Title: Transfection of CHO-K cells with pEGFP-Actin and pDsRed2-Mito

Approvals:

Preparer: William H Woodruff Date 14 May14
Reviewer: Date
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1. Purpose: To transfect CHO-K cells with non-viral plasmids carrying EGFP protein with an affinity to actin and DsRed2 protein with an affinity to mitochondria

2. Scope: Transfecting CHO-K cells with GFP to see actin, and RFP to see mitochondria.

3. Responsibilities:
   - 3.1. It is the responsibility of the supervisor to ensure that this SOP is performed as directed and to update the procedure when necessary.
   - 3.2. It is the responsibility of the technician to follow the SOP as described and to inform the supervisor about any deviations or problems that may occur while performing the procedure.

4. References:
   - 4.1. Basics of Cell Culture Manual, Afshar, Golnar, PhD (pages 44-47)

5. Definitions:
   - 5.1. Transfection: to transfer non-viral recombinant DNA into mammalian cells.
     - 5.1.1. Transient transfection: foreign DNA molecule stays independent of the host genome and is eventually lost during subsequent cell division.
     - 5.1.2. Stable transfection: foreign DNA molecule inserts into the host cell genome and is passed to subsequent generations
   - 5.2. pDsRed2: plasmid vector containing the gene for red fluorescent protein conjoined to a peptide molecule with an affinity to mitochondria (a subunit of cytochrome C), resulting in tagging mitochondria with florescent red for viewing purposes
   - 5.3. pEGFP: plasmid vector containing the gene for green fluorescent protein conjoined to actin, resulting in tagging cytoskeleton actin with fluorescent green for viewing purposes

6. Precautions:
   - 6.1. None specific to this protocol

7. Materials:
   - 7.1. 24 well plate
   - 7.2. 70-80% Confluent CHO-K cells

7.3. Laminar Flow Hood
7.4. 70% Ethyl Alcohol
7.5. Pipettes (sterile 1 or 2 mL, 5 mL , 10 mL)
7.6. Pipette Aid
7.7. 10 – 100 ul micropipette
7.8. Sterile pipette tips
7.9. Growth Media (RPMI 1640 + 10% FBS)
7.10. pDsRed2 (1ug/ul)
7.11. pEGFP (1ug/ul)
7.12. Sterile Microcentrifuge tubes (sMCT)
7.13. Fugene HD
7.14. PBS
7.15. 4% paraformaldehyde (optional)
7.16. Waste container for paraformaldehyde
7.17. Fluorescent microscope with
    7.17.1. GFP Filter Set
    7.17.2. RFP Filter Set
    7.17.3. DAPI Filter Set

8. Procedure:

MONDAY

8.1. General: Preparing CHO-K cells for transfection
    8.1.1. Obtain sterile tissue culture grade 24 well plates
    8.1.2. Harvest 85%-95% confluent CHO-K cells from a healthy growing culture
    8.1.3. Count the harvested cells and dilute to an appropriate number to result in 70%-80% confluence on the day of transfection.
    8.1.4. Plate the CHO-K cells in 12 of the wells using 4 rows X 3 columns

TUESDAY

8.2. Prepare plasmids for use
    8.2.1. Obtain 4 sterile microcentrifuge tubes
    8.2.2. Label 1 microcentrifuge tube as the Control Tube
        8.2.2.1. Add 100ul of serum-free RPMI 1640 stock media
    8.2.3. Label 1 microcentrifuge tube as the pDsRed2 Mito Tube
        8.2.3.1. Add 98ul of serum-free RPMI 1640 stock media
        8.2.3.2. Add 2ul pDsRed2 (1ug/ul)
    8.2.4. Label 1 microcentrifuge tube as the Actin Tube
        8.2.4.1. Add 98ul of serum-free RPMI 1640 stock media
        8.2.4.2. Add 2ul of pEGFP (1ug/ul)
    8.2.5. Label 1 microcentrifuge tube as the Mixed Tube
        8.2.5.1. Add 98ul of serum-free RPMI 1640 stock media

8.2.5.2. Add 1ul of pDsRed2 (1ug/ul)
8.2.5.3. Add 1ul of pEGFP (1ug/ul)
8.2.6. Add 8ul of Fugene to each of the 4 solutions in the microcentrifuge tubes
8.2.7. Mix by vortex and allow all solutions to incubate at room temperature for 15 minutes.

8.3. While waiting change the media in the cell culture plate adding 1ml of fresh growth media to all 12 wells before continuing

8.4. Add 25ul of transfection mixture to each well drop by drop, following the table guide below.
8.4.1.

<table>
<tr><td>Control</td><td>O</td><td>O</td><td>O</td><td rowspan="4">X</td></tr>
<tr><td>Actin</td><td>O</td><td>O</td><td>O</td></tr>
<tr><td>Mito</td><td>O</td><td>O</td><td>O</td></tr>
<tr><td>Actin/Mito</td><td>O</td><td>O</td><td>O</td></tr>
</table>

8.4.2. Mix plate by moving it back and forth, left and right gently.

8.5. Place plate in 37^{o} C incubator for 48 hours

THURSDAY

8.6. Fixing Cells – after 48 hours incubation (optional for long term storage)
NB: If you do not fix the cells at least wash with PBS for a clearer fluorescence
8.6.1. Remove growth media with plasmids from all wells
8.6.2. Wash wells with 0.5ml of PBS, discard
8.6.3. Add 0.5ml of 4% paraformaldehyde per well
8.6.4. Wait 15 minutes
8.6.5. Transfer paraformaldehyde to a waste container
NOTE: (this solution is toxic and should **not** be discarded in the sink)
8.6.6. Wash wells with 0.5ml of PBS, discard
8.6.7. Place another 0.5ml of PBS in wells leaving this solution in the wells.

8.6.8. If desired, the plates may be stored for future viewing. Wrap plates in foil and keep them in the dark or place cells in a refrigerator for storage and future viewing.

8.7. Viewing cells

8.7.1. Place plate on the fluorescent microscope.

8.7.2. Set filters to view red fluorescence

8.7.3. Set filters to view green fluorescence

8.8. Record results

Control				X
Actin				
Mito				
Actin/Mito				