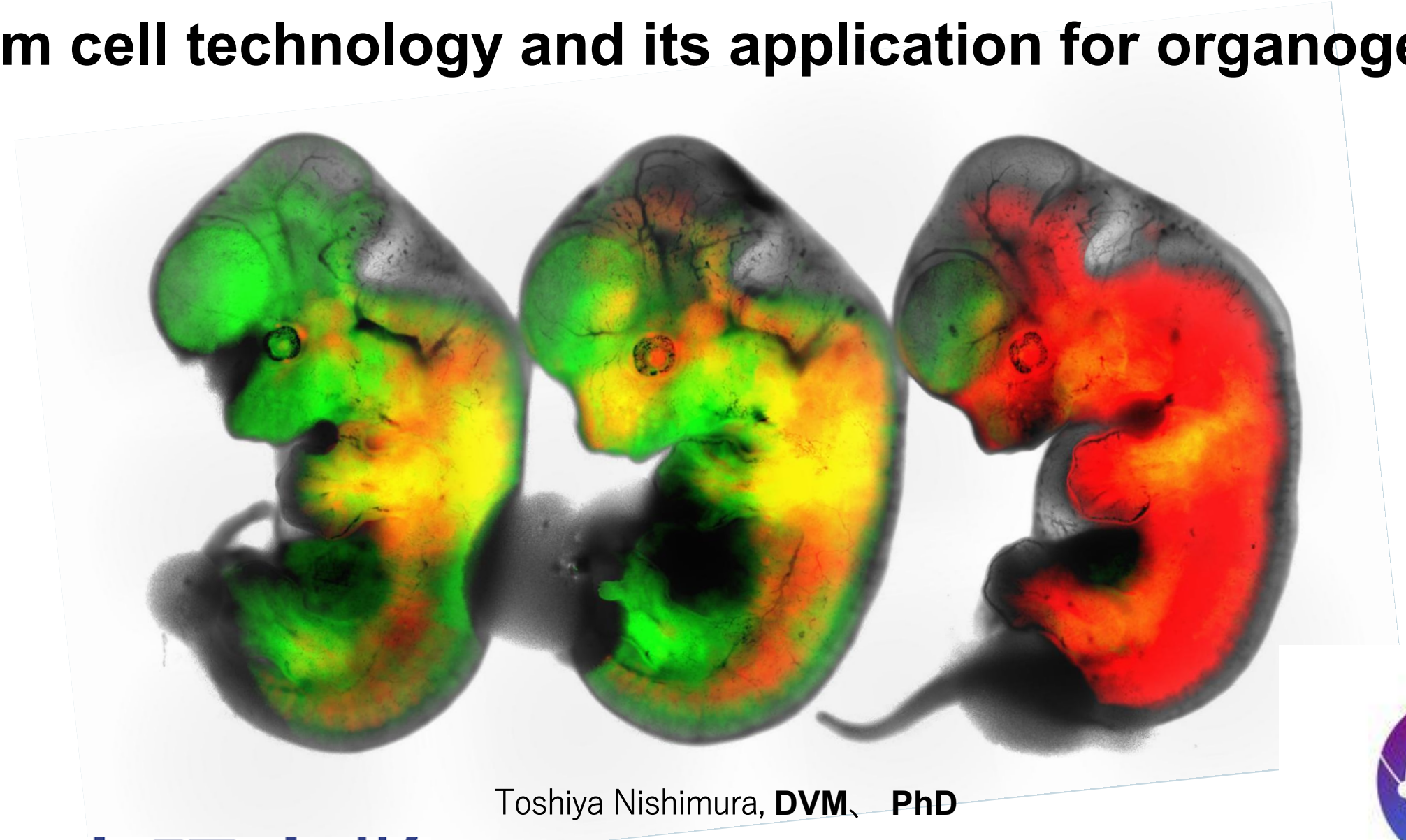
Stem cell technology and its application for organogenesis



Toshiya Nishimura, **DVM、 PhD**

WPI-PRIMe, Osaka University

大阪大学
OSAKA UNIVERSITY

PRIMe
WPI Osaka University

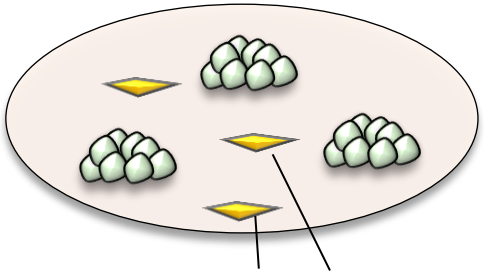
Boosting Cardiac Differentiation Efficiency

Optimized Culture of Canine iPS Cells

Dogs naturally develop cardiovascular diseases, tumors, and diabetes similar to humans, making them valuable disease models. However, stable and efficient generation of canine iPS cells has been challenging. Here, we successfully established stable culture of canine iPS cells using **AR medium containing activin and Wnt inhibitors**. The derived iPS cells showed high-efficiency differentiation into cardiomyocytes, with formation of beating cardiac tissue confirmed. This technology offers a new platform for canine heart disease models, regenerative medicine applications, and potential clinical veterinary therapies.

Canine iPS Cell Culture Is Unstable

Thawing from Frozen Stock

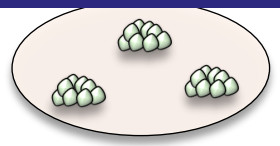


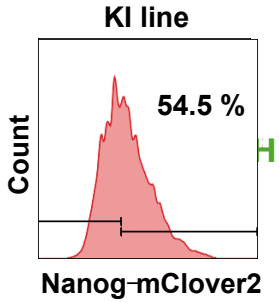
Differentiated Cells

Canine iPS Cells Rapidly Differentiate, Losing Pluripotency

Experimental Overview

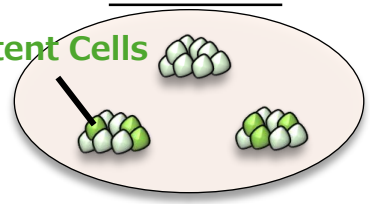
Pluripotency Gene (Nanog) Fluorescent Reporter Cells株 (Green)





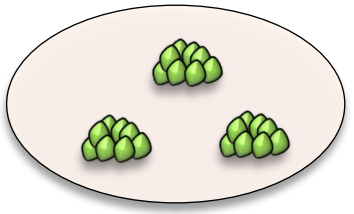
Highly Pluripotent Cells

Eatablised Method

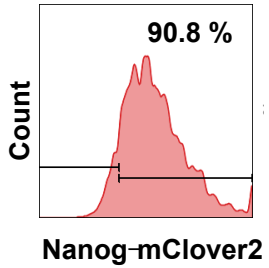


Heterogeneous expression of pluripotency genes within the cell population

Newly developed A+R medium



Homogeneous population: Over 90% of cells show high pluripotency gene expression



Cardiomyocyte Differentiation

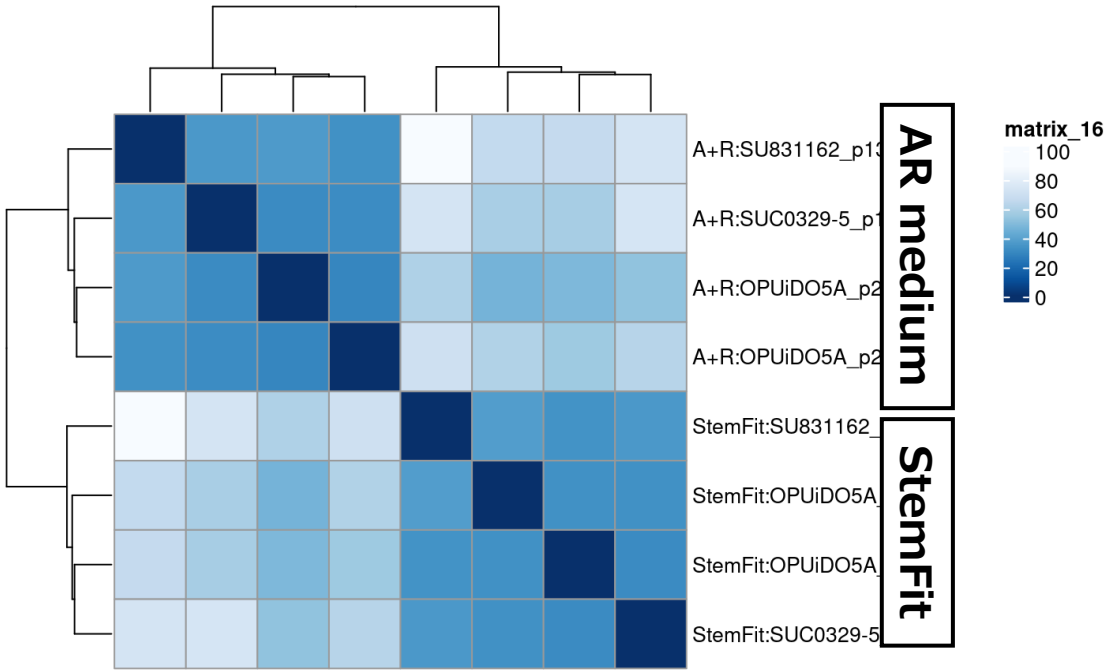
Beating Heart Cells Made Easy:
A+R Medium Alone

Benefits of A+R Medium

- **Stable during freezing and thawing**
- **Loss of differentiation gene expression**
- **Easy culture of difficult cell lines**
- **Efficient induction of cardiomyocyte differentiation**
- **Homogeneous cell population during differentiation**

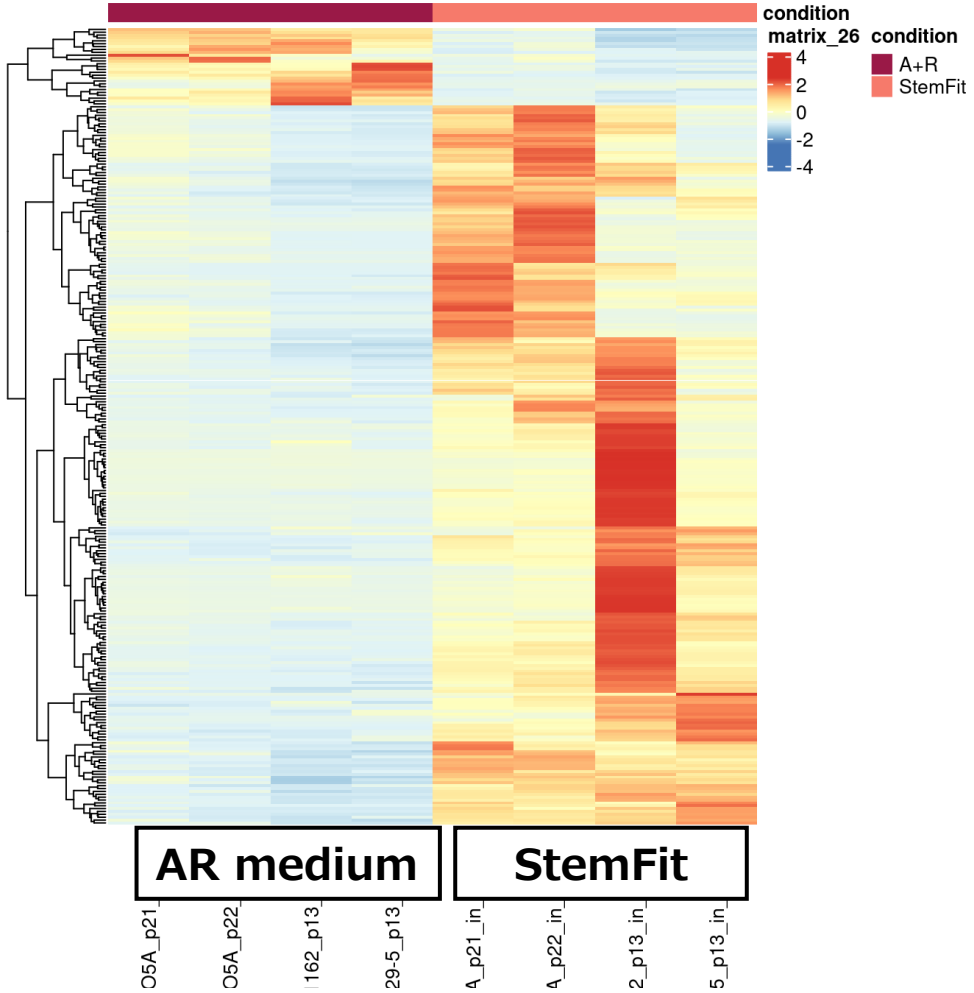
"Aligning" iPS Cell Gene Expression: AR Medium

Different Gene Expression in Canine iPS Cells Cultured with AR vs. Conventional Media



AR Medium Modifies and Homogenizes Gene Expression Patterns in Canine iPS Cells

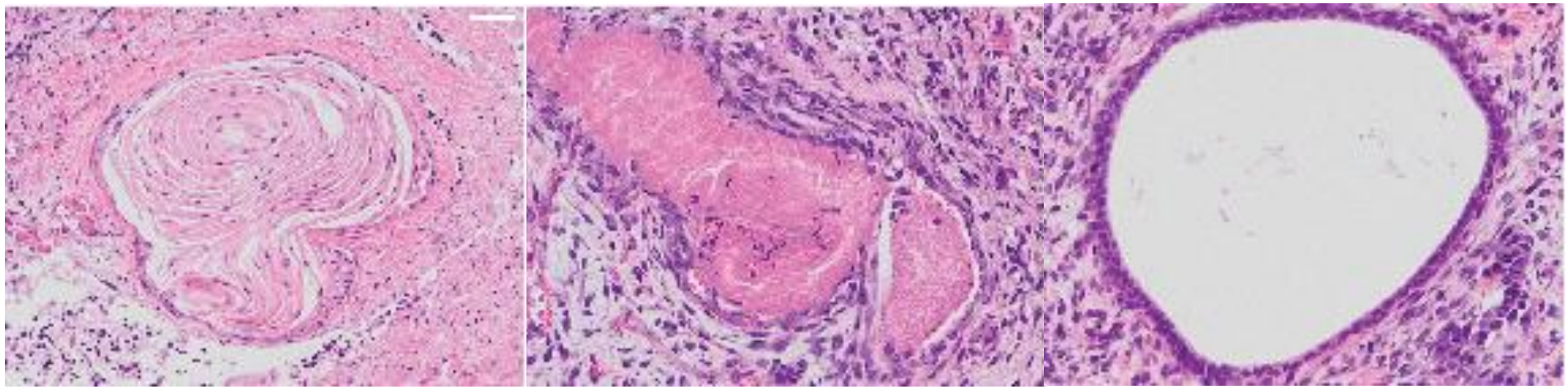
Impact of Media Differences on Gene Expression Profiles



AR Medium Enables Stable Culture of Canine iPS Cells

In Vivo Differentiation Potential

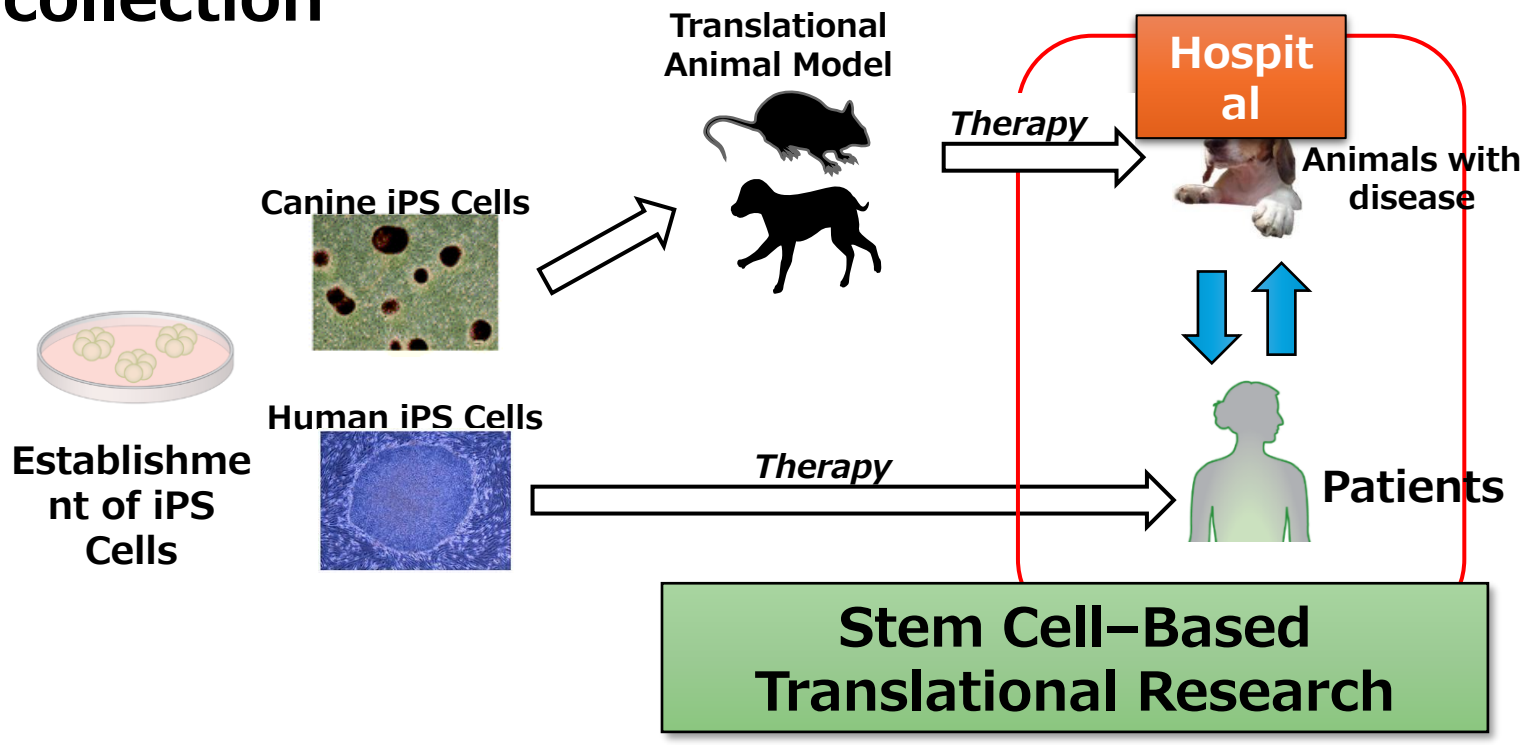
Ectoderm Mesoderm Endoderm



AR Medium Enables In Vivo Differentiation Even in Previously Non-Differentiable Cell Lines

Dogs as Valuable Translational Models for Human Stem Cell Therapy

- Share living environments and many common diseases with humans
- Longer lifespan compared to rodents and other lab animals
- Serve as both experimental and companion animals, allowing for both experimental and clinical data collection



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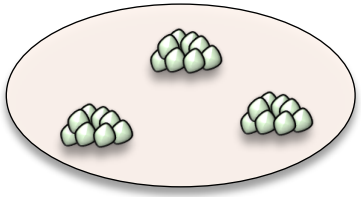


Canine iPS Cells

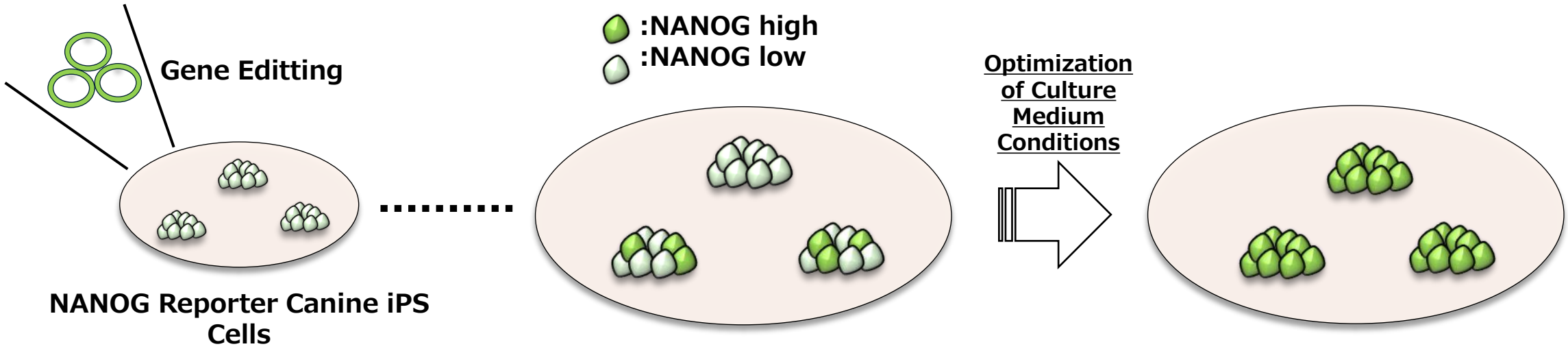
No Existing Human Stem Cell Therapy Model Using Canine iPS Cells

Optimizing Medium Conditions for Canine iPS Cells

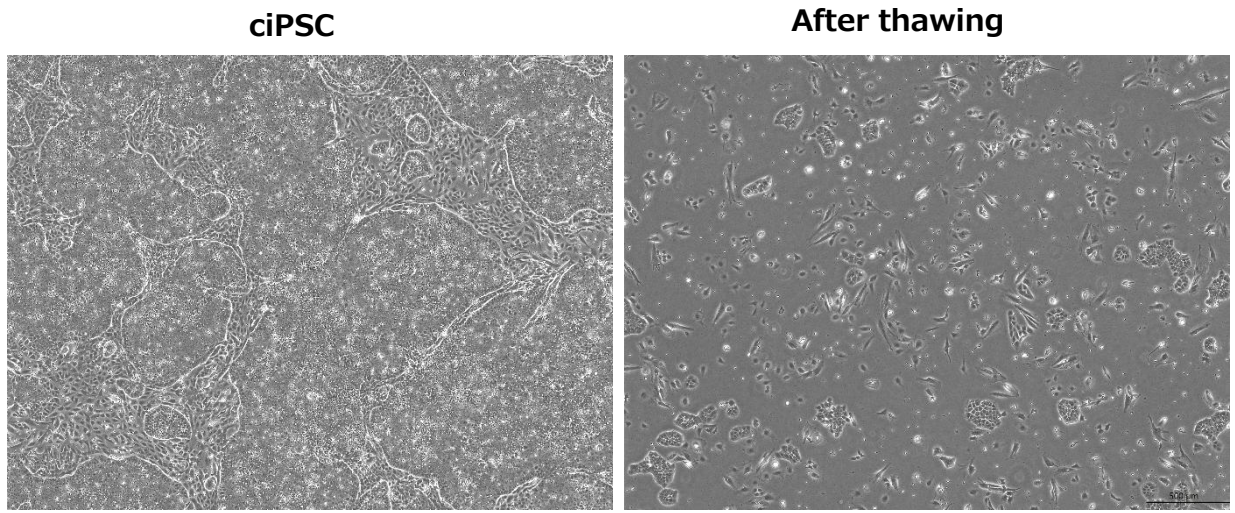
1. Fluorescent Protein Gene Introduced into Pluripotency Gene (NANOG)

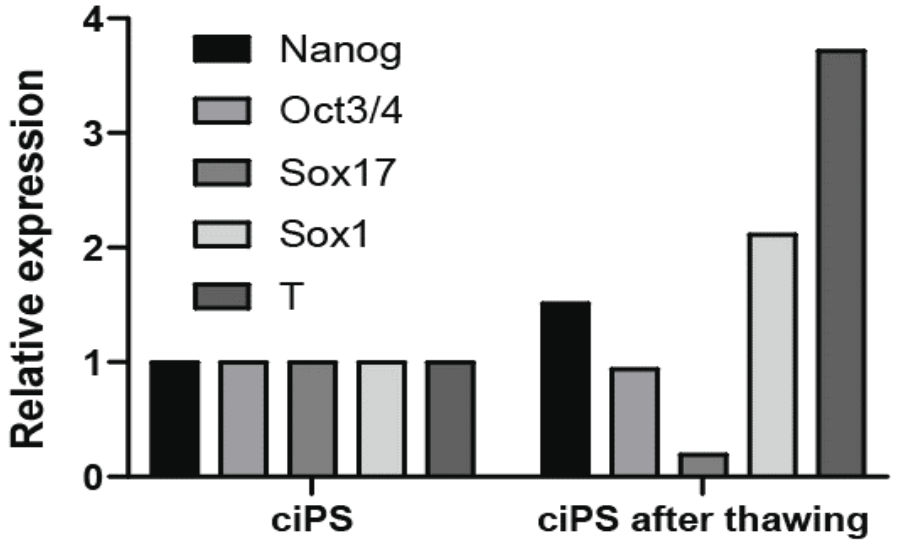
2. Optimizing Culture Conditions for Highly Pluripotent Canine iPS Cells Using Fluorescence

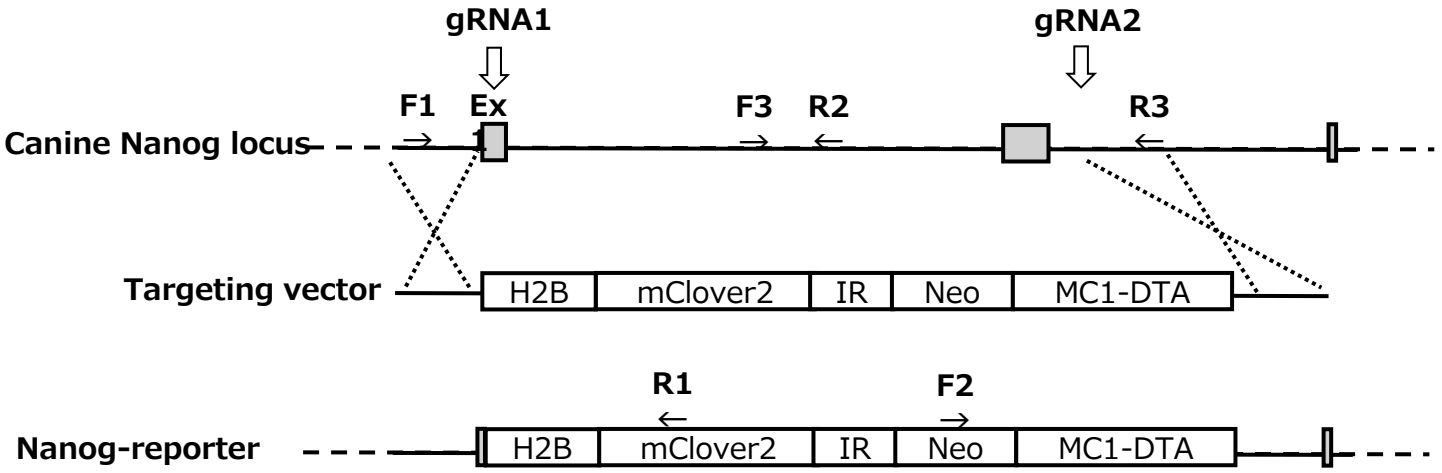
3. valuating Functionality of Canine iPS Cells in New Culture Medium

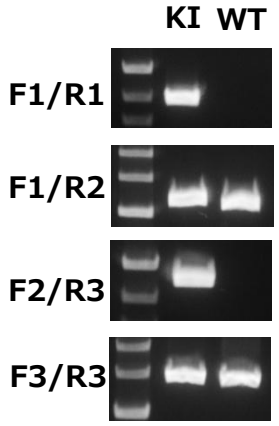


Generation of Nanog Reporter Cell Line

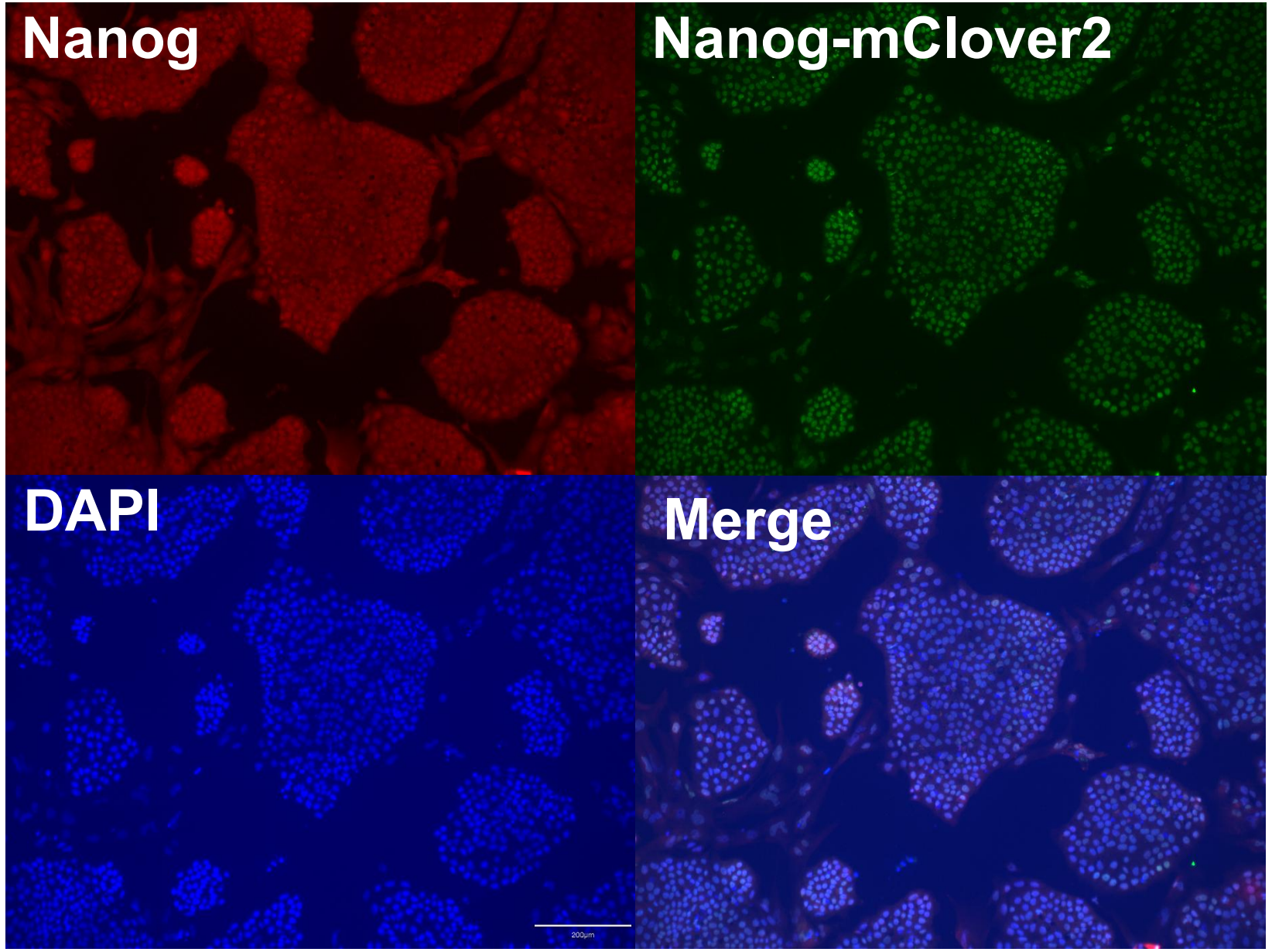








Generation of Nanog Reporter Cell Line

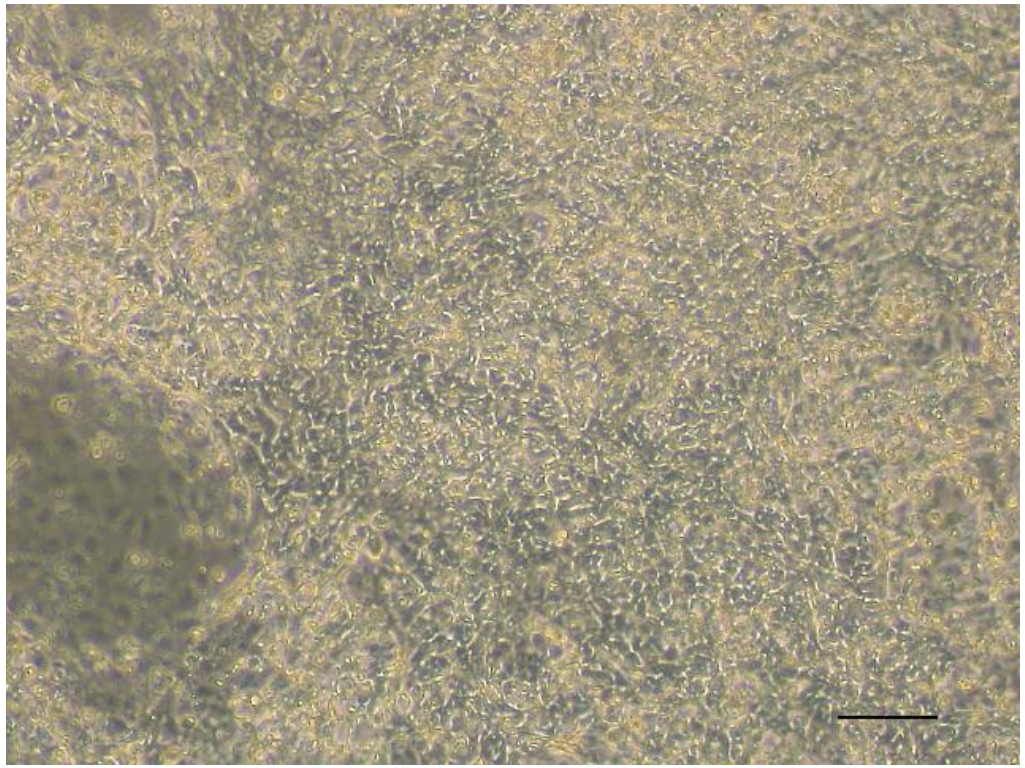


Differentiation of Canine iPS Cells into Functional Cardiomyocytes

Day 9 of Cardiomyocyte Differentiation (AR Medium)

X10 （Movie）

X40 （Movie）



AR medium enabled induction of uniformly beating cardiomyocytes, while existing media failed to produce beating cells.

Differentiation of Canine iPS Cells into Functional Cardiomyocytes

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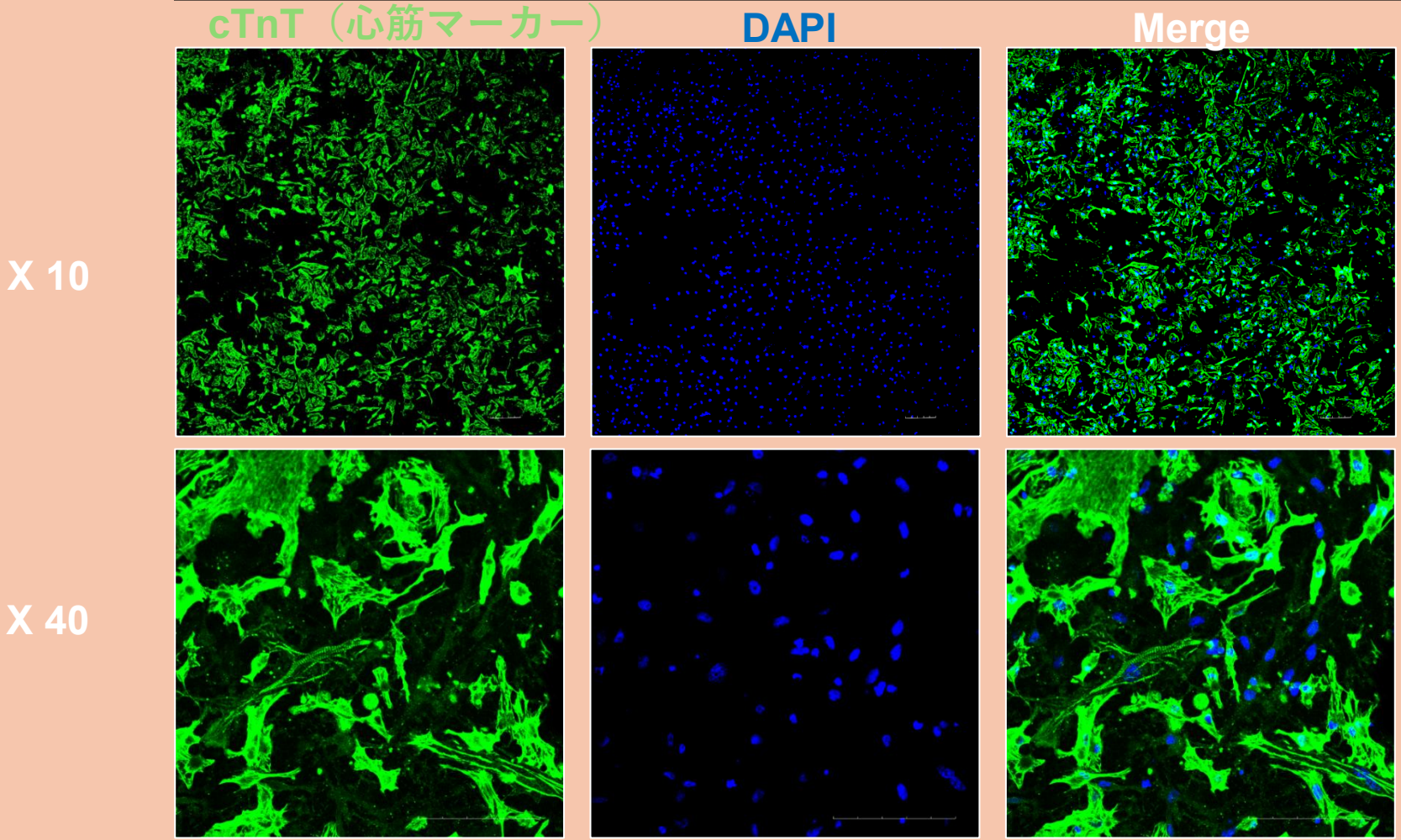


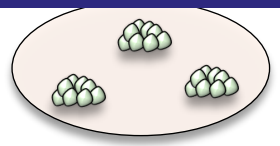
Most cardiomyocytes from A+R cultured canine iPS cells expressed cTnT.

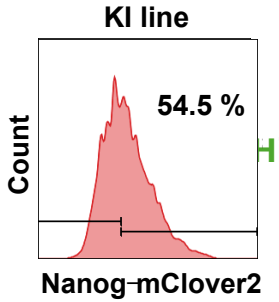
Summary

- **Culturing canine iPS cells in Activin + Wnt inhibitor medium (A+R) enabled more stable culture compared to conventional media.**
- **A+R eliminated differentiated cells appearing after freeze-thaw and reduced expression of differentiation genes.**
- **Effective across all tested cell lines, A+R is broadly applicable for canine iPS cell culture.**
- **Canine iPS cells cultured in A+R showed gene expression patterns similar to those of conventional cells.**
- **A+R medium did not affect iPS cell function and enabled uniform differentiation.**
- **Cells cultured in A+R differentiated more efficiently into cardiomyocytes than with conventional methods.**
- **Cardiomyocytes derived from A+R cultured cells beat with uniform rhythm.**

Experimental Overview

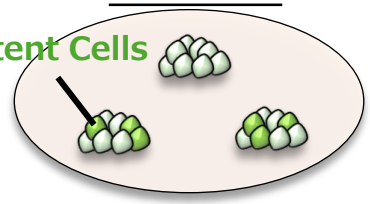
Pluripotency Gene (Nanog) Fluorescent Reporter Cells株 (Green)





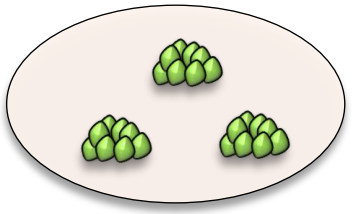
Highly Pluripotent Cells

Eatablised Method

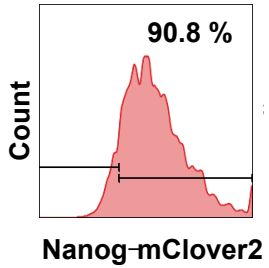


Heterogeneous expression of pluripotency genes within the cell population

Newly developed A+R medium



Homogeneous population: Over 90% of cells show high pluripotency gene expression



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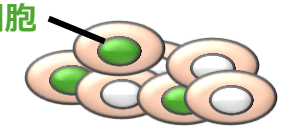
Conclusion

Schematic

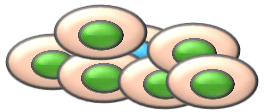
Established method

多能性が高い細胞



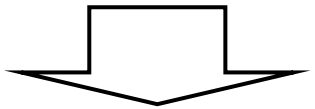
Heterogeneous expression of pluripotency genes within the cell population

Newly developed AR medium



Homogeneous population: Over 90% of cells show high pluripotency gene expression

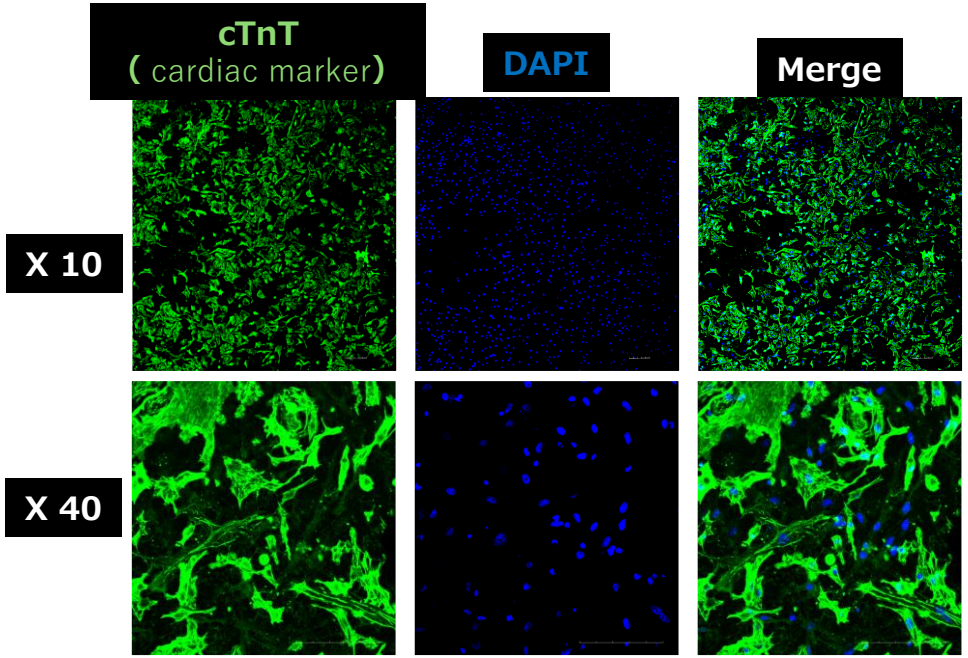
Efficient Canine iPS Cell Culture with AR Medium



Beating Heart Cells Observed

Opening New Doors in Canine Regenerative Medicine

AR medium enables stable and efficient generation and maintenance of canine iPS cells with improved freeze-thaw stability, reduced differentiation, and enhanced cardiomyocyte induction.



Many cardiomyocytes derived from canine iPS cells cultured in AR medium expressed the cardiac marker cTnT and showed spontaneous beating.