Confidential

Process Development for Motor Neuron Differentiation from Human iPS Cells Using a Small-Scale Bioreactor

CiRA Foundation

Junya Sakamoto, Yuko Kitano, Masayoshi Tsukahara

CiRA Foundation

公益財団法人
京都大学 iPS細胞研究財団

CiRA Foundation



Turn Key Lab
https://www.krp.co.jp/turnkeylab/lp/

CiRA

Nakanoshima Qross
https://www.nakanoshima-qross.jp/

myIPS

R&D Center
staff：30

Closed-, Automated-, cell manufacturing



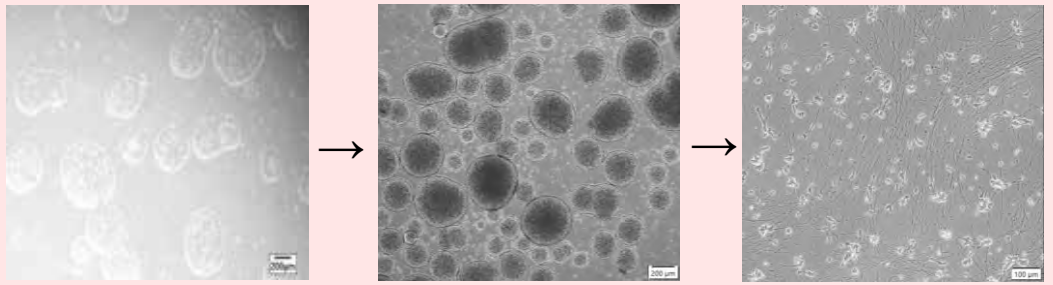


Our Challenge:
Motor Neuron Differentiation in a DASbox mini Bioreactor

Why Motor Neurons?

Complex, multi-step differentiation

A suitable model for evaluating culture automation



hiPSC → MN progenitor aggregates → Motor Neuron (MN)

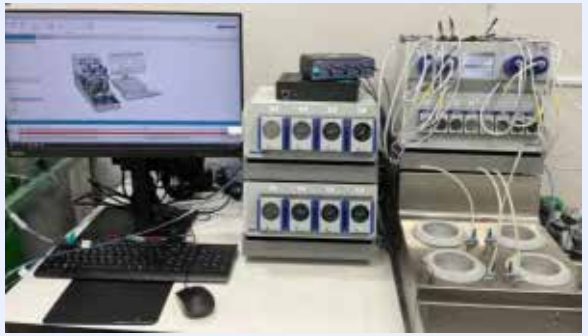
Why DASbox mini?

Automation & process control at a development-friendly scale

A suitable platform for process development and optimization



BioBLU 0.3sc



DASbox mini Bioreactor system (Eppendorf)

Our Objective

Automated MN differentiation + In-line monitoring based control

Establish a model process for automated hiPSC differentiation

Experimental Workflow

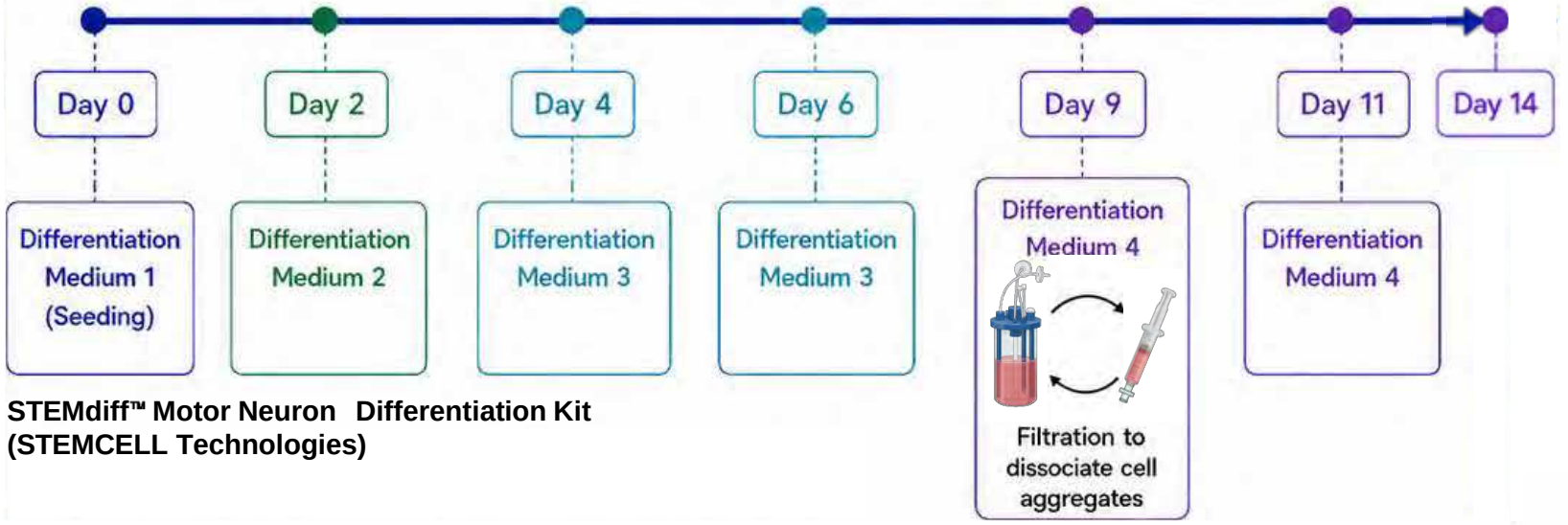
Overall workflow

Seeding (Day 0)





Culture (Days 0～14)



STEMdiff™ Motor Neuron Differentiation Kit (STEMCELL Technologies)

Harvest (Day14)



➡ FCM (NKX6.1/ISL1)

Process development : from manual to automated culture

Manual



- manual medium exchange

Automated



- Hands-free culture controlled by a pre-programmed script
- Automated medium exchange at preset time points

Automated + Two-Step Culture Conditioning



- Culture environment stabilization
- Conditioned medium for seeding

Automated + Two-Step Culture Conditioning + pH trigger



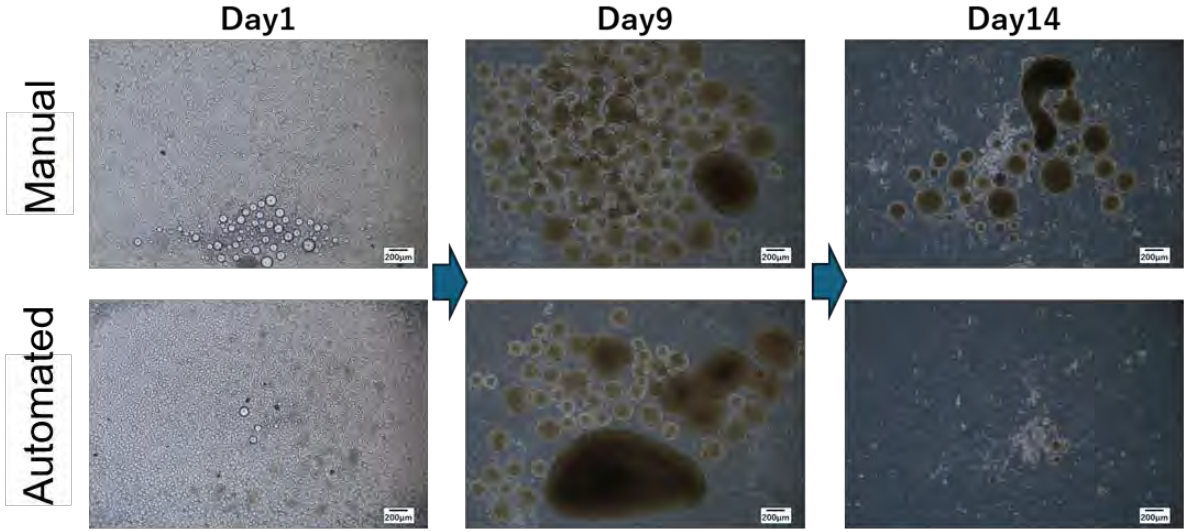
- Medium changes at protocol-defined transitions
- Additional exchanges triggered by pH < 7.0

Evaluation of Automated Cell Culture

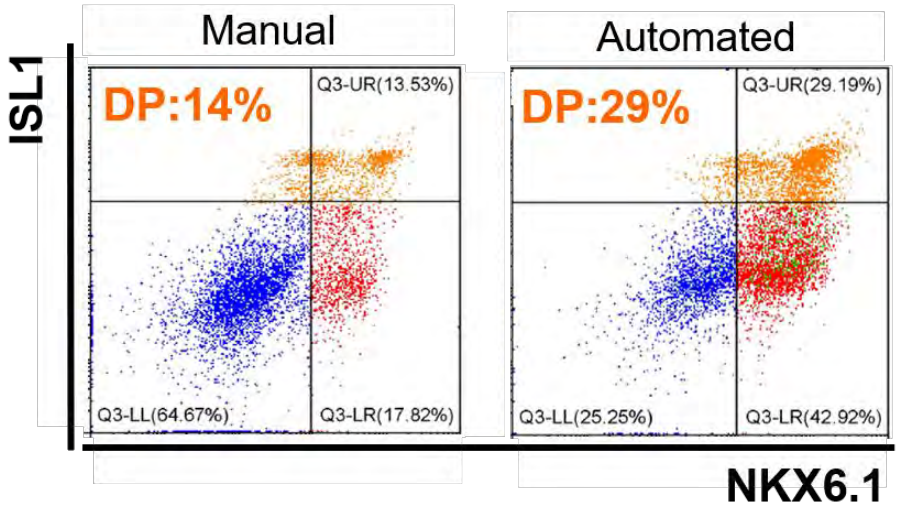
○Comparison of Medium change methods

	Reactor handling	Volume（mL）	duration（min）	Medium Removal position	Removal Rate	Temperature Control	Gas Supply
Manual	Remove from systems	100	45	Surface-following	Variable	—	—
Automated	Remains in place	100	35	Fixed by reactor design	360 mL/h	Off	On

○Cell Morphology



○FCM（Day14）



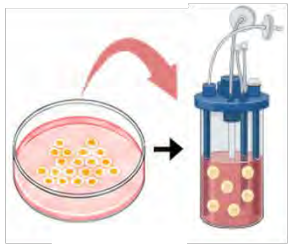
Key message | **Automated culture generated MN marker-positive cells with higher differentiation efficiency than manual culture.**

Evaluation of Two-Step Culture Conditioning

Two-Step Culture Conditioning



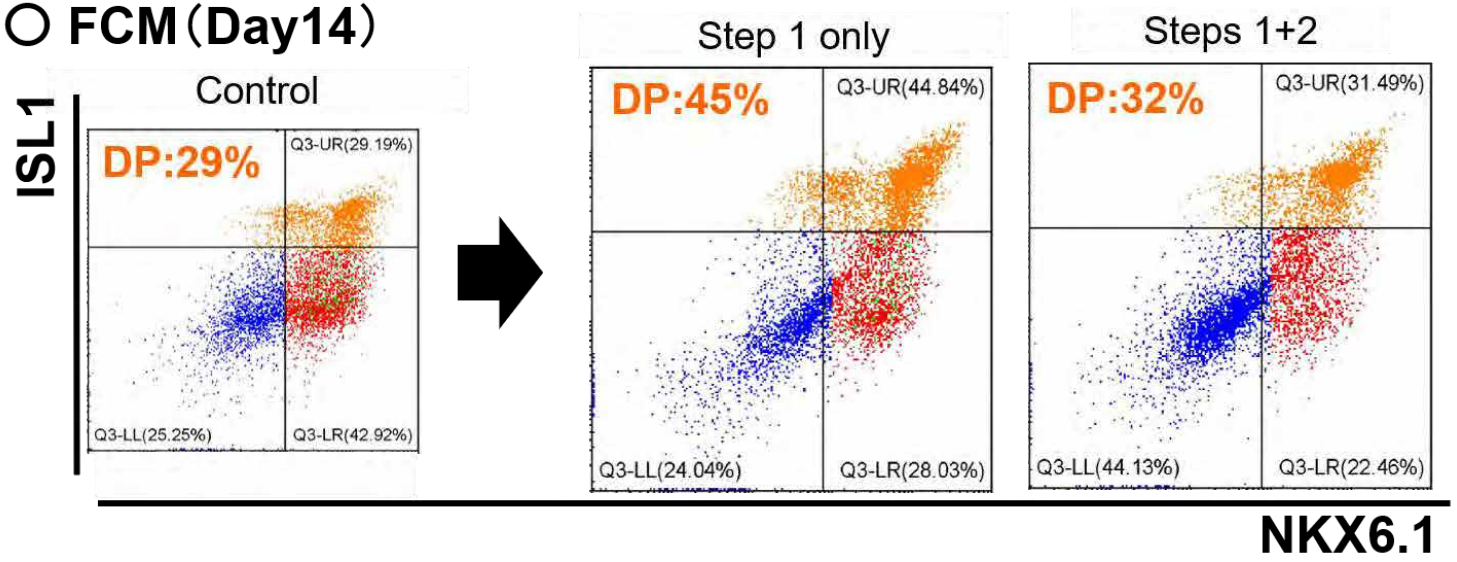
Culture Condition Stabilization
- Start Gas supply
- Start temperature control



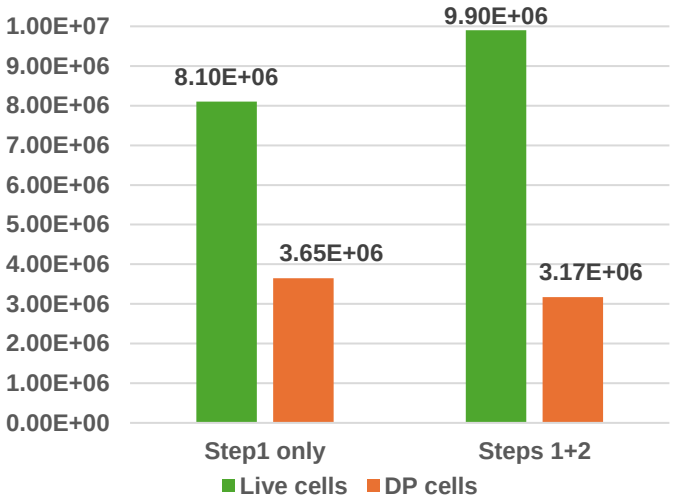
Use of Conditioned Medium
- Differentiation Medium 1 conditioned with cells overnight
- Used for reactor seeding

Step 1 (Day -1) — Step 2 (Day 0)

○ FCM（Day14）

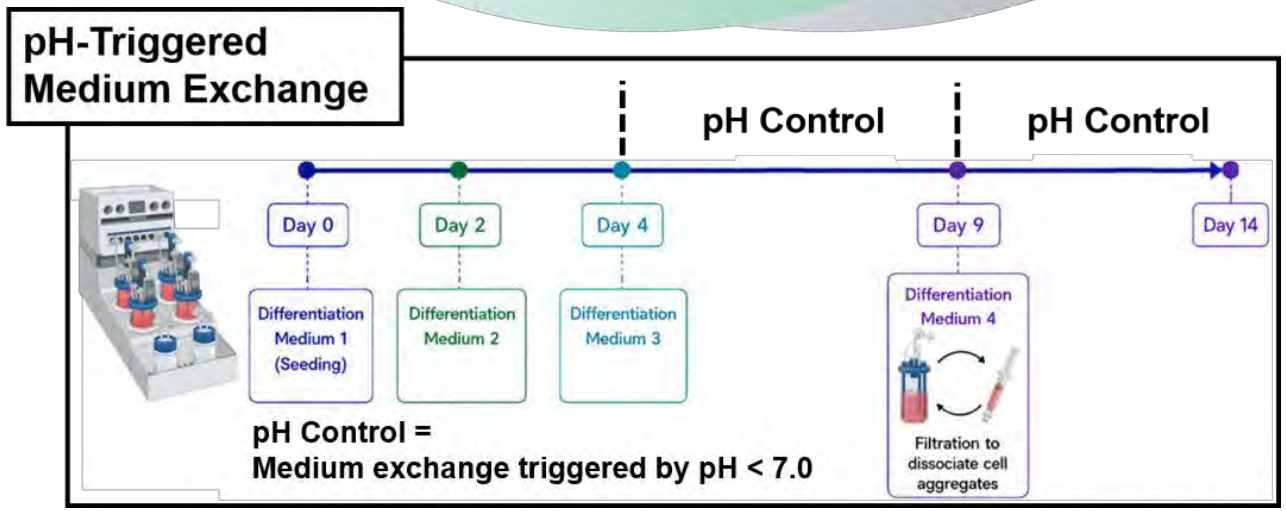


○ Cell Recovery (Day 14)

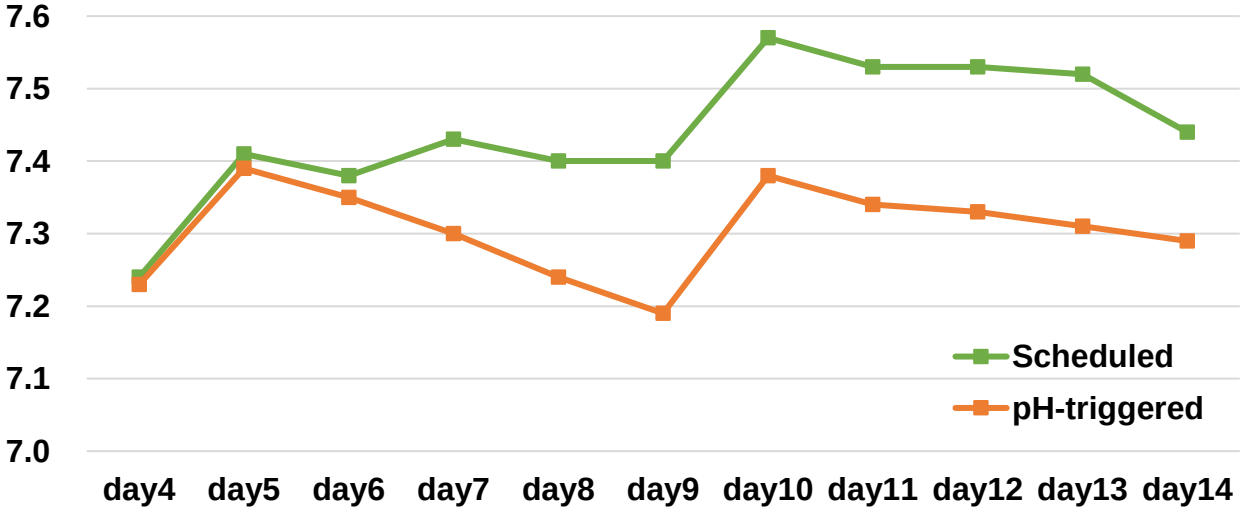


Key message | **Culture environment stabilization improved MN differentiation efficiency, while the use of conditioned medium increased viable cell recovery.**

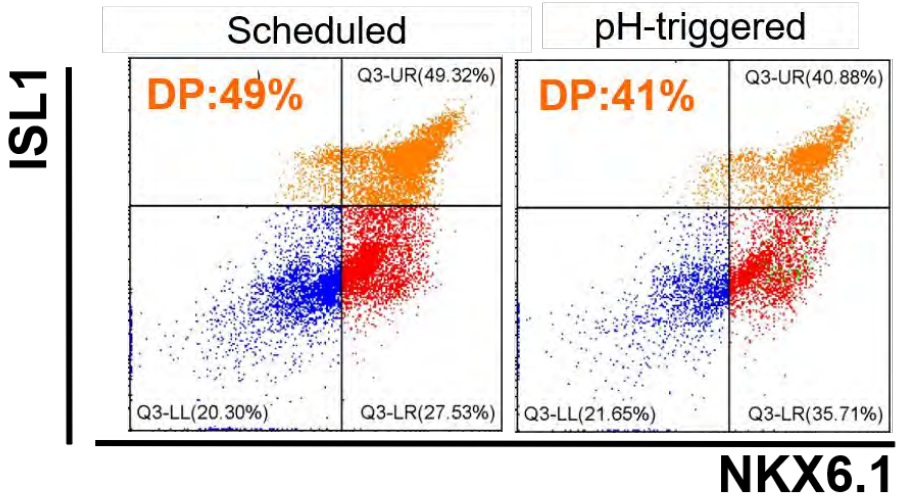
Evaluation of pH-Triggered Medium Exchange



○pH Profile



○ FCM（Day14）



Key message | pH-triggered medium exchange based on in-line monitoring enabled MN differentiation without unnecessary medium exchanges.

Summary & Future Directions

Our Objective

Establish a model process for automated hiPSC differentiation

What we achieved

- Automated MN differentiation was implemented in a small-scale bioreactor.
- Culture conditioning improved differentiation performance and viable cell recovery.
- In-line pH monitoring enabled needs-based medium exchange.

Future directions

- Validate reproducibility across runs and cell lines.
- Expand in-line monitoring and optimize feedback control.

A model process was established; improving robustness and advancing feedback control are the next steps.

CiRA Foundation

www.cira-foundation.or.jp

公益財団法人
京都大学iPS細胞研究財団
606-8397 京都市左京区聖護院川原町53番地

Public Interest Incorporated Foundation
CiRA Foundation
53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8397, Japan.