

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		
Spontaneous differentiation	hESC	MEF	KO-DMEM, 20% FBS	Collagenase IV	EB	20% FBS KO-DMEM												8% OF EBs	Kahari 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	Actin 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	Actin A BMP4 RPMI+B27												30% MYH1+	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, Actin A, FGF2 DKK1, VEGFA StemPro-34, ascorbic acid												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/catenin modulation	hESC	Matrigel	MEF-CM, FGF2	Collagenase IV	Monolayer	Actin 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, Actin A, FGF2 DKK1, VEGFA VEGFA, FGF2 StemPro-34, ascorbic acid, L-glutamine, 1TG												50-70% TNNT2+	Katman et al., 2011
Optimization of chemically-defined media leads to higher efficiency	hiPSC	Gettex	MEF-CM	TrypLE	EB	BMP4, FGF2 20% FBS or HSA RPMI+PVA RPMI, ascorbic acid 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 KO-DMEM, NEAA, BME												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, Actin A, FGF2 IWR-1 IWR-1, FGF2, VEGFA LIF2, VEGFA 2% FBS StemPro-34 DMEM												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, Actin A, VEGFA, SCF LI-APEL (MDMF12, PFHM-II, PVA, BSA, lipids, ITS, 1TG), ascorbic acid LI-AEL (MDMF12, PFHM-II, 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 DMEM/F12+N2+B27 RPMI+B27												70-80% NKX2-6+	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 IMDM, NEAA, BME, HSA or BSA												98% TNNT2+	Minami et al., 2012
Defined pluripotent media, small molecule-based differentiation	hESC, hiPSC	Matrigel or Synthemax	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	Actin 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	Synthemax 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	RelLeSR	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	Synthemax	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