TOTO-1 nucleic acid labeling protocol

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Abstract

A modified TOTO-1 iodide labeling protocol to enable visualization of nucleic acid loading efficiency, as a means to verify that nucleic acid constructs are entering the cells. With additional cleanup steps to decrease background staining. A convenient assay to use in conjunction with transfection optimization. (Modified from Golzio, M., Teissie, J. & Rols, M.P. 2002. Direct visualization at the single-cell level of electrically mediated gene delivery. Proc Natl Acad Sci USA 99, 1292–1297.)

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Guidelines

DNA Copy Number Calculation and Dye Binding Ratio

DNA Size, bp	enter value
DNA Conc, ug/uL	enter value
DNA Abundance, copies/uL	=((DNAConc, ug/uL)*6.023*1E+23)/(DNA Size, bp*1000000*650)
bp per vol	=(DNA Abundance, copies/uL)*(DNA Size, bp)
Binding Target	5 bp/dye
Dye molecules needed	=(bp per vol)/(Binding Target)
Dye Conc, M	enter value
molecules/L	=Dye Conc, M*6.023E+23
Dye Volume Needed	=(Dye molecules needed/molecules per L)*1000000 uL Dye per uL DNA

Materials

TOTO™-1 Iodide (514/533) - 1 mM Solution in DMSO T3600 by Thermo Fisher Scientific

Microcon-30kDa Centrifugal Filter Unit with Ultracel-30 membrane MRCF0R030 by Emd Millipore

Sheared Salmon Sperm DNA 10mg/mL AM9680 by Thermo Fisher Scientific

Protocol

Preparing the Millipore Column

Step 1.

Hydrate Microcon-30 kDa centrifugal filter columns at least 1h to overnight with nuclease free sterile water.

Step 2.

Spin columns at 3000xg for 20 minutes, repeat as many times as needed until all water has been pushed through column to collection tube (in older columns, this may require extended spin time. Column must be fully hydrated before proceeding).

Labeling nucleic acids with TOTO-1 dye

Step 3.

On ice, aliquot sufficient volume of DNA or RNA for experiments, want at least 1 ug/uL conc.

Step 4.

Add TOTO-1 dye from stock (1 mg/mL, in DMSO) to desired concentration (see guidelines for dye concentration calculation).

Step 5.

Mix well by gentle pipetting.

Step 6.

Shield from light and incubate on ice for 1 hour

Clean up dye labeled nucleic acids

Step 7.

Transfer TOTO-1 labeled nucleic acid mixture to Millipore spin filter column.

Step 8.

Centrifuge for 20 minutes at 3,000xg in microcentrifuge. If filter intact, there should be ca. 20 µL remaining in filter cup.

Step 9.

Add 200 µL TE pH8 to filter cup and centrifuge as in step 8. Repeat if background fluorescence is an issue in procedural blanks.

Step 10.

The stained nucleic acid is retained in filter cup of the hydrated spin column in ca 20 µL of TE and will be visible. The eluate and collection tube can be discarded.

Step 11.

Resuspend filter retentate in TE buffer, adjust volume added to yield [DNA] ≥ 1 µg/µl (. Gently pipette up and down around the filter to collect all the dye bound dna.

Proceed to Transfection

Step 12.

Add dye bound nucleic material to cells prepared for electroporation (in electroporation buffer).

Incubate on ice for 10 minutes.

Electroporation

Step 13.

Electroporation steps (follow preferred electroporation protocol)

Step 14.

Allow electroporated cells to incubate at room temperature for 10 minutes in cuvette. This will allow nucleic acid adhered to extracellular wall to enter cellular pores generated during electroporation.

Step 15.

Visualize aliquots (drops) 10 min post electroporation in same buffer. Border of cells should show signal on GFP channel but background staining will be high due to residual TOTO-1:DNA complex in solution. (*This is an optional QC step to view staining progress*).

Step 16.

Gently transfer electroporated cells from cuvette to 30 mL falcon tube in addition of 10mL sterile seawater for wash post electroporation. Spin at 3,000xg for no more than 5 minutes to minimize extracellular adhesion loss. The wash step will remove unbound stained nucleic material from the media.

Remove supernatant and gently resuspend cell pellet in 0.5mL sterile seawater(adjust final volume

based on the desired density of your cell sample) .

Image cells

Step 17.

TOTO-1 nucleic acid complex fluorescence overlaps with standard FITC / GFP filter sets (e.g. GFP Light Cube on our Thermo-Fisher EVOS FL inverted microscope).

Tips and Tricks

Step 18.

Filtering transfection samples onto black filters (Isopore HTBP01300, 0.4um pore or equivalent) followed by seawater rinses provides a low noise, high contrast background for TOTO-1 nucleic acid imaging (see below, however, this does not hold true for the Cy5 light cube used to stimulate chlorophyll fluorescence).

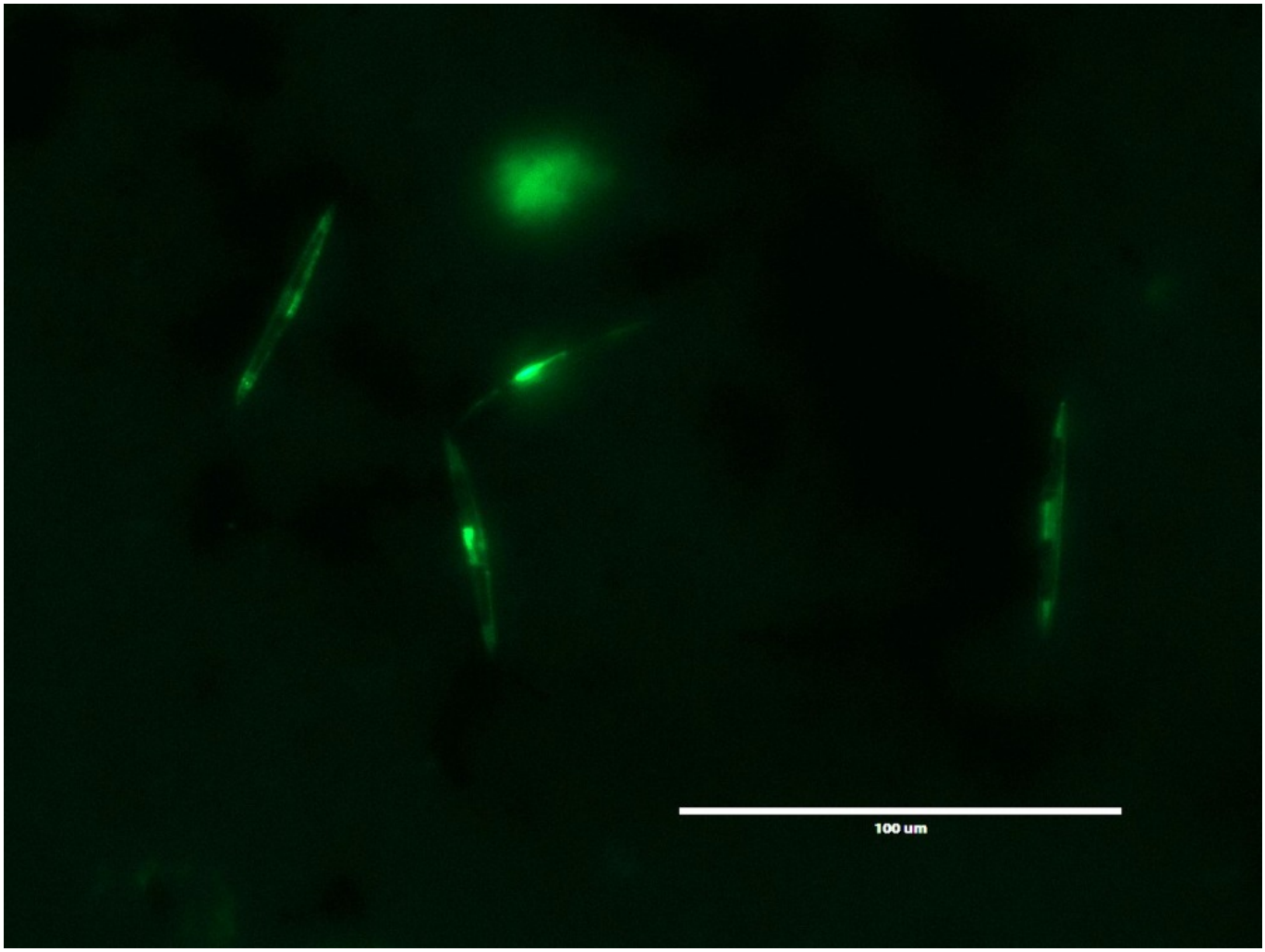
Tips and Tricks

Step 19.

Carrier DNA (e.g Salmon sperm DNA, Thermo-Fisher AM9680 at 1-10ug/mL) may be added to electroporation step to aid in transfection of nucleic material.

TOPO-1 DNA accumulates along raphe with strong intrafrustule labeling in proximity to the nuclear compartment in Pseudo-nitzschia multiseries cells.

Step 20.



Intracellular labeling is diffuse and not appearent in chloroplasts.

Step 21.