorescent Activity Detection Kit (Sigma-Aldrich)

#### BERTHOLD instruments:

- LB 970 Microplate Fluorometer
- LB 940 Mithras Multimode Reader

### Luminescence technology:

#### Kit:

- Luciferase reporter Gene Assay (Roche)
- β-Galactosidase Reporter Gene Assay (Roche)
- SEAP Reporter Gene Assay (Roche)
- Dual-Glow™ Luciferase Assay (Promega)

#### BERTHOLD instruments:

- LB 940 Mithras Multimode Reader
- LB 960 Centro Microplate Luminometer
- LB 9507 Tube Luminometer
- LB 953/955 Automated Tube Luminometers

### Spectral Luminescence technology:

#### Kit:

- Chroma-Glo™ Luciferase Assay (Promega)

#### BERTHOLD instruments:

- LB 940 Mithras Multimode Reader

## Publications:

Dasgupta et al. (2005): Functional Genomic Analysis of the *Wingless/Wnt* Signaling Pathway

Pillon et al. (2005): Binding of Estrogenic Compounds to Recombinant Estrogen Receptor-α: Application to Environmental Analysis

Hong et al. (2004): Aberrant expression and function of TCF4 in the proliferation of hepatocellular carcinoma cell line BEL-7402

Fragnet et al. (2003): The RNA Subunit of Telomerase Is Encoded by Marek's Disease Virus

Yin et al. (2004): Human RAD9 checkpoint control/proapoptotic protein can activate transcription of p21

Habu et al. (2005): Inhibition of HIV-1 gene expression by retroviral vector-mediated small-guide RNAs that direct specific RNA cleavage by tRNase ZL

With this abstract BERTHOLD TECHNOLOGIES likes to give a short introduction and some information about available kits. BERTHOLD TECHNOLOGIES will not be in no way responsible for the validity of information given on these pages. Chroma-Glo, DLR and DLReady logo are trademarks of Promega Corporation.