

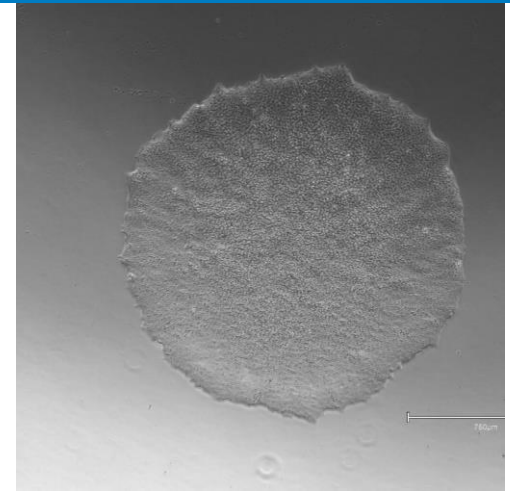


Leiden University  
Medical Center

**LUMC0296iOAT03, 07,08**

## Characterization of the hiPSC line

LUMC hiPSC Hotel  
Anatomy & Embryology  
MIRAIHOUSE -- LEIDEN



# LUMC0296iOAT

Results RT-qPCR analysis for EBNA Presence, indicating episomal integration

Cq values	03	07	08	pos. CTRL	neg. CTRL
HK2P	22,51	22,16	22,99	22,1	23,3
EBNA	N/A	N/A	N/A	16,1	30,8
	N/A	N/A	N/A	15,6	30,8
	N/A	N/A	N/A	13,4	N/A

Conclusion: *EBNA* was undetectable at passage 5 in the 3 tested clones. This indicates integration of complete plasmids didn't take place. However, the assay is unable to detect integration of plasmid fragments.

# LUMC0296iOAT

Results STR analysis, matching primary material with gained hiPSCs

- WK15071 = Primary donor cells

	Your Sample	Your Sample	Your Sample	Your Sample
Name	LUMC0296OAT #3	LUMC0296OAT#7	LUMC0296OAT#8	WK15071
FLDO-code	X23-030-28	X23-030-29	X23-030-30	X23-030-42
N° Markers	22	22	22	22
Score				
Amel	X,Y	X,Y	X,Y	X,Y
CSF1PO	10,12	10,12	10,12	10,12
D1S1656	16,16.3	16,16.3	16,16.3	16,16.3
D2S1338	17,20	17,20	17,20	17,20
D2S441	11,13	11,13	11,13	11,13
D3S1358	15,18	15,18	18	15,18
D5S818	11,13	11,13	11,13	11,13
D7S820	10,12	10,12	10,12	10,12
D8S1179	12,14	12,14	12,14	12,14
D10S1248	12,16	12,16	12,16	12,16
D12S391	17,20	17,20	17,20	17,20
D13S317	8	8	8	8
D16S539	11,12	11,12	11,12	11,12
D18S51	12,14	12,14	12,14	12,14
D19S433	14,18.2	14,18.2	14,18.2	14,18.2
D21S11	29,31.2	29,31.2	29,31.2	29,31.2
D22S1045	16,17	16,17	16,17	16,17
FGA	21,23	21,23	21,23	21,23
Penta D	8,12	8,12	8,12	8,12
Penta E	7,15	7,15	7,15	7,15
TH01	9.3	9.3	9.3	9.3
TPOX	8,11	8,11	8,11	8,11
vWA	14,19	14,19	14,19	14,19

Conclusion: There is a match between primary material and gained clones, indicating that clones stem from primary material.

# LUMC0296iOAT03

Results Karyotyping with ddPCR, indication of genetic integrity. At indicated passage

## SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS

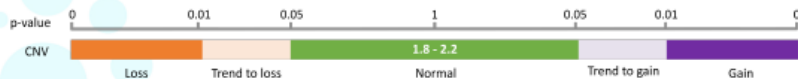
Sample	Passage	dsDNA (ng/ $\mu$ L)	dsDNA quantity	CNVs	Sex*
LUMC0296iOAT03	p7	27.7	Good	Not detected	M

The results of this test are for research use only.

To cite the iCS-digital™ test in your article, please select the most appropriate option from sample texts available [here](#).

\* If unknown, sex is deduced from the ChrX copy number; note that one ChrX can be detected in female cells that lost one ChrX, and two ChrX in male cells that gained one copy.

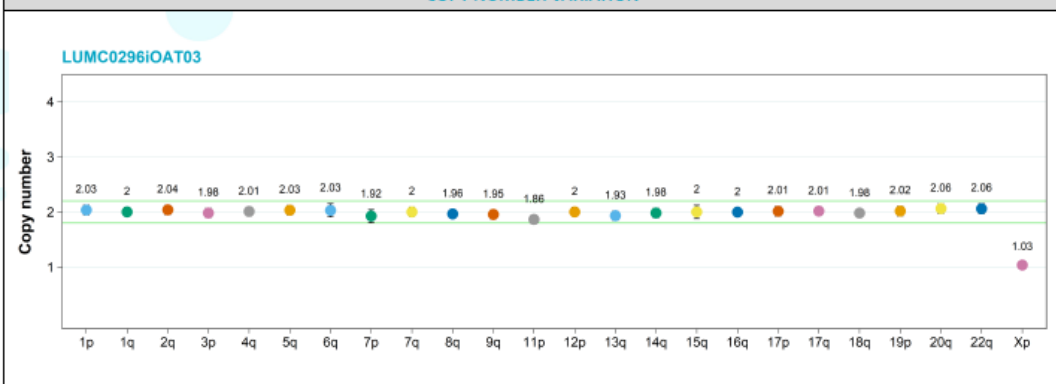
Copy number values:



## SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS

CHROMOSOME	1p	1q	2q	3p	4q	5q	6q	7p	7q	8q	9q	11p	12p	13q	14q	15q	16q	17p	17q	18q	19p	20q	22q	Xp	
LUMC0296iOAT03																									

## COPY NUMBER VARIATION



Conclusion: No genetic abnormalities were found.

# LUMC0296iOAT07

Results Karyotyping with ddPCR, indication of genetic integrity. At indicated passage

## SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS

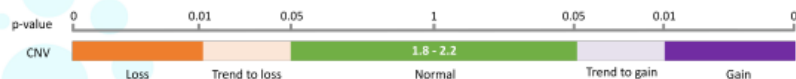
Sample	Passage	dsDNA (ng/ $\mu$ L)	dsDNA quantity	CNVs	Sex *
LUMC0296iOAT07	p7	29.3	Good	Not detected	M

The results of this test are for research use only.

To cite the iCS-digital™ test in your article, please select the most appropriate option from sample texts available [here](#).

\* If unknown, sex is deducted from the ChrX copy number; note that one ChrX can be detected in female cells that lost one ChrX, and two ChrX in male cells that gained one copy.

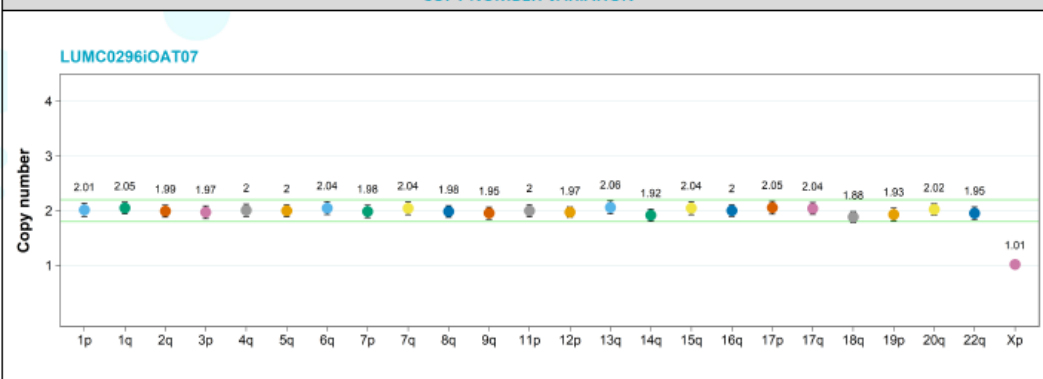
Copy number values:



## SUMMARY OF THE DETECTED COPY NUMBER VARIATIONS

CHROMOSOME	1p	1q	2q	3p	4q	5q	6q	7p	7q	8q	9q	11p	12p	13q	14q	15q	16q	17p	17q	18q	19p	20q	22q	Xp	
LUMC0296iOAT07																									

## COPY NUMBER VARIATION



Conclusion: No genetic abnormalities were found.

# LUMC0296iOAT

Flow Cytometry Results for Pluripotency Markers (p2)

Clone	Oct 3/4	Nanog	SSEA-4	SSEA-5	TRA-1-60	CD15 (neg. ctrl)
03	>95%	>91%	>99%	>99%	>99%	1%
07	>96%	>92%	>98%	>98%	>98%	1%
08	>97%	>84%	>99%	>99%	>98%	1%

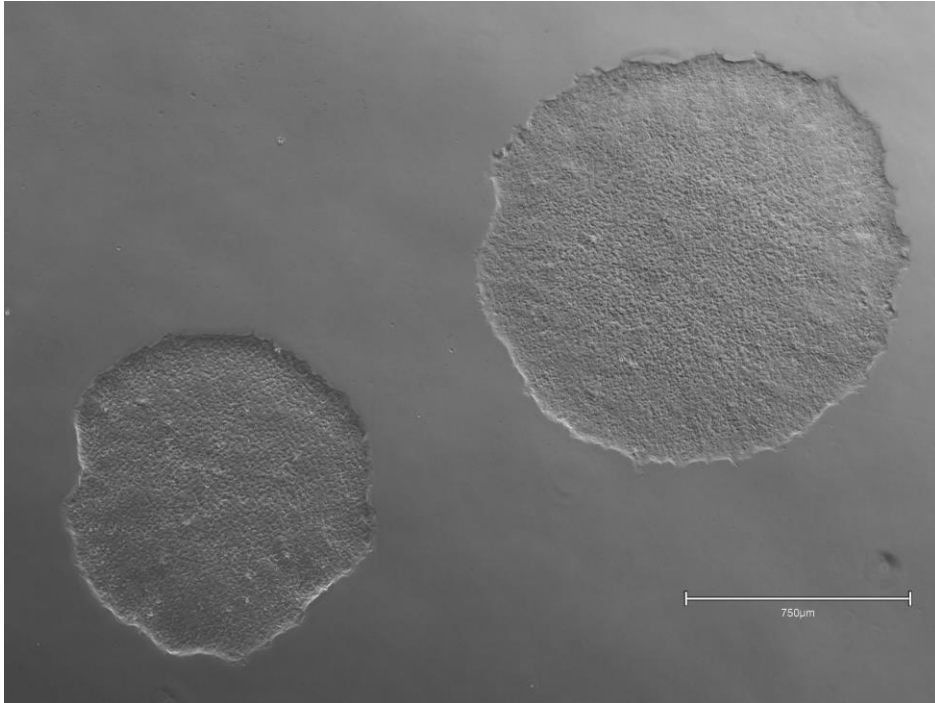
**Conclusion:**  $\geq 75\%$  of cells express pluripotency markers OCT3/4, Nanog, SSEA-4, SSEA-5 and TRA-1-60. In general, hiPSCs don't express CD15 (negative control).



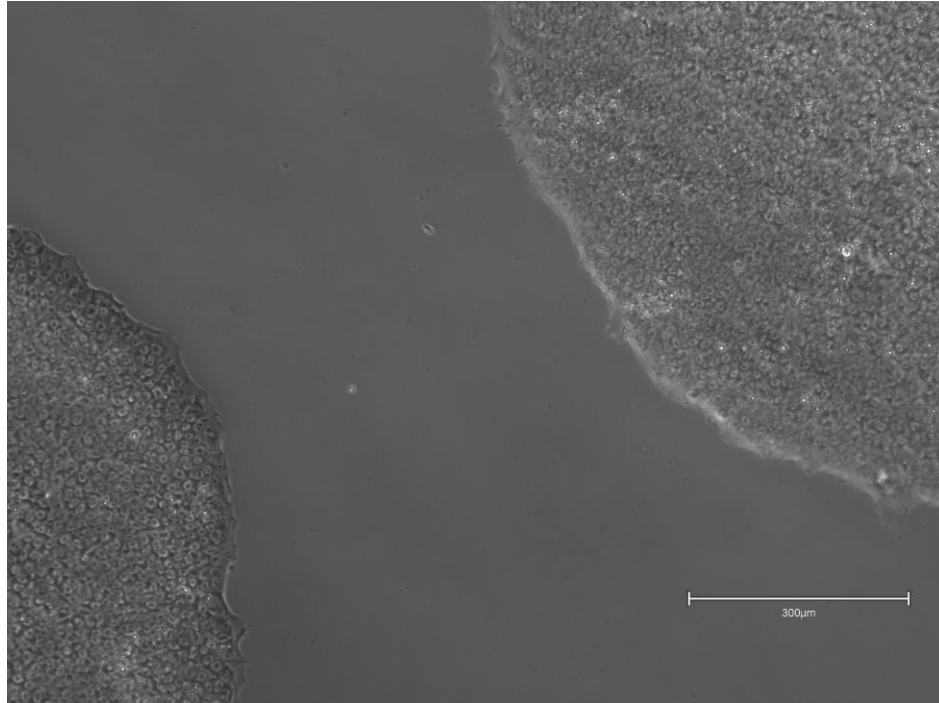
# LUMC0296iOAT03, p5

Result morphology: Brightfield pictures of representative hiPSC colony on day 7 after passaging

40x



100x

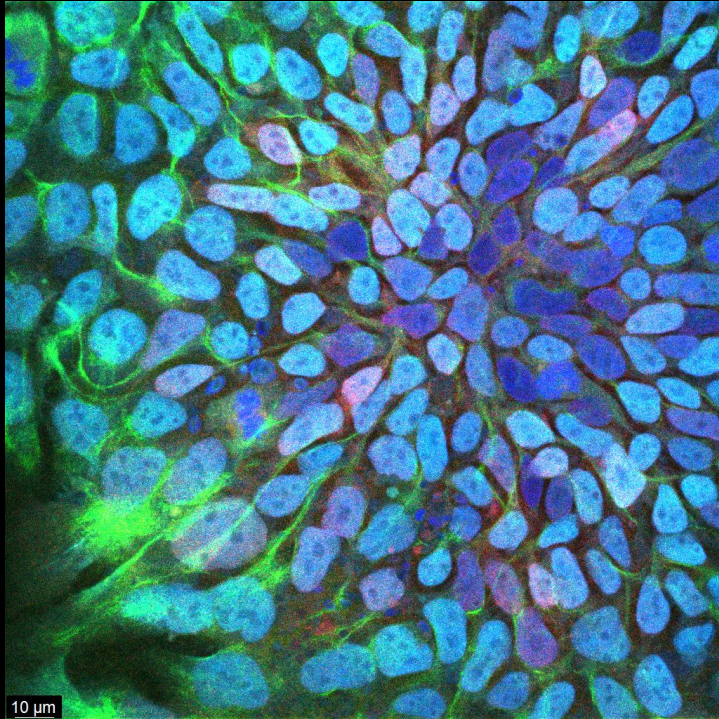


**Conclusion:** hiPSC colony displays the typical morphology of undifferentiated hPSCs.

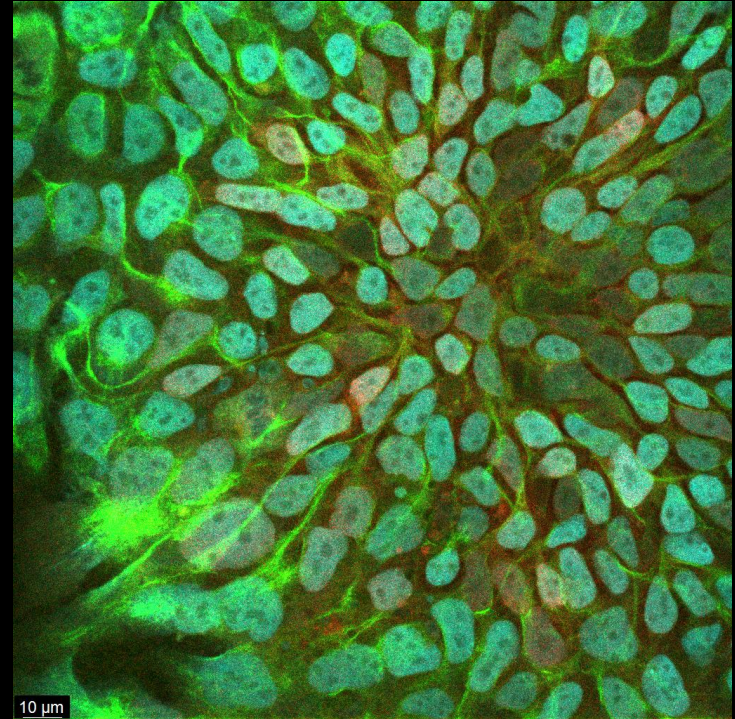
# LUMC0296iOAT03, p5

7 days Ectoderm Differentiation

Nestin FABP7 PAX6 DAPI



Nestin FABP7 PAX6

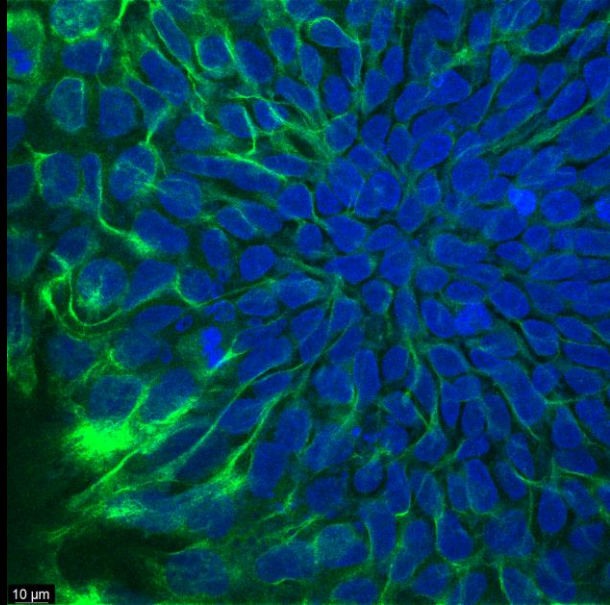




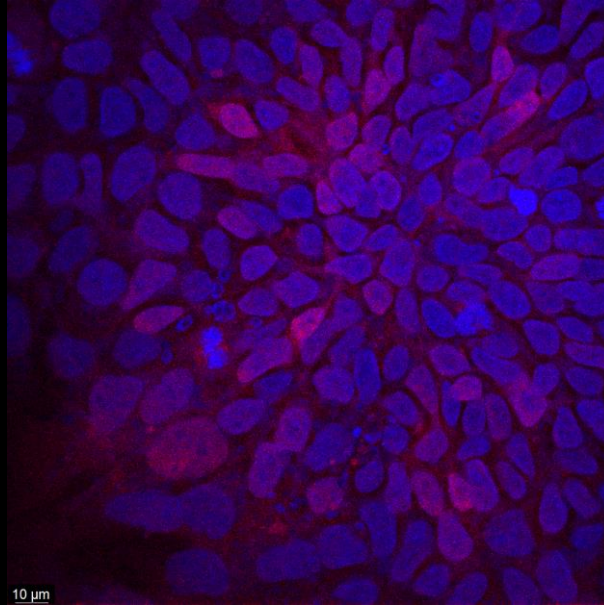
# LUMC0296iOAT03, p5

7 days Ectoderm Differentiation

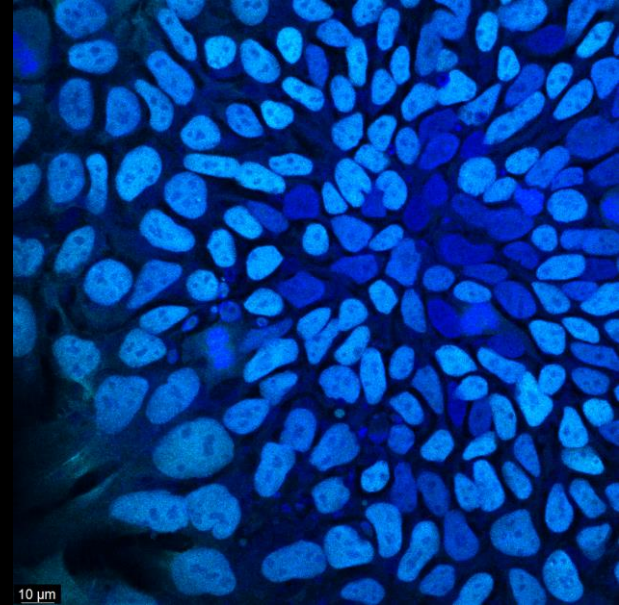
Nestin DAPI



FABP7 DAPI



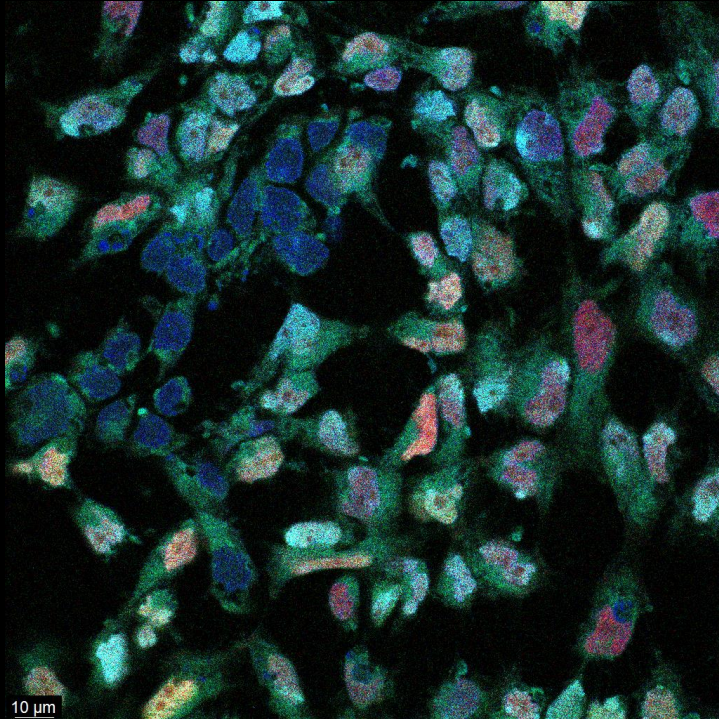
PAX6 DAPI



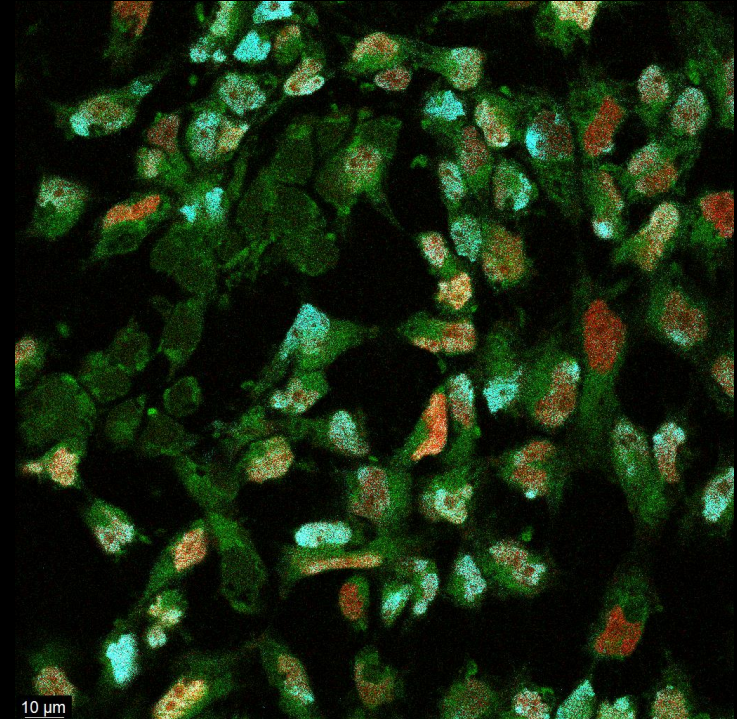
# LUMC0296iOAT03, p5

5 days Endoderm Differentiation

EOMES FOXA2 GATA-4 DAPI



EOMES FOXA2 GATA-4

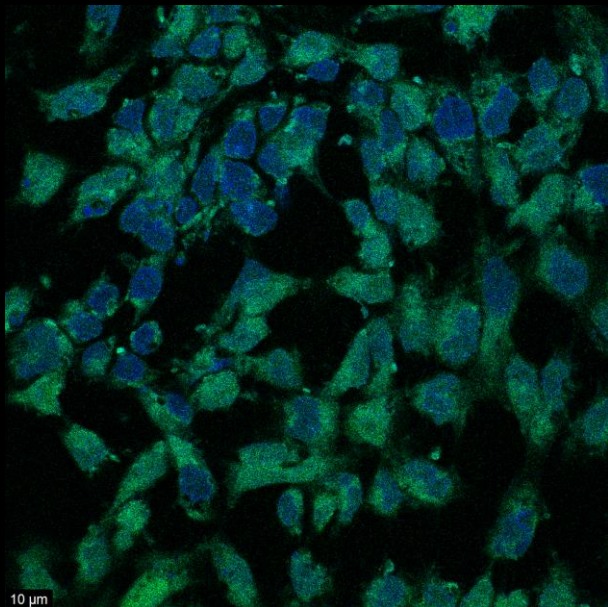




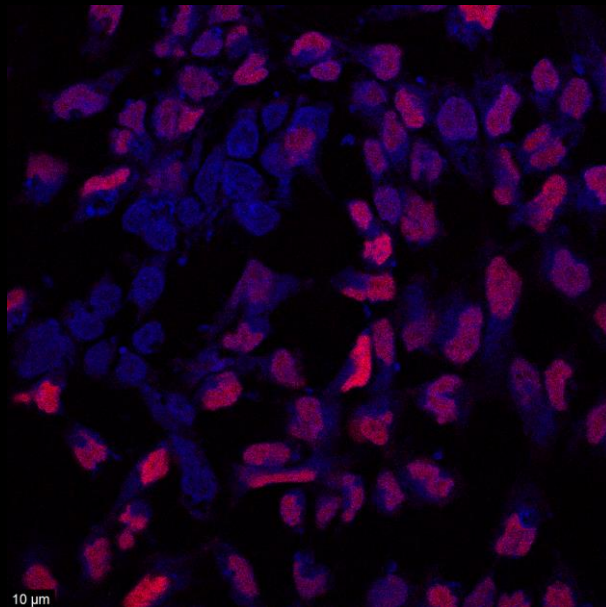
# LUMC0296iOAT03, p5

5 days Endoderm Differentiation

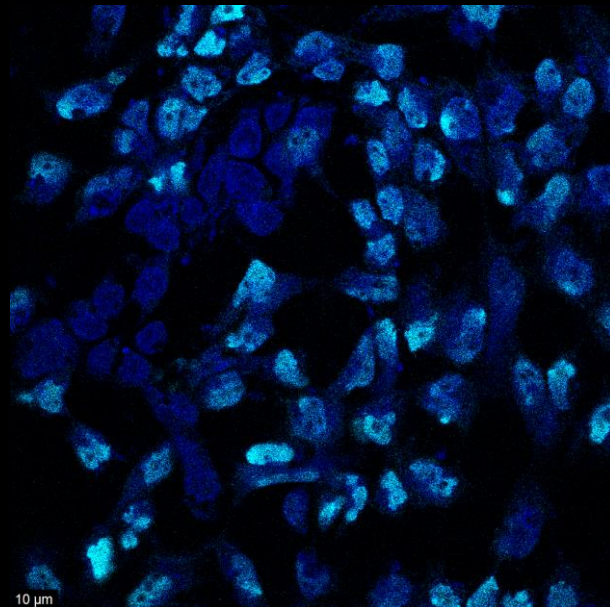
EOMES DAPI



FOXA2 DAPI



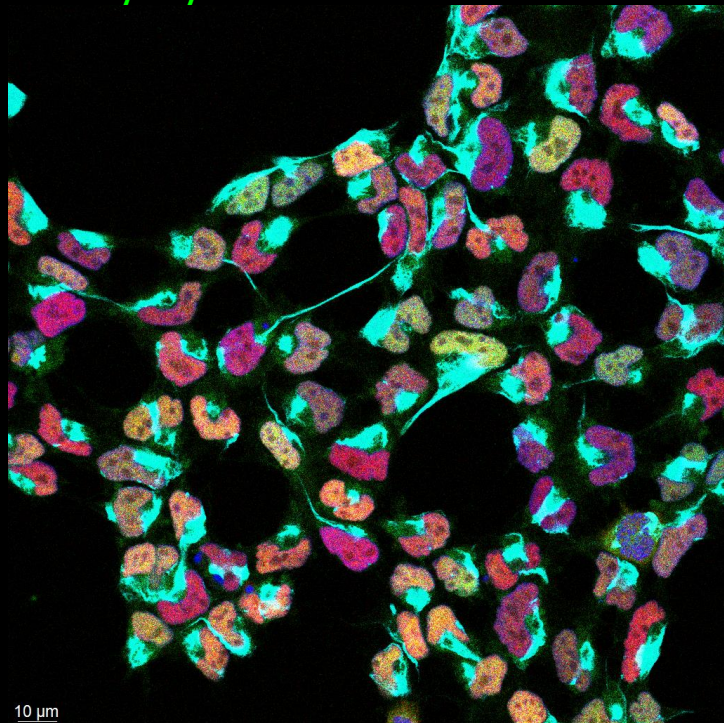
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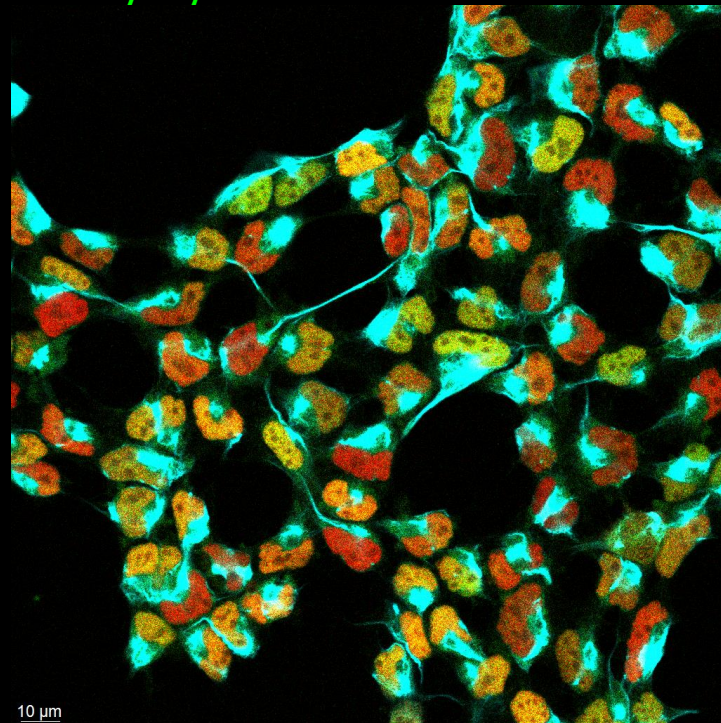
# LUMC0296iOAT03, p5

5 days Mesoderm Differentiation

Brachyury CDX2 Vimentin DAPI



Brachyury CDX2 Vimentin

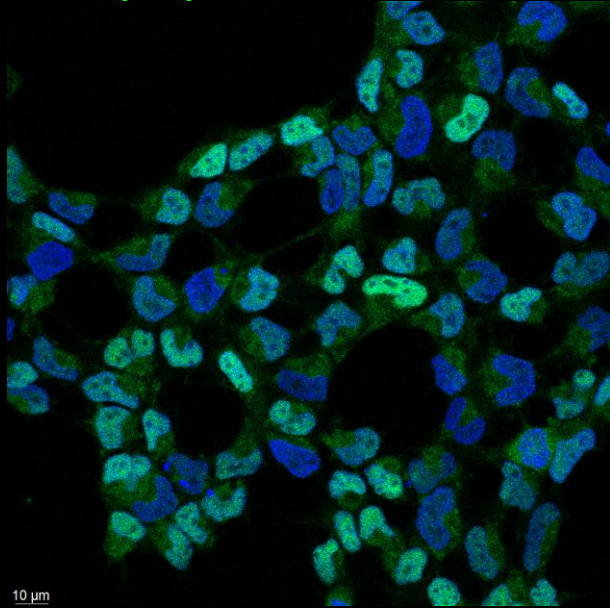




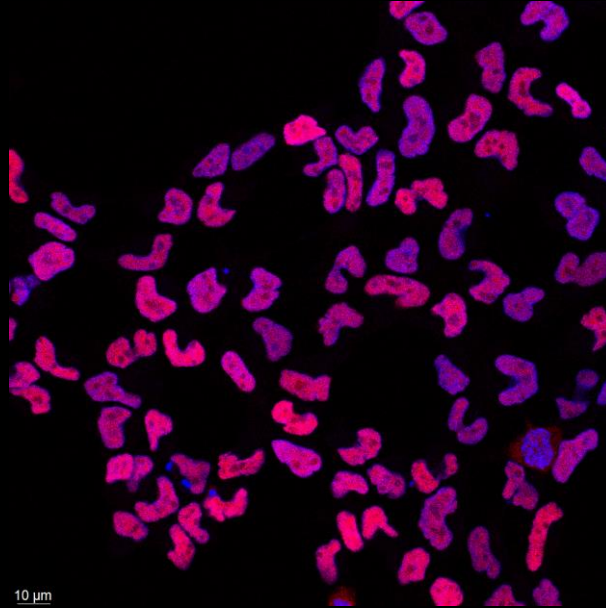
# LUMC0296iOAT03, p5

5 days Mesoderm Differentiation

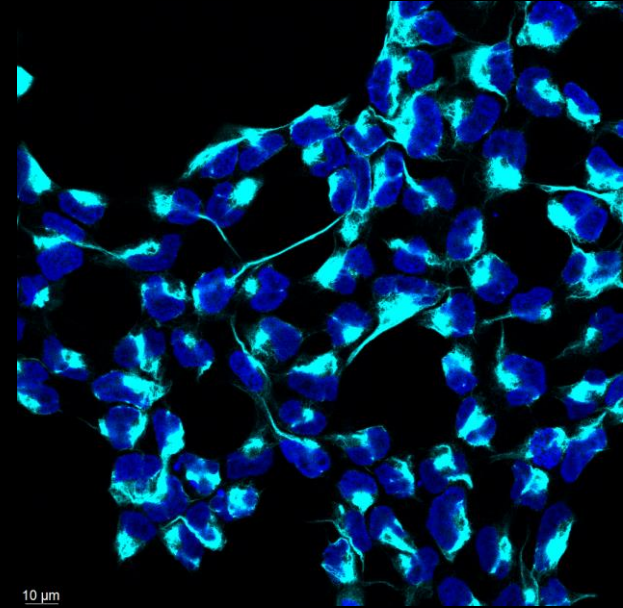
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CDX2 DAPI



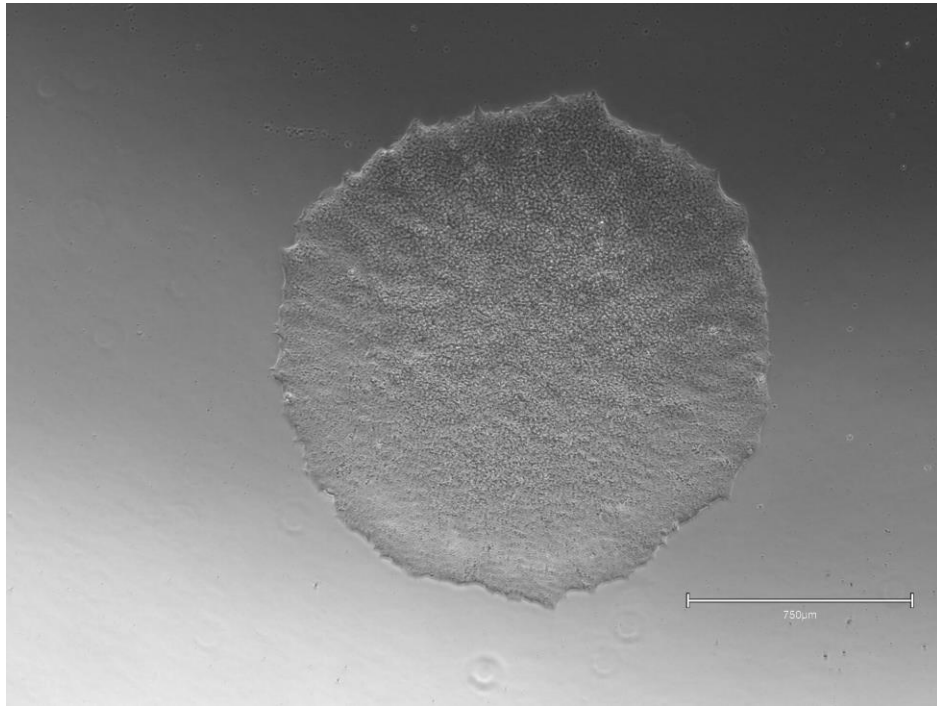
Vimentin DAPI



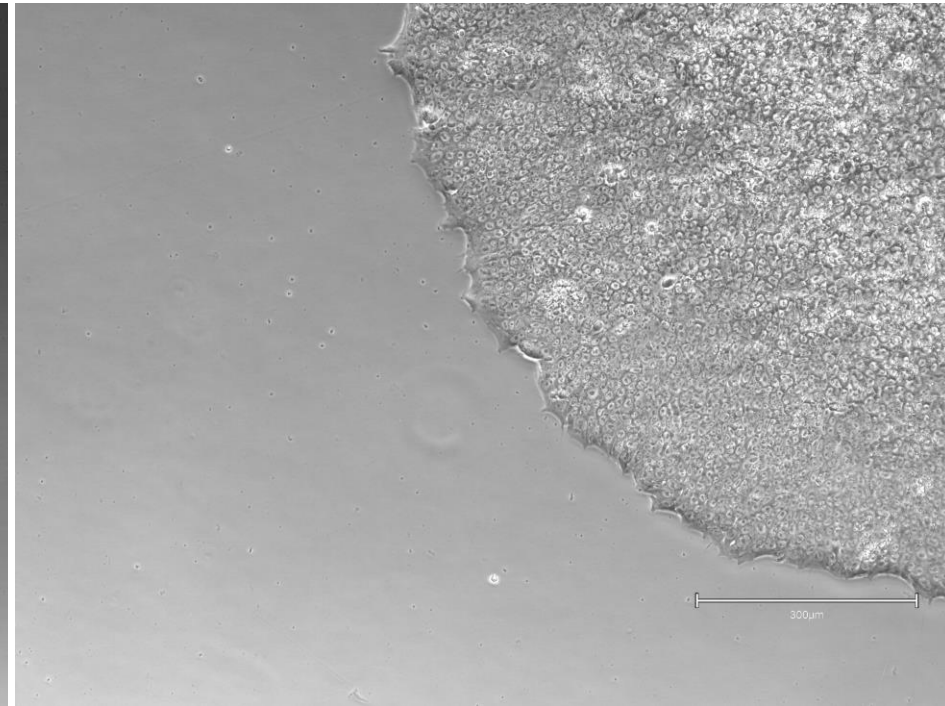
# LUMC0296iOAT07, p5

Result morphology: Brightfield pictures of representative hiPSC colony on day 7 after passaging

40x



100x



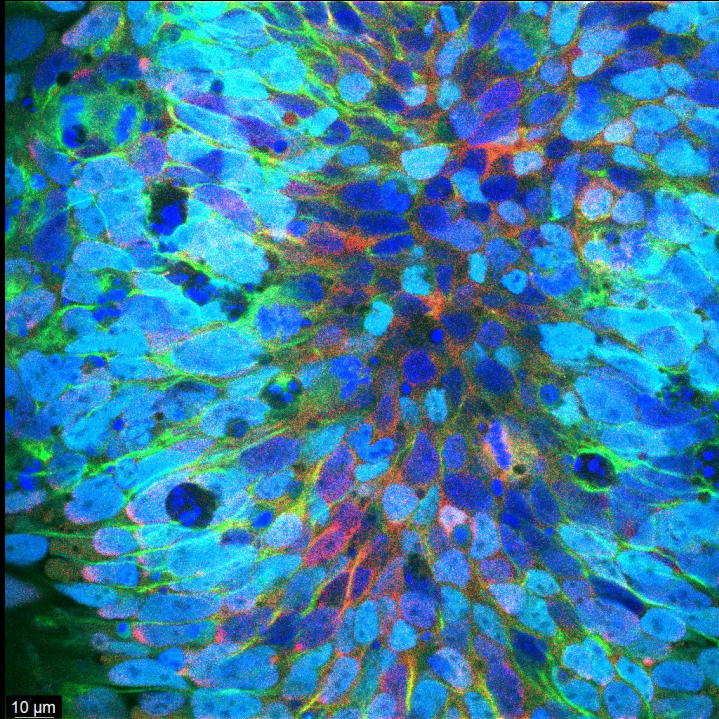
**Conclusion:** hiPSC colony displays the typical morphology of undifferentiated hPSCs.



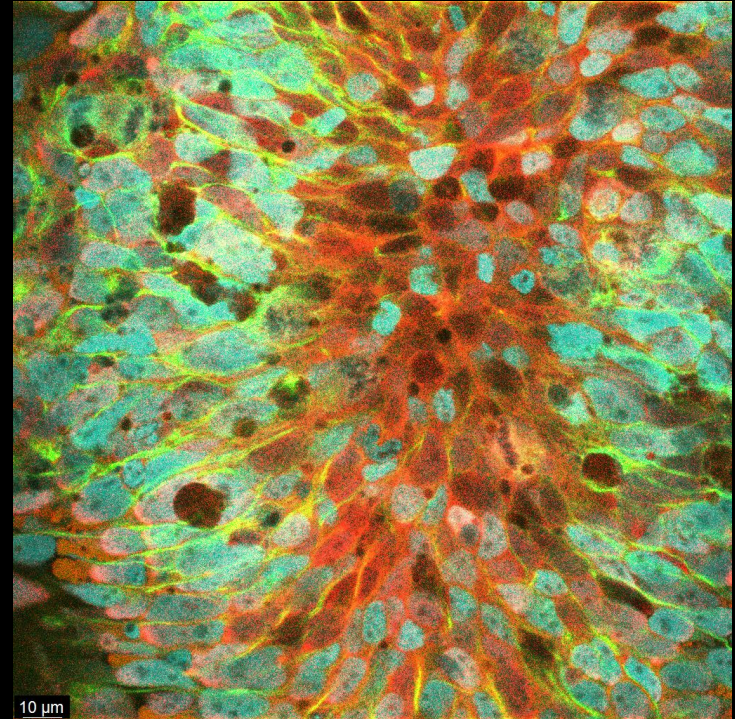
# LUMC0296iOAT07, p5

7 days Ectoderm Differentiation

Nestin FABP7 PAX6 DAPI



Nestin FABP7 PAX6

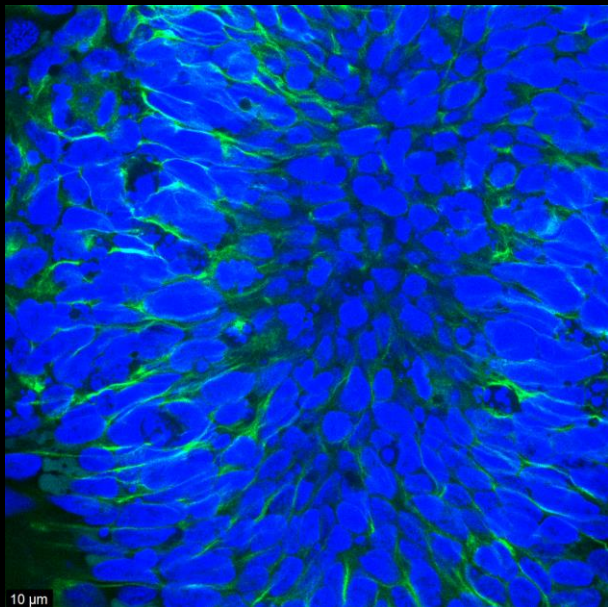




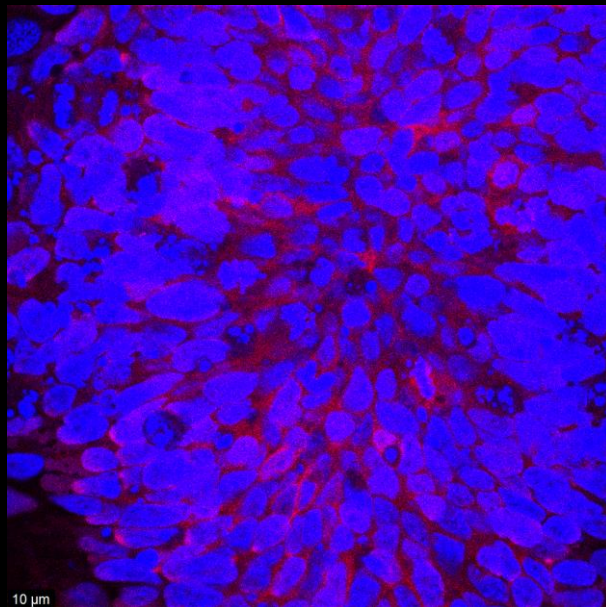
# LUMC0296iOAT07, p5

7 days Ectoderm Differentiation

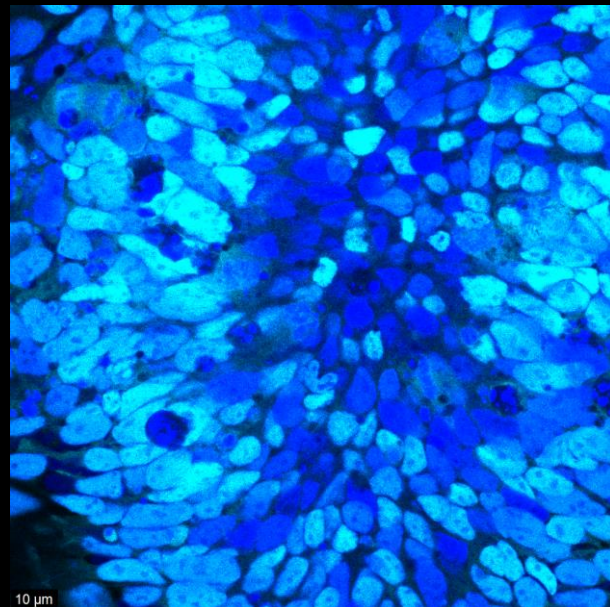
Nestin DAPI



FABP7 DAPI



PAX6 DAPI

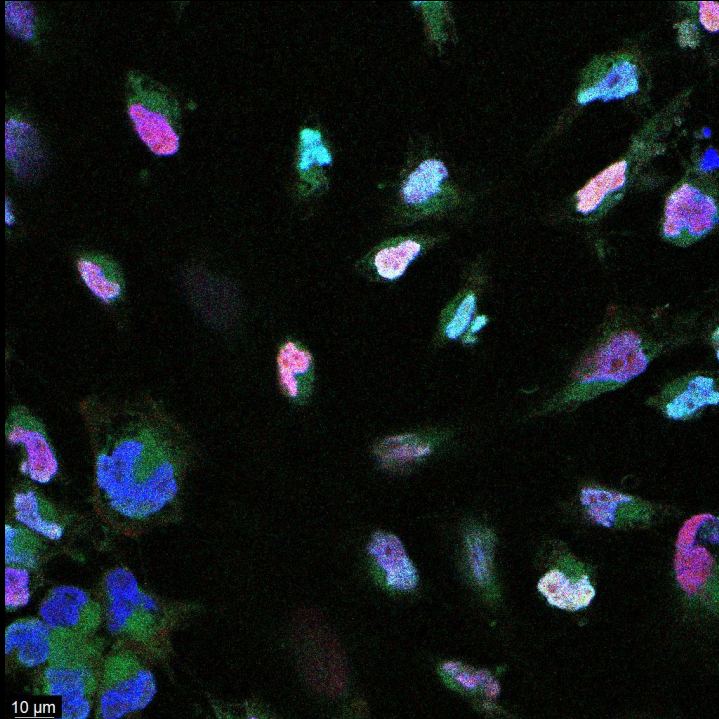




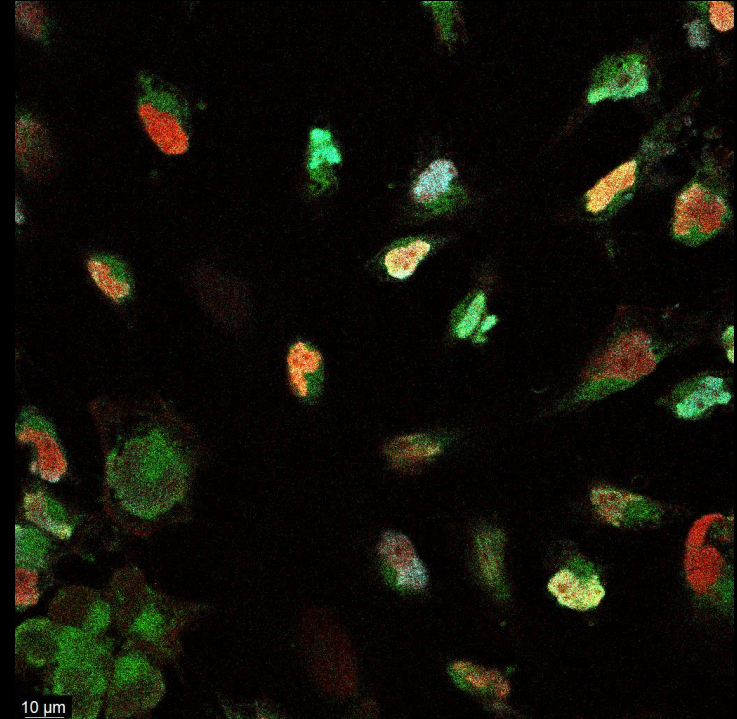
# LUMC0296iOAT07, p5

5 days Endoderm Differentiation

EOMES FOXA2 GATA-4 DAPI



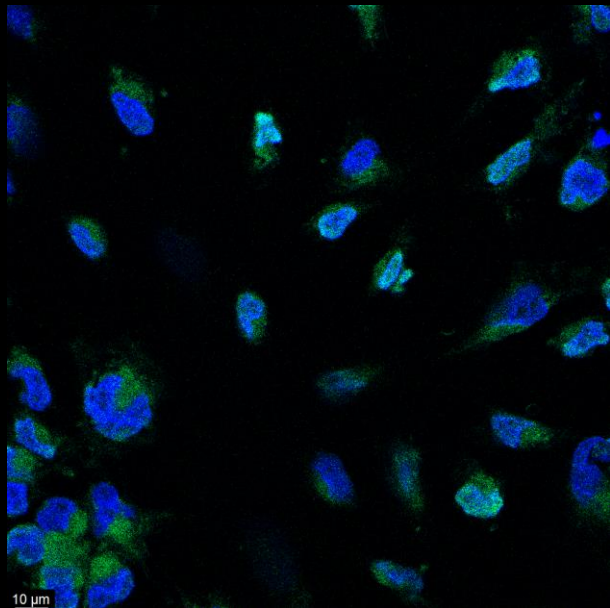
EOMES FOXA2 GATA-4



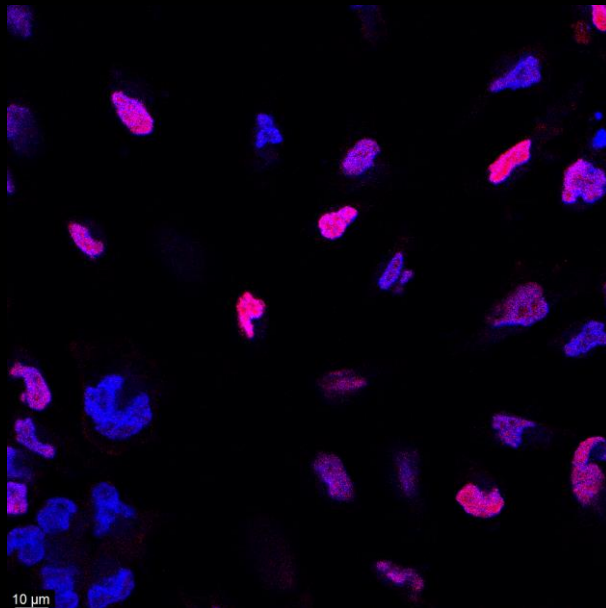
# LUMC0296iOAT07, p5

5 days Endoderm Differentiation

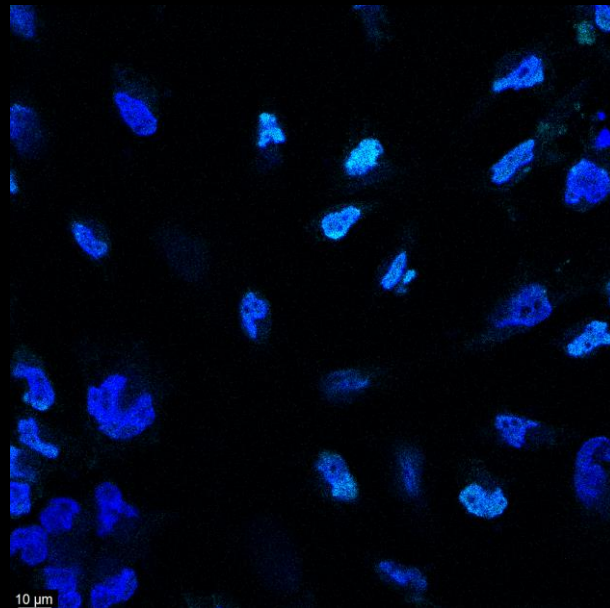
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FOXA2 DAPI



GATA-4 DAPI

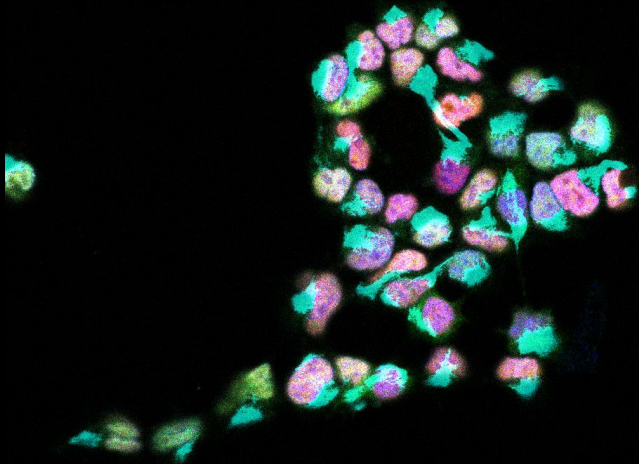


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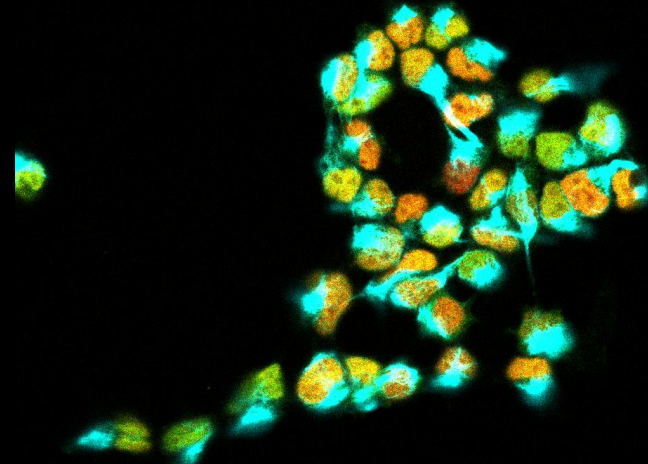
5 days Mesoderm Differentiation

Brachyury CDX2 Vimentin DAPI

Brachyury CDX2 Vimentin



10  $\mu$ m

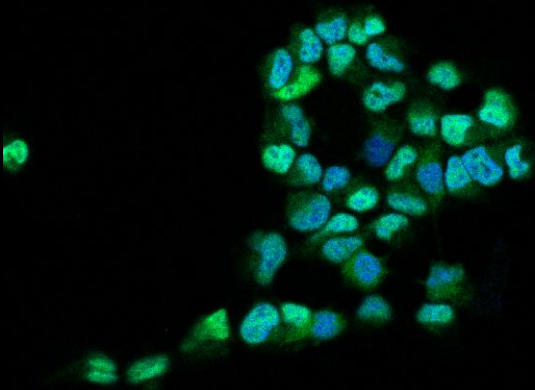


10  $\mu$ m

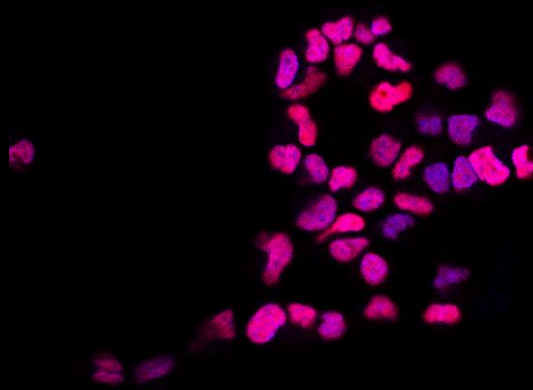
# LUMC0296iOAT07, p5

5 days Mesoderm Differentiation

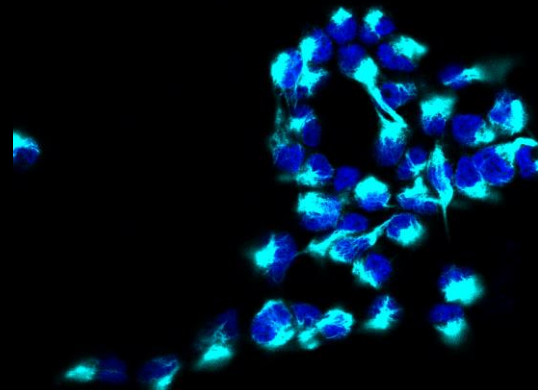
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CDX2 DAPI



Vimentin DAPI

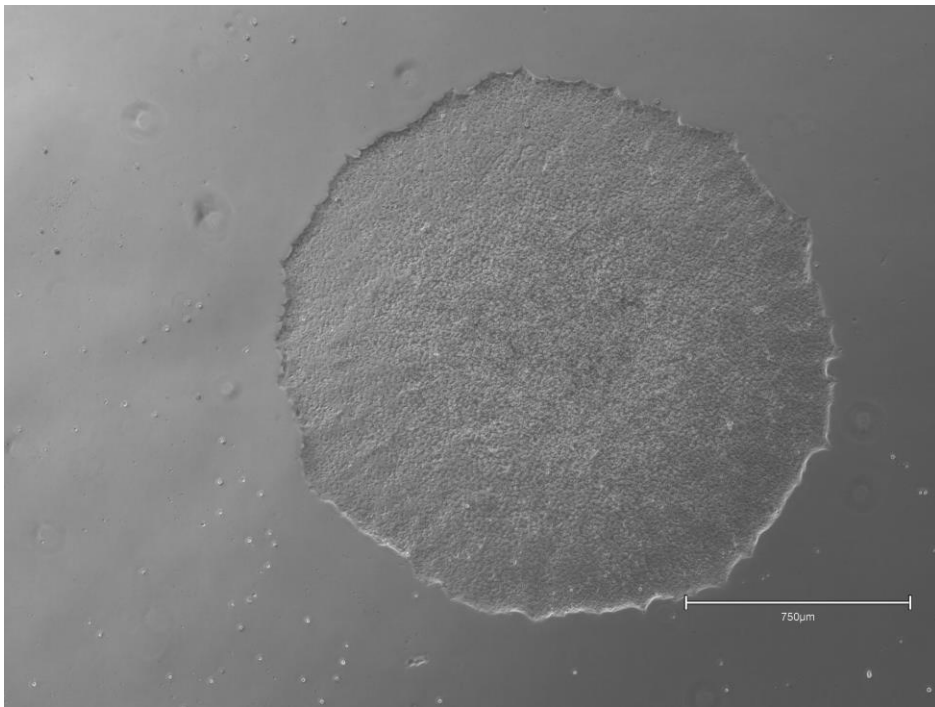




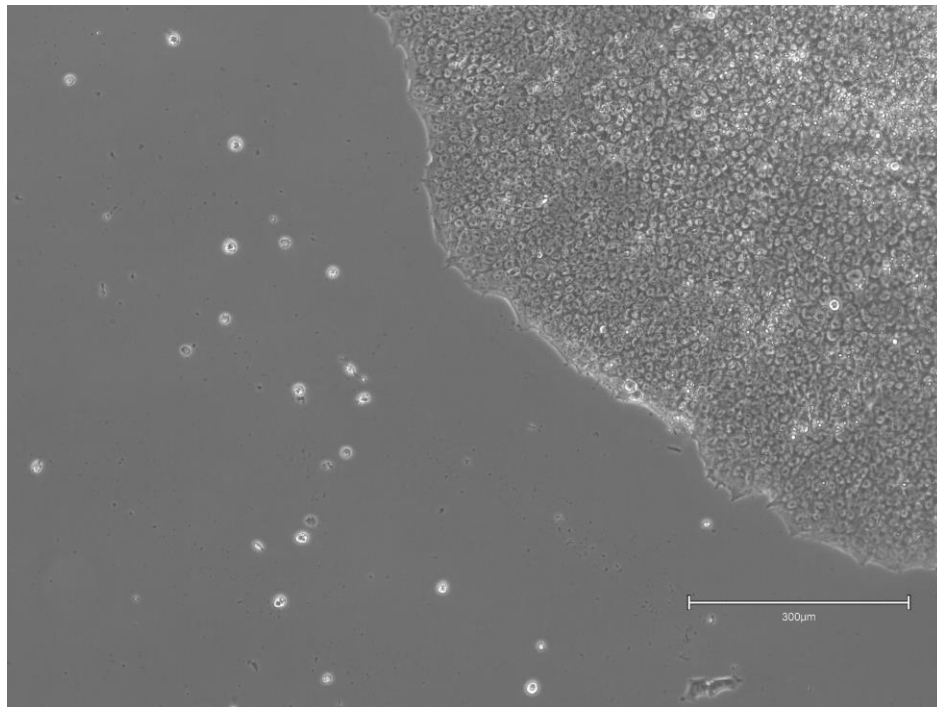
# LUMC0296iOAT08, p5

Result morphology: Brightfield pictures of representative hiPSC colony on day 7 after passaging

40x



100x

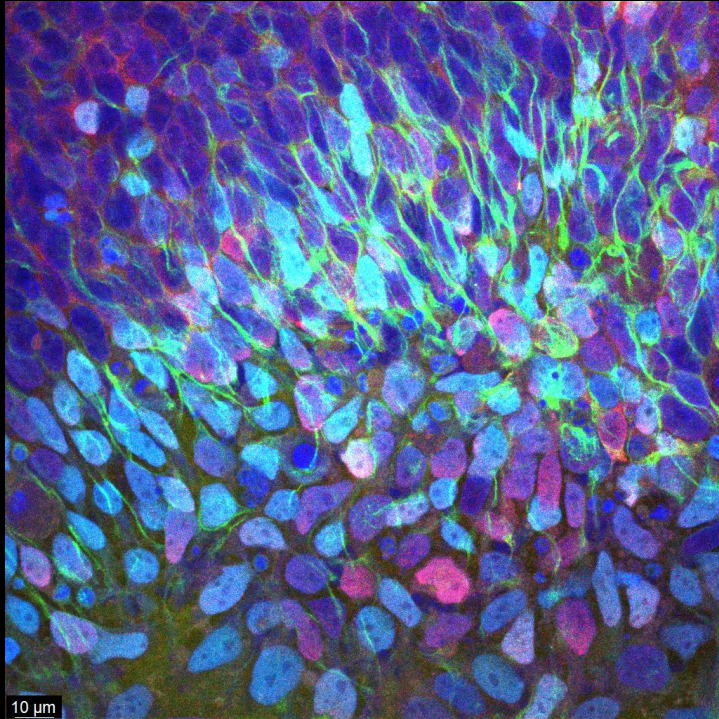


**Conclusion:** hiPSC colony displays the typical morphology of undifferentiated hPSCs.

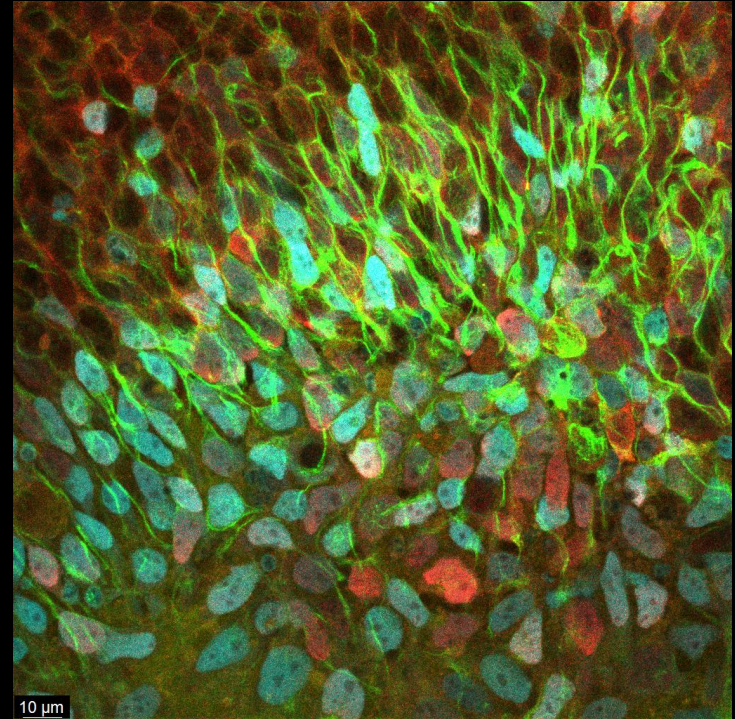
# LUMC0296iOAT08, p5

7 days Ectoderm Differentiation

Nestin FABP7 PAX6 DAPI



Nestin FABP7 PAX6

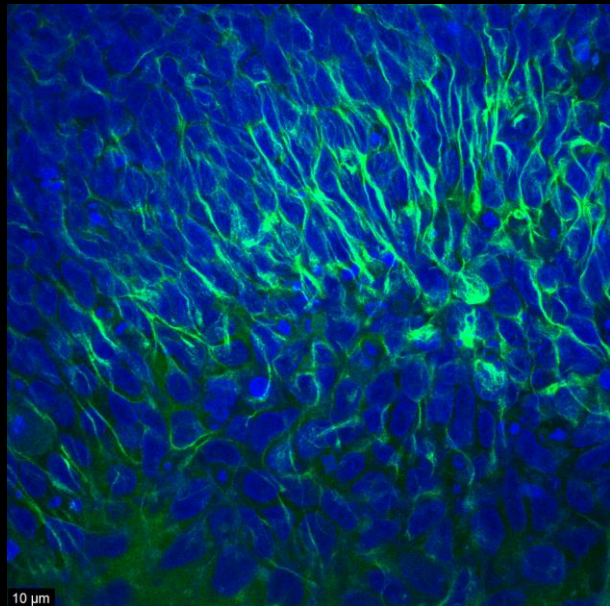




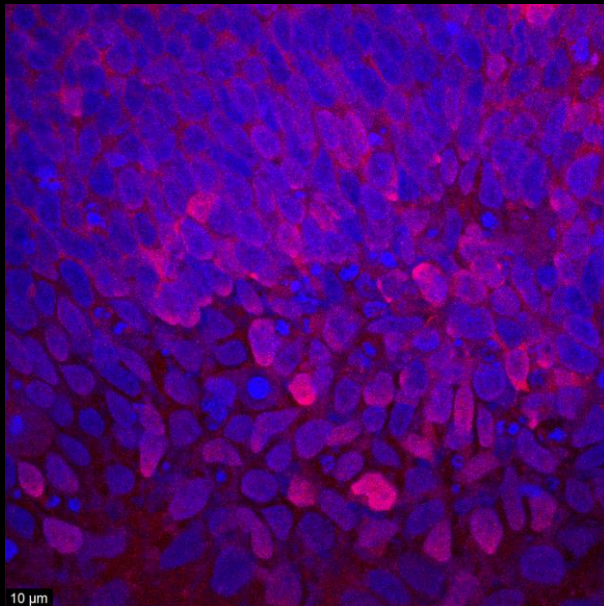
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7 days Ectoderm Differentiation

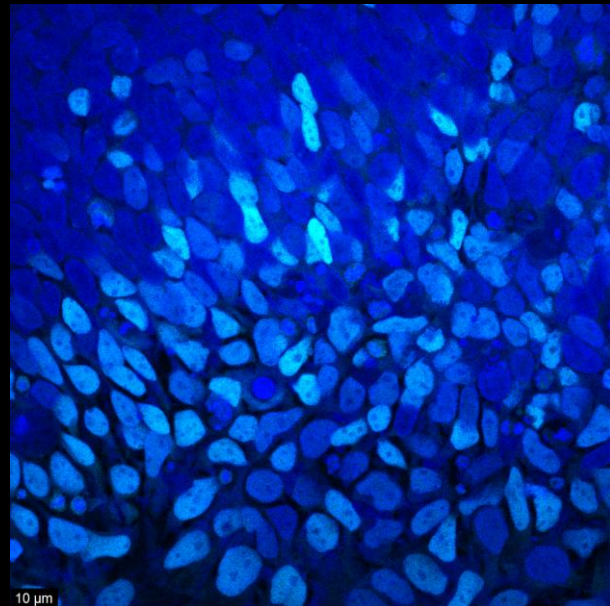
**Nestin** DAPI



**FABP7** DAPI



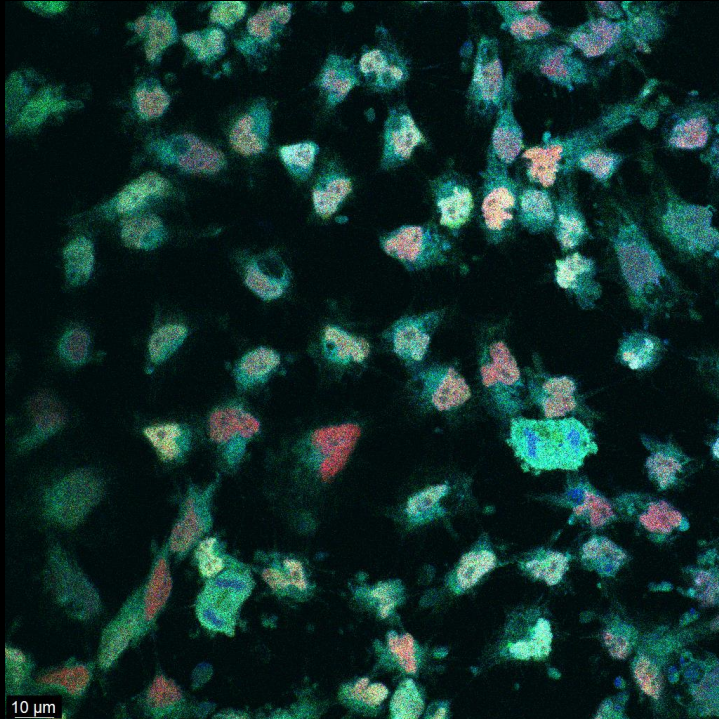
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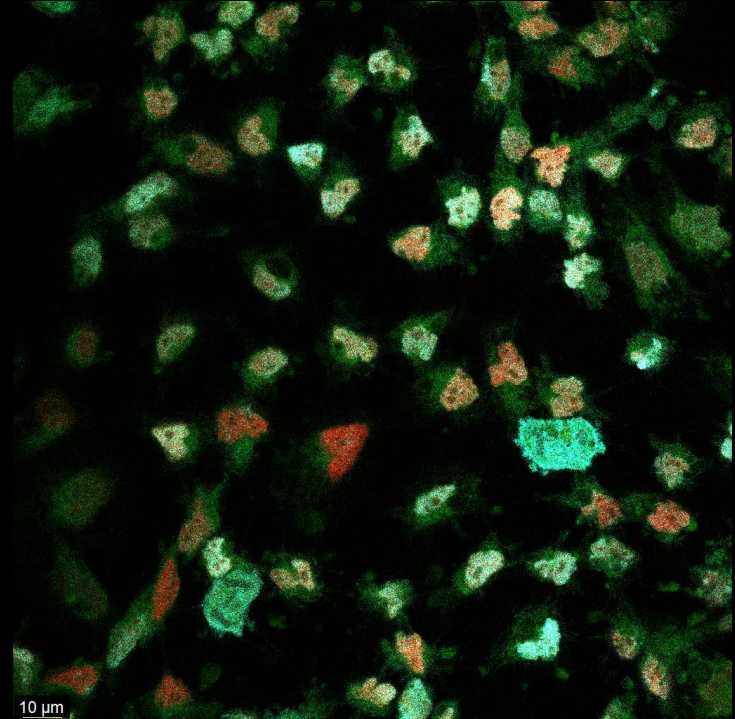
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5 days Endoderm Differentiation

EOMES FOXA2 GATA-4 DAPI



EOMES FOXA2 GATA-4

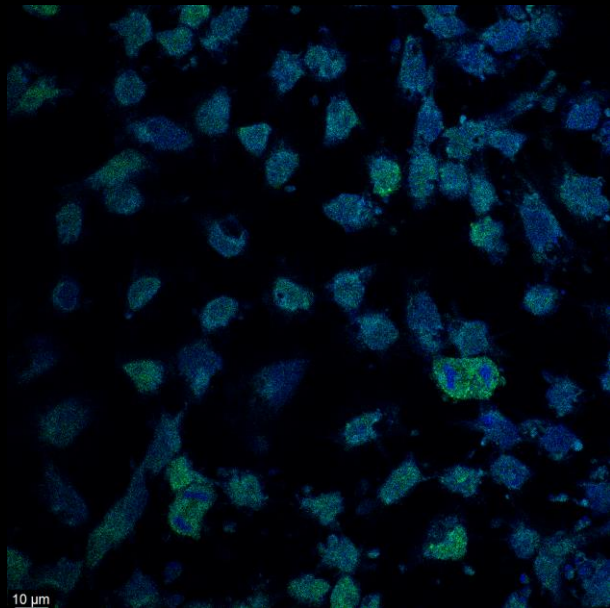




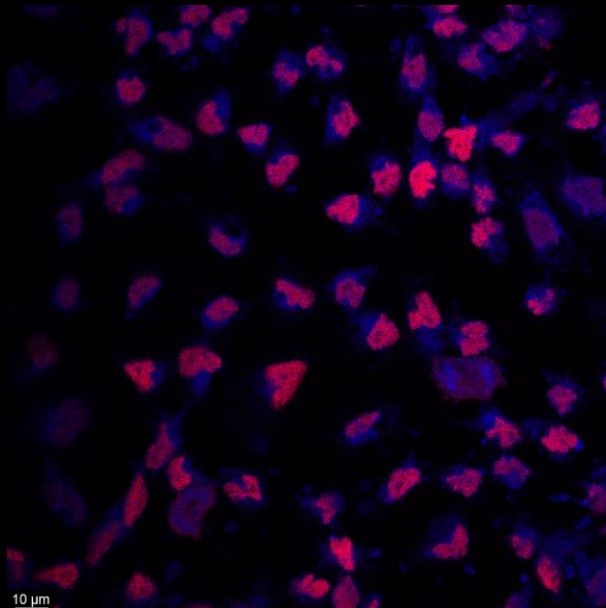
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5 days Endoderm Differentiation

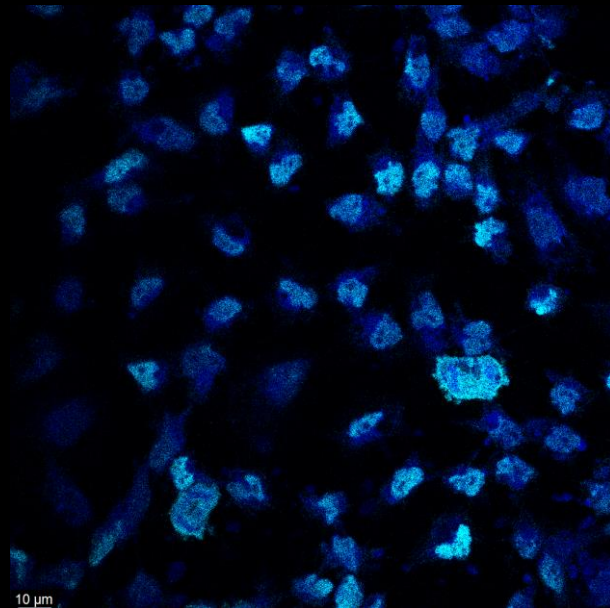
EOMES DAPI



FOXA2 DAPI



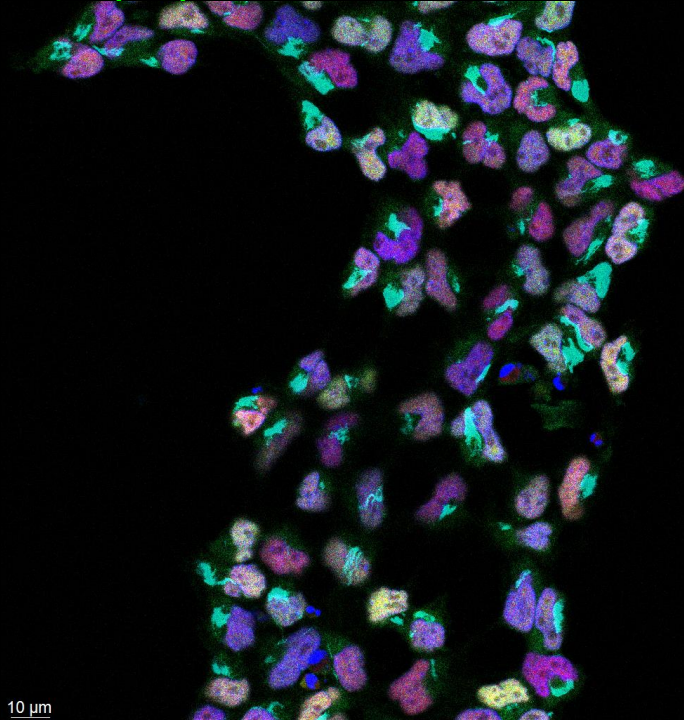
GATA-4 DAPI



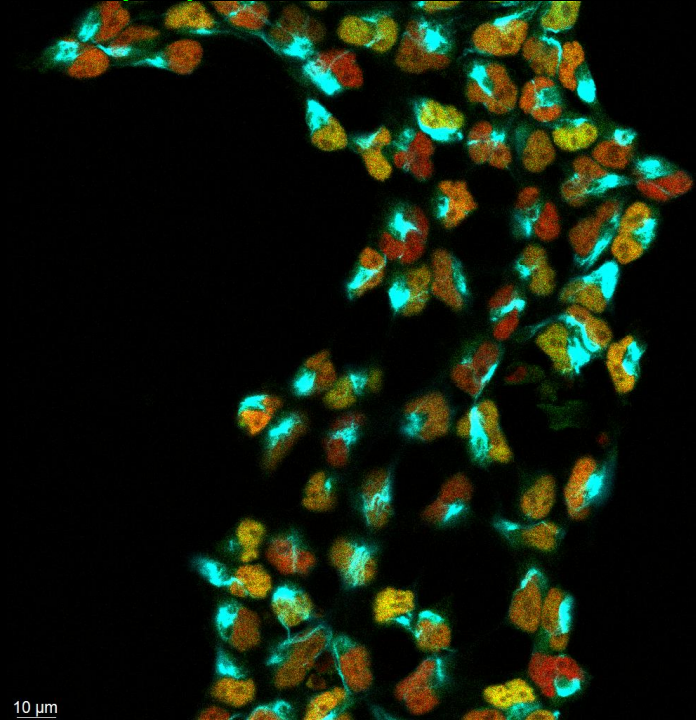
# LUMC0296iOAT08, p5

5 days Mesoderm Differentiation

Brachyury CDX2 Vimentin DAPI



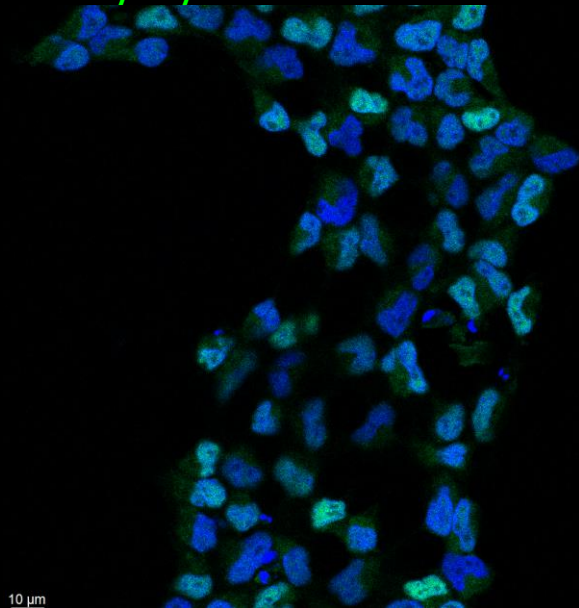
Brachyury CDX2 Vimentin



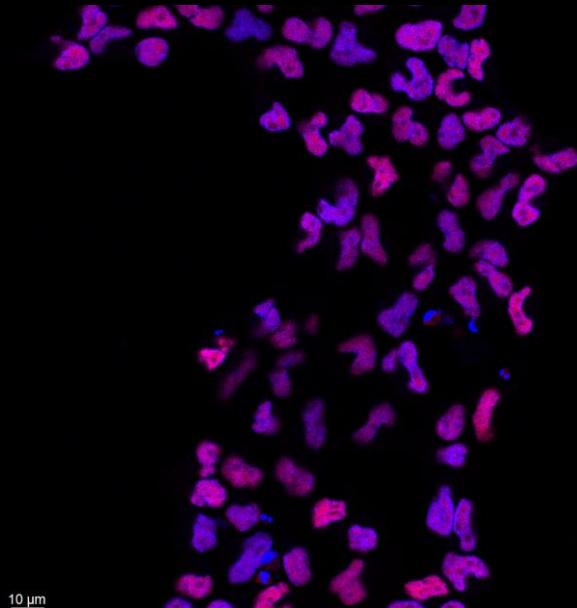
# LUMC0296iOAT08, p5

5 days Mesoderm Differentiation

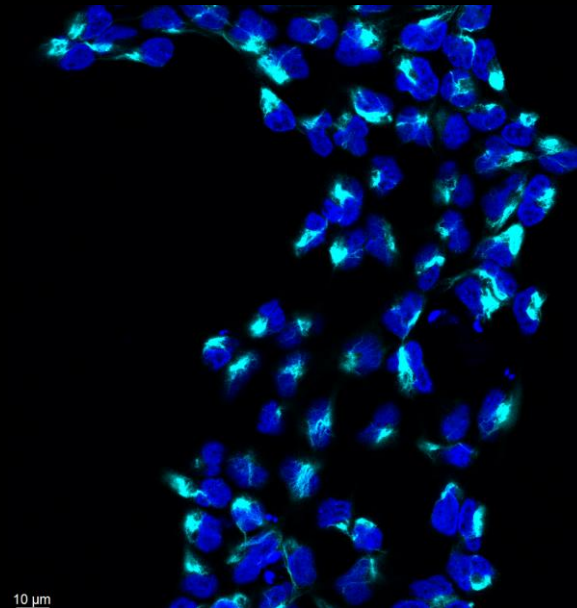
Brachyury DAPI



CDX2 DAPI



Vimentin DAPI





# LUMC0296iOAT - Summary conclusions

Clone	SeV Absence	EBNA Absence	CLA	Karyotyping	Pluripotency markers	Morphology	Ectoderm	Endoderm	Mesoderm
03	✓	✓	✓	✓	✓	✓	✓	✓	✓
07	✓	✓	✓	✓	✓	✓	✓	✓	✓
08	✓	✓	✓	✓	✓	✓	✓	✓	✓
	undetectable	undetectable	identity match between primary cells and hiPSCs	No abnormalities detected	≥75% positive for all markers, Except for CD15, this should not exceed 10%	Typical hiPSC morphology	≥2 markers clearly present	≥2 markers clearly present	≥2 markers clearly present



Leiden University  
Medical Center

#### Contact information LUMC Hotel:

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The Netherlands  
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