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Protocol for Image processing and analysis of VPS13D recruitment to mitochondria

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ABSTRACT

This protocol details the image processing and analysis of VPS13D recruitment to mitochondria as it was performed in https://doi.org/10.1083/jcb.202010004. The first part details the analysis of acute optogenetic recruitment, and the second part of basal recruitment under different conditions.

ATTACHMENTS

dn3wbgtzx.pdf

DOI

dx.doi.org/10.17504/protocols.io.bvgnn3ve

EXTERNAL LINK

https://doi.org/10.1083/jcb.202010004

PROTOCOL CITATION

Marianna Leonzino, Andrés Guillén-Samander, Ni Tang, Pietro De Camilli 2021. Protocol for Image processing and analysis of VPS13D recruitment to mitochondria. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvgnn3ve

KEYWORDS

Image processing, VPS13D recruitment, Mitochondria, ASAPCRN

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MATERIALS TEXT

Protocol for methods described in https://doi.org/10.1083/jcb.202010004

Fluorescence images were processed using FIJI (ImageJ; NIH) software. Gaussian blur filters were applied on some of the images presented.

For the analysis of optogenetic experiments:

1 Build kymographs tracing a line ROI across the mitochondria that was illuminated with Blue Light.

2 Measure an intensity profile by tracing a line ROI across the center of the mitochondrial signal on the kymograph. Take a background profile using the same line ROI outside the illumination area.

3 Normalize the fluorescence intensities to the min and max values using the following formula:

$$\text{Normalized } f[t] = \frac{f[t]-f_{min}[t]}{f_{max}[t]-f_{min}[t]}$$

where $f[t] = F_{mito}[t] - F_{bkgd}[t]$.

For the analysis of VPS13D mitochondrial recruitment:

4 Analyze the cells coexpressing VPS13D^EGFP and mitoBFP individually. Store the each cell outline as an ROI in the ROI Manger of Fiji.

5 Apply the “Gaussian Blur” tool and “Enhance Local Contrast” tool to the MitoBFP channel to average the brightness of the whole image and to clarify the shape and margin of mitochondria.

6 Use an automated macro designed in Fiji to measure VPS13D^EGFP signal overlapping with mitochondria and that localized in the cytosol surrounding them.

7 Calculate the ratio between the intensity of VPS13D^EGFP on mitochondria and the intensity of VPS13D^EGFP in the cytosol surrounding mitochondria for each cell and plot a graph indicating the enrichment of VPS13D^EGFP on mitochondria.

The macro (which can be adapted to analyse recruitment of any given protein to mitochondria) is included below:

```
macro "ROI in batches auto measure VPS13 binding with mitochondrial " {

Dialog.create("reminder");//create an interface

        Dialog.addMessage("choose saving path");//an interactive interface for user to set the
saving directory

        Dialog.show();//an interactive interface for user to set the saving directory
```


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```
	dir_saving = getDirectory("Choose a Directory to save");//an interactive interface for user to set the saving directory

count = roiManager("Count");//save the information from ROI manager into the term "count"


for (i = 0; i < count; i ++){

	name = getTitle;

	selectWindow(name);

	roiManager("Select", i);//save the selected ROIs in the ROI manager in batch

	Stack.getPosition(channel, slice, frame);//save the selected ROIs in the ROI manager in batch

	run("Duplicate...", "duplicate slices=" + slice);//save the selected ROIs in the ROI manager in batch

	saveAs("Tiff", dir_saving + "ROIslice"+i+name); //save the selected ROIs in the ROI manager in batch

	name = replace(name, ".tif", "");//motify the name of images

	C1 = "C1-ROIslice"+i+name+".tif";//motify the name of images

	C2 = "C2-ROIslice"+i+name+".tif";//motify the name of images

	run("Split Channels");//split the integrated image into single channel

	Dialog.create("Content of the channel");//create an interface

		Dialog.addMessage("Make sure the content of the channels. If same as described, click OK. If different, click Cancel and make changes.\n");

		Dialog.addMessage("Channel 1 is VPS13D channel. Channel 2 is Mitochondrial channel.");
		Dialog.show();//make sure the content of each channels

	selectWindow(C1);//choose the cytosol slice

	run("Duplicate...", " ");//duplicate a seperate image to process

	run("Brightness/Contrast...");//preprocess the VPS13D signal in the cytosol area


//measure the intensity of VPS13D signal in mitochondria area

	selectWindow(C2);//choose the mitochondria slice

	run("Duplicate...", " ");//duplicate a seperate image to process

	run("Gaussian Blur...", "sigma=2 slice");//balance the brightness of image

	run("Enhance Local Contrast (CLAHE)", "blocksize=127 histogram=256 maximum=3 mask=*None* fast_(less_accurate)");//clearify the outline of mitochondria

	run("Enhance Local Contrast (CLAHE)", "blocksize=127 histogram=256 maximum=3
```


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```
mask=*None* fast_(less_accurate)");//clearify the outline of mitochondria

        run("Enhance Local Contrast (CLAHE)", "blocksize=127 histogram=256 maximum=3
mask=*None* fast_(less_accurate)");//clearify the outline of mitochondria

        run("Threshold...");//choose the mitochondria area

        run("Convert to Mask");//generate an accurate mitomask

        saveAs("Tiff", dir_saving + "ROI"+i+" mitomask "+name);//generate an accurate mitomask

        imageCalculator("AND create", mitomask, C1);//map C1 onto the mitomask

        setThreshold(1, 255);//choose the calculated area

        saveAs("Tiff", dir_saving + "ROI"+i+" result of mitochondria intensity "+name);

        mitointensity=getTitle;

        run("Measure");//measure the intensity of VPS13D signal in mitochondria area

//measure the VPS13D intensity in the cytosol area


        run("Dilate");

        run("Dilate");

        run("Dilate");//duplicate three times to eliminate the interference from mitochondria signal

        run("Convert to Mask");

        run("Invert");//invert the enlarged mitomask

        saveAs("Tiff", dir_saving + "ROI"+i+" mitomask x3 "+name);//generate the enlarged inverted        mitomask

        mitomask3 = getTitle;

        imageCalculator("AND create", mitomask, mitomask);//map the enlarged inverted mitomask
onto the original mitomask to get the enlarged outline of mitochondria in the cytosol area

        saveAs("Tiff", dir_saving + "ROI"+i+" cytomask "+name);//generate outline profile of mitomask,
which refers to mask of cytosol area

        cytomask = getTitle;

        imageCalculator("AND create", cytomask, C1);//map the cytomask onto cytosol

        setThreshold(1,255);

        saveAs("Tiff", dir_saving + "ROI"+i+" result of cytosolic intensity "+name);

        cytointensity=getTitle;

        run("Measure");//measure the VPS13D intensity in the cytosol area

        saveAs("Results", dir_saving + "ROI"+i+" measurement results of VPS13 binding to
mitochondrial.csv");
```


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```
        close()

        }

        Dialog.create("reminder");

            Dialog.addMessage("Mission accomplished.Congratulations!");

            Dialog.show();

}
```


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