http://www.brc.riken.jp/lab/cell/aes/

遺伝子トラップ法による変異を有するマウスES細胞
http://www2.brc.riken.jp/lab/mouse_es/

受注生産品ですので、受注してからご提供までに数カ月を要しますこと、ご了承下さい。

ヒトES細胞(HES)
http://www.brc.riken.jp/lab/cell/hes/

動物iPS細胞(APS)
http://www.brc.riken.jp/lab/cell/aps/

ヒトiPS細胞(HPS)
http://www.brc.riken.jp/lab/cell/hps/

2. 申込先、問合せ先

〒305-0074 茨城県つくば市高野台3-1-1
独立行政法人理化学研究所 バイオリソースセンター受付
電話:029-836-9184 FAX:029-836-9182
E-mail:brc-gate@brc.riken.jp

3. その他

細胞の培養方法や細胞の特性などに関するご質問は、下記宛にメールを下さい。
E-mail:cellqa@brc.rikne.jp

第6章　各種サービスのご案内

1．技術研修のご案内

当室では「ヒトES細胞に係わる技術研修会」及び「ヒト iPS 細胞の凍結保存法（簡易ガラス化法）に係わる技術研修会」を定期的に実施しております。下記のホームページをご参照下さい。

http://www.brc.riken.jp/inf/kensyu/index.shtml#cell3

http://www.brc.riken.jp/lab/cell/hps/seminar0904-06.shtml

2. ホームページのご案内

http://www.brc.riken.jp/lab/cell/

3. ニュースレター配信のご案内

新規に提供を開始した細胞の情報、既存細胞の付随情報に関する新しい情報、提供日の変更等の情報を、月に1回程度、配信しております。配信をご希望の方は、下記宛にメールを下さい。

E-mail：cellbank@brc.riken.jp

4. その他

利用者登録について：

利用者登録は、本カタログの情報解析技術室ページをご参照頂き、上記の当細胞バンクホームページからご登録下さい。利用者登録の際にご希望を頂けば、その後、カタログ、ニュースレターなどの情報をお届け致します。

第7章　よくある質問

＜使用機関における倫理審査は必要か？＞

「ヒトゲノム・遺伝子解析研究に関する倫理指針」における対象試料の定義は次です。「ヒトゲノム・遺伝子解析研究に用いようとする血液、組織、細胞、体液、排泄物及びこれらから抽出したDNA等の人の体の一部並びに提供者の診療情報（死者から提供されたものを含む。）をいう。ただし、学術的な価値が定まり、研究実績として十分に認められ、研究用に広く一般に利用され、かつ、一般に入手可能な組織、細胞、体液及び排泄物並びにこれらから抽出したDNA等は、含まれない。」

当室で扱っている全ての細胞は連結不可能匿名化されており、ほとんどの細胞が上記の対象外試料の定義に該当しますので、使用機関における倫理審査は不要です。ただし、臍帯血、間葉系幹細胞等、一部の細胞につきましては使用機関の倫理審査が必要となりますので、ホームページ等でご確認をお願い致します。

＜細胞の増殖が悪い！＞

細胞をご提供した後に最も多い質問が、「細胞の増殖が悪い」又は「細胞が起きない」というものです。ご提供している細胞の中には、細胞特性として増殖能力がきわめて低い細胞もあります。細胞をご使用になる前に、ホームページ等で細胞特性をご確認のうえ、寄託者指定の培養液、培養方法にて培養をして頂きたく存じます。

＜発送日＞

発送日は原則として毎週火曜日です。火曜日に当細胞バンクから発送しますので、近隣のユーザーには翌水曜日、遠方のユーザーには2日後の木曜日又は3日後の金曜日にお届けできます。しかしながら、当方の都合によりまして発送しない週もございますので、下記ホームページにてご確認下さい。

http://www.brc.riken.jp/lab/cell/plan/

＜提供手続きに関して＞

各細胞の提供手続きに関しましては、ホームページ TOP 画面のサイドバー（http://www.brc.riken.jp/lab/cell/distribution/cell_order.shtml）の他、同じく TOP 画面の、各細胞毎のアイコンをクリックして頂くと、具体的な流れや必要書類のダウンロードが可能ですので併せてご参照下さい。

＜使用培地に関して＞

ご提供しております細胞の培地・添加物のメーカー・品番等に関しましては、下記ホームページにてご確認下さい。

http://www.brc.riken.jp/lab/cell/rcb/med_table.shtml

尚、血清(FBS)のメーカー・Lot に関しましては、当バンクで使用中のものをお答えすることは可能ですが、当バンクより分譲してはおりませんので、ご了承下さい。

＜培養方法に関して＞

ご購入いただいた細胞の、融解操作を含めた培養方法に関しましては、ホームページの下記サイトをご参照下さい。

http://www.brc.riken.jp/lab/cell/manual/

＜培養条件に関して＞

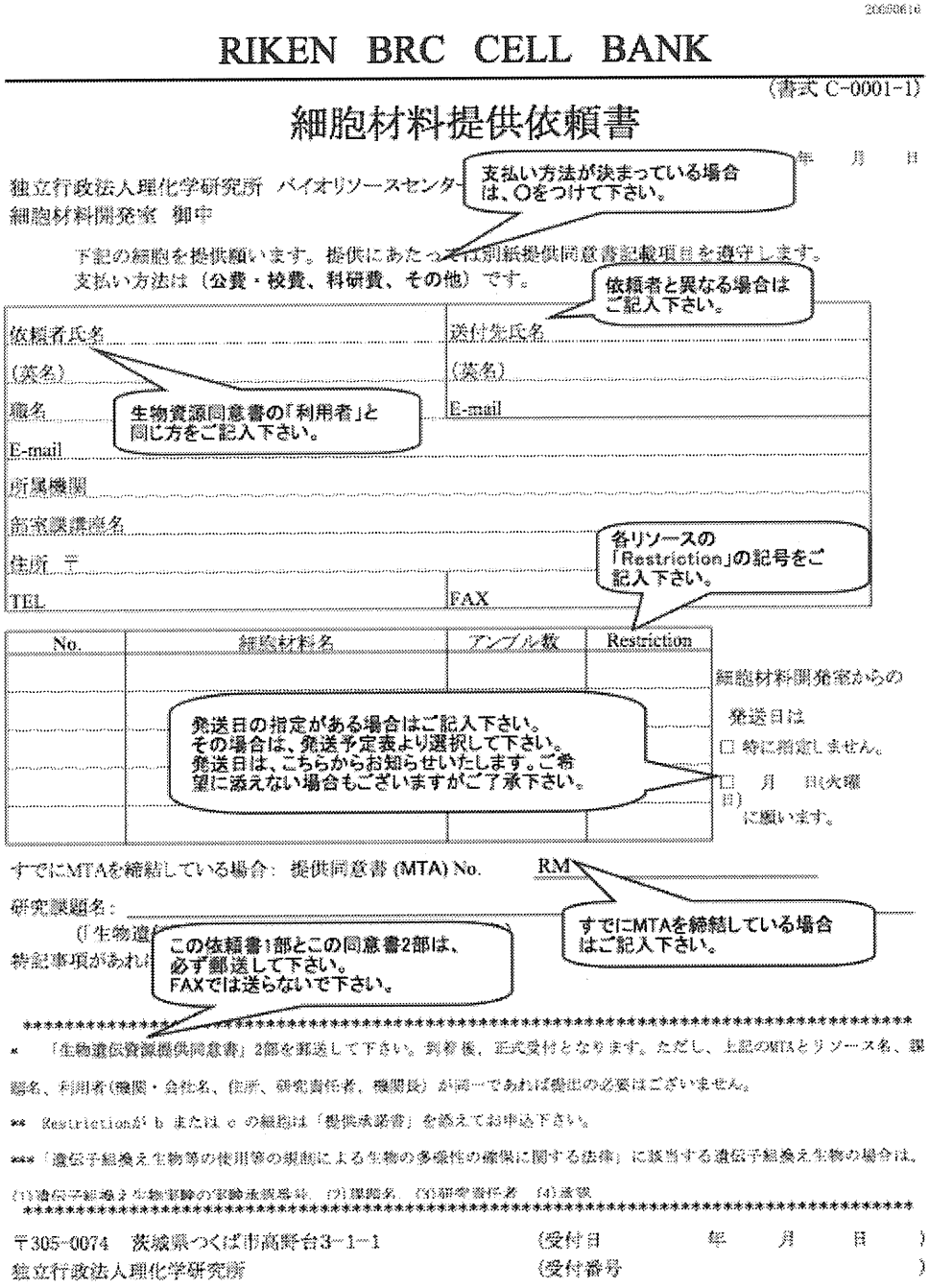
各細胞の培養条件に関しましては、それぞれのデータ画面をご参照下さい。また、当細胞バンクでは原則として寄託時の条件で培養を行っており、それ以外の条件での増殖能等の確認は行っておりません。従いまして、他の培地で培養する場合は、必要量だけ実験用培地で培養し、残りの細胞は維持用培地で培養することをお勧め致します。

補足:申込書記入方法およびオンライン入力

1. 申込書類の記入方法

一般細胞(RCB)の書式の記入方法です。

・ 細胞材料提供依頼書(書式 C-0001)



20050616

RIKEN BRC CELL BANK

(書式 C-0001-1)

細胞材料提供依頼書

年 月 日

独立行政法人理化学研究所 バイオリソースセンター
細胞材料開発室 御中

支払い方法が決まっている場合は、○をつけて下さい。

下記の細胞を提供願います。提供にあたっては別紙提供同意書記載事項を遵守します。
支払い方法は (公費・校費、科研費、その他) です。

依頼者と異なる場合はご記入下さい。

依頼者氏名	送付先氏名
(英名)	(英名)
職名	E-mail
E-mail	
所属機関	
前室課課長名	
住所 〒	
TEL	FAX

生物資源同意書の「利用者」と同じ方をご記入下さい。

各リソースの「Restriction」の記号をご記入下さい。

No.	細胞材料名	アンプル数	Restriction

細胞材料開発室からの発送日は
□ 特に指定しません。
□ 月 日(火曜日)
に願います。

発送日の指定がある場合はご記入下さい。
その場合は、発送予定表より選択して下さい。
発送日は、こちらからお知らせいたします。ご希望に添えない場合もございますがご了承下さい。

すでにMTAを締結している場合: 提供同意書 (MTA) No. RM

研究課題名:

(「生物遺
特記事項があれ

すでにMTAを締結している場合はご記入下さい。

この依頼書1部とこの同意書2部は、必ず郵送して下さい。
FAXでは送らないで下さい。

****

* 「生物遺伝資源提供同意書」2部を郵送して下さい。到着後、正式受付となります。ただし、上記のMTAとリソース名、課題名、利用者(機関・会社名、住所、研究責任者、機関長)が同一であれば提出の必要はございません。

** Restrictionが b または c の細胞は「提供承諾書」を添えてお申込下さい。

*** 「遺伝子組換え生物等の使用等の規制による生物の多様性の確保に関する法律」に該当する遺伝子組換え生物の場合は、
(1)遺伝子組換え生物等実験の実験承認番号、(2)課題名、(3)研究責任者、(4)承認

****

〒305-0074 茨城県つくば市高野台3-1-1
独立行政法人理化学研究所

(受付日 年 月 日)
(受付番号)

・ 細胞材料提供承諾書(書式 C-0002)

20070507

RIKEN BRC CELL BANK

(書式 C-0002)

細胞材料提供承諾書

申込日　　年　　月　　日

独立行政法人理化学研究所　バイオリソースセンター
細胞材料開発室　御中

生物遺伝資源同意書と同じ記載をして下さい。

<<利用者>>
住所：〒
機関名・会社名：
機関長：
研究責任者：
担当者：
E-mail：
TEL：
FAX：

利用者は、下記の条件で寄託者から提供承諾を受けたことを理研ＢＲＣに報告します。

記

課題名（生物遺伝資源提供同意書と同一内容でご記入下さい。）

リソース名（No.）

同一の提供条件の場合は、この書式で複数の細胞材料の承諾を得ることができます。

提供条件（カタログ及びホームページに掲載された条件をご記入下さい。）

カタログ、ホームページに記載された提供条件を文言でご記入下さい。

《寄託者》
寄託者は、上記の条件で利用者への提供を承諾いたします。

住所：〒
機関名・会社名：
研究責任者：
担当者：

申込日が承諾した日付より6ヶ月以上たった場合は、再度、承諾を得て下さい。

承諾した日付をご記入下さい。

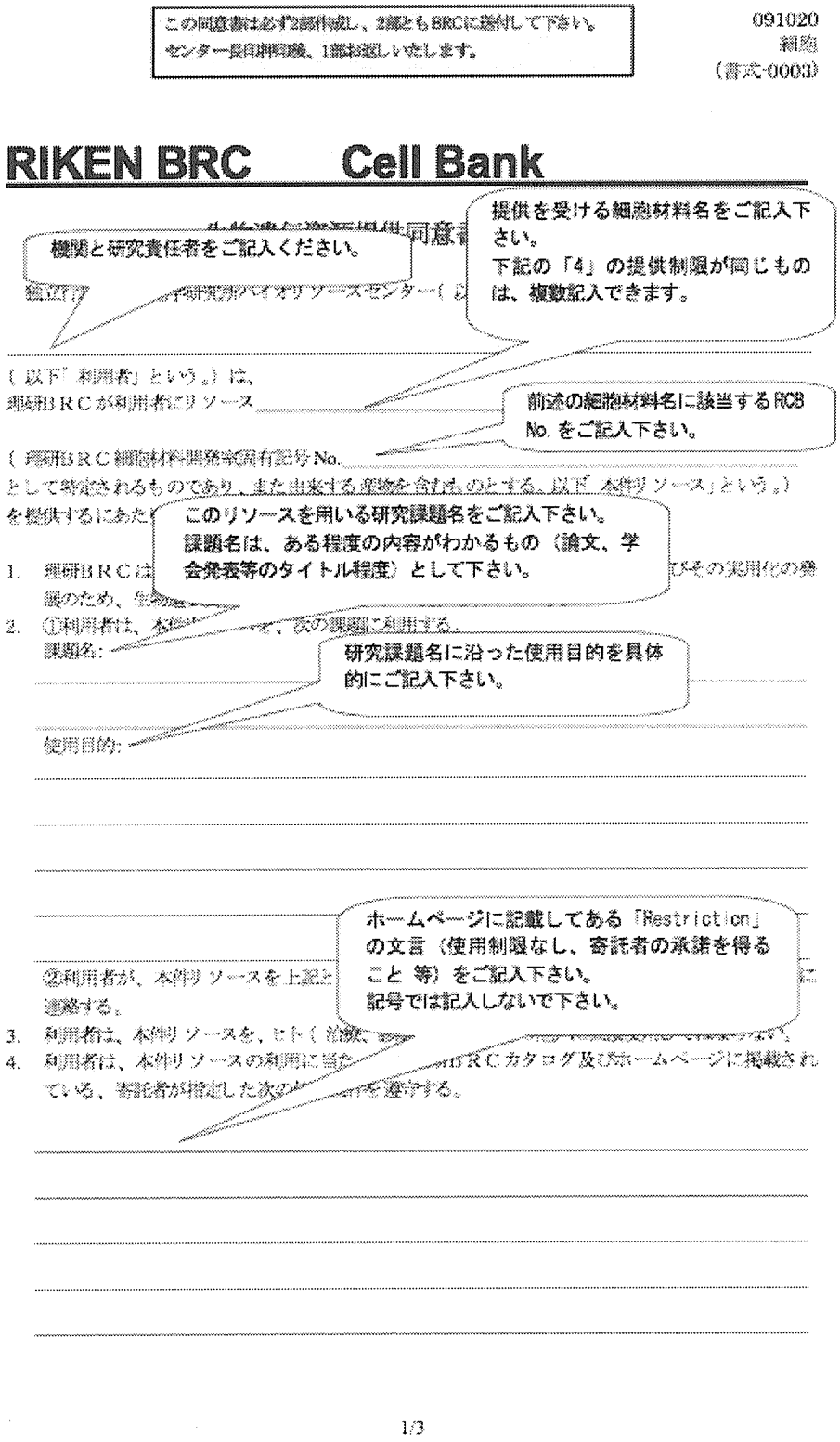
年　　月　　日

なお、本承諾書の有効期限は、本書の日付から６ヶ月以内とします。

〒305-0074 茨城県つくば市高野台３－１－１
独立行政法人理化学研究所　筑波研究所
研究推進部　企画課

(理研記入)
(受付日　　年　　月　　日)
(受付番号　　)
(User No.　　)

・ 生物遺伝資源提供同意書(書式 C-0003)



この同意書は必ず2部作成し、2部ともBRCに送付して下さい。
センター長署押印後、1部お返しいたします。

091020
細胞
(書式-0003)

RIKEN BRC　　Cell Bank

機関と研究責任者をご記入ください。

提供を受ける細胞材料名をご記入下さい。
下記の「4」の提供制限が同じものは、複数記入できます。

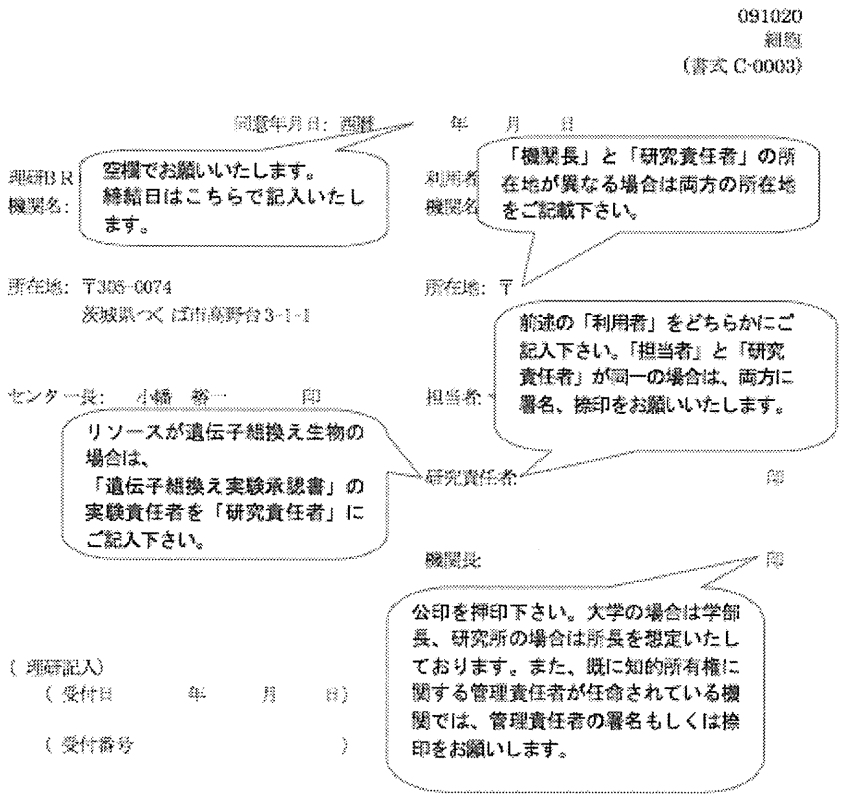
前述の細胞材料名に該当するRCB No. をご記入下さい。

このリソースを用いる研究課題名をご記入下さい。
課題名は、ある程度の内容がわかるもの（論文、学会発表等のタイトル程度）として下さい。

研究課題名に沿った使用目的を具体的にご記入下さい。

ホームページに記載してある「Restriction」の文言（使用制限なし、寄託者の承諾を得ること 等）をご記入下さい。
記号では記入しないで下さい。

1/3



3/3

2. オンライン入力による提供依頼

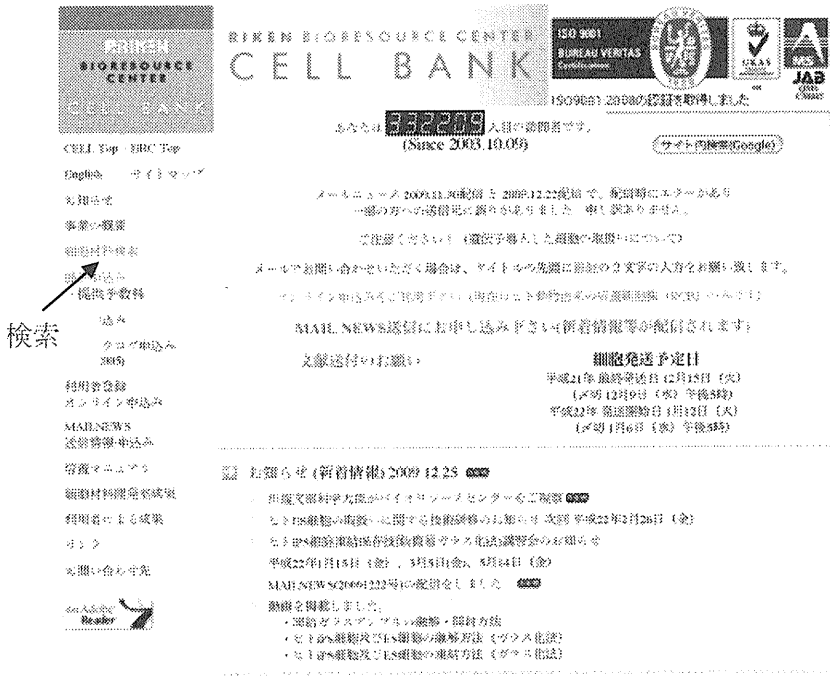
一般細胞(RCB)については、オンラインによる書類の作成が可能です。カタログの情報解析室のページおよび下記のホームページをご参照下さい。

http://www.brc.riken.jp/lab/cell/rcb/houto_online.shtml

補足:ホームページでの検索方法

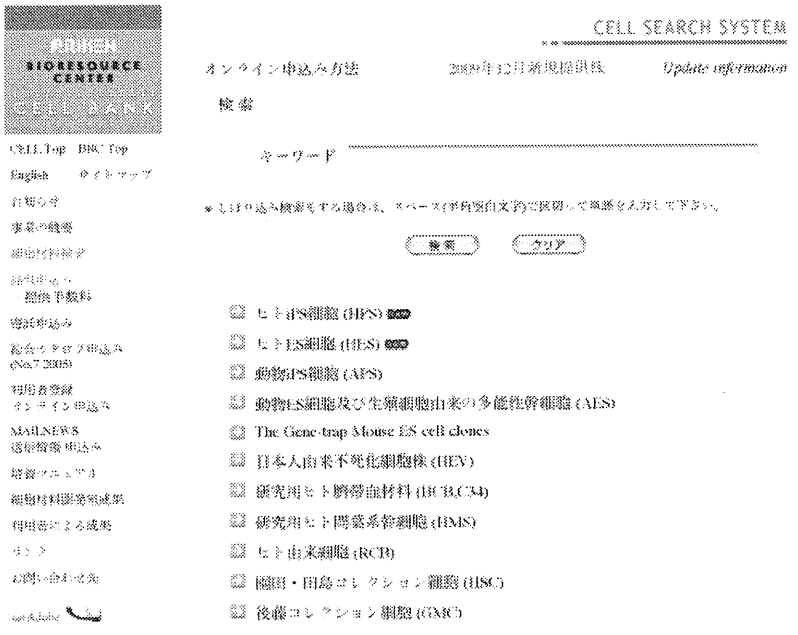
1. ホームページによる検索

http://www.brc.riken.jp/lab/cell/



2. 検索方法

リソースを検索する場合は、左のメニューの「細胞材料検索」をクリックして下さい。



すべての細胞材料が検索対象です。

3. キーワードを入力

(1) キーワードをご入力下さい。

例:「iPS」と入力した場合

Items 6 Search

細胞番号	細 胞 名
APS0001	iPS-MEF-Ng-20D-17
APS0002	iPS-MEF-Ng-178B-5
APS0003	iPS-MEF-Fb-Ng-440A-3
APS0004	iPS-MEF-Ng-492B-4
HPS0001	201B7
HPS0002	253G1

(2) さらに条件を絞り込む場合。

例:「iPS　human」

Items 2 Search

細胞番号	細 胞 名
HPS0001	201B7
HPS0002	253G1

TOP

View Cart

Search キーワード iPS human

検索 クリア

＊　初めから、スペース(半角)で区切っての検索も可能です。

＊　各細胞の特性(日)に含まれる単語であれば、日本語での検索も可能です。

例:「iPS human 4 因子」と入力した場合

Items 1 Search

細胞番号	細 胞 名
HPS0001	201B7

(3) 細胞番号クリックで詳細情報が表示されます。

HPS0001 : 201B7

特性(日)	ヒト人工多能性幹（iPS）細胞株。レトロウイルスベクターにより4因子（Oct3/4, Sox2, Klf4, c-Myc）を導入。
動物種	human

II. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
中村幸夫	iPS細胞と細胞の品質管理	小幡裕一、城石俊彦、芹川忠夫、田中啓二、米川博通	生物機能モデルと新しいリソース・リサーチツール	エル・アイ・シー社	東京	2011	659-671

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nakamura, Y., Hiroyama, T., Miharada, K., and Kurita, R.	Red blood cell production from immortalized progenitor cell line.	*Int. J. Hematol.*	93	5-9	2011
Masuya, H., Makita, Y., Kobayashi, N., Nishikata, K., Yoshida, Y., Mochizuki, Y., Doi, K., Takatsuki, T., Waki, K.,Tanaka, N., Ishii, M., Matsushima, A., Takahashi, S., Mizoguchi, R., Kozaki, K., Furuichi, T., Kawaji, H., Wakana, S., Nakamura, Y., Yoshiki, A., Murata, T., Fukami-Kobayashi, K., Mohan, S., Ohara, O., Hayashizaki, Y., Obata, Y., and Toyoda, T.	The RIKEN integrated database of mammals.	*Nucl. Acids Res.*	39	D861-870	2011
Danjoh, I., Saijo, K., Hiroyama, T., and Nakamura, Y.	The Sonoda-Tajima Cell Collection, a human genetics research resource with emphasis on South American indigenous populations.	*Genome Biology and Evolution*	Open access journal	doi:10.1093/gbe/evr014	2011

Nakamura, Y.	ES cell-derived erythroid cell lines able to produce mature red blood cells.	*InTech* "Embryonic Stem Cells-Recent Advances in Pluripotent Stem Cell-Based Regenerative Medicine" (edited by Craig Atwood)	Chapter 15	273-288	2011
Hiroyama, T., Miharada, K., Kurita, R., and Nakamura, Y.	Plasticity of cells and *ex vivo* production of red blood cells.	*Stem Cell Int.*	Article ID 195780, open access journal, doi:10.4061/2011/195780		2011
Matsui, H., Nagano, Y., Shimokawa, O., Kaneko, T., Rai, K., Udo, J., Hirayama, A., Nakamura, Y., Indo, H.P., Majima, H.J., and Hydo, I.	Gastric acid induces mitochondrial superoxide production and lipid peroxidation in gastric epithelial cells.	*J. Gastroenterol.*	46	1167-1176	2011
Nagano, Y., Matsui, H., Tamura, M., Shimokawa, O., Nakamura, Y., Kaneko, T., and Hyodo, I.	NSAIDs and acidic environment induce gastric mucosal cellular mitochondrial dysfunction.	*Digestion*	85	131-135	2012

III. 研究成果の刊行物・別刷

Int J Hematol (2011) 93:5–9
DOI 10.1007/s12185-010-0742-2

Red blood cell production from immortalized progenitor cell line

Yukio Nakamura · Takashi Hiroyama · Kenichi Miharada · Ryo Kurita

Received: 21 November 2010 / Revised: 24 November 2010 / Accepted: 30 November 2010 / Published online: 25 December 2010

Abstract The supply of transfusable red blood cells (RBCs) is not sufficient in many countries. If immortalized erythroid progenitor cell lines able to produce transfusable RBCs in vitro were established, they would be valuable resources. However, such cell lines have not been established. We have developed a robust method to establish immortalized erythroid progenitor cell lines following the induction of hematopoietic differentiation of mouse embryonic stem (ES) cells and have established many immortalized erythroid progenitor cell lines so far. Although their precise characteristics varied among cell lines, each of these lines could differentiate in vitro into more mature erythroid cells, including enucleated RBCs. Following transplantation of these erythroid cells into mice suffering from acute anemia, the cells proliferated transiently, subsequently differentiated into functional RBCs, and significantly ameliorated the acute anemia. Considering the number of human ES cell lines that have been established so far and the number of induced pluripotent stem cell lines that will be established in future, the intensive testing of a number of these lines for establishing immortalized erythroid progenitor cell lines may allow the establishment of such cell lines similar to the mouse erythroid progenitor cell lines.

Keywords Erythrocyte · Erythropoiesis · Red blood cell · Transfusion therapy

Y. Nakamura (✉) · T. Hiroyama · K. Miharada · R. Kurita
Cell Engineering Division, RIKEN BioResource Center, Koyadai 3-1-1, Tsukuba, Ibaraki 305-0074, Japan
e-mail: yukionak@brc.riken.jp

1 Introduction

Organ and cell transplantation therapy is now a standard therapy. However, the supply of organs or cells is not necessarily sufficient all over the world. Hence, production of artificial organs and in vitro production of transplantable cells have been studied in earnest. At the moment, transfusion therapies involving red blood cells (RBCs), platelets, and neutrophils depend on the donation of these cells from healthy volunteers. Unpredictable adverse results derived from the current transfusion therapy system such as contamination of hazardous viruses and transfusion-related acute lung injury (TRALI) may be eliminated by the development of new technologies in the future. On the other hand, lack of supply of transfusable materials by the current system will become a severe problem in advanced nations including Japan, since in those countries the ratios of aged generations who have more opportunities to require transfusion therapies are increasing while the ratios of younger generations who can donate transfusable materials are decreasing. In this situation, research and development to produce transfusable blood materials is very important and should be carried out more earnestly. In particular, since RBC transfusion is now routine and indispensable for many clinical purposes, in vitro production of transfusable RBC is an urgent theme.

2 RBC production from hematopoietic stem cells

The hematopoietic stem cells present in bone marrow and umbilical cord blood are promising materials for in vitro production of RBCs and this has stimulated interest in the development of in vitro procedures for the generation of functional RBCs from these tissues [1–3]. Umbilical cord

blood cells are of particular interest as they are readily available but are usually discarded. Provided the mother of a neonate consents to use of the umbilical cord blood, this material can provide a useful resource without any further complicating critical or ethical concerns.

It was reported that human erythroid cells (nucleated cells) produced on a large scale ex vivo could differentiate in vivo into enucleated RBCs [1]. This study demonstrated that erythroid progenitor cells produced in vitro from hematopoietic stem and progenitor cells could have a clinical application as an alternative method for transfusing terminally differentiated RBCs. More recently, the same group described an ex vivo methodology for producing fully mature human RBCs from hematopoietic stem cells [2]. The enucleated RBCs produced by this approach are potentially even more valuable as they should be functional immediately after transfusion without requiring the long latency period for enucleation normally necessary for erythroid cells.

The mechanism of erythroblast enucleation, a critical step in RBC production, has not yet been fully elucidated [4, 5]. The role of interactions between erythroblasts and other cells, such as macrophages, in this process is a controversial topic [6–10]. Macrophages in retinoblastoma gene (Rb)-deficient embryos are unable to physically interact with erythroblasts and RBC production is impaired in these embryos [9]. In addition, in vitro production of enucleated RBCs from immature hematopoietic stem/progenitor cells proceeds efficiently in the presence [2] but not in the absence [1] of feeder cells.

Of note, however, enucleation can apparently be initiated in vitro in erythroblasts that have been induced to differentiate in vivo to a developmental stage that is competent for nuclear self-extrusion [10, 11]. Moreover, we have developed a method to produce enucleated RBCs efficiently in vitro without use of feeder cells [3]. The culture system has allowed erythroid cells to differentiate to a developmental stage competent for nuclear self-extrusion [3]. Taken together, although it has generally been thought that efficient enucleation of erythroblasts is largely dependent on signals mediated by cells in their local environment [6–9], the interaction of erythroblasts with other cells is not necessary for efficient erythroblast enucleation [3]. Signals mediated by humoral factors appear to be sufficient for the efficient autonomous completion of erythroblast enucleation [12].

Since culture without the use of feeder cells is technically easier and less expensive, the method we established [3] has the potential to be a cost-effective means of producing transfusable RBCs on a large scale from immature hematopoietic stem/progenitor cells. Currently, however, cost factors mean that it is not yet realistic to produce RBCs on a large scale, approximately 200 ml or more, using our in vitro culture system. In particular, patents on the growth factors used in the culture system are a major obstacle, because these growth factors are very expensive, at least at the moment. After the relevant patents expire, our in vitro culture system will become a more realistic scenario.

3 RBC production from embryonic stem (ES) cells

ES cells possess the potential to produce various differentiated cells able to function in vivo and thus represent another promising resource for RBC production. Furthermore, since ES cell lines are immortalized, they can be used repeatedly and have potential to produce abundant differentiated cells in the quantities required for clinical use. However, it will be important to carry out routing screening of the ES cell lines for de novo chromosomal aberrations and/or genetic mutations that may arise in vitro, before these long-term cell cultures are applied in the clinic. Unsurprisingly, there is now a widespread and enthusiastic debate on standardization of the characteristics of ES cells for regenerative medicine protocols that exploit these cell lines. In my opinion, since chromosomal aberrations and genetic mutations are inevitable in long-term cell cultures, only ES cell lines that have been cultured for a limited period, e.g., less than 30 passages, should be selected for clinical use.

Hematopoietic cells, including those in the erythroid lineage, have been generated from mouse ES cells [13–16], non-human primate ES cells [17–19], and human ES cells [20–25]. We have also established a long-term in vitro method for culturing hematopoietic cells derived from ES cells of the non-human primate, the common marmoset [26]. Recently, abundant production of enucleated RBCs from human ES cells was reported [27].

Taken together, we can now produce mature RBCs by in vitro culture of ES cells or the hematopoietic stem/progenitor cells present in umbilical cord blood. In practice, however, the efficiency of RBC generation varies with the quality of the ES cell line or the umbilical cord blood sample. Since ES cell lines can be utilized repeatedly, derivation of RBCs from ES cells appears to be more practical. However, even with optimal experimental procedures and the most appropriate ES cell line, the generation of abundant RBCs directly from primate ES cells is a costly and time-consuming process [26, 27]. If human erythroid progenitor cell lines can be established that have efficient production of mature RBCs, they would provide a much more useful resource than ES cell lines.

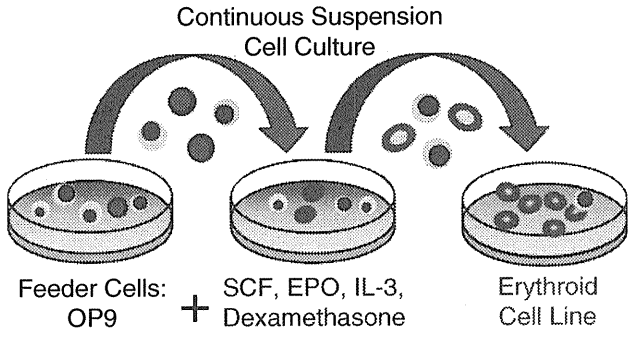


Fig. 1 Culture protocol to establish immortalized erythroid progenitor cell lines. Hematopoietic cells derived from ES or iPS cells are cultured on feeder cells, OP9, in the presence of stem cell factor (SCF), erythropoietin (EPO), interleukin-3 (IL-3) and dexamethasone

4 Establishment of mouse RBC progenitor cell lines able to produce transfusable RBCs

Several mouse and human erythroid cell lines have been established. However, to the best of our knowledge, there is no cell line that can efficiently differentiate into enucleated RBCs. It is generally difficult to establish hematopoietic cell lines from adult hematopoietic stem and progenitor cells as both are sensitive to DNA damage and are unable to maintain the lengths of telomere repeats on serial passage [28]. In contrast, ES cells are relatively resistant to DNA damage and maintain telomere lengths on serial passage [28]. Therefore, these characteristics of ES cells may be advantageous for the establishment of cell lines since differentiated cells derived from ES cells may retain them.

Recently, we developed a robust method to obtain differentiated cell lines following the induction of hematopoietic differentiation of mouse ES cells (Fig. 1), and established five independent hematopoietic cell lines using this method [29]. Three of these lines exhibited characteristics of erythroid cells, and they were designated mouse ES cell-derived erythroid progenitor (MEDEP) cell lines. Although their precise characteristics varied, each of the MEDEP lines could differentiate in vitro into more mature erythroid cells, including enucleated RBCs (Fig. 2). Following transplantation into mice suffering from acute anemia, MEDEP cells proliferated transiently and subsequently differentiated into functional RBCs. Treated mice showed a significant amelioration of acute anemia. In addition, MEDEP cells did not form tumors following transplantation into mice. This report was the first to demonstrate the feasibility of establishing erythroid cell lines able to produce mature RBCs [29]. At present, the mechanism underlying the establishment of differentiated cell lines from ES cells has not been elucidated. Nevertheless, our data clearly indicate that useful erythroid cell lines can be reproducibly obtained from mouse ES cells.

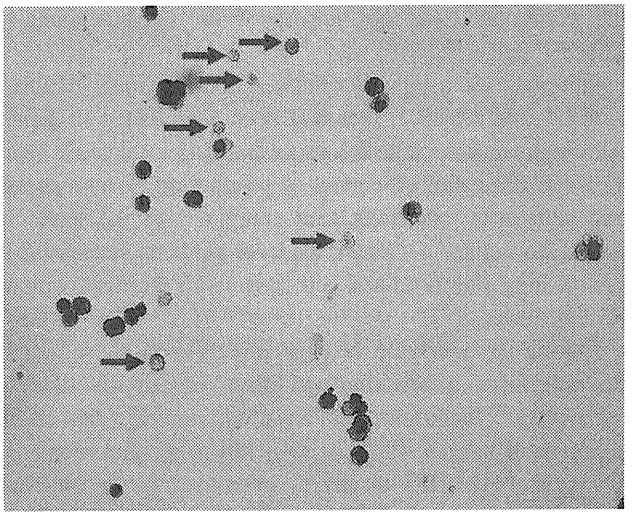


Fig. 2 Red blood cells (RBCs) produced from immortalized mouse progenitor cell line. *Arrows* indicate enucleated RBCs

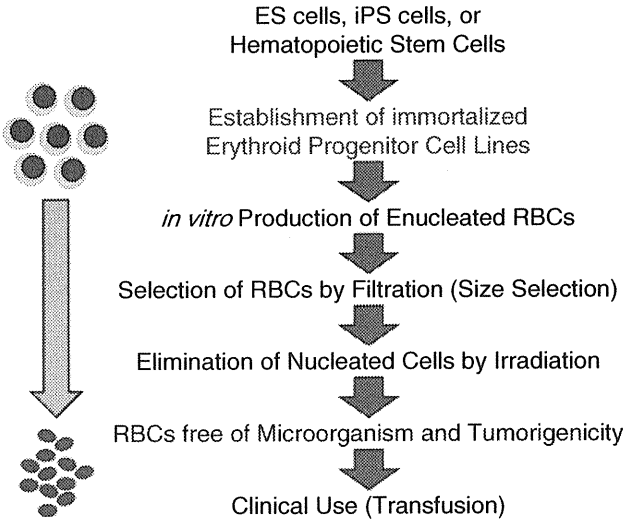


Fig. 3 Strategy to utilize red blood cells (RBCs) produced in vitro from immortalized erythroid progenitor cell lines in the clinic

5 Establishment of RBC progenitor cell lines from human ES cells or human induced pluripotent stem (iPS) cells

The reproducible establishment of MEDEP cell lines described above strongly suggests that similar erythroid cell lines could also be established from human ES cells. We, therefore, sought to establish human erythroid progenitor cell lines. The methods used to induce hematopoietic cells from ES cells and to culture the induced hematopoietic cells are similar to those established for MEDEP cell lines, with the exception that the corresponding human factors were applied and IL-3 was not used at all. Exclusion of IL-3 was based on our finding that

the compound was not necessary for establishment of MEDEP cell lines [29].

Initially, we used three human ES cell lines, KhES-1, KhES-2 and KhES-3, that had been established in Japan. Although we were able to induce hematopoietic cells from all three lines, the efficiency of production of hematopoietic cells was extremely low compared to the cases of mouse ES cells. As a result, we have yet been successful in establishing immortalized cell lines from the three human ES cell lines.

During the course of the experiments using these human ES cell lines, a breakthrough discovery in the field of regenerative medicine was reported, namely, the establishment of human iPS cells [30] following that of mouse iPS cells [31]. This discovery prompted us to establish human iPS cells, since the characteristics of pluripotent stem cells, such as ES cells, differ among cell lines. In other words, we speculated that we could obtain iPS cell lines that could have the ability to differentiate into hematopoietic cells efficiently. We were able to establish a number of human iPS cell lines using fibroblast-like cells derived from neonatal tissues [32]. Fortunately, we were able to induce abundant numbers of hematopoietic cells from some of these iPS cell lines and also to establish immortalized hematopoietic cell lines from the induced hematopoietic cells (data not shown). Currently, we are investigating the characteristics of these immortalized hematopoietic cell lines. Some seem to be erythroid cell lines.

6 Clinical application of human RBC progenitor cell lines

We reported that MEDEP cells did not exhibit tumorigenicity in vivo [29]. Nevertheless, the tumorigenic potential of any human erythroid cell line will need to be thoroughly analyzed prior to clinical use [33, 34]. In general, immortalized cell lines are not necessarily homogenous in karyotype, even after cloning. The emergence of cells possessing abnormal karyotypes is often observed following continuous culture of immortalized cell lines. Indeed, although the vast majority of the MEDEP cells in each cell line could differentiate into mature erythroid cells and transplantation of these cells significantly ameliorated anemia, the MEDEP lines included many cells possessing abnormal karyotypes [29]. Hence, it may be advisable to engineer the cells in such a way that they are eliminated if a malignant phenotype arises for any reason [35].

Alternatively, the use of terminally differentiated cells that no longer have the capability of proliferating should allow clinical applications of ES cell derivatives without the associated risk of tumorigenicity. Thus, e.g., RBCs lack nuclei following terminal differentiation, and are highly unlikely to exhibit tumorigenicity in vivo. As such, even if the original ES/iPS cells or the immortalized erythroid progenitor cell lines derived from them possessed abnormal karyotypes and/or genetic mutations, they might nonetheless be useful for clinical applications, provided that they can produce enucleated RBCs. Since enucleated RBCs are much smaller than normal nucleated cells, enucleated RBCs produced in vitro could be selected by size prior to use in the clinic so as to exclude nucleated cells, e.g., by filtration. In addition, X-ray irradiation might be useful for eradicating any contaminating nucleated cells without affecting the enucleated RBCs (Fig. 3).

Another potential obstacle to the clinical use of ES cell derivatives is that of immunogenicity [36, 37]. Transplanted MEDEP cells could not ameliorate acute anemia in mouse strains other than those from which each individual cell line was derived or in immunodeficient mice [29], suggesting immunological rejection in heterologous strains. Hence, the direct clinical application of immortalized erythroid cell lines will require use of many cell lines that express different major histocompatibility (MHC) antigens. However, in vitro-generated mature RBCs need to be compatible with ABO and RhD antigens alone (8 types in total). Moreover, RBCs lacking all of A, B and RhD antigens can be transfused into the vast majority of patients around the world. Hence, the establishment of a human erythroid cell line lacking the genes to produce A, B and RhD antigens would be a very useful resource for clinical application.

7 Conclusion

We propose that by utilizing ES cells or iPS cells it will be possible to establish human erythroid progenitor cell lines able to produce enucleated RBCs. RBCs produced by in vitro culture of such erythroid cell lines could be applied in the clinic following size selection and elimination of nucleated cells by irradiation (Fig. 3).

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The RIKEN integrated database of mammals

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ABSTRACT

The RIKEN integrated database of mammals (http://scinets.org/db/mammal) is the official undertaking to integrate its mammalian databases produced from multiple large-scale programs that have been promoted by the institute. The database integrates not only RIKEN's original databases, such as FANTOM, the ENU mutagenesis program, the RIKEN Cerebellar Development Transcriptome Database and the Bioresource Database, but also imported data from public databases, such as Ensembl, MGI and biomedical ontologies. Our integrated database has been implemented on the infrastructure of publication medium for databases, termed SciNetS/SciNeS, or the Scientists' Networking System, where the data and metadata are structured as a semantic web and are downloadable in various standardized formats. The top-level ontology-based implementation of mammal-related data directly integrates the representative knowledge and individual data records in existing databases to ensure advanced cross-database searches and reduced unevenness of the data management operations. Through the development of this database, we propose a novel methodology for the development of standardized comprehensive management of heterogeneous data sets in multiple databases to improve the sustainability, accessibility, utility and publicity of the data of biomedical information.

INTRODUCTION

Securing the sustainability of databases is one of the most important issues for research institutes, funding agencies and research communities, because the accumulated cost of maintenance becomes a serious burden on the responsible institutes and communities (1). Moreover, the development of technology for biomedical analyses has brought about a dramatic increase in the amount and variety of data and information. The outdating of isolated data is also a serious problem. The association with public data records broadly used in the research community is crucially important to improve the usability and accessibility of data. If data are isolated in the application software without updates from external data, then the data will become increasingly difficult to retrieve by external retrieval systems and will become useless, unnecessarily occupying the storage resource. On the contrary, the integration of a datum with external data will generally increase its usability and value, often promoting unexpected uses and knowledge discovery. In the community of mammalian research, authoritative data are provided by the Mouse Genome Informatics Database (MGI), HUGO

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