

# CMPortal Protocol Upload Form

## Purpose

This form is available at CMPortal (<http://palpantlab.com/cmportal>). It acts as a standardised tool for researchers to upload and benchmark their cardiac stem cell protocols. Users can select protocol features, reported  $\geq 3$  times in 322 published protocols, to compare against key characteristics of maturity indicators, and those of other parameters of interest revealed in our data mining. Your experimental data can be uploaded with another PDF file.

## How to Use

- Review each Feature Category (starting from page 2).
- Under each category, you'll find feature labels grouped logically (e.g., media type, time points). Select all feature options applicable to your protocol. The number in brackets is the number of studies out of 322 reporting that variable. All options were reported  $\geq 3$  times.
- Use the check-boxes to select all options that apply to your protocol. For example, check BMP4 regardless of your dosage of Bone Morphological Protein 4. Our study design, and algorithms consider variable effects as long there is a significant relationship between feature and outcome/application. This includes context-dependent effects, including concentration.
- If a specific item is not listed, please skip the fields that are unavailable, not applicable, unknown, or undesired for your benchmarking.

## Outcome

Data will be used in CMPortal to support comparative analytics, trends, and benchmark performance indicators. It will only be used for research purposes. Users are encouraged to send a copy of the dataset shown in "Database Viewer" if they want to integrate their protocol into CMPortal.

## Submission

Please complete the form and upload it via the CMPortal submission interface under 'Protocol Benchmarking'. None of this or the experimental data form will be stored on CMPortal.

## Support

For adding your protocol to CMPortal, or for technical support and questions, contact [n.palpent@uq.edu.au](mailto:n.palpent@uq.edu.au) or [c.s.y.chow@uq.edu.au](mailto:c.s.y.chow@uq.edu.au)

# Feature Category: Protocol Variable

## hiPSC Matrix Coating

- ☐ EBs (18)
- ☐ Geltrex (33)
- ☐ Matrigel (163)
- ☐ MEF feeder cells (8)
- ☐ Vitronectin (10)

## hiPSC Backbone Media

- ☐ Conditioned (12)
- ☐ DMEM/F12 (10)
- ☐ Embryonic Stem Cell (127)
- ☐ Essential 8 (82)
- ☐ mTeSR (106)
- ☐ StemFit (5)
- ☐ StemFlex (5)

## hiPSC-CM Backbone Media

- ☐ Commercial CM Kit (12)
- ☐ Cor.4U Complete (6)
- ☐ DMEM (18)
- ☐ iCell Maintenance (86)
- ☐ RPMI-1640 (167)
- ☐ StemPro-34 (14)

## hiPSC-CM Media Supplement

- ☐ 1-thioglycerol (14)
- ☐ Albumin (28)
- ☐ Ascorbic Acid (41)
- ☐ B27 (180)
- ☐ bFGF (3)
- ☐ FBS (16)
- ☐ GlutaMax (8)
- ☐ HEPES (16)
- ☐ iCell Maintenance Medium (41)
- ☐ L-glutamine (19)
- ☐ Lipid Mix (5)
- ☐ Lipids (9)
- ☐ Mercaptoethanol (10)

# Feature Category: Protocol Variable

## hiPSC-CM Media Supplement

- ☐ Nonessential Amino Acids (8)
- ☐ Polyvinylalcohol (6)
- ☐ Selenium (7)
- ☐ Transferrin (11)
- ☐ VEGF (4)

## Wnt Induction

- ☐ Activin A (80)
- ☐ bFGF (45)
- ☐ BMP4 (74)
- ☐ CHIR99021 (184)
- ☐ StemCell Diff Kit (4)
- ☐ Wnt3a (3)

## Seeding Confluency Regardless of 2D or 3D (%)

- ☐ 70 to 79 (11)
- ☐ 80 to 84 (12)
- ☐ 85 to 89 (43)
- ☐ 90 to 94 (26)
- ☐ 95 to 100 (23)

## Seeding Confluency Specifically for 2D Protocols (%)

- ☐ 70 to 79 (6)
- ☐ 80 to 84 (5)
- ☐ 85 to 89 (26)
- ☐ 90 to 94 (12)
- ☐ 95 to 100 (6)

## Seeding Confluency Specifically for 3D Protocols (%)

- ☐ 70 to 79 (3)
- ☐ 80 to 84 (5)
- ☐ 85 to 89 (12)
- ☐ 90 to 94 (13)
- ☐ 95 to 100 (13)

## Feature Category: Protocol Variable

### Wnt Induction Duration (days)

- ☐ 3 days (38)
- ☐ 4 days (15)
- ☐ 5 days (8)

### Wnt Induction Duration (days) Quantiles

- ☐ Q1 ( $>2$  and  $\leq 5$ ) (61)
- ☐ Q2 ( $>1$  and  $\leq 2$ ) (75)
- ☐ Q3 ( $>0$  and  $\leq 1$ ) (186)

### Wnt Inhibitor

- ☐ bFGF (8)
- ☐ BMP4 (7)
- ☐ DS-I-7 (9)
- ☐ IWP (112)
- ☐ IWR (56)
- ☐ KY02111 (7)
- ☐ VEGF (3)
- ☐ Wnt-C59 (30)
- ☐ XAV939 (24)

### Wnt Inhibitor Duration (days)

- ☐ 3 days (17)
- ☐ 4 days (19)
- ☐ 5 days (6)
- ☐ 6 days (4)
- ☐  $>6$  days (12)

### Wnt Inhibitor Duration (days) Quantiles

- ☐ Q1 ( $>2$  and  $\leq 9$ ) (58)
- ☐ Q2 ( $>1$  and  $\leq 2$ ) (156)
- ☐ Q3 ( $>1$  and  $\leq 1$ ) (108)

### Insulin Start Day

- ☐ 0 (7)
- ☐ 1 (19)
- ☐ 2 (4)
- ☐ 3 (5)

## Feature Category: Protocol Variable

### Insulin Start Day

- ☐ 4 (11)
- ☐ 5 (14)
- ☐ 6 (20)
- ☐ 7 (85)
- ☐ 8 (15)
- ☐ 9 (10)
- ☐ 10 (6)
- ☐ 11 (3)
- ☐ After 11 (7)

### Insulin Withdrawal Duration (days) Quantiles

- ☐ Q1 ( $>4$  and  $\leq 10$ ) (11)
- ☐ Q2 ( $>2$  and  $\leq 4$ ) (25)

### Insulin Withdrawal Duration (days)

- ☐ 3 days (6)
- ☐ 4 days (18)
- ☐ 6 days (3)
- ☐ 8 days (3)

### Purification Protocol

- ☐ Antibiotic (4)
- ☐ Cell Sorting (7)
- ☐ Glucose and Lactate (85)
- ☐ Metabolic (8)

### hiPSC-CM Purification Duration (days)

- ☐ 3 days (13)
- ☐ 4 days (29)
- ☐ 5 days (6)
- ☐ 6 days (10)
- ☐ 7 days (6)
- ☐ 8 days (4)
- ☐  $<3$  days (31)
- ☐  $>9$  days (5)

## Feature Category: Protocol Variable

### hiPSC-CM Purification Duration (days) Quantiles

- ☐ Q1 (>4 and ≤20) (31)
- ☐ Q2 (>1 and ≤4) (61)

### Differentiation Purity (%) Quantiles

- ☐ Q1 (>95 and ≤99) (22)
- ☐ Q2 (>90 and ≤95) (27)
- ☐ Q3 (>85 and ≤90) (34)
- ☐ Q4 (>79 and ≤85) (40)
- ☐ Q5 (>30 and ≤79) (32)

### New Media for Maturation

- ☐ Commercial Kit (5)
- ☐ DMEM (21)
- ☐ F12 (7)
- ☐ RPMI-1640 (30)

### hiPSC-CM Maturation Media

- ☐ Commercial Kit (27)
- ☐ Cor.4U Complete (6)
- ☐ DMEM (35)
- ☐ F12 (10)
- ☐ iCell Maintenance (83)
- ☐ RPMI-1640 (153)
- ☐ StemPro-34 (14)

### Coating for Replating

- ☐ Fibronectin (32)
- ☐ Gelatin (43)
- ☐ Geltrex (10)
- ☐ Laminin (5)
- ☐ Matrigel (65)
- ☐ Synthemax (3)
- ☐ Vitronectin (3)

### Maturation Strategy

- ☐ Cell Alignment (59)
- ☐ ECM (21)

# Feature Category: Protocol Variable

## Maturation Strategy

- ☐ Elastomeric (33)
- ☐ Electrical (39)
- ☐ Mechanical (36)
- ☐ Metabolic (33)
- ☐ Other Cells (80)
- ☐ Tension (64)

## Metabolic Maturation Component

- ☐ Albumax (3)
- ☐ Ascorbic Acid (3)
- ☐ B27 (3)
- ☐ Biotin (3)
- ☐ Creatine (7)
- ☐ Dexamethasone (7)
- ☐ Fatty Acid (13)
- ☐ Galactose (4)
- ☐ IGF-1 (3)
- ☐ Insulin-Transferrin-Selenium (3)
- ☐ KOSR (3)
- ☐ L-carnitine (6)
- ☐ Lactate (4)
- ☐ Nonessential Amino Acids (6)
- ☐ Palmitic Acid (11)
- ☐ T3 (14)
- ☐ Taurine (7)
- ☐ Vitamin B12 (3)

## Metabolic Maturation Component Category

- ☐ Amino Acids and Derivatives (9)
- ☐ Fatty Acids and Lipids (21)
- ☐ Hormonal Stimulation (14)
- ☐ Kinase Inhibitors (3)
- ☐ Metabolic Modulation (20)
- ☐ Signaling Pathway Regulators (6)
- ☐ Sugars and Carbohydrates (9)

# Feature Category: Protocol Variable

## 2D Surface

- ☐ Decellularized ECM (3)
- ☐ ECM-coated (115)
- ☐ Electrospun (13)
- ☐ Hydrogel (17)
- ☐ Microelectrode Array (9)
- ☐ Microparticle/fluid (3)
- ☐ Micropatterned (27)
- ☐ Nanotopography (6)

## 3D Platform

- ☐ 3D printed (9)
- ☐ Collagen (38)
- ☐ Extracellular Scaffold (18)
- ☐ Fibrin (50)
- ☐ Fibronectin (3)
- ☐ Gelatin (6)
- ☐ Matrigel (33)
- ☐ Nanotechnology (3)
- ☐ Polyethylene Glycol (8)
- ☐ Scaffold Free (43)

## 3D Tissue Media

- ☐ Commercial Kit (21)
- ☐ DMEM (53)
- ☐ Growth Factor (12)
- ☐ High-glucose DMEM (9)
- ☐ iCell Maintenance (12)
- ☐ Iscove (5)
- ☐ MEM- $\alpha$  (60)
- ☐ RPMI-1640 (72)



## Feature Category: Analysis Method

### Differentiation Purity Assessment

- ☐ Flow Cytometry a-actinin+ (9)
- ☐ Flow Cytometry cTnI+ (3)
- ☐ Flow Cytometry cTnT+ (135)
- ☐ Flow Cytometry SIRPA+ (4)
- ☐ Flow Cytometry VCAM1+ (4)
- ☐ IHC a-actinin (8)
- ☐ IHC cTnT (7)
- ☐ Visual Inspection (6)

### Immunofluorescent Imaging

- ☐ Yes (268)

### Electron Imaging

- ☐ Scanning (22)
- ☐ Transmission (62)

### Sacromere or Cellular Alignment Analysis

- ☐ Yes (72)

### Contractile Analysis Method

- ☐ Deflection (39)
- ☐ Force Transducer (27)
- ☐ Motion Tracking (93)
- ☐ Traction Force Microscopy (9)

### Calcium Handling Analysis Method

- ☐ Genetic (23)
- ☐ Visual (104)

### Electrophysiology Analysis Method

- ☐ Genetic (3)
- ☐ Microelectrode (31)
- ☐ Motion-Contrast Reconstruction (5)
- ☐ Optical Mapping (39)
- ☐ Patch Clamp (59)

## Feature Category: Analysis Method

### Metabolic Analysis Method

- ☐ Flux Rates (13)
- ☐ Genetic (3)
- ☐ Mitochondrial (4)
- ☐ Seahorse (35)

### Fatty Acid Metabolism Assessed

- ☐ Yes (20)

### Gene Analysis Method

- ☐ RNA (169)

## Feature Category: Cell Profile

### Cell Line

- ☐ 201B6 (3)
- ☐ 201B7 (9)
- ☐ 253G1 (10)
- ☐ ATCC (5)
- ☐ BJ1 (7)
- ☐ BJ RiPS (4)
- ☐ C25 (6)
- ☐ Cellapy (4)
- ☐ Cor.4U (16)
- ☐ DF19-9-11T.H (16)
- ☐ Gibco episomal (10)
- ☐ iCell2 (8)
- ☐ iCell (47)
- ☐ IMR90 (19)
- ☐ PGP1 (11)
- ☐ SCVI-273 (8)
- ☐ WTC11 (30)

### Number of Cell Lines

- ☐ 1 (225)
- ☐ 2 (50)
- ☐ 3 (29)
- ☐ 4 (11)
- ☐ >5 (9)

### Cell Line Sex

- ☐ Both (118)
- ☐ Female (40)
- ☐ Male (64)

### Cell Line Ancestry

- ☐ Asian (28)
- ☐ Caucasian (41)

### Cell Coculture

- ☐ Cardiomyocyte (157)
- ☐ Endothelial Cell (35)

# Feature Category: Cell Profile

## Cell Coculture

☐ Stromal Cell (78)

## 3D CM Ratio (CM-EC-SC) Quantiles

☐ Q1 ( $>91$  and  $\leq 100$ ) (74)

☐ Q2 ( $>75$  and  $\leq 91$ ) (28)

☐ Q3 ( $>9$  and  $\leq 75$ ) (48)

## 3D EC Ratio (CM-EC-SC) Quantiles

☐ Q1 ( $>0$  and  $\leq 91$ ) (31)

☐ Q2 ( $>0$  and  $\leq 0$ ) (119)

## 3D SC Ratio (CM-EC-SC) Quantiles

☐ Q1 ( $>10$  and  $\leq 50$ ) (47)

☐ Q2 ( $>0$  and  $\leq 10$ ) (29)

☐ Q3 ( $>0$  and  $\leq 0$ ) (74)

## 3D Stromal Cell Source

☐ Cardiac Fibroblast (32)

☐ Dermal Fibroblast (7)

☐ hiPSC-CardiacF (8)

☐ hiPSC-MuralC (3)

☐ hiPSC-SmoothMC (3)

☐ Human Fibroblast (38)

☐ Mesenchymal Stem Cell (12)

☐ Stromal Cell (35)

## 3D Endothelial Cell Source

☐ Cardiac Microvascular EndothelialC (5)

☐ hiPSC-EndothelialC (16)

☐ Umbilical Vein EndothelialC (10)