Protocol for hPSC Differentiation to Skeletal Muscle

This protocol describes conversion of human pluripotent stem cells to skeletal muscle myotubes, through myogenic precursor (Pax7) and myoblast (MyoD) transitions.

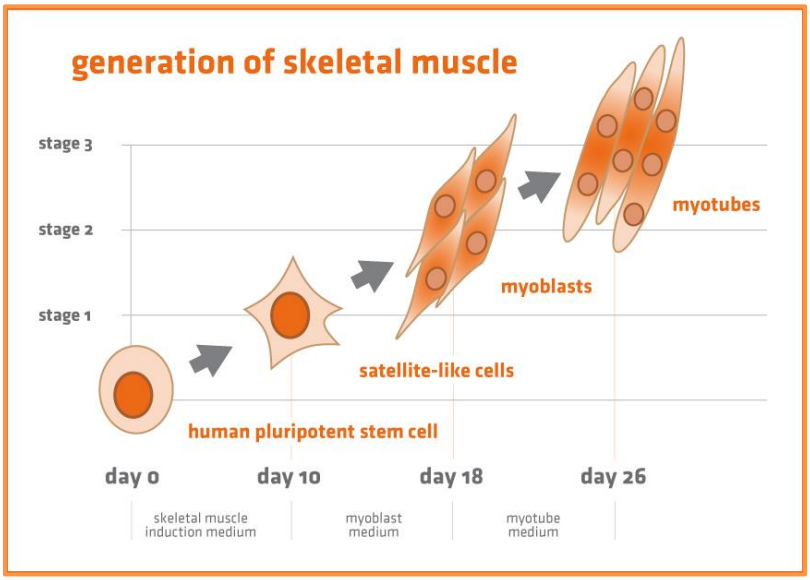

Figure 1. Genea Biocells Skeletal Muscle differentiation as per Caron et. Al. 2016. Schematic of differentiation.

Reagents:

SKM-KITM – Skeletal Muscle Differentiation Kit

– provided by Genea Biocells comprises:

- SKM-01 | 250mL Skeletal Muscle Induction Medium (Stage I)
- SKM-02 | 250mL Skeletal Myoblast Medium (Stage II)
- SKM-03 | 250mL Myotube Medium (Stage III, Option 1)

  And
- SKM-03+ | 250mL Myotube Fusion Medium (Stage III, Option 2)

Media Handling – We recommend thawing and storage of media at 4°C then warming the required amount of media just before use to 37°C. For storage longer than two weeks, aliquot media and store at -80°C

Note: after thawing media can be frozen and thawed one additional time.

Collagen I coated plates – We recommend using the Corning Biocoat™ Collagen I range of pre-coated plates and flasks for best performance. Alternatively, plates can be self-coated with Collagen I solution (Gibco).

0.05% Trypsin-EDTA – Mixed from commercially available solutions.

The differentiation is performed at 37°C in 5% CO_2 and atmospheric O_2 levels.

Approximate media volumes:

Plate/Flask Format				
96 well plate	100uL/well		T-25 Flask	7mL to 8mL
24 well plate	0.5mL/well		T-75 Flask	15mL to 20mL
12 well plate	1mL to 2mL		T-175 Flask	35mL to 50mL
6 well plate	3mL to 4mL			

Protocol Index

Cat #	Description
SKM01-250mL	SkM. Induction Medium, 250mL Bottle
SKM02-250mL	SkM. Myoblast Medium, 250mL Bottle
SKM03-250mL	SkM. Myotube Medium, 250mL Bottle
SKM03+-250mL	SkM. Myotube Fusion Medium, 250mL Bottle

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Differentiation Protocol

Stage I – Conversion of human PSC to myogenic precursors

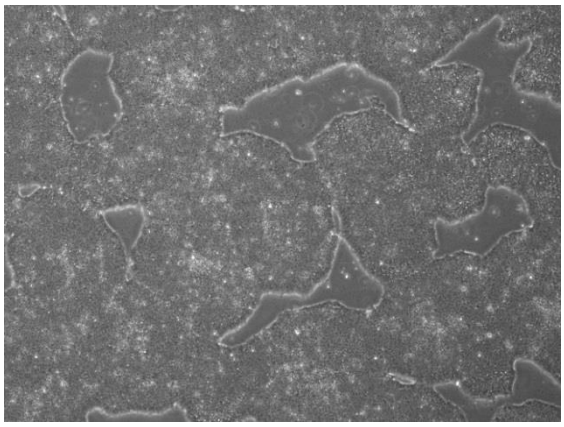
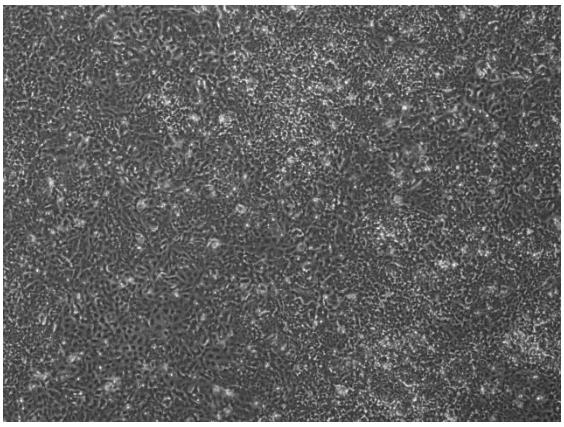
1.1. Myogenic Precursor induction from human Pluripotent Stem Cells

1.1.1. PBS rinse then dissociate mTESR hPSCs to single cells with Accutase for ~5 min at 37°C
1.1.2. Harvest the cells in Skeletal Muscle Induction Medium
1.1.3. Centrifuge the cells for 4 mins at 300 X g
1.1.4. Aspirate medium without disturbing the cell pellet and resuspend cells in warm Skeletal Muscle Induction Medium.
1.1.5. Count the number of viable cells.
1.1.6. Plate the cells at 5000 cells/cm^2 onto a collagen I coated plate.

Note: Successful skeletal muscle differentiation has been observed in difficult cell lines plated at densities from 2500 to 10,000 cells/cm^2.
Note: Growth formats smaller than 12 well plates are not recommended for Stage I.

1.1.7. Perform a media change every 2 days with Skeletal Muscle Induction Medium.
1.1.8. Cells are maintained for 6-10 days in Skeletal Muscle Induction Medium or until confluent.
1.1.9. Use for assay requirements, or continue to next stage.

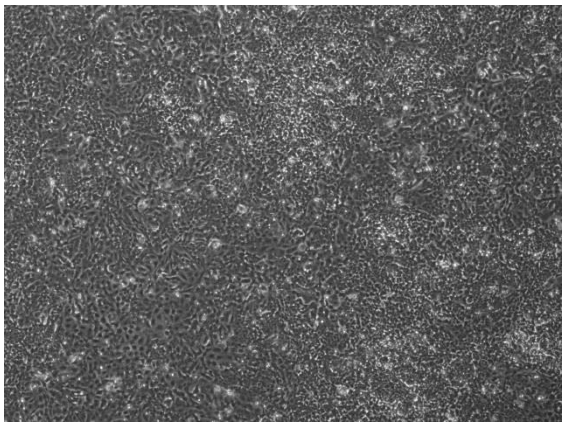
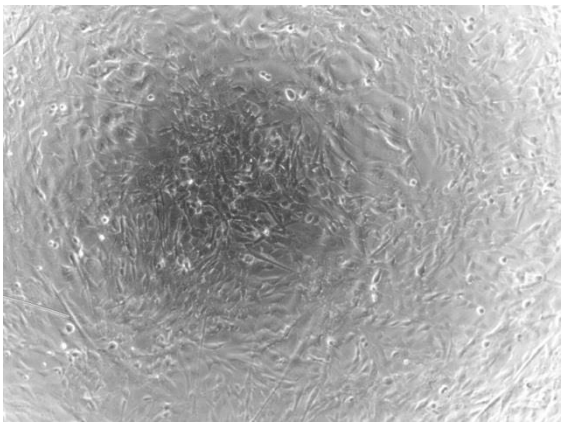
1.2. Representative image of Pluripotent Stem Cell to Myogenic Precursor transition

		
hESC / iPSC Line	**Stage I**	**Myogenic Precursor**

1.3. Conversion of Myogenic Precursors to Myoblasts

1.3.1. After 6-10 days in Skeletal Muscle Induction Medium, dissociate Myogenic Precursors with 0.05% Trypsin-EDTA for 5 min at 37°C.
1.3.2. Harvest the cells in medium containing 10% serum
1.3.3. Centrifuge the cells for 4 mins at 300 X g
1.3.4. Aspirate media without disturbing the cell pellet and resuspend cells in warm Skeletal Myoblast Medium.
1.3.5. Count the number of viable cells.
1.3.6. Plate the cells at 5000 cells/cm^2 onto a collagen I coated plate.
1.3.7. Perform a media change every 2 days with Skeletal Myoblast Medium.
1.3.8. Myoblasts are maintained for 6-8 days in Skeletal Myoblast Medium or until the cells reach confluence.
1.3.9. Use for assay requirements or continue to next stage.

1.4. Representative image of Myogenic Precursor to Myoblast transition

		
Myogenic Precursor	**Stage II**	**Myoblasts**

1.5. Conversion of Myoblasts to Skeletal Muscle Myotubes

1.5.1. If Continuing Cultured Cells From Step 1.3.9

1.5.1.1. When myoblasts are confluent from Stage II (usually 6-8 days) switch the media to either Myotube Medium or Myotube Fusion Medium.

Note: Myoblasts are not dissociated at this stage; ensure the cells have formed a confluent monolayer to fully differentiate into Myotubes

Note: Use of Myotube/Myotube Fusion Medium will depend on end user assay requirements

1.5.1.2. Perform a media change every 3 to 4 days with Myotube/Myotube Fusion Medium.

1.5.2. If Using Myoblast Cells provided by Genea Biocells

1.5.2.1. Prepare a 15mL Falcon tube with 3mL warm Skeletal Myoblast Medium for each vial thawed.

1.5.2.2. Remove vial from LN2 storage and thaw in a 37°C water bath.

1.5.2.3. As soon as the vial is thawed, use a 1mL pipette to slowly transfer cells to the warm Skeletal Myoblast Medium.

1.5.2.4. Mix by gentle inversion.

1.5.2.5. Centrifuge the cells for 4 mins at 300 X g

1.5.2.6. Aspirate media without disturbing the cell pellet and resuspend cells in 5mL warm Skeletal Myoblast Medium.

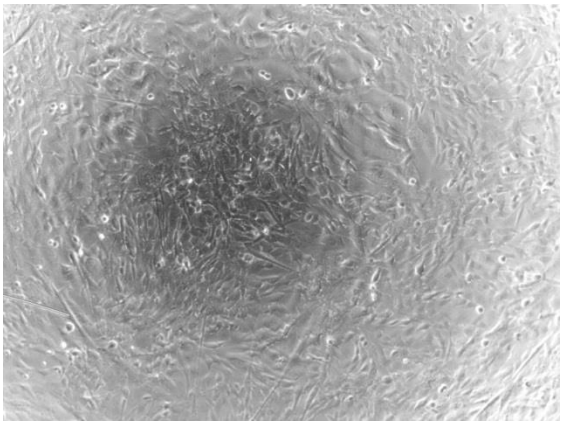
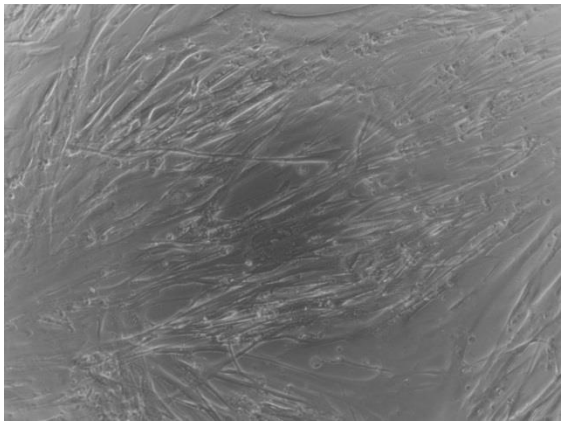
1.5.2.7. Assess the number of viable cells.

1.5.2.8. Plate at 30,000 – 60,000 cells/cm^2

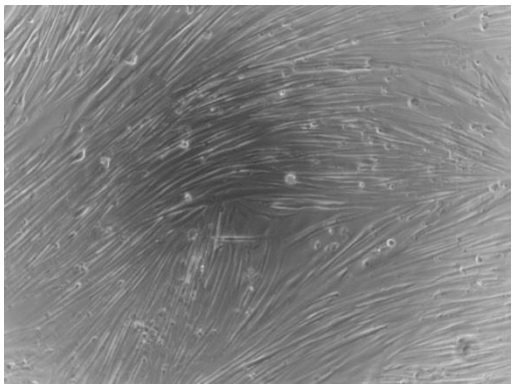
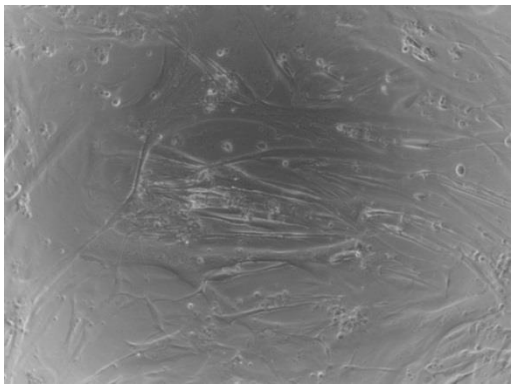
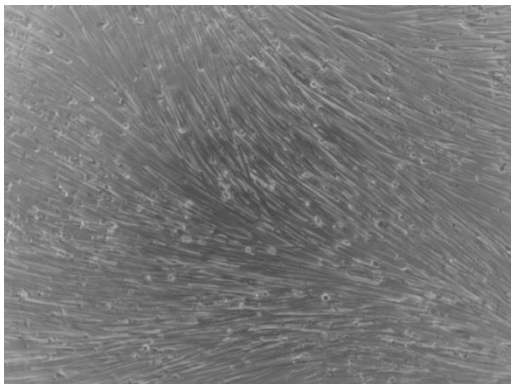
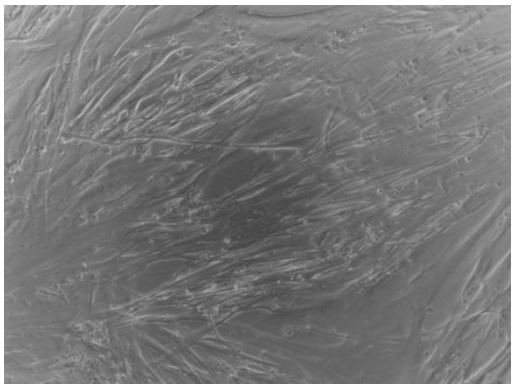
1.5.2.9. Change media every 2 days with Skeletal Myoblast Medium.

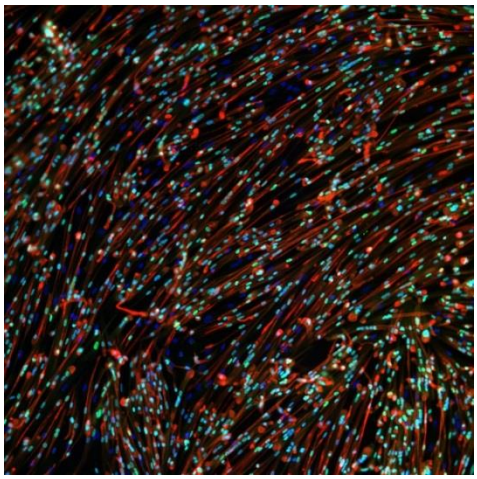
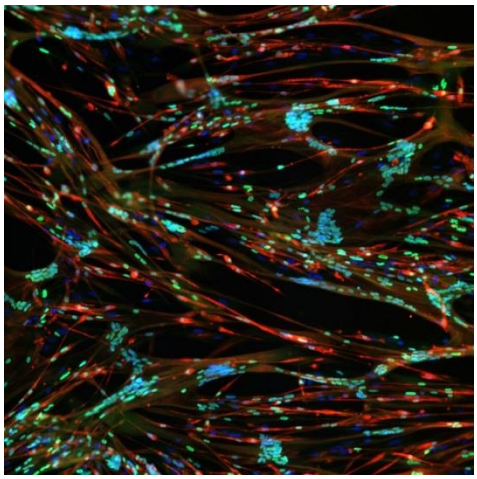
1.5.2.10. When confluent (usually 3-4 days) change the medium to Myotube Medium or Myotube Fusion Medium.

1.5.3. Representative image of myoblast to myotube transition

		
Myoblasts	**Stage III**	**Myotubes in Myotube Fusion Medium**

1.5.4. Comparison between Myotube Medium and Myotube Fusion Medium

	**Cell Line 1**	
	**Cell Line 2**	
Myotubes in SkM. Myotube Medium (SKM-03)	**Stage 3**	**Myotubes in SkM. Myotube Fusion Medium (SKM-03+)**

	MyoG MHC	
Myotubes in SkM. Myotube Medium (SKM-03)	**Stage 3**	**Myotubes in SkM. Myotubue Fusion Medium (SKM-03+)**