I-Sce1 / DR-GFP repair assay

Background:
This assay is used to test the influence of siRNAs/shRNAs/inhibitors/overexpression on DNA double strand break repair through homologous recombination. The pDR-GFP HR reporter system was originally described by the lab of Maria Jasin (Pierce et al).

Cluture monoclonal HeLa-pDR-GFP or MCF—pDR-GFP cells. Culture medium for HeLa cells is Hams F-12:DMEM (1:1), 10% FCS, pen/strep + 1ug/ml puromycin. Culture medium for MCF-7 cells is RPMI, 10% FCS, pen/strep + 1ug/ml puromycin.

I-Sce1 +/- inhibitors (As a positive control we use the CDK inhibitor Roscovitine (20mM).

- Day 1: culture 300,000 cells in 6-well dishes (results in approximately 40% confluency)
- Day 2: transfect cells with I-Sce (1ugr per well) using X-tremegene/Fugene

  Depending on the transfection reagent: replace medium for DMEM without antibiotics 1 hour before transfection

  transfection (per well):
  - 100 ul optimum
  - 1 ul I-Sce1 (1ug/ul maxiprep)
  - 3ul X-tremegene/Fugene transfection reagent

- Day 2: add inhibitors at 2 hours after transfection
- Day 4: trypsonize cells, and determine GFP-content in live cells using flow cytometry

I-Sce1 +/- inhibitors (As a positive control we use BRC2 siRNA)

- Day 1: culture 200,000 cells in 6-well dishes (results in approximately 40% confluency)
- Day 2: transfect cells with siRNA (30 nM) using oligofectamine according to manufacturer’s protocol. Depending on the transfection reagent: replace medium for DMEM without antibiotics 1 hour before transfection
- Day 3: transfect cell with I-Sce1 (1ugr per well) using X-tremegene/Fugene.
transfection (per well):
- 100 ul optimum
- 1 ul I-Sce1 (1ug/ul maxiprep)
- 3ul X-tremegene/Fugene transfection reagent

- Day 5: trypsonize cells, and determine GFP-content in live cells using flow cytometry