Variant functionalization by localization

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Cell culture and transfection

1 Clean, sterilize and dry coverslips. You may need to treat the coverslips with poly-L-lysine to encourage cell adhesion. Place the coverslips in the appropriate plasticware.

For example, we used 22x22mm glass coverslips (Fisher) in 6-well plates (Corning).

2 Grow cells to sub confluent.

For example, using MCF10A cells, seed cells at $2x10^5$ cells per well in a 6-well plate 24h before transfection and incubate overnight.

3 Transfect cells according to manufacturer's recommendations.

For example, we used lipofectamine 2000.

4 The next day, gently wash the coverslips with PBS. Verify transfection efficiency by checking under a fluorescent microscope. If the transfection efficiency is satisfactory (i.e. > 10%), continue.

Immunofluorescence 1d 0h 45m

5 This section shows the steps of a typical immunofluorescence experiment. It generally involves fix -> permeabilize -> block -> primary antibody -> secondary antibody ->mount. All steps are at room temperature unless stated otherwise. 10m

5.1 Wash the cells 3x with PBS. Fix cells by adding 4% PFA (in PBS) and incubate for 10 min. 10m
Room temperature

5.2 Aspirate the PFA and wash 3x with PBS. Permeabilize cells by adding 0.1% Triton X-100 (in PBS) and incubate for 5 min. 5m **Room temperature**

5.3 Aspirate the Triton X-100 and wash 3x with PBS. Block by using the appropriate blocking solution e.g. 10% goat serum or BSA (in PBS). Incubate for 10 min. 10m **Room temperature**

5.4 Aspirate the blocking solution and wash 3x with PBS. Add primary antibody (in blocking solution). In our experiments, we used rabbit anti-PTEN antibody (138G6, Cell Signaling Technology) at 1:100 in blocking solution. Incu **4 °C** bate overnight. 1d

5.5 Wash the coverslips 3x with PBS. Add secondary antibody (in blocking solution). In our experiments, we used mouse anti-rabbit Alexa Fluor 568-conjugated antibody (Invitrogen) at 1:100 in blocking solution. Incubate for 1 hr. **Room temperature**

5.6 Wash the coverslips 3x with PBS. Incubate the coverslips with DAPI (in PBS) if you want to stain the nuclei for 5 min then wash with PBS. 5m

5.7 Mount the coverslips onto glass microscopy slides by using your preferred mounting media. We used ProLong Gold antifade mountant (Thermo Fisher Scientific). 30m

5.8 After waiting ~30min or more, seal the slides with clear nail polish. 5m

Microscopy

6 Acquire images with your favorite microscopy setup. An automated microscope is not required to proceed to image analysis using our MAPS software, but will certainly accelerate image acquisition. In our experiments, we used a Cellomics Arrayscan (Cellomics Inc.). using a 20x objective. 500 images were acquired per coverslip at 3 channels (green/ red/ blue) per image.

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