

Supplementary Information

for

Flow cytometry-based quantification of targeted knock-in events in human cell lines using a GPI-anchor biosynthesis gene *PIGP*

Md. Lutfur Rahman, Toshinori Hyodo, Muhammad Nazmul Hasan, Yuko Mihara, Sivasundaram Karnan, Akinobu Ota, Shinobu Tsuzuki, Yoshitaka Hosokawa, and Hiroyuki Konishi*

Department of Biochemistry, Aichi Medical University School of Medicine, Nagakute, Aichi 480-1195, Japan

* Corresponding author

Hiroyuki Konishi

Department of Biochemistry, Aichi Medical University School of Medicine

1-1 Yazako Karimata, Building #2, Room 362,

Nagakute, Aichi 480-1195, Japan

Phone: +81-561-62-3311 ext. 12362

Fax: +81-561-61-4056

E-mail: hkonishi@aichi-med-u.ac.jp

- **Supplementary Figures 1 and 2**
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Supplementary Figure 1

Donor-*PIGP*ex3

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-174          #1          -154          #2          -127          -75
--aaacgagcta gaagaactga aaggaggaaa aaatcatttc tgagttaatc agggacactt caaagagct tttctgtttt atctgaatct taatagattg
--tttgctcgat cttcttgact ttctctcttt tttagtaaag actcaattag tccctgtgaa gttttctcga aaagacaaaa tagacttaga attatctaac

-74
atagattaga tgtatgtctt actgacttct cttcctcttt tttttctttt tctttttctac agTACTTTAC CTCGTGTGGG CCTTTATTCC TGAATCTTGC
tatctaactc acatacagaa tgactgaaga gaaggagaaa aaaaagaaaa agaaaagatg tcATGAAATG GAGCACCCC GGAAATAAGG ACTTAGAAC
                                     I L Y L V W A F I P E S W

+26
CTAAACTCTT TAGGTTTAACT CTATTGGCCT CAAAAGtaag ttttaatatat ttctttaaag aggatataat taatgctggt ttgaggagtc atatcatttc--
GATTTGAGAA ATCCAAATG GATAACCGGA GTTTTcattc aaattatata aagaatttac tccatatta attacgacaa aactcctcag tatagtaaag--
L N S L G L T Y W P Q K

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Genomic region surrounding *PIGP* exon 3

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-174          #1          -154          #2          -127          -75
--aaacgagcta gaagaactga aaggaggaaa aaatcatttc tgagttaatc agggacactt caaagagct tttctgtttt atctgaatct taatagattg
--tttgctcgat cttcttgact ttctctcttt tttagtaaag actcaattag tccctgtgaa gttttctcga aaagacaaaa tagacttaga attatctaac

-74
atagattaga tgtatgtctt actgacttct cttcctcttt tttttctttt tctttttctac agTACTTTAC CTCTAGAGGG CCTTTATTCC TGAATCTTGC
tatctaactc acatacagaa tgactgaaga gaaggagaaa aaaaagaaaa agaaaagatg tcATGAAATG GAGATCTCCC GGAAATAAGG ACTTAGAAC
                                     E -1 ±0 I L Y L * R A F I P E S W

+26
CTAAACTCTT TAGGTTTAACT CTATTGGCCT CAAAAGtaag ttttaatatat ttctttaaag aggatataat taatgctggt ttgaggagtc atatcatttc--
GATTTGAGAA ATCCAAATG GATAACCGGA GTTTTcattc aaattatata aagaatttac tccatatta attacgacaa aactcctcag tatagtaaag--
L N S L G L T Y W P Q K

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Supplementary Figure 1 DNA sequences surrounding the knock-in site at *PIGP* exon 3 in the Donor-*PIGP*ex3 plasmid (top) and the DLD-1-derived *PIGP* reporter clone (bottom). Target sequences of a Cas9 nuclease and Cas9 nickases are shaded in blue and green, respectively, with darker shading on their neighboring protospacer adjacent motifs (PAMs). Numbers with plus or minus signs near Cas9 target sites indicate the distance from the knock-in site (± 0) to theoretical cleavage sites within individual Cas9 target sites. Nucleotides shown by red and green bold letters represent a *PIGP* truncating mutation in the DLD-1-derived *PIGP* reporter clone and its corresponding wild-type sequence in Donor-*PIGP*ex3, respectively. Upper case and lower case letters indicate exonic and intronic sequences, respectively.

Supplementary Figure 2

Donor-*PIGPex2*

-134 #5 -93 **-35**
 --gtaccgcg cggagcgagg aggcgagaat **ggatcaatgg** **tgcca****cgga** cacatcgctg acgctgattg tgttcctttt ccacagATTG TCTAAAGCCC
 --catgggcgc gcctcgctcc tccgctctta cctagttacc ac**ggtgcctc** **gtgtagcgac** **tcgactaac** acaaggaaaa ggtgtcTAAC AGATTTCGGG
-86 #6

-34 **±0** **+65**
 CAGGAAAAAT GGTGGAAAAAT TCACCGTCGC **CAT****TGCC**AAGA AAGAGCGATT TATGGCTTTG TTCTTTTCTT AAGCTCCCAA TTTGGCTTCA gtaagtactt
 GTCCTTTTTA CCACCTTTTA AGTGGCAGCG **GTA****ACGG**TCT TTCTCGCTAA ATACCGAAAC AAGAAAAGAA TTCGAGGGTT AAACCGAAGT cattcatgaa
 M V E N S P S P **L P** E R A I Y G F V L F L S S Q F G F

+66 #8 +118 **+165**
 tcctgatgga tggccagggg ctttttctgg cgttcagtga **caaggaaga** **atacat****tggt** tagactgtgc acacatgaac tgttgattct ttctccctat--
 aggactacct acc**ggtcccc** **gaaaaagacc** **gcaagt**cact gttcccttct tatgtaacca atctgacacg tgtgtacttg acaactaaga aagagggata--
+85 #7

Genomic region surrounding *PIGP* exon 2

-134 #5 -93 **-35**
 --gtaccgcg cggagcgagg aggcgagaat **ggatcaatgg** **tgcca****cgga** cacatcgctg acgctgattg tgttcctttt ccacagATTG TCTAAAGCCC
 --catgggcgc gcctcgctcc tccgctctta cctagttacc ac**ggtgcctc** **gtgtagcgac** **tcgactaac** acaaggaaaa ggtgtcTAAC AGATTTCGGG
-86 #6

-34 F -6 **±0** **+65**
 CAGGAAAAAT GGTGGAAAAAT TCACCGTCGC **CAT****TG****TA**AAGA AAGAGCGATT TATGGCTTTG TTCTTTTCTT AAGCTCCCAA TTTGGCTTCA gtaagtactt
 GTCCTTTTTA CCACCTTTTA AGTGGCAGCG **GTA****CC****AT**TCT TTCTCGCTAA ATACCGAAAC AAGAAAAGAA TTCGAGGGTT AAACCGAAGT cattcatgaa
 M V E N S P S P **W *** E R A I Y G F V L F L S S Q F G F

+66 #8 +118 **+165**
 tcctgatgga tggccagggg ctttttctgg cgttcagtga **caaggaaga** **atacat****tggt** tagactgtgc acacatgaac tgttgattct ttctccctat--
 aggactacct acc**ggtcccc** **gaaaaagacc** **gcaagt**cact gttcccttct tatgtaacca atctgacacg tgtgtacttg acaactaaga aagagggata--
+85 #7

Supplementary Figure 2 DNA sequences surrounding the knock-in site at *PIGP* exon 2 in the Donor-*PIGPex2* plasmid (top) and the HCT116-derived *PIGP* reporter clone (bottom). For annotations of the numbers and colors shown in this figure, see the legend for Supplementary Figure 1.

Supplementary Table 1: Cas9 target sites with PAMs

Target name	Sequence
<i>Creation of the initial deletion</i>	
<i>PIGP-nuclease-A</i>	ACTGTTGTGAGGATTAACGGGG <u>GGG</u>
<i>PIGP-nuclease-B</i>	AAGGGATGACTAGTTCACAT <u>TGG</u>
<i>Extension of the deletion in HCT116</i>	
<i>TTC3-nuclease-C</i>	TAAATCCATAGGCTGACACAG <u>GGG</u>
<i>Junction-nuclease-D</i>	CTGTTGTGAGGATTAACAT <u>TGGG</u>
<i>PIGP correction assay using the DLD-1-derived PIGP reporter clone</i>	
<i>PIGP-nickase-1</i>	GAGCTAGAAGAAGACTGAAAGG <u>AGG</u>
<i>PIGP-nickase-2</i>	AATCATTTCTGAGTTAATCAG <u>GGG</u>
<i>PIGP-nickase-3</i>	GGGCCTTTATTCCTGAATCT <u>TGG</u>
<i>PIGP-nickase-4</i>	TTAATATATTTCTTAAATG <u>AGG</u>
<i>PIGP-nuclease-E</i>	TACAGTACTTTACCTCTAGAG <u>GGG</u>
<i>PIGP correction assay using the HCT116-derived PIGP reporter clone</i>	
<i>PIGP-nickase-5</i>	AGAATGGATCAATGGTGCCAC <u>GGG</u>
<i>PIGP-nickase-6</i>	AGCGTCAGCGATGTGCTCCG <u>TGG</u>
<i>PIGP-nickase-7</i>	TGAACGCCAGAAAAAGCCCT <u>TGG</u>
<i>PIGP-nickase-8</i>	GTGACAAGGGAAGAATACAT <u>TGG</u>
<i>PIGP-nuclease-F</i>	TGGAAAATTCACCGTCGCCAT <u>TGG</u>

PAM, protospacer adjacent motif

Underlining indicates PAM sequences.

Supplementary Table 2: PCR primers generated in this study

Primer name	Sequence
<i>Creation of dual sgRNA vectors</i>	
U6 promoter-F	5'-CCTAGGT <u>CTAGAG</u> GAGGGCCTATTTCCCATGATTCC
CAG-R	5'-CCATTTACCGTAAGTTATGTAAC
<i>Creation of Donor-PIGPex3</i>	
PIGPint2-F2	5'-CTTTTAGAGCTCATTTCGTGAGCACTTGTAAC
PIGPint3-R	5'-TTTTCTGGATCCTGCATTATCTCTATTTTCCTT
<i>Creation of Donor-PIGPex2</i>	
TTC3ex1-R	5'-CTCGGCTGCAGCGGGACAA
PIGPint2-R1	5'-CCTACCCCATAGAACTGTTTCATGCGTC
<i>Analytical PCRs probing deletions</i>	
PIGPint2-F1	5'-TGGCTGACTTCACTCTGCTG
PIGPint2-R2	5'-AGCTTCTAATCCTTGACATACTCTT
PIGPdownstream-F1	5'-CAGGGTGAGGCAACTAAGCA
PIGPdownstream-R1	5'-CCAGCCTACAAAGCCCTTCA
TTC3int1-F	5'-GCCACCTTGGCTTCTCTTGT
TTC3int1-R	5'-CACCTCACCTGCCACTAAAG
<i>Production of PIGP Southern probe</i>	
PIGPdownstream-F2	5'-CTGAGTTTTGGTCAAGCAGGG
PIGPdownstream-R2	5'-ACACTAAACTGATCATGACCCTCA

PCR, polymerase chain reaction

Underlining indicates the restriction enzyme sites used for the incorporation of PCR products into a plasmid.