## Supporting Online Material



## Supporting Text

### Explanation of Informed Consent Process and Regulatory Oversight

Prior to beginning any experiments, we obtained approval for this study from the Institutional Review Board (IRB) on Human Subjects Research and Ethics Committee at Hanyang University Hospital, Seoul, Korea. Oocyte and somatic cell donors were counseled by two IRB members to ensure that the donors were fully aware of the scope of the investigation and that no immediate clinical benefits were anticipated. Each donor signed an informed consent form as summarized below. See last section of the SOM for copies of the informed consent forms, in Korean and English. Parents of children (under eighteen years old) that were donating somatic cells were similarly counseled, and both parents signed informed consent forms on behalf of their child. The risks associated with oocyte donation were described to donors, including side effects of injection, of hormone stimulation, of anesthesia and the risk of unanticipated complications.

Regarding cell donation and informed consent, individuals voluntarily donated oocytes and/or somatic cells for research on the potential applications of therapeutic cloning. Donors understood that neither they nor their relatives would benefit from this research. Donations were made without coercion or financial payment. Although expenses for public transportation and injections administered by medical personnel could have been provided, none of the donors requested this reimbursement. Donations were confidential in that donor identity was encoded by the responsible clinician and donor identity was unknown to the investigators and others. At any point up to cell donation, donors could cancel their participation in the study for any reason. Oocytes, embryos, and unused somatic cells would also be destroyed according to the guidelines of the Korean Society

for Obstetrics and Gynecology, which are identical to those of the American Society for Reproductive Medicine. Finally, it was understood that although intellectual property and commercial rights might be derived from the study, donors relinquished all rights and benefits that might be derived from the cell donations. See final section of the SOM for Korean and translated copies of the informed consent forms.

On January 1, 2005, the Republic of Korea's new regulation entitled: 'Bioethics and Biosafety Act – Act No. 7150,' became effective. This Act codified the guidelines of the Korean Society of Obstetrics and Gynecology into law. Whereas all donors and their parents were fully counseled by two IRB members that neither they nor their family members could benefit from this basic research on patient-specific NT-hESC establishment, the Korean Network for Organ Sharing Regulation Code 18-1 states that first degree relatives (spouses and children) receive priority followed by second degree relatives (cousins, aunts and uncles) for transplantation. Consistent with Code 18-1, the IRB approval for this study required that when relatives donated oocytes or somatic cells, donors were informed that family members would theoretically receive priority, but only if or when NT-hESCs were demonstrated to be safe, effective and tolerated. In addition, the use of these cells for patients would only occur if or when the donor specific cells were proven to be of any medical value.

The Republic of Korea's regulation 'Bioethics and Biosafety Act – Act No. 7150' requires governmental licensing of somatic cell nuclear transfer using human oocytes and subsequent derivation of NT-hESCs (therapeutic cloning). On Jan 12, 2005, we received government approval, in accordance with this new law. This law also required IRB approval from the College of Veterinary Medicine, Seoul National University, which was granted on January 25, 2005. See penultimate section in the SOM.

With regard to the new 'Guidelines for Human Embryonic Stem Cell Research' just announced by the National Academy of Sciences (NAS) on April 26, 2005 (*3*), the University of Pittsburgh and the Magee-Womens Research Institute (the institutional home of the Pittsburgh Development Center) had previously convened a panel similar to the NAS guidelines' description of an institutional Embryonic Stem Cell Research Oversight Committee (ESCRO). This panel's guidance was sought by the US scientists (GS and JHP) with respect to permissible roles for their advisory involvement in the preparation of this manuscript in the context of Federal and state hESC policies. The US scientists also filed an application, seeking concurrence that their

involvement did not involve human subjects research, with the University IRB, consistent with their role in the analysis of anonymized data. See penultimate section in the SOM.

## Materials and Methods

### Ovarian Stimulation Protocols

Three protocols were followed for ovarian stimulation: 1) gonadotropin releasing hormone (GnRH) antagonist, 2) GnRH agonist (GnRHa) long and 3) GnRHa short. The majority underwent the first protocol, i.e. 225-300 IU/day recombinant follicle stimulating hormone (rFSH, Gonal-F; Serono Korea, Seoul, Korea) starting from Day 3 of the cycle. On Day 7 ultrasonography was performed and serum $E_2$ assayed to monitor follicle growth. Depending on follicular response, the dosage of rFSH was adjusted and then follicle growth assessed on Days 10 or 11. Donors received daily doses of 0.25 mg GnRH antagonist (Cetrotide, Serono) starting Days 8-10.

For the GnRHa long protocol, 400 μg/day buserelin (Suprefact, Handok Pharmaceuticals, Seoul, Korea) was administered from the mid-luteal phase to Day 2 of the next cycle. On Day 3, donors received 200 μg/day buserelin and 225-300 IU/day rFSH. Responses were assessed as previously described.

For the GnRHa short protocol, donors received 200 μg buserelin every 12 hours starting from Day 2. Gonadotropins (150 IU/day Metrodin and 150 IU/day Pergonal; Serono) were started on Day 3 for four days. Responses were assessed on Day 7 as before. Some donors received only Pergonal. 10,000 IU human chorionic gonadotropin (hCG; Profasi, Serono) was administered in all three protocols when at least three follicles reached >18mm in diameter. Ultrasound-guided oocyte retrieval was performed 36 hours after hCG administration.

### Preparation of donor cells

An abdominal skin biopsy (1.5 cm x 0.5 cm x 0.5 cm) was obtained from patients under local anesthesia with lidocane and transferred to DMEM (Life Technologies, Rockville, MD) supplemented with 10% (v: v) FBS (Hyclone, South Logan, UT), 1% (v: v) non-essential amino acids (Life Technology) and 10 μg/mL penicillin-streptomycin (Sigma-Aldrich Corp, St Louis, MO). Tissue fragments were washed twice with DMEM supplemented with 10% FBS, 1% non-essential amino acids and 10 μg/mL penicillin-streptomycin solution, and mechanically minced with a surgical blade on a 100-mm culture dish (Becton Dickinson, Lincoln

Park, NJ). The minced tissue was centrifuged at 300 x G for 3 min, and supernatant was discarded. The tissue pellet was resuspended in 4.5 ml DMEM supplemented with 10% FBS, 1% non-essential amino acids and 10 µg/mL penicillin-streptomycin solution, and 500 µl (2000 unit/ml) of collagenase type II (Life Technologies) were added. The medium was overlaid onto 60-mm tissue culture dishes (Becton Dickinson) and incubated overnight. After incubation, the medium was centrifuged at 300 x G for 3 min, and the cell pellet was resuspended in DMEM with 10% FBS and seeded into a 60-mm culture dish. Seeded cells were subsequently cultured until confluent in DMEM supplemented with 10% FBS, 1% non-essential amino acids and 10 µg/mL penicillin-streptomycin solution at 37°C in a humidified atmosphere of 5 % $CO_2$ and 95 % air. Prior to NT, individual cells were retrieved from the monolayer by trypsinization with 0.25% (v/v) trypsin-EDTA (Life Technologies) for 30 sec at 37°C and subsequently used for NT.

**Somatic cell nuclear transfer**

Except derivation of a NT-hESC-8, heterologous SCNT was performed, i.e. the donor fibroblasts were transferred back into the enucleated oocytes from either biologically-unrelated relatives (i.e. spouses) or anonymous donors. For establishment of NT-hESC-8 line, autologous NT was performed, i.e. the donor's own fibroblasts were transferred back into her own enucleated oocytes. Enucleation, confirmation of removal of the oocyte's DNA, NT, and electrical fusion were performed as previously described *(1)*. The ten previously noted procedural improvements were, in addition to the five noted in the text are: 1. Fresh, prime oocytes; 2. Donation by fertile women; 3. Korea's enabling oversight for stem cell research, while criminalizing reproductive cloning; 4. Enucleation by gentle pressure (not aspiration) through a slit in the zona pellucida; 5. Barely mature oocytes (not fully matured or aged); 6. Two-hour reprogramming time between fusion and activation; 7. Electric fusion separated from artificial oocyte activation using calcium ionophore and 6-dimethylaminopurine; 8. Sequential culture media; 9. Synthetic protein; and 10. Human serum albumin.

**Activation and Culture of Human SCNT embryos**

After electrical fusion, reconstructed oocytes were incubated for 2 hrs before chemical activation. Fused donor oocytes and somatic cells were activated in 10 µM calcium ionophore A23187 (Sigma) in 25 µl G1 ver.3 medium (Vitro Life, Goteborg, Sweden) at 37°C for 5 min. Oocytes were then rinsed with fresh G 1 ver.3 medium, placed in G 1 ver.3 medium containing 2 mM 6-DMAP (Sigma), and incubated at 37°C in 5% $CO_2$, 5% $O_2$, 90% $N_2$ for 4 hrs. After incubation, oocytes were washed with fresh G 1 ver.3 medium and cultured in 10 µl drops of G 1 ver.3 medium at 37°C in 5% $CO_2$, 5% $O_2$, 90% $N_2$ for 48 hrs. At 48 hrs of activation,

cleaved embryos were transferred to human modified synthetic oviductal fluid (SOF) with amino acids (hmSOFaa)-2 containing glucose (1.5 mM), and human serum albumin (10 mg/ml), instead of bovine serum albumin (8 mg/ml), and cultured for another 6 days at 37°C in 6% $CO_2$, 5% $O_2$, 89%.

**Isolation of Inner Cell Mass Cells**

For immunosurgical isolation of ICMs, after zona pellucida (ZP) digestion with 0.1% pronase (Sigma), cloned blastocysts were first incubated with 100% anti-human serum antibody (Sigma) for 20 min, followed by an additional 15 min exposure to the guinea pig complement (Life Technologies) in 20 µl droplets at 37 °C in 5% $CO_2$. In some experiments, intact blastocysts, with or without ZP, after digestion with 0.1% pronase were directly mounted on feeder cells without ICM isolation.

**Establishment and Culture of NT-hESC lines**

Isolated ICMs or intact blastocysts with or without ZP were cultured on mitomycin C mitotically inactivated primary human fibroblast as used for donor cells of NT-hESC-2 line in 0.1% gelatin-coated 4-well tissue culture dishes. The culture medium consisted of Dulbecco's modified Eagle's Medium (DMEM)/DMEM F12 (1:1) (Life Technologies) supplemented with 20% Knockout Serum Replacement (Life Technologies), 0.1 mM β-mercaptoethanol (Sigma), 1% nonessential amino acids, 100 units/ml penicillin, 100 µg/ml streptomycin, and 4 ng/ml basic fibroblast growth factor (bFGF; R & D Systems, Minneapolis, MN).

**Immunohistochemical staining for Cell Surface Markers**

Alkaline phosphatase activity was measured, and cell surface markers for SSEA-1, SSEA-3, SSEA-4, TRA-1-60, TRA-1-80 and Oct-4 were immunostained in NT-hESC colonies as previously described *(2)*.

**DNA Fingerprinting Analyses**

DNA fingerprinting analyses were performed with genomic DNA from the donor cells, SCNT-hES cells and lymphocytes from unrelated donors, and human STR markers using a STR Amp FLSTR PROFILER Kit (Perkin Elmer Corp., Wellesley, MA) on an automated ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

**HLA Typing**

HLA-A, -B, -C, -DRB1, and -DQB1 DNA-based typing was performed using the Dynal RELI$^{TM}$ typing kits (Dynal Biotech, Ltd., Bromborough, UK) according to the manufacturer's instructions with polymerase chain reaction.

**Formation of Embryoid Bodies and Teratomas**

Embryoid bodies (EBs) were formed, placed into a small drop of 1% molten low melting point agarose, fixed in 4% paraformaldehyde in PBS and embedded in paraffin as previously described *(2)*. Individual (2-μm) sections were placed on slides and immunohistochemical analysis was carried out. The antibodies used are as follows: Neurofilament (18-0171), glial fibrillary acidic protein (GFAP, 18-0063), S-100 (18-0046), Cytokeratin-7 (18-0234), and Desmin (18-0016) were supplied by Zymed (South San Francisco, CA); Pax3/7 (SC-7748), N-CAM (123C3), MAP-2 (SC-5359), HNF-4α (SC-6556), ANP (atrial natriuretic peptide, SC16867), GATA-4 (SC-9053), Myo D (SC-760), BMP-4 (SC-6896), MHC (SC-12117), and MLC2 (SC-9449) were purchased from Santa Cruz Biotechnology; CD34 (MS-363-P) and actin muscle (MS-1296-P) were purchased from Lab Vision Corporation (Fremont, CA).Primary antibodies were localized with biotinylated secondary anti-rabbit, anti-mouse, or anti-goat and then with avidin-conjugated alkaline phosphatase complex. For teratoma formation, clumps consisting of about 100 cells with undifferentiated morphology were inoculated into the testis of six- to eight-week-old SCID mice and the resulting teratomas sections were stained with hematoxylin and eosin staining as previously described *(2)*.

**Statistical analysis**

Using the statistical analysis system (SAS)-FREQ procedure, data was analyzed by chi-square homogeneity test. In order to confirm the results of the homogeneity test, the data was incorporated to log linear model using SAS-CATMOD procedure. Differences of $P < 0.05$ were considered statistically significant.

**Figure S1. Phase contrast photographs of NT-hESC lines and expression of characteristic cell surface markers**. The NT-hESC-2 and -3 (A) expressed another cell surface markers for SSEA-3 and TRA-160. The NT-hESC-4 to -11 display similar morphology to that reported previously for hSCNT-ES-1 and IVF-derived hES cells (B and C, top panels) and expressed cell surface markers including alkaline phosphatase (AP), SSEA-

3, SSEA-4, TRA-1-60, TRA-1-81, and Oct-4, but not SSEA-1 (B and C). Magnification = x 100. Scale bars = 100 μm.

**Figure S2. DNA fingerprinting analysis of NT-hESCs.** (A) DNA fingerprinting analysis of the remaining eight NT-hESCs cells (-5 to –12) demonstrates genetic identities with donor patient nuclei (DNA fingerprinting of NT-hESC-2 through NT-hESC-4 are shown in paper's Fig. 2A. Isogenic analysis in loci amelogenin (chromosome location: X: p22.1-22.3, Y: p11.2); D5S818 (chromosome location: 5p22-31); FGA (chromosome location: 4q28). The boxed numbers and corresponding peaks represent locations of polymorphisms for each short tandem repeat marker; loci amelogenin (peak: X, Y); D5S818 (peak no.: 9-13); FGA (peak no.: 18-26). (B) Isogenic analysis in loci; D19S433 (chromosome location: 19q12); vWA (chromosome location: 12p12-pter); TPOX (chromosome location: 2p23-2per); D18S51 (chromosome location: 18q21.2-21.3). (C) Isogenic analysis in loci; D3S1358 (chromosome location: 3p); THO1 (chromosome location: 11p15.5); D13S317 (chromosome location: 13q22-31); D16S539 (chromosome location: 16q24-qter); D2S1338 (chromosome location: 2q35-36). (D) Isogenic analysis in loci; D8S1179 (chromosome location: 8q23-24); D21S11 (chromosome location: 21q11.2-22.1); D7S820 (chromosome location: 7q11.21-22); CSF1PO (chromosome location: 5q33.3-34). The boxed numbers and corresponding peaks represent locations of polymorphisms for each short tandem repeat marker; loci D19S433 (peak no.: 12-16.2); vWA (peak no.: 14-20); TPOX (peak no.: 8-12); D18S51 (peak no.: 13-22); D3S1358 (peak no.: 14 to 17); THO1 (peak no.: 6-9); D13S317 (peak no.: 8-14); D16S539 (peak no.: 9-12); D2S1338 (peak no.: 17 -27); D8S1179 (peak no.: 10-16); D21S11 (peak no.: 28-32.2); D7S820 (peak no.: 8-12); CSF1PO (peak no.: 8-13).

**Figure S3. *In vitro* differentiation of embryoid bodies (EBs) generated from NT-hESC lines**. Immunohistochemical staining was performed for neurofilament (A and P), GFAP (B), S-100 (C and R), HNF-4α (D and N), CD34 (E), cytokeratin 7 (F and V), ANP (G), GATA-4 (H and W), MyoD (I and U), actin muscle (J and S), Desmin (K), MHC (L), MLC2 (M), N-CAM (O), Pax 3/7 (Q) and BMP-4 (T). The EBs differentiated into all three germ layers expressing ectodermal (A to C and O to R), mesodermal (D to H, N, V and W) and endodermal (I to M and S to U) markers. Magnification = x 200. Scale bar = 100 μm.

**Table S1. Successful Establishment of Patient-Specific Human Embryonic Stem Cell Lines After Transfer of Either Female or Male Donor Nuclei.**

| Sex of cell donors | # injected oocytes | # fused oocytes (%) | Blastocyst formation | | | Establishment of NT-hESC lines | | |
|---|---|---|---|---|---|---|---|---|
| | | | # blastocysts | % Blastocysts/ fused oocytes | # NT-hESC lines | % line/ blastocysts | Mean # injected oocytes/line | Mean # fused oocytes/line |
| XX | 46 | 30 (65.2) | 10 | 33.3 | 4 | 40.0 | 11.5 | 7.5 |
| XY | 139 | 99 (71.2) | 21 | 21.2 | 7 | 33.3 | 19.8 | 14.1 |
| Total | 185 | 129 (69.7) | 31 | 24.0 | 11 | 35.4 | 16.8 | 11.7 |

No statistically significant differences in the rates of oocyte fusion, blastocyst formation or NT-hESC derivations are observed.

**Table S2. Successful Establishment of Patient-Specific Human Embryonic Stem Cell Lines After Transfer of Nuclei Donated by Children (2-10), Younger (23-30) or Older Adults (≥31).**

| Age of cell donors | # injected oocytes | # fused oocytes (%) | Blastocyst formation | | | Establishment of NT-hESC lines | | |
|---|---|---|---|---|---|---|---|---|
| | | | # blastocysts | % Blastocysts/ fused oocytes | # NT-hESC lines | % lines/ blastocysts | Mean # injected oocytes/line | Mean # fused oocytes/ line |
| ≤10 | 35 | 18 (51.4)[a] | 6 | 33.3 | 3 | 50.0 | 11.7 | 6.0 |
| 23-30 | 63 | 45 (71.4)[b] | 9 | 20.0 | 3 | 33.3 | 21.0 | 15.0 |
| ≥31 | 87 | 66 (75.8)[b] | 16 | 24.2 | 5 | 31.2 | 17.4 | 13.2 |
| Total | 185 | 129 (69.7) | 31 | 24.0 | 11 | 35.4 | 16.8 | 11.7 |

[ab] Within the same column, values with different superscripts were significantly different (P<0.01).

No statistically significant differences in rates of blastocyst formation or NT-hESC derivations are observed.

**Table S3. Effect of Age of Oocyte Donors on Establishment of Patient-Specific Human NT-hESC lines.**

| Age of oocyte donors (#) | # injected oocytes | # fused oocytes (%) | Blastocyst formation | | | Establishment of NT-hESC lines | | |
|---|---|---|---|---|---|---|---|---|
| | | | # blastocysts | % Blastocysts /fused oocytes | # NT-ESC lines | % lines/ blastocysts | Mean # injected oocytes/line | Mean # fused oocytes/line |
| <30 (10) | 125 | 90 (72.0) | 22 | 24.4 | 9 | 40.9 | 13.9 | 10.0 |
| ≥30 (8) | 60 | 39 (65.0) | 9 | 23.0 | 2 | 22.2 | 30.0 | 19.5 |
| Total | 185 | 129 (69.7) | 31 | 24.0 | 11 | 35.4 | 16.8 | 11.7 |

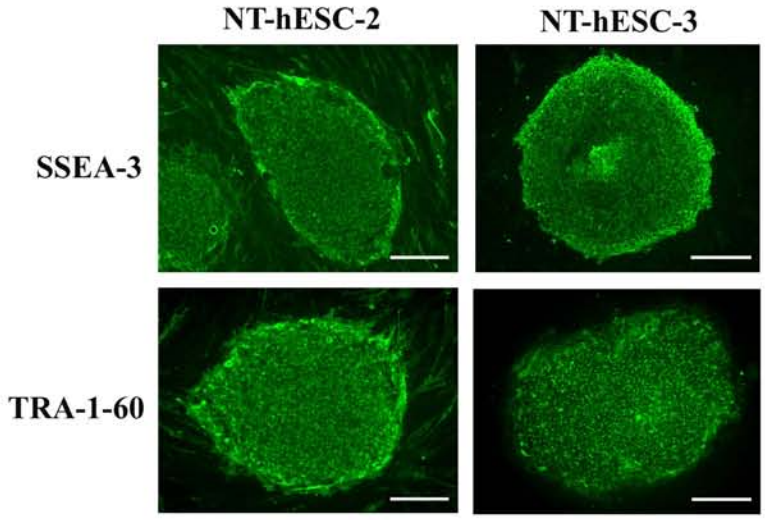
While the number of NT-hESC lines/blastocysts (%) might seem to differ (40.9% vs. 22.2%), they are not yet statistically different due to different number of blastocysts (22 vs. 9).

**Table S-IV. Identical Matches of MHC-HLA Isotypes among NT-hESC lines –5 to –12 with Donors. A, B, C, DRB and DQB represent gene locus. *, indicates genotyping analysis.**

| | MHC-I | | | MHC-II | |
|---|---|---|---|---|---|
| | HLA-A | HLA-B | HLA-C | HLA-DRB | HLA-DQB |
| Donor 5 (♂) | A*02 | B*60 | Cw*03 | DRB1*09 | DQB1*0303 |
| | A*11 | B*62 | Cw*08 | DRB1*15 | DQB1*0602 |
| NT-hESC-5 | A*02 | B*60 | Cw*03 | DRB1*09 | DQB1*0303 |
| | A*11 | B*62 | Cw*08 | DRB1*15 | DQB1*0602 |
| Donor 6 (♀) | A*02 | B*44 | Cw*01 | DRB1*07 | DQB1*02 |
| | A*33 | B*62 | Cw*07 | DRB1*15 | DQB1*0602 |
| NT-hESC-6 | A*02 | B*44 | Cw*01 | DRB1*07 | DQB1*02 |
| | A*33 | B*62 | Cw*07 | DRB1*15 | DQB1*0602 |
| Donor 7 (♀) | A*02 | B*44 | Cw*01 | DRB1*07 | DQB1*02 |
| | A*33 | B*62 | Cw*07 | DRB1*15 | DQB1*0602 |
| NT-hESC-7 | A*02 | B*44 | Cw*01 | DRB1*07 | DQB1*02 |
| | A*33 | B*62 | Cw*07 | DRB1*15 | DQB1*0602 |
| Donor 8 (♀) | A*11 | B*35 | Cw*01 | DRB1*12 | DQB1*0302 |
| | A*24 | B*46 | Cw*03 | DRB1*15 | DQB1*0602 |
| NT-hESC-8 | A*11 | B*35 | Cw*01 | DRB1*12 | DQB1*0302 |
| | A*24 | B*46 | Cw*03 | DRB1*15 | DQB1*0602 |
| Donor 9 (♂) | A*02 | B*39 | Cw*07 | DRB1*09 | DQB1*0301 |
| | A*31 | B*51 | Cw*14 | DRB1*14 | DQB1*0303 |
| NT-hESC-9 | A*02 | B*39 | Cw*07 | DRB1*09 | DQB1*0301 |
| | A*31 | B*51 | Cw*14 | DRB1*14 | DQB1*0303 |
| Donor 10 (♂) | A*26 | B*46 | Cw*01 | DRB1*04 | DQB1*0302 |
| | A*26 | B*61 | Cw*03 | DRB1*09 | DQB1*0303 |
| NT-hESC-10 | A*26 | B*46 | Cw*01 | DRB1*04 | DQB1*0302 |
| | A*26 | B*61 | Cw*03 | DRB1*09 | DQB1*0303 |
| Donor 11 (♂) | A*24 | B*46 | Cw*01 | DRB1*08 | DQB1*0303 |
| | A*24 | B*51 | Cw*14 | DRB1*12 | DQB1*0601 |
| NT-hESC-11 | A*24 | B*46 | Cw*01 | DRB1*08 | DQB1*0303 |
| | A*24 | B*51 | Cw*14 | DRB1*12 | DQB1*0601 |
| Donor 12 (♂) | A*02 | B*35 | Cw*03 | DRB1*04 | DQB1*0302 |
| | A*24 | B*62 | Cw*04 | DRB1*14 | DQB1*0503 |
| NT-hESC-12 | A*02 | B*35 | Cw*03 | DRB1*04 | DQB1*0302 |
| | A*24 | B*62 | Cw*04 | DRB1*14 | DQB1*0503 |

## References


1. J. Kwun *et al.*, *Mol. Reprod. Dev.* **65**, 167 (2003).
2. W. S. Hwang *et al.*, *Science* **303**, 1669 (2004) Supporting Online Material (www.sciencemag.org).
3. *"Guidelines for Human Embryonic Stem Cell Research"*, published by the National Research Council Institute of Medicine, of the National Academies (2005). Available online at http://www.nap.edu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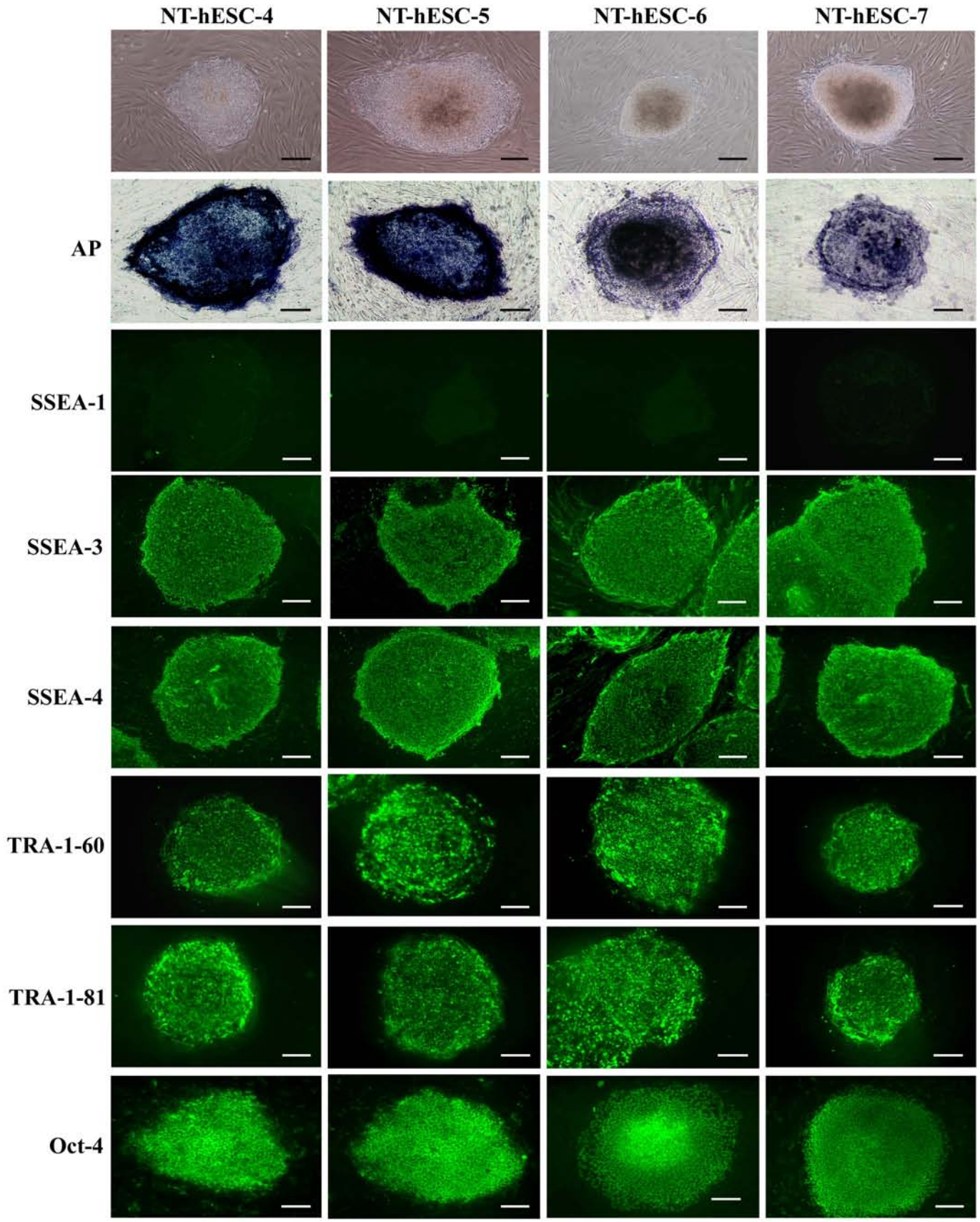
SSEA-3
TRA-1-60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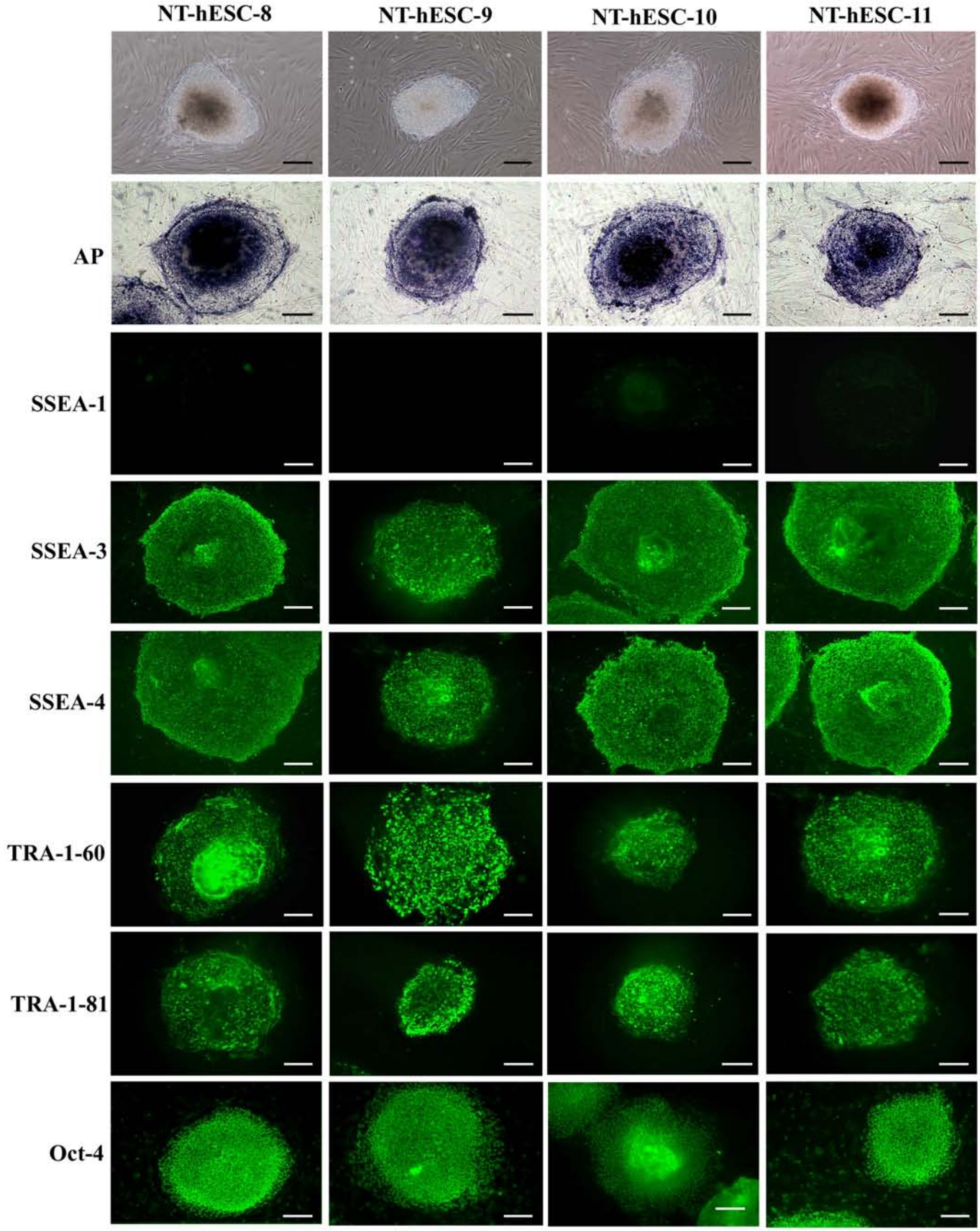
TRA-1-81
Oct-4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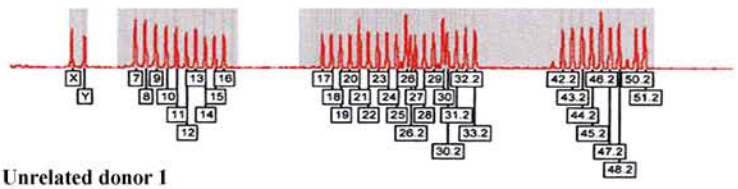
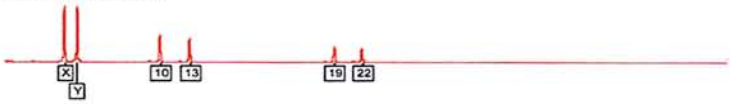



Unrelated donor 1


Unrelated donor 2




Unrelated donor 3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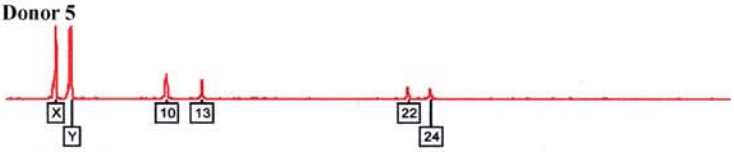
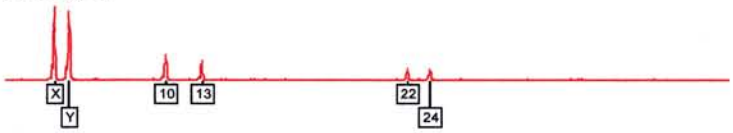
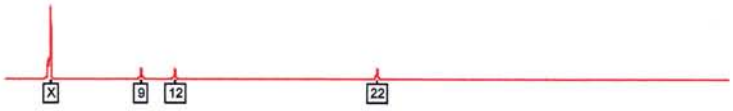



NT-hESC-5




Donor 6


NT-hESC-6

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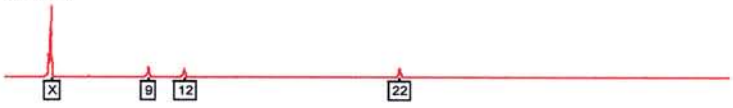


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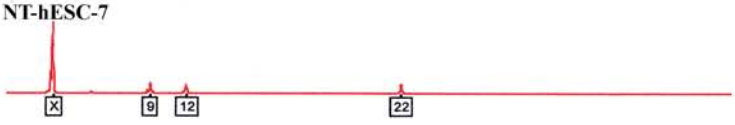
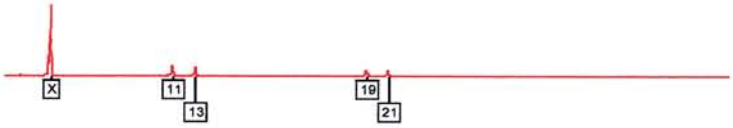
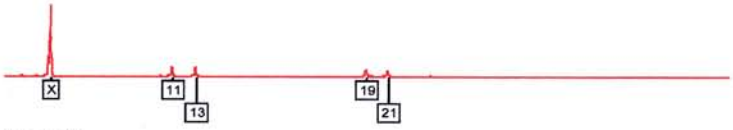



Donor 8




NT-hESC-8

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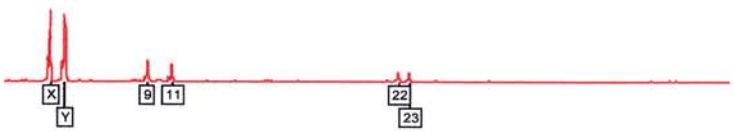


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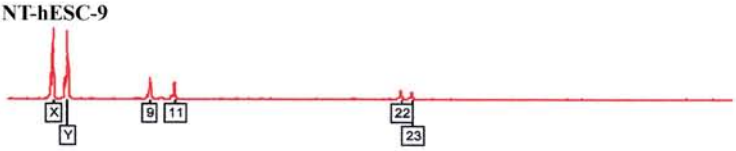


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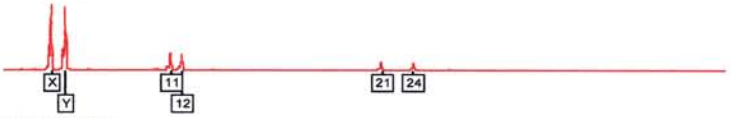
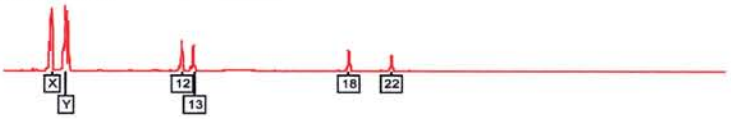



NT-hESC-10


Donor 11


NT-hESC-11

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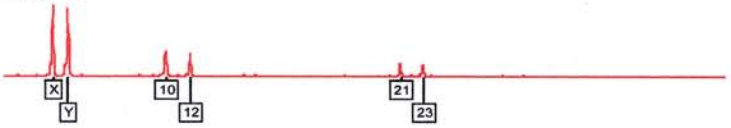


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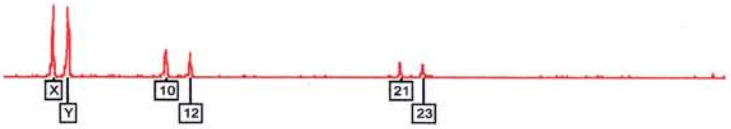

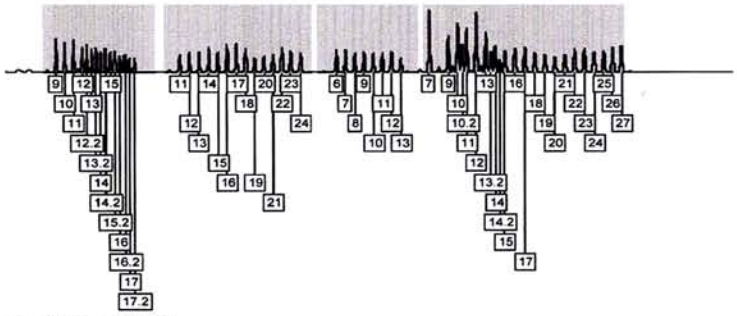

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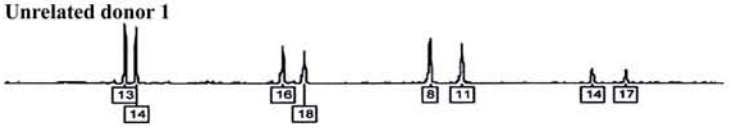

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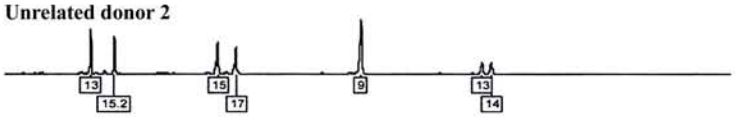

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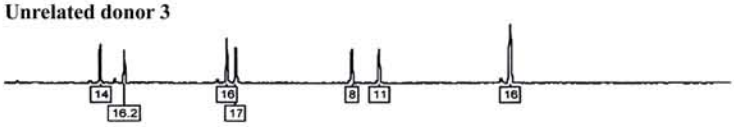

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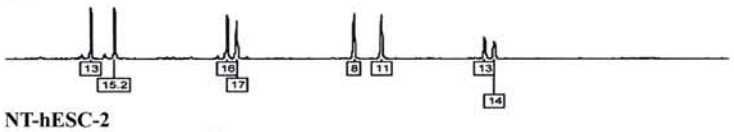

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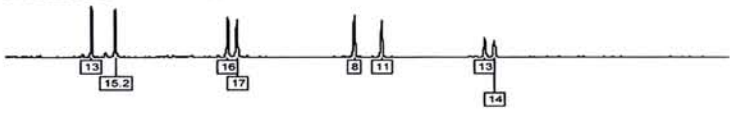

Donor 3

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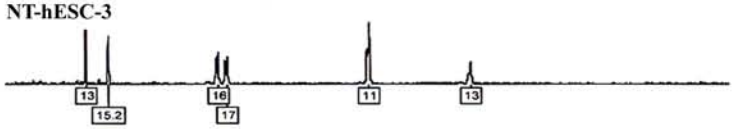

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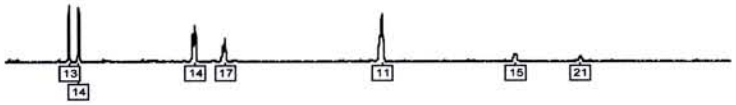

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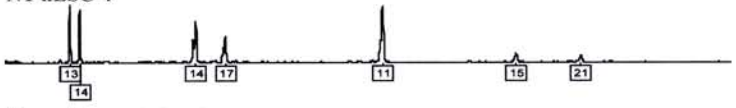

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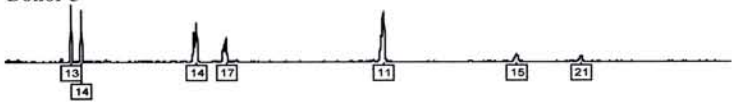

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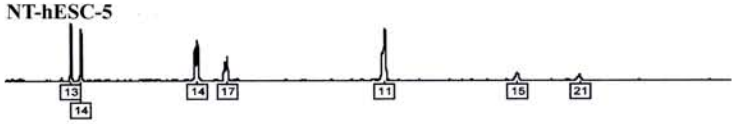

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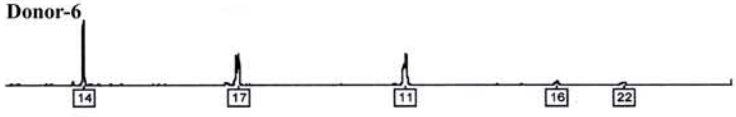

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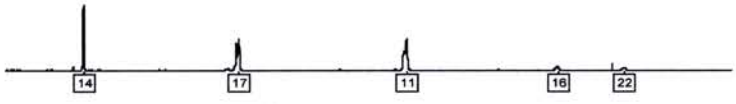

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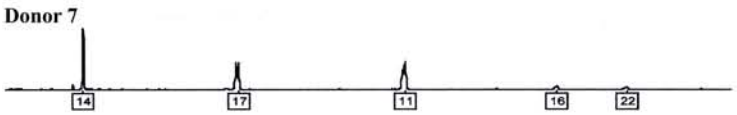

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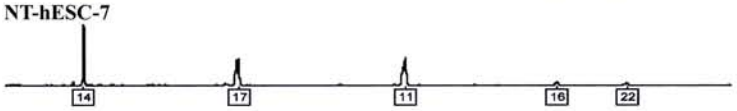
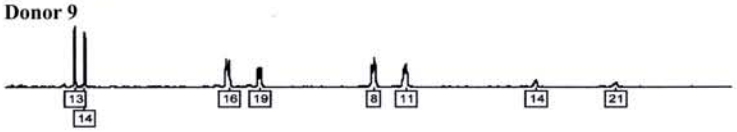


Donor 8

NT-hESC-8

Donor 9



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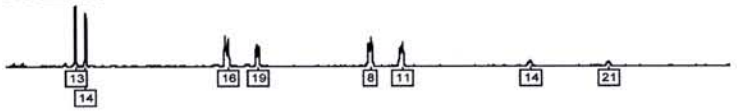

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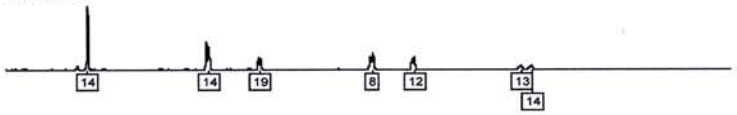

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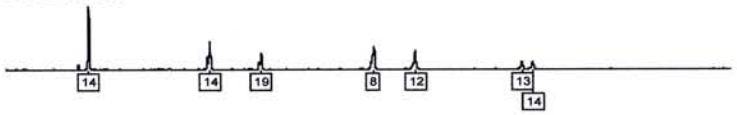

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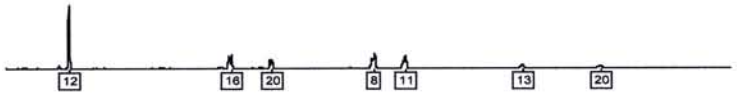

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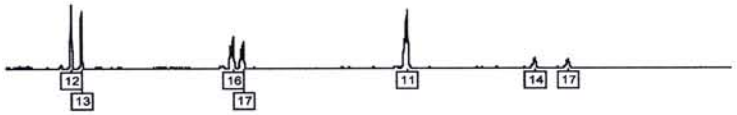

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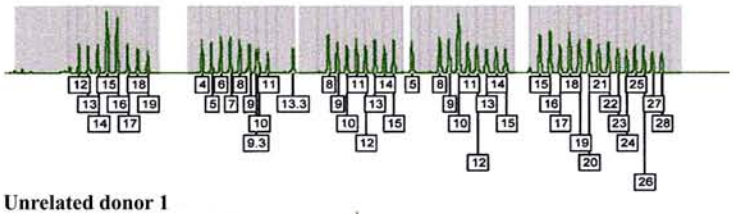

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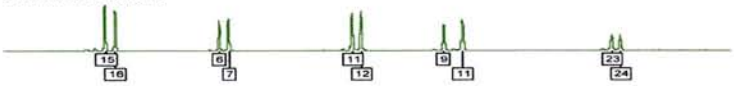

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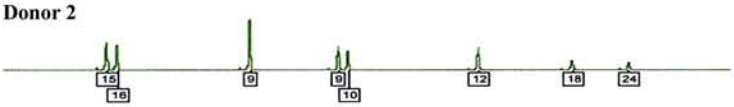

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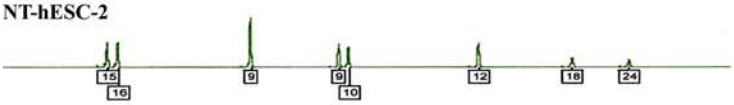

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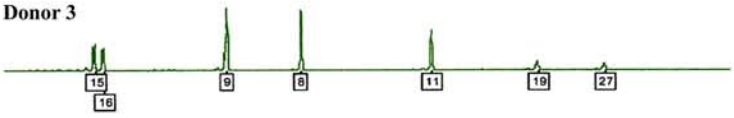

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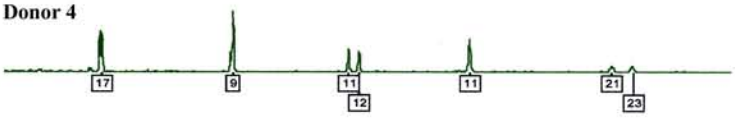

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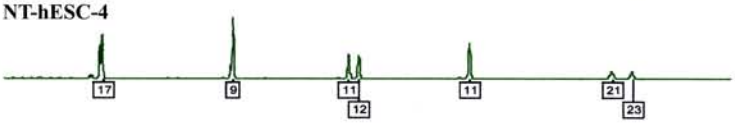

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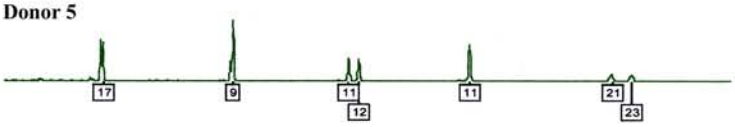

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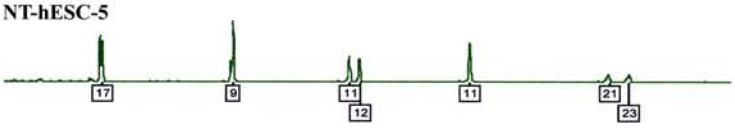

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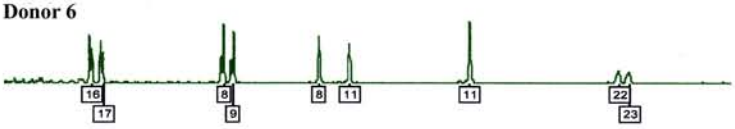

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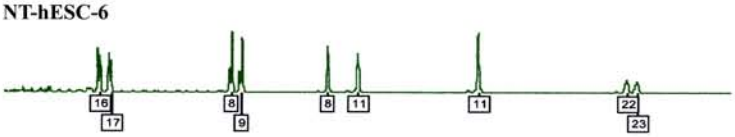

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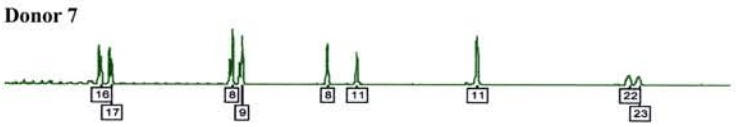

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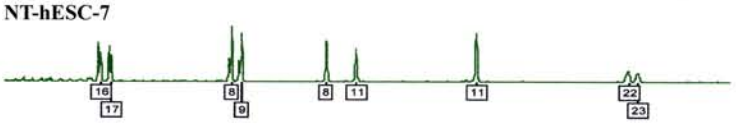

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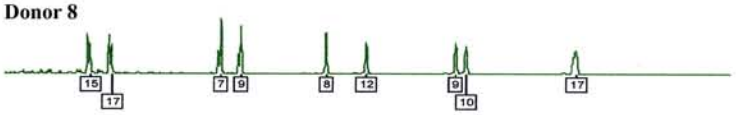

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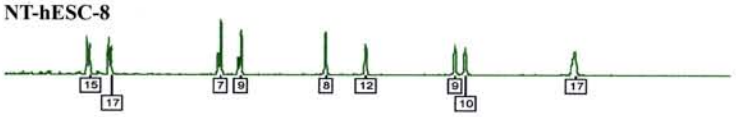

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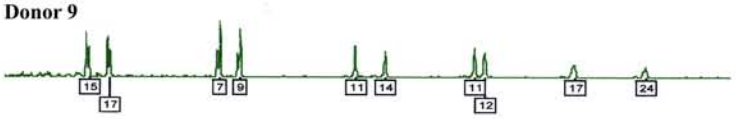

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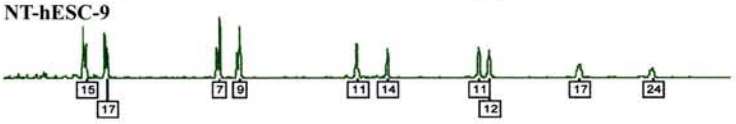

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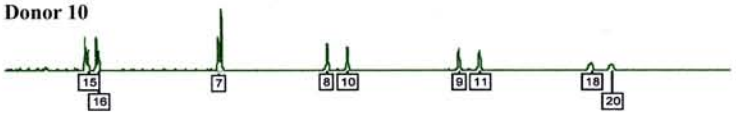

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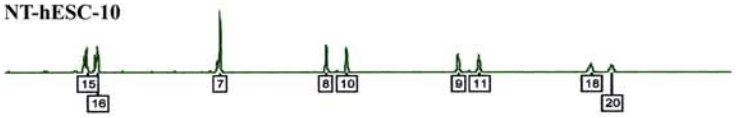

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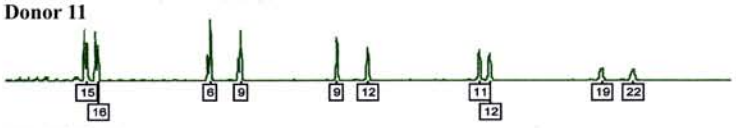

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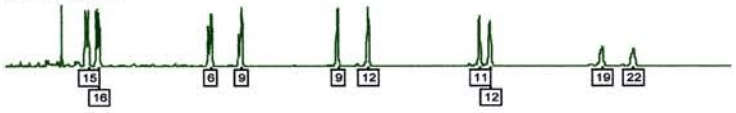

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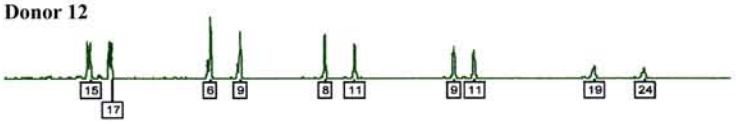

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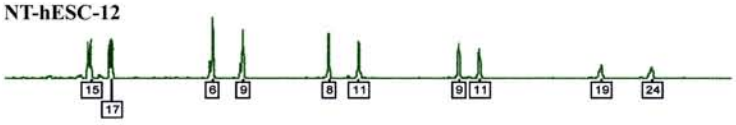

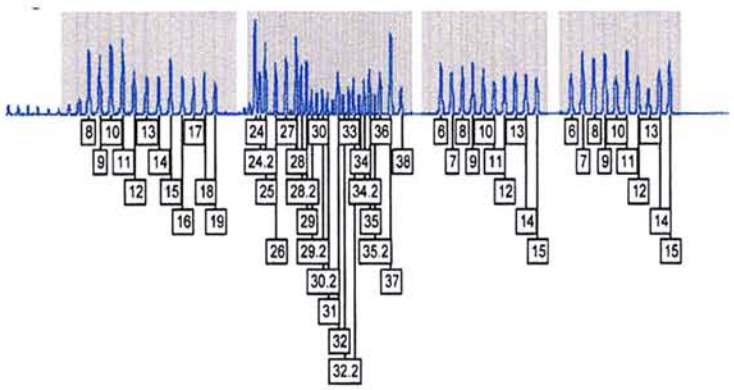

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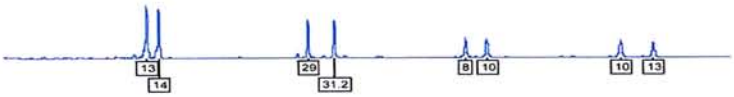

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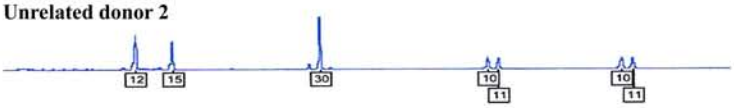

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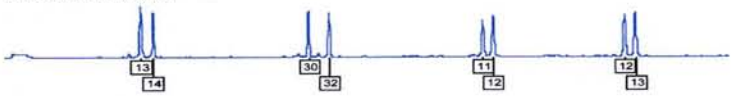

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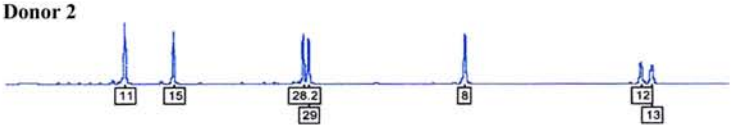

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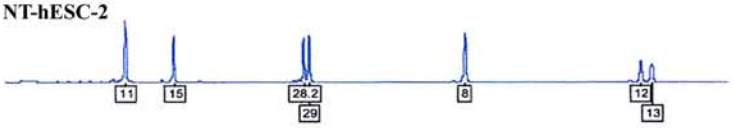
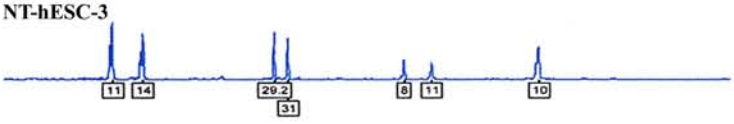


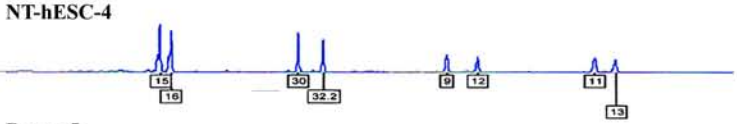
Donor 3

NT-hESC-3

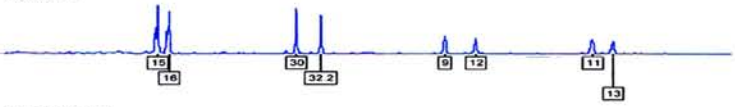


Donor 4

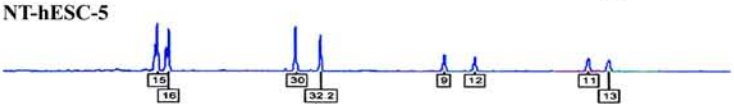
NT-hESC-4



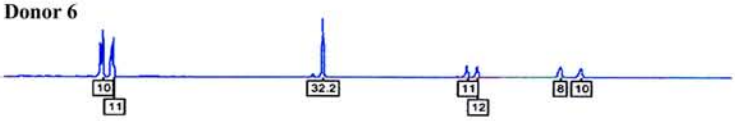
Donor 5



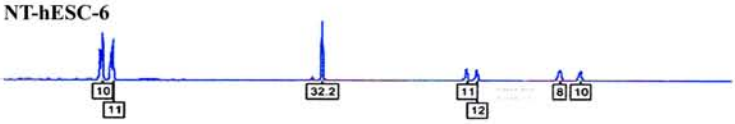
NT-hESC-5



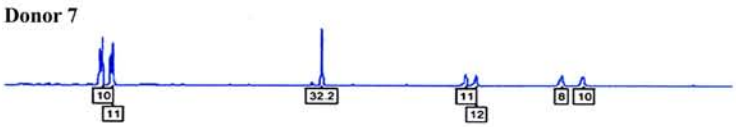
Donor 6



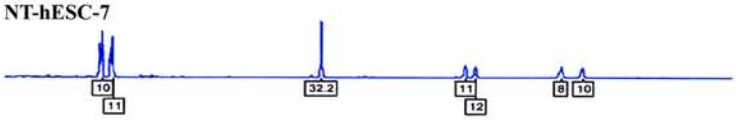
NT-hESC-6



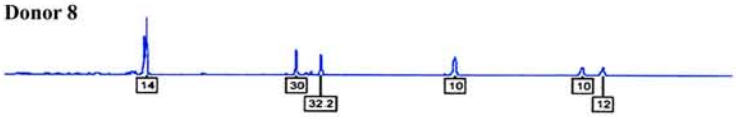
Donor 7



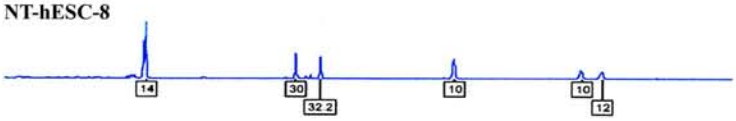
NT-hESC-7



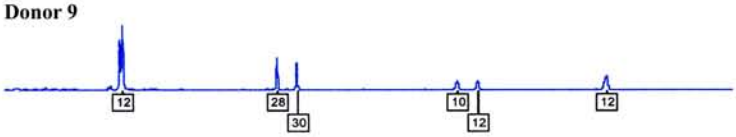
Donor 8



NT-hESC-8



Donor 9



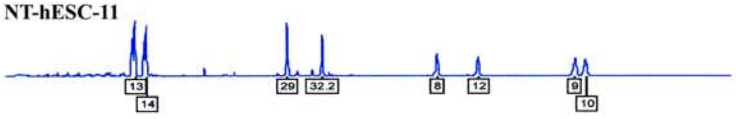
NT-hESC-9

Donor 10

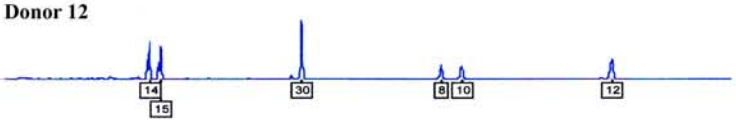
NT-hESC-10

Donor 11

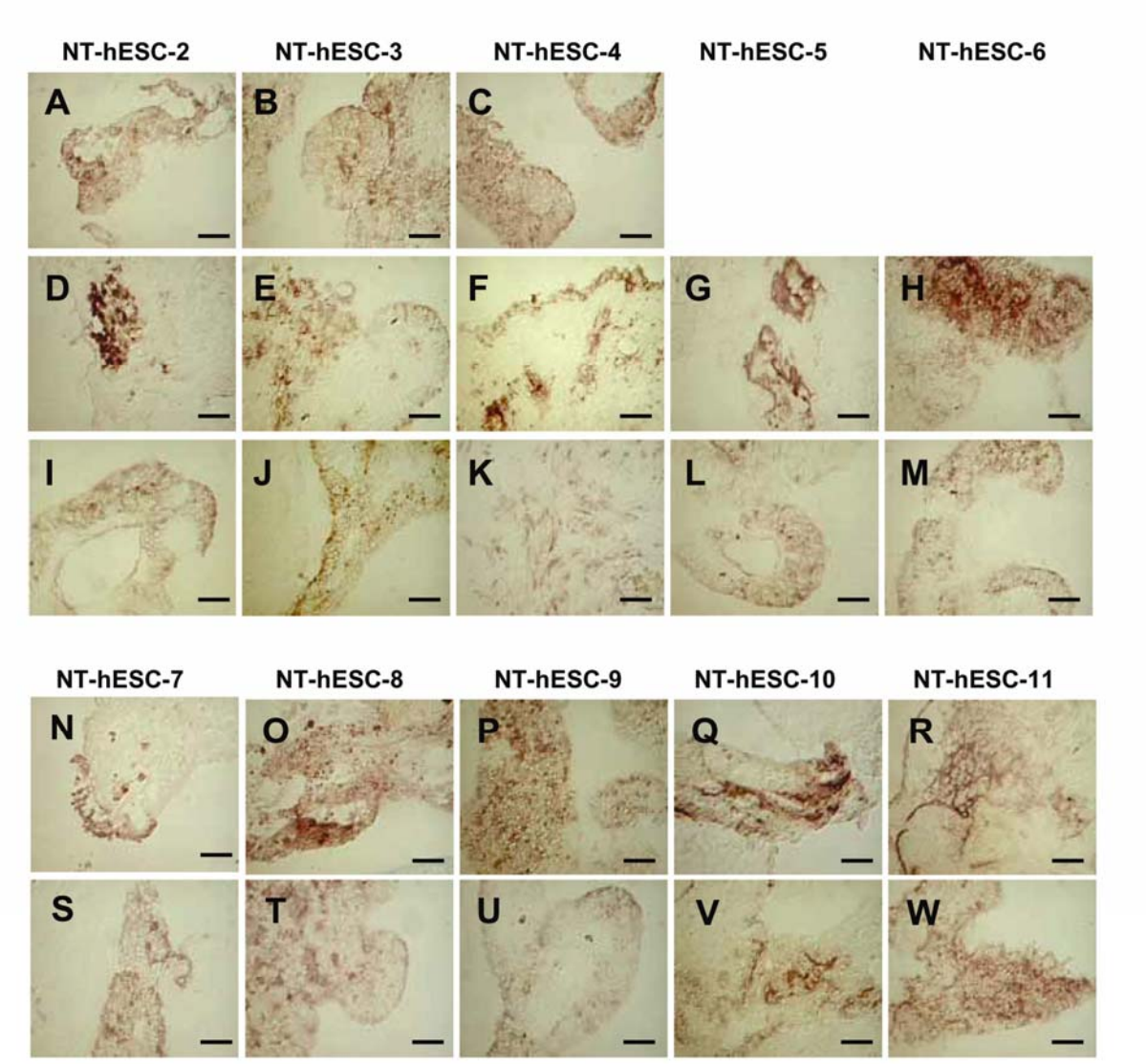
NT-hESC-11



Donor 12



NT-hESC-12


NT-hESC-2
NT-hESC-3
NT-hESC-4
NT-hESC-5
NT-hESC-6
A
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NT-hESC-7
NT-hESC-8
NT-hESC-9
NT-hESC-10
NT-hESC-11
N
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**Figure S3**

The IRB approvals and informed consent forms are provided in the original Korean and as English versions, translated in Korea under the Korean authors' direction. In cases of ambiguity, the original Korean document should be consulted.

# 임상연구위원회 연구계획 심의결과 통지서

| | |
|---|---|
| 연구 과제명 | 체세포 핵이식 기술을 이용한 치료복제에 의한 줄기세포주 수립 및 분화연구 |
| 연구 책임자 | 황 윤 영교수 |
| 연구 의뢰자 | 한양대학교 의과대학 산부인과 |
| 위 원 회 결 의 사 항 | 원안대로 통과 |

상기 임상시험을 본 위원회의 내규에 따라 승인함.

2003년 02월 10일

한 양 대 학 교 병 원 장

# Approval for the study from the Institutional Review Board on Human Subjects Research and Ethics Committees

| | |
|---|---|
| Research Title | Research on the establishment and differentiation of pluripotent human embryonic stem cells by somatic cell nuclear transfer |
| Director of the research | Hwang, Youn Young, M.D. Associate Professor |
| Institute for the Research | Department of Obstetrics & Gynecology, Hanyang University Hospital |
| Decision from the Committee | Approval of the original protocol |

Above mentioned proposed research has been approved in accordance with the IRB bylaws.

10 February 2003

Chairman, Institutional Review Board
of Hanyang University Hospital

*I verify that this english document is the exact translation of the original Korean document.*

*14 April 2005*

*Moon-il Park, M.D.*
*Chairman of IRB, Hanyang University Hospital*

# 임상연구위원회 연구계획 심의결과 통지서

| | |
|---|---|
| 연구 과제명 | 체세포 핵이식 기술을 이용한 치료목적에 의한 줄기 세포주 수립 및 분화연구 (2단계) |
| 연구 책임자 | 부교수 황 정 혜 |
| 연구 의뢰처 | 한양대학교 의과대학 산부인과 |
| 위 원 회 결 의 사 항 | 원안대로통과 |

상기 임상시험을 본 위원회의 내규에 따라 승인함.

2004년 10월 19일

한 양 대 학 교 병 원 장

# Approval for the study from the Institutional Review Board on Human Subjects Research and Ethics Committees

| | |
|---|---|
| Research Title | Research on the establishment and differentiation of pluripotent human embryonic stem cells by somatic cell nuclear transfer |
| Director of the research | Hwang, Jung Hye, M.D. Associate Professor |
| Institute for the Research | Department of Obstetrics & Gynecology, Hanyang University Hospital |
| Decision from the Committee | Approval of the original protocol |

Above mentioned proposed research has been approved in accordance with the IRB bylaws.

19 October 2004

Chairman, Institutional Review Board
of Hanyang University Hospital

*I verify that this english document is the exact translation of the original Korean document.*

*14 April 2005*

*Moon-il Park, M.D.*
*Chairman of IRB, Hanyang University Hospital*

# 연구윤리심의위원회 연구계획 심의결과 통지서

| | |
|---|---|
| 연구과제명 | 체세포 핵이식 기술을 이용한 치료복제에 의한<br>줄기세포주의 수립, 분화 연구 및 전임상 연구 |
| 연구책임자 | 황 우 석 교수 |
| 연구의뢰자 | 서울대학교 수의과대학 생물공학연구팀 |
| 위 원 회<br>결 정 사 항 | 원안대로 통과 |

상기 연구계획을 본 위원회의 내규에 따라 승인함

2005년 1월 25일

서울대학교 수의과대학장

| Approval for the study from the Institutional Review Board on Human Subject Research and Ethics Committee | |
|---|---|
| Title of the Study | Study of Establishment and Differentiation of Human Embryonic Stem Cell Lines by Therapeutic Cloning, and Pre-clinical Trials |
| Director of the research | Prof. Hwang, Woo Suk (DVM, PhD) |
| Institute for the Research | Department of Theriogenology and Biotechnology, College of Veterinary Medicine, Seoul National University |
| Decision from the Committee | Approve the study as proposed |

After thorough review by the IRB on Human Subject Research and Ethics Committees, this letter certifies that the proposed research is approved.

2005. 1. 25.

Dean. College of Veterinary Medicine
Seoul National University

* English translation of original Korean approved by Prof. Yong Soon Lee 01/05/25

# University of Pittsburgh
## *Institutional Review Board*

Exempt and Expedited Reviews
Christopher M. Ryan, Ph.D., Vice Chair

3500 Fifth Avenue
Suite 105
Pittsburgh, PA 15213
Phone: 412.383.1480
Fax: 412.383.1508
e-mail: irbexempt@upmc.edu


TO: Gerald Schatten

FROM: Christopher M. Ryan, Ph.D., Vice Chair

DATE: March 16, 2005

PROJECT: Study of Establishment and Differentiation of Human Embryonic Stem Cell Line by Therapeutic Cloning, and Pre-Clinical Trials

IRB Number: 0503142

The above-referenced protocol has been reviewed by the University of Pittsburgh Institutional Review Board. Based on the information provided to the IRB, this project includes no involvement of human subjects, according to the federal regulations [§46.102(f)]. That is, the investigator conducting research will not obtain data through intervention or interaction with the individual, or will not obtain identifiable private information.

CR:tmr

## Informed Consent Form for Donor Cell Donation

# 체세포 기증 동의서

| |
|---|
| 등록번호(hSCNT_ES_SCI ID): _____ |
| 본인성명:_____ |
| 주 소: _____ |
| 연 락 처: 집) _____핸드폰)_____ |

1) 본 동의서는 치료목적의 인간복제 배아 및 줄기세포 생산을 위해 연구용을 체세포를 제공하는 데 대한 동의서입니다.
2) 본 체세포 기증은 전적으로 자의에 의한 정상적 판단에 따른 제공으로 어떠한 강요도 없었습니다.
3) 본인은 체세포의 기증이 금전 등 어떠한 이해 관계도 없이 무상으로 제공됨을 주지하고 이에 동의합니다.
4) 제공된 체세포는 체세포 핵 이식을 하는 데 사용될 수 있으며, 그 결과 줄기세포의 추출이 가능한 배아 줄기 세포주를 확립할 수 있습니다. 연구 수행 후 적법한 절차에 의해 폐기함을 원칙으로 합니다.
5) 본인은 기증된 체세포를 이용한 연구 및 결과물에 대하여 지적 재산권 또는 여타의 부가 가치가 창출될 수도 있음을 설명 들었으며, 그것은 결코 본인의 권리가 아님을 인정하고, 향후 이에 대한 권리를 주장할 수 없음을 인정합니다.
6) 본인은 본인에 관한 인명 및 신상 정보 등 일체의 개인 정보가 보호됨을 설명 들었습니다.
7) 본인은 체세포의 채취 전에는 언제든지 체세포의 기증을 취소할 수 있음을 설명 들었습니다.

본인은 이상의 내용을 충분히 인지하고 기증된 체세포를 연구 목적으로 제공하는데 동의합니다.

200 년 월 일

| | |
|---|---|
| 본 인: _____(서명/인) | 담당 주치의:_____ (서명/인) |
| 대리인:_____(서명/인) | 소속: |

# Informed Consent Form

## Informed Consent for Somatic Cell Donation

| |
|---|
| No. of Registration (NT-hESC_SCI ID): _____ |
| Name of donor: _____ |
| Address: _____ |
| Contact: (H) _____ (Cell)_____ |

1) This informed consent form is to certify the volunteer subject's willful consent to donate human somatic cells with intention to produce human cloned embryos and embryonic stem cells for the purpose of researching their potential therapeutic applications.

2) I confirm that the donation of my somatic cells for participation in this research is of my own will and entirely voluntary. No one has forced, persuaded, or recommended my participation.

3) I acknowledge and confirm that my participation in this research and donation of my somatic cells is free of element of any financial reward or conflict-of-interest**.

4) I acknowledge and confirm that somatic cells donated herein can be used for the transferring the cellular nucleus material to oocytes. I acknowledge that unused donated cells will be destroyed according to the matter as defined by the Korean legal code***.

5) I acknowledge that the potential benefits/commercial value, including the intellectual property, of the outcome from the research was fully explained to me. I hereby agree that I do not/will not reserve, file, or claim any right on the outcome of the research activities resulting from my donation.

6) I acknowledge that the act of this donation as well as my private information are entirely protected and will not be disclosed or divulged under any circumstances.

7) I acknowledge and confirm that I reserve my right to refuse to participate, redraw from the study, cancel the intention of donation at any time prior to the actual donation without any penalty or loss of benefits, which otherwise I am entitled to.

I confirm that I have read this consent form. All my questions have been answered including the alternatives to my participation in this research. My signature below indicates my willingness to participate in this research and authorization to use and share the donated somatic cells for research.

Date:

| | |
|---|---|
| Donor:_____ signature | Physician in charge: _____ signature |
| Study Representative:_____ signature | Title: |

Biomedical and Biotechnology Research Team,
Seoul National University

# Informed Consent Form for Oocyte Donation

# 난자 기증 동의서 (기증자가 환자와 혈연관계가 없을 때)

1. 본 동의서는 치료 목적의 줄기세포 생산을 위해 연구용으로 난자를 제공하는 데 대한 동의서입니다.

2. 본 난자기증은 전적으로 자의에 의한 정상적 판단에 따른 기증으로 어떠한 강요도 없었습니다.

3. 본인은 난자의 기증이 금전 등 어떠한 이해 관계 없이 무상으로 제공됨을 주지하고 이에 동의합니다. 단, 난자 기증을 위하여 소요된 왕복 교통비, 시술비 등 실비에 한하여 제공될 수 있음을 설명 들었습니다.

4. 본인이 기증하는 난자는 본인과 혈연 관계가 없으며 서로 알지 못하는 환자에게 사용되도록 순수하게 기탁함을 서약합니다.

5. 본인은 난자 기증 시 필요한 수술 및 과정에 대해 충분한 설명을 들었으며 그에 따른 합병증 가능성에 대해서도 충분히 숙지하고 있습니다. 연구수행 후 적법한 절차에 의해 폐기함을 원칙으로 합니다.

6. 본인은 기증된 난자를 이용한 연구 및 결과물에 대하여 지적 재산권 또는 여타의 부가가치가 창출될 수도 있음을 설명 들었으며, 그것은 결코 본인의 권리가 아님을 인정하고, 향후 이에 대한 권리를 주장할 수 없음을 인정합니다.

7. 본인은 본인에 관한 인명 및 신상 정보 등 일체의 개인 정보가 보호됨을 설명 들었습니다.

8. 본인은 난자의 채취 전에는 언제든지 난자의 기증을 취소할 수 있음을 설명 들었습니다.

본인은 이상의 내용을 충분히 인지하고 기증된 난자를 연구 목적으로 제공하는데 동의합니다.

200 년 월 일

| | |
|---|---|
| 본 인: _____ (서명/인) 주민등록 번호<br>주소: | |
| 소속 IRB 위원<br><br>_____(서명/인) | 소속 연구원<br><br>_____(서명/인) |

# Informed Consent Form for Oocyte Donation

# 난자 기증 동의서 (기증자가 환자의 가족일 때)

1. 본 동의서는 치료 목적의 줄기세포 생산을 위해 연구용으로 난자를 제공하는 데 대한 동의서입니다.

2. 본 난자기증은 전적으로 자의에 의한 정상적 판단에 따른 기증으로 어떠한 강요도 없었습니다.

3. 본인은 난자의 기증이 금전 등 어떠한 이해 관계 없이 무상으로 제공됨을 주지하고 이에 동의합니다.

4. 본인이 기증하는 난자는 본인과 가족 관계에 있는 환자에게 우선적으로 사용하고, 사용 후 남는 난자에 대해서는 혈연관계도 없으며 서로 알지 못하는 다른 환자에게 사용되도록 순수하게 기탁함을 서약합니다.

5. 본인은 난자 기증 시 필요한 수술 및 과정에 대해 충분한 설명을 들었으며 그에 따른 합병증 가능성에 대해서도 충분히 숙지하고 있습니다. 연구 수행 후 적법한 절차에 의해 폐기됨을 원칙으로 합니다.

6. 본인은 기증된 난자를 이용한 연구 및 결과물에 대하여 지적 재산권 또는 여타의 부가가치가 창출될 수도 있음을 설명 들었으며, 그것은 결코 본인의 권리가 아님을 인정하고, 향후 이에 대한 권리를 주장할 수 없음을 인정합니다.

7. 본인은 본인에 관한 인명 및 신상 정보 등 일체의 개인 정보가 보호됨을 설명 들었습니다.

8. 본인은 난자의 채취 전에는 언제든지 난자의 기증을 취소할 수 있음을 설명 들었습니다.

본인은 이상의 내용을 충분히 인지하고 기증된 난자를 연구 목적으로 제공하는데 동의합니다.

200 년 월 일

| | |
|---|---|
| 본 인: _____ (서명/인) 주민등록 번호<br>주소: | |
| 소속 IRB 위원<br><br>_____(서명/인) | 소속 연구원<br><br>_____(서명/인) |

# Informed Consent Form

**Informed Consent for Oocytes* Donation**
(Without blood or familial relationship between the donor and the recipient)

1) This informed consent form is to certify the volunteer subject's willful consent to donate human oocytes with intention to produce human cloned embryos and embryonic stem cells for the purpose of researching their potential therapeutic applications.

2) I confirm that the donation of my oocytes for participation in this research is of my own will and entirely voluntary. No one has forced, persuaded, or recommended my participation.

3) I acknowledge and confirm that my participation in this research and donation of my oocytes is free of element of any financial reward or conflict-of-interest**. I was fully informed that costs incurred for the oocytes donation procedures, including cost for public transportation for the study participation, could be provided.

4) I acknowledge and confirm that oocytes donated herein can be used for the treatment of anonymous patient(s), who is (are) not related to me in any way.

5) I confirm that the purpose of this research, the study procedures including the surgical procedures for oocytes retrieval, the possible risks, and discomforts relating to this research have been fully explained to me. I acknowledge that the embryos and oocytes, after the study end point, will be destroyed according to the manner as defined by the Korean legal codes***.

6) I acknowledge that the potential benefits/commercial value, including the intellectual property, of the outcome from the research was fully explained to me. I hereby agree that I do not/will not reserve, file, or claim any right on the outcome of the research activities resulting from my donation.

7) I acknowledge that the act of this donation as well as my private information are entirely protected and will not be disclosed or divulged under any circumstances.

8) I acknowledge and confirm that I reserve my right to refuse to participate, redraw from the study, cancel the retrieval of oocytes at any time prior to the donation without any penalty or loss of benefits, which otherwise I am entitled to.

I confirm that I have read this consent form. All my questions have been answered including the alternatives to my participation in this research. My signature below indicates my willingness to participate in this research and authorization to use and share the donated oocytes for research.

Date:

| |
|---|
| Donor: _____ signature<br>Address:<br>NO. of Identification: |

| | |
|---|---|
| IRB member in charge:<br>_____ signature | Researcher in charge:<br>_____ signature |

Translation Notes
* In Korean, the word 'oocyte (s)' is preferred choice of word over 'human egg(s)', and is commonly used.
* In Korean, plurality and singularity of certain object is often omitted and understood in the contexts.
** Conflict-of-interest (COI) describes a relationship, commitment or financial interest that could results in moral hazard and breach of ethical obligations.
*** Korean Legal Code refers to the bill and code for Korean Legal Codes for Biomedical Ethics and Safety - Code 7150

Biomedical and Biotechnology Research Team,
Seoul National University

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2) I confirm that the donation of my oocytes for participation in this research is of my own will and entirely voluntary. No one has forced, persuaded, or recommended my participation.

3) I acknowledge and confirm that my participation in this research and donation of my oocytes is free of element of any financial reward or conflict-of-interest**.

4) I acknowledge and confirm that oocytes donated herein will be used for the patient(s) who is/are in blood or familial relationship to me with priority. However, any unused oocytes can be used for the treatment of anonymous patient(s), who is (are) not related to me in any way.

5) I confirm that the purpose of this research, the study procedures including the surgical procedures for oocytes retrieval, the possible risks, and discomforts relating to this research have been fully explained to me. I acknowledge that the embryos and oocytes, after the study end point, will be destroyed according to the manner as defined by the Korean legal codes***.

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