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Supplemental Information

Engineering of PEDF-Expressing Primary Pigment Epithelial Cells by the *SB* Transposon System Delivered by pFAR4 Plasmids

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Supplemental Figures and Legends

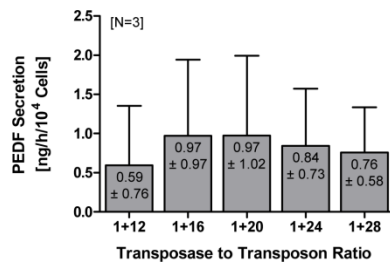


Figure S1. ELISA quantification of total PEDF secreted by primary human RPE cells transfected with varying ratios of SB100X transposase plasmid to PEDF transposon plasmid. For each donor sample, 2 transfections of 1×10^5 cells with 0.038 μg to 0.017 μg pFAR4-CMV SB100X SV40 transposase and 0.462 μg to 0.483 μg pFAR4-ITRs CMV PEDF BGH plasmid DNA were carried out. PEDF secretion was analyzed in 3 human donor eyes (age: 61.7 ± 13.3 years; gender: 2 males and 1 female; time postmortem: 20.1 ± 1.4 hours; cultivation time before transfection: 45.7 ± 17.0 days). Data are presented as mean \pm SD. Statistical analysis by one-way ANOVA with Bonferroni's Multiple Comparison Test showed no significant differences.

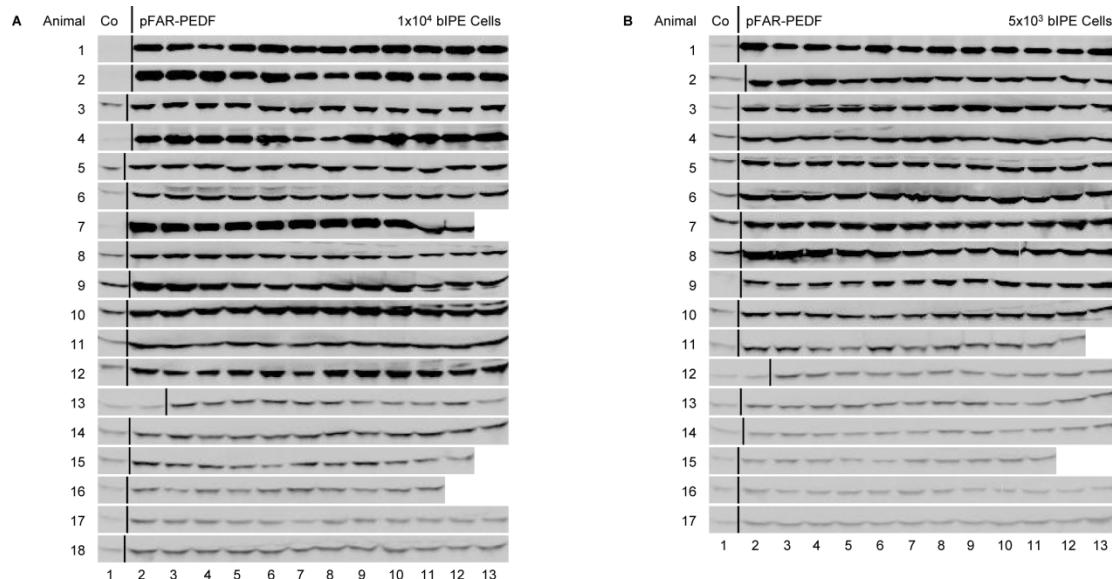


Figure S2. Western blot analysis of PEDF secretion by primary bovine IPE cells transfected with the pFAR4-ITRs CMV PEDF BGH transposon miniplasmid. Each analysis included 1 or 2 control transfections without the addition of miniplasmid DNA (column 1-2) and 10-12 transfections using 0.03 μg pFAR4-CMV SB100X SV40 transposase and 0.47 μg pFAR4-ITRs CMV PEDF BGH transposon miniplasmid DNA (columns 2/(3)-13). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. (A) Western blots of PEDF secreted by 1×10^4 transfected IPE cells isolated from 18 bovine eyes (cultivation time before transfection: 26.9 ± 9.76 days). (B) Western blots of PEDF secreted by 5×10^3 transfected IPE cells isolated from 17 bovine eyes (cultivation time before transfection: 27.9 ± 13.6 days). Note that the same exposure time was used for all chemiluminescence reactions.

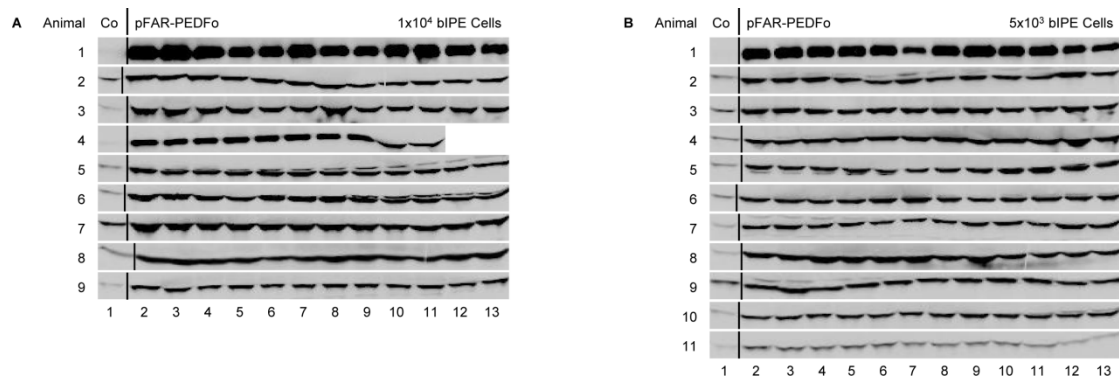


Figure S3. Western blot analysis of PEDF secretion by primary bovine IPE cells transfected with the pFAR4-ITRs CMV PEDFOptimized BGH transposon miniplasmid. Each analysis included 1 control transfection without the addition of miniplasmid DNA (column 1) and 10-12 transfections using 0.03 μg pFAR4-CMV SB100X SV40 transposase and 0.47 μg pFAR4-ITRs CMV PEDFOptimized BGH transposon miniplasmid DNA (columns 2-13). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. **(A)** Western blots of PEDF secreted by 1×10^4 transfected IPE cells isolated from 9 bovine eyes (cultivation time before transfection: 22.6 ± 7.15 days). **(B)** Western blots of PEDF secreted by 5×10^3 transfected IPE cells isolated from 11 bovine eyes (cultivation time before transfection: 29.2 ± 15.2 days). Note that the same exposure time was used for all chemiluminescence reactions.

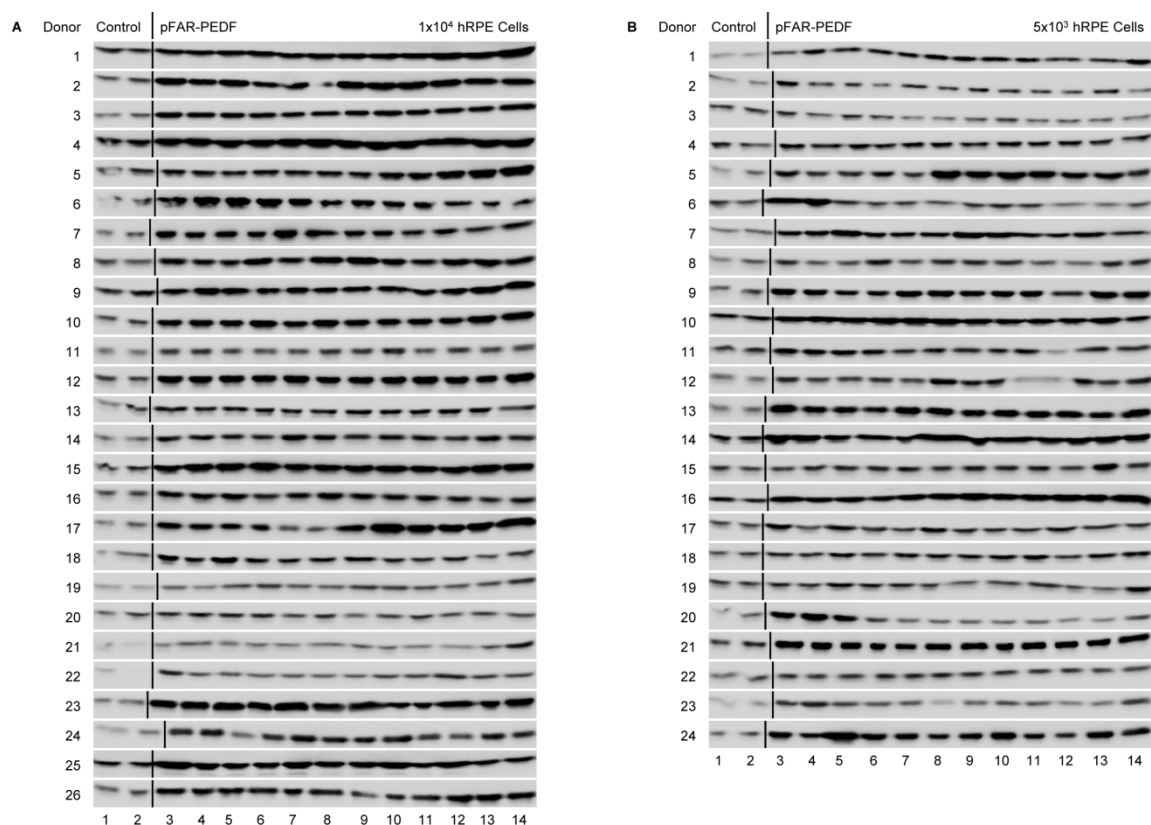


Figure S4. Western blot analysis of PEDF secretion by primary human RPE cells transfected with the pFAR4-ITRs CMV PEDF BGH transposon miniplasmid. Each analysis included 2 control transfections without the addition of miniplasmid DNA (columns 1-2) and 12 transfections using 0.03 μg pFAR4-CMV SB100X SV40 transposase and 0.47 μg pFAR4-ITRs CMV PEDF BGH transposon miniplasmid DNA (columns 3-14). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. **(A)** Western blots of PEDF secreted by 1×10^4 transfected RPE cells isolated from 26 human donor eyes (age: 67.5 ± 12.0 years; gender: 13 males and 13 females; time postmortem: 31.7 ± 14.4 hours; cultivation time before transfection: 42.0 ± 14.4 days). **(B)** Western blots of PEDF secreted by 5×10^3 transfected RPE cells isolated from 24 human donor eyes (age: 66.2 ± 14.6 years; gender: 14 males and 10 females; time postmortem:

32.9 ± 14.5 hours; cultivation time before transfection: 45.2 ± 15.8 days). Note that the same exposure time was used for all chemiluminescence reactions.

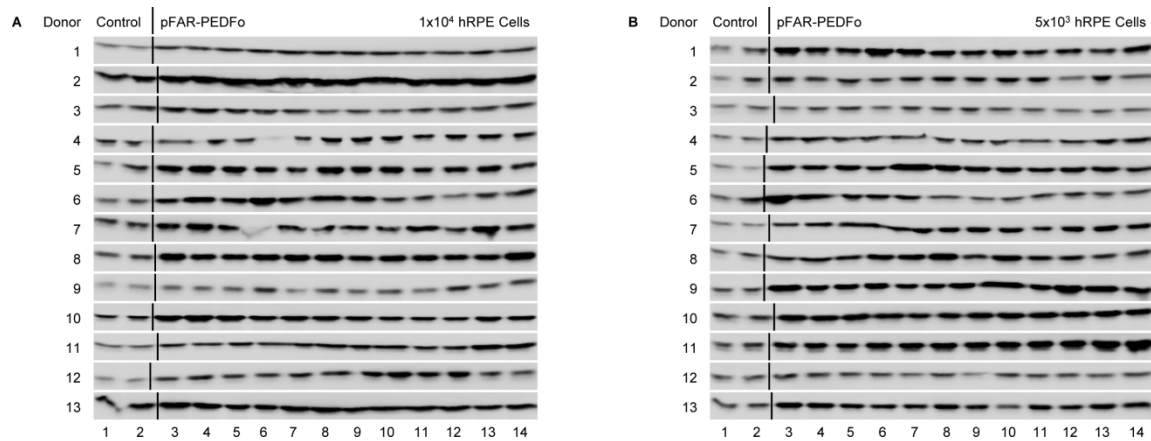


Figure S5. Western blot analysis of PEDF secretion by primary human RPE cells transfected with the pFAR4-ITRs CMV PEDFOptimized BGH transposon miniplasmid. Each analysis included 2 control transfections without the addition of miniplasmid DNA (columns 1-2) and 12 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDFOptimized BGH transposon miniplasmid DNA (columns 3-14). Culture supernatants were analyzed for total PEDF secretion using anti-PEDF antibodies 21 days after transfection. **(A)** Western blots of PEDF secreted by 1x10⁴ transfected RPE cells isolated from 13 human donor eyes (age: 61.1 ± 16.3 years; gender: 7 males and 6 females; time postmortem: 33.5 ± 16.2 hours; cultivation time before transfection: 39.8 ± 17.1 days). **(B)** Western blots of PEDF secreted by 5x10³ transfected RPE cells isolated from 13 human donor eyes (age: 61.1 ± 16.3 years; gender: 8 males and 5 females; time postmortem: 32.5 ± 16.1 hours; cultivation time before transfection: 42.6 ± 18.6 days). Note that the same exposure time was used for all chemiluminescence reactions.

Supplemental Tables

Table S1. Western blot-based quantification of long-term PEDF secretion by primary bovine IPE cells after SB100X-mediated transfection. Each analysis included 2 control transfections without the addition of miniplasmid DNA and 2 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDF BGH or pFAR4-ITRs CMV PEDFoptimized BGH miniplasmid DNA. Culture supernatants were analyzed for total PEDF secretion at times from 5 to 20 weeks. Western blot signal intensities of PEDF-transfected and PEDFo-transfected cells were normalized to the signal intensities of the control cells. Using an unpaired two-tailed t test, a statistical difference was observed between control and transfected cells, but not between cells transfected with pFAR-PEDF and pFAR-PEDFo at any time period.

| | | Time [Days] | N [Samples] | Signal Intensities | | | pFAR-PEDF versus pFAR-PEDFo | |
|--|--|----------------|----------------|--------------------|-------------|-----------------|--------------------------------|-----------------|
| | | | | Mean ± SD | Range | | | |
| 1x10⁴ bIPE Cells | Control | 49 ± 1 | 18 | 1.00 ± 0.25 | 0.54-1.46 | | | |
| | pFAR-PEDF | | | 19.9 ± 22.8 | 1.95-74.9 | **P=0.0013 | | |
| | pFAR-PEDFo | | | 17.1 ± 16.1 | 2.29-58.1 | ***P=0.0002 | Not significant | |
| | Control | 102 ± 9 | 18 | 1.00 ± 0.41 | 0.24-1.76 | | | |
| | pFAR-PEDF | | | 17.4 ± 13.2 | 1.34-42.6 | ****P<0.0001 | | |
| | pFAR-PEDFo | | | 22.6 ± 18.8 | 0.89-58.5 | ****P<0.0001 | Not significant | |
| 1x10⁴ bIPE Cells | Control | 136 ± 10 | 18 | 1.00 ± 0.30 | 0.44-1.56 | | | |
| | pFAR-PEDF | | | 15.2 ± 14.5 | 2.93-65.6 | ***P=0.0002 | | |
| | pFAR-PEDFo | | | 13.5 ± 8.74 | 2.98-29.7 | ****P<0.0001 | Not significant | |
| | Control | 221 ± 10 | 16 | 1.00 ± 0.36 | 0.30-1.70 | | | |
| | pFAR-PEDF | | | 5.97 ± 4.20 | 1.16-18.4 | ****P<0.0001 | | |
| | pFAR-PEDFo | | | 6.00 ± 4.54 | 1.53-16.9 | ***P=0.0001 | Not significant | |
| 1x10⁴ bIPE Cells | Control | 365 ± 3 | 16 | 1.00 ± 0.24 | 0.49-1.51 | | | |
| | pFAR-PEDF | | | 13.5 ± 23.7 | 0.50-73.8 | *P=0.0499 | | |
| | pFAR-PEDFo | | | 12.2 ± 22.1 | 0.19-76.3 | Not significant | Not significant | |
| | 5x10³ bIPE Cells | Control | 49 ± 1 | 11 | 1.00 ± 0.24 | 0.54-1.46 | | |
| | | pFAR-PEDF | | | 15.2 ± 14.4 | 1.75-55.6 | ****P<0.0001 | |
| | | pFAR-PEDFo | | | 12.4 ± 12.5 | 1.28-57.0 | ****P<0.0001 | Not significant |
| Control | | 103 ± 9 | 11 | 1.00 ± 0.40 | 0.24-1.76 | | | |
| pFAR-PEDF | | | | 13.9 ± 12.3 | 2.56-43.9 | ****P<0.0001 | | |
| pFAR-PEDFo | | | | 19.1 ± 17.3 | 1.00-50.6 | ****P<0.0001 | Not significant | |
| 5x10³ bIPE Cells | Control | 136 ± 9 | 11 | 1.00 ± 0.29 | 0.44-1.56 | | | |
| | pFAR-PEDF | | | 10.2 ± 6.87 | 2.08-25.1 | ****P<0.0001 | | |
| | pFAR-PEDFo | | | 8.60 ± 7.55 | 1.84-27.8 | ****P<0.0001 | Not significant | |
| | Control | 221 ± 9 | 10 | 1.00 ± 0.33 | 0.30-1.70 | | | |
| | pFAR-PEDF | | | 7.92 ± 7.18 | 1.09-30.2 | ***P=0.0001 | | |
| | pFAR-PEDFo | | | 7.88 ± 8.28 | 0.52-26.1 | ***P=0.0007 | Not significant | |
| 5x10³ bIPE Cells | Control | 365 ± 3 | 10 | 1.00 ± 0.39 | 0.00-2.00 | | | |
| | pFAR-PEDF | | | 8.64 ± 11.1 | 0.44-37.8 | **P=0.0040 | | |
| | pFAR-PEDFo | | | 8.79 ± 19.2 | 0.20-86.8 | Not significant | Not significant | |

Table S2. Western blot-based quantification of long-term PEDF secretion in primary human RPE cells after SB100X-mediated transfection. Each analysis included 2 control transfections without the addition of miniplasmid DNA and 2 transfections using 0.03 µg pFAR4-CMV SB100X SV40 transposase and 0.47 µg pFAR4-ITRs CMV PEDF BGH or pFAR4-ITRs CMV PEDFoptimized BGH miniplasmid DNA. Culture supernatants were analyzed for total PEDF secretion at the times indicated. Western blot signal intensities of PEDF-transfected and PEDFo-transfected cells were normalized to the signal intensities of the control cells. Statistical analysis by unpaired two-tailed t test showed a significant difference between transfected and non-transfected cells. No statistical significant difference was observed between cells transfected with pFAR-PEDF and pFAR-PEDFo for the first 110 days after transfection. However, at 160 days, a statistical significant decrease in PEDF secretion by pFAR-PEDF transfected cells compared to pFAR-PEDFo transfected cells was observed.

| | | Time [Days] | N [Samples] | Signal Intensities | | | pFAR-PEDF versus pFAR-PEDFo |
|---------------------------------|------------|----------------|----------------|--------------------|-----------------|--------------|--------------------------------|
| | | | | Mean ± SD | Range | | |
| 1x10 ⁴ hRPE Cells | Control | 31 ± 5 | 10 | 1.00 ± 0.15 | 0.74-1.26 | | |
| | pFAR-PEDF | | | 6.36 ± 3.27 | 2.08-11.3 | ****P<0.0001 | |
| | Control | 27 ± 1 | 4 | 1.00 ± 0.22 | 0.73-1.27 | | |
| | pFAR-PEDFo | | | 10.7 ± 4.86 | 5.07-16.7 | **P=0.0071 | Not significant |
| | Control | 61 ± 10 | 30 | 1.00 ± 0.25 | 0.56-1.44 | | |
| | pFAR-PEDF | | | 6.03 ± 4.57 | 0.93-19.3 | ****P<0.0001 | |
| | Control | 64 ± 8 | 14 | 1.00 ± 0.27 | 0.61-1.39 | | |
| | pFAR-PEDFo | | | 8.07 ± 4.14 | 1.71-15.5 | ****P<0.0001 | Not significant |
| | Control | 107 ± 8 | 30 | 1.00 ± 0.24 | 0.43-1.57 | | |
| | pFAR-PEDF | | | 6.84 ± 7.03 | 0.41-27.1 | ****P<0.0001 | |
| | Control | 108 ± 7 | 14 | 1.00 ± 0.23 | 0.51-1.49 | | |
| | pFAR-PEDFo | | | 8.13 ± 4.98 | 1.13-15.5 | ****P<0.0001 | Not significant |
| | Control | 162 ± 14 | 20 | 1.00 ± 0.15 | 0.75-1.25 | | |
| | pFAR-PEDF | | | 2.51 ± 1.72 | 0.34-6.03 | ***P=0.0004 | |
| Control | 154 ± 4 | 8 | 1.00 ± 0.17 | 0.76-1.24 | | | |
| pFAR-PEDFo | | | 4.83 ± 2.49 | 1.66-8.12 | ***P=0.0007 | **P=0.0098 | |
| Control | 215 ± 20 | 10 | 1.00 ± 0.10 | 0.85-1.15 | | | |
| pFAR-PEDF | | | 2.61 ± 2.12 | 0.78-7.33 | *P=0.0278 | | |
| Control | 193 ± 0 | 2 | 1.00 ± 0.08 | 0.94-1.06 | | | |
| pFAR-PEDFo | | | 8.10 ± 3.92 | 5.32-10.9 | Not significant | *P=0.0135 | |
| 5x10 ³ hRPE Cells | Control | 33 ± 5 | 14 | 1.00 ± 0.24 | 0.65-1.35 | | |
| | pFAR-PEDF | | | 7.51 ± 4.84 | 2.13-19.4 | ****P<0.0001 | |
| | Control | 31 ± 6 | 8 | 1.00 ± 0.28 | 0.65-1.35 | | |
| | pFAR-PEDFo | | | 6.88 ± 4.86 | 3.30-16.7 | **P=0.0042 | Not significant |
| | Control | 62 ± 12 | 30 | 1.00 ± 0.23 | 0.56-1.44 | | |
| | pFAR-PEDF | | | 7.29 ± 6.92 | 1.70-33.2 | ****P<0.0001 | |
| | Control | 64 ± 13 | 20 | 1.00 ± 0.19 | 0.76-1.24 | | |
| | pFAR-PEDFo | | | 4.79 ± 2.75 | 0.81-10.7 | ****P<0.0001 | Not significant |
| | Control | 109 ± 10 | 26 | 1.00 ± 0.23 | 0.55-1.45 | | |
| | pFAR-PEDF | | | 8.66 ± 11.4 | 1.32-55.8 | **P=0.0012 | |
| | Control | 111 ± 11 | 16 | 1.00 ± 0.17 | 0.75-1.25 | | |
| | pFAR-PEDFo | | | 8.47 ± 8.03 | 0.98-26.7 | ***P=0.0008 | Not significant |
| | Control | 162 ± 18 | 20 | 1.00 ± 0.25 | 0.56-1.44 | | |
| | pFAR-PEDF | | | 3.20 ± 2.01 | 0.73-7.79 | ****P<0.0001 | |
| Control | 153 ± 12 | 10 | 1.00 ± 0.29 | 0.56-1.44 | | | |
| pFAR-PEDFo | | | 7.33 ± 7.90 | 1.35-23.8 | *P=0.0208 | *P=0.0379 | |
| Control | 216 ± 20 | 12 | 1.00 ± 0.40 | 0.43-1.57 | | | |
| pFAR-PEDF | | | 2.87 ± 2.02 | 0.63-6.61 | **P=0.0048 | | |
| Control | 201 ± 11 | 4 | 1.00 ± 0.47 | 0.43-1.57 | | | |
| pFAR-PEDFo | | | 7.53 ± 1.32 | 6.33-9.40 | ****P<0.0001 | ***P=0.0010 | |

Table S3. Primer sequences for integration site profiling of the pFAR4-ITRs CMV PEDF BGH transposon in human RPE cells.

| | |
|------------------------|--|
| SB_20_hmr | AACTTAAGTGTATGTAAACTTCCGACT |
| 4mer primers | |
| atcc_asPE | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTHNNHNNNatcc |
| ggat_asPE | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTBBBNNNggat |
| gaca_asPE | GGACTGGAGTTCAGACGTGTGCTCTTCCGATCTVVVNNNgaca |
| gcaa_asPE | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTVNVNVNgcaa |
| tgte_asPE | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTHHHNNNtgte |
| ttgc_asPE | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTHHHNNNttgc |
| SB-7 | cttAAGTGTATGTAAACTTCCGACTTCA |
| PE_first | Gtgactggagttcagacgtg |
| SB_PE_noTA_BC_1 | ACACTCTTTCCTACACGACGCTCTTCCGATCTATCACGatGTAAACTTCCGACTTCAACTG |
| SB_PE_noTA_BC_2 | ACACTCTTTCCTACACGACGCTCTTCCGATCTCGATGTatGTAAACTTCCGACTTCAACTG |
| SB_PE_noTA_BC_3 | ACACTCTTTCCTACACGACGCTCTTCCGATCTTTAGGCatGTAAACTTCCGACTTCAACTG |
| SB_PE_noTA_BC_4 | ACACTCTTTCCTACACGACGCTCTTCCGATCTTGACCAatGTAAACTTCCGACTTCAACTG |
| PE nest | CAAGCAGAAGACGGCATAACGAGAT_reverse_complement_of_Illumina_Truseq_barcode_GTGACTGG AGTTCAGACGTGTGCTCTTCCGATCT |
| Illumina 1 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCTACACGACGCTCTTCCGATCT Oligonucleotide sequences© 2006-2010 Illumina, Inc All rights reserved. |