Unleash the Power of Luciferase to Analyze miRNA Activity in Cancer

November 12, 2018

Presenter:

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Senior Application Scientist
GeneCopoeia, Inc.

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Functional Genomics & Cell Biology

Clones	*Viral systems*	*Stable Cell Lines*	*Kits & reagents*	*Fluorescent detection*
ORF	Lentivirus	CRISPR-Cas9 stable cell lines	Transfection	Cell function assays
Promoter	AAV	Labeled cancer cell lines	Luciferase	Nucleic acid detection
miRNA		Cancer biomarker mutant cell lines	FISH probes	Cell structure probes
CRISPR			Indel detection	Fluorescent dyes
shRNA			Cloning	

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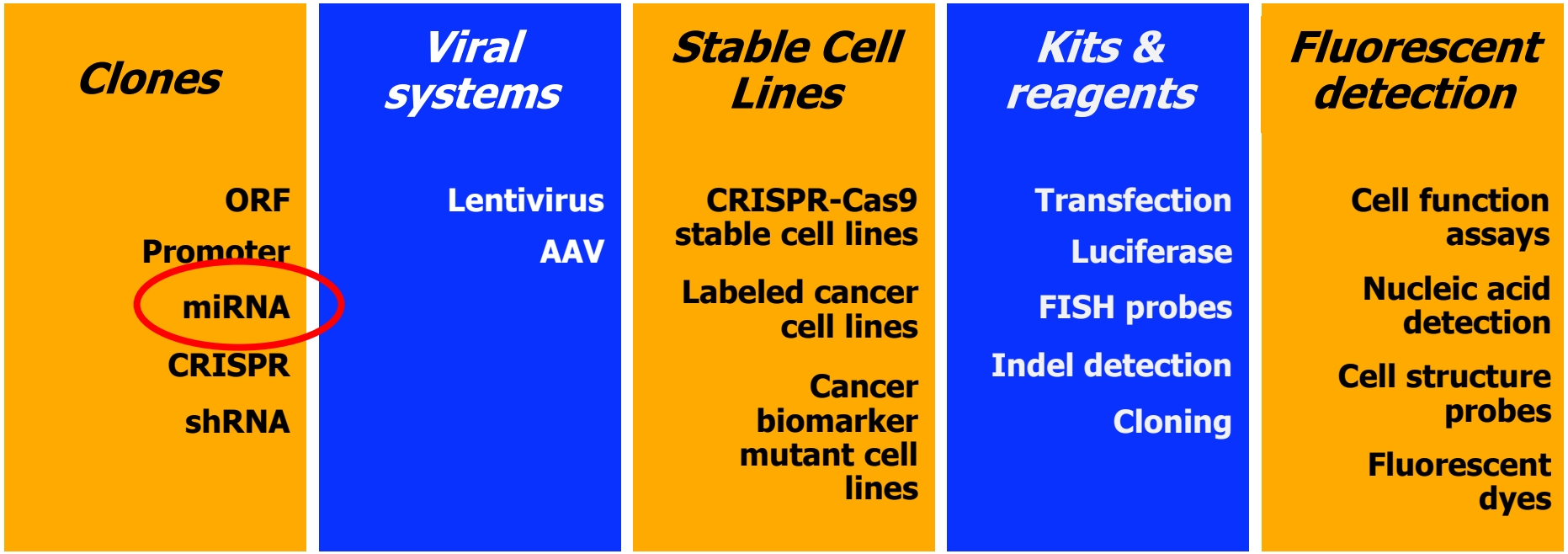

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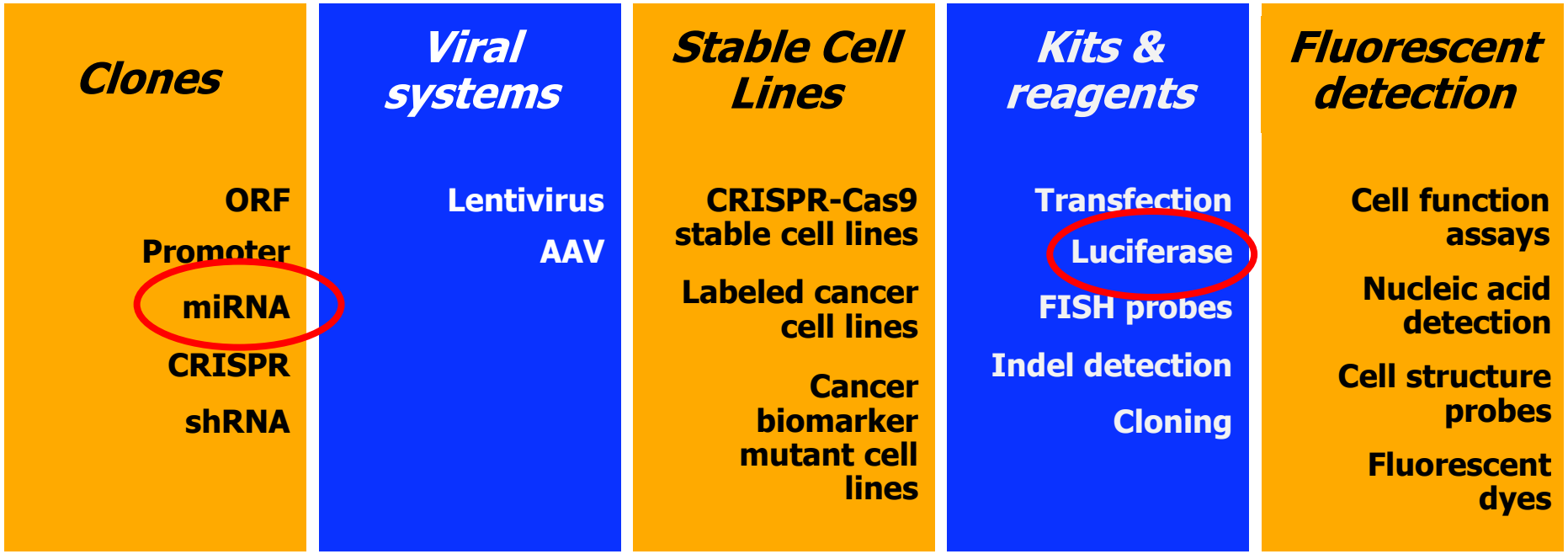

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Outline

- miRNA biology
- Introduction to luciferases
- Studying miRNA in cancer. Example 1
- Studying miRNA in cancer. Example 2
- Other ways of analyzing miRNAs

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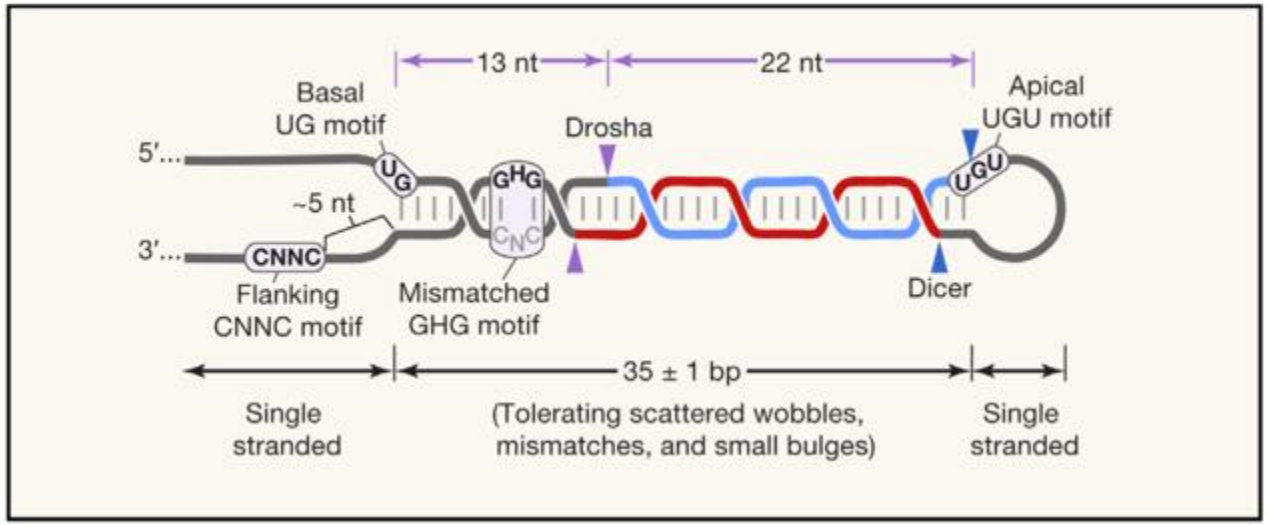
Micro RNAs (miRNAs)

Small, non-protein-coding RNAs

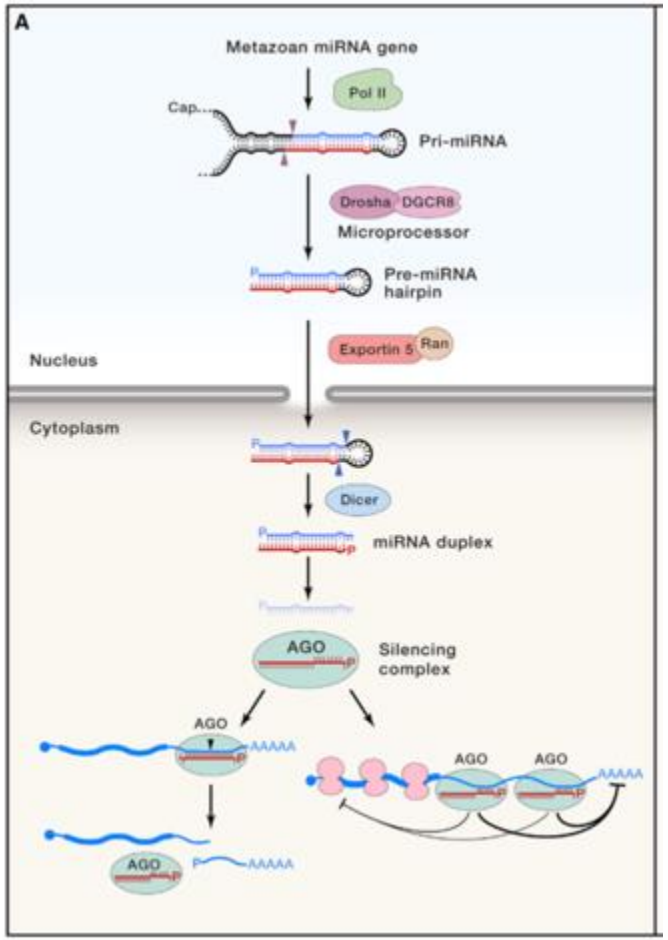


Bartel (2018). *Cell* **173**, 20

- Class of small, non-protein-coding RNA molecules ~21 nucleotides in length
- Present in most known metazoans, including humans and mice
- Approximately 1,900 known miRNAs are known to exist in humans
- Major known function is to post-transcriptionally regulate levels of protein-coding gene expression by targeting the 3' untranslated region (3' UTR) of mRNAs

miRNA biogenesis

Small, non-protein-coding RNAs



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Luciferases

Bioluminescent enzymes

- Enzymes that convert a substrate to light
- Found in many beetles, such as fireflies, sea pansies such as *Renilla*, copepods such as *Gaussia*, and others
- Very high signal-to-noise ratio due to virtually complete absence of background in mammalian cells

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miRNAs in cancer

Dysregulation can be pathogenic

- miRNAs fist shown to be involved in cancer in a deletion occurring in chromic lymphocytic leukemia (CLL)
- Loss of miRNA function in CLL leads to overexpression of BCL2, an inhibitor of apoptosis
- Have since been found to be involved in every stage of many cancers
- Stable presence in body fluids make miRNAs good predictors of prognosis

miRNAs in cancer

Dysregulation can be pathogenic

Table 1. Well-characterized microRNAs and their validated targets in cancer according to Tarbase [79]

MicroRNA	Mechanism		Targets
miR-17~92	Oncogene/tumor suppressor gene		E2F1, HBP1, CDKN1A, NCOA3, ERa, PTEN, MECP2, HOXA5, VPS4B, MYCN, RAB14, DPYSL2, TGFBR2, TSG101, ARHGAP12, BACE1,
miR-21	Oncogene	✔	PDCD4, PTEN, RECK, PPARa, TIMP3, FasL, TGFBR2, SERINB5, CDK2AP1, TPM1
miR-221/222	Oncogene	✔	CDKN1B, KIT, PPP2R2A, p27kip1, CDKN1C, ERa, KIT, DDIT4, BNIP3L, ZEB2, TBK1, CREBZF, MYBL1, DKK2
let-7	Tumor suppressor gene	✔	NIRF, NF2, CASP3, TRIM71
miR-15/16	Tumor suppressor gene		BACE1, DMTF1, C22orf5, BCL2, ARL2, CCNT2, TPPP3, VEGFA, RARS, FGF2, ZNF622, DNAJB4, PURA, SHOC2, LUZP1, FNDC3B, ITGA2, ATG9A, CA12, TMEM43, YIF1B, TMEM189, VTI1B, RTN4, TOMM34, NAA15, PNP, SRPR, IPO4, NAPg, PFAH1B2, SLC12A2, SEC24A, NOTCH2, PPP2R5C, KCNN4, UBE4A, KPNA3, RAB30, ACP2, SRPRB, EIF4E, ABCF2, TPM3, ARHGDIA, GALNT7, LYPLA2, CHORDC1, TMEM109, LAMC1, EGFR, GPAM, ADSS, PPIF, RFT1, TNFSF9, IGF2R, TXN2, GFPT1, SLC7A1, SQSTM1, PANX1, UTP15, NPR3, SLC16A3, PTGS2, HARS, LAMTOR3, HSPA1B
miR-200	Tumor suppressor gene	✔	ZEB1, CTNNB1, BAP1, GEMIN2, PTPRD, WDR37, KLF11, SEPT9, HOXB5, ERBB2IP. KLHL20, FOG2, RIN2, RASSF2, ELMO2, TCF7L1, VAC14, SHC1, SEPT7, FOG2
miR-34	Tumor suppressor gene	✔	SIRT1, BCL2, YY1, MYC, CDK6, CCND1, FOXP1, HNF4a, CDKN2C, ACSL4, LEF1, ACSL1, MTA2, AXL, LDHA, HDAC1, CD44, BCL2, E2F3

Hayes, *et al.* (2014). *Trends in Molecular Medicine* **20**, 460

Using luciferases to study miRNAs in cancer

Example 1: Latonen, et al. (2018): Prostate cancer

- Prostate cancer is the most common cancer among men in Western countries and second most common among men worldwide
- No cure for castration-resistant prostate cancer (CRPC)
- Found, compared with untreated primary prostate cancer (PC), 728 proteins differentially expressed in benign prostate hyperplasia (BPH), and 382 proteins differentially expressed in CRPC.
- 153 proteins were common between the 2 sets

Latonen, et al. (2018). *Nature Communications* doi: 10.1038/s41467-018-03573-6

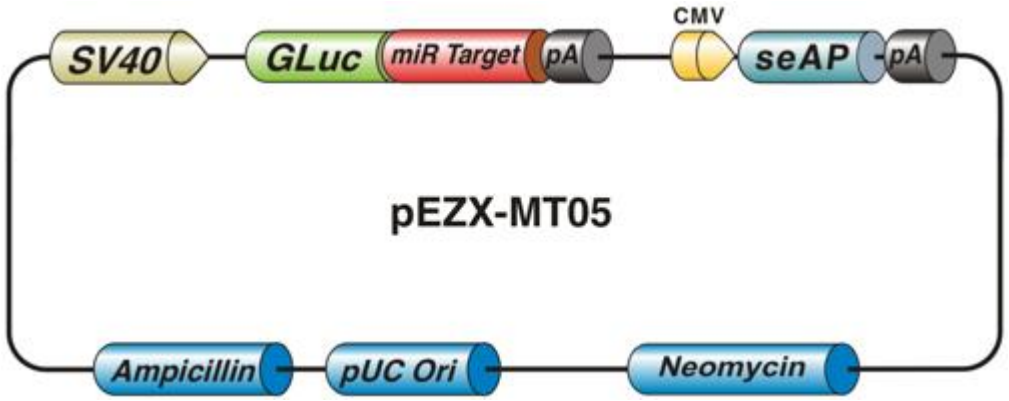
Using luciferases to study miRNAs in cancer

Example 1: Latonen, et al. (2018): Prostate cancer

- Further showed that 95 miRNAs were differentially expressed between CRPC and PC
- Pathway analysis indicated that the tricarboxylic acid (TCA) cycle was strongly differentially expressed in CRPC vs. PC
- MDH2 protein levels are differentially expressed in CRPC vs. PC, while mRNA levels are not. Found that two miRNAs, miR-22 and miR-205, are differentially expressed and negatively correlate with MDH2 protein levels.
- Tested effect of miR-22 and miR-205 on MDH2 mRNA expression

Latonen, et al. (2018). *Nature Communications* doi: 10.1038/s41467-018-03573-6

GeneCopoeia miTarget™ 3’ UTR clones



Features

- 3’ UTRs from any human, mouse, or rat gene fused to luciferase reporter
- miRNA activity will cause decrease in reporter signal
- Dual-reporter vector system. Enables transfection normalization for accurate across-sample comparison
- Ready for transfection of mammalian cells

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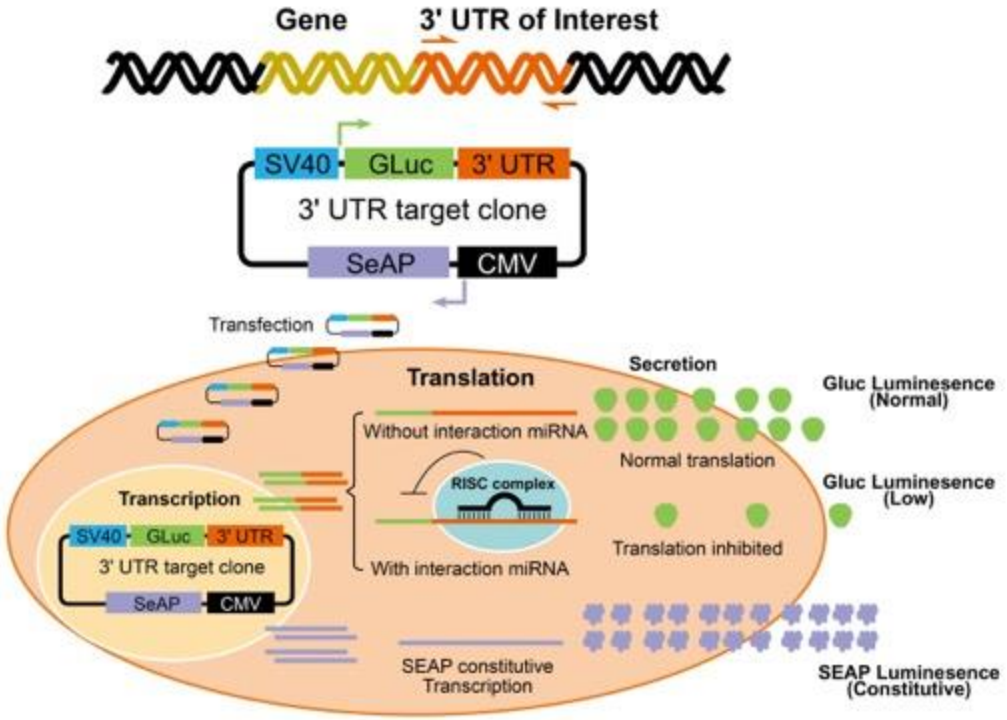
GeneCopoeia Secrete-Pair™ Luciferase Kits



Features

- Secreted reporter system for live cell assays. Use cell culture medium, Lysis unnecessary.
- Dual-reporter detection. SEAP allows normalization of GLuc signal for greater accuracy.
- Sensitive and robust system. GLuc is 1,000 times more sensitive than firefly and Renilla luciferases.
- Flexible assay conditions. Two robust buffer conditions are provided for GLuc assays

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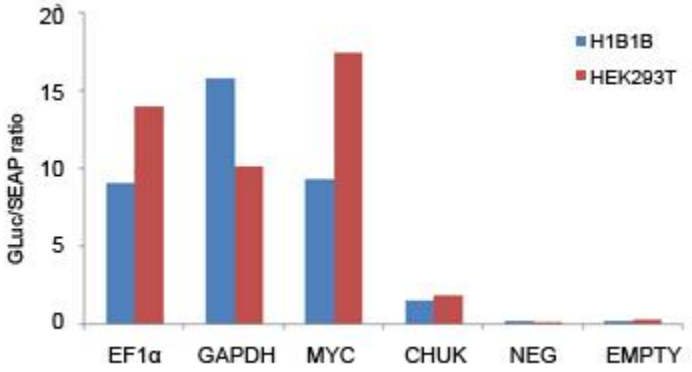
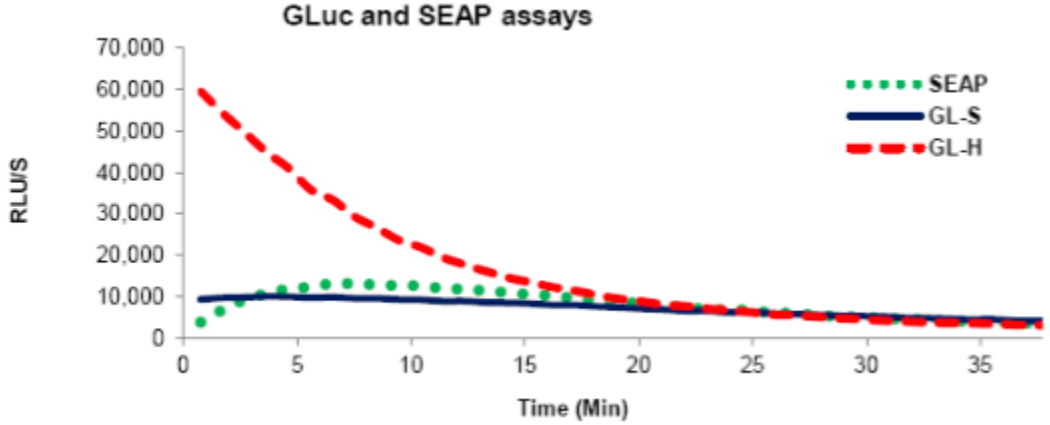
GeneCopoeia Secrete-Pair™ Luciferase Kits





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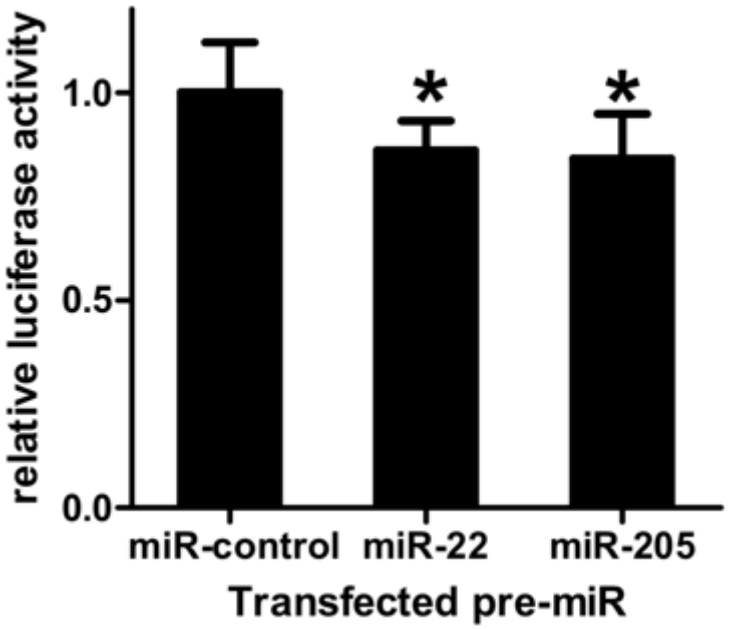
Using luciferases to study miRNAs in cancer

Example 1: Latonen, et al. (2018): Prostate cancer



- Co-transfected PC-3 cells with miRNA plasmids and GeneCopoeia miTarget™ MDH2 3'UTR clone
- Observed small but statistically significant reduction in luciferase activity using Secrete-Pair™
- Result suggests that miRNA activities might play a major role in prostate cancer progression

Latonen, et al. (2018). *Nature Communications* doi: 10.1038/s41467-018-03573-6

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Using luciferases to study miRNAs in cancer

Example 2: Mohr, et al. (2017): Acute myeloid leukemia

- Acute myeloid leukemia (AML) is an aggressive blood cancer.
- Begins in bone marrow
- Characterized by enhanced proliferation, blocked differentiation, and dysregulated apoptosis
- Hox genes play major role in AML development and progression

Mohr, *et al.* (2017). *Cancer Cell* **31**, 549

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Using luciferases to study miRNAs in cancer

Example 2: Mohr, et al. (2017): Acute myeloid leukemia

- Hoxa9 and Meis1, when overexpressed in lineage-derived bone marrow cells, transformed them and killed mice
- Proteomics analysis showed 1,810 proteins differentially expressed after transformation
- Syk is one of the most upregulated proteins in Hoxa9/Meis1 transformed cells
- Syk mRNA not upregulated by either qPCR or RNA-seq
- Also found Syk protein, but not mRNA, overexpressed in patient AML samples

Mohr, *et al.* (2017). *Cancer Cell* **31**, 549

Using luciferases to study miRNAs in cancer

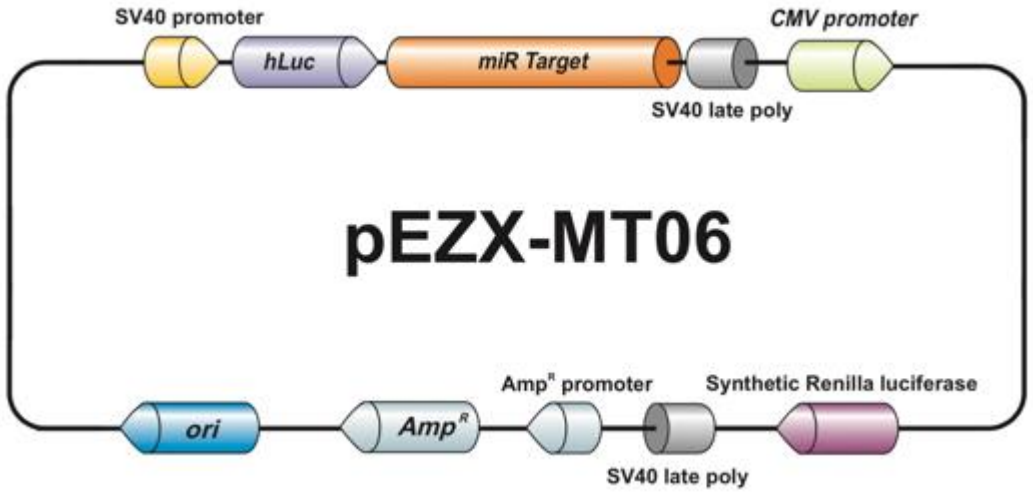
Example 2: Mohr, et al. (2017): Acute myeloid leukemia

- Syk protein overexpression in absence of mRNA elevation suggests that miRNAs might be involved
- Global miRNA expression profiling found that 8 miRNAs are significantly downregulated in cells overexpressing Hoxa9 and Meis1
- One of these 8 miRNAs, miR-146a, is predicted to bind Syk 3'UTR at two sites

Mohr, *et al.* (2017). *Cancer Cell* **31**, 549

GeneCopoeia miTarget™ 3’ UTR clones

Firefly/*Renilla* reporters



Features

- 3’ UTRs from any human, mouse, or rat gene fused to luciferase reporter
- miRNA activity will cause decrease in reporter signal
- Dual-reporter vector system. Enables transfection normalization for accurate across-sample comparison
- Ready for transfection of mammalian cells
- Compatible with any Firefly/*Renilla* luciferase assay system

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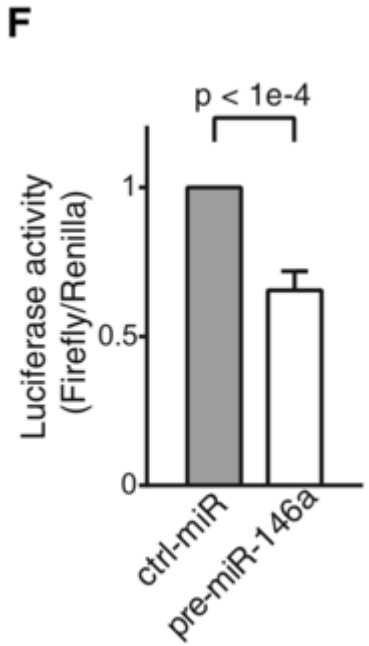
Using luciferases to study miRNAs in cancer

Example 2: Mohr, et al. (2017): Acute myeloid leukemia



- Co-transfected HEK293T cells with miRNA plasmids and GeneCopoeia miTarget™ SYK 3'UTR clone
- Observed small but statistically significant reduction in Firefly luciferase activity normalized to *Renilla* luciferase
- Result suggests that miRNA activities might play a major role in AML formation and/or progression

Mohr, *et al.* (2017). *Cancer Cell* **31**, 549

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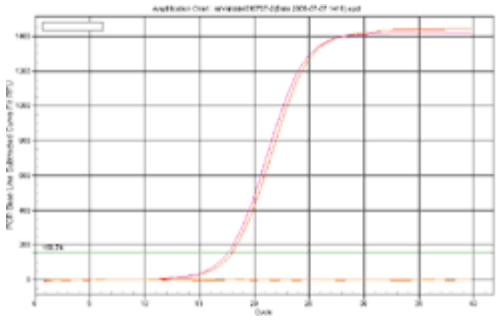
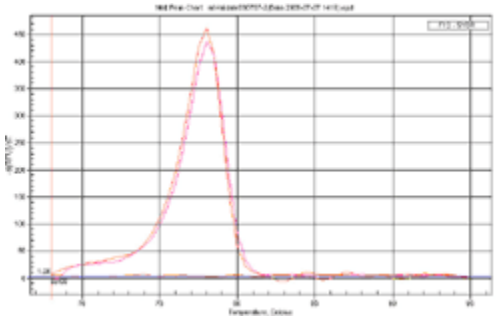
Other ways to study miRNAs in cancer

Measure miRNA abundance using GeneCopoeia All-in-One™ miRNA qPCR primers





- Designed using a proprietary algorithm
- Pre-validated
- Best when used in combination with the All-in-One™ SYBR® Green miRNA qRT-PCR kit

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Other ways to study miRNAs in cancer

GeneCopoeia miRNA qPCR arrays

- For high-throughput profiling of miRNA expression
- Designed using a proprietary algorithm
- Primer are-validated
- 43 pre-made arrays available in including whole-miRNome (5), cancer (27), and disease and focus group (11). Custom arrays also available.
- Best when used in combination with the All-in-One™ SYBR® Green miRNA qRT-PCR kit

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Other ways to study miRNAs in cancer

GeneCopoeia All-in-One™ miRNA qPCR reagents

Buy	Catalog#	Product	Description
☐	QP015	All-in-One™ miRNA qRT-PCR Detection Kit* (20 RT and 200 qPCR reactions)	Poly A Polymerase, RTase Mix, qPCR Mix, ROX Reference Dye, Universal Adaptor PCR Primer and other buffers (for use with miRNA qPCR primers)
☐	QP016	All-in-One™ miRNA qRT-PCR Detection Kit* (60 RT and 600 qPCR reactions)	Poly A Polymerase, RTase Mix, qPCR Mix, ROX Reference Dye, Universal Adaptor PCR Primer and other buffers (for use with miRNA qPCR primers)
☐	QP017	All-in-One™ miRNA First-Strand cDNA Synthesis Kit for miRNA qPCR array (20 RT reactions)	Poly A Polymerase, RTase Mix, PAP/RT buffer, spike-in control (for use with miRNA qPCR arrays)
☐	QP018	All-in-One™ miRNA First-Strand cDNA Synthesis Kit for miRNA qPCR array (60 RT reactions)	Poly A Polymerase, RTase Mix, PAP/RT buffer, spike-in control (for use with miRNA qPCR arrays)

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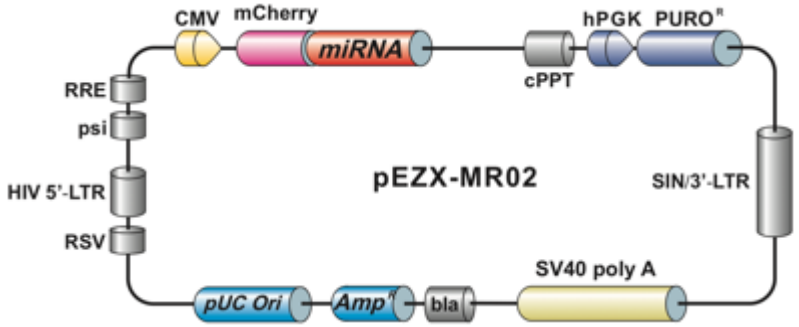
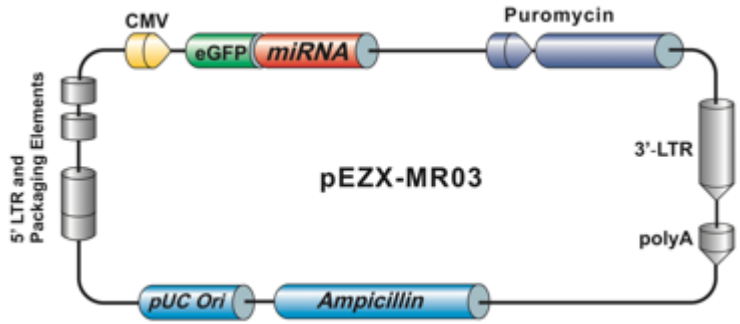
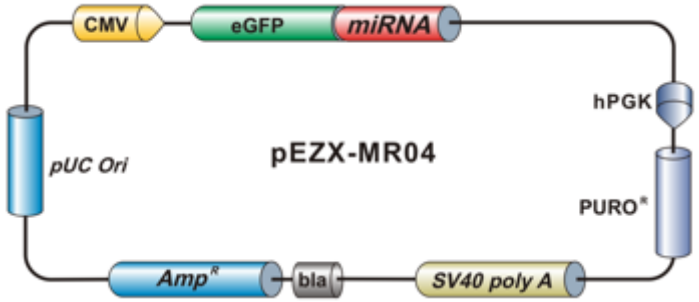
Other ways to study miRNAs in cancer

GeneCopoeia miExpress™ miRNA precursor clones







- Ideal for validating miRNA binding sites in 3’ UTR clones
- Full coverage. All known human, mouse and rat miRNAs in the current miRBase covered.
- Flexible delivery systems. Available in lentiviral or non-viral formats.
- Also available as ready-to-use lentiviral particles
- Synthetic dsRNA mimics also available

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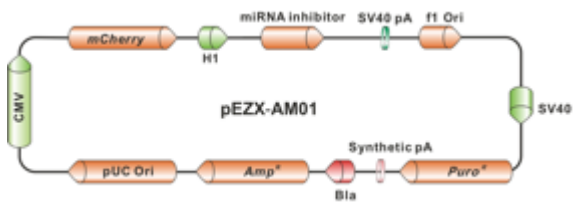
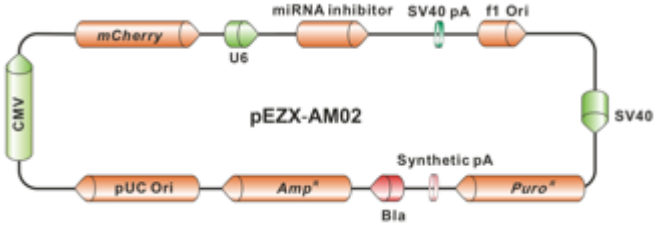
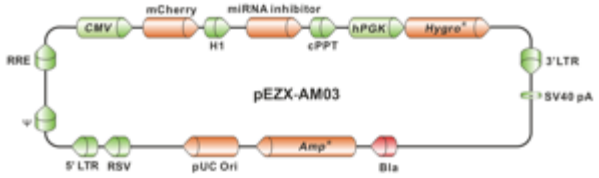
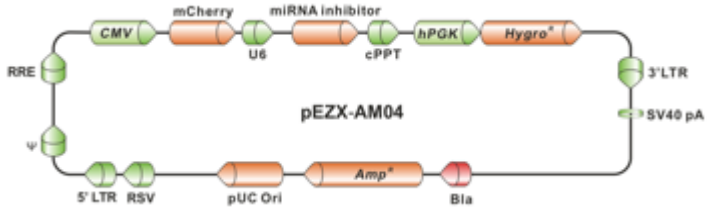
Other ways to study miRNAs in cancer

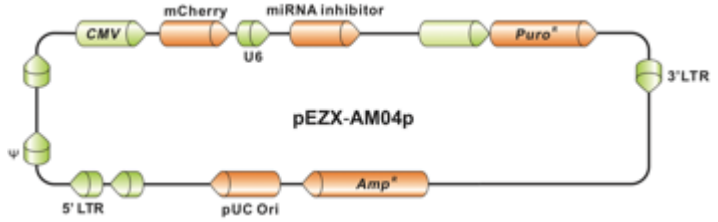
GeneCopoeia miArrest™ miRNA inhibitor clones











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- Also available as ready-to-use lentiviral particles
- Synthetic RNA also available

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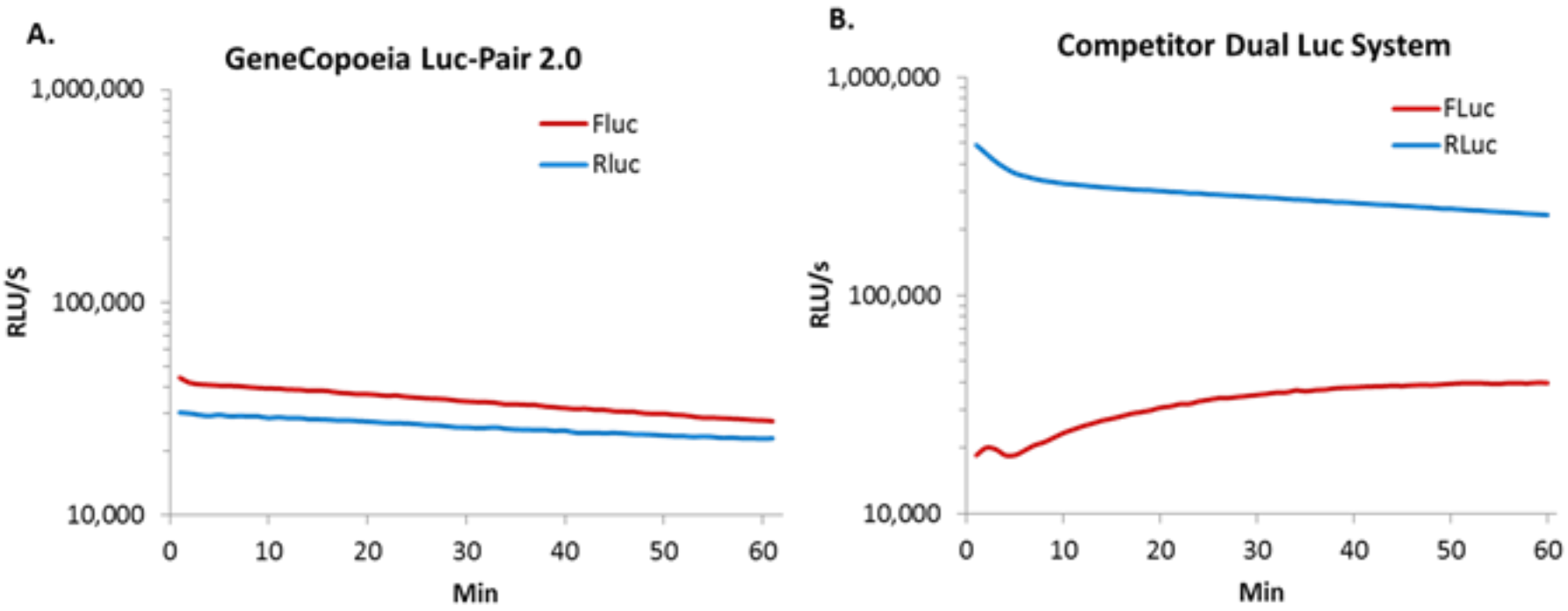
Other GeneCopoeia luciferase products

Luc-Pair™ Firefly/*Renilla* luciferase assay kits

Product	Purpose
Luc-Pair™ Duo-Luciferase Assay Kit 2.0	Assays both Firefly and *Renilla* luciferases. Designed for enhanced stability
Luc-Pair™ Duo-Luciferase HS Assay Kit	Assays both Firefly and *Renilla* luciferases. Designed for enhanced signal intensity.
Luc-Pair™ Duo-Luciferase HT Assay Kit	Assays both Firefly and *Renilla* luciferases. Designed for enhanced signal intensity. Ideal for use with injectors.
Luc-Pair™ Firefly Luciferase HS Assay Kit	Assays Firefly luciferase only. Designed for enhanced signal intensity.
Luc-Pair™ Firefly Luciferase HT Assay Kit	Assays Firefly luciferase only. Designed for enhanced signal intensity. Ideal for use with injectors.
Luc-Pair™ *Renilla* Luciferase HS Assay Kit	Assays *Renilla* luciferase only. Designed for enhanced signal intensity.
Luc-Pair™ *Renilla* Luciferase HT Assay Kit	Assays *Renilla* luciferase only. Designed for enhanced signal intensity. Ideal for use with injectors.

Other GeneCopoeia luciferase products

GeneCopoeia Luciferase vs. leading competitor



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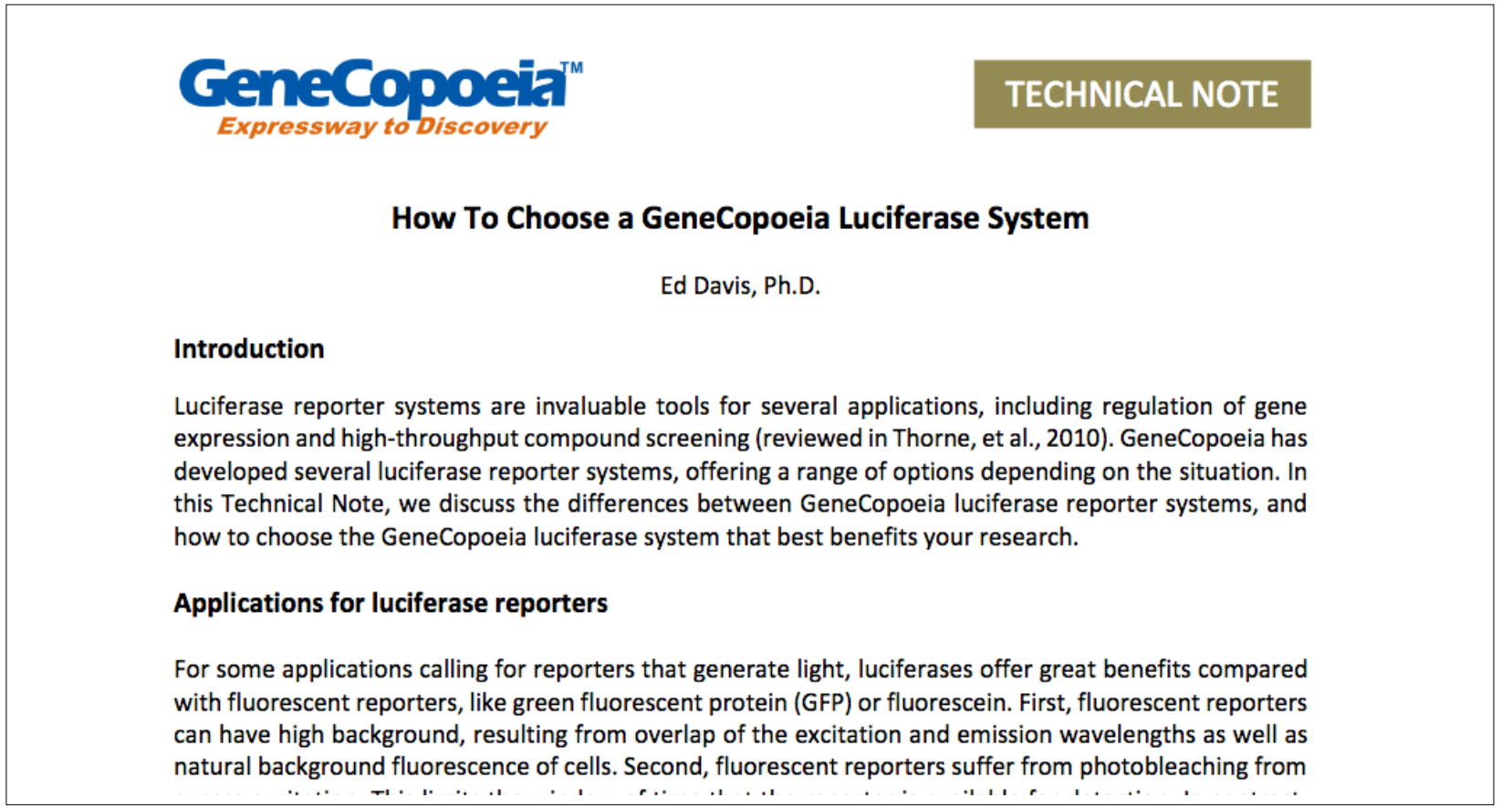
CRISPR-Cas9 genome editing technology

Technical Note: How to Choose a GeneCopoeia Luciferase System



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TECHNICAL NOTE

How To Choose a GeneCopoeia Luciferase System

Ed Davis, Ph.D.

Introduction

Luciferase reporter systems are invaluable tools for several applications, including regulation of gene expression and high-throughput compound screening (reviewed in Thorne, et al., 2010). GeneCopoeia has developed several luciferase reporter systems, offering a range of options depending on the situation. In this Technical Note, we discuss the differences between GeneCopoeia luciferase reporter systems, and how to choose the GeneCopoeia luciferase system that best benefits your research.

Applications for luciferase reporters

For some applications calling for reporters that generate light, luciferases offer great benefits compared with fluorescent reporters, like green fluorescent protein (GFP) or fluorescein. First, fluorescent reporters can have high background, resulting from overlap of the excitation and emission wavelengths as well as natural background fluorescence of cells. Second, fluorescent reporters suffer from photobleaching from

Download from:

https://www.genecopoeia.com/wp-content/uploads/2017/06/GeneCopoeia-Technical-Note-How-to-Choose-a-Luciferase-System-04-2017.pdf

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Summary

- miRNAs are important, post-transcriptional regulators of gene expression, and their dysregulation can lead to many diseases, including cancers
- GeneCopoeia 3’ UTR plasmid clones have been used extensively to help demonstrate the critical nature of miRNA function in the formation and progression of cancers
- Luciferases are bioluminescent enzymes, with high signal intensity and low background, which makes them ideal reporters for detecting miRNA activity in cancer progression
- GeneCopoeia offers many products, from clones for 3’UTRs, miRNA precursors, and inhibitors, to powerful secreted and Firefly/Renilla luciferase assay kits, to greatly facilitate your cancer miRNA studies

Thank You!

If you have any additional questions, please call

1-866-360-9531 x227

Email: edavis@genecopoeia.com

Or visit us on the web:

www.genecopoeia.com

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