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Preparation and imaging of enriched Golgi from GolgiTAG-IP using Transmission Electron Microscopy

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ABSTRACT

Transmission electron microscopy (TEM) is a tool used to image, in good resolution, the structure of organelles within the cell. Available protocols are designed to image structures within fixed intact cells. Here, we described a protocol where Golgi, isolated from cells by GolgiTAG immunoprecipitation (IP) (as described in ddx.doi.org/10.17504/protocols.io.6qpvrdjrogmk/v1), can be prepared and imaged using TEM. This protocol can also be used to image any organelles isolated using various organelle-IP protocols that are available.

ATTACHMENTS

ivgnbgrdf.docx

DOI

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MATERIALS TEXT

Materials:

- ☒ Paraformaldehyde **Sigma**
- **Aldrich Catalog #158127**
- ☒ Glutaraldehyde EM Grade 25% **Sigma**
- **Aldrich Catalog #G5882-50ML**
- ☒ Sodium cacodylate trihydrate **Merck Millipore**
- **Sigma Catalog #C0250**
- Osmium tetroxide (Agar. Catalog # R1023)
- ☒ Sodium ferrocyanide decahydrate **Sigma**
- **Aldrich Catalog #13425**
- Tannic acid (BDH. Catalog # 30337)
- Uranyl acetate (Agar. Catalog # R1260A)
- ☒ Durcupan™ ACM **Sigma**
- **Aldrich Catalog #44611**
- ☒ Lead(II) citrate tribasic trihydrate **Sigma**
- **Aldrich Catalog #15326**
- ☒ Sodium citrate monobasic **Sigma**
- **Aldrich Catalog #71497**
- ☒ Sodium hydroxide (NaOH) **Sigma**
- **Aldrich Catalog #S5881**
- Potassium chloride (KCl) (VWR. Catalog# 267264)
- ☒ Potassium phosphate monobasic **Sigma –**
- **Aldrich Catalog #P5379**
- Calcium chloride (Calbiochem. Catalog# 208291)
- ☒ (R)-( )-Propylene oxide **Sigma**
- **Aldrich Catalog #540048**
- ☒ Ethyl alcohol, 200 proof, anhydrous, ≥99.5% **Sigma**
- **Aldrich Catalog #459836**

Buffer:

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- KPBS ([M]136 millimolar (mM) KCl, [M]10 millimolar (mM) KH_2PO_4. Adjust to pH 7.25 with KOH).
- [M]0.4 Molarity (M) sodium cacodylate buffer (17.12 g sodium cacodylate dissolved in water. Adjust to pH7.4 with HCl).
- Reynold lead citrate (1.33 g lead citrate, 1.76 g sodium citrate; 8 mL 1N NaOH in 50 mL water).

Equipment:

- Pierce™ Anti-HA Magnetic Beads **Thermo Fisher Catalog #88837**
- DynaMag™- Spin Magnet **Thermo Fisher Catalog #12320D**
- Microcentrifuge with thermostat (VWR Micro Star 17R. S/N 42209232)
- Scalpel
- P1000 pipette tips
- Pasteur pipette
- Oven (TAAB. I064)
- Leica UCT ultramicrotome (Leica)
- Clear glass with solid top black phenolic 14B rubber lined cap **VWR international Ltd Catalog #215-3571**
- JEOL 1200EX TEM using SIS III camera

Method 2d 5h 4m

1 Perform GolgiTAG-IP as previously described in dx.doi.org/10.17504/protocols.io.6qpvrdjrogmk/v1

2 1h

After last wash of beads with KPBS, fix the beads in 1 mL solution containing 4% (w/v) paraformaldehyde and 2.5% (v/v) glutaraldehyde in [M]0.1 Molarity (M) sodium cacodylate buffer dilute in water for 01:00:00 at Room temperature .

3 Pellet beads with magnet DynaMagTM Spin Magnet (or equivalent) and remove the supernatant.

4

Wash beads pellet three times in 1 mL [M]0.1 Molarity (M) sodium cacodylate buffer, pelleting the beads

with magnet after each wash.

5 1m

After third wash, spin down beads in tabletop centrifuge at **1000 x g** for **00:01:00** . Pellet should be tightly packed.

6

Use a needle to gently dislodge the pellet and use a Pasteur pipette to remove the pellet to a clean glass vial. (Note: All steps are carried out in a glass vial).

7 1h

To ensure rapid dehydration and embedding, cut pellet with scalpel into approximately $1mm^3$ pieces, then post-fix in **1 mL** 1% (w/v) Osmium tetroxide with 1.5% (w/v) sodium ferrocyanide in **0.1 Molarity (M)** sodium cacodylate buffer for **01:00:00** at **Room temperature** .

8

Wash pellets three times in **0.1 Molarity (M)** sodium cacodylate buffer, by adding the buffer, allowing the pellets to settle and gently taking out supernatant with P1000 tip.

8.1 1m

Wash pellets three times in **0.1 Molarity (M)** sodium cacodylate buffer for **00:01:00** (1/3).

8.2 1m

Wash pellets three times in **0.1 Molarity (M)** sodium cacodylate buffer for **00:01:00** (2/3).

8.3 1m

Wash pellets three times in **0.1 Molarity (M)** sodium cacodylate buffer for **00:01:00** (3/3).

9 After the third wash, wash with water three times (as in step 8), then resuspend in 1% w/v tannic acid and 1% w/v uranyl acetate diluted in water for **01:00:00** . Remove supernatant. 1h

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10 1h 10m

Dehydrate pellet through alcohol series:
50% ethanol for **00:10:00**
70% ethanol for **00:10:00**
80% ethanol for **00:10:00**
90% ethanol for **00:10:00**
95% ethanol for **00:10:00**
then twice in 100% ethanol for **00:10:00** (1/2)
100% ethanol for **00:10:00** (2/2)

Use a P1000 pippete to gently remove ethanol after each series.

11 Further dehydrate in 100% propylene oxide twice for 10 min.

11.1 Further dehydrate in 100% propylene oxide twice for **00:10:00** (1/2). 10m

11.2 Further dehydrate in 100% propylene oxide twice for **00:10:00** (2/2). 10m

12 10m

Resuspend pellet into 50% v/v propylene oxide and 50% v/v Durcupan resin **Overnight** .

13 Open glass vial to allow propylene oxide to evaporate (This will take approximately **01:00:00**). 1h

14 To embed pellet in resin, resuspend in 100% Durcupan resin.

15 Embed polymerise resin in an oven at **60 °C** for **48:00:00** . 2d

Cut sections of polymerised embedded resin with Leica UCT ultramicrotome.

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16 Sections should be 70-100nm thick

17 Stain sections with 3% aqueous uranyl acetate for **00:20:00** . 20m

18 Stain with Reynold lead citrate for **00:20:00** . 20m

19

Image stained sections on JEOL 1200EX TEM using SIS III camera.

Note: Due to the cutting and many centrifugation steps, it is expected that the magnetic particles dissociate from the plastic bead core.)

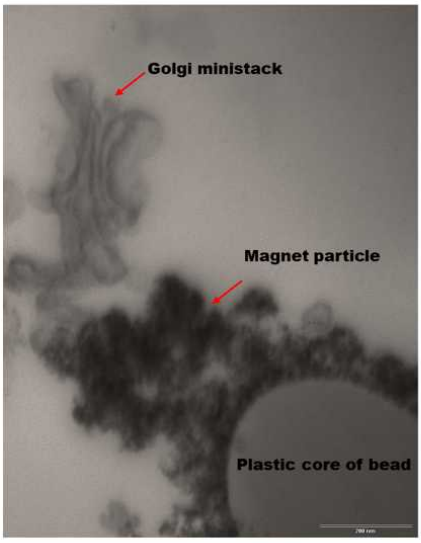

Figure 1: Transmission electron micrograph of enriched Golgi. Intact Golgi ministack is shown to be captured by GolgiTAG-IP. Scale bar 200nm

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