

# Assessment of DNA Double Strand Break Repair Activity Using High-throughput and Quantitative Luminescence-based Reporter Assays

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## Citation

Grande, D., Rajendra, E., Mason, B., Galbiati, A., Boulton, S.J., Smith, G.C.M., Robinson, H.M.R. Assessment of DNA Double Strand Break Repair Activity Using High-throughput and Quantitative Luminescence-based Reporter Assays. *J. Vis. Exp.* (208), e66969, doi:10.3791/66969 (2024).

## Date Published

June 14, 2024

## DOI

10.3791/66969

## URL

jove.com/video/66969

## Materials

| Name  | Company            | Catalog Number | Comments   |
|---|--------------------|----------------|--|
| 10x TBE buffer  | Thermo Fisher      | AM9863         |  |
| 20% TBE-acrylamide gel                                | Invitrogen         | EC6315BOX      |  |
| 50x TAE buffer  | Fisher Scientific  | BP13321        |  |
| 6x Gel Loading Dye, Purple                            | NEB                | B7024S         |  |
| 96-well white plate (with transparent bottom and lid) | Porvair            | 204012         |  |
| Agarose   | Cleaver Scientific | CSL-AG500      |  |
| AMPure XP beads                                       | Beckman Coulter    | A63881         | For bead-based purification of DNA                     |
| Antarctic phosphatase and 10X buffer                  | NEB                | M0289L         |  |
| CLARIOstar  | BMG Labtech        | 430-101        | Plate reader   |
| Countess II Automated Cell Counter                    | Thermo Fisher      | AMQAX1000      | Cell counter   |
| Custom oligonucleotides                               | Sigma-Aldrich      | Custom order   | For resection-independent MMEJ substrate. See Table 2. |
| D300e   | Tecan              | 30100152       | DMSO-based compound dispenser                          |
| D300e D4+ cassette                                    | Tecan              | 30097371       | High volume cassette for D300e compound dispenser      |
| D300e T8+ cassette                                    | Tecan              | 30097370       | Low volume cassette for D300e compound dispenser       |
| Dimethyl sulfoxide, cell culture grade                | Sigma-Aldrich      | D2650-100ML    | Vehicle for compounds, used in Figure 4 and Figure 5   |
| DSBR reporter source plasmids                         | Artios             |                | Available to the academic community upon request       |
| EcoRV-HF and 10x CutSmart buffer                      | NEB                | R3195M         |  |
| Ethanol absolute                                      | VWR                | 20821.365      |  |

|   |                   |                       |  |
|---|-------------------|-----------------------|--|
| Foetal Bovine Serum                                   | PAN-Biotech       | P30-3031              |  |
| GeneRuler Ultra Low Range DNA Ladder                  | Thermo Fisher     | SM1211                | Low molecular weight DNA ladder  |
| HEK-293 cells   | ATCC              | CRL-1573              | Example cell line used in protocol   |
| HindIII-HF and 10x CutSmart buffer                    | NEB               | R3104M                |  |
| HyClone Molecular Biology grade water                 | Fisher Scientific | 10275262              |  |
| I-SceI and 10x Tango Buffer                           | Invitrogen        | ER1771                |  |
| Isopropanol   | VWR               | 20842.33              |  |
| JetPRIME reagent and buffer                           | PolyPlus          | 114-15                | Lipid-based transfection reagent   |
| MegaStar 1.6  | VWR               | 521-1749              | Centrifuge for 15 or 50 mL tubes   |
| MEM Eagle   | PAN-Biotech       | P04-08056             | Culture medium for HEK-293 cells   |
| Microplate shaker                                     | Fisherbrand       | 15504070              | Microplate orbital shaker  |
| MicroStar 17R   | VWR               | 521-1647              | Centrifuge for 1.5 or 2 mL tubes   |
| MultiDrop   | Thermo Fisher     | 5840300               | Cell or water-based reagent dispenser  |
| MultiDrop Standard Cassette                           | Thermo Fisher     | 24072670              | Cassette for MultiDrop reagent dispenser   |
| NanoDLR Stop & Glo Buffer and Substrate               | Promega           | N1630 or N1650        | Reporter luciferase (NanoLuc) reagent to quench Firefly luminescence and detect NanoLuc luminescence. Part of the Nano-Glo Dual-Luciferase Reporter Assay System kit |
| ONE-Glo EX Luciferase Assay Buffer and Substrate      | Promega           | N1630 or N1650        | Control luciferase (Firefly) assay reagent to detect Firefly. Part of the Nano-Glo Dual-Luciferase Reporter Assay System kit   |
| PBS (without calcium or magnesium)                    | PAN-Biotech       | P04-36500             |  |
| pGL4 Firefly plasmid (or similar)                     | Promega           | E1310 (or equivalent) | Firefly control plasmid  |
| pNL1.1 NanoLuc plasmid (or similar)                   | Promega           | N1091 (or equivalent) | NanoLuc control plasmid, for adjustment of equipment settings/ optimisation experiments  |
| QIAquick Gel Extraction Kit                           | Qiagen            | 28706                 |  |
| Quick-Load Purple 1 kb DNA Ladder                     | NEB               | N0552S                | High molecular weight DNA ladder   |
| Sodium Acetate (3 M), pH 5.5                          | Thermo Fisher     | AM9740                | For pH adjustment during gel extraction with QIAquick Gel Extraction Kit   |
| SYBR Gold (10,000x)                                   | Invitrogen        | S11494                | For visualisation of ssDNA and dsDNA   |
| SYBR Safe (10,000x)                                   | Invitrogen        | S33102                | For visualisation of dsDNA only  |
| T4 DNA Ligase and 10x Ligase buffer                   | NEB               | M0202L                |  |
| Trypan Blue stain 0.4%                                | Invitrogen        | T10282                | For measurement of viability during cell counting  |
| Trypsin-EDTA solution; 0.25% Trypsin and 0.53 mM EDTA | Sigma-Aldrich     | T4049-100ML           |  |
| XhoI and 10x CutSmart buffer                          | NEB               | R0146M                |  |