

Milestone	Cellular model	Culture substrate	Pluripotency media	Dissociation reagent	Differentiation format	Timeline, growth factors, small molecules, and basal media												Efficiency	Reference		
						d0	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	d11			d12	
Spontaneous differentiation	hESC	MEF	KO-DMEM, 20% FBS	Collagenase IV	EB	20% FBS KO-DMEM												8% OF EBs	Kehat et al., 2001		
Co-culture differentiation	hESC	MEF	DMEM, 20% FBS	Dispase	Colonies	20% FBS, END-2 cells DMEM												N/S	Mummary et al., 2003		
Growth-factor Ebs	hESC	Matrigel	MEF-CM	Trypsin	EB	Activin A, FGF2		20% FBS CDM-PVA (MDMF12, ascorbic acid, 1TG, insulin, transferrin, PVA)										24% of EBs	Burridge et al., 2007		
Growth factor monolayer	hESC	Matrigel	MEF-CM	Collagenase IV or EDTA	Monolayer	Activin A	BMP4			RPMI+B27									30% MYH7+	Lafamme et al., 2007	
First application of a small molecule (SB203580)	hESC	HF	KO-DMEM, 20% KSR, FGF2	Collagenase IV	EB	SB203580 END-2 conditioned: DMEM, NEAA, insulin, transferrin, sodium selenite, 2ME												22% MYH6+	Graichen et al., 2008		
First chemically-defined medium, insulin has negative effects early in differentiation	hESC	MEF	KO-DMEM, 20% KSR, FGF2	Collagenase IV	EB	PGI2, SB203580 DMEM, NEAA, transferrin, sodium selenite, 2ME												11% MYH6+	Xu et al., 2008		
Increased efficiency via Wnt inhibition	hESC	MEF	DMEM/F12, 20% KSR, FGF2	Trypsin	EB	BMP4	BMP4, Activin A, FGF2			DKK1, VEGFA			DKK1, VEGFA, FGF2			50% TNNT2+	Yang et al., 2008				
Concentration optimization leads to increased efficiency	hESC	MEF	DMEM, 20% KSR, FGF2	ReproCELL Dissociation Solution	EB	BMP4, 20% FBS				20% FBS				DMEM, NEAA, 2ME				98% of EBs	Takei et al., 2009		
Stage specific effects of Wnt/β-catenin modulation	hESC	Matrigel	MEF-CM, FGF2	Collagenase IV	Monolayer	Activin A, WNT3A	BMP4			DKK1				RPMI+B27				49% MYH1+	Paige et al., 2010		
Identification of developmentally-relevant stage-specific signaling requirements	hESC, hiPSC	MEF, then Matrigel	DMEM/F12, 20% KSR, FGF2	Collagenase B, trypsin	EB	BMP4	BMP4, Activin A, FGF2			DKK1, VEGFA			VEGFA, FGF2			50-70% TNNT2+	Katman et al., 2011				
Optimization of chemically-defined media leads to higher efficiency	hiPSC	Gelrex	MEF-CM	TrypLE	EB	BMP4, FGF2		20% FBS or HSA		RPMI, insulin, lipids							94% of EBs	Burridge et al., 2011			
Wnt inhibition via small molecule	hiPSC	MEF	DMEM/F12, 20% KSR, FGF2	Collagenase IV	EB	20% FBS, BMP4				20% FBS	20% FBS, IWR-1		20% FBS		2.5% FBS		16% TNNT2+	Ren et al., 2011			
Serum-free, small molecule-based Wnt inhibition	hESC	MEF	KO-DMEM, 20% KSR, FGF2	Collagenase IV	EB	BMP4	BMP4, Activin A, FGF2			IWR-1	IWR-1, FGF2, VEGFA			FluPZ, VEGFA	2% FBS		30% MYH6+	Willems et al., 2011			
Differentiation in both EB and monolayer formats	hESC	Low density MEF	DMEM/F12, 20% KSR, FGF2	TrypLE	EB and monolayer	BMP4, WNT3A, Activin A, VEGFA, SCF LI-APEL (MDMF12, PFHM-II, PVA, BSA, lipids, ITS, 1TG), ascorbic acid												~25% GFP+	Elliott et al., 2011		
First protocol utilizing solely small molecules	hESC	Matrigel	MEF-CM	Accutase	Monolayer	CHIR99021		IWR-1, SB431542, pumorphamine										70-80% NKX2-5+	Gonzalez et al., 2011		
Use of dual GSK3β inhibitors and a novel Wnt-inhibitor	hESC, hiPSC	Gelatin or laminin-211	DMEM/F12, 20% KSR	CTK dissociation solution	EB	CHIR99021, BIO		KY02111, XAV939										98% TNNT2+	Minami et al., 2012		
Defined pluripotent media, small molecule-based differentiation	hESC, hiPSC	Matrigel or Synthmax	mTeSR1	TrypLE	Monolayer	CHIR99021	IWP-4			RPMI+B27 without insulin						RPMI+B27	85% TNNT2+	Lian et al., 2012 Lian et al., 2013			
Protein transduction-based differentiation	hESC	Suspension	MEF-CM	Trypsin	EB	Activin A	ISL1, BMP4			ISL1			RPMI+B27						75% TNNT2+	Fonoudi et al., 2013	
First chemically-defined, small molecule-based differentiation in multiple lines and various matrices	hESC, hiPSC	Synthmax or laminin-521	E8	EDTA	Monolayer	CHIR99021		Wnt-C59		CDM3 (RPMI, recombinant HSA, ascorbic acid 2-phosphate)										95% TNNT2+	Burridge et al., 2014
BMP inhibition-based differentiation	hESC, hiPSC	Matrigel	mTeSR1	ReLeSR	Monolayer	CHIR99021		DMH1		DMEM/F12+B27							75% TNNT2+	Aguilar et al., 2015			
Albumin-free	hESC, hiPSC	Matrigel	FTDA	Accutase	EB and monolayer	BMP4, FGF2, CHIR		IWP-2 or Wnt-C59		KO-DMEM, ascorbic acid, transferrin, sodium selenite (insulin and Y27632 for first day)							80-95% TNNT2+	Zhang et al., 2015			
Albumin-free, small molecule-based	hESC, hiPSC	Synthmax	E8 or mTeSR1	Accutase	Monolayer	CHIR99021		IWP-2		RPMI, putrescine, progesterone, sodium selenite			RPMI, insulin, ascorbic acid 2-phosphate				88-98% TNNT2+	Lian et al., 2015			
Use of heparin in lieu of albumin, EB-based	hESC, hiPSC	Matrigel	E8	EDTA	Monolayer	CHIR99021		IWP-2		DMEM/F12, ascorbic acid, lipids, transferrin, sodium selenite, sodium bicarbonate (heparin from d1-d7)							94% TNNT2+	Lin et al., 2017 Lin and Zou, 2020			

Subtype differentiation	Cellular model	Pluripotency media	Differentiation format	Differentiation protocol, growth factors, small molecules, and media by day of protocol													Efficiency	Reference
				d0	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12		
Atrial	hESCs	MEF-CM	Monolayer	BMP4, FGF2	Activin A		Noggin	retinoic acid, DKK1	DKK1							50% TNNT2+	Zhang et al., 2011	
				RPMI+B27														
	hESCs	BPEL	EB	Activin A, BMP4, stem cell factor, VEGFA, CHIR99021			retinoic acid									50% NKX2-5+	Devalla et al., 2015	
				BPEL														
	hESC, hiPSC	StemPro-34	EB	Activin A, BMP4, FGF2	retinol, IWP-2, VEGFA	VEGFA										85% TNNT2+	Lee et al., 2017	
			StemPro-34, L-glutamine, ascorbic acid, transferrin, 1TG, hypoxia															
	iPSC	E8 /Stem MACS iPS-Brew XF /Stem Flex	Monolayer	CHIR99021	IWP-2	IWP-2, retinoic acid	retinoic acid									87% TNNT2+	Kleinsorge et al., 2020	
			RPMI, ascorbic acid 2-phosphate, recombinant HSA															
			RPMI+B27															
Nodal	hESC, hiPSC	StemPro-34	EB	BMP4	Activin A, BMP4, FGF2	BMP4, SB-431542, retinoic acid, IWP-2, VEGFA, PD173074	VEGFA									50% TNNT2+ /SHOX2+	Protze et al., 2017	
				StemPro-34, L-glutamine, ascorbic acid, transferrin, 1TG, hypoxia														
	hESC, hiPSC	hiPSC Medium BioCISO	Monolayer	CHIR99021		IWP-2	BMP4, PD173074, BMS189453									55 % TNNT2+ /NKX2-5-	Liu et al., 2020	
				RPMI+B27 without insulin							RPMI+B27							
	hESC, hiPSC	E8	Monolayer	CHIR99021		IWP-2	CHIR99021									50% TNNT2+ /NKX2-5-	Ren et al., 2019	
			RPMI+B27 without insulin							RPMI+B27								
	hESC, hiPSC	E8	Monolayer	CHIR99021		Wnt-C59										20-30% TNNT2+ /NKX2-5-	Liang et al., 2020	
			RPMI+B27 without insulin															

Features	Cell types		Cocktails	Efficiency	Reference
	From	To			
GMT-based cocktail	CF, TTF	MYH6-GFP+	GMT	~ 20%	Ieda et al., 2010
microRNA	CF, MEF	Beating cells	GMT , miR-133	7 fold more	Muraoka et al., 2014
	HCF	TNNT2+	GMT , MESP1, MYOCD, miR-133	23-27%	
	CF	MYH6-GFP+	GMT	~ 30%	Mohamed et al., 2017
	HCF	TNNT2-GCaMP5+	GMT , MYOCD, ESRRG, MESP1, ZFPM2	~ 12%	
Chemical compound	MEF, TTF	Beating cells	GMT or GHMT	100 fold	Yamakawa et al., 2015
	TTF	Beating cells	GMT or GHMT	4 fold	Muraoka et al., 2019
Polycistronic puro selection	CF	Beating cells	polycistronic MGT	10 fold	Wang et al., 2014
Optimized GHMT-based cocktail	CFs, TTFs	MYH6-GFP+/TNNT2+	GHMT	5-20%	Song et al., 2012
	MEF	Beating cells	GHMT Akt1	50.0%	Zhou et al., 2015
	CF, TTF			~0.8%	
	MEF, CF, TTF	MYH6-GFP+/TNNT2+ Beating cells	GHMT , Akt1, PHF7	> 20% ~2 fold	Garry et al., 2021
	HCF	MYH6-GFP+/TNNT2+	GHMT , Myocd, PHF7	~ 3%	
Chamber-specific subtype	MEF, TTF	Sarcomere+/HCN4-GFP+	GHMT	1% x ~32%	Nam et al., 2014
		Sarcomere+/HCN4-GFP-/MYL2+		1% x ~22%	
		Sarcomere+/HCN4-GFP-/NPPA+		1% x ~35%	
	MEF	MYL7+/TNNT3+	Polycistronic M-G-T-H	~26%	Zhang et al., 2019, 2021
		MYL2+/TNNT3+ Sarcomere+, beating cells		~16% ~5-6 fold	
Human Fibroblasts	H9F	MYH6-mCherry+/TNNT2+	GMT, ESRRG, MESP1, MYOCD, ZFPM2 (7F)	13.0% ± 9.3%	Fu et al., 2013
	HCF, HDF	ACTN2+/TNNT2+		1-4%	
	HFF, AHCF, AHDF	TNNT2+	GHT, Myocd, miR-1, miR-133 (6F)	~10-35%	Nam et al., 2013
	HCF, HDF	ACTN2+ or TNNT2+	GMT, Mesp1, Myocd (GMTMM)	~ 5%	Wada et al., 2013
	H9F, HCF	TNNT2+	Polycistronic hMGT + miR-133 (MGT133)	40-60%	Garbutt et al., 2020
MicroRNA combo	MEF, CF	MYH6-CFP+	miR-1, miR-133, miR-208, miR-499 (miRcombo)	~13-27%	Jayawardena et al., 2012
Chemical only approach	MEF, TTF	ACTN2+ or MYH6+	small-molecule combination CRFVPT	~10-15%	Fu et al., 2015
	HFF	TNNT2+ cells	9 chemical compounds (9C)	6.6 ± 0.4%	Cao et al., 2016
	HLF			5.5%	

Cell source	Pre-reprogramming media	Reprogramming protocol, viral infection, small molecules and medium																		Reference							
		d0	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	d13	d14	3w	4w	5w		6w						
CF, TTF	DMEM, 10% FBS	Infection								Flow								Ca ²⁺ flux	Beating		Ieda et al., 2010						
		iCM medium (high glucose DMEM/199(4:1), 10% FBS)																									
CF, MEF	DMEM, 10% FBS	Infection							Flow					Beating					Ca ²⁺ flux / Beating		Muraoka et al., 2014						
HCF		iCM medium																									
CF	IMDM, 20% FBS	Infection	SB431542	XAV939					Beating						Flow			Ca ²⁺ flux		Mohamed et al., 2017							
		iCM medium, NEAA, sodium pyruvate, Glutamax																									
HCF	IMDM, 20% FBS	Infection	SB431542	XAV939													Flow/Ca2+ flux										
		iCM, NEAA, sodium pyruvate, Glutamax				iCM:RPMI+B27 (3:1)				iCM:RPMI+B27 (1:1)				iCM:RPMI+B27 (3:1)				iCM									
MEF, TTF	DMEM, 10% FBS	Infection																		Beating							Yamakawa et al., 2015
	IMDM, 20% FBS	iCM medium																		FFV medium (StemPro-34, Glutamax, ascorbic acid, FGF2, FGF10, VEGFA)							
TTF	IMDM, 20% FBS	Infection	one dose diclofenac d0-d4																Flow		Beating	Muraoka et al., 2019					
		iCM medium																		FFV medium							
CF	IMDM,15% FBS	Infection	puromycin				puromycin				Flow												Wang et al., 2014				
		iCM medium																									
CF, TTF	DMEM, 15% FBS	Infection													Flow			Flow	Beating				Song et al., 2012				
	ICM, 15% FBS	Cardiac induction medium (DMEM/199 (4:1), 10% FBS, 5% horse serum, NEAA, EAAs, B-27, ITS, vitamin mixture, sodium pyruvate), 10% conditioned medium obtained from neonatal rat/mouse CM culture																									
MEF		Infection 1 & 2							Flow					Flow	Flow /Beating						Zhou et al., 2015						
CF, TTF	DMEM, 10% FBS	Cardiac induction medium, 10% conditioned medium obtained from neonatal rat/mouse CM culture																									
MEF, CF, TTF		Infection 1 & 2							Flow					Ca ²⁺ flux /Beating													
	DMEM, 10% FBS	Cardiac induction medium																									
HCF		Infection																	Flow								Garry et al., 2021
		iCM, NEAA				iCM:RPMI+B27 (3:1)				iCM:RPMI+B27 (1:1)				iCM:RPMI+B27 (1:3)				RPMI+B27+FFV									
MEF, TTF	DMEM, 10% FBS	Infection									Flow					Flow	Flow /Beating					Nam et al., 2014					
		Cardiac induction medium, 10% conditioned medium obtained from neonatal rat/mouse CM culture																									
MEF	DMEM, 10% FBS	Infection 1 & 2																	Flow /Beating					Zhang et al., 2019, 2021			
		Cardiac induction medium, SB431542, A83-01																									
H9F		Infection																	Flow			Ca ²⁺ flux	AP (10w)	Fu et al., 2013			
HCF, HDF	DMEM, 10% FBS	iCM medium, NEAA																		RPMI+B27							
HFF, AHCF, AHDF	DMEM, 10% FBS	Infection 1 & 2																					Flow	Ca ²⁺ (8w)		Nam et al., 2013	
		Cardiac induction medium, 10% conditioned medium obtained from neonatal rat CM culture																									
HCF, HDF	IMDM, 20% FBS	Infection	iCM medium																								
H9F, HCF	DMEM, 20% FBS	Infection					puromycin 1ug/ml								Flow			Ca ²⁺ flux		Garbutt et al., 2020							
		iCM medium										hiCM medium (RPMI1640, B27, 2% FBS, 0.05% BSA, ascorbic acid 2-phosphate, Glutamax, NEAA)															
MEF, CF	DMEM, 15% FBS	transfection	JAK inhibitor I (5-8 days)												Flow							Jayawardena et al., 2012					
		DMEM, 15% FBS																									
		CHIR99021 (C), RepSox (R), Forskolin (F), VPA (V), Pamate (P), TTNPB (T)																									
MEF, TTF	DMEM, 15%FBS, Glutamax, NEAA	CRM (DMEM, 15% FBS, 5% KSR, N2, B27, Glutamax, NEAA, 2ME, ascorbic acid 2-phosphate)																Beating (d16) CMM (DMEM, 15% FBS, CHIR: PD0325901, LIF: ascorbic acid, insulin) (d22-24)								Fu et al., 2015	
HFF	DMEM, 10%FBS, Glutamax, NEAA	CHIR99021, BIX01294, AS8351, SC1, Y27632, OAC2, SU16F, JNJ10198409, A83-01								CHIR99021, BMP4, activin A, VEGFA											Cao et al., 2016						
HLF		DMEM, 5% FBS, 10% KSR, ITS, ascorbic acid 2-phosphate, 2ME, Glutamax, NEAA								RPMI+B27 without insulin, Glutamax, 1TG						hiCM medium											

Cells	Overexpressed Genes	Virus	Backbone	Transfection	Polybrene	Positive selection	Induction system	Reference
Platinum E cells	GMT	Retrovirus	pMXs	FuGene6 (Roche)				Ieda et al., 2010
Platinum E cells	Myc-tagged GHMT	Retrovirus	pBabeX	FuGene6 (Roche)	6 µg/mL			Song et al., 2012
HEK293 FT	GMT , ESRRG, MESP1, MYOCD, ZFPM2 (7F)	Lentivirus	pLVX-tetO-cDNA pLVX-Tight-Puro pMXs	Lipofectamine 2000	4 µg/mL		1 µg/mL Dox	Fu et al., 2013
		Retrovirus	Retro-X-Tet-ON pRetro-X-Tight-cDNA	FuGene6 (Promega)	5 µg/mL	puromycin & neomycin	100 ng/mL Dox	
Platinum A cells	GMT , Mesp1, Myocd	Retrovirus	pBabeX	FuGene6 (Promega)	6 µg/mL			Nam et al., 2013
PLAT-GP cells	GMT , MESP1, MYOCD (GMTMM)	Retrovirus	pMXs	TremeGene 9 (Roche)	4 µg/mL			Wada et al., 2013
293FT cells		Lentivirus	pLVX-Tight-Puro pLVX-rtTA pLVX-tetO-cDNA	Lipofectamine 2000	4 µg/mL		1 µg/mL Dox	
Platinum A cells	Myc-tagged GHMT	Retrovirus	pBabeX	FuGene6 (Promega)	6 µg/mL			Nam et al., 2014
Platinum E cells	GMT	Retrovirus	pMXs	FuGene6 (Roche)				Muraoka et al., 2014
HEK293	human GMT , MESP1, MYOCD	Lentivirus	CSII-CMV-RfA	Lipofectamine 2000				
Platinum E cells	Polycistronic M-G-T	Retrovirus	pMXs	Lipofectamine 2000	4 µg/mL	puromycin 2 µg/mL		Wang et al., 2014
Platinum A cells	GHMT Akt1	Retrovirus	pBabeX pMxs-MyrAkt1	FuGene6 (Promega)	6 µg/µL			Zhou et al., 2015
Platinum E cells	GHMT , MESP1, MYOCD	Retrovirus	pMXs	FuGene6 (Promega)				Yamakawa et al., 2015
Platinum E cells	GMT	Retrovirus	pMXs	FuGene HD (Roche)	0.6 µg/mL			Mohamed et al., 2017
Platinum E cells	GMT or GHMT	Retrovirus	pMXs	FuGene6			10 µM diclofenac	Muraoka et al., 2019
Platinum E cells	polycistronic M-G-T-H	Retrovirus	pBabeX	FuGene6 (Promega)	6 µg/mL			Zhang et al., 2019, 2021
293T cells	Polycistronic human M-G-T , miRNA-133	Retrovirus	pMXs pBabe	Nanofect	8 µg/mL	puromycin 1 µg/mL		Garbutt et al., 2020
Platinum E cells	GHMT , Myocd, PHF7	Retrovirus	pMXs	FuGene6 (Promega)	8 µg/mL			Garry et al., 2021