Stable Luciferase-Expressing Cell Lines for *In Vivo* Xenograft and Syngeneic Tumor Model Bioluminescence Imaging

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Abstract

Human and mouse cancer cell lines are used in xenograft and syngeneic models, respectively, for studying *in vivo* tumor formation and development, evaluating metastasis, measuring tumor burden in whole animals, and monitoring response to therapeutic treatment. Whole animal *in vivo* imaging has been widely applied by researchers due to the ease of operation in visualizing *in vivo* biological events while eliminating the requirement for animal subject sacrifice, allowing for continual monitoring/imaging of a single individual animal, and reducing the amount of inter-animal variation. Luciferase reporters provide a relatively simple, robust, and highly sensitive means to measure biological processes and to assess drug efficacy in animal models through *in vivo* bioluminescence imaging. Here, we report on the generation of a panel of cell lines which express high levels of luciferase and have broad applications for *in vitro* and *in vivo* studies. The panel includes human and mouse cell lines that represent various common cancer and tissue types such as breast, colon, lung, pancreas, prostate, and skin. After introduction of a Lenti-LUC2 luciferase reporter into the parental cell lines, single cell cloning was performed to isolate stable clones with high luciferase expression. We quantified *in vitro* bioluminescence signals within the cells and analyzed the signal-to-noise ratio. In addition, the cell lines were characterized and authenticated by using cell morphology, growth kinetics, and STR analysis. Luciferase-labeled human cell lines were administered into immune-deficient mice either by subcutaneous or orthotropic injection to establish xenograft models. Luciferase labeled mouse cell lines were tested in the associated breed of mice to evaluate tumor growth in the syngeneic mouse model. The bioluminescence live imaging was performed using the Xenogen IVIS® imaging system and the *in vivo* bioluminescent radiance was analyzed. Although growth can be evaluated by both traditional caliper measurement and bioluminescence in subcutaneous mouse models, only live bioluminescent imaging enables continuous monitoring tumor metastasis or analysis of tumor growth of the intracranial brain tumor models. In summary, luciferase-expressing cell lines are valuable tools for elucidating mechanisms involved in tumorigenesis, studying tumors *in vivo*, and screening anti-cancer compounds for drug discovery and development.

Results

Table 1. ATCC luciferase-expressing cell lines

Parental cell line	ATCC® No.	Species	Tissue/disease	Photons/ cell/sec*	*In vivo* model
B16-F1	CRL-6323™	Mouse	Melanoma	336,669	Syngeneic
B16-F10	CRL-6475™	Mouse	Melanoma	207,727	Syngeneic
4T1	CRL-2539™	Mouse	Breast cancer	2,564,375	Syngeneic
EL4	TIB-39™	Mouse	Lymphoma	277,331	Syngeneic
LL/2 (LLC1)	CRL-1642™	Mouse	Lung cancer	29,104	Syngeneic
A549	CCL-185™	Human	Lung cancer	22,325,000	Xenograft
EML4-ALK A549	CCL-185IG™	Human	Lung cancer	19,356,250	Xenograft
A375	CRL-1619™	Human	Melanoma	491,308	Xenograft
KRAS G13D A375	CRL-1619IG-1™	Human	Melanoma	347,619	Xenograft
NRAS Q61K A375	CRL-1619IG-2™	Human	Melanoma	1,192,822	Xenograft
A-431	CRL-1555™	Human	Epidermoid carcinoma	603,085	Xenograft
TF-1	CRL-2003™	Human	Leukemia	112,759	Xenograft
IDH2 mutant TF-1	CRL-2003IG™	Human	Leukemia	64,768	Xenograft
HL-60	CCL-240™	Human	Leukemia	123,330	Xenograft
U-87 MG	HTB-14™	Human	Glioblastoma	8,601,250	Xenograft
IDH1 mutant U-87MG	HTB-14IG™	Human	Glioblastoma	8,165,625	Xenograft
HT-1080	CCL-121™	Human	Fibrosarcoma	2,125,000	Xenograft
HCT 116	CCL-247™	Human	Colon cancer	2,770,000	Xenograft
PANC-1	CRL-1469™	Human	Pancreatic cancer	2,175,533	Xenograft
PC-3	CRL-1435™	Human	Prostate cancer	397,640	Xenograft
LNCaP clone FGC	CRL-1740™	Human	Prostate cancer	1,187,513	Xenograft

* The *in vitro* bioluminescence (photons/cell/sec) was quantified by Xenogen IVIS Spectrum (subject to imaging and cell culture condition).

Generation of Luciferase-expressing Cell Lines

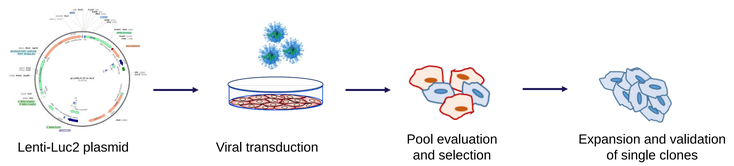

Figure 1. Scheme of developing a stable cell line containing the Luc2 gene.

Characterization of mouse B16-F10-Luc2 cell line *in vitro*

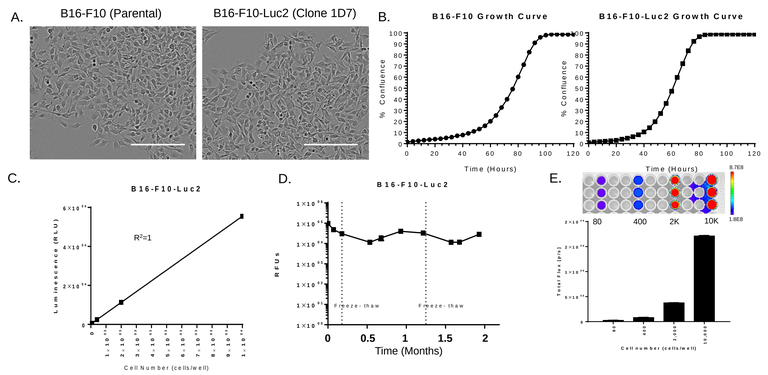

Figure 2. Characterization of luciferase expressing cell lines. (A) Cell morphology of the B16-F10 and B16-F10-Luc2 (ATCC® CRL-6475-LUC2™) cell lines was observed under microscopy and images were captured via digital camera (scale bar = 200 µm). (B) Cells were seeded in 96-well plates at 1000 cells per well. Cell growth kinetics were captured by a live imaging system measuring cell confluences. (C) Luciferase assay was performed by using Bright-Glo™ (Promega®) Luciferase Assay System and a luminescence plate reader. Data showed a linear correlation between bioluminescence intensity and cell number. (D) To verify the stability of luciferase expression, the cells were maintained in culture for 2 months plus two freeze-thaw cycles. The luciferase expression was monitored every week by using the luciferase assay. (E) The *in vitro* absolute bioluminescence was quantified using a Xenogen IVIS® Spectrum (PerkinElmer) to measure the photons emitted per cell.

Syngeneic tumor model *in vivo* bioluminescence imaging

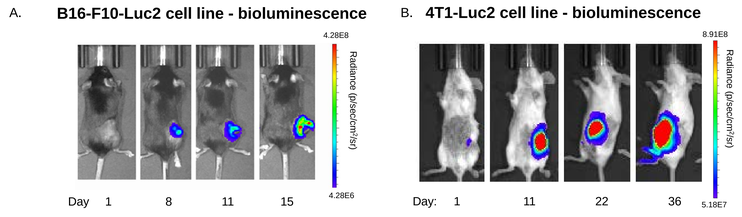

Figure 3. *In vivo* detection of luciferase activity of syngeneic tumor model mouse cell lines. (A) B16-F10-Luc2 cells (1x10^6) were subjected to subcutaneous injection into the dorsal region near the thigh of C57BL/6J mice. (B) 4T1-Luc2 cells (ATCC® CRL-2539™; 3x10^4) were injected into the mammary fat pad of BALB/cfC3H mice. Tumor growth was monitored using a Xenogen IVIS® Spectrum. *In vivo* bioluminescence imaging demonstrated the progression of tumors.

Characterization of luciferase-expressing glioma cell line *in vitro*

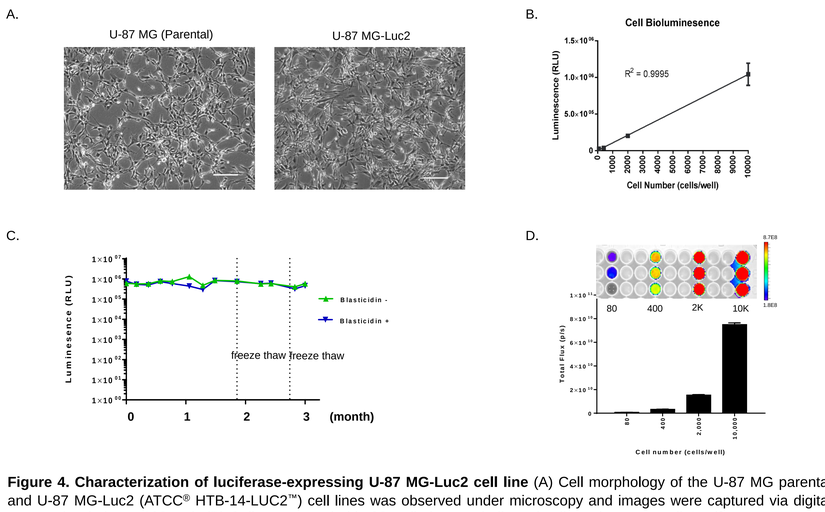

Figure 4. Characterization of luciferase-expressing U-87 MG-Luc2 cell line (A) Cell morphology of the U-87 MG parental and U-87 MG-Luc2 (ATCC® HTB-14-LUC2™) cell lines was observed under microscopy and images were captured via digital camera (scale bar = 100 µm). (B) Cells were seeded in 96-well plates at indicated cell numbers per well. Luciferase assay was performed by using Bright-Glo™ Luciferase Assay System and luminescence plate reader. Data showed a linear correlation between bioluminescence intensity and cell number. (C) To verify the stability of luciferase expression, the cells were maintained in culture media with or without blasticidin for 3 months plus three freeze-thaw cycles; luciferase expression was monitored every week for 3 months via luciferase assay. (D) The *in vitro* absolute bioluminescence was quantified by using a Xenogen IVIS® Spectrum to measure the photons emitted per cell.

Orthotopic xenograft model *in vivo* bioluminescence Imaging

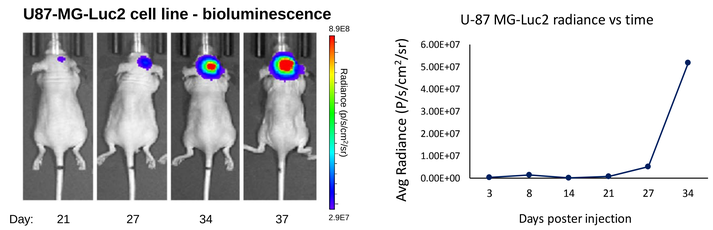

Figure 5. *In vivo* detection of luciferase activity of U-87 MG-Luc2. U-87 MG-Luc2 cells (4x10^5) were subjected to intracranial injection into the brain of nude mice. Tumor growth was monitored weekly using a Xenogen IVIS® Spectrum. Bioluminescence *in vivo* imaging demonstrated the progression of tumors.

Conclusion

- Animal models play an important role in biological mechanism studies and drug development.
- ATCC® luciferase-expressing cell lines were derived from commonly used tumorigenic human or mouse tumor cell lines and were developed by single cell cloning.
- The cell lines were extensively validated for cell growth and stable expression of high bioluminescent signals both *in vitro and in vivo.*
- These murine pathogen-free and fully authenticated reporter cell lines provide new versatile tools for *in vitro* luminescent assays and *in vivo* live animal bioluminescent imaging.

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