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

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Protocol status: Working
We use this protocol and it's working

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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

DOCX

ivgnbgrdf.docx
804KB

Materials

Materials:

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

Buffer:

- KPBS ([M] 136 millimolar (mM) KCl, [M] 10 millimolar (mM) KH_2PO_4. Adjust to pH 7.25 with KOH).
- [M] 0.4 Mass Percent 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 (Avantor) 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 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 Mass Percent sodium cacodylate buffer dilute in water for 01:00:00 at Room temperature . 1h

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 Mass Percent sodium cacodylate buffer, pelleting the beads with magnet after each wash.

5 After third wash, spin down beads in tabletop centrifuge at 1000 x g for 00:01:00 . Pellet should be tightly packed. 1m

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 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 [M] 0.1 Mass Percent sodium cacodylate buffer for 01:00:00 at Room temperature . 1h

8 Wash pellets three times in [M] 0.1 Mass Percent sodium cacodylate buffer, by adding the buffer, allowing the pellets to settle and gently taking out supernatant with P1000 tip.

8.1 Wash pellets three times in [M] 0.1 Mass Percent sodium cacodylate buffer for 00:01:00 (1/3). 1m

8.2 Wash pellets three times in [M] 0.1 Mass Percent sodium cacodylate buffer for 00:01:00 (2/3). 1m

8.3 Wash pellets three times in [M] 0.1 Mass Percent sodium cacodylate buffer for 00:01:00 (3/3). 1m

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

10 Dehydrate pellet through alcohol series: 1h 10m
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)

Note

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 Resuspend pellet into 50% v/v propylene oxide and 50% v/v Durcupan resin Overnight . 10m

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

16 Cut sections of polymerised embedded resin with Leica UCT ultramicrotome.

Note

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

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

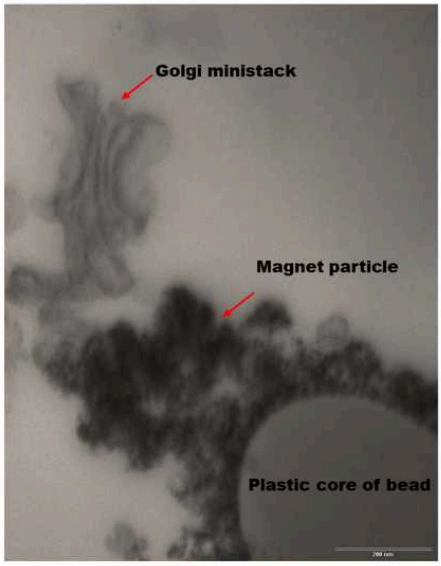

Figure 1: Transmission electron micrograph of enriched Golgi. Intact Golgi ministack is shown to be captured by GolgiTAG-IP. Scale bar 200nm