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Evaluating BEST1 mutations in pluripotent stem cell-derived retinal pigment epithelial cells

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Abstract

Bestrophin-1 (BEST1) is a calcium-activated chloride channel (CaCC) predominantly expressed at the basolateral membrane of the retinal pigment epithelium (RPE). Over 250 mutations in the *BEST1* gene have been documented to cause at least five retinal degenerative disorders, commonly termed bestrophinopathies, to which no treatment is currently available. Therefore, understanding the influences of *BEST1* disease-causing mutations on the physiological function of BEST1 in RPE is critical for deciphering the pathology of bestrophinopathies and developing therapeutic strategies for patients. However, this task has been impeded by the rarity of *BEST1* mutations and limited accessibility to native human RPE cells. Here, we describe a pluripotent stem cell (PSC)-based pipeline for reproducibly generating RPE cells expressing endogenous or exogenous mutant BEST1, which provides us with a powerful "disease-in-a-dish" approach for studying *BEST1* mutations in physiological environments.

1. Introduction

In 1905, German physician Friedrich Best discovered a condition characterized by progressive vision loss, which was thereafter called "Best disease." Today, this genetic disease (officially called Best vitelliform macular dystrophy, or BVMD) is known to arise from mutations in the *BEST1* gene located at chromosome 11q13 (Forsman et al., 1992; Petrukhin et al., 1998; Stone et al., 1992; Sun et al., 2002). *BEST1* encodes a calcium-activated chloride channel, namely Bestrophin-1 (BEST1), which is predominantly expressed at the basolateral membrane of the retinal pigment epithelium (RPE) (Hartzell et al., 2008; Li et al., 2017; Marmorstein et al., 2000; Tsunenari et al., 2003). BEST1 is believed to be the ion channel that generates the "light peak," a corneoretinal potential change when the eye is suddenly exposed to light after dark adaptation. The defective light peak in Best disease patients can be measured by a decrease in their electrooculogram (Boon et al., 2009; Marmorstein et al., 2006; Marmorstein, Cross, & Peachey, 2009). Other clinical phenotypes of the disease include retinal detachment and observable yellow lesions (vitelliform), which are presumably caused by abnormalities in the fluid and/or electrolyte homeostasis between photoreceptors and RPE. While Best disease is distinct as

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an early-onset and debilitating form of macular degeneration, mutation of the *BEST1* gene also results in several other retinal disorders including autosomal recessive bestrophinopathy (ARB) (Burgess et al., 2008), autosomal dominant vitreoretinochoridopathy (ADVIRC) (Burgess et al., 2009; Yardley et al., 2004), adult-onset vitelliform dystrophy (AVD) (Allikmets et al., 1999; Krämer, Stöhr, & Weber, 2004), adult-onset faveomacular vitelliform dystrophy (AFVD) (Seddon et al., 2001), and retinitis pigmentosa (RP) (Davidson et al., 2009). Collectively, these *BEST1*-associated diseases fall under the umbrella term "bestrophinopathies," while over 250 distinct patient-derived mutations have been reported to date.

Understanding how each mutation influences the physiological activity of BEST1 is essential for elucidating the disease-causing mechanisms and developing treatments for bestrophinopathy patients. Historically, transient overexpression model systems such as HEK293 cells have been used in characterizing BEST1 function (Barro-Soria et al., 2010; Boon et al., 2009; Ji et al., 2019a,b; Li et al., 2017; Milenkovic et al., 2009, 2011; O'Driscoll et al., 2009; Owji et al., 2020; Park et al., 2009; Qu et al., 2007; Qu & Hartzell, 2008; Reichhart et al., 2010; Sun et al., 2002; Vaisey & Long, 2018; Xiao et al., 2009; Yang et al., 2014; Yu et al., 2008; Zhang et al., 2018), but these cell lines do not recapitulate the native RPE environment in which Best1 is canonically expressed. While isolated RPE cells from postmortem donors can be used to test BEST1 in its native habitat, the very limited resource of these cells and the rarity of a specific *BEST1* mutation in the population makes it infeasible for research purposes. To circumvent this difficulty, the recent advancements in stem cell technologies have opened up a door for investigating *BEST1* mutations. As stem cells have unlimited renewability and preservation of the pluripotent state, they can be reliably maintained and differentiated into RPE cells. Depending on the cell origin, there are now two avenues of stem cell-based *BEST1* research in RPE: (1) skin fibroblasts can be biopsied from bestrophinopathy-inflicted patients, reprogrammed to induced pluripotent stem cells (iPSCs), and subsequently differentiated into RPE cells (iPSC-RPE); (2) established human embryo-derived pluripotent stem cells (hPSC) provide a standardized lineage which can be differentiated into RPE (hPSC-RPE). Each of these cell types can be manipulated by endogenous or exogenous methods to study desired *BEST1* mutations.

Bestrophinopathy patient-derived iPSC-RPEs naturally carry *BEST1* disease-causing mutations, and thus provide a platform to study endogenous BEST1 mutants. On the other hand, standard hPSC lines such as the NIH-approved H1 cells normally contain the WT *BEST1* gene, so that WT BEST1 protein will be expressed in the resultant hPSC-RPEs. In order to study endogenous *BEST1* mutations in hPSC-RPE, direct manipulation at the DNA level is required. Recent advancements in CRISPR/Cas9-mediated genome editing have answered this call. A doxycycline-inducible Cas9 expression cassette was integrated into H1 cells, developing a highly efficient and adaptable system (iCas9) for generating endogenous *BEST1* mutations (Soh & Huangfu, 2017).

Alternatively, RPE cells that do not express endogenous BEST1 can be used to study desired BEST1 mutants after exogenous expression. This includes $BEST1^{-/-}$ hPSC-RPE cells generated from the iCas9 system and iPSC-RPE cells derived from a patient carrying

a mutation in the 5′ untranslated region (UTR) of the *BEST1* gene that completely abolishes endogenous BEST1 expression (Fung et al., 2015). An exemplary tool for studying exogenously expressed *BEST1* mutations in these cells with a blank BEST1$^{-/-}$ background is the BacMam expression virus system (Kost, Condreay, & Jarvis, 2005), which uses a modified insect cell virus (baculovirus) as a vehicle to deliver gene(s) of interest of up to 38 kb into mammalian cells with limited cytotoxicity. The DNA encompassed by the BacMam system can be mutated by conventional molecular biology methods, making the BacMam system highly versatile and a powerful tool for studying exogenously expressed *BEST1* mutants.

Collectively, the iPSC-RPE and hPSC-RPE methods provide us with a powerful "disease-in-a-dish" approach for studying *BEST1* mutations in physiological environments.

2. iPSC-RPE vs. hPSC-RPE

There are pros and cons of using each of these two cell types. iPSCs are beneficial in their relevance to the respective patients, as the fibroblast cells obtained from a given patient contain the patient's individualized genetic background. Thus, the iPSC-RPEs are inherently representative of each patient, and ideal for testing personalized medicine and therapeutics in vivo. However, it is worth to note that there are several limitations: (1) as genetic mutation in the *BEST1* gene is rare, it is logistically difficult to access a full spectrum of patient samples, and thus there is no guarantee to have a desired mutation on demand; (2) non-specific phenotypes may result from different genetic backgrounds of the patients; (3) the reprogramming and differentiation efficiencies of different donors' skin cells vary such that in some cases it can be technically challenging to obtain iPSC-RPEs; (4) it is hard to perform genomic manipulation in either iPSC or iPSC-RPE. In contrast, the hPSC-based iCas9 system is capable of generating any desired mutations from the same parental cell line, providing an opportunity to study BEST1 mutations in isogenic hPSC-RPEs. Furthermore, the iCas9 platform allows simultaneous generation of heterozygous, homozygous, and compound homozygous BEST1 mutations. Therefore, the iPSC-RPE route is more clinically relevant when patient samples are available, whereas the hPSC-RPE route provides better accessibility for mutations harder to obtain from the population and offers a more controlled comparison between different mutations.

3. Methods—iPSC

3.1 Generating iPSCs from fibroblast cells

To generate iPSC-RPEs, the first step is to obtain patient fibroblasts. Consent from a knowledgeable, willing patient must be procured through the appropriate channels. The skin biopsy must be performed by a licensed dermatologist or other appropriately trained physician under human subjects' ethical guidelines. Once obtained, the skin cells will be cultured and reprogrammed into iPSCs.

3.1.1 Materials and equipment

- Discussed in (Li, Nguyen, & Tsang, 2016).

3.1.2 Protocol—The protocol for performing the skin biopsy, extracting fibroblast cells from the skin biopsy tissue, and reprogramming these cells into iPSCs is described in detail in (Li et al., 2016).

3.2 Differentiating iPSCs into iPSC-RPEs

Once patient-derived iPSCs are obtained, the iPSCs will be differentiated into iPSC-RPEs which express endogenous BEST1 protein and other RPE cell markers such as RPE65 and CRALBP. The protocol for differentiating iPSCs into iPSC-RPEs takes approximately 3–4months (Kittredge et al., 2018).

3.2.1 Materials and equipment

- Laminar flow biosafety cabinet*
- 70% EtOH*
- Sterile incubator, 37°C and 5% CO_2*
- 9” glass Pasteur pipette*
- Bunsen burner*
- Patient-derived iPSCs
- Dulbecco’s Phosphate-Buffered Saline (DPBS) without calcium or magnesium*
- Dulbecco’s Modified Eagle Medium (DMEM)*
- Rho-associated protein kinase (ROCK) Y-27632 inhibitor*
- Activin-A*
- Trypsin*
- Collagenase*
- KnockOut-DMEM (KO-DMEM)*
- KO-DMEM serum replacement*
- Nonessential amino acids*
- Glutamine*
- Penicillin-streptomycin*
- Nicotinamide*
- Matrigel*
- Ethylenediaminetetraacetic acid (EDTA) 0.5M, pH8.0*
- 6-well tissue culture plates*

*Also used in hPSCs methods for differentiation into hPSC-RPEs.

3.2.2 Protocol

1. Plate cells to be ~60–70% confluent on a 6-well plate coated with Matrigel basement membrane the day before differentiation begins. On the first day of the differentiation protocol, cells should be tightly packed within each well
    a. Cells are kept in a sterile incubator set to 37°C and 5% CO_2
    b. For adhering Matrigel membrane to the plate, thaw an aliquot of Matrigel on ice for at least 30min. Once liquid, dilute Matrigel in DMEM at a 1:50 (v/v) ratio and place the mixture in the wells of the 6-well dish, enough to cover the entirety of the bottom of the well (1–2mL/well). Place the plate in the 37°C incubator for at least 1 h before use. Aspirate the solution before adding media. This method for coating the wells with Matrigel membrane is used any time hPSC, hPSC-RPE, or iPSC-RPE cells are split
2. On the first day of the differentiation protocol, exchange the stem cell media with differentiation media. The recipe for differentiation media is given in Table 1. Change media 3 times per week (e.g., on Monday, Wednesday and Friday)
3. On the 15th day of culture in the differentiation media, supplement the growth media with 100ng/mL human Activin-A. Continue with the Activin-A additive for 2 weeks
4. On the 29th day of culture, stop Activin-A supplementation. Continue growing cells for at least an additional 10–12 weeks in the differentiation media, continuing to change media 3 times per week. Small pigmented clusters of cells should be visible to the naked eye around this time. Continue culturing cells until the number of pigmented cells plateaus
    a. See Fig. 1 for a schematic of the differentiation protocol
5. On the day of harvesting the pigmented, differentiated cells, manufacture glass pipette cell scrapers. Do this by holding a long 9” glass Pasteur pipette horizontally above a flaming Bunsen burner by its two ends, and when the glass begins to melt, quickly pull apart the pipette. The resultant cell scraper should have a thin, yet durable and inflexible tip. Generate at least 10 scrapers, spray them with 70% EtOH, and let them air-dry in the biosafety cabinet before use
    a. Note: Be careful with the pulled pipettes. They are sharp and can result in punctured skin and bleeding if accidentally poked
6. Using 1U/μL collagenase and 0.05% trypsin, digest the desired wells for approximately 20–30min and pick the pigmented colonies. Pick at least 5000 cells/well of a Matrigel-coated 12-well dish
7. Once enough cells are picked in each well, add 1mL extra RPE media into each well. Do not change media for at least 2days so that cells can adhere to the Matrigel membrane

8. Grow the cells in RPE media (see Table 1 for recipe) until cells display the typical RPE cobblestone morphology. Partially change media 3 times per week, replacing approximately 2/3 of the total volume (e.g., 1 mL of 1.5 mL) each time. As the cells age, they may appear to generate “bubbles” or “domes” and lift up from the plate. This suggests the RPE cells are transporting fluids and is indicative of mature RPE cells, referred to as P0 iPSC-RPE

4. Methods—hPSCs

To generate a given mutation in hPSCs, an inducible CRISPR/Cas9 (iCas9) system is used.

4.1 gRNA and ssDNA design and generation for iCas9 mutagenesis

The H1-iCas9 hPSC line expresses Cas9 upon doxycycline treatment. Co-transfecting H1-iCas9 cells with a gRNA and an ssDNA template allows site-directed mutagenesis at the DNA sequence of choice. The protocol for designing/generating these oligonucleotides is explained in (Soh & Huangfu, 2017). The resultant gRNA and ssDNA may be saved in −80°C and −20°C freezers, respectively, until transfection.

4.1.1 Materials and equipment

- Oligonucleotides
- MEGAshortscript T7 Transcription kit
- MEGAclear Transcription Clean-up kit
- Gel electrophoresis apparatus and gel imaging equipment^
- TAE buffer^
- 1% agarose gel^
- Loading dyê
- 1 kb DNA ladder^
- Thermal cycler^

Âlso used in knockin/knockout mutation generation.

4.1.2 Protocol—The original protocol for the design and generation of gRNA and ssDNA for iCas9 mutagenesis is described in (Soh & Huangfu, 2017).

4.2 Knockin/knockout endogenous mutation generation with iCas9

The protocols for generating knockin and knockout mutations in H1-iCas9 cells are almost identical to one another. The only major difference is the presence of ssDNA serving as a template when generating knockin mutations. H1 cells integrated with an iCas9 cassette are transfected with gRNA (and ssDNA for making knockin mutations) to create double-stranded DNA breaks. Cells from the transfected wells are lysed and the cutting efficiency of each gRNA is determined by PCR. Cells from wells with the highest cutting efficiency

are seeded in large plates, and individual clones are manually picked and sequenced for determining the precise mutation.

4.2.1 Materials and equipment

- Laminar flow biosafety cabinet
- 70% EtOH
- H1-iCas9 cells
- mTeSR1 media and supplement
- Opti-MEM I reduced serum medium
- Dulbecco's Phosphate-Buffered Saline (DPBS) without calcium or magnesium
- EDTA 0.5 M, pH8.0
- Rho-associated protein kinase (Rock) inhibitor, Y-27632 dihydrochloride
- Dimethyl Sulfoxide (DMSO), cell culture reagent
- Lipofectamine RNAiMax reagent
- Doxycycline
- Primers flanking the cut site of interest
- PCR enzyme kit
- T7E1 endonuclease kit
- 10 × lysis buffer (0.45% NP-40, 0.45% Tween 20 in 10 mM Tris pH8.0)
- Proteinase K
- TrypLE Select Enzyme, no phenol red
- Thermal cycler
- Primers flanking cut site of choice

4.2.2 Protocol

1. Culture hPSCs in mTeSR1 media, adhering cells to plates using Matrigel coating
2. Perform the gRNA (+ssDNA for knockin mutants) transfection as previously described in (Soh & Huangfu, 2017), and determine the cutting efficiency of each gRNA using the T7E1 kit
3. Using TrypLE Select Enzyme to dissociate the cells, seed the hPSCs from the most efficient gRNA transfection to 2 × 10 cm dishes at 1000 cells/plate and 2000 cells/plate. Do not change media the following day
    a. Note: Harvest the rest of the cells and save in liquid nitrogen
4. 10–12days later, when colonies are visible to the naked eye, pick individual clones and replate them to their own wells of a 96-well dish. Grow duplicates

for genetic extraction and amplification via lysis and PCR and submit each DNA extract for Sanger sequencing

5. Analyze Sanger sequencing using the raw traces
6. Save and grow clones with the desired mutation(s)

4.3 Differentiate hPSCs into hPSC-RPEs

The materials and protocol for differentiating hPSCs into hPSC-RPEs are identical to those for differentiating iPSCs into iPSC-RPEs.

5. Exogenous viral expression of *BEST1* mutations in BEST1$^{-/-}$ RPE

RPE cells with a clean BEST1 null background can be differentiated from *BEST1* knockout H1-iCas9 cells or a BEST1 null iPSC cell line derived from a patient with a mutation in the 5′ UTR region of the *BEST1* gene that abolishes endogenous BEST1 expression (Fung et al., 2015). On a modified pEG BacMam vector containing the cDNA of *BEST1*, desired BEST1 mutations are generated by conventional mutagenesis protocols. After baculovirus production and amplification (Goehring et al., 2014), the resultant virus concentration will be determined by titration and used to infect *BEST1*$^{-/-}$ hPSC-RPE or iPSC-RPE cells in order to exogenously express mutant BEST1. A schematic for the production of baculovirus and its transduction into PSCs is shown in Fig. 2.

5.1 Materials and equipment

- Sf9 cells
- BacMam expression vector
- Sf9/S media
- Cellfectin II
- Incubator set to 28°C and 150rpm
- Tissue culture flasks, various sizes
- Laminar Flow Biosafety Cabinet
- PBS
- Matrigel
- Trypsin
- Collagenase
- Fluorescence microscope
- iPSC-RPE or hPSC-RPE
- Dry bath set to 37°C

5.2 Protocol

1. Obtain cDNA of the *BEST1* gene and insert the sequence into the pEG BacMam expression vector by conventional methods
2. Perform mutagenesis on this *pEG BacMam-BEST1-GFP* plasmid by conventional methods to obtain the desired mutation(s)
3. Transform the *pEG BacMam-BEST1(mut)-GFP* plasmid into DH10Bac cells to generate baculovirus DNA (bacmid)
4. Obtain baculovirus as previously described (Goehring et al., 2014)
    a. Note: Use Sf9 cells as the virus-producing host, and Sf-900 II SFM media supplemented with 10mL/L (10,000units/mL) penicillin-streptomycin and 1 mL Amphotericin B/L (Sf9/S media) prewarmed at room temperature. Cells are incubated at 28°C and rotated at 150 rpm
5. Prepare 0.5×10^6 Sf9 cells/well of a 12-well dish (1 mL total volume) for determining the viral titer. Incubate cells at 28°C for 30–60 min until cells are attached
6. Perform 1:10 serial dilutions of the P3 virus in Sf9/S media in the biosafety cabinet
7. Verify that the cells have attached to the wells and remove the media from each well. Add 0.9 mL fresh media warmed to room temperature and add 0.1 mL of serial diluted samples from 10^8 to 10^2 per well, leaving 1 well for a negative control
8. Incubate in a 28°C humidified incubator for 48 h and then view the cells under a fluorescent microscope with a GFP filter. Count the number of fluorescent cells to determine an approximate titer (infectious units):

$$Infectious\ units = (\#of\ fluorescent\ cells)(serial\ dilution)(dilution\ factor)$$

*dilution factor is 10 because 100 μL of virus is diluted in 900 μL media/well.

9. Split iPSC-RPE or hPSC-RPE as previously described (Kittredge et al., 2018). In brief:
    a. Digest a fully confluent well of a 12-well plate of mature P0 PSC-RPE cells by aspirating the media, washing with PBS, and digesting the cells with 1mL of 0.05% trypsin plus 1U/μL collagenase, incubating at 37°C for 8 min
    b. Wash the plate with a 1 mL micropipette and transfer the cells to a 15 mL conical tube, incubating the solution in a 37 °C dry bath for 8 min. Gently pipette up and down 10–15 times and repeat the 8 min incubation in the conical tube once more

c. Add 5 mL of pre-warmed RPE media to the tube, and spin at 200 × g for 5 min. Resuspend and seed cells on a Matrigel-coated 35 mm dish at 10–20% confluency with RPE media

10. Infect P3 virus at multiplicity of infections (MOIs) ranging from 50 to 200 onto the cells to induce exogenous expression of the mutant BEST1 protein

$$mL\ of\ inoculum = \frac{(MOI)(total\ number\ of\ cells)}{infectious\ units}$$

6. Testing BEST1 mutants in downstream experiments

Testing mutant BEST1 function in vivo is critical for verifying not only that all previously mentioned steps were performed correctly, but also to determine the disease-causing mechanism(s) of a given mutation.

6.1 Materials and equipment

- mRNA extraction kit
- RPE65, CRALBP, BEST1, and β-actin primary antibodies
- Fluorophore conjugated secondary IgG
- Confocal microscope
- Patch clamp rig

6.2 Protocol

1. mRNA can be extracted from these cell types by a commercially available RNA extraction kid. Downstream experiments, such as reverse transcriptase (RT)-PCR, can be performed using the extracted mRNA to determine BEST1 transcription level
2. BEST1 protein levels and RPE differentiation validation can be acquired by immunoblotting using primary antibodies again BEST1 (1:1000 dilution) and other RPE markers including RPE65 (1:1000 dilution) and CRALBP (1:1000 dilution).
3. BEST1 subcellular localization can be validated by immunostaining using BEST1 primary antibody (1:200 dilution) and a fluorophore-conjugated secondary IgG (1:1000 dilution), followed by confocal microscopy
4. Conduct whole-cell patch clamp experiments to examine the channel function of mutant BEST1 in hPSC-RPEs and iPSC-RPEs
    a. The protocol for preparing hPSC-RPE cells for whole-cell patch clamp analysis is explained in further detail in (Kittredge et al., 2018).

7. Potential pitfalls and solutions

7.1 Cell death and cutting ratio

Cell death is expected to be very high after gRNA transfection of the H1 cells, especially when the initial confluency is too low (30% or less). Likewise, the cutting ratio of the Cas9 decreases significantly when the confluency is too high. Therefore, along with testing multiple gRNAs for transfection, multiple cell confluencies may be required to find an optimum set of conditions. Repeating the transfection with 30%/60% and 40%/80% duplicates may be beneficial for this. In addition, the low confluence (30% and 40%) wells may take much longer to grow to confluence than the high confluence (60% and 80%) wells, yet may have higher cutting efficiencies.

7.2 Stem cells not differentiating

Some H1 hPSC CRISPR clones may differentiate more or less efficiently than others, and H1 cells generally differentiate into RPEs less efficiently than other hPSC cell lines. Thus, it is advised to differentiate multiple wells and to test multiple constructs simultaneously. This should provide sufficient numbers of fully differentiated hPSC-RPEs for future analysis. Testing multiple constructs simultaneously also circumvents the potential problem of having observable off-target effects caused by the Cas9 protein with a particular gRNA.

8. Summary

In summary, this protocol describes an adaptable technique to obtain iPSC-RPEs and hPSC-RPEs with specific BEST1 mutations. Using patient-derived iPSC-RPEs, we are able to observe BEST1 patient mutations in their natural RPE environment. Likewise, using CRISPR/iCas9 technology to generate specific mutations in H1 hPSCs, subsequently differentiating these cells into BEST1-expressing hPSC-RPE cells recapitulates any desired BEST1 mutation. The generation of baculovirus harboring a *BEST1* mutation of choice is also an incredibly versatile method for exogenous expression of desired BEST1 mutations in RPE cells with a null *BEST1* background. Downstream experiments such as RT-PCR, immunoblotting, immunostaining, and whole-cell patch clamp provide a wide variety of applications, enabling researchers to dissect the disease-causing mechanisms of BEST1 mutations in a physiological setting. A summary of the overall process and possible routes of experimentation are shown in Fig. 3.

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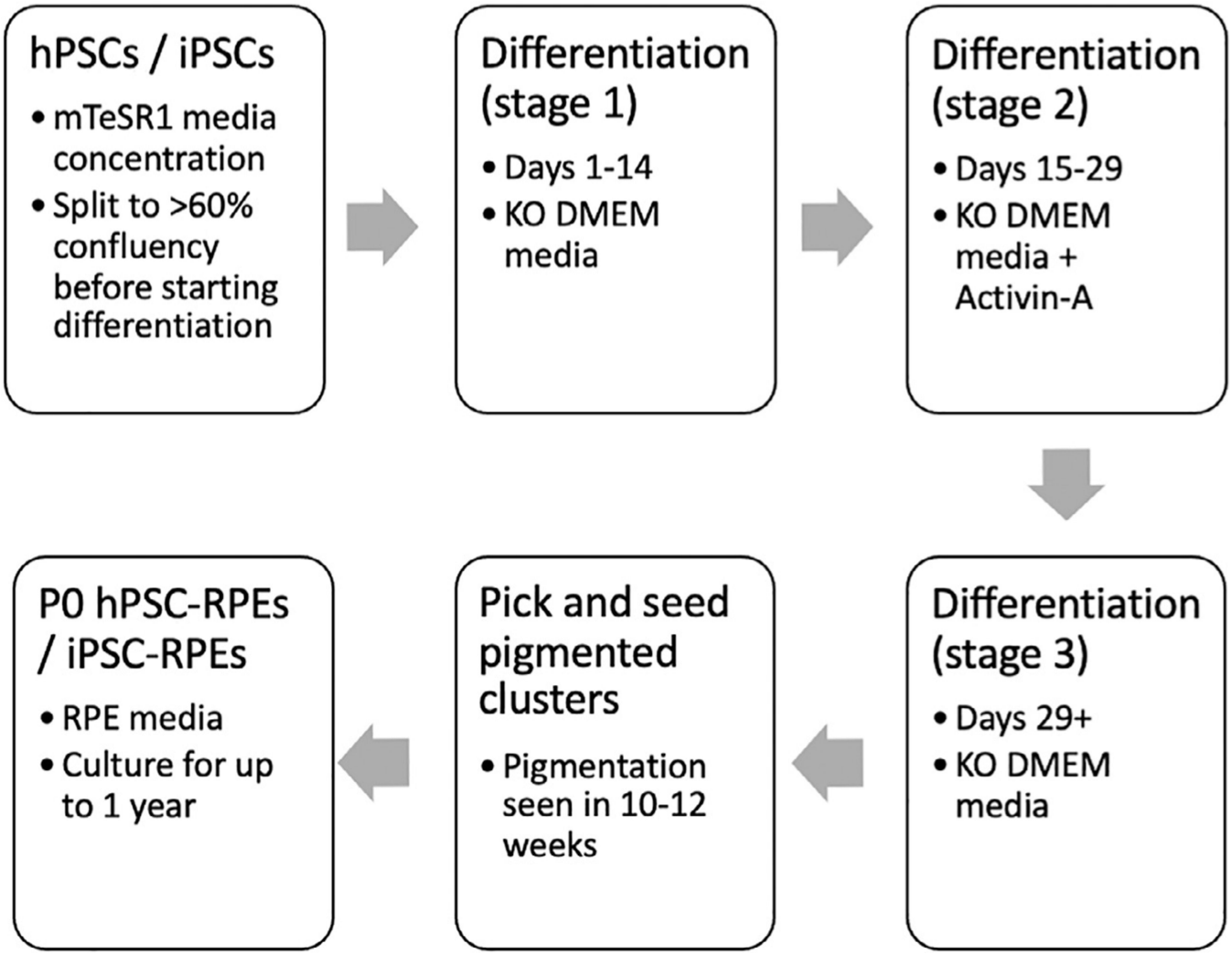

Fig. 1.

Schematic of differentiation protocol. hPSCs or iPSCs are grown to confluency in mTeSR1 media. For the first two weeks of differentiation, mTeSR1 media is replaced with differentiation media without Activin-A supplementation. The supplement is added for the next two weeks, and then stopped. Pigmentation should be observed in 10–12weeks, and the pigmented cells are manually picked and grown in RPE media until fully mature.

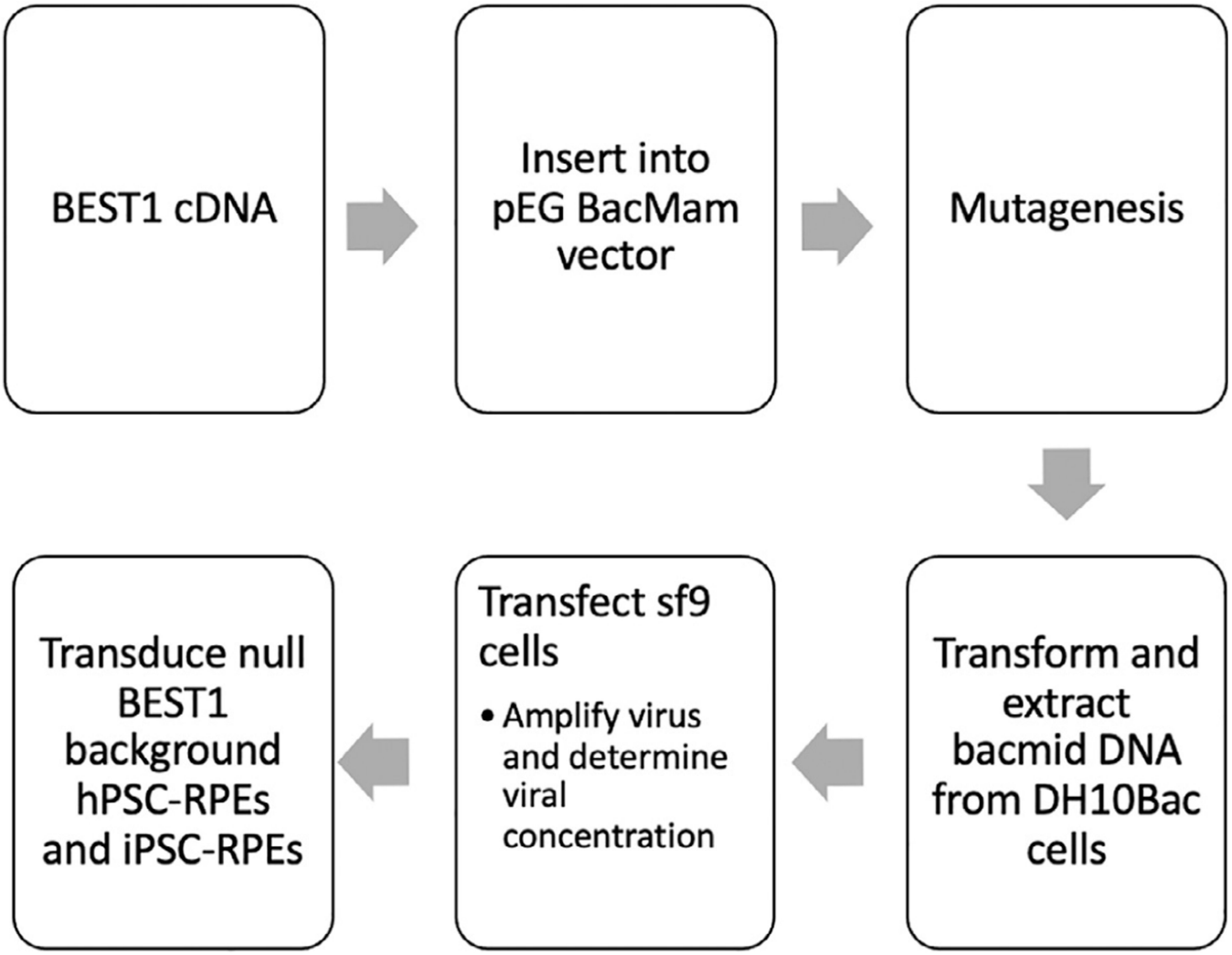

Fig. 2.

Schematic of BEST1 exogenous expression in RPE cells. BEST1 cDNA is inserted into a BacMam expression vector for mutagenesis by conventional molecular biology methods. Plasmids containing mutant BEST1 are transformed into DH10Bac cells to generate bacmids, which are extracted and transfected into Sf9 insect cells for baculovirus production. After amplification and determination of the virus titer, baculoviruses carrying mutant BEST1 are transduced into iPSC-RPEs or hPSC-RPEs with a BEST1 null background.

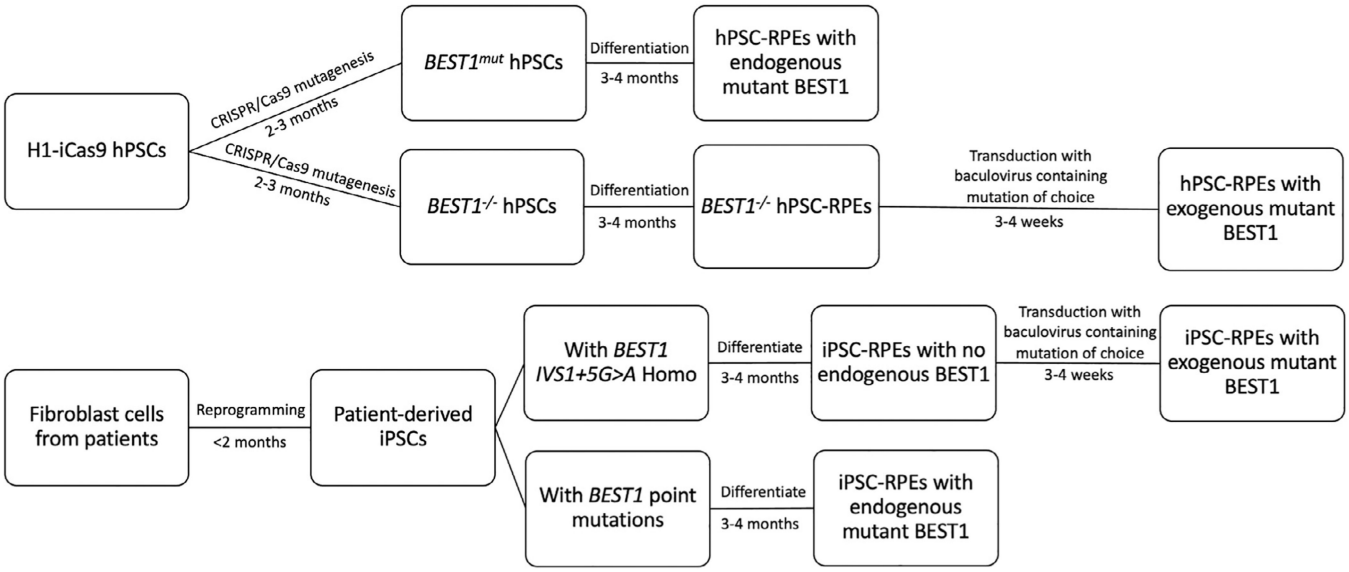

Fig. 3.

Workflow summary. Endogenous mutations in the *BEST1* gene are generated via the iCas9 system in hPSC or naturally inherited in iPSC derived from patient fibroblast cells. Exogenous expression of mutant BEST1 is mediated by baculovirus transduction of hPSC-RPEs and iPSC-RPEs with a BEST1 null background. hPSC-RPEs and iPSC-RPEs expressing mutant BEST1 are subjected to downstream assays such as immunostaining, immunoblotting, and whole-cell patch clamp.

Table 1

Recipes for differentiation and RPE medium.

	Reagent	Amount
RPE differentiation medium	KO DMEM	500 mL
	KO serum replacement	15% (75 mL)
	Nonessential amino acids	1% (5 mL)
	Glutamine	1% (5 mL)
	Penicillin-streptomycin	1% (5mL)
	Nicotinamide	10 mM
	Human Activin-A[a]	100 ng/mL
RPE culture medium	MEM (α-modification)	500 mL
	Fetal Bovine Serum	5% (25 mL)
	N1 supplement	1% (5 mL)
	Glutamine-penicillin-streptomycin	1% (5 mL)
	Nonessential amino acids	1% (5 mL)
	Taurine	125 mg
	Hydrocortisone	10 μg
	Triiodothyronine	0.0065 μg

[a]Human Activin-A is supplemented to media on days 15–28 of the differentiation protocol.

The reagents to make media necessary for the differentiation protocol and for RPE maintenance are listed.