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Paraffin embedding of tissue specimen | HuBMAP | JHU-TMC V.2

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A.M. Braxton, A.L. Kiemen, M.P. Grahn, A. Forjaz, J. Parksong, J.M. Babu, J. Lai, L. Zheng, N. Niknafs, L. Jiang, H. Cheng, Q. Song, R. Reichel, S. Graham, A.I. Damanakis, C.G. Fischer, S. Mou, C. Metz, J. Granger, X.-D. Liu, N. Bachmann, Y. Zhu, Y.Z. Liu, C. Almagro-Pérez, A.C. Jiang, J. Yoo, B. Kim, S. Du, E. Foster, J.Y. Hsu, P.A. Rivera, L.C. Chu, D. Liu, E.K. Fishman, A. Yuille, N.J. Roberts, E.D. Thompson, R.B. Scharpf, T.C. Cornish, Y. Jiao, R. Karchin, R.H. Hruban, P.-H. Wu, D. Wirtz, and L.D. Wood, "3D genomic mapping reveals multifocality of human pancreatic precancers", Nature (2024)

A.L. Kiemen, A. Forjaz, R. Sousa, K. Sang Han, R.H. Hruban, L.D. Wood, P.H. Wu, and D. Wirtz, "High-resolution 3D printing of pancreatic ductal microanatomy enabled by serial histology", Advanced Materials Technologies 9, 2301837 (2024)

T. Yoshizawa, J. W. Lee, S.-M. Hong, D.J. Jung, M. Noe, W. Sbijewski, A. Kiemen, P.H, Wu, D. Wirtz, R.H. Hruban, L.D. Wood, and K. Oshima. "Three-dimensional analysis of ductular reactions and their correlation with liver regeneration and fibrosis", Virchows Archiv (2023).

A.L. Kiemen, A.I. Damanakis, A.M. Braxton, J. He, D. Laheru, E.K. Fishman, P. Chames, C. Almagro Perez, P.-H. Wu, D. Wirtz, L.D. Wood, and R. Hruban, "Tissue clearing and 3D reconstruction of digitized, serially sectioned slides provide novel insights into pancreatic cancer", Med 4, 75-91 (2023)

A. Kiemen, Y. Choi, A. Braxton, C. Almagro Perez, S. Graham, M. Grahm, N., N. Roberts, L. Wood, P. Wu, R. Hruban, and D. Wirtz, "Intraparenchymal metastases as a cause for local recurrence of pancreatic cancer", Histopathology 82: 504-506 (2022)

A.L. Kiemen, A.M. Braxton, M.P. Grahn, K.S. Han, J.M. Babu, R. Reichel, A.C. Jiang, B. Kim, J. Hsu, F. Amoa, S. Reddy, S.-M. Hong, T.C. Cornish, E.D. Thompson, P. Huang, L.D. Wood, R.H. Hruban, D. Wirtz and P.H. Wu, "CODA: quantitative 3D reconstruction of large tissues at cellular resolution", Nature Methods 19: 1490-1499 (2022)

K.S.Han, I. Sander, J. Kumer, E. Resnick, C. Booth, B. Starich, J. Walston, A.L. Kiemen, S. Reddy, C. Joshu, J. Sunshine, D. Wirtz, P.-H. Wu "The microanatomy of human skin in aging." bioRxiv (2024): 2024-04.

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We use this protocol and it's working

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Abstract

The standard for tissue embedding is to ensure tissue samples are appropriately oriented and embedded on the "cut edge" or flattest edge available. Histology laboratories that receive mostly soft tissues prefer a "softer" paraffin as opposed to laboratories that receive hard specimens, such as skin, which would require a "harder" paraffin.



Troubleshooting



Orientation

1 The standard for tissue embedding is to ensure tissue samples are appropriately oriented and embedded on the "cut edge" or flattest edge available

Equipments

2 Paraffin embedding equipment includes paraffin pots for melting paraffin pellets, the embedding console unit and the cryo console unit

Type of paraffin

- the type of paraffin used in your laboratory should be optimal for your needs. Paraffin comes in many types. These types are differentiated by how much (if any) polymer is added to the paraffin, which determines the final quality of the paraffin block.
- 4 Histology laboratories that receive mostly soft tissues prefer a "softer" paraffin as opposed to laboratories that receive hard specimens, such as skin, which would require a "harder" paraffin.
- Each paraffin type comes with a specification sheet which identifies what the "melting point" range is for that particular paraffin. It is important to match this temperature range with the proper temperature settings used in the tissue processor, paraffin pot and embedding center. Your paraffin vendor can provide valuable information to help you make this decision.

Melting and replenishing paraffin

- Most paraffin is provided in bags of "pellets". Most laboratories have one or more "paraffin pots" used to melt the hard pellets into liquid form by heating. Regulations require that the temperatures of the paraffin pots be monitored and recorded on a daily basis. This ensures that the paraffin in use is neither under heated, nor over heated, which could result in sub-optimal results.
- The melted paraffin in the paraffin pots is used to replace paraffin used in tissue processors, and to replenish paraffin in the embedding console. Some laboratories may use different paraffins for processing and embedding; however, most laboratories use the same paraffin type in both locations. Alternatively, paraffin pellets may be added directly to the paraffin holding tank of the embedding console. In every scenario, temperature ranges must be determined and the temperatures monitored, as stipulated by the federal CLIA regulations and the College of American Pathologists (CAP: COM.30775).



Maintenance

- 8 During the annual preventative maintenance program, both paraffin pots and embedding console paraffin holding tanks must be inspected for properly functioning heating elements. These, in turn, provide an accurate temperature range, set specifically for your laboratory's paraffin type. In addition, the on board thermometer measuring device for each of the units must also be calibrated, to ensure the resulting temperature reading is accurate (CAP: COM.30700, 30725). These readings must be recorded daily and is a regulatory requirement (CAP: COM.30750).
- 9 In a similar fashion, the cryo console temperature measurement mechanism must be inspected and calibrated to ensure accurate temperature readings. The service technician will also examine the wiring and switches to ensure safe operation by laboratory personnel. Melting ice from the cryo console poses a hazard if allowed to drain into the electrical connections of the equipment.
- 10 The service technician will also examine the integrity of the paraffin holding tank, combined with the dispensing switch and related tubing. Sometimes a leak can occur in one of these connections. The result is a paraffin buildup inside the dispensing console, out of view of the operator. If allowed to leak, the paraffin can build up to a point that causes failure of the unit.
- 11 Light sources on the dispensing unit will also be examined by the service technician. In the event that there are light bulbs which can easily be replaced, the technician can leave spare bulbs in the event that one is needed. This can be an easy operation carried out by laboratory personnel.

Protocol references

Avantik, April 3 2024, Part 4: Embedding | Avantik (avantik-us.com)