Jun 15, 2019

Scanning electron microscopy

PLOS One

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Working dx.doi.org/10.17504/protocols.io.zz4f78w

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EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0218029

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Lin S, Miao Y, Su S, Xu J, Jin L, Sun D, Peng R, Huang L, Cao J (2019) Comprehensive analysis of Ogura cytoplasmic male sterility-related genes in turnip (*Brassica rapa* ssp. *rapifera*) using RNA sequencing analysis and bioinformatics. PLoS ONE 14(6): e0218029. doi: 10.1371/journal.pone.0218029

MATERIALS TEXT

2.5% glutaraldehyde in phosphate buffer (pH7.0), phosphate buffer (pH7.0), 1% OsO_4 in phosphate buffer (pH7.0), ethanol (50%, 70%, 80%, 90%, 95% and 100%), iso-amyl acetate, liquid CO_2, gold-palladium.

1 Double fixation: The specimen was first fixed with 2.5% glutaraldehyde in phosphate buffer (pH7.0) for more than 4 hours; washed three times in the phosphate buffer; then postfixed with 1% OsO_4 in phosphate buffer (pH7.0) for 1 hour and washed three times in the phosphate buffer.

2 Dehydration: The specimen was first dehydrated by a graded series of ethanol (50%, 70%, 80%, 90%, 95% and 100%) for about 15 to 20 minutes at each step, transferred to the mixture of alcohol and iso-amyl acetate (v:v=1:1) for about 30 minutes, then transferred to pure iso-amyl acetate for about 1 hour. In the end, the specimen was dehydrated in Hitachi Model HCP-2 critical point dryer with liquid CO_2.

3 Coating and observation: The dehydrated specimen was coated with gold-palladium in an Eiko Model IB5 ion coater (Eiko Engineering Company, Ibaraki, Japan) and observed in a Hitachi Model TM-1000 scanning electron microscope (Tokyo, Japan).