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TurboID-phosphoenrichment workflow protocol

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Jasmin Jansen[1,2,3]

[1]University of Konstanz, Department of Biology; [2]Aligning Science Across Parkinson's Disease; [3]Konstanz Research School of Chemical Biology (KoRS-CB)

Jasmin Jansen
University of Konstanz

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Protocol status: Working
We use this protocol and it has worked in our previous experiments.

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Grant ID: 496470458
German Research Foundation
Grant ID: 516836828

Abstract

This protocol is meant to accompany the manuscript "Probing the proteome-wide interactomes of Leucine-rich Repeat Kinases 1 and 2 and alterations in their phosphorylation activity" and its methods section.
The protocol describes all steps of the experimental workflow starting from cell culture over TurboID, streptavidin enrichment and phosphopeptide enrichment to mass spectrometric analysis using Data-Independent acquisition (DIA)-based quantification and data analysis.

The aim is to get a comprehensive dataset of interactors together with changed phosphosites of this interactome based on TurboID (Cho et al., 2020) and TiO2-based phosphoenrichment.

All steps are described per sample.

Guidelines

When preparing samples for mass spectrometry, work carefully, always wear appropriate gloves to minimize sample contamination that impedes your analysis. For downstream applications like mass spectrometry, it is crucial to wash cell pellets carefully to minimize protein contamination through fetal bovine serum.

For cell culture, please check your cell line(s) routinely for mycoplasma and other contaminations to avoid artefacts introduced thereby.

Materials

- Anti-LRRK2 (phospho S935) antibody [UDD2 10(12)] **Abcam** **Catalog #**ab133450
- Primary Antibody anti-LRRK2 c41-2 **Abcam** **Catalog #**ab133474
- Lactic acid solution, 85 % **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**252476
- Glycolic acid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**124737
- Titansphere TiO, 5 µm, 500 mg **GL Science** **Catalog #**020-75000
- Sequencing Grade Modified Trypsin **Promega** **Catalog #**V5113

Equipment

Sep-Pak tC18 1 cc Vac Cartridge, 50 mg Sorbent per Cartridge, 37 - 55 µm NAME

C18 column TYPE

Sep-Pak BRAND

WAT054960 SKU

https://www.waters.com/nextgen/de/de/shop/sample-preparation--filtration/wat054960-sep-pak-tc18-1-cc-vac-cartridge-50-mg-sorbent-per-cartridge-37--.html?srsltid=AfmBOooKGYIlKjNbsCZb0q4yTMnBi0q3z56np309jwnnm-1F51XaphIA LINK

Equipment

Pierce™ C18-Spin columns NAME

C18 column TYPE

Pierce BRAND

84850 SKU

https://www.thermofisher.com/order/catalog/product/de/de/84850 LINK

- Trypsin/Lys-C Mix, Mass Spec Grade, 5 × 20ug **Promega** **Catalog #**V5073
- Iodoacetamide **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**I1149-5G
- Tris(2-carboxyethyl)phosphin -hydrochlorid **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C4706
- Pierce™ Streptavidin Magnetic Beads **Thermo Fisher** **Catalog #**88817
- cOmplete™, EDTA-free protease inhibitor cocktail **Roche** **Catalog #**11873580001
- Formaldehyede 37% solution **Carl Roth** **Catalog #**7398
- Dulbeccos Phosphate-buffered saline **Gibco - Thermo Fisher Scientific** **Catalog #**21300058
- IN04 **AOBIOUS Inc** **Catalog #**AOB13355
- MLi-2 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**SML3101
- Biotin **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**B4639
- Polyethylenimine, Linear, MW 25000, Transfection Grade **Polysciences, Inc.** **Catalog #**23966
- Opti-MEM™ I Reduced Serum Medium **Thermo Fisher** **Catalog #**31985070
- Dulbeccos modified eagle medium (DMEM), high glucose **Gibco - Thermo Fisher Scientific** **Catalog #**41965062
- Fetal bovine serum (FBS) Superior **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S0615
- DMSO **Supelco** **Catalog #**1.09678.0100
- HRP Anti-Rabbit IgG **Dianova** **Catalog #**AKC080
- Anti-RAB10 (phospho T73) antibody [MJF-R21] **Abcam** **Catalog #**ab230261
- Recombinant Anti-RAB10 antibody [MJF-R23] **Abcam** **Catalog #**ab237703
- HiPPR™ Detergent Removal Spin Column Kit **Thermo Fisher** **Catalog #**88305
- Sep-Pak tC18 1 cc Vac Cartridge 50 mg Sorbent per Cartridge **Waters** **Catalog #**WAT054960
- 3M™ Empore™ C18 47 mm Extraction Disc Model 2215 20 pack 3 packs per case **3M corporation** **Catalog #**2215
- Acclaim™ PepMap™ 100 C18 HPLC Columns **Thermo Scientific** **Catalog #**164945

Equipment

Concentrator Plus	NAME
Centrifugal vacuum concentrator	TYPE
Eppendorf	BRAND
5305000568	SKU
https://www.eppendorf.com/gb-en/eShop-Products/Centrifugation/Concentrator/Concentrator-plus-p-5305000568	LINK

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- Polyethylenimine, Linear, MW 25000, Transfection Grade **Polysciences, Inc.** **Catalog #**23966
- HRP Anti-Rabbit IgG **Dianova** **Catalog #**AKC080
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- Pierce™ Streptavidin Magnetic Beads **Thermo Fisher** **Catalog #**88817
- DMSO **Supelco** **Catalog #**1.09678.0100
- Anti-RAB10 (phospho T73) antibody [MJF-R21] **Abcam** **Catalog #**ab230261
- Opti-MEM™ I Reduced Serum Medium **Thermo Fisher** **Catalog #**31985070

Safety warnings

Before performing the experiment, please optimize the incubation of biotin with TurboID-transfected cells to avoid overlabelling. This can be done by SDS-PAGE and Western blotting or mass spectrometry.

Please do not skip the detergent removal step as it is crucial for an optimal mass spectrometric analysis. Detergents like NP-40 will interfer with C18 nano-HPLC and lead to non-optimal resolution of peptides. Also, they can contaminate the mass spectrometers and cause ion suppression.

After performing the phosphopeptide enrichment, make sure that the exposition to basic pH is as short as possible because this can cause a loss of phosphorylations
Perform the enrichment shortly before measurement to keep native phosphorylation intact.

Before start

Please read the protocol carefully before you start the experiment to prepare all materials and methods and plan time and resources accordingly.

Cell culture

1d 2h 15m

1 HEK293T wt cells are cultured in 10 cm dishes in

Dulbeccos modified eagle medium (DMEM), high glucose **Gibco - Thermo Fisher Scientific Catalog** #41965062

with [M] 10 % (v/v)

Fetal bovine serum (FBS) Superior **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #S0615

(hereafter "medium") at 37 °C , 95 % and [M] 5 % (v/v) CO2 and passaged upon confluency.

2 For transient transfection and proximity labeling, cells were grown in 15 cm Petri dishes to 50-60 % confluency. For 1 replicate, 4× 15 cm dishes were used to have enough material for the interactome enrichment.

Transient transfection

2.1 Change medium and add 12.5 mL fresh medium to each plate.

2.2 For one replicate (4× 15cm dish), mix 7.5 µg of plasmid carrying TurboID-fusion construct with 5 mL

Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Catalog** #31985070 .

Add 90 µL

Polyethylenimine, Linear, MW 25000, Transfection Grade **Polysciences, Inc. Catalog** #23966

([M] 1 mg/mL stock solution).

2.3 Mix well by pipetting.

2.4 Incubate for 00:15:00 at Room temperature . 15m

2.5 Add 1.25 mL of the mix to each of the four plates. Tilt the plates and carefully add dropwise into medium on the side to not disturb adherent cells.

2.6 Incubate for 24:00:00 at 37 °C , 95 % and [M] 5 % (v/v) CO2 . 1d

Addition of inhibitors

3 Change medium and add DMSO/inhibitors with this medium change

3.1 Add 5 µL of either [M] 10 millimolar (mM) MLi-2 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**SML3101 , [M] 50 millimolar (mM) IN04 **AOBIOUS Inc Catalog #**AOB13355 (both in DMSO) or DMSO to 50 mL medium for [M] 1 micromolar (µM) final MLi-2 or [M] 5 micromolar (µM) final IN04 inhibitor concentration.

3.2 Add 12.5 mL of the prepared 50 mL mix to each of the four plates of 1 replicate.

3.3 Incubate for 01:00:00 at 37 °C , 95 % and [M] 5 % (v/v) CO2 1h

TurboID proximity labelling 1h

4 Add 6.25 µL [M] 100 millimolar (mM) Biotin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**B4639 in DMSO carefully to each of the plates to start proximity labelling.

4.1 Incubate for 01:00:00 at 37 °C , 95 % and [M] 5 % (v/v) CO2 . 1h

Harvest 20m

5 Adherent cells were washed carefully with 2x Dulbeccos Phosphate-buffered saline **Gibco - Thermo Fisher Scientific Catalog #**21300058 that was equilibrated to 37 °C prior to washing.

6 Add 5 mL warm PBS with [M] 0.025 % (v/v) Formaldehyede 37% solution **Carl Roth Catalog #**7398 to each plate.

6.1 Incubate for 00:10:00 at Room temperature . 10m

6.2 Remove liquid carefully

7 Add 5 mL warm [M] 50 millimolar (mM) Tris-HCl pH 7.4 to the adherent cells.

7.1 Incubate for 00:05:00 at Room temperature 5m

7.2 Remove liquid carefully.

8 Place cell dishes on ice and harvest cells with 10 mL ice-cold 1x Dulbeccos Phosphate-buffered saline **Gibco - Thermo Fisher Scientific Catalog #**21300058 per plate.

8.1 Transfer ice-cold cell suspension to 50 mL Falcon tubes and pool the suspensions from 4× 15 cm dishes for each replicate (same transfection/inhibitor/biotin mix for each of the 15 cm dishes).

9 Wash cells twice with 10 mL ice-cold 1x Dulbeccos Phosphate-buffered saline **Gibco - Thermo Fisher Scientific Catalog #**21300058 .
Pellet cells by centrifugation 800 x g, 4°C for 00:05:00 in between.

9.1 Pellet cells by centrifugation 800 x g, 4°C for 00:05:00 and snap freeze in liquid N_2. 5m

10 Store cell pellets at -80 °C until further processing.

Cell lysis

35m

11 Place frozen cell pellets on ice. When working with cells and cell lysate please always work on ice until proteins are denatured or the protocol indicates to do otherwise.

12 Resuspend cell pellets in 16 mL lysis buffer ([M] 25 millimolar (mM) Tris-HCl pH 7.4 , [M] 150 millimolar (mM) NaCl, [M] 1 % (v/v) NP-40, [M] 5 millimolar (mM) $MgCl_2$, [M] 1 millimolar (mM) Na_3VO_4, [M] 5 millimolar (mM) NaF, [M] 5 millimolar (mM) β-glycero phosphate, 1x cOmplete™, EDTA-free protease inhibitor cocktail **Roche Catalog #**11873580001 , [M] 1 millimolar (mM) DTT).

12.1 Incubate for 00:15:00 at 4 °C while gently mixing/rolling. 15m

13 Pellet cell debris by centrifugation at 16000 x g, 4°C for 00:20:00 . 20m

14 Take supernatant and continue with streptavidin enrichment. Discard the pellet.

Streptavidin enrichment

20h 3m

15 Equilibrate 0.5 mL Pierce™ Streptavidin Magnetic Beads **Thermo Fisher Catalog #**88817 (5 mg per sample) with 3x 500 µL lysis buffer in 1.5 mL tube using a magnetic rack. 3m

16 Add the equilibrated beads to the supernatant from go to step #14 (see previous section).

16.1 Incubate for 20:00:00 at 4 °C and gentle agitation to allow binding of biotinylated proteins to streptavidin beads. 20h

17 Pellet beads by centrifugation 2000 x g, 4°C for 00:15:00 .

17.1 Use a magnet to carefully decant "flow-through" of streptavidin beads without disturbing the bead pellet.

17.2 If necessary, repeat centrifugation to retain as many beads as possible.

18 Resuspend beads with 1 mL lysis buffer and transfer to a fresh 2 mL reaction tube.

19 Wash beads with 1 mL lysis buffer using a magnetic rack.

19.1 Repeat washing step.

On-bead digestion 22h

20 Equilibrate streptavidin beads twice with 1 mL [M] 50 millimolar (mM) ammonium bicarbonate to prepare samples for on-bead digestion.

21 Resuspend beads in 150 µL [M] 8 Mass Percent urea.

22 Add TCEP
Tris(2-carboxyethyl)phosphin -hydrochlorid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C4706
for [M] 3 millimolar (mM) final concentration to reduce cysteines.

22.1 Incubate for 00:30:00 at 37 °C and 650 rpm .

23 Add IAA (Iodoacetamide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**I1149-5G) to [M] 6 millimolar (mM) final concentration to alkylate cysteines.

23.1 Incubate for 00:30:00 at Room temperature and 650 rpm **in the dark**. 30m

24 Dilute urea to [M] 4 Mass Percent using [M] 50 millimolar (mM) ammonium bicarbonate.

25 Add 3 µg Trypsin/Lys-C Mix, Mass Spec Grade, 5 × 20ug **Promega Catalog #**V5073 to sample.

25.1 Incubate for 03:30:00 at 37 °C and 650 rpm . 3h 30m

26 Dilute urea concentration to [M] 1 Mass Percent using [M] 50 millimolar (mM) ammonium bicarbonate.

27 Add 1.5 µg Sequencing Grade Modified Trypsin **Promega Catalog #**V5113 .

27.1 Incubate for 18:00:00 at 37 °C and 650 rpm . 18h

28 Remove residual detergents with the HiPPR™ Detergent Removal Spin Column Kit **Thermo Fisher Catalog #**88305 (200 µg per sample) according to the manufacturer's instructions.

29 Acidify flow-through by adding formic acid to a final concentration of [M] 2 % (v/v) .

29.1 After mixing, check if pH 2 or lower. This is important since acidic pH stops trypsin digestion and is crucial for a successful C18 desalting.

30 Add [M] 1 % (v/v) acetonitrile (ACN) final concentration and desalt your sample using

Sep-Pak tC18 1 cc Vac Cartridge 50 mg Sorbent per Cartridge **Waters Catalog #**WAT054960

and a vacuum manifold.

30.1 Wet cartridges with 1 mL pure ACN.

30.2 Equilibrate cartridges with 2 mL [M] 1 % (v/v) ACN, [M] 0.1 % (v/v) formic acid.

30.3 Add sample and apply it slowly to the C18 resin. Don't open the valve fully to apply slowly.

30.4 Wash peptides with 2 mL [M] 1 % (v/v) ACN, [M] 0.1 % (v/v) formic acid.

30.5 Elute peptides into low-binding 1.5 mL tube with 1 mL [M] 50 % (v/v) ACN, [M] 0.1 % (v/v) formic acid.

31 For further analysis, split sample for peptide and phosphopeptide analysis. Therefore, use 10 % of sample for "normal" peptide analysis (**Fraction 1**), and 90 % of sample for subsequent phosphopeptide enrichment and phosphopeptide analysis (**Fraction 2**).

Dry both fractions in a

Equipment

NAME	TYPE	BRAND	SKU	LINK
Concentrator Plus	Centrifugal vacuum concentrator	Eppendorf	5305000568	https://www.eppendorf.com/gb-en/eShop-Products/Centrifugation/Concentrator/Concentrator-plus-p-5305000568

.

31.1 For Fraction 1, continue directly to "Mass spectrometric analysis".

31.2 For Fraction 2, continue with "Phosphopeptide enrichment".

Phosphopeptide enrichment

1h 2m

32 Weigh 0.5 mg beads per sample (if 50 µg peptides) Titansphere TiO, 5 µm, 500 mg **GL Science Catalog #**020-75000 and resuspend in **Loading buffer 1** ([M] 0.1 Molarity (M) Glycolic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**124737 , [M] 70 % (v/v) ACN, [M] 5 % (v/v) trifluoroacetic acid (TFA)) so that 10 µL per 1 mg beads .

32.1 To minimize unspecific binding, incubate TiO_2 beads in **Loading buffer 1** for 00:20:00 at Room temperature and 1200 rpm prior to peptide incubation

20m

33 Resuspend approx. 50 µg of dried peptides in 200 µL **Loading buffer 1**.

33.1 Incubate for 00:10:00 at 37 °C for proper resuspension. 10m

34 Add equilibrated beads to peptides at a **peptide:bead ratio of 1:10**. If prepared as described, add 5 µL per sample.

34.1 Incubate for 00:20:00 at Room temperature in rolling incubator. 20m

35 Prepare self-made, single-layered C8 Stage tips. See

Citation

Rappsilber J, Mann M, Ishihama Y (2007)
. Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips.

https://doi.org/ LINK

for details.
Use

3M™ Empore™ C18 47 mm Extraction Disc Model 2215 20 pack 3 packs per case **3M corporation Catalog #**2215

to make the tips.

35.1 Equilibrate C8 Stage tips with **Loading buffer 1**.

36 Settle incubated TiO_2 beads by centrifugation 10000 x g for 00:02:00. 2m

36.1 Transfer 150 µL of supernatant to fresh low-binding tube and keep for second enrichment step, see .

37 Use residual supernatant to transfer beads to equilibrated C8 Stage tips.

37.1 Centrifuge for 00:02:00 at 500 x g and add flow-through to supernatant fraction from step go to step #36.1 . 2m

38 Wash beads that were retained by C8 Stage tips with 50 µL **Wash buffer 1** ([M] 80 % (v/v) ACN, [M] 1 % (v/v) TFA) and centrifuge at 500 x g for 00:02:00 . 2m

39 Wash beads with 50 µL **Wash buffer 2** ([M] 10 % (v/v) ACN, [M] 0.2 % (v/v) TFA) and centrifuge at 500 x g for 00:02:00 . Transfer tips to new vial. 2m

40 Prepare low-binding tubes with 60 µL [M] 10 % (v/v) formic acid for phosphopeptide elutions to directly acidify them and minimize loss of phosphorlyation by ammonium hydroxide.

41 Elute bound phosphopeptides with 30 µL **Elution buffer 1** ([M] 1 % (v/v) NH_4OH) by centrifugation at 500 x g for 00:02:00 and directly transfer elution to prepared tubes (go to step #40). 2m

42 Elute residual bound phosphopeptides with 30 µL **Elution buffer 2** ([M] 5 % (v/v) NH_4OH, [M] 25 % (v/v) ACN) by centrifugation at 500 x g for 00:02:00 and directly transfer elution to prepared tubes (go to step #40). 2m

43 Repeat phosphopeptide protocol with retained supernatant from go to step #36.1 and repeat protocol with **Loading buffer 2** ([M] 20 % (v/v) Lactic acid solution, 85 % **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**252476 , [M] 70 % (v/v) ACN, [M] 5 % (v/v) TFA) instead of **Loading buffer 1**.

This second enrichment step maximizes yield of phosphopeptide by repeating the enrichment and changing the specificity by using lactic acid instead of glycolic acid.

44 Combine phosphopeptide elutions and dry them by vaccuum evaporation.

Mass spectrometric analysis

6h 8m

45 Resuspend samples in **mass spectrometric (MS) buffer** ([M] 3 % (v/v) ACN, [M] 0.1 % (v/v) formic acid), measure peptide concentration by Nanodrop and adjust concentration to [M] 0.25 µg/µL if possible.

46 Load 500 ng peptides onto a 50 cm Acclaim™ PepMap™ 100 C18 HPLC Columns **Thermo Scientific Catalog #**164945 connected to an EASY-nLC 1200 nano-LC system coupled to a QExactive HF mass spectrometer.

47 For normal peptides (**Fraction 1**), resolve peptides across a 03:38:00 active gradient at a 150 nL/min flow rate. 6h 8m
For phosphopeptides (**Fraction 2**), resolve peptides across a 02:30:00 min active gradient at a 150 nL/min flow rate.

48 Perform mass spectrometric analysis in data-independent acquisition mode with 24 (Fraction 1) or 20 (Fraction 2) variable windows with 1 m/z overlaps.

48.1 Record full mass spectra in the Orbitrap at a resolution of 120K in the range of 300-1650 m/z (Fraction 1) or 350-1600 m/z (Fraction 2) with a maximum injection time of 60 ms and an AGC target of 3e6.

48.2 Isolate precursor ions in the quadrupole and fragment them with stepped HCD at 28 ±3 % NCE (normalized collision energy).

48.3 Record fragment mass spectra in the Orbitrap at a resolution of 30K, an AGC target of 1e6 and maximum injection time set to auto.

Data analysis

49 Analyze raw data with Spectronaut in directDIA mode.

49.1 For Fraction 1, normal peptides, use default BGS settings except for minimal peptide lengths 6.

For Fraction 2, phosphopeptides, change default BGS settings to also include variable modifications phosphorylation at S, T, and Y, deamidation at N and Q as well as glutamine to pyro-glutamine modification. Set the Normalization Type Filter to include phosphorylated residues. Enable the PTM workflow and set the PTM localization filter to 0.75.

49.2 Use Swissprot database containing protein sequences from *homo sapiens* and additionally, a contaminant database like this one:

Citation

Frankenfield AM, Ni J, Ahmed M, Hao L (2022)
. Protein Contaminants Matter: Building Universal Protein Contaminant Libraries for DDA and DIA Proteomics.

https://doi.org/10.1021/acs.jproteome.2c00145 LINK

49.3 Retain only proteins or peptides that were confidently identified with a Q-value ≤ 0.01.

50 Export quantification results as pivot report for statistical analysis and hypothesis testing.

51 Use an appropriate tool for statistical analysis. For example, you can use Perseus.

Citation

Tyanova S, Cox J (2018)
. Perseus: A Bioinformatics Platform for Integrative Analysis of Proteomics Data in Cancer Research.

https://doi.org/10.1007/978-1-4939-7493-1_7 LINK

52 For statistical analysis of Fraction 1:

52.1 Load data from Spectronaut into your tool.

52.2 Filter out Contaminants

52.3 Log2 transform quantities.

52.4 Group all replicates of one condition so you can use these groups for subsequent filtering and analysis.

52.5 Filter out proteins that have been identified and quantified inconsistently in less than 3 or 4 of 4 biological replicates across all conditions, i.e. if a protein has been identified sparsely in all conditions, filter it out, but if it is identified in 3 or 4 of 4 replicates in one condition or more, keep it.

52.6 Filter out proteins that have been identified and quantified based on only 1 precursor.

52.7 Impute missing values using a tail-based imputation. This step is crucial to perform hypothesis tests.

52.8 Use two-sample t-tests to determine significant differences between two conditions and multiple sample t-tests (ANOVA-based) to determine significant differences between more than 2 conditions.

52.9 Filter results for significantly changed proteins

52.10 You can also perform a post-hoc Tukey test based on the results from the ANOVA to determine between which conditions significant differences were identified. Then, use these results to filter out hits that are enriched in the control or filter hits that are significantly changed between certain conditions.

52.11 Normalize values to z-scores and average replicates of the same condition.

52.12 Perform hierarchical cluster analysis to identify and visualize cluster of protein that behave similarly and are enriched or depleted for one or more conditions.

52.13 Use clusters from previous step to perform further interactome analysis.

53 For statistical analysis of Fraction 2

53.1 Use the phosR package to process phosphosites.

Citation

Kim HJ, Kim T, Hoffman NJ, Xiao D, James DE, Humphrey SJ, Yang P (2021)
. PhosR enables processing and functional analysis of phosphoproteomic data.

https://doi.org/10.1016/j.celrep.2021.108771 LINK

53.2 Keep only phosphosites that were identified in more than 2 of 4 replicates for at least one condition.

53.3 Impute missing values first using a site- and condition specific imputation, and then a paired-tail imputation and normalize resulting values.

53.4 Export data from phosR and continue in your statistical tool of choice or continue in R.

53.5 Perform hypothesis tests for phosphosites as described for proteins above.

Protocol references

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Bruderer, R. *et al.* (2017) 'Optimization of Experimental Parameters in Data-Independent Mass Spectrometry Significantly Increases Depth and Reproducibility of Results', *Molecular & Cellular Proteomics*, 16(12), p. 2296. Available at: https://doi.org/10.1074/MCP.RA117.000314.

Utriainen, M. and Morris, J.H. (2023) 'clusterMaker2: a major update to clusterMaker, a multi-algorithm clustering app for Cytoscape', *BMC Bioinformatics*, 24(1), pp. 1–28. Available at: https://doi.org/10.1186/S12859-023-05225-Z/FIGURES/10.

Citations

Step 51

Tyanova S, Cox J. Perseus: A Bioinformatics Platform for Integrative Analysis of Proteomics Data in Cancer Research.
https://doi.org/10.1007/978-1-4939-7493-1_7

Step 53.1

Kim HJ, Kim T, Hoffman NJ, Xiao D, James DE, Humphrey SJ, Yang P. PhosR enables processing and functional analysis of phosphoproteomic data.
https://doi.org/10.1016/j.celrep.2021.108771

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