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**Parkinson’s Progression Marker Initiative (PPMI)**

**Cell Lines Manual of Procedures**

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**Parkinson’s Progression Marker Initiative (PPMI)**

**Goals and Background of PPMI Cell Lines**

While the initial goal of PPMI was to collect standardized longitudinal samples with corresponding clinical data for use by qualified researchers to advance biomarker validation studies, PPMI has since expanded to generate fibroblasts and induced pluripotent stem cell (iPSC) lines from a subset of PPMI subjects.

The primary objective is to develop a bank of fibroblasts and iPSC lines from skin biopsies and blood samples obtained from PPMI study subjects in order to create a cellular disease model in Parkinson’s disease (PD) with the goals of 1) furthering understanding of disease origin and underlying causes of PD and 2) enabling future studies of disease-modifying therapies. The Michael J. Fox Foundation (MJFF) is confident that PPMI cell lines and clinical data can be leveraged together for biomarker research, drug screening, and disease modeling.

Cells lines are derived from participants who have idiopathic PD, healthy control (HC) subjects, participants with clinical risk factors for PD, and participants with and without PD who have genetic risk factors for PD (GBA1, LRRK2, and SNCA mutations). This diversity of sources, and the corresponding clinical, imaging, and biosample data, make these cell lines an unparalleled resource for the study of PD.

**Cell Line Derivatives**

**Pilot Study**

Fibroblasts and iPSCs derived from skin biopsies from 20 PD subjects and 5 HC subjects are available from an initial pilot study performed with the New York Stem Cell Foundation (NYSCF). Skin biopsies obtained and shipped to NYSCF, where these tissues were transformed into cell lines resulting in fibroblasts as well as multiple iPSC clones per subject. See Table 1. iPSC Validation Metrics, for data available for NYSCF specimens.

For NYSCF cell line expansion recommendations, see Appendix A: NYSCF iPSC Recovery and Expansion.

**PPMI Study Amendment 10**

In Amendment 10, PPMI expanded to include the generation of iPSC lines from reprogrammed peripheral blood mononuclear cells (PBMCs) through a sub-study with Cellular Dynamics International (CDI). PPMI anticipates completing blood collections from 135 PPMI subjects by the end of 2017. Blood draws were performed at approved sites and samples are shipped overnight to CDI, where the iPSC lines were generated, and multiple clones per subject were shipped to IU. See Table 1. iPSC Validation Metrics, for data available for CDI specimens.

For CDI cell line expansion recommendations, see Appendix B: CDI User’s Guide.

**Cell line expansions**

**iPSC Expansion - WiCell**

PPMI partnered with WiCell Research Institute to complete the expansion of iPSCs from NYSCF. iPSCs shipped from the PPMI Biorepository at Indiana University to WiCell where they were thawed, expanded, and cryopreserved. They were shipped back to Indiana University for storage and distribution. See Table 1. iPSC Validation Metrics, for data available on specimens expanded by WiCell.

For WiCell cell line expansion recommendations, see Appendix C: Insert Here.

**Fibroblast Expansion – Rutgers University**

PPMI partnered with the Rutgers University Cell and DNA Repository (RUCDR) to complete the expansion of fibroblasts from NYSCF. Fibroblasts shipped from the PPMI Biorepository at Indiana University to Rutgers University where they were thawed, expanded, and cryopreserved. They were then shipped back to Indiana University for storage and distribution. See Table 1. iPSC Validation Metrics, for data available on specimens expanded by Rutgers.

For Rutgers cell line expansion recommendations, see Appendix D: Insert Here.

**Indiana University**

**Cell Line Expansion – Indiana University**

The Indiana University department of Medical & Molecular Genetics laboratory is responsible for all other expansion of stock vials of cell lines from NYSCF, CDI, WiCell, and Rutgers, as well as subsequent expansion. See Table 1. iPSC Validation Metrics, for data available on specimens expanded by Indiana University.

For Indiana University cell line expansion recommendations, see Appendix E: Insert Here.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **QC Metric** | **NYSCF** | **CDI** | **WiCell** | **IUGB** |
| **Method of Reprogramming** | mRNA (5 factors + GFP during transfection) | Episomal (7 factors) | N/A | N/A |
| **Growth Medium** | Freedom | E8 | mTeSR1 | mTeSR1 |
| **Growth Serum** | None | None | None | None |
| **Substrate/Matrix** | Cultrex | Matrigel | Matrigel | Matrigel |
| **Subcultivation Method** | Accutase (leaves single cells) | EDTA (leaves cells in small clumps) | EDTA (leaves cells in small clumps) | ReLeSR/ EDTA (leaves cells in small clumps)  |
| **Identity Test Procedure** | Fluidigm SNP | Proprietary SNP genotyping method | STR | Fluidigm SNP |
| **Sterility Test Procedure** | SteriQuot | None | Outsource to Biotest Laboratories | None |
| **Mycoplasma Test Procedure** | Lonza MycoAlert Detection Kit | Lonza MycoAlert for starting material; PCR assay for iPSCs | Lonza MycoAlert Detection Kit | Lonza MycoAlert Detection Kit (from Baylor) |
| **Karyotype Test Procedure** | Nanostring Karyotyping Assay | G-banding karyotype (from WiCell) | G-banding karyotype | G-banding karyotype (from Baylor) |
| **QC Thaw Test Procedure** | Thaw 1 vial into single well of 12 well plate | Thaw 1 vial into 3 wells of a 6-well plate (Thaw test all lines and check visually for confluence and viability) | Depends on cell line | Thaw one vial into a 100mm dish (thaw test all lines and check visually for confluence, viability and contamination) |
| **Pluripotency Test Procedure** | Nanostring Pluripotency Scorecard Analysis | Gene expression assay | Flow cytometry | Gene expression assay (from Baylor) |
| **Differentiation Capacity Test** | Nanostring 3 Germ Layer Scorecard Analysis | None | None | STEMdiff Trilineage Differentiation Kit (from Baylor) |
| **Split Ratio** | 1:10 | 0.5 mM EDTA method at 1:4, 1:6, or 1:9 depending on the line | 1:10 to 1:20 if using EDTA depending on the line | 1:6 to 1:20 depending on the cell line |
| **Temperature (⁰C)** | 37 | 37 | 37 | 37 |
| **Percent CO2** | 5 | 5 | 5 | 5 |
| **Recommended Thaw Ratio** | 1:1 (12-well plate) | 1:1 (6-well plate) | N/A | 1:1 (6-well plate) |

**Table 1. iPSC Validation Metrics**