Applied StemCell

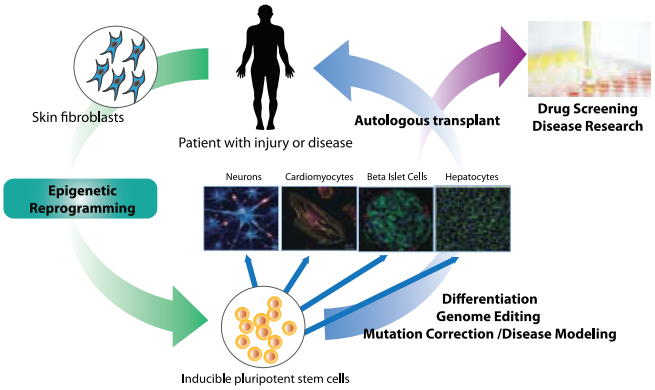
Genome editing *in vitro* and *in vivo*

iPSC Generation, Disease Modeling, Mutation Correction and Differentiation

Comprehensive suite of premium quality stem cell related services and products for affordable and efficient modeling of "disease-in-a-dish"

Why work with ASC?

- ASC is one of the earliest licensees and most experienced service provider of both the iPSC (from iPS Academia, Japan) and the CRISPR/Cas9 technology (from the Broad Institute), worldwide.
- ASC's CRISPR and iPSC disease modeling platforms have been featured in top peer-reviewed journals such as Nature, and PNAS, as well as in biotechnology magazines.
- ASC's genome editing services include engineering cell lines from healthy and diseased donors.
- iPSC genome editing portfolio includes customizable deliverables, including a full panel of pluripotency characterization and teratoma analysis.



Footprint-Free iPSC Generation Service

ASC has successfully reprogrammed hundreds of iPSCs from both healthy and disease patient samples, using its proprietary footprint-free, non-integrating protocols. Some key features include:

- Reprogramming from patient specific fibroblasts or blood cells
- No genomic footprint: suitable for drug discovery and cell therapy applications
- High reprogramming efficiency
- Safe to handle: no viral particles

Case Study: Generation of human iPSCs from dermal fibroblasts of a patient with a rare disorder. The dermal fibroblasts obtained by skin biopsy from a patient were reprogrammed into iPSC using ASC's proprietary protocols. High quality colonies were isolated and characterized by immunocytochemical staining for pluripotency markers.

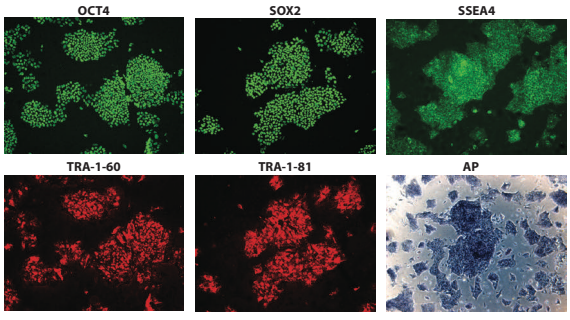


Figure. iPSC reprogrammed from patient-derived fibroblasts and cultured using ASC's proprietary protocols. The reprogrammed iPSC colonies stained positive for pluripotency markers OCT4, SOX2, SSEA4, TRA-1-60, and TRA-1-81, and alkaline phosphatase (AP).

You need to provide

- 1 x 10^6 cells of patient fibroblast or PBMC
- Pathogen-free report

You will receive

- At least 3 clones with 2 vials of each clone (> 2 x 10^5 cells/vial)
- Full report with high resolution images
- Clones are characterized by pluripotency markers
- Teratoma analysis, EB formation, qPCR, RNA-seq (optional)

Timeline: 2 months

Cell recovery and quarantine

▼

Transfection with episomal DNA vectors

▼

Colony formation

▼

Colony expansion

▼

Characterization via antibody staining and RT-PCR (Teratoma and EB analysis optional)

▼

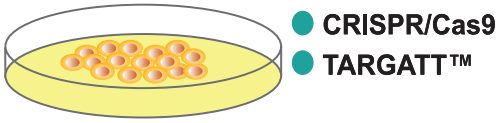
Cell Banking/further differentiation

iPSC Genome Editing/Disease Modeling Service (Knock-in, Knockout, Point Mutation)

ASC's acclaimed iPSC gene editing service provides precise engineering of iPSCs to your specifications, using highly optimized iPSC and CRISPR/Cas9 transfection conditions to achieve >90% success rate. With our unique expertise in genome engineering and stem cells, our custom iPSC engineering offers the following advantages:

- Isogenic control and disease cell lines for reliable comparison of results without genetic variability
- Unlimited resource of *in vitro* models of human genetics and diseases
- Patient-related research and drug discovery

iPSC Genetic Modification



ASC provides services under licensing agreement with the Broad Institute for CRISPR/Cas9 Gene-Editing technology and iPS Academia, Japan for iPSC technology along with ASC's proprietary TARGATT ™

You need to provide

- 1 x 10^6 cells of your iPSC or using ASC's iPSC lines
- Gene information

You will receive

- Clones with desired mutation
- Full report with publishable data

Timeline: 3-5 months

Cell line validation

↓

Targeting DNA vector construction and validation

↓

Transfection of CRISPR/Cas9 constructs

↓

Cell confirmation and expansion

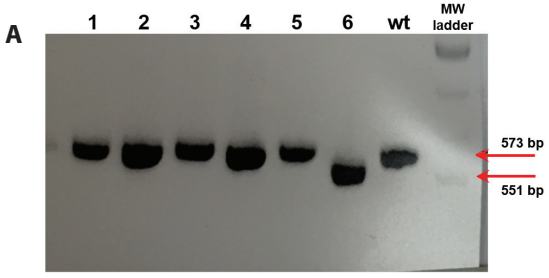
Customized Deliverables:

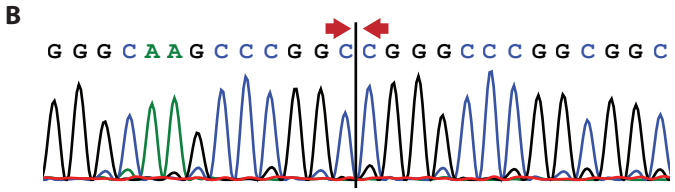
- Choice of homozygous and/or heterozygous clones
- Choice to use silent mutations
- Choice to use feeder-free or feeder-dependent protocols
- Foot-print free editing
- Gene knockout, insertion, and replacement
- Reporter gene insertion
- Gene correction using your patient-derived iPSCs, or ASC's characterized iPSCs

Case Studies

1. Knockout Mutation

Goal: To generate a frameshift knockout (KO) mutation in ASC's human iPSC cell line using CRISPR/Cas9. ASC's iPSC line was used for this study. After gRNA validation and transfection, the iPSCs were screened and potential KO iPSC colonies were subjected to genotyping PCR and sequencing.





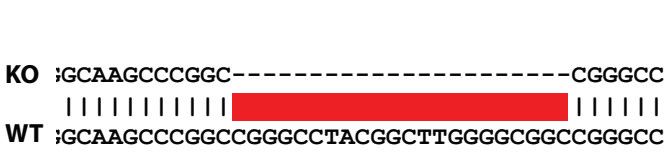


Figure 1. Genotyping analysis and sequence confirmation of KO iPSC clones. PCR amplification of either side of the gRNA cut site indicated the presence of a 551 bp PCR product with a 22 bp deletion clone #6; wildtype (WT) PCR product = 573 bp. (B) Sequence chromatogram showing deletion. (C) Sequence alignment between and wildtype.

2. Point Mutation Correction

Goal: To correct a point mutation found in a mutant allele of a gene in patient-derived iPSCs using CRISPR/Cas9.

Patient-derived iPSCs were transfected with CRISPR/Cas9 elements (Cas9/gRNA vector and single stranded oligodeoxynucleotide donor) using optimized CRISPR-iPSC protocols. After screening selection, one corrected clone was identified and confirmed by sequencing (Figure 2).

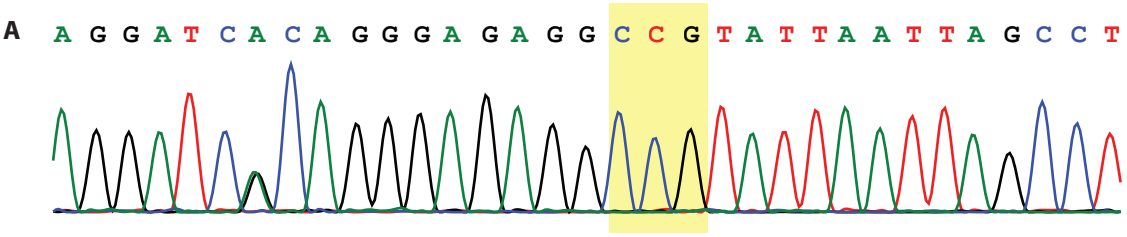


Figure 1. Sequencing result of corrected clone (CTG > CCG). Red arrow indicates the nucleotide correction from a CTG to CCG.

Figure 2. Sequencing and alignment of corrected clone (CTG > CCG). (A) The corrected codon (CCG) is highlighted by a yellow box. (B) The corrected sequence (Clone) is the top sequence while the point mutation (locus) is the bottom sequence. Two silent mutations were introduced by donor ssODN (CGC > CGT; GGG > GAG) while the corrected.

3. GFP Reporter Knock-in

Goal: To insert a GFP reporter gene into human iPSCs at a specified locus "A" using CRISPR.

Validated gRNA, Cas9 and donor vectors were co-transfected into ASC's human iPSC cell line. Colonies were isolated and screened for GFP reporter gene knock-in (Figure 3).

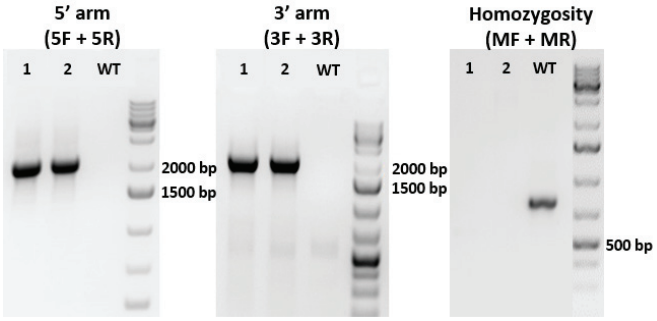


Figure 3. PCR genotype screening of GFP-in at locus "A". Three sets of PCR primers were designed to amplify PCR fragments flanking left homology region (5' arm), right homology region (3' arm), and reporter gene insertion region (M) to identify homologous clones. Both clone #1 and #2 were homozygous as attested by absence of PCR band (when compared to WT).

CRISPRCLEAR™

CRISPRCLEAR™ Kits

ASC's CRISPR iPSC Gene Editing kits are designed for your disease-specific study. The kits contain an optimized and validated set of reagents that are customized to your specifications. Our scientists will work closely with you to design the most efficient CRISPR components to be provided in the kit, thereby ensuring a fast turnaround and quality deliverables.

ASK-7020S	CRISPR Point Mutation Kit for iPSCs
ASK-7010S	CRISPR Knockout Kit for iPSCs
ASK-7030S	CRISPR Knock-in Kit for iPSCs
ASK-7040	hH11 Safe Harbor Locus Knock-in Kit
ASK-7041	hRosa26 Safe Harbor Knock-in Kit
ASK-7042	hAAVS1 Safe Harbor Knock-in Kit

Kit Contains
- gRNA plasmid (with validation report)
- Cas9-puro plasmid
- Donor DNA
- Human iPSC (from healthy tissue) - optional

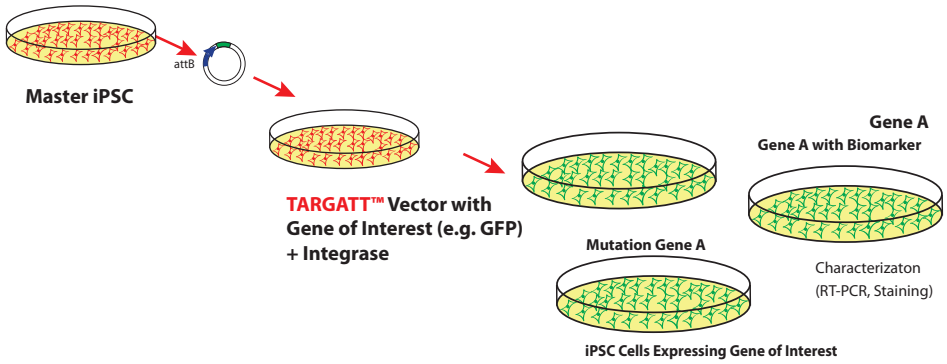
TARGATT™

iPSC Disease Cell Line Model Generation Service

Based on our proprietary site-specific TARGATT™ technology, ASC developed a human TARGATT™ iPSC line with pre-enginered attB sites for introducing gene(s) of interest into human iPS cells. The unique advantage of this system is that any gene of interest can be inserted efficiently into a defined, transcriptionally-active locus with high gene expression in a single copy fashion.

Start from iPSC with TARGATT™ site:
TARGATT™ iPSCs allow easy insertion of any gene of interest into a safe harbor locus in the genome.

Site-specific rapid knockin:
- High knock-in efficiency
- Single transgene copy
- Minimal off-target



Timeline: 3-5 months

Cell recovery and quarantine

TARGATT DNA vectors construction and validation

Transfection of TARGATT constructs

Cell confirmation and expansion

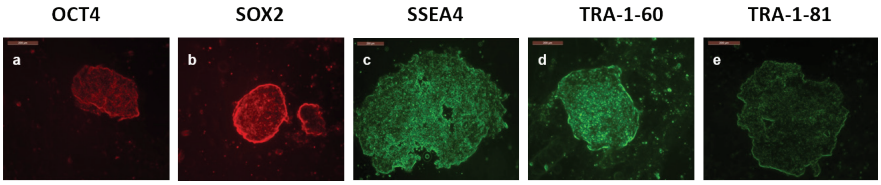


Figure 2. Staining of TARGATT™ iPSC master cell line with standard iPSC expression markers.
(a) Oct4, (b) Sox2, (c) SSEA4, (d) TRA-1- 60, (e) TRA-1-81.

You need to provide	You will receive
- Gene of interest - Project details	- Clones with desired gene knock-in - Full report with publishable results

TARGATT™ iPSC Master Cell Line & Quick Knock-in Kit

ASC's TARGATT™ iPSC Quick Knock-in Kit is a complementary kit that works with our TARGATT™ iPSC Master Cell Line to insert large fragment DNA into preselected "attP" docking sites that have been engineered into the iPSC Master Cell Line. This kit is an excellent tool for generating cell line models for gene overexperssion, and to generate reporter lines in hiPS and differentiated cells. The TARGATT™ iPSC Genotyping Kit provides a convenient method to genotype the TARGATT™ iPSC Master Cell Line or Knock-in cells generated from a Master Cell Line.

AST-1100	iPSC Master Cell Line
AST-1101	Quick knockin kit
AST-1102	Genotyping kit now available

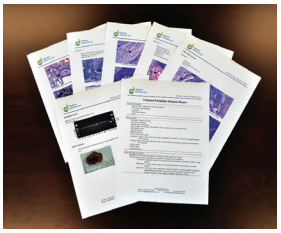
Knock-in Kit Contains
- Non-replicable PhiC31 integrase plasmid
- Positive control plasmid with GFP
- TARGATT™ cloning plasmids (with different promoters)

Genotyping Kit Contains
- DNA extraction buffers
- Two sets of genotyping PCR primers flanking integration sites and inserted fragment
- Nuclease free water
- Positive control DNA

iPSC Characterization Services

Teratoma Formation Analysis Service

Teratoma Analysis is one of the most stringent and accurate quality control assessments for stem cells, in addition to other methods to characterize stemness such as immunohistochemistry and RT-PCR detection of pluripotency markers, in vitro embryoid body formation and karyotyping. It provides a functional assessment of pluripotency of the stem cells, including iPSCs, by analyzing the ability of the cells to form all three embryonic germ cell layers when xenografted into mice. ASC's teratoma formation analysis service has >97% success rate and has been acknowledged in > 30 peer-reviewed publications.



You will need to provide

- Two T75 flasks containing cells for testing (note: total count of 3-6 x 10^6 cells for mouse iPSCs).

Timeline: 1-3 months

Cell injection
(2 sites: kidney and testis)

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Teratoma harvesting

▼

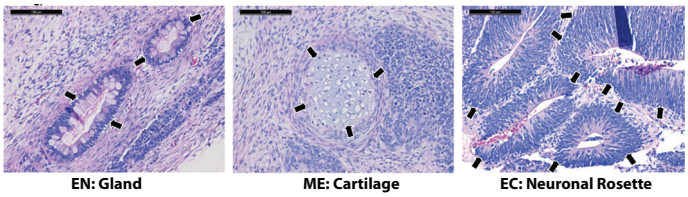
Tisuue sectioning

▼

H&E staining

▼

Histology analysis of teratoma sections



EN: Gland | ME: Cartilage | EC: Neuronal Rosette

Figure 1. Histological analyses using H&E staining of kidney and testis teratomas from mice injected with an iPSC line. Differentiated tissues representing the three germ layers are shown and indicated by arrow heads. Abbreviations: EN: endoderm; ME: mesoderm; EC: ectoderm. Magnification: 200x.

You will receive

- Complete report with histological analysis and high quality/ publication grade images of EN, ME and EC formation.
- Tissue blocks and H&E stained tissue section slides.

Success Rate

Key points	ASC's methods			Traditional methods
Cell Type	mESC/miPSC	hESC	hiPSC	hESC
Teratoma Formation Rate	100%	100%	93.7%	25-40%
Differentiation	distinctive	distinctive	distinctive	poor
Turnaround Time	3-5 weeks	5-8 weeks	10-14 weeks	12-18 weeks
Cells needed	0.5-1 million/site	0.5-2 million/site	1-2 million/site	3-5 million/site

Antibody Pluripotency Marker Staining:
- Human: OCT4, SOX2, SSEA4, TRA-1-60, TRA-1-81
- Mouse: OCT4, SOX2, SSEA1

Pluripotency & lineage-specific marker detection qPCR, RNA-seq

Karyotyping: Chromosome counting; G-banding)

Embryoid Body Formation and Characterization

Germline Transmission Evaluation

mESC Derivation Service

Custom iPSC Culture Service

iPSC Banking Service

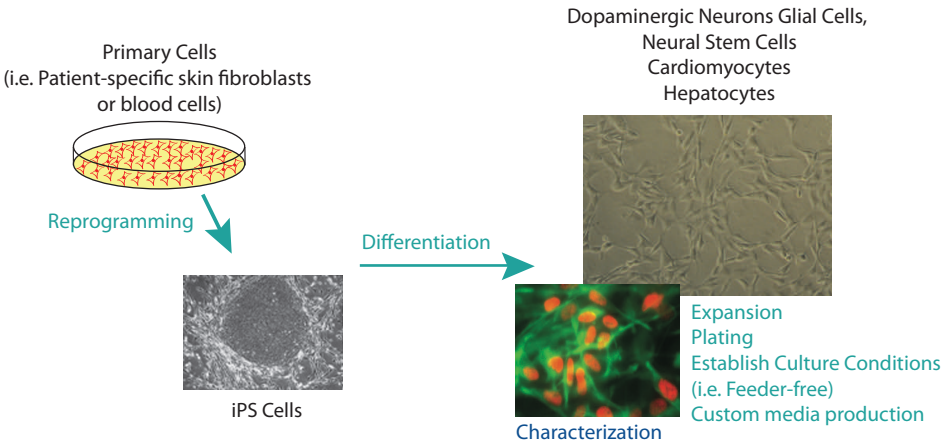
Related Services* (*additional fees may apply)

Cell Immortalization Service
- Patient fibroblasts, primary cells, and more
- Transduction, colony selection (up to 10 passages)
- Characterization (transgene expression by RT-PCR)

Vector Cloning, Virus Packaging Services
- RNAi and inducible vectors/ BAC recombineering
- Retro- and lentivirus packaging service in 10 days (regular, high, ultra-high titers)

iPSC Diifferentiation Service

ASC offers various iPSC differentiation services for ESC / iPSC differentiation into more specialized cells including multipotent stem cells and fully differentiated somatic cells. Using proprietary protocols and reagents, we can differentiate your ESCs or patient-derived or healthy iPSCs into various lineage cells: neural stem cells (NSC), neurons, cardiomyocytes, and hepatocytes



You need to provide

- Cells to be differentiated: 2 vials of frozen stocks on dry ice or 1 T25 flask of live cells per sample
- Pathogen-free report

You will receive

- Fresh or frozen cells
- Comprehensive characterization of differentiated cells
- Full report with high resolution images

Human Neural Stem Cell Differentiation Service

ASC will use proprietary protocols and optimized reagents to differentiate of your iPSCs into ready-to-use multipotent neural stem cells. Simply thaw the cells we ship and you can use them directly for your neuroscience research or further differentiate into motor neurons and/or oligodendrocytes.

- High purity (>90%) NSCs in 1 week using ASC's proprietary neural induction protocol
- Derived NSCs are proliferating cells that can be frozen and cultured over a prolonged period of time while retaining phenotype
- Derived NSCs retain phenotype under feeder-free culture conditions
- NSCs can be further differentiated into multiple neural cell types.

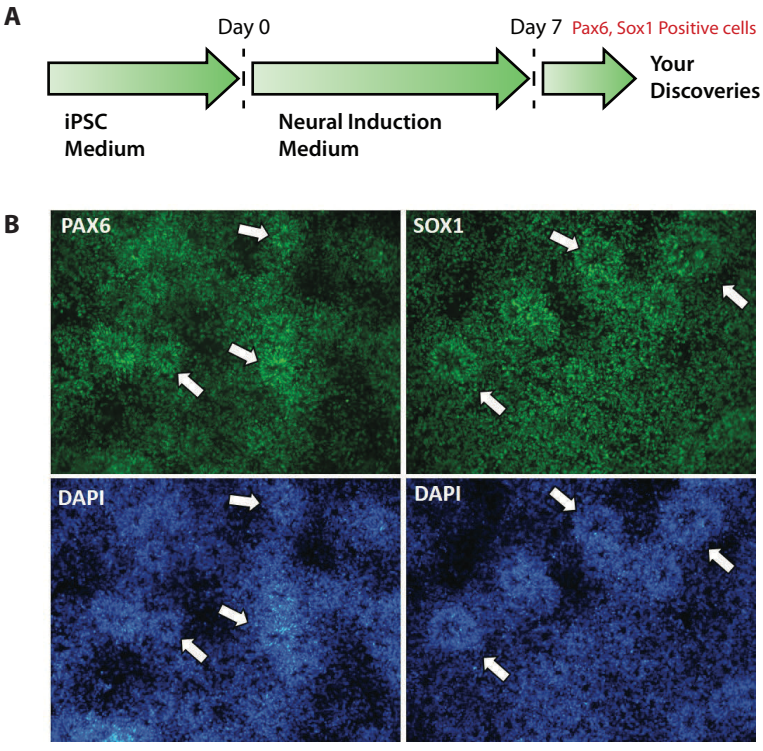


Figure. iPSC differentiation service. (A) Time line to show NSC differentiation workflow. (B) iPSCs differentiation to NSCs in feeder-free condition with high efficiency using ASC's proprietary neural induction protocol and Neural Differentiation media (ASM-4013) in < 1 week. The differentiated NSCs retain neural stem cell phenotype even after passaging and freeze-thaw under feeder-free conditions as evidenced by staining for NSC markers PAX6 and SOX2 and nuclear DNA (DAPI). Note: White arrows indicate distinctive neuronal rosettes.

NSC-related products: Neural Stem Cells and Medium

ASE-9303 Human iPSC-derived Neural Stem Cells

ASM-4013 NeuroSure™ Neural Differentiation Medium

ASM-4014 NeuroSure™ Neural Stem Cell Culture Medium

Human Cardiomyocyte Differentiation Service

ASC provides custom service to differentiate iPSCs into functional cardiomyocytes from your iPSCs directly, or after re-programming your patient-derived fibroblasts and PBMCs (optional custom service). Our proprietary protocols yield high purity (>90%), beating cardiomyocytes that express typical markers such as TNNT/cTnT and α-Actinin (see Figure 1), and are validated for functional viability by patch-clamp and Fluo-4TM Direct Calcium Assay. These cardiomyocytes can be used for electrophysiological and biochemical assays (Figure 2). For example, the effect of compounds on the heart rate can be assayed by impedance-based measurements.

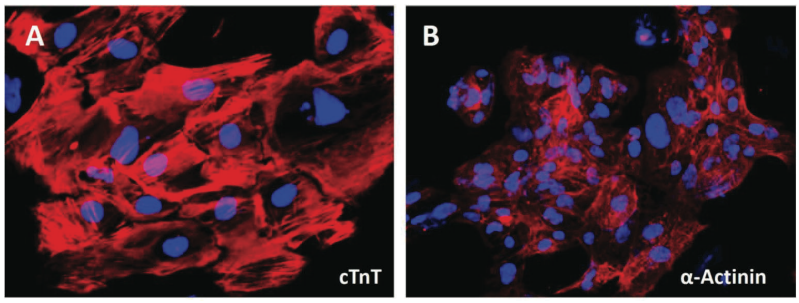


Figure 1. After replating in Cardiomyocyte Maintenance Medium on the Matrigel-coated plates for 2 days, more than 85% of the iPSC-derived cardiomyocytes express cTnT and α-Actinin (Figure A and B).

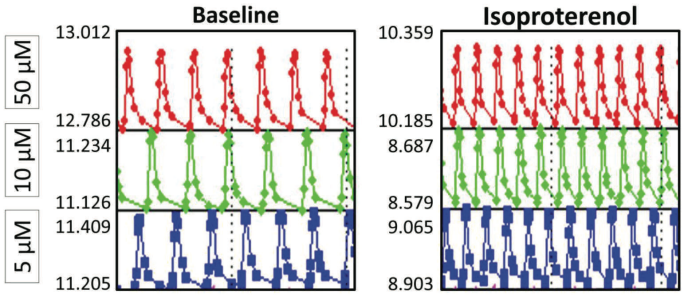
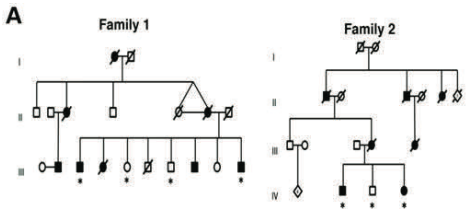


Figure 2. Beating rate of iPSC-derived cardiomyocytes measured with xCELLigence RTCA Cardio System (ACEA Biosciences and Roche Applied Science). Cells were treated with various dosages of Isoproterenol. (Left) Before Treatment (50 µM; 10 µM; 5 µM); (Right) 30 min after treatment (50 µM; 10 µM; 5 µM).

iPSC Cells from ALS8 Patients and Non-Carrier Siblings

ASC's iPSC catalog now includes fully characterized iPSCs reprogrammed from fibroblasts of 5 individual ALS8 patients (with P56S mutation) and their non-affected siblings belonging to two families affected with the autosomal dominant form of familial ALS (ALS8). This series of iPSC lines is a very useful and new tool to study ALS with reduced genetic variability and can be used to develop early diagnostic tools, and to identify possible targets for drugs and therapies.



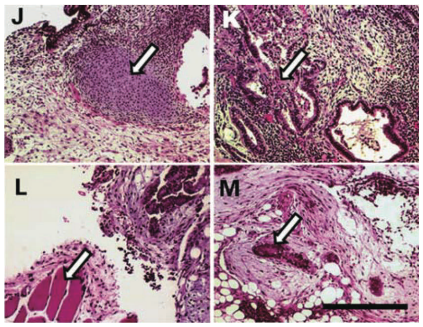


Figure. Characterization of controls and ALS8-patients iPSCs. (A) Heredograms of the two ALS8 families. ALS8 patients: dark symbols; non-affected individuals: white; *: Skin biopsies from affected and control individuals. (J-M) In vivo characterization by teratoma induction identified tissue from the 3 germ layers.

Ref: Mitne-Neto, et al. (2011). Downregulation of VAPB expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. Human molecular genetics, 20(18), 3642-3652.

iPSC Cell Culture Products

MEF Cells

- Strict quality control tested for long term culture of either mouse or human ESC/iPSC lines (>168 passages)
- Our MEFs are used by more than 200 clients in academic and industrial labs worldwide
- DR4, CF-1, Neo, SNL 76/7 cells available (Untreated, irradiated, mitomycin C-treated)

We provide high-quality MEF cells!
Average viability >95%

Cell Culture Products

- Serum and feeder free medium
- Conditioned medium for human ESC/iPSC culture
- Freezing medium

Easy-to-use Kits

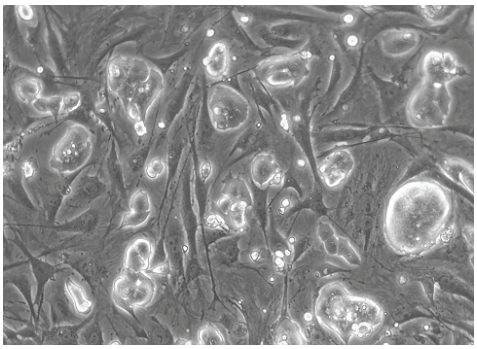
- EZ iPSC generation kit (Episomal/Retroviral)
- iPSC characterization kit
- Immortalization kit

Primary Cells

- Fibroblasts (Healthy/Disease)
- Cardiomyocytes, Cardiac Progenitor Cells

ESC-Sure™ FBS

- High quality, affordable ESC-grade serum for ES and iPS cell culture
- Supports undifferentiated growth of mESCs (QC report provided)



ESC-Sure™ FBS is extensively tested for supporting undifferentiated growth of mpuse ESCs (mouse embryonic stem cells). The image shows healthy mESC that were cultures in MEF-feeder cell dependent system along with ASC's ES-grade FBS.

My EZ Gel™ CDX Models

- Protein-based solubilized base membrane
- Synthesized from commonly used amino acids
- Compatible with various adhesion proteins and cell growth factors
- Powerful reagent to package and deliver cancer cells, primary cancer cells, or stem cells

ESC/ iPSC Cell Line Catalog

Human iPS Cells from Healthy and Patient Samples

ASE-9203	Human iPSCs (Episomal, Healthy, Fibroblasts)
ASE-9028	Human iPSCs (Episomal, Parkinson's Disease, Fibroblasts)
ASE-9029	Human iPSCs (Episomal, Parkinson's Disease, Adipocytes)
ASE-9030	Human iPSCs (Episomal, Parkinson's Disease, PBMCs)
ASE-9031	Human iPSCs (Episomal, Diabetes II, Fibroblasts)
ASE-9033	Human iPSCs (Episomal, Diabetes II, PBMCs)

iPSCs & ESCs: Mouse, Rat, Pig, Guinea Pig

ASE-9005	Germline mESC; C57Bl/6
ASE-9006	Germline mESC; 129-EZ
ASE-9007	Germline mESC C57Bl/6 EZ
ASE-9008	Germline mESC BALB/c EZ
ASE-9009	mESC C57Bl/6 Albino
ASE-9106	Pig iPSCs
ASE-9107	Mouse iPSCs
ASE-9108	Guinea Pig iPSCs

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