A xeno-free slow-freezing cryopreservation medium for primate ES/iPS cells

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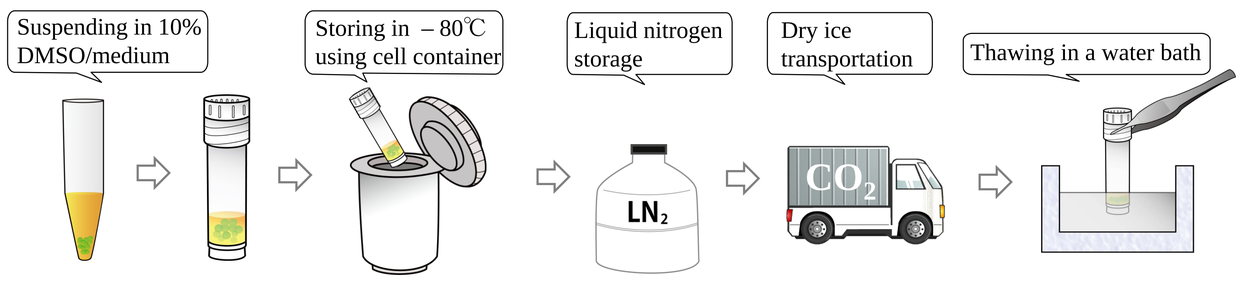
Abstract

Cryopreservation of primate ES/iPS cells is severe and difficult compared to murine ones. It can be described that vitrification method might be adequate for primate ES/iPS cells at this moment, however it needs special skills and has to avoid dry ice transportation. To address these problems, we developed a new xeno-free freezing medium for primate ES/iPS cells using slow-freezing, and evaluated its recovery and dry ice transportation.

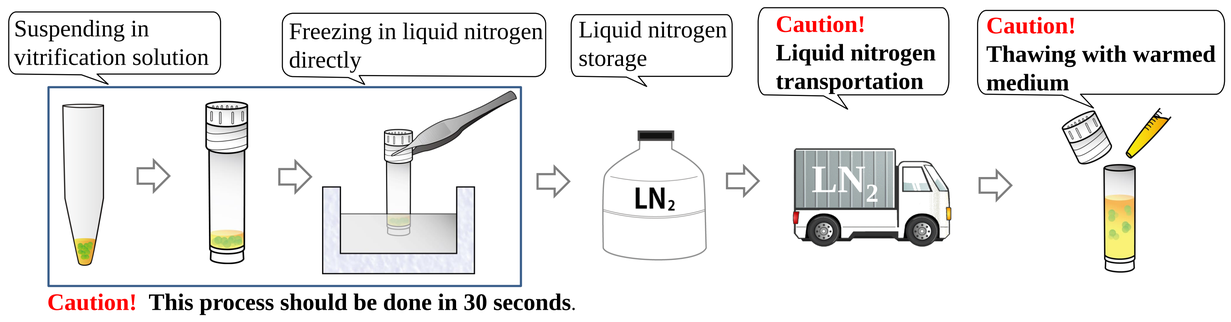
Introduction

A flowchart of cryopreservation, transportation and thawing for primate ES/iPS cells

A) Slow freezing



B) Vitrification



Caution! This process should be done in 30 seconds.

The recovery of primate ES/iPS cells after cryopreservation with the conventional slow-freezing is quite low .Vitrification is not an easy option because the steps required are more complicated than those of slow-freezing. In addition, frozen cells using vitrification have to be avoided dry ice transportation. Therefore more efficient freezing method or freezing medium are needed.

Materials & method

Common marmoset ES cell line, CMEM40 and human iPS cell line, ATCC-HYR0103 were used to evaluate the efficiency of slow-freezing or vitrification for cryopreservation of primate ES/iPS cells. The ES cells were dissociated into several small clumps and frozen in our freezing medium or 10% DMSO/medium by slow-freezing or in a vitrification solution by quick-freezing. After thawing, cells were cultured for 4 days. The number of alkaline phosphatase-positive colonies was counted as recovery points.

The ES cells were frozen as described above. The frozen cells were transferred to a box filled with dry ice and stored for 24 hours. After thawing, ES cells were cultured for 4 days. The recovery was estimated as described above.

10% DMSO/medium, Our freezing medium

Vitrification solution

The iPS cells were cultured in the presence or absence of Y-27632(ROCK inhibitor) before freezing. 1 hour later, colonies were dissociated into single cells and cells were frozen in our freezing medium using slow-freezing. After thawing, 20,000 cells were seeded and cultured in the presence or absence of Y-27632. 18 hours later, culture medium was changed in the absence of Y-27632 and cells were cultured for 8 days. The recovery was estimated as described above.

Result

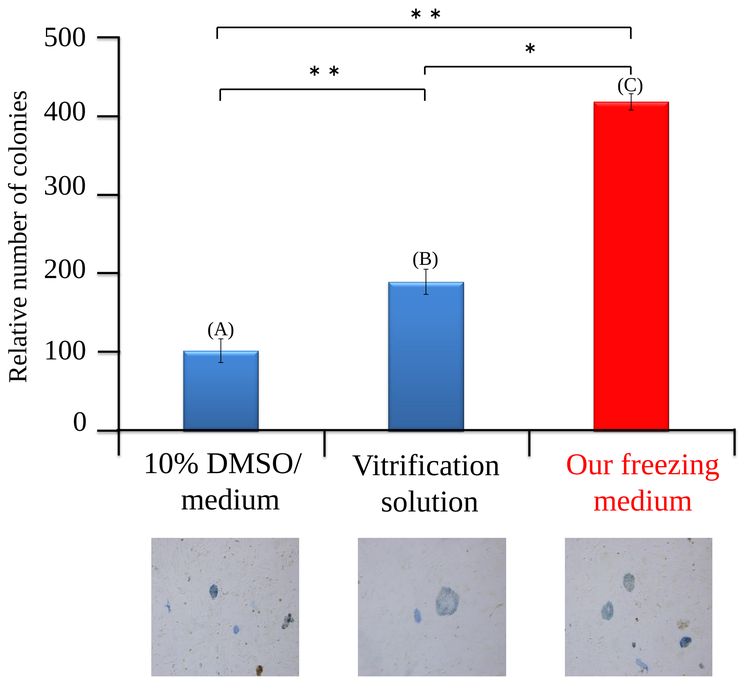


Figure 1. The recovery of monkey ES cells after cryopreservation and thawing

The ES cells were cryopreserved in 10% DMSO/ medium(A) or vitrification solution(B) or our freezing medium(C). The recovery efficiency was estimated by counting the number of alkaline phosphatase-positive colonies at 4 days after thawing. Results are expressed as means ±SEM for n=3.*P<0.05, **P<0.01

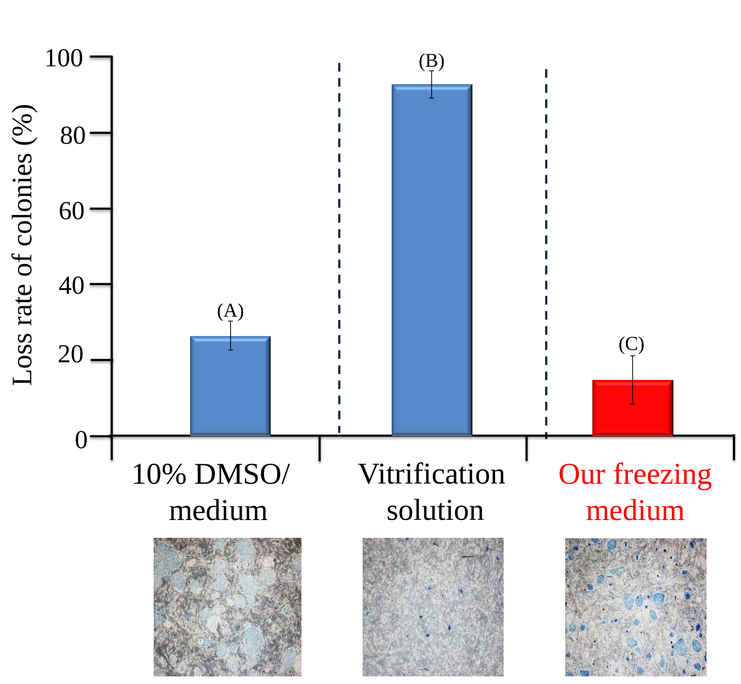


Figure 2. The loss rate of colonies after dry ice storage

The ES cells were cryopreserved in 10% DMSO/medium(A) or vitrification solution(B) or our freezing medium(C) then stored in a box filled with dry ice for 24 hours. The loss rates of colony were calculated by comparing the number of colonies after dry ice storage/liquid nitrogen storage at 4 days after thawing. Results are expressed as means ±SEM for n=3.

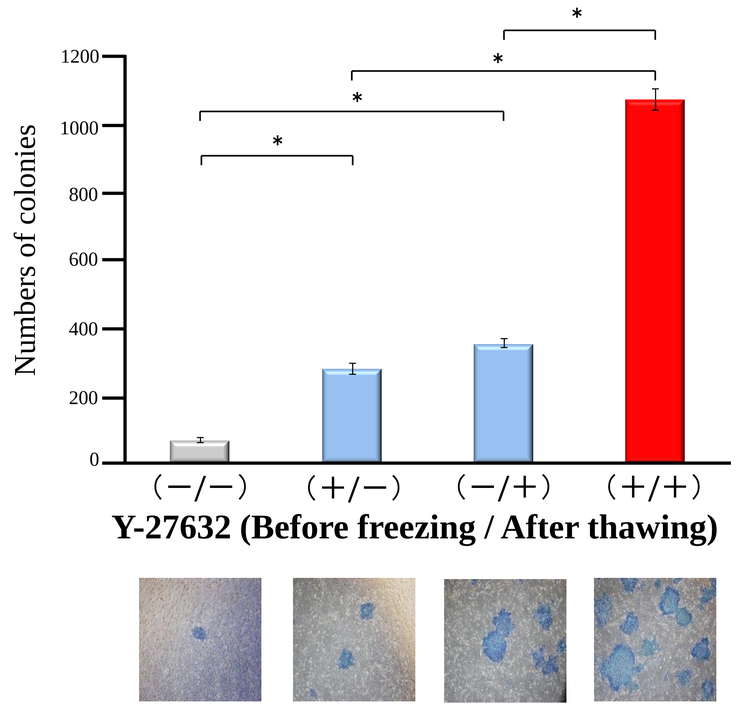


Figure 3. The recovery of human iPS cells in the presence of the Y-27632 (ROCK inhibitor)

The iPS cells were cultured in the presence or absence of Y-27632 before freezing.1 hour later, colonies were dissociated into single cells and cells were frozen in our freezing medium using slow-freezing. Cells were thawed and 20,000 cells were seeded in the presence or absence of Y-27632 . The recovery was estimated by counting the number of alkaline phosphatase-positive colonies at 8 days after thawing. Results are expressed as means ±SEM for n=3. *P<0.001

Conclusion

- **We have developed an efficient freezing medium for primate ES/iPS cells.**
- **Cells frozen with our medium can be transported using a container filled with dry ice.**
- **Our medium is quite useful as xeno-free freezing medium especially in medical application.**