What is FRET?

Fluorescence resonance energy transfer (FRET) is a process involving the radiationless transfer of energy from a "donor" fluorophore to an appropriately positioned "acceptor" fluorophore. The distance over which FRET can occur is limited to between 1-10 nm, and hence this technique is used to demonstrate whether two types of molecules, labelled with a donor-fluorophore and a receptor fluorophore, occur within 10nm of each other. Measuring FRET by confocal imaging enables the intracellular locations of the molecular interaction to be determined. This is far more powerful than demonstrating "colocalisation" which is limited by the resolution of a light microscope, and at best can show that two species of fluorophore-labelled molecules co-exist within a volume of 200x200x400nm.

FRET can occur when the emission spectrum of a donor fluorophore significantly overlaps (>30%) the absorption spectrum of an acceptor. The combination of CFP and YFP labelled fusion proteins has been widely used for FRET measurements in living cells. Other donor and acceptor fluorophore pairs which have been used for FRET include CFP and dsRED, BFP and GFP, GFP or YFP and dsRED, Cy3 and Cy5
(see below), Alexa488 and Alexa555, Alexa488 and Cy3, FITC and Rhodamine (TRITC), YFP and TRITC or Cy3. Positive controls of labelled proteins which are known to interact (and therefore should show FRET) and negative controls (eg only the acceptor or the donor fluorophore is present, respectively) are required.

Dr Koenig has recently developed and published a technique for performing FRET on fixed specimens by immunofluorescence (Lab Invest 2006 pp1-12), I have attached this paper in case anyone would like to prepare samples using this technique, for example with Cy3- and Cy5- conjugated antibodies. Again, positive and negative controls are needed. In this scenario, a positive control
for FRET could be the use of a primary rabbit antibody detected with two anti-rabbit IgG secondardy antibodies, one conjugated to Cy3 and the other conjugated to Cy5 respectively. Negative FRET controls would need to include a sample with the primary antibody and the Cy3-conjugated secondary (no Cy5 present), and a sample with the primary antibody and the Cy5-conjugated secondary (no Cy3 present).