Alzheimer's In a Dish™ Clonal FAD ReNcell® VM Human Neural Stem Cell Lines

Datasheet for Catalog Nos.

SCC008FAD1
SCC008FAD2
SCC008FAD3
SCC008FAD4
SCC008FAD5
SCC008FAD6
SCC008FAD7

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INTRODUCTION

Amyloid-β (Aβ) plagues and neurofibrillary tangles are key pathological features observed in the brains of Alzheimer's patients. Familial, early onset forms of AD (FAD) are caused by autosomal dominant inherited genetic mutations and offer an opportunity to study the effects of key mutations on the disease's progression and pathology[1]. To date, approximately 200 FAD mutations in amyloid β precursor protein (APP) and/or presenilin I (PSEN1) have been reported[2]. Recently a 3D model using genetically engineered human neural stem cells that overexpress FAD mutations was reported to recapitulate the two pathological hallmarks of AD – β-amyloid plagues and neurofibrillary tangles[3,4,5]. Importantly, single cell derived clonal FAD neural stem cells were used to demonstrate that high Aβ42/40 ratio and not total Aβ level directly correlated with accelerated accumulations of insoluble Aβ aggregates and pathogenic phospho-tau levels[5]. Increased cell death was also detected in 3D-differentiated FAD clones with high Aβ42/40 ratio[5].

Alzheimer's in a Dish™ is a proprietary collection of immortalized single cell derived ReNcell® VM human neural progenitor cells that express stable levels of fluorescently tagged AD genes with multiple FAD mutations. The clonal FAD neural progenitor cells secrete different amounts of total Aβ and differ in the Aβ42/40 ratios. The clonal lines are notable in the homogeneity and relative stability of APP expression levels and pathogenic Aβ levels across multiple passages. The Alzheimer's in a Dish™ collection of clonal NSC lines allows the direct assessment of the role of Aβ42/40 ratio in regulating phospho-tau accumulations and neurofibrillary tangles pathology in AD.

Source: Lentiviruses expressing the FAD mutations in the full length β-amyloid precursor protein (APP) with both the K670N/M671L (Swedish) and V717I (London) mutations (APP(Swe/Lon)) and/or PSEN1 with the ΔE9 mutation (PS1(ΔE9)) and APP(Swe/Lon)/PS1(ΔE9) along with fluorescent proteins as reporters for viral infection, were used to transfect ReNcell® VM human neural stem cells[3,4,5]. Single cell derived clonal FAD neural stem cells were selected by FACS-assisted 96-well single-cell sorting based on GFP and/or mCherry signals and screened for pathogenic Aβ secretion using Western blot and ELISA[5].

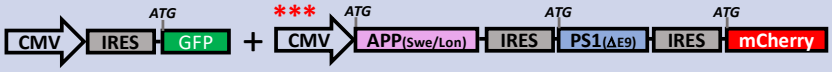
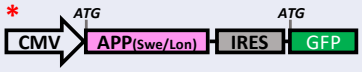
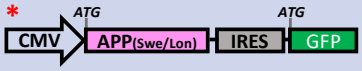
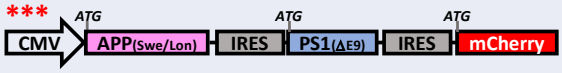
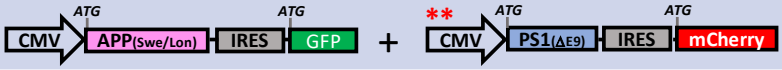
Cat. No.	Description	Expressed Genes	A42/40 ratio
SCC008FAD1	HReN-mGAP Clone A4H1		3rd highest Aβ42/40 ratio
SCC008FAD2	ReN-mGAP10 Clone D4		2nd highest Aβ42/40 ratio
SCC008FAD3	ReN-G2 Clone B2		Negative control – no FAD mutations
SCC008FAD4	ReN-GA2 Clone 3C1		Low Aβ42/40 ratio; higher APP levels than Clone A5
SCC008FAD5	ReN-GA2 Clone A5		Low Aβ42/40 ratio
SCC008FAD6	ReN-mAP4 Clone E6F4		Highest level of total Aβ species, but low Aβ42/40 ratio
SCC008FAD7	ReN-mGAP2 Clone H10		Highest Aβ42/40 ratio

Table 1. A list of lentiviral DNA constructs expressed in the clonal neural stem cells. The expression levels of APPSL (*) determine total Aβ levels while PS1ΔE9 (**) levels affect Aβ42/40 ratio by altering PS1/γ-secretase complex in clonal AD NSCs. In case of NSCs with APPSL-PS1ΔE9 construct (***), the Aβ42/40 ratio cannot be changed since transcription of both APPSL and PS1ΔE9 are controlled by a single CMV promoter. Clonal FAD neural stem cell lines with high Aβ42/40

ratio (SCC008FAD2 and SCC008FAD7) exhibited robust accumulations of detergent-resistant Aβ42 aggregates and phospho-tau and had elevated levels of Caspase-3 indicative of increased neuronal cell death[5].

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SHORT TANDEM REPEAT (STR) PROFILE

D3S1358: 15, 16	D16S539: 10, 11
TH01: 6, 9.3	CSF1PO: 11, 12
D21S11: 28, 32	Penta D: 9, 13
D18S51: 10, 17	vWA: 14, 17
Penta E: 7, 21	D8S1179: 13, 16
D5S18: 10, 11	TPOX: 8, 11
D13S317: 10, 12	FGA: 20, 25
D7S820: 10	AMEL: X, Y

Immortalized cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passage

QUALITY CONTROL TESTING

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested by PCR and are negative for HPV-16, HPV-18, Hepatitis A, C, and HIV-1 & 2 viruses as assessed by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

STORAGE & HANDLING

Alzheimer's in a Dish™ Clonal FAD Neural Stem Cells (SCC008FAD1-7) should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

MATERIALS REQUIRED BUT NOT SUPPLIED:

1. ReNcell® NSC Maintenance Medium (Cat. No. SCM005)
2. ReNcell® NSC Freezing Medium (Cat. No. SCM007)
3. Basic fibroblast growth factor (bFGF; FGF-2; Specific Activity $\geq$ 2 X 10^6 Units/mg. GF003)
4. Epidermal growth factor (EGF; Specific Activity $\geq$ 1 x 10^7 Units/mg; Cat. No. GF001)
5. Laminin (Cat. No. L2020)
6. DMEM/F12 w/o HEPES, w/ L-Glutamine (Cat. No. DF-042-B)
7. Accutase™ (Cat. No. SCR005)
8. Tissue culture-ware
9. Phosphate-Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
10. Penicillin/Streptomycin/Amphotericin B Solution (100X) (Cat. No. 516104)

PREPARATION OF COATED FLASKS:

We recommend coating tissue culture plastic- or glassware that are used to culture clonal FAD ReNcell® NSC cells with laminin. Tissue culture flasks should be coated on the same day that the cells are thawed from liquid nitrogen or on the same day that the cells need to be passage. The following procedure is recommended:

1. Thaw the laminin (L2020) in the morning at 2-8°C. Dilute laminin with DMEM/F12 (Cat. No. DF-042-B) to 20 μg/mL.
2. Add enough of the diluted laminin solution to cover the whole surface of the tissue culture-ware. Use 3 mL volume for 6-cm plates and 6.5 mL volume for 10-cm plates and T75 flasks. Incubate in a 37°C, 5% CO_2 incubator for at least 4 hours.
3. Just before use, aspirate the laminin solution in the coated flasks and rinse the flasks once with 1X PBS.
4. Prepare the Complete ReNcell® NSC Medium by adding 20 – 40 ng/mL FGF-2 and 20 – 40 ng/mL EGF (final concentrations) to ReNcell® NSC Maintenance Medium (Cat. No. SCM005).
5. Add 10 mL of the freshly made Complete ReNcell® NSC Medium to the laminin coated T75 flasks. Incubate in a 37°C, 5% CO_2 incubator. The laminin-coated flasks are now ready to receive the cells.

THAWING OF CELLS

1. Do not thaw the cells until the recommended medium and appropriately coated laminin plasticware and/or glassware are on hand.
2. Remove a vial of clonal ReNcell® FAD NSCs from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. **IMPORTANT: Do not vortex the cells.**
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of ReNcell® NSC Maintenance Medium (Cat. No. SCM005) (pre-warmed to 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole volume of medium at once to the cells. This may result in decreased cell viability due to osmotic shock.**
6. Gently mix the cell suspension by slow pipetting up and down twice. Be careful to not introduce any bubbles. **IMPORTANT: Do not vortex the cells.**
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in a total volume of 5 mL of ReNcell® NSC Maintenance Medium (Cat. No. SCM005) (pre-warmed to 37°C) containing freshly added 20 – 40 ng/mL FGF-2 and 20 – 40 ng/mL EGF.
   Note: *FGF-2 and EGF should always be added fresh to the ReNcell® NSC Maintenance Medium.*
10. Plate the cell mixture onto the laminin coated T75 tissue culture flask that was pre-incubated in the 37°C incubator. The laminin coated T75 flask should already have 10 mL of Complete ReNcell® Neural Stem Cell Medium (i.e. ReNcell® NSC Maintenance Medium containing 20 – 40 ng/mL FGF-2and 20 - 40 ng/mL EGF).
11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.
12. The next day, exchange the medium with fresh ReNcell® NSC Maintenance Medium (Cat. No. SCM005) (pre-warmed to 37°C) containing 20 - 40 ng/mL FGF-2 and 20 - 40 ng/mL EGF. Exchange with fresh medium containing FGF-2 and EGF every other day thereafter.
13. When the cells are approximately 80% confluent, they can be dissociated with Accutase™ and passaged or alternatively frozen for later use.

SUBCULTURING:

1. Prepare fresh laminin-coated flasks (refer to Preparation of Coated Flasks).
2. Carefully remove the medium from the laminin coated T75 flasks containing the confluent layer of clonal FAD NSCs.
3. Rinse the flask once with 1X PBS. ***Note:*** *Add the PBS slowly from the side to avoid detaching the cells.*
4. Aspirate the PBS.
5. Apply 3-5 mL of Accutase™ and incubate in a 37°C incubator for 3-5 minutes.
6. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
7. Apply 5 mL of ReNcell® NSC Maintenance Medium (Cat. No. SCM005) (pre-warmed to 37°C) to the flask.
8. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
9. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
10. Discard the supernatant.
11. Apply 2 mL of ReNcell® NSC Maintenance Medium (Cat. No. SCM005) containing 20 – 40 ng/mL FGF-2 and 20 – 40 ng/mL EGF to the conical tube and resuspend the cells thoroughly. ***Note:*** *Do not vortex the cells.*
12. Count the number of cells using a hemocytometer.
13. Plate the cells to the desired density into the appropriate fresh laminin-coated flasks, plates or wells in ReNcell® NSC Maintenance Medium (Cat. No. SCM005) containing 20 – 40 ng/mL FGF-2 and 20 – 40 ng/mL EGF. We typically plated the cells at ~1.5 million cells on laminin coated T75 flasks.
14. The next day, exchange the medium with fresh ReNcell® NSC Maintenance Medium (Cat. No. SCM005) containing 20 - 40 ng/mL FGF-2 and 20 - 40 ng/mL EGF. Exchange with fresh medium containing FGF-2 and EGF every other day thereafter. The cells should be ready for passaging or harvesting 2 to 3 days after this step.

CRYOPRESERVATION

Clonal FAD ReNcell® NSC lines may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

ANALYSIS OF Aβ LEVELS IN UNDIFFERENTIATED CLONAL AD CELL LINES

1. Bring cells to confluence in T25 flasks.
2. Detach cells using Accutase® Cell Dissociation Reagent (Cat. No. SCR005) and determine cell counts.
3. Plate 3 x 10^6 cells/well in 3 wells of a 6-well plate in ReNcell® NSC Maintenance Medium (Cat. No. SCM005) containing 20 ng/mL FGF-2 (Cat. No. GF003), 20 ng/mL EGF (Cat. No. GF001) and 1X penicillin/streptomycin/amphotericin-B solution (Cat. No. 6516104).
4. After 24 h, drain media and add 1 ml of fresh ReNcell® NSC Maintenance Medium containing 20 ng/mL FGF-2 and 20 ng/mL EGF-2.
5. After growing cells for additional 24 h, collect both media and cell pellets (see #8).
6. Centrifuge media samples at 20,000 x g for 10 minutes at 4°C in order to remove cell debris.
7. Collect supernatant in new tubes and store at -20°C until further analysis.
8. For protein extraction from cell pellets, wash cells three times with ice-cold PBS (Cat. No. BSS-1005-B) and scrape cells using cell scraper. Pellets can be stored at -20°C or -80°C. Otherwise directly dissolve cell pellets in RIPA lysis buffer (Cat. No. R0278).

9. Measure levels of Aβ38, Aβ40 and Aβ42 in media simultaneously by multi-array electrochemiluminescence assay kit (V-PLEX Aβ Peptide Panel 1 (6E10) kit) (*Meso Scale Diagnostics (MSD), #K15200E-2*) on triplicate media samples collected
10. Measure protein concentration by BCA assay on the triplicate cell pellets samples collected
11. Calculate Aβ levels. Values are normalized by protein amount for each sample.
12. Run statistical analysis (n = 3).

REFERENCES

1. *Biomark Med*. 2010; 4(1): 99-112.
2. *Bioassays* 2015; 37(10): 1139-1148.
3. *Nature* 2014; 515(7526): 274-278.
4. *Nature Protocol* 2015; 10(7): 985-1006.
5. *Nature Commun.* 2020; 11(1): 1377.

REPRESENTATIVE IMAGES

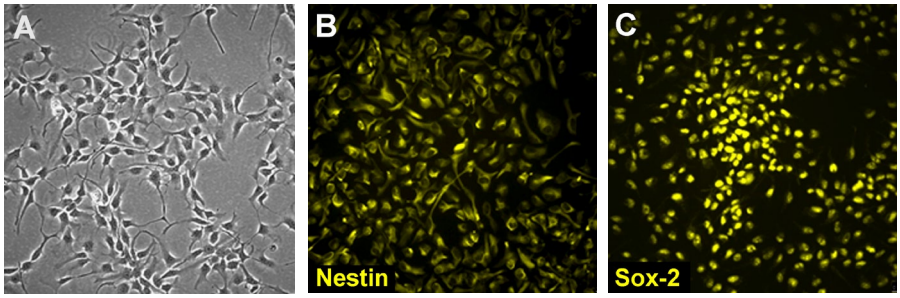

Figure 1. Representative bright-field image of clonal FAD NSC lines one day after thaw (**A**). All clonal FAD NSCs express Nestin (**B**) and Sox-2 (**C**).

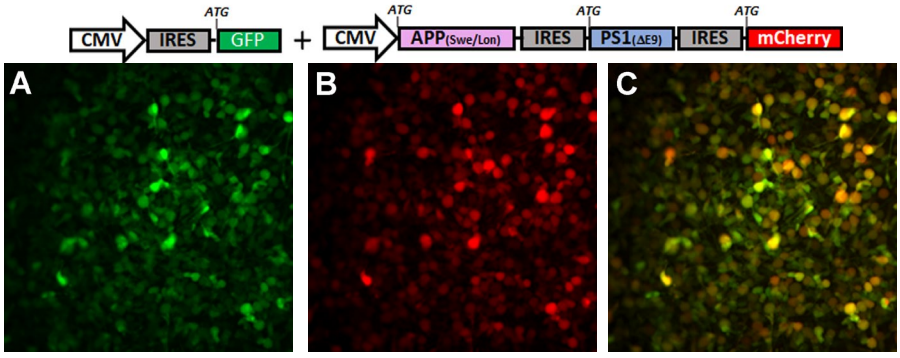

Figure 2. HReN-mGAP Clone A4H1 (SCC008FAD1) expresses the 3rd highest Aβ42/40 ratio of the clonal FAD NSC lines[5]. Cells were infected with lentiviruses in which GFP expression is driven by the CMV promoter (**A**) along with a polycistronic lentivirus in which the APP(Swe/Lon), PS1 (ΔE9) FAD mutations and mCherry (**B**) are expressed and separated by IRES sequences[3,4,5]. Merged images (**C**).

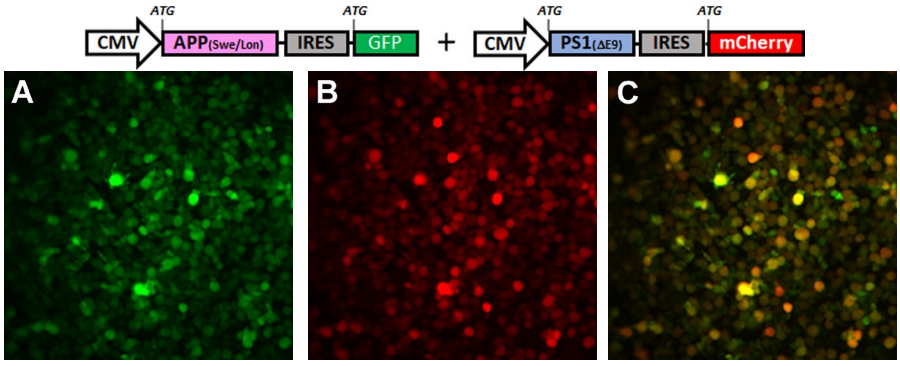

Figure 3. ReN-mGAP10 Clone D4 (SCC008FAD2) expresses the 2nd highest Aβ42/40 ratio of the clonal FAD NSC lines[5]. Cells were double infected with polycistronic lentiviruses expressing APP (Swe/Lon) and GFP and PS1 (ΔE9) and mCherry. GFP (**A**) and mCherry (**B**) expressions. Merged images (**C**).

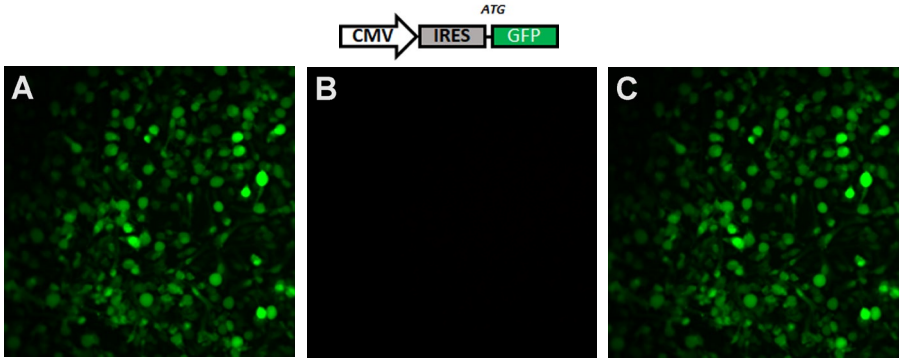

Figure 4. ReN-G2 Clone B2 (SCC008FAD3) expresses GFP driven by the CMV promoter (**A**). The cells do not express mCherry (**B**). Merged images (**C**).

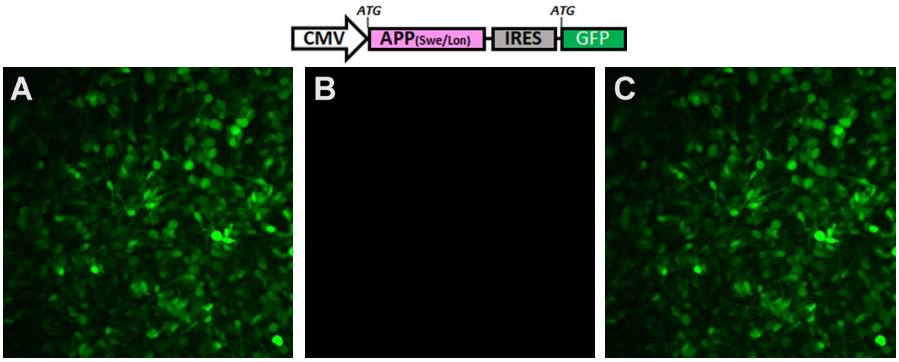

Figure 5. ReN-GA2 Clone 3C1 (SCC008FAD4). Cells were infected with polycistronic lentiviruses expressing APP (Swe/Lon) and GFP separated by IRES sequences (**A**). The cells do not express mCherry (**B**). Merged images (**C**).

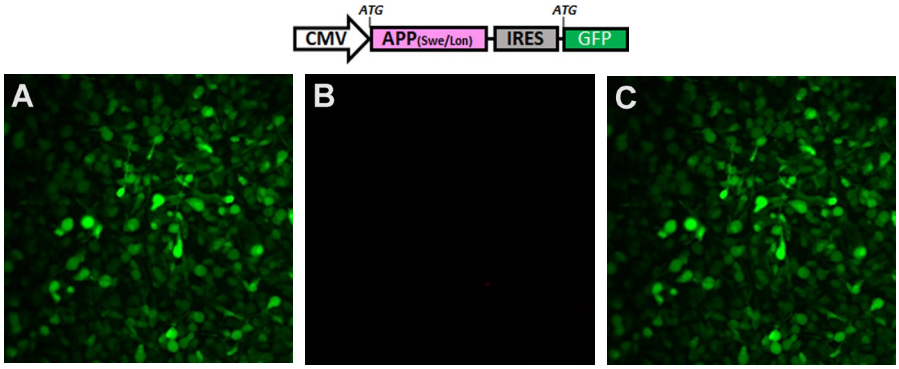

Figure 6. ReN-GA2 Clone A5 (SCC008FAD5). Cells were infected with polycistronic lentiviruses expressing APP (Swe/Lon) and GFP separated by IRES sequences (**A**). The cells do not express mCherry (**B**). Merged images (**C**).

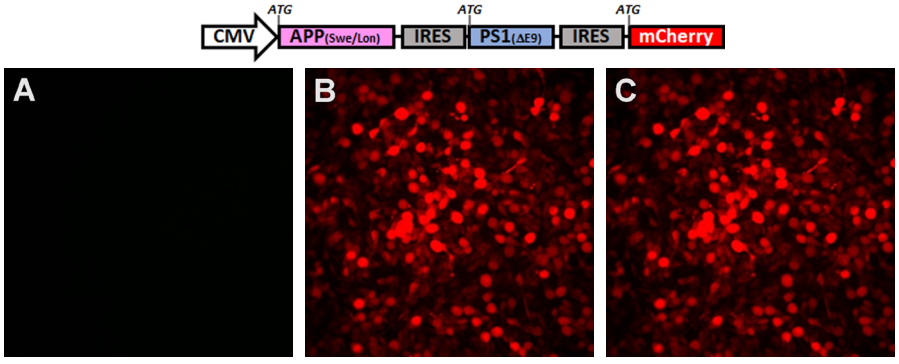

Figure 7. ReN-mAP4 Clone E6F4 (SCC008FAD6). Cells were infected with polycistronic lentiviruses expressing APP(Swe/Lon), PS1(ΔE9), and mCherry separated by IRES sequences (**B**). Cells do not express GFP (**A**). Merge images (**C**).

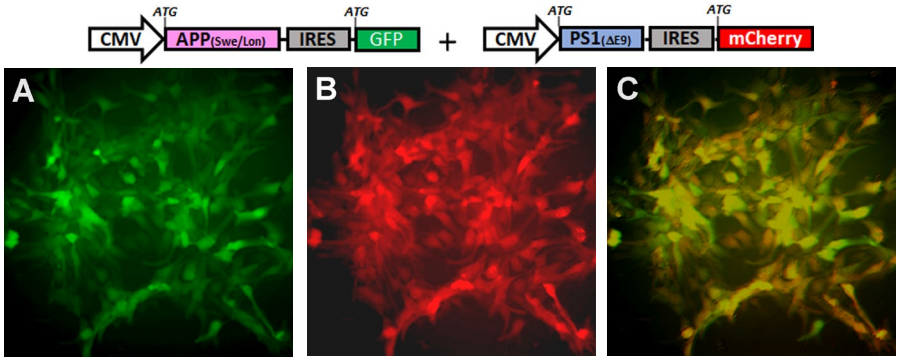

Figure 8. ReN-mGAP2 Clone H10 (SCC008FAD7) expresses the highest Aβ42/40 ratio of the clonal FAD NSC lines[5]. Cells were double infected with polycistronic lentiviruses expressing APP (Swe/Lon) and GFP and PS1 (ΔE9) and mCherry. GFP (**A**) and mCherry (**B**) expressions. Merged images (**C**).

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