



**FIGURE 1.** Imaging of protein interactions in living animals. (A) Diagrammatic cross section of a black box cooled CCD imaging apparatus (Xenogen IVIS) that can be used to measure bioluminescent and fluorescent signals from both live cultured cells and cells within living animals. The culture plates, or animals, can be placed on the height-adjustable stage in the temperature-controlled chamber and the light emitted from the source can be captured, using the highly sensitive cooled CCD camera. The stage can be moved up or down, depending on the area required for scanning. The 6-position filter wheel is optional and can be used for imaging any specific wavelength of light by installing filter sets. The operation of this camera box is completely automated, controlled by Living Image software. (B) There are, fundamentally, two different types of optically based imaging systems: fluorescence imaging, which uses emitters such as GFP, wavelength-shifted GFP mutants, RFP, “smart” probes, and NIRF probes, and bioluminescence imaging, which utilizes systems such as firefly luciferase/D-luciferin or *Renilla* luciferase/coelenterazine. Emission of light from fluorescent markers requires external light excitation, whereas bioluminescent systems generate light de novo, when the appropriate substrates/cofactors are made available. In both cases, light emitted from either system can be detected with a thermoelectrically cooled CCD camera, since the light emitted is in the visible (400–700 nm) to near-infrared (~800 nm) range. Top center is a representative image obtained from a glioma tumor model, which expresses RFP. Top right is a typical image obtained with this bioluminescence technology (reprinted from Bhaumik and Gambhir 2002). This image was obtained after i.v. injection of coelenterazine into a mouse containing intraperitoneal hRluc-expressing tumor cells. Significant bioluminescence is detected from the region of the xenograft.

*Protein–Protein Interactions: A Molecular Cloning Manual*, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 35, Figure 1.