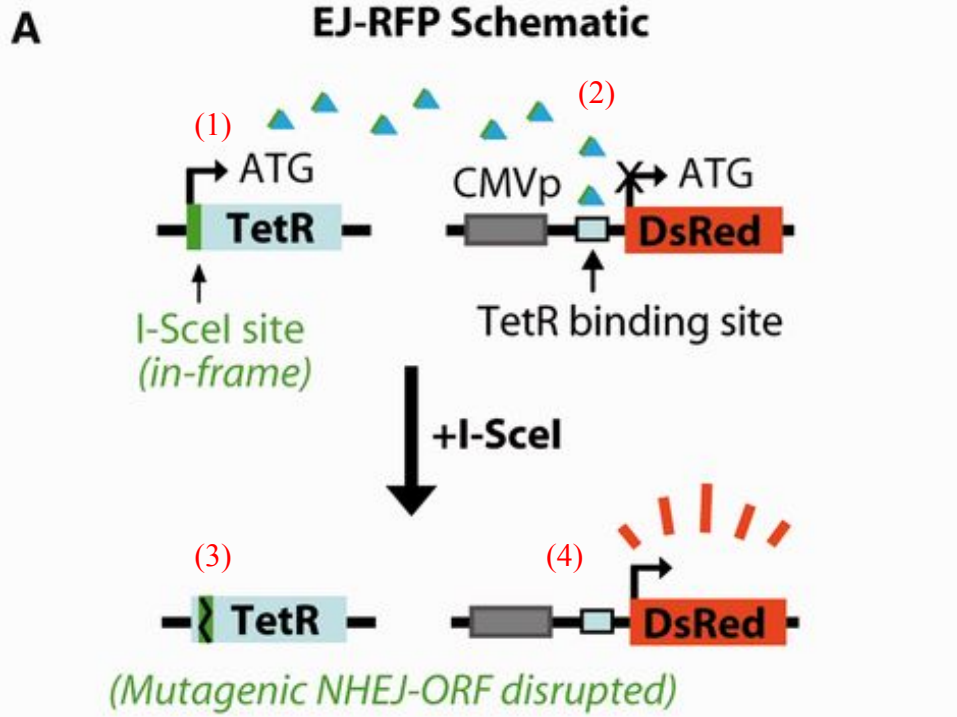


Modified Fig 1 from Bindra et al. *Nuc Acids Res* 2013, Vol. 41:11

5′...TAGGGATAACAGGGTAAT...3′
3′...ATCCCTATTGTCCCATTA...5′

FYI: this is the I-SceI recognition site. Why do you think this site, in particular, is useful for creating sensors?

Description of Goglia et al. mutagenic NHEJ reporter. Two plasmids are linearized and transfected into mammalian cells. By linearizing (cutting) the plasmid one increases the chances of the plasmid DNA integrating into the chromosome of the host cell. This creates a "stable" system that can be used for many cell passages.

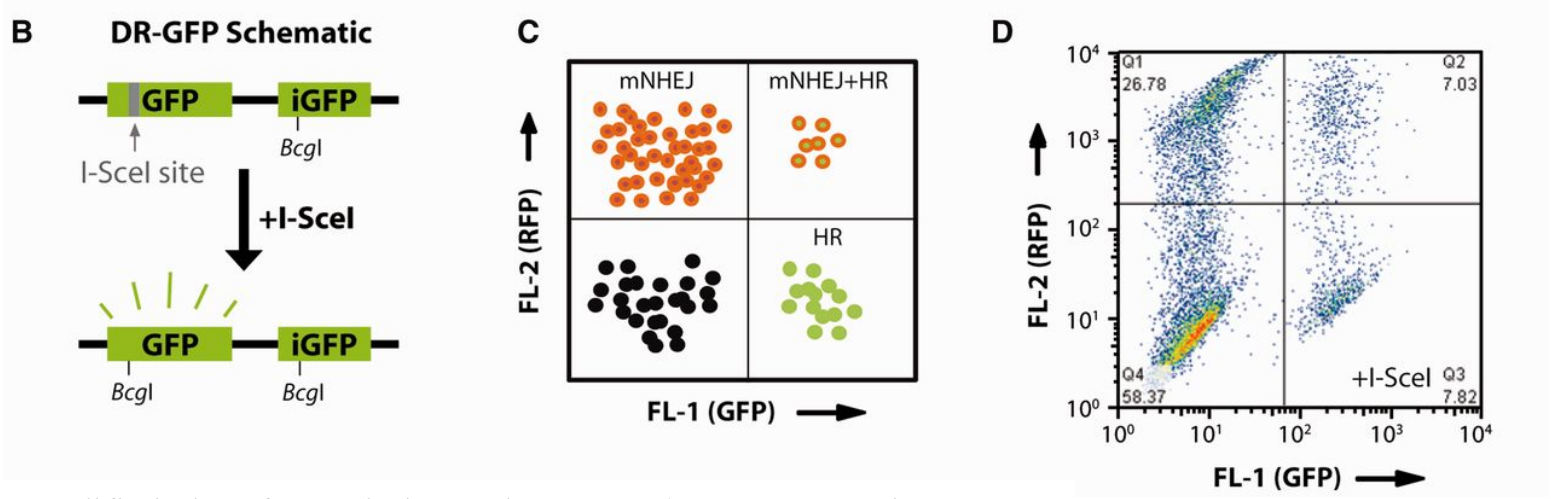
(1) The first plasmid encodes a Sce-TetR transgene that contains an I-SceI restriction enzyme recognition site just after the start codon (ATG) in frame with the gene that produces the protein TetR. When the restriction enzyme site is intact (uncut), the TetR protein is produced (little blue triangles).

(2) The TetR protein binds to the tet operator (tetO) and suppresses gene expression. Therefore, when TetR is present there is no DsRed (aka RFP) expression.

(3) However, when I-SceI is present, a double strand break is introduced and TetR gene transcription is interrupted. If the double strand break is repaired "perfectly" (no mutations or errors are made), TetR can turn back on and the situation shown in (1) occurs. If the DSB is repaired by an error-prone process (here called "Mutagenic NHEJ") no TetR is made due to a frame shift in the gene.

(4) If no TetR protein is produced, tetO cannot suppress gene expression and the cell fluoresces red.

Therefore, the Goglia et al. NHEJ sensor measures only repair that is error-prone (or mutagenic). Think about how this is different from the sensor that we are using in class.



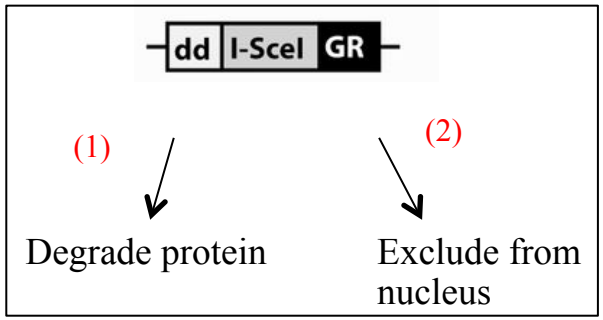
Modified Fig 2 from Bindra et al. *Nuc Acids Res* 2013, Vol. 41:11

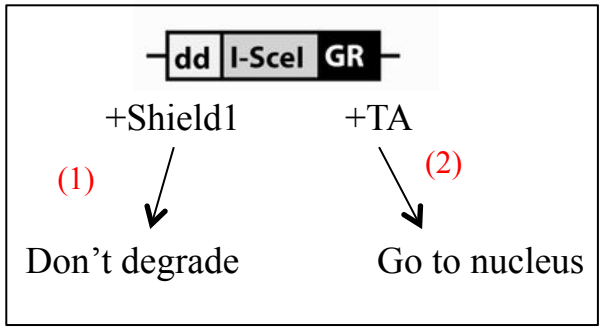
Description of Goglia et al. HR reporter – the Direct Repeat GFP (DR-GFP). One plasmid is linearized and transfected into mammalian cells to create a stable sensor.

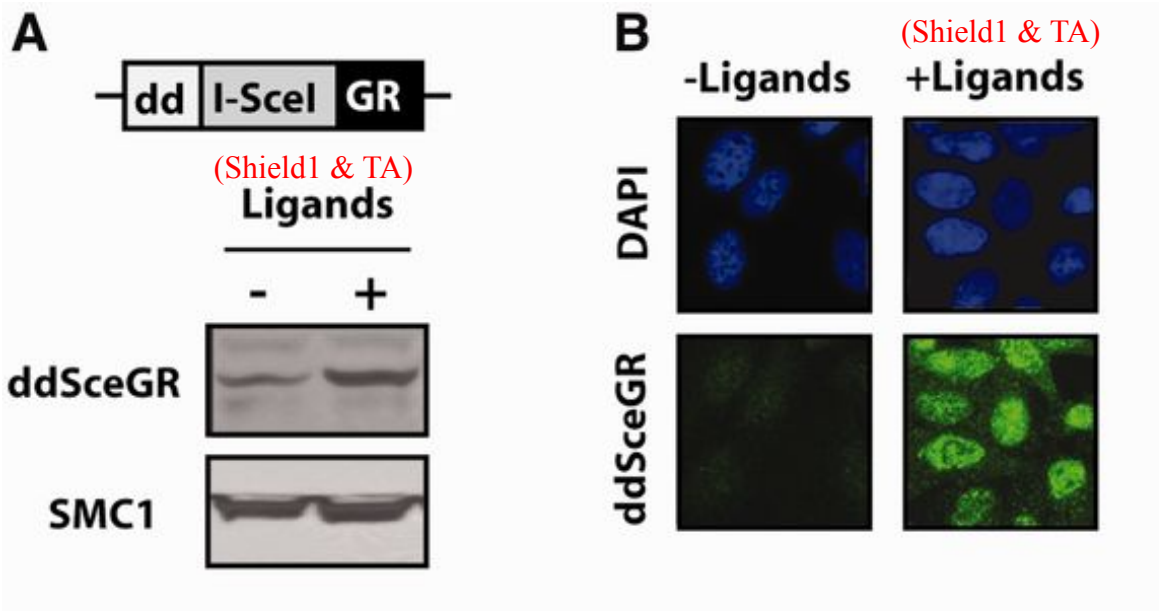
(B) The plasmid encodes a full length GFP gene with a I-SceI recognition site inserted near the 5' end. A bit downstream of the GFP is another mini-GFP that contains homologous sequence to that surrounding the I-SceI site in the first GFP. When I-SceI is added, a double strand break is produced in the full-length GFP. The break is repaired using the homologous sequence that is downstream using the process of **homologous recombination**.

(C) When both reporters (EJ-RFP and DR-GFP) are stably expressed in cells and I-SceI is present many double strand breaks are created. Just like we did, the authors used flow cytometry to determine which repair pathways were active. This cartoon flow cytometry plot illustrates what data looks like when only (mutagenic)NHEJ, mNHEJ + HR, or only HR occur.

(D) Think about what is happening in the cells that appear in each one of the quandrants – including the lower left one.







Modified Fig 3 from Bindra et al. *Nuc Acids Res* 2013, Vol. 41:11

Inducible control of double strand breaks. The final aspect of the Goglia et al system addresses the way that DSB are introduced in in the DR-GFP and EJ-RFP systems. As you might imagine, you can't just add restriction enzymes to cell culture media – how would they get inside the cell? There are a couple choices, the most straightforward is to introduce the DNA that encodes the restriction enzyme using a plasmid (again, similar to what you have done). But that doesn't offer much control over when, where, and how much damage is done.

To overcome this, Bindra et al. designed a plasmid that contained the gene for I-SceI and flanked it with two control systems.

(1) First, they added a destabilizing domain (dd) on the 5' end of the gene. When this the dd is transcribed the I-SceI protein is tagged by ubiquitin and degraded. If you add a small chemical ligand called Shield1 it blocks the dd amino acid sequence and prevents the protein from being degraded.

(2) Second, they added a portion of the rat glucocorticoid receptor (GR) to the 3' of the I-SceI gene. When the GR binds to another ligand, triamcinolone acetonide (TA), it translocates the I-SceI to the nucleus so that the restriction enzyme can introduce DSB into the DR-GFP and EJ_RFP reporters.