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# Applying reverse genetics to study measles virus interactions with the host

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## Abstract

The study of virus-host interactions is essential to achieve a comprehensive understanding of the viral replication process. Commonly used methods are yeast two-hybrid approach, and transient expression of a single tagged viral protein in host cells followed by affinity-purification of interacting cellular proteins and mass spectrometry analysis (AP-MS). However, by these approaches, virus-host protein-protein interactions are detected in the absence of a real infection, not always correctly compartmentalized, and for the yeast two-hybrid approach performed in a heterologous system. Thus, some of the detected protein-protein interactions may be artificial. Here we describe a new strategy based on recombinant viruses expressing tagged viral proteins to capture both direct and indirect protein partners during the infection (AP-MS in viral context). This way, virus-host protein-protein interacting co-complexes can be purified directly from infected cells for further characterization.

**Key words:** reverse genetics, recombinant virus, virus-host interactions, measles virus, tagged proteins, affinity purification.

## 1 Introduction

Measles virus (MV) is a negative-sense single-stranded RNA virus from the *Paramyxoviridae* family that encodes eight proteins, two of which (V and C) are virulence factors that are transcribed from the phosphoprotein (P) gene. Characterization of host protein partners of

these factors is of major importance as they are susceptible to exert multiple regulatory functions during the viral replication cycle.

Reverse genetics makes it possible to express tagged viral proteins directly from a viral genome. Numerous reverse genetics protocols allow the generation of infectious viruses from plasmids coding for genomic cDNA [1-7]. For MV, a noteworthy and pioneer work performed in the Billeter laboratory allowed to clone an infectious cDNA corresponding to the antigenome of MV and established a reverse genetics procedure to rescue the virus [8]. We adapted this protocol by engineering an infectious cDNA from the Schwarz/Moraten strain (pTM-MV Schw), a widely used MV vaccine [9]. pTM-MV Schw plasmid was further modified to introduce additional transcription units (ATUs) at different positions of the genome for the expression of foreign genes [9]. ATUs enable the cloning of supplementary sequences into the MV genome. Three different positions of ATUs on the MV genome can be considered, ATU1 (before the N gene), ATU2 (between P and M genes), and ATU3 (between H and L genes) (**Figure 1a**) (see **Note 1**) [4].

We used a reverse genetic protocol to generate recombinant MV strains expressing tagged viral proteins. This provided MV-V and MV-C purification directly from infected cells using a modified one-step affinity purification approach. In this protocol, viral proteins of interest (baits) are labeled with a recombinant fusion tag, which is then used to purify baits and their respective interacting partners (preys). Examples of tags frequently used in virus-host protein-protein interactions studies are FLAG-tag, 6-His-Tag, Myc-Tag, HA-tag, and STREP-Tag [10-13]. We set a protocol to study virus-host protein-protein interactions in infected cells using recombinant MVs expressing One-STREP tagged proteins (**Figure 2**). We chose STREP-Tag because being a short peptide, none or minimal interference with viral replication is expected [14-18]. If little knowledge of a viral protein of interest is available, N-terminal (N-ter) and C-terminal (C-ter) recombinant fusion tags should be considered. We successfully cloned and produced recombinant MVs expressing N-terminally tagged V protein and nucleoprotein (N) from ATU2 [19], and N- and C-terminally tagged MV-C protein from ATU3 [20] (**Note 1**). Upon infection with MV recombinant tagged viruses, cells are lysed and viral protein-specific co-complexes are purified by affinity chromatography for further study by bottom-up mass spectrometry-based proteomics (LC-MS/MS) and protein-protein interaction validation by western blotting (WB).

This powerful approach can be applied to different cell lines, primary cells, organ cultures, or even animal model organisms to study protein-protein interactions in a viral infectious context (**Figure 3**). Moreover, this protocol can be adjusted to study specific RNA-protein interactions [21]. Finally, by taking samples at specific time points, the kinetics of interactions can be analyzed giving important insights into host-viral interplay during a viral replication cycle.

## 2 Materials

### 2.1 Cell culture

#### 2.1.1 Cell lines

1. Helper HEK293-T7-NP cells stably expressing N and P proteins from MV and phage T7 RNA polymerase [9].
2. Vero-NK cells (ATCC #CCL-81).
3. HEK293T (ATCC #CRL-3216) (see **Note 2**).

#### 2.1.2 Media culture

1. Dulbecco's modified Eagle's medium (DMEM) high glucose.

2. 100X Penicillin-Streptomycin (#15140122, Gibco).
3. Fetal bovine serum (FBS) (#MT35015CV, Corning).
4. Opti-MEM Reduced Serum Medium, GlutaMAX™ Supplement (#51985026, Gibco).
5. 1X DPBS.

## 2.2 Production of recombinant MV

### 2.2.1 Plasmids

1. pTM2-STrEP-V: Plasmid encoding for MV-V protein with an amino-terminal One-STrEP tag inserted into the pTM-MV Schw plasmid containing MV genome [19] (see **Note 3**).
2. pTM2-STrEP-CH: Plasmid encoding for mCherry protein with an amino-terminal One-STrEP tag inserted into the pTM-MV Schw plasmid containing MV genome [19] (see **Note 3 and 4**).
3. pEMC-La expressing the MV polymerase L gene [9].

### 2.2.2 Transfection Reagents

1. HEPES 2X (#51558, Sigma) passed by a 0.2 µm filter.
2. CaCl<sub>2</sub> 1M.
3. DNase/RNase-Free Distilled Water.

## 2.3 Extraction of protein co-complexes

1. Recombinant tagged MV strains: rMV STrEP-V and rMV STrEP-CH.
2. Lysis buffer: 20 mM MOPS-KOH pH7.4, 120 mM of KCl, 0.5% Igepal, 2 mM β-Mercaptoethanol, supplemented with Complete Protease Inhibitor Cocktail (#11873580001, Roche) (see **Note 5**).
3. StrepTactin High Performance Sepharose beads (#28935599, GE Healthcare).
4. Washing buffer: 20 mM MOPS-KOH pH7.4, 120 mM KCl, 2 mM β-Mercaptoethanol supplemented with Complete Protease Inhibitor Cocktail (#11873580001, Roche) (see **Note 5**).
5. 10X StrepTactin elution buffer with Biotin (#2-1000-025, IBA): Prepare 1X solution by diluting 1 volume of 10X StrepTactin elution buffer with 9 volumes of DNase/RNase-Free distilled water.
6. 12% Trichloroacetic acid (TCA).
7. Acetone.
8. 8M urea solution.
9. 4% SDS solution.
10. DNase/RNase-Free Distilled Water.

## 2.4 Mass Spectrometry Analysis and database search

1. Denaturation buffer: 4M Urea (#U4883-6X25ML, Sigma-Aldrich), 100 mM NH<sub>4</sub>HCO<sub>3</sub> (#09830, Fluka).
2. Sonicator: Ultrasound Technology UP200St equipped with the Vialtweeter Sonotrode, Hielscher.
3. Reduction of disulfides bond: 500-mM tris (2-carboxyethyl) phosphine, TCEP (#646547, Sigma-Aldrich).
4. Alkylation of Cysteine: 500-mM iodoacetamide (#1114, Sigma-Aldrich).
5. Digestion 1: ratio 50:1 (Protein:Enzyme) rLys-C (#V1671, Promega).

6. Digestion 2: ratio 50:1 (Protein: Enzyme) Sequencing Grade Modified Trypsin (#V5111, Promega).
7. Thermostatic enclosure: ThermoMixer® C equipped with a ThermoTop®, Eppendorf.
8. Stop solution: 4 % Formic Acid (FA) (#94318, Fluka).
9. Elution buffer: 80% acetonitrile (ACN) (#412042, Carlo Erba Reagents), 20% water (#412092, Carlo Erba Reagents), 0.1% Formic Acid (FA) (#94318, Fluka).
10. Loading buffer: 2 % acetonitrile (ACN) (#412042, Carlo Erba Reagents): 0.1 % Formic Acid (FA) (#94318, Fluka): 98% water (#412092, Carlo Erba Reagents).
11. Speedvac: Concentrator plus, Eppendorf.
12. C18 Spin Columns Pierce™ (#89870, ThermoFisher Scientific).
13. Q Exactive™ Plus Mass Spectrometer (ThermoFisher Scientific).
14. Proxeon EASY-nLC 1000 (Thermo Fisher Scientific).
15. Column oven for Nano-ESI (#PRSO-V1, Sonation).
16. Protein LoBind® 1.5 ml tubes (#0030108116, Eppendorf).
17. C18 column home-made (#ReproSil-Pur Basic C18, 1.9 µm, Dr. Maisch GmbH).
18. Maxquant software using Andromeda as a search engine (freely available on <https://www.maxquant.org/>).
19. R packages: cp4p, imp4p, DAPAR and limma, freely available on:
  - <https://cran.rstudio.com/web/packages/cp4p/index.html>,
  - <https://cran.rstudio.com/web/packages/imp4p/index.html>,
  - <https://www.bioconductor.org/packages/release/bioc/html/DAPAR.html>,
  - <https://www.bioconductor.org/packages/release/bioc/html/limma.html>

## 2.5 Protein Extraction Validation

1. SDS-PAGE on 4 to 12% NuPAGE Bis-Tris gels (#WG1401BOX, Thermo Fisher Scientific).
2. Protein Ladder 10 to 250 kDa (#26619, Thermo Fisher Scientific).
3. MOPS Migration buffer (#NP0001, Thermo Fisher Scientific).
4. NuPAGE LDS Sample Buffer 4X (#NP0007, Thermo Fisher Scientific).
5. Antioxidant NuPAGE (#NP0005, Thermo Fisher Scientific).
6. NuPAGE Sample Reducing agent 10X (#NP0004, Thermo Fisher Scientific).
7. Cellulose membranes (#10600018, GE Healthcare).
8. Whatman paper Grade 3MM Chr (#3030-917, GE Healthcare).
9. TBS solution: 20 mM Tris pH 7.4, 140 mM NaCl, 0.1% Tween20.
10. Blocking buffer: TBS solution with 5% of skimmed milk.
11. Transfer buffer: 25 mM Tris pH 8.3, 192 mM glycine, 20% ethanol. Pre-chill transfer buffer to 4°C.
12. High-Current Power Supply.
13. Vertical protein electrophoresis chamber.
14. Ice block.
15. Magnetic stir bar.
16. Magnetic stirrer.
17. Wet transfer chamber with cassettes for the gel/membrane set up.
18. Fiber pads.
19. Monoclonal Anti-β-Actin antibody (#A5441, Sigma).
20. Strep-tag antibody (#34850, Qiagen).
21. Goat Anti-Mouse Immunoglobulins/HRP (#P0447, Dako).

22. SuperSignal West Pico PLUS Chemiluminescent Substrate (#34580, Thermo Fisher Scientific).

### 3 Methods

#### 3.1 Rescue of recombinant MVs by CaCl<sub>2</sub> transfection

1. The day before transfection, plate HEK293T7-NP helper cells into 6-well plates. Cells need to be around 60% confluence the day of transfection (see **Note 6**).
2. Place all the reagents at room temperature before starting transfection.
3. Prepare the transfection reaction in a 1.5 ml Eppendorf tube by mixing:
  - a. 20 ng of pEMC-La plasmid and 5 µg of pTM2-STrEP-V or pTM2-STrEP-CH plasmid in a final volume of 131 µl with 44 µl of CaCl<sub>2</sub> 1M (125 mM final concentration).
  - b. add 175 µl of HEPES 2X.
4. Incubate for ≈ 1 min at room temperature.
5. Add the transfection mixture dropwise to the cells and incubate overnight (15h) at 37°C in a 5% CO<sub>2</sub> atmosphere.
6. The day after transfection replace media culture with fresh DMEM supplemented with 10% FBS and 1% PS.
7. Perform a heat shock by incubating the cells at 42°C for 3h and immediately return them to a 37°C incubator.
8. Two-days post transfection prepare two 100-mm dishes with Vero cells to obtain 70-80% confluency the day after seeding.
9. Three-days post transfection proceed to a co-culture step and transfer HEK293T7-NP cells to 100-mm dishes with monolayers of Vero cells and incubate for 2-7 days before collecting virus stock as soon as viral syncytia are observed in Vero cells (**Figure 1b**).

#### 3.2 Extraction of virus-host protein co-complexes

1. Seed 2 T150 flasks per condition one day before the experiment.
2. Infect HEK293T cells ( $3 \times 10^7$  cells per flask) with recombinant MV at multiplicity of infection (MOI) of 1 for 24 h (see **Note 7**). Include 2 T150 flasks with mock-infected cells (treated with media alone). Incubate at 37°C in a 5% CO<sub>2</sub> atmosphere.
3. 24h post infection place T150 flasks on ice, eliminate growth media and wash cells twice with 10 ml of cold 1X DPBS.
4. Collect the infected cells by scrapping them into 4 ml of lysis buffer per 1x T150 flask, combine lysates for each sample in one 15 ml Falcon tube. Incubate tubes on ice for 20 min with gentle mixing every 5 min.
5. Clarify cell lysates by centrifugation at 16,000g for 15 min at 4°C and transfer clarified lysates to a new 15 ml Falcon tube. Take two 100 µl aliquots for SDS-PAGE analysis and mass-spectrometry analysis of the input protein samples (see **Note 8**). Store samples at -80°C.
6. Incubate clarified cell lysates for 2h on a spinning wheel at 4°C with 200 µl of StrepTactin Sepharose High Performance beads for  $6 \times 10^7$  lysed cells (2x T150 flasks).
7. Collect Sepharose beads by centrifugation (1,600g for 5 min at 4 °C) and wash twice for 5 min on a spinning wheel with 5 ml of washing buffer.
8. After the last wash, discard supernatant leaving to 2ml of it in the tube with the beads. Resuspend beads and transfer to 2 mL Eppendorf tubes, then centrifuge again and proceed with elution step.

9. Perform a first elution with 600  $\mu\text{L}$  of elution buffer per one 2 mL Eppendorf. Incubate 15 min at 4°C on a spinning wheel.
10. Centrifuge at 1,600g for 5 minutes at 4°C to collect the eluate.
11. Perform a second elution with 600  $\mu\text{L}$  of elution buffer per one 2 mL Eppendorf. Repeat the previous steps and then mix the two eluates (total volume=1200  $\mu\text{L}$ )
12. Aliquot protein samples into three 1.5 mL Eppendorf tubes (400  $\mu\text{L}$  in each tube).
13. Precipitate eluted proteins overnight at 4°C with 55  $\mu\text{L}$  of trichloroacetic acid (12% final concentration).
14. Wash protein pellets twice with ice-cold acetone and store on -80°C (output protein samples for SDS-PAGE and mass spectrometry).

### 3.3 Assessment of protein interactors by LC-MS/MS

#### 3.3.1 Digestion

1. Add 90  $\mu\text{L}$  of denaturing buffer to the pellets to denature proteins and sonicate them.
2. Reduce with 10  $\mu\text{L}$  of the reduction buffer and alkylate with 11,1  $\mu\text{L}$  of the alkylation buffer for 1h each at room temperature in the dark.
3. Digest proteins with digestion buffer 1 for 3h / 1,000 rpm / 37°C and then digest with digestion buffer 2 overnight / 1,000 rpm / 37°C.
4. Stop digestion with 22,2  $\mu\text{L}$  of the stop buffer.
5. Desalt resulting peptides using C18 Spin Columns Pierce™ and centrifuge 3 min at 1500g.
6. Elute peptides twice with 50  $\mu\text{L}$  of Elution buffer in 1.5ml Protein LoBind® Eppendorf tubes and and centrifuge 3 min at 1500g.
7. Dry peptides using the speedvac and then resuspend in 15  $\mu\text{L}$  of Loading buffer.

#### 3.3.2 MS analysis

1. Perform an LC-MS/MS analysis of protein co-complexes on a Q Exactive™ Plus Mass Spectrometer coupled with a Proxeon EASY-nLC 1000. Set the column temperature to 60°C on the column oven for Nano-ESI.
2. Inject peptides onto a home-made 50 cm C18 column and elute with a multi-step gradient from 2 to 27% ACN in 105min, 27 to 50% ACN in 40min and 50 to 60% ACN in 10min, at a flow rate of 250 nL/min over 185 min.
3. Acquire MS data using Xcalibur software with a Top10 data-dependent method.
4. Set MS scans at a resolution of 70,000 with an AGC of 3E+06 and a maximum injection time of 20ms.
5. Set MS/MS scans at a resolution of 17,500 with an AGC of 1E+06 and a maximum injection time of 60ms. The isolation window is set to 1.6 m/z and normalized collision energy of 28 for HCD fragmentation.
6. Set an underfill ratio of 1.0% for an intensity threshold of 1.7E+05.
7. Reject unassigned precursor ion charge states as well as 1, 7, 8 and >8 charged states.
8. Disable peptide match.
9. Set a dynamic exclusion of 45s.

#### 3.3.3 Database search

1. Analyze RAW data using MaxQuant software including the appropriate database of the virus and the host. Proteomes fasta files are available on Uniprot: UP000180902 for



MV Schwarz vaccine strain (<https://www.uniprot.org/proteomes/UP000180902>) and UP000005640 for human reference proteome (<https://www.uniprot.org/proteomes/UP000005640>).

2. Set variable (methionine oxidation and N-terminal acetylation) and fix (cysteine carbamidomethylation) modifications.
3. Allow a maximum of two missed cleavages for theoretical tryptic peptides with a minimum peptide length of 7 amino acids.
4. Adjust the false discovery rate (FDR) for peptide and protein identification to 0.01. A unique peptide per protein group is required for the identification of protein.
5. Set the main search peptide tolerance to 4.5 ppm and to 20 ppm for the MS/MS match tolerance.
6. Enable the second peptides to identify co-fragmentation events.
7. Select the match between runs (MBR) option with a match time window of 0.7 min for an alignment time window of 20 min (see **Note 9**).
8. Perform the quantification using the XIC-based LFQ algorithm with the Fast LFQ mode. Accept unique and razor peptides, included modified peptides, with at least 2 ratio counts for quantification (see **Note 10**).

#### 3.3.4 Statistical analysis

1. Remove the proteins categorized as 'reverse' and 'potential contaminants' in MaxQuant outputs.
2. Only consider proteins with at least 3 quantified values in an experimental condition for further analysis (see **Note 11**).
3. Process intensities of proteins using a log<sub>2</sub> transformation, and normalize them by median centering within conditions, or any other normalization method available in the R package DAPAR.
4. Consider proteins that are quantified in only one condition out of the two compared conditions as 'differentially abundant proteins'.
5. Fill missing values of the remaining proteins using the R package imp4p.
6. Perform the statistical analysis using a paired t-test with the limma R package.
7. Perform an adaptive Benjamini–Hochberg correction of the P values using the R package cp4p.
8. Consider proteins that are associated to a fold change above 2.0 and to an adjusted p-value inferior to FDR of 1% as 'significantly differentially abundant proteins'.
9. Generate the lists of 'differentially abundant proteins': the proteins that appear to be only quantified in a condition (Step 4) and the proteins that meet the fold change and FDR thresholds (Step 8).

#### 3.4 Protein-protein interaction validation by WB analysis

SDS-PAGE analysis of protein extracts before (input: sample acquired in section 3.2 after step 5) and after (output: sample acquired in section 3.2 after step 14) affinity chromatography purification.

1. Resuspend output sample in 30 µl 8 M urea, 4% SDS final solution.
2. Prepare protein samples for loading by mixing 5 µl of sample buffer with 2 µl of reducing agent and 13 µl of the output or input samples (20 µl final volume).
3. Heat for 5 min at 98°C.



4. Load 20  $\mu\text{l}$  of the sample onto 4 to 12% NuPAGE Bis-Tris gel and 10  $\mu\text{l}$  of a Protein Ladder.
5. Add 1 ml of antioxidant for each 400 ml of MOPS 1X running buffer and run the gel at 100-120V for 1-1.5 h.
6. Transfer proteins to cellulose membrane at 100V for 1h. Pre-wet in chilled transfer buffer each component before setting up the gel/membrane. Set up the gel/membrane sandwich by placing into the cassette in this order (from cathode to anode): a fiber pad, a whatman paper, the gel, the cellulose membrane, a whatman paper and a fiber pad (**Figure 4**) (see **Note 12**). Place the transfer chamber with a magnetic stir bar and an ice block inside, on the top of a magnetic stirrer and proceed to transfer.
7. Block membranes in TBS buffer with 5% skimmed milk for 30 min at room temperature.
8. Incubate one hour with  $\alpha$ -Strep-Tag antibody (1:10,000 dilution in TBS 5% skimmed milk) at room temperature and gentle agitation (see **Note 13**)
9. Wash membranes twice with TBS buffer for 5 min at room temperature.
10. Incubate with HRP-conjugated secondary antibody (1:10,000 dilution in TBS 5% skimmed milk) for 1 h at room temperature and gentle agitation.
11. Wash membranes twice with TBS buffer for 5 min at room temperature.
12. Prepare SuperSignal West Pico PLUS Chemiluminescent Substrate by mixing equal parts of both components.
13. Incubate the membrane with the substrate working solution for 5 minutes at room temperature.
14. Image the membrane using an imaging system or an X-ray film.

#### 4 Notes

**Note 1:** ATU positions differ in their expression level due to the paramyxovirus (and other mononegavirales[22,23]) mechanism of transcription. Viral genes are transcribed in succession from the 5' to 3' extremities. L polymerase stops and restarts transcription at intergenic regions. However, as the efficiency of re-initialization is not 100%, mRNAs are produced in decreasing amounts from the 5' to 3' end of the genome. Consequently, a gradient of viral mRNA transcription is observed from N to L genes [24,25]. For this reason, cloning in the ATU1 position can lead to gene overexpression and interfere with the replication of the recombinant MV (lower viral fitness compared to the parental Schwarz strain). ATU2 position is higher expressed than ATU3 but less than ATU1 and these aspects need to be considered when deciding position of a gene insertion into the MV vector.

**Note 2:** The protocol can be adapted to other cell lines as HeLa cells (ATCC #CCL-2) or A549 cells (ATCC #CCL-185).

**Note 3:** Measure DNA concentration by spectrophotometry and adjust to about 1  $\mu\text{g}/\mu\text{l}$ . It is important to obtain high-purity transfection-grade plasmids, since residual ethanol, solvents or other compounds can inhibit the successful rescue of recombinant viruses.

**Note 4:** Negative control virus expressing a non-relevant protein (e.g., mCherry) should be used in parallel in each experiment to assess non-specific protein-protein interactions.

**Note 5:** MOPS Lysis and Washing buffers should be kept at 4°C protected from the light.  $\beta$ -Mercaptoethanol and Complete Protease Inhibitor Cocktail should be added just before the experiment.

**Note 6:** The confluence of HEK293T7-NP cells during transfection for virus rescue should be between 50-70%. This ensures that they can still grow exponentially.

**Note 7:** Additional setting experiments to determine the % of infected cells with different MOIs should be performed to have from 50 to 90% infected cells 24h post-infection (see also **Note 2**).

**Note 8:** The input sample for mass spectrometry should be treated following steps 13 and 14 of 3.2 section.

**Note 9:** Set a distinct fraction number between the 2 conditions to avoid misleading identification with MBR option.

**Note 10:** Because the bait and Mock conditions are not similar, it is better to use skip normalization.

**Note 11:** Each experimental condition (peptides derived from rMV STrEP-V-infected cells, peptides derived from rMV STrEP-CH-infected cells, and peptides derived from non-infected cells) should be tested in triplicate and proteins with at least 3 quantified intensity values should be retained for analysis.

**Note 12:** When putting the cellulose membrane on top of the gel, be sure to eliminate any air bubbles that may be trapped underneath.

**Note 13:** Use Anti- $\beta$ -Actin at 1:10,000 as a total protein loading control.

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Conflict of Interest: The authors declare no conflicts of interest.

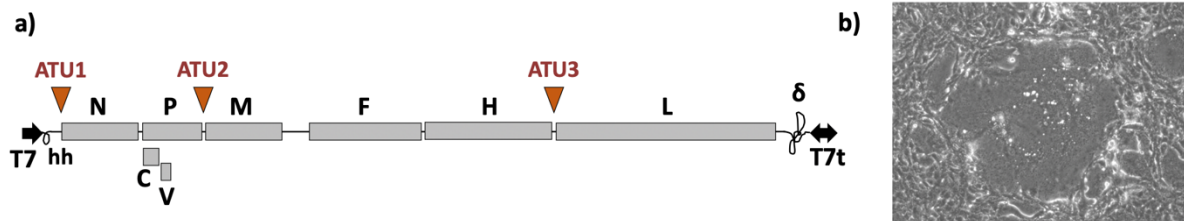
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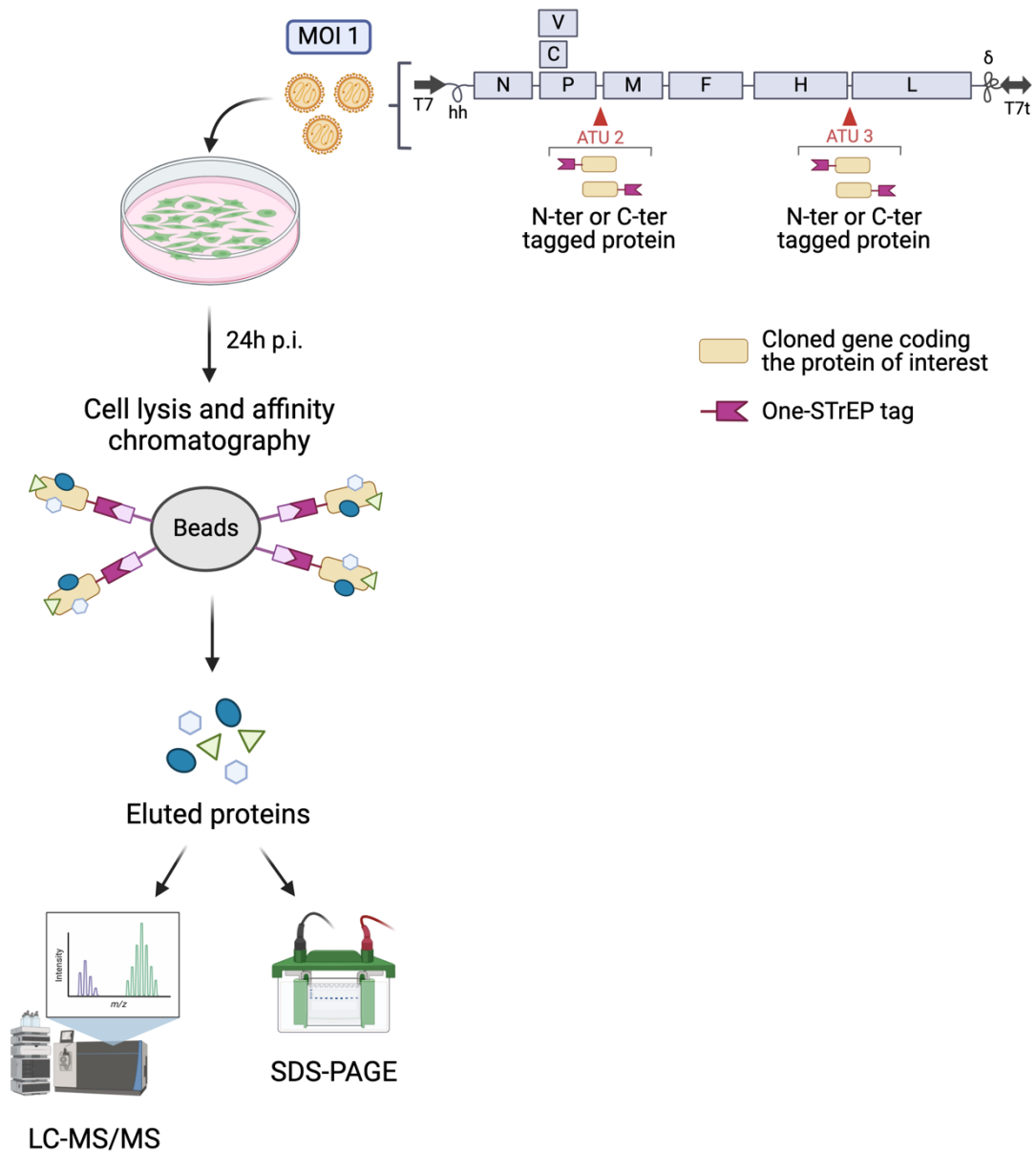
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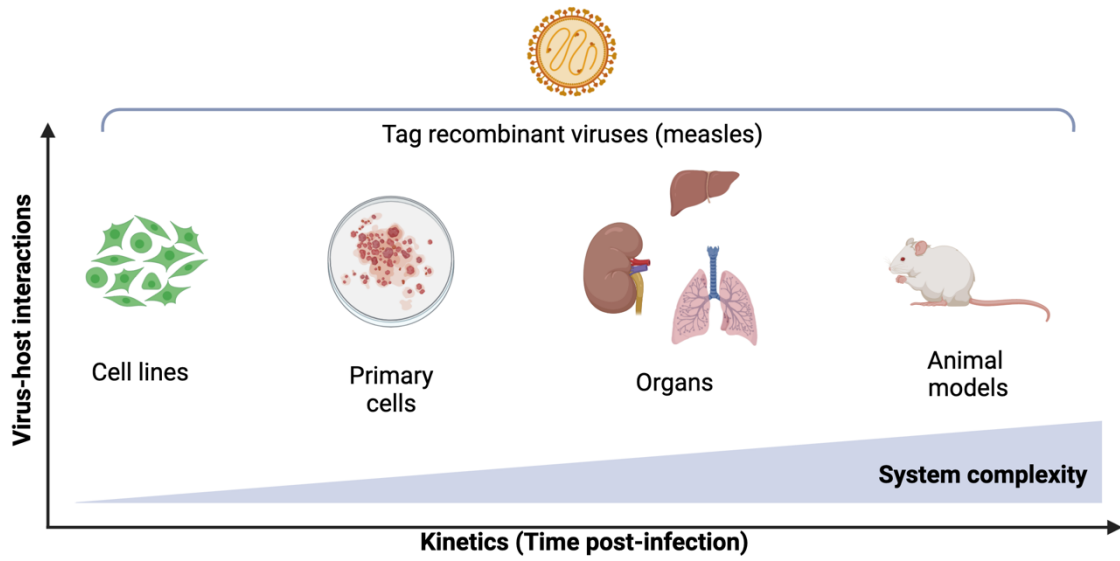
## Figures



**Figure 1: a) Schematic representation of cloned measles virus vector genome (pTM-MV Schw) containing additional transcription units (ATU).** Structural proteins genes (N, nucleoprotein; P, phosphoprotein; M, matrix protein; F, fusion protein; H, hemagglutinin; L, large polymerase), and accessory proteins genes (C and V proteins) are shown. T7 is a T7 RNA polymerase promoter sequence, hh is a hammerhead ribozyme, T7t is a T7 RNA Polymerase termination signal,  $\delta$  is a hepatitis delta ribozyme. **b) Syncytia formation on Vero cells infected with a recombinant measles virus.**

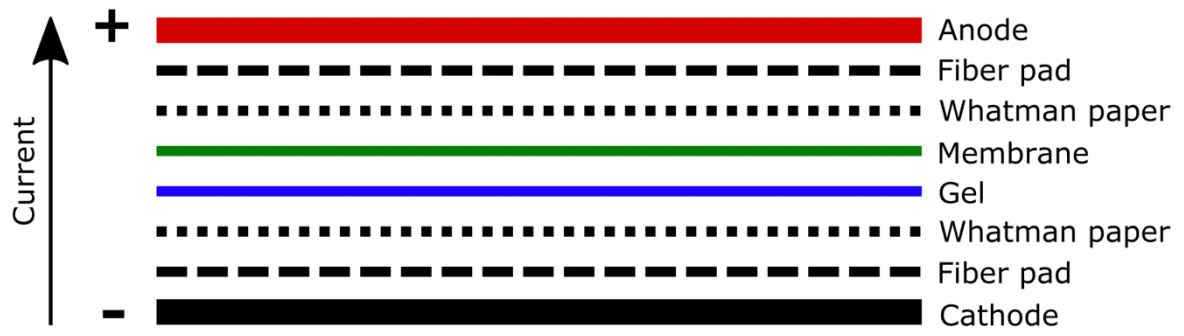


**Figure 2: Summary of the protocol used to detect protein-protein interactions.** Suitable cell lines are infected at MOI 1 with recombinant measles virus expressing One-STrEP tagged proteins. 24 h post-infection, protein co-complexes are purified by affinity chromatography using StrepTactin Sepharose beads. Protein complexes are released from the beads using biotin buffer. Eluted proteins are analyzed by LC-MS/MS or resolved by SDS-PAGE.



**Figure 3:** Global diagram illustrating the variety of biological samples that can be used to study virus-host interactions with AP-MS described method following infection with recombinant viruses expressing One-STrEP tagged proteins of interest.





**Figure 4:** Diagram of Western Blot transfer stack