GFP and luciferase dual reporter cell lines for non-invasive in vivo fluorescence and bioluminescence imaging in mouse tumor xenograft and syngeneic models

Poster 77

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Abstract

Background: Whole animal in vivo optical imaging is widely used for the ease of operation in visualizing in vivo biological events, eliminating the requirement for animal subject sacrifice, allowing for continuous monitoring/imaging of a single individual animal, and reducing the amount of interanimal variation. Although xenograft and syngeneic models are both useful in vivo models for studying tumor formation, development, and metastases and measuring tumor burden in whole animals, syngeneic models are particularly valuable for studying the interplay between tumor cells and host immune system and monitoring responses to immunotherapy. Here, we report on the generation of dual reporter syngeneic cell lines that stably express GFP and luciferase with broad applications for in vitro and in vivo cancer immunology studies. These GFP and luciferase dual reporters provide a relatively simple, robust, and highly sensitive means to measure biological processes and to assess therapeutic efficacy in animal models through non-invasive in vivo fluorescence and bioluminescence imaging.
Methods: These dual reporter cell lines were derived from mouse breast and colon cancer cell lines. After the introduction of Lenti-GFP-LUC2 dual reporter into the parental cell lines and antibiotic selection, single cell cloning was performed to isolate stable clones with high GFP and luciferase expression. The isolated clones were characterized by cell morphology, growth kinetics, and stable expression of GFP and luciferase. The established cell lines were tested for their tumorigenicity in immunodeficient mice and subsequent whole-body in vivo bioluminescent and fluorescent imaging were performed by Xenogen IVIS imaging system. At the endpoint, the tumors were excised for additional ex vivo bioluminescent and fluorescent imaging.
Results: We confirmed high level of GFP and luciferase expression in selected clones via fluorescence and luminescence imaging and flow cytometric analysis. The fluorescence and bioluminescence intensity showed positive linear correlation with the cell numbers. The GFP and luciferase expression remained high after 30 population doublings. The morphology and the growth rate were comparable to the parental cell lines. Fluorescence and bioluminescence imaging of mouse xenograft models displayed positive correlation of fluorescence or bioluminescence intensity to tumor size. Ex vivo imaging of tumors also showed high intensity GFP and bioluminescence.
Conclusions: In vivo bioluminescence and fluorescence imaging provide complementary non-invasive approaches for real-time monitoring and studying of immune responses and tumor progression in preclinical models. The newly developed dual reporter syngeneic cell lines offer a powerful imaging tool for studying multiple aspects of complex cellular interactions during preclinical investigation and facilitating development of more effective immunotherapeutic strategies.

Results

Generation of GFP- and luciferase-expressing dual reporter cell lines for advanced imaging

Table 1: GFP- and luciferase-expressing dual reporter cell lines.

Parental cell line	Dual Reporter cell line	Dual Reporter ATCC® No.	Species	Tissue/ Disease
4T1	4T1-GFP-Luc2	CRL-2539-GFP-LUC2™	Mouse	Breast Cancer
CT26.WT	CT26.WT-GFP-Luc2	CRL-2638-GFP-LUC2™	Mouse	Colon Cancer

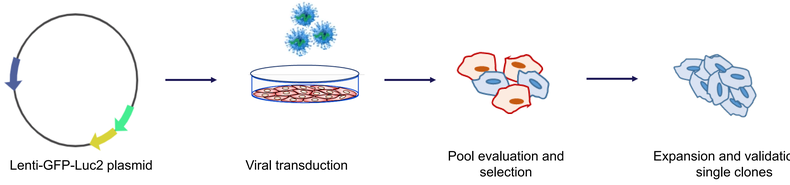
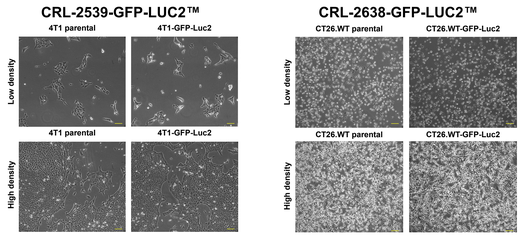


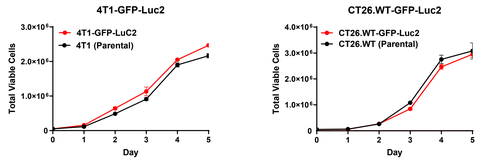
Figure 1: Scheme of developing a stable cell line containing the Luc2 gene. Selected mouse cell lines were transduced with lentiviral-GFP-Luc2 plasmids in the presence of 50 µg/mL protamine sulfate (Millipore Sigma) for 24 hours. The cells were then enriched by puromycin selection and single cells were isolated by automatic cell sorting (SH800, Sony). Expanded single cell clones were evaluated for luciferase and GFP expression. The clones that yielded the highest GFP and luciferase signal were selected for future experiments.

Characterization of GFP- and luciferase-expressing dual reporters in vitro

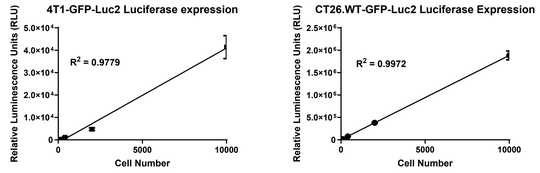
A. Morphology of mouse dual reporter cell lines is similar to the parental cell line



B. Growth Curves of mouse dual reporter cell lines are similar to the parental cell line



C. Luciferase activity shows a linear correlation between bioluminescence intensity and cell number



D. Over 95% of cells are GFP-positive

E. GFP imaging in GFP-Luc2 dual reporters

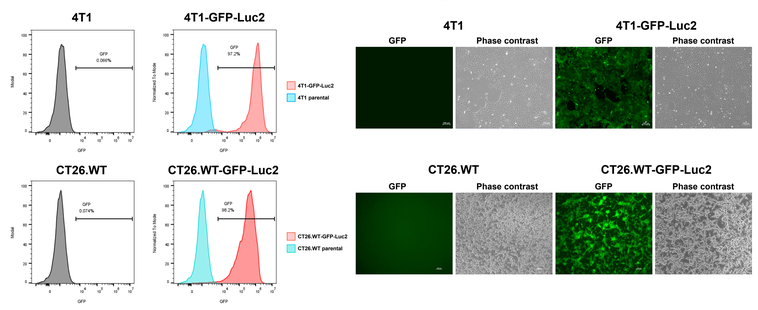
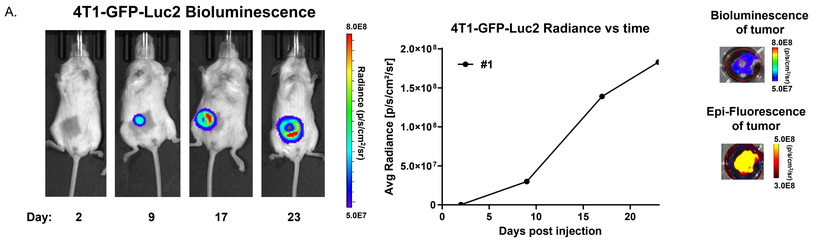


Figure 2: Characterization of GFP- and luciferase-expressing cell lines in vitro. (A) Cell morphology of the 4T1, 4T1-GFP-Luc2, CT26.WT, and CT26.WT-GFP-Luc2 cell lines was observed under microscopy and images were captured via digital camera (scale bar = 100 µm). (B) Cell growth kinetics were captured by Cytation 1 (Agilent) after seeding cells in 96-well plates at 50,000 cells per well. The cell growths were monitored for 7 days. (C) Luciferase expression was quantified by using Bright-Glo Luciferase Assay System (Promega). Luminescence intensity was measured by SpectraMax i3x (Molecular Devices). Data showed a linear correlation between bioluminescence intensity and cell number. (D) GFP expression was measured by flow cytometry (CytoFLEX, Beckman Coulter) and (E) captured via digital camera (Zyla, Andor).

Xenograft model in vivo bioluminescence and epi-fluorescence imaging

A. 4T1-GFP-Luc2 Bioluminescence



B. CT26.WT-GFP-Luc2 Bioluminescence

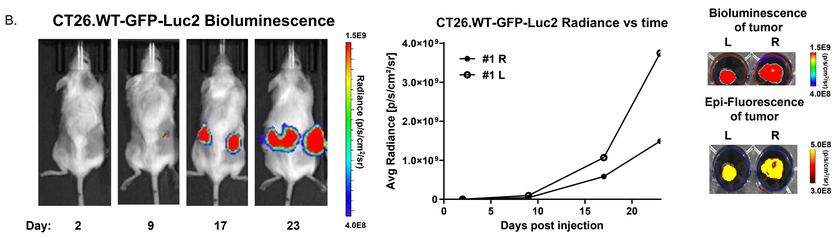


Figure 3: In vivo detection of luciferase activity of 4T1-GFP-Luc2 and CT26.WT-GFP-Luc2. (A) 4T1-GFP-Luc2 cells (5.0 x 10^5) were subjected to subcutaneous injection into the mammary fat pad of NSG mice. (B) CT26.WT-GFP-Luc2 cells (5.0 x 10^5) were subjected to subcutaneous injection into the right and left flank of NSG mice. Tumor growth was monitored weekly using a Xenogen IVIS Spectrum. On day 23, tumors were excised and imaged for the bioluminescence and epi-fluorescence.

Syngeneic model in vivo bioluminescence and epi-fluorescence imaging

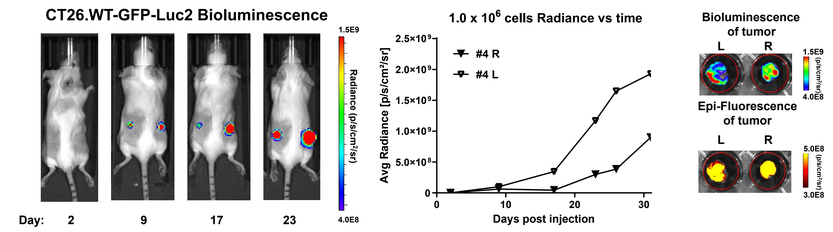


Figure 4. In vivo detection of luciferase activity of CT26.WT-GFP-Luc2. CT26.WT-GFP-Luc2 cells (1.0 x 10^6) were subjected to subcutaneous injection into the right and left flank of Balb/c mice. Tumor growth was monitored weekly using a Xenogen IVIS Spectrum. On day 36, tumors were excised and imaged for the bioluminescence and epi-fluorescence.

Conclusion

- Bioluminescence and fluorescence imaging in vivo provide complementary approaches for tracking and investigating immunotherapy in preclinical models.
- By enabling non-invasive, real-time tracking of immune cell activity and tumor behavior, these methods enhance our understanding of cancer immunology and support the development of more effective treatments.
- The newly developed dual reporter cell lines express both luciferase and GFP, offering a versatile platform for advanced imaging for examining diverse and complex cellular interactions during preclinical studies.

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