NCI Patient-Derived Models Repository (PDMR) Material Information

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Please check for revision status of the SOP at

https://pdmr.cancer.gov/sops/

PDMR NCI Patient-Derived Models Repository
An NCI Precision Oncology Initiative℠ Resource

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Information on Patient-Derived Xenograft (PDX) Material

- Upon receipt of viably cryopreserved material, vial(s) should be **immediately placed in LN2 Vapor Phase** (-180°C)**; Do not store at -80°C**.
- A single viably cryopreserved PDX fragment vial (sufficient tissue to implant into 2-5 NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice. Distribution lots are comprised of multiple tumor-bearing mice with the maximum passage for the distribution lot defined in the PDMR database.
    - Material cannot be requested from a specific PDX (sample) lineage.
    - Viably cryopreserved PDX tumor fragment distribution lots will be provided for those models that (1) are proven to be of human origin, (2) have histopathology consistent with the model, (3) have at least 95% concordance among the nonsynonymous NCI Cancer Gene Panel variants with an allele frequency > 10% with P0 (NCI defines P0 as the first mouse passage implanted with the original human specimen), (4) are tumorigenic following cryopreservation, (5) are free of a panel of human and rodent pathogens, and (6) have STR sequence homology with the P0 passage.
- DNA, RNA, and fresh-frozen tumor fragments for protein extraction are generated from tumors initiated from the distribution lot.
- A flash-frozen DNA vial (single vial from a PDX tumor no higher than the listed maximum passage for the distribution lot; approximately 2-3 µg DNA in at least 10 µL prepared using Qiagen's DNA/RNA AllPrep Mini Kit [cat#: 80204])
- A flash-frozen RNA vial (single vial from a PDX tumor no higher than the listed maximum passage for the distribution lot; approximately 2-3 µg RNA in at least 10 µL prepared using Qiagen's DNA/RNA AllPrep Mini Kit [cat#: 80204]). RNA quality is periodically assessed using an Agilent 2100 Bioanalyzer and vials are maintained if the RIN is >5. For those interested in doing RNASeq, we would recommend requesting a flash-frozen fragment and performing an independent extraction.
- A fresh-frozen PDX fragment vial for protein extraction (single vial with a 30-60 mg fresh-frozen PDX tumor no higher than the listed maximum passage for the distribution lot).

PDX Fragment Limitations and Caveats:

- The PDMR SOP page (https://pdmr.cancer.gov/sops/default.htm) contains the required instructional documentation for thawing, implanting, passaging, banking, and cryopreservation of PDX material.
- PDX material can grow more slowly than the growth curves of viably passaged material posted in the PDMR database when implanted from a cryopreserved fragments; some models can take as long as 200-300 days from cryopreservation before tumor is of sufficient size for passage (1000-2000 mm3).

- Some models have stably low tumor content (e.g., 30%); the remainder being murine stroma. The Pathology Report view in the PDMR database can be used to view human versus stromal content in representative PDX tumors for the model: https://pdmdb.cancer.gov/pls/apex/f?p=101:54.
- NCI PDMR passages tumors using PDX fragments; therefore, tumor heterogeneity can be observed across different PDX lineages within a model. The PDMR cannot guarantee which fragment a site will receive. For instance, there may be variation in differentiation level of the tumor (e.g., for a stomach adenocarcinoma in the signet ring cell content).
- NCI PDMR provided PDX pathology, WES, RNASeq, etc in the PDMR database are **representative** of the model. Recipients should perform their own analysis and characterization on PDXs they generate. STR profiles are provided for all models in the PDMR database and should be used for model authentication.
- DNA, RNA, and protein vials are from PDX tumors which are a mixture of human tumor and mouse stroma; these are not pure human extractions. The mouse strain used for PDX growth is a NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) strain; the whole exome sequence (*.vcf) for the colony of NSG mice used by NCI PDMR can be found on the SOP page under the Genomics section.
- Recipients should perform their own model validation once cells have been expanded at their site to ensure the model matches that is distributed by the PDMR. STR profiles are provided for all models in the PDMR database at the patient record level and should be used for model authentication.

Information on In Vitro Material

- Upon receipt of viably cryopreserved material, vial(s) should be **immediately placed in LN2 Vapor Phase** (-180°C); **Do not store at -80°C**.
- All NCI Patient-Derived Models Repository (PDMR) in vitro and organoid cultures are tested for sterility and confirmed mycoplasma free.
- Viably cryopreserved Patient/PDX-Derived Organoid cultures (PDOrg) contain a minimum of 1-5 x 105 cells.
    - PDOrgs are guaranteed for experimental use for ≥10 passages when maintained in the recommended defined Media + Y compound + basement matrix following the PDMR SOPs. The PDMR has not taken most cultures beyond this point due to limited incubator space; this does not mean they will not grow for additional passages.
- Viably cryopreserved Patient/PDX-Derived Tumor Cultures (PDCs) contain a minimum of 7.5 x 105 cells.
    - PDCs are guaranteed for experimental use for ≥20 passages when maintained in the recommended defined Media + Y compound following the PDMR SOPs. The PDMR has not taken most cultures beyond this point due to limited incubator space; this does not mean they will not grow for additional passages.
- Viably Cancer-Associated Fibroblasts (CAFs) cultures contain a minimum of 7.5 x 105 cells.
    - **CAFs have a finite lifespan in vitro**. CAFs are guaranteed for experimental use for up to 3 passages when maintained on Matrigel-coated surface in the recommended defined Media + Y compound. Additional population doublings and subcultures are possible, but overall fitness of culture may deteriorate with subsequent passages.
- PDOrgs, PDCs, and CAFs are non-transformed cells. Cultures are maintained at early passage and no clonal selection is performed so that the maximum heterogeneity possible is maintained in the culture within the limitations of model generation.
- All PDOrgs and PDCs are tested for ability to generate a cell-line derived xenograft (CLX) in NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice, though as with traditional cell line models, we expect that some models will be unable to generated CLXs. CAFs are also tested for to ensure they fail to form a CLX when implanted into NSG mice.
- Model Derivation – Order of Complexity
    - In vitro/organoid culture derivation is always attempted from patient material. If this fails, we move downward in complexity and attempt to generate in vitro/organoid models from multiple PDX sources.
    - Occasionally this also fails, and if needed we will attempt to generate a PDC from a PDOrg; never in the reverse order. This is all in an attempt to generate all model types for each original patient tumor specimen.

- We clearly define the origin of all cultures in the PDMR database so that researchers can make an informed decision on what will best address their research goals. A schematic of the model development process is outlined in more detail on the PDMR Models page (https://pdmr.cancer.gov/models/default.htm).

In Vitro Culture Limitations and Caveats

- The PDMR SOP page (https://pdmr.cancer.gov/sops/default.htm) contains the required instructional documentation for thawing, culturing, passaging, banking, and cryopreservation of PDC, PDOrg, and CAF cultures.
- A model-specific Certificate of Analysis (COA) will be provided with the distributed material which contains the growth characteristics, subculturing conditions, and defined Media requirements.
- The COA and the PDMR SOPs should be used to ensure culture success – these models should not be treated like traditional in vitro cultures (e.g., HeLa, MCF7).
- Do not thaw cells until the defined Media has been prepared and PDMR SOPs for thawing, banking, and cryopreserving material have been read in total. Failure to follow these SOPs will likely result in loss of the cell culture and a free replacement vial will not be provided.
- Alternate media conditions or sub-culture conditions should not be attempted until a cryopreserved master cell stock (MCS) in the recommended defined media has been established. Details on how to establish a MCS are provided in the SOPs.
- Recipients should perform their own model validation once cells have been expanded at their site to ensure the model matches that is distributed by the PDMR. STR profiles are provided for all models in the PDMR database at the patient record level and should be used for model authentication.

Contact Us

- NCI Patient-Derived Models Repository - NCI_PDM_Repository@mail.nih.gov