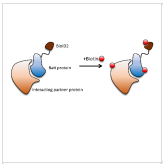

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Identification of interacting proteins using proximity-dependent biotinylation with BioID2 in *Trypanosoma brucei*

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bdrri556

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ABSTRACT

A range of recently developed techniques that take advantage of proximity biotinylation, such as BioID, TurboID, and APEX, are particularly suitable for the studies of protein-protein interactions. As compared to classical co-immunoprecipitation, their advantage is higher reproducibility and capacity to identify stable complexes and also capture transient interactions. Briefly, the protein of interest is fused with modified biotin ligase, which promiscuously biotinylates proteins in its proximity. However, until now BioID has been used in *T. brucei* only in just a handful of studies. Here, we took advantage of the recently developed advanced biotin ligation-based approach named BioID2, which we have successfully adapted for *T. brucei*. In contrast to the classical BioID technique, BioID2 biotin ligase is smaller (26 kDa), more specific, and attached to the protein of interest by several nm-long linker arm, which improves protein folding and the biotinylation range. When applied to *T. brucei*, BioID2 labeling produced a highly specific output.

ATTACHMENTS

ProtocolsIO.pptx

GUIDELINES

As compared to classical co-immunoprecipitation, advantage of this protocol is a higher reproducibility and capacity to identify stable complexes, and also capture transient interactions. Same protocol can be applied for the identification of interaction partners of variety (e.g. membrane-anchored, soluble) proteins. This protocol is designed to identify interacting partners in mitochondria but can be adapted to other organelles.

Briefly, the protein of interest is fused with modified biotin ligase (BioID2), which promiscuously biotinylates proteins in its proximity. Then, purified mitochondria of the procyclic stage of *T. brucei* were dissolved in 1% SDS-containing buffer and incubated at 80 °C for 10 min, after which solubilized biotinylated proteins were affinity-purified by streptavidin-coated Dynabeads. Protein composition was measured and compared among samples by label-free quantitative proteomics.

Note that this experimental design can also be used for the identification of organellar proteomes, if appropriate controls are used.

MATERIALS

NAME	CATALOG #	VENDOR
Roche Complete Protease Inhibitor EDTA-Free tablets	5056489001	Sigma Aldrich
Dynabeads™ MyOne™ Streptavidin C1	65001	Thermo Fisher Scientific
Streptavidin, Alexa Fluor™ 488 conjugate	S11223	Thermo Fisher

BEFORE STARTING

Experimental design one:
Identification of interacting partners of our protein of interest (POI) in mitochondria

1. Create a cell line, where your POI is fused with BioID2 protein.
2. Prepare at least two additional negative control (NC) cell lines. For example, you can use BioID2 protein with just mitochondrial leader sequence, or any random BioID2 tagged mitochondrial protein, which you expect does not interact with your POI.

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3. Isolate biotinylated proteins and analyse them with label free quantitative proteomics
4. Interacting proteins are the ones enriched in your POI datasets, compared to NC datasets

notes

- In our hands, the number of identified putative interaction partners ranged from 1 to 9 proteins depending on the bait protein.
- It is important, that the level of expression of proteins in the NC and POI is similar. For example, in the situation when BioID2 fusion protein in NC cell line will be twice more expressed than your POI BioID2 fusion, you need to have two times higher enrichments for your POI protein datasets in order to be able to specifically identify the interaction partners.

Experimental design two:
Identification of mitochondrial proteome

1. Create at least two cell lines, where a mitochondrial protein is fused with BioID2 protein.
2. Prepare one or two additional negative control cell lines. For example, you can use BioID2 protein only, or BioID2 protein fused to some cytoplasmic protein.
3. Isolate biotinylated proteins and analyse them with label free quantitative proteomics
4. Organellar proteins are the ones enriched in your mitochondrial protein datasets, compared to negative controls

notes

- In our hands, the number of identified mitochondrial proteins was 117, therefore the did not managed to identify the whole organellar proteome
- among the identified proteins there was not a single non-mitochondrial hit (114 proteins were already experimentally localized to the mitochondrion, 3 have strong predictions to be mitochondrial), therefore purity was excellent.
- This technique is ideal for the identification of highly expressed proteins, therefore, it is suitable for some poorly defined organelles in derived protists, rather than in *T. brucei.*

Prior steps - cloning

1 Tag you protein of interest with linker_BioID2 tag sequence according to attached pptx file **ProtocolsIO.pptx**

2 Screen for a successful biotinylation in the cells supplemented with biotin on IFA using fluorescent Alexa-488-streptavidin

Biotin treatment

3 Supplement SDM-79 media with 100 uM Biotin over night

Hypotonic mitochondria isolation

4 Spin down 3 x10^9 cells for each replicate – 1500x g, 15min, 4°C

5 resuspend cell pellets in 50 ml of SBG and spin again at same condition

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5.1 SBG recipe:
150 mM NaCl
20 mM Glucose
20 mM NaH_2PO_4
pH 7.9

6 resuspend cell pellet in 3 ml of DTE

6.1 DTE recipe:
TRIS 1 mM
EDTA 1 mM
pH 8.0

7 Dounce – 10 strokes

8 Add 0,5 ml of 60% Sucrose, mix the sample in dounce and transfer it into 3 eppendorf tubes

9 Spin down: 16,000x g, 10min, 4°C

10 Resuspend all 3 pellets in 1,5 ml of STM (merge everything in 2 ml eppendorf tubes)

10.1 STM recipe
Sucrose 250 mM
Tris, pH 8.0 20 mM
$MgCl_2$ 2mM

11 Pipette
$MgCl_2$ to a final concentration 5 mM,
$CaCl_2$ to a final concentration 0.5 mM
and
DNase to a final concentration 5ug/ml

12 Incubate on ice for 20 minute

13 STE Wash 1,5 ml, 3x, 12,000x g, 10 min, 4°C

14 snap freeze with liquid nitrogen and store in -80°C until needed or proceed directly to Dynabeads protein isolation

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Dynabeads protein isolation

15 Resuspend pellets in 1,8 ml of Boiling Buffer, 10 minutes, 80°C

15.1 Boiling buffer recipe:
TRIS 50 mM
EDTA 1mM
SDS 1%
pH 7.4

16 Spin down: 16,000x g, 10min, 20°C

17 Meanwhile wash 50 ul of dynabeads per sample 2x times with 1ml of Incubation buffer, each time 5 minutes

17.1 Incubation buffer recipe:
NaCl 150 mM
TRIS 50 mM
EDTA 5mM
TRITON X-100 1%
pH 7.4

18 Mix supernatants from step **14** with 18 ml of Incubation Buffer (supplemented with ROCHE Complete protease inhibitors EDTA free)

19 Add 50 uL of washed dynebeads resuspended in incubation buffer from step **15** per sample (final 0.5 mg per sample) (Dynabeads™ MyOne™ Streptavidin C1 -10 mg/ml)

20 Roll for 2 hours at RT at falcon tubes, then place overnight at fridge

21 Wash the beads 3 times with 1,5 ml of Boiling buffer

22 Wash the beads 3 times with 1,5 ml of Incubation buffer

23 Remove all liquid, put dry dynabeads into to the -80 freezer

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