LARGE-SCALE EXPANSION OF HUMAN iPS CELLS IN CliniMACS PRODIGY® ADHERENT CELL CULTURE SYSTEM

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公益財団法人
京都大学 iPS 細胞研究財団

INTRODUCTION

MANUFACTURING AND BANKING OF CLINICAL GRADE iPS CELLS

EFFICIENT AND SCALABLE CELL EXPANSION PROTOCOL

MAINTAINING CELLS MORPHOLOGY, IMMUNOPHENOTYPE, GENETIC STABILITY AND PLURIPOTENCY
CURRENT GOOD MANUFACTURING PRACTICE (CGMP) REQUIREMENTS

TRANSITION FROM MANUAL PROTOCOLS ONTO CLOSED CULTURE PLATFORM

iMatrix-511/StemFit AK03N CULTURE SYSTEM

CliniMACS PRODIGY ADHERENT CELL CULTURE SYSTEM

GMP-COMPLIANT AND SCALABLE iPSC CULTIVATION

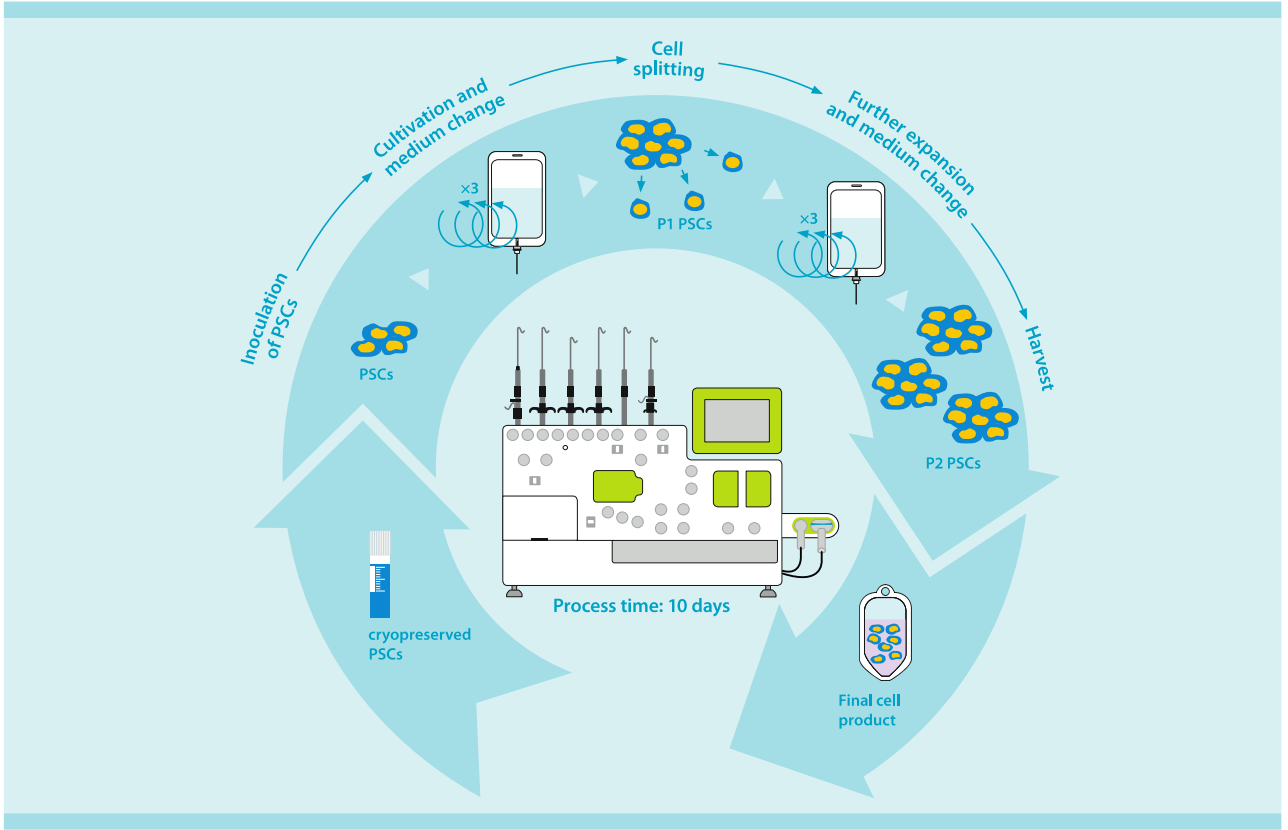
PROCESS DEVELOPMENT PROGRESS

INITIAL EXPANSION ON THE STANDARD 6-WELL TISSUE CULTURE PLATE
HARVEST AND INOCULATION INTO THE CliniMACS PRODIGY TS 730 TUBING SET CHAMBER
CULTURE UNDER THE ADHERENT CELL CULTURE PROCESS
HARVEST AND INOCULATION INTO THE CellSTACK-1 OR CellSTACK®-5 CHAMBER (CORNING)
FINAL HARVEST AND BANKING

SEMI-AUTOMATED EXPANSION OF IPSCS

FROM 1E+05 UP TO 3E+08 CELLS
10 ~ 14 DAYS

Principle of the PSC expansion process using the CliniMACS Prodigy®



THAWING CELLS | 6-WELL PLATE

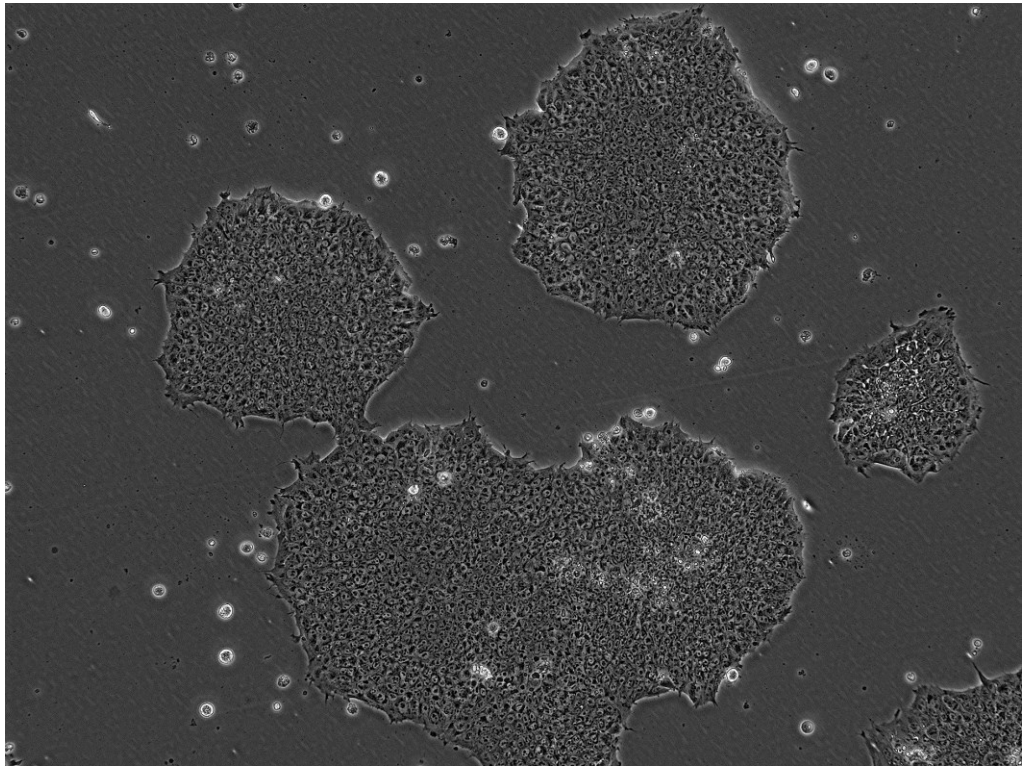
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CULTURE SUBSTRATE
- iMatrix-511 (MATRIXOME) | 0.25 µg/cm^2

INOCULATION
- 6-WELL PLATE | 1 WELL
- 1E+05 CELLS

DAILY MEDIUM CHANGE
- StemFit AK03N (AJINOMOTO)

HARVEST
- DAY 4 ~ 5
- ~ 2E+06 CELLS



D05 | 10X

CELL EXPANSION | PRODIGY TS 730 CHAMBER

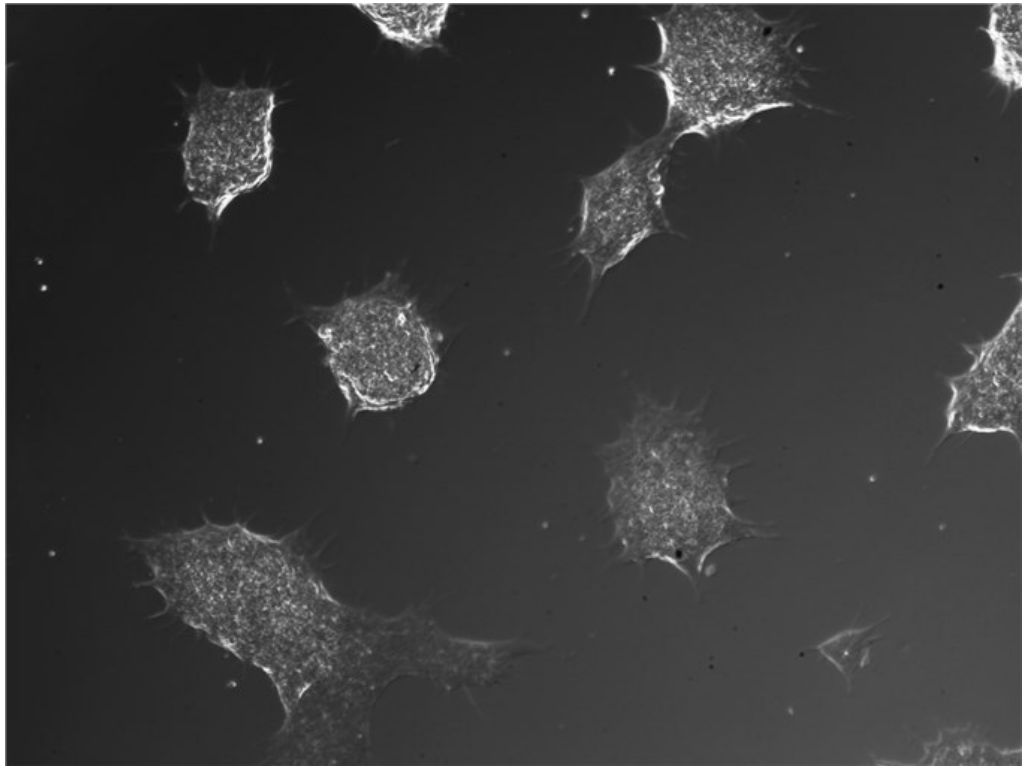
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CHAMBER COATING
- iMatrix-511 (MATRIXOME) | 4.5 µg/mL | 25 mL
- ~ 1 µg/cm^2

INOCULATION
- 2E+06 CELLS

DAILY MEDIUM CHANGE
- StemFit AK03N (AJINOMOTO)

HARVEST
- DAY 5
- 1.2E+07 CELLS



D02

CHALLENGE: OPTIMIZING iMatrix-511 CONCENTRATION

CELL EXPANSION | PRODIGY TS 730 | CellSTACK-1

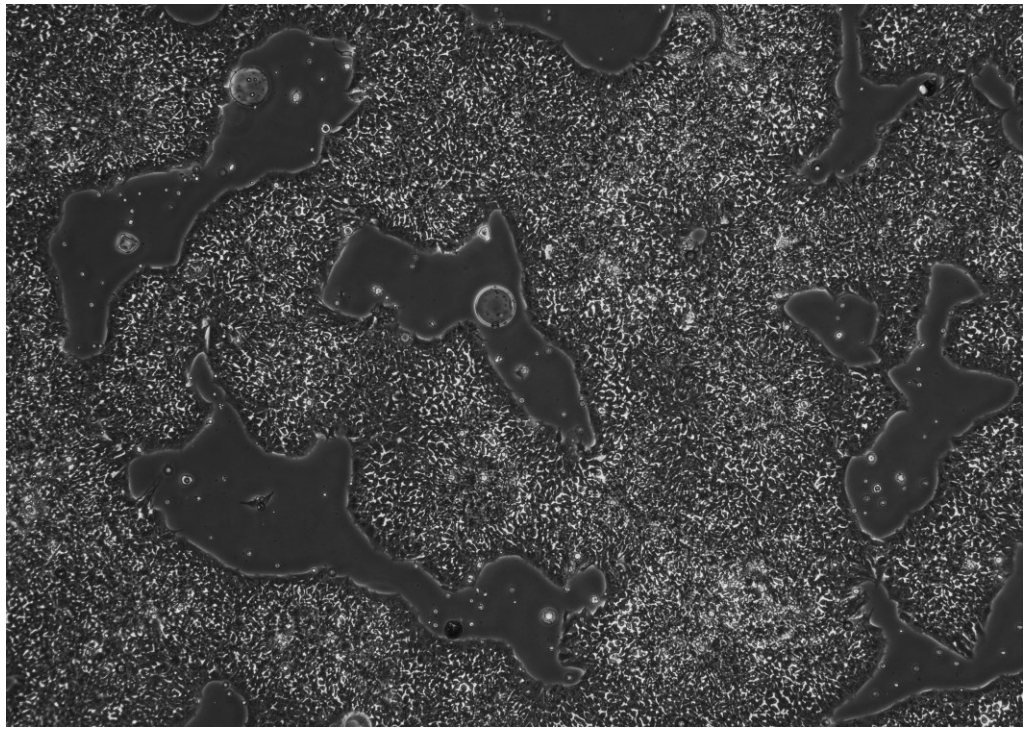
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CULTURE SUBSTRATE
- iMatrix-511 (MATRIXOME) | 2 µg/mL | 100 mL
- ~ 0.25 µg/cm^2

INOCULATION
- 2.5E+06 CELLS

DAILY MEDIUM CHANGE
- StemFit AK03N (AJINOMOTO)

HARVEST
- DAY 5
- 1.2E+08 CELLS



D04 | 4X

CELL EXPANSION | PRODIGY TS 730 | CellSTACK-5

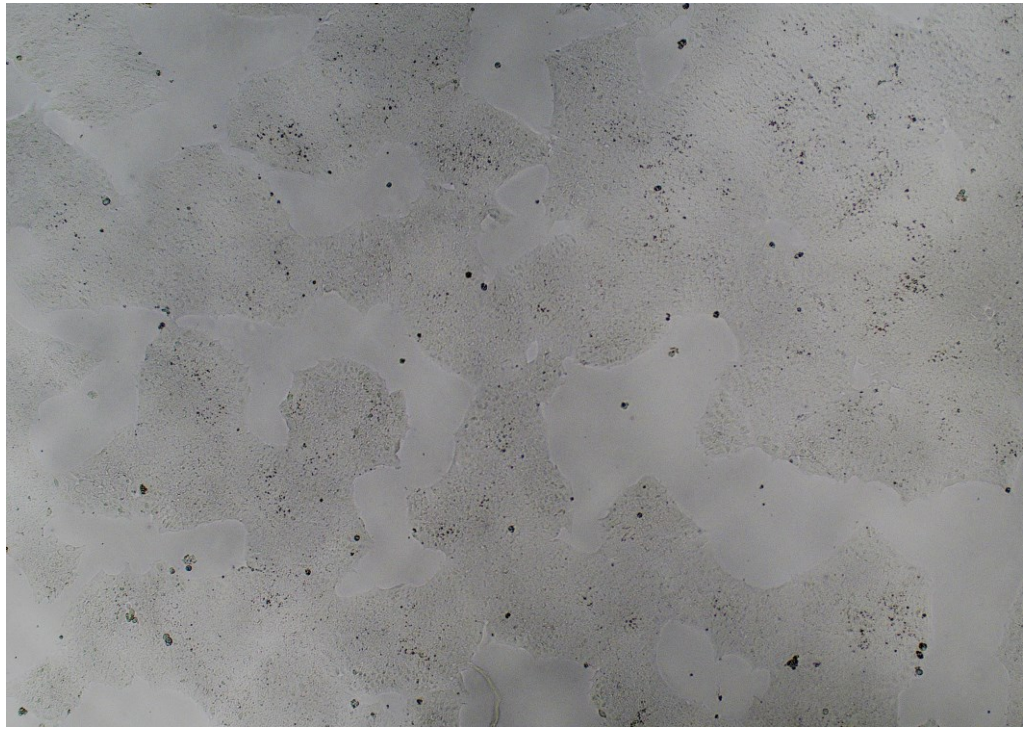
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CULTURE SUBSTRATE
- iMatrix-511 (MATRIXOME) | 2 µg/mL | 500 mL
- ~ 0.25 µg/cm^2

INOCULATION
- 2.2E+07 CELLS

DAILY MEDIUM CHANGE
- StemFit AK03N (AJINOMOTO)

HARVEST
- DAY 5
- 4E+08 CELLS

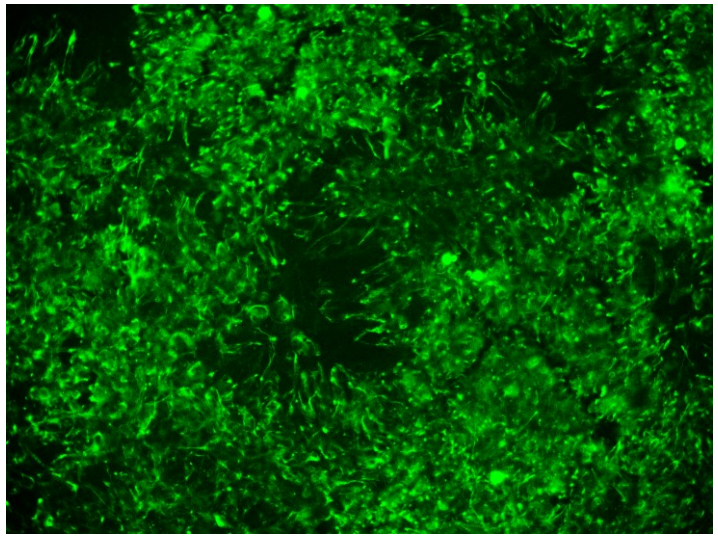


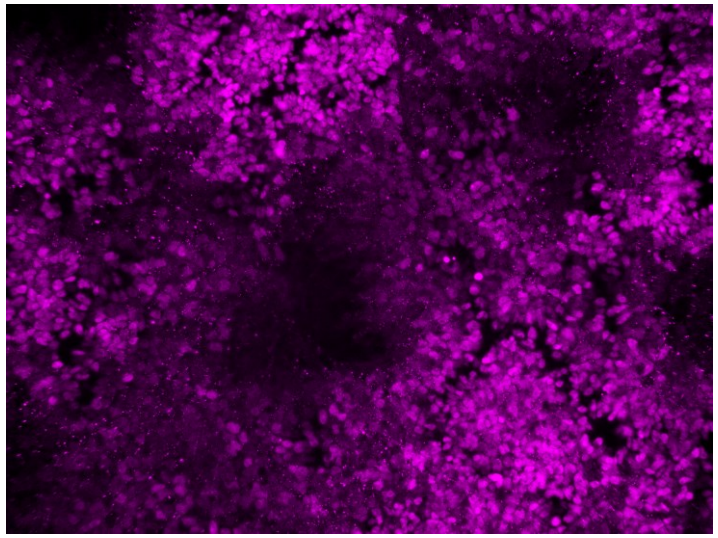
D03 | 4X

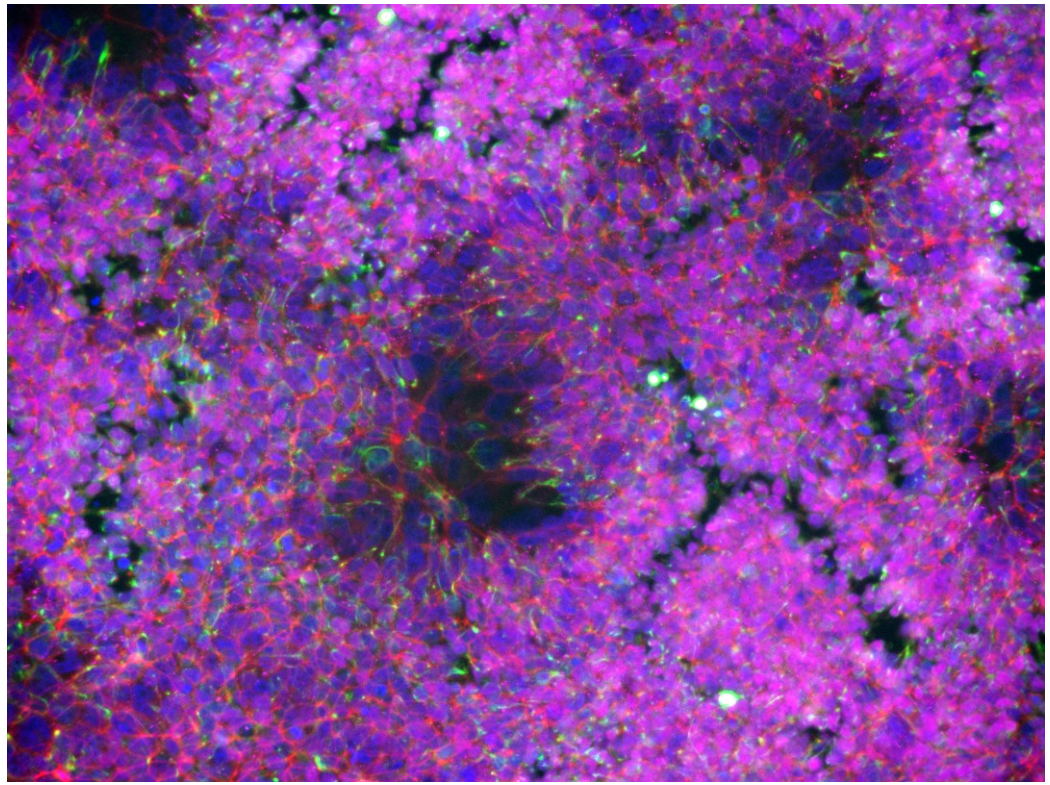
CHALLENGE: OPTIMIZING HARVEST PROTOCOL

CELL QUALITY EVALUATION

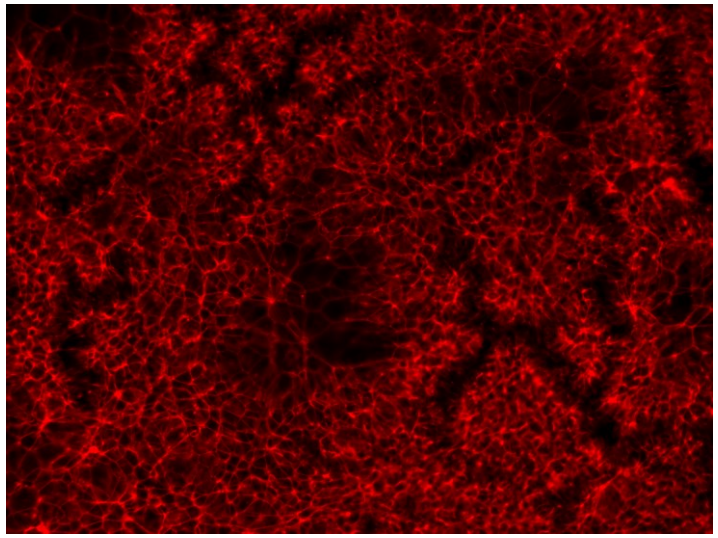
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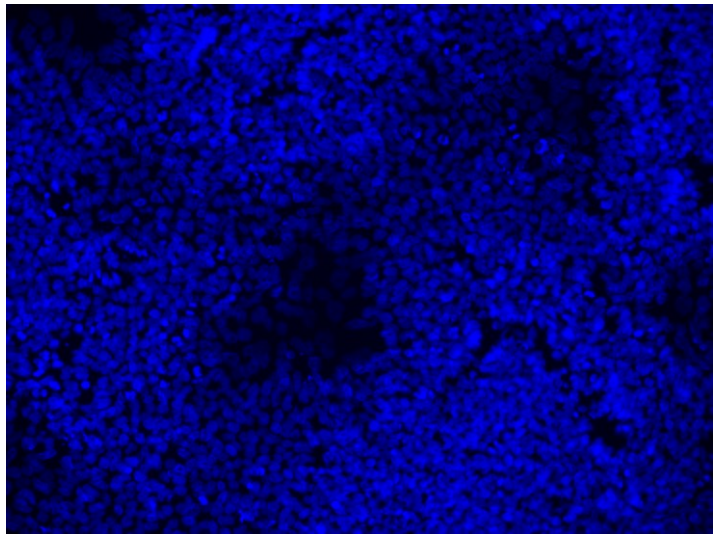






20X

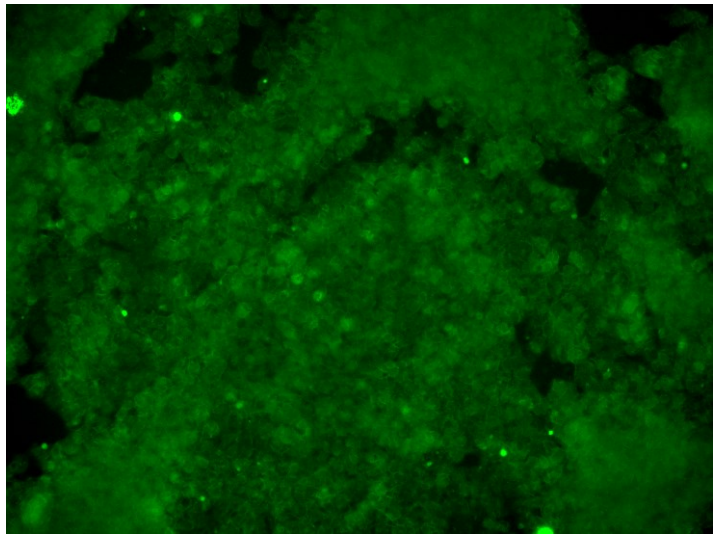


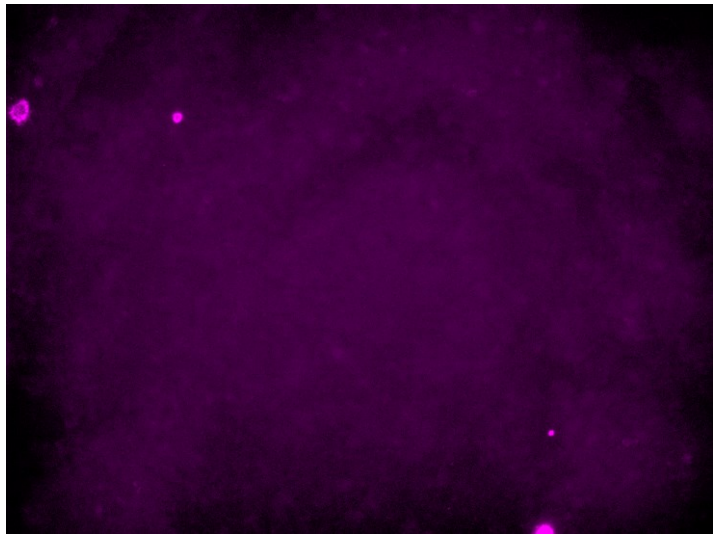


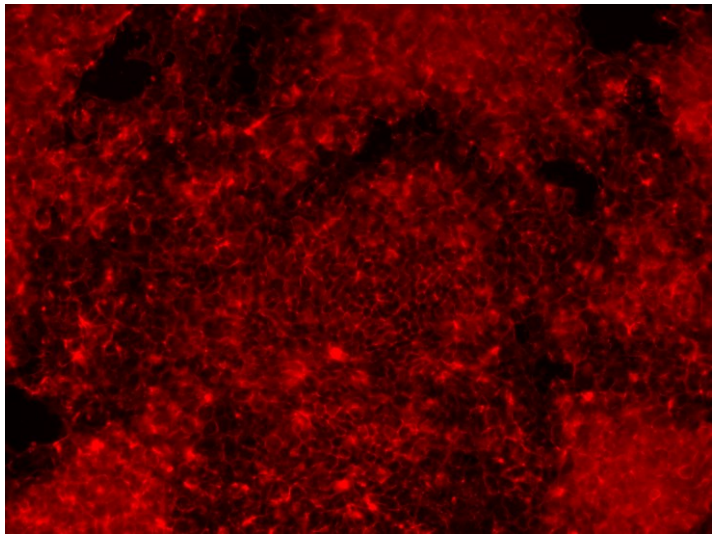
TRILINEAGE DIFFERENTIATION | ECTODERM

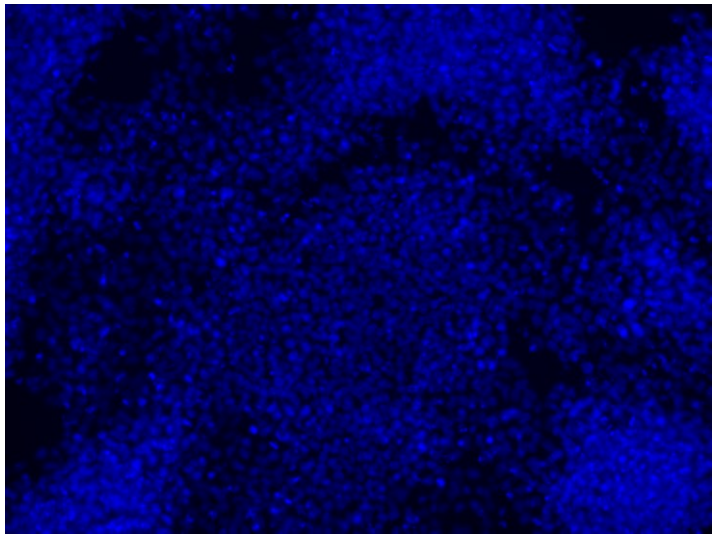
NESTIN	ECTODERM MARKER
PAX6	ECTODERM MARKER
ACTIN	COUNTERSTAINING
DAPI	NUCLEAR COUNTERSTAINING

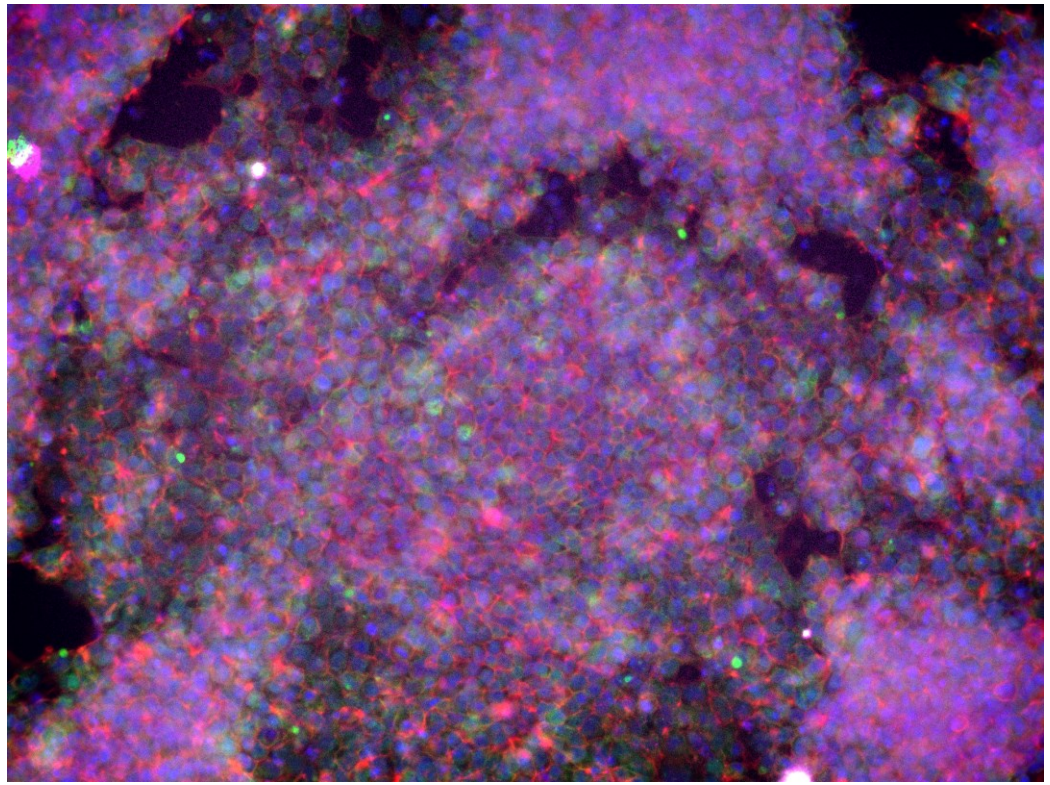
CELL QUALITY EVALUATION









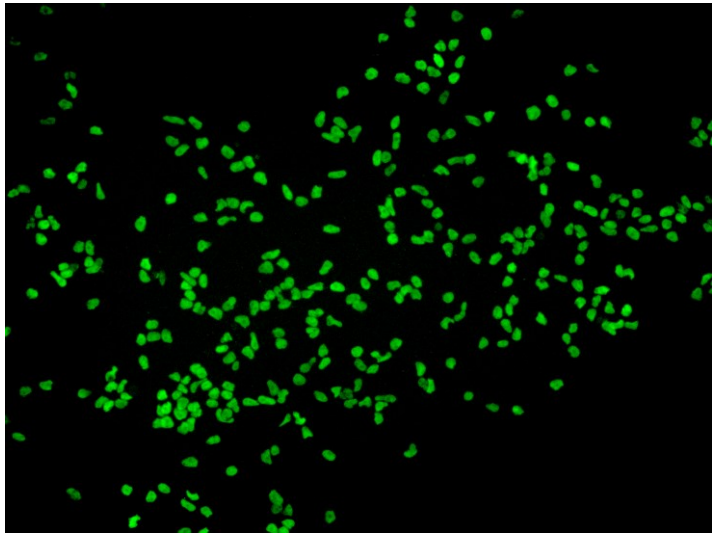


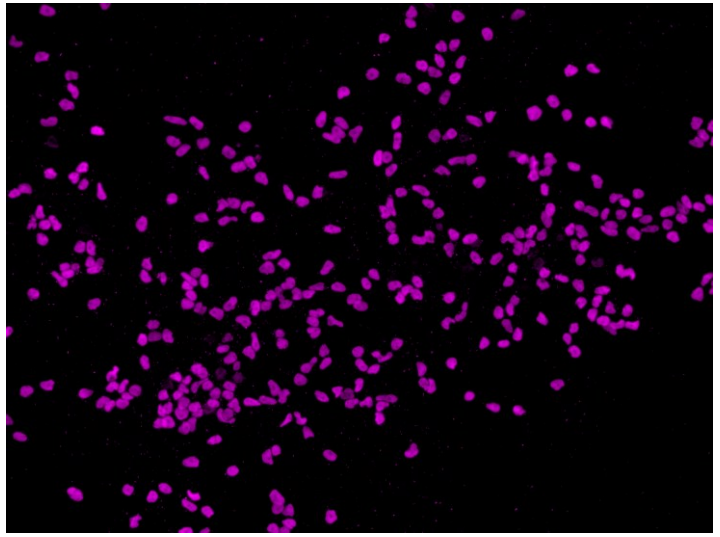
20X

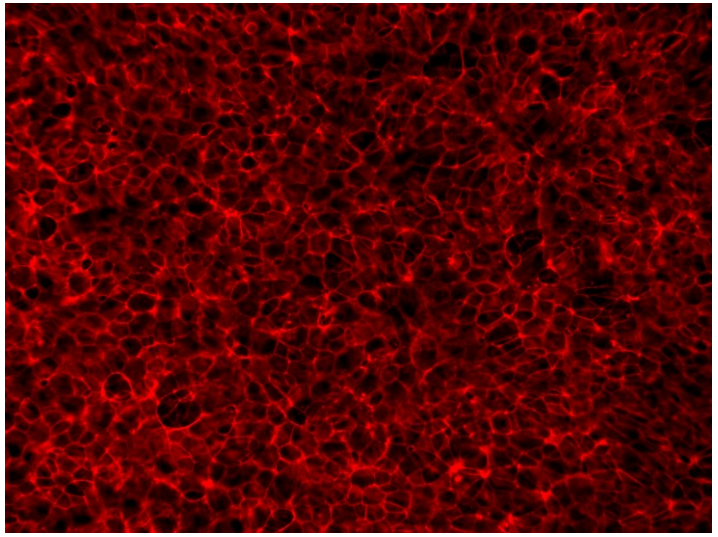
TRILINEAGE DIFFERENTIATION | MESODERM

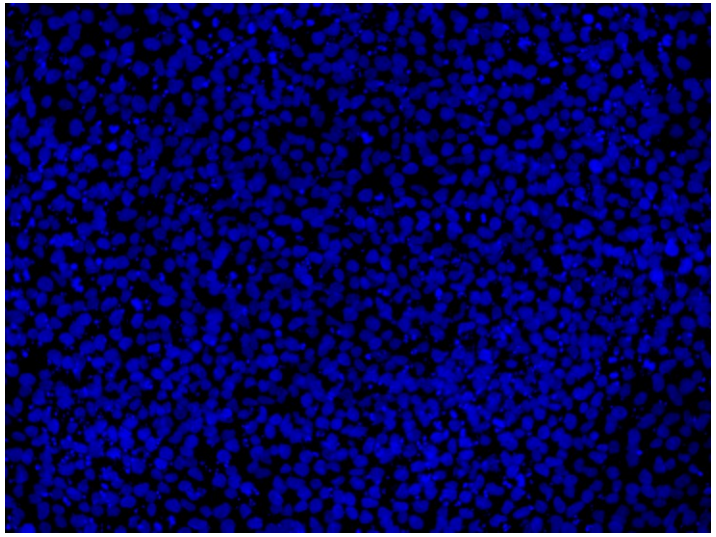
BRACHYURY	MESODERM MARKER
NCAM1	MESODERM MARKER
ACTIN	COUNTERSTAINING
DAPI	NUCLEAR COUNTERSTAINING

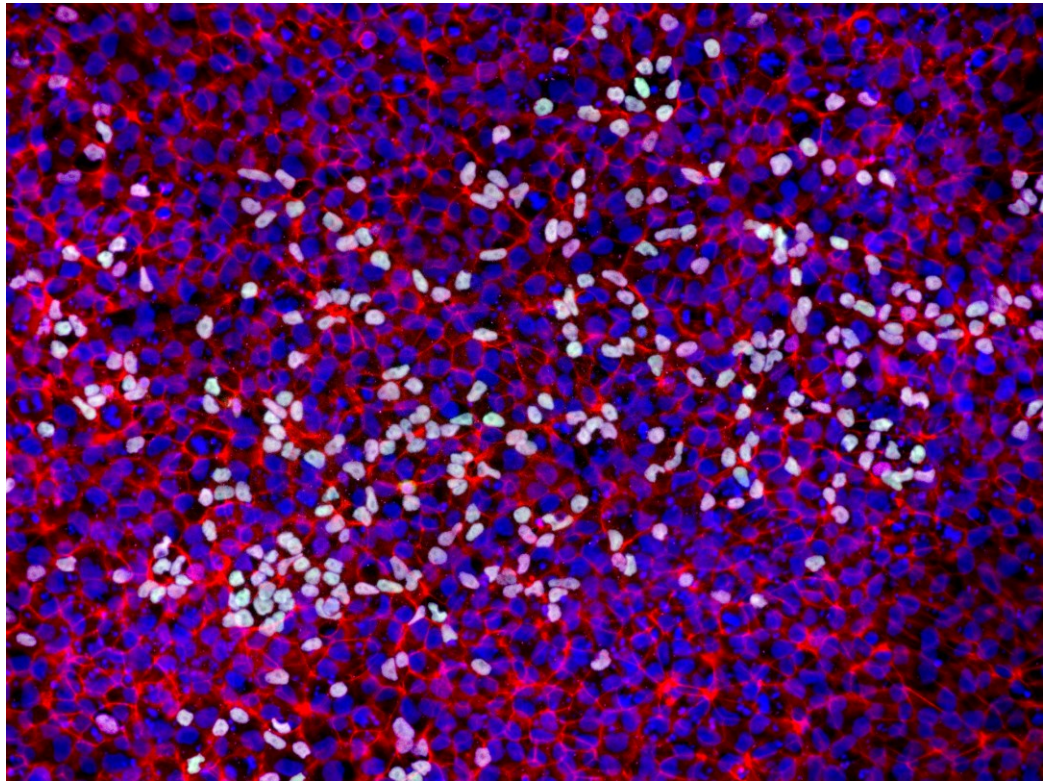
CELL QUALITY EVALUATION











20X

TRILINEAGE DIFFERENTIATION | ENDODERM

SOX17	ENDODERM MARKER
FOXA2	ENDODERM MARKER
ACTIN	COUNTERSTAINING
DAPI	NUCLEAR COUNTERSTAINING

CONCLUSIONS

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- CliniMACS PRODIGY ADHERENT CELL CULTURE PLATFORM ALLOWS HIGHLY SCALABLE iPSC EXPANSION IN iMatrix-511 / StemFit AK03N CULTURE SYSTEM
- TRANSITION FROM MANUAL iPSC EXPANSION SYSTEM TO CliniMACS PRODIGY HAS ITS CHALLENGES, BUT THEY CAN BE MITIGATED WITHOUT MUCH DIFFICULTY
- WE ARE WORKING ON FINALIZING THE PROCESS AND PLANNING THOROUGH QUALITY ASSESSMENT OF THE CELLS EXPANDED WITH CliniMACS PRODIGY PLATFORM

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