CherryPicker™ Assay Kit Protocol-At-A-Glance (PT5164-2)

Use the CherryPicker Assay Kit (Cat. Nos. 632570 and 632571) to capture and isolate cells expressing the CherryPicker fluorescent protein.

I. Additional Materials Required

- Low-retention microcentrifuge tubes, 1.5 ml (e.g., Sarstedt, Cat. No. 72690)
- Cells
- Cell culture medium
- Hemocytometer
- G418
- Cell dissociation buffer (tissue-culture-grade for attached cells; e.g., Sigma, Cat. No. C5914)
- PBS without Mg^{2+} or Ca^{2+}
- Cell strainer (e.g., BD Falcon, 70 µm; from BD Biosciences, Cat. No. 352350)
- Magnetic Stand (Cat. No. 631964).

II. Protocol

1. Transfect or transduce your cells with the CherryPicker construct of your choice. *Transfected* cells should be collected 24−36 hr post-transfection to allow an appropriate amount of transient CherryPicker expression, which can be checked by fluorescence microscopy or flow cytometry.
2. Collect the transfected/transduced cells. For *attached* cells, perform steps a−i, below; for cells *in suspension*, perform steps g−i.

   NOTE: If you plan to re-culture the cells after capture, ensure that all materials (i.e., buffers, H2O, plastic wear, etc.) are sterile.

   a. Aspirate the medium from the culture plate(s).
   b. Rinse the plate(s) gently with warm, tissue-culture-grade PBS free of Mg^{2+} and Ca^{2+}, then aspirate off the PBS.
   c. Pre-warm the cell dissociation buffer to 37°C and add an appropriate amount to each plate; gently swirl the plate to ensure all of the cells are covered. **Do not use trypsin**—it might cleave the extracellular portion of the CherryPicker protein.
   d. Aspirate off most of the cell dissociation buffer, leaving a thin layer on top of the cells.
   e. Place the plate(s) back into the incubator for 5−10 min.
   f. Take the plates out of the incubator and add enough medium to resuspend the cells; resuspend the cells well.
   g. Centrifuge the cells at 1,000−1,200 rpm for 5 min at 4°C.
   h. Aspirate off the medium and resuspend the cells in 5–10 ml of cold PBS.
   i. Run the cells through a cell strainer, and count using a hemocytometer.
3. For each sample, aliquot between 0.1×10^6 – 1×10^6 cells into a sterile, 1.5 ml, low-retention microcentrifuge tube (we use 0.5×10^6 cells per sample). After aliquoting the cells, if the volume in each tube is less than 1 ml, bring the volume of each up to 1 ml with PBS.

   NOTE: Prepare enough samples to allow for at least two negative controls: a no-antibody control and a no-bead control.
4. Place the samples on ice and add 10 µl of 0.5 mg/ml CherryPicker Antibody to each tube (except the 'no antibody' control). Mix gently by inverting each tube and incubate on ice for 30 min. During this 30 min incubation, mix the cells gently by inverting each tube every 10 minutes.

   NOTE: The amount of antibody recommended is sufficient for the capture of 5×10^5 cells. If your samples contain more cells, adjust the amount of antibody accordingly.
5. While the cells are incubating on ice, calculate the amount of the Mag Capture Bead stock you'll need to use in your experiment: Use 40 µl of the Mag Capture Bead stock per 5×10^5 cells in your sample.

EXAMPLE: If you have 5 samples with 0.5 x 10^6 cells per sample, you will need to use 200 µl of the Mag Capture Bead stock in Step 6.

6. Transfer the required amount of Mag Capture Bead stock (calculated in Step 5) into a sterile, 1.5 ml, low-retention microcentrifuge tube and wash the beads twice with 1 ml of 1X Wash Buffer. After each wash, place the microcentrifuge tube on a Magnetic Stand and allow the magnet to pull the magnetic beads to the wall of the tube before removing the wash buffer.
7. After the second wash, prepare the 'washed bead suspension' (to be used in Step 10) by resuspending the Mag Capture Beads in 1X Wash Buffer. To determine the volume of 1X Wash Buffer you will need in this step, multiply the number of your samples by 100. This will allow you to add 100 µl of the washed bead suspension to each sample in Step 10.
8. After the cells have completed their 30 min incubation with the antibody (Step 4, above), centrifuge the cells at 500 x g for 4 min at 4°C.
9. Remove the supernatant from each sample and wash the cells twice with 1ml cold PBS. After the second wash, gently resuspend each cell pellet; use1 ml cold 1X Wash Buffer for every 5x10^5 cells in the sample.
10. Add 100 µl of the washed bead suspension (Step 7, above) to each sample.
11. Place the tubes on a shaker or rotator (at slow speed) at room temperature for 30 min.
12. Place the tubes on the Magnetic Stand. Wait until the beads have been pulled to the wall of the tube, then **remove and save the supernatant**—it contains non-captured cells that you may want to analyze later. Wash the beads once with cold 1X Wash Buffer by gently pipetting up and down 5 times with a P1000 pipette.
13. Place the tubes on the Magnetic Stand. Wait until the beads have been pulled to the wall of the tube and then remove the supernatant.

    At this point, the cells captured on the beads can either be analyzed (e.g., by Western analysis, PCR, qPCR, etc.), or transferred into one well of a 6-well tissue culture dish and re-cultured in 2 ml of tissue culture medium. Magnetic beads in the culture can be removed the next time cells are fed or split: for attached cells, the beads will simply be aspirated off with the medium; for cells in suspension, the beads can be removed from the culture with a magnet.

NOTE: If you are going to determine the capture efficiency by flow cytometry, set the flow cytometer to collect 60 sec of data. This will insure that all of the data will be collected for a set time and volume, allowing you to determine the percentage of captured cells expressing CherryPicker. Also, make sure that the samples are all collected at the same flow rate (i.e., low, medium, or high). Analyze the samples by either creating a histogram with markers M1/M2, or drawing regions on an FL1/FL2 plot.

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