**Supplementary File 2 – List of plasmids used in this study**

|  |  |  |
| --- | --- | --- |
| **Plasmid name** | **Reference** | **Comment** |
|  |  |  |
| **General** |  |  |
| pCMV-PE2  | Anzalone et al. | Addgene #132775 |
| pX330-Flag-wtSpCas9-H840A | Unpublished | Welker Lab, Addgene #80453 |
| pX330-Flag-dSpCas9 | Kulcsar et al. | Addgene #92113 |
|  |
| **PEAR target plasmids** |
| pDAS12124\_PEAR-GFP-1-preedited | This study |  |
| pDAS12125\_PEAR-GFP-1 | This study |  |
| PEAR-GFP-2 | Tálas et al. | pAT9651-BEAR-GFP (Addgene #162989)  |
| PEAR-mScaret | Tálas et al. | pAT9752-BEAR-mScarlet (Addgene #162991) |
| pAT9624-BEAR-cloning | Tálas et al. | Addgene #162986 |
| 12420\_PEAR-GFP\_insGT-AAGT | This study |  |
| 12421\_PEAR-GFP\_insG-GT | This study |  |
| 12422\_PEAR-GFP\_insCAG\_subGT | This study |  |
| 12423\_PEAR-GFP\_insGT\_subAAGT | This study |  |
| 12424\_PEAR-GFP\_insAG\_subGT | This study |  |
| 12425\_PEAR-GFP\_del\_CAGA\_to\_CAG | This study |  |
| 12426\_PEAR-GFP\_del\_CAGAA\_to\_CAG | This study |  |
| 12427\_PEAR-GFP\_del\_CTAG\_to\_CAG | This study |  |
| 12428\_PEAR-GFP\_del\_GAT\_to\_GT | This study |  |
| 12429\_PEAR-GFP\_del\_GACT\_to\_GT | This study |  |
| 12430\_PEAR-GFP\_del\_CACAG\_to\_CAG | This study |  |
| 12431\_PEAR-GFP\_delTGA | This study |  |
| 12432\_PEAR-GFP\_CAG-CT-AAGT | This study |  |
| 12433\_PEAR-GFP\_CAG-AC-TGCG | This study |  |
| 12434\_PEAR-GFP\_CAG-CT-TGCG | This study |  |
| 12435\_PEAR-GFP\_CAA-AC-AAGT | This study |  |
| 12436\_PEAR-GFP\_CAA-CT-AAGT | This study |  |
| 12439\_PEAR-GFP\_CAG-AC | This study |  |
| 12440\_PEAR-GFP\_CAG-CT | This study |  |
| 12443\_PEAR-GFP\_CAG-GT-AAGT | This study |  |
|  |  |  |
| **2in1 PEAR plasmids** |
| pDAS12342\_PEAR-GFP\_2in1 | This study |  |
| 12488\_2in1\_EGFPs7\_9 PBS 24 RT  | This study |  |
| 12489\_2in1\_EGFPs7\_12 PBS 24 RT  | This study |  |
| 12490\_2in1\_EGFPs7\_15 PBS 24 RT  | This study |  |
| 12488\_2in1\_EGFPs2\_9 PBS 24 RT  | This study |  |
| 12489\_2in1\_EGFPs2\_12 PBS 24 RT  | This study |  |
| 12490\_2in1\_EGFPs2\_15 PBS 24 RT  | This study |  |
|  |  |  |
| **pegRNA plasmids** |
| 12069-U6-pegRNA-mCherry | This study | target cloning plasmid |
| 12222-U6-pegRNA-BFP | This study | target cloning plasmid |
|  |  |  |
| 12227\_U6-pegRNA-PEAR-GFP\_10PBS-16RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12228\_U6-pegRNA-PEAR-GFP\_13PBS-16RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12229\_U6-pegRNA-PEAR-GFP\_16PBS-16RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12230\_U6-pegRNA-PEAR-GFP\_10PBS-24RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12231\_U6-pegRNA-PEAR-GFP\_13PBS-24RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12232\_U6-pegRNA-PEAR-GFP\_16PBS-24RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12233\_U6-pegRNA-PEAR-GFP\_10PBS-33RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12234\_U6-pegRNA-PEAR-GFP\_13PBS-33RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
| 12235\_U6-pegRNA-PEAR-GFP\_16PBS-33RT\_mCherry | This study | targeting the PEAR-GFP plasmid |
|  |  |  |
| 12248\_U6-pegRNA-PEAR-GFP-2\_10PBS-22RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12249\_U6-pegRNA-PEAR-GFP-2\_13PBS-22RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12250\_U6-pegRNA-PEAR-GFP-2\_16PBS-22RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12251\_U6-pegRNA-PEAR-GFP-2\_10PBS-26RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12252\_U6-pegRNA-PEAR-GFP-2\_13PBS-26RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12253\_U6-pegRNA-PEAR-GFP-2\_16PBS-26RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12254\_U6-pegRNA-PEAR-GFP-2\_10PBS-33RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12255\_U6-pegRNA-PEAR-GFP-2\_13PBS-33RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
| 12256\_U6-pegRNA-PEAR-GFP-2\_16PBS-33RT\_mCherry | This study | targeting the PEAR-GFP-2 plasmid and the PEAR-GFP cell line |
|  |  |  |
| 12257\_U6-pegRNA-PEAR-mScarlet-target1\_10PBS-31RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12258\_U6-pegRNA-PEAR-mScarlet-target1\_13PBS-31RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12259\_U6-pegRNA-PEAR-mScarlet-target1\_16PBS-31RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12260\_U6-pegRNA-PEAR-mScarlet-target1\_10PBS-35RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12261\_U6-pegRNA-PEAR-mScarlet-target1\_13PBS-35RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12262\_U6-pegRNA-PEAR-mScarlet-target1\_16PBS-35RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12263\_U6-pegRNA-PEAR-mScarlet-target1\_10PBS-39RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12264\_U6-pegRNA-PEAR-mScarlet-target1\_13PBS-39RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
| 12265\_U6-pegRNA-PEAR-mScarlet-target1\_16PBS-39RT\_BFP | This study | targeting the PEAR-mScarlet - target1 plasmid and the PEAR-mScarlet cell line |
|  |  |  |
| 12266\_U6-pegRNA-PEAR-mScarlet-target2\_10PBS-38RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12267\_U6-pegRNA-PEAR-mScarlet-target2\_13PBS-38RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12268\_U6-pegRNA-PEAR-mScarlet-target2\_16PBS-38RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12269\_U6-pegRNA-PEAR-mScarlet-target2\_10PBS-42RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12270\_U6-pegRNA-PEAR-mScarlet-target2\_13PBS-42RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12271\_U6-pegRNA-PEAR-mScarlet-target2\_16PBS-42RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12272\_U6-pegRNA-PEAR-mScarlet-target2\_10PBS-46RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12273\_U6-pegRNA-PEAR-mScarlet-target2\_13PBS-46RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
| 12274\_U6-pegRNA-PEAR-mScarlet-target2\_16PBS-46RT\_BFP | This study | targeting the PEAR-mScarlet - target2 |
|  |  |  |
| 12410\_EMX1 pegRNA\_15 PBS 13 RT\_TagBFP | This study | Targeting EMX1 genomic target |
| 12411\_RNF2 pegRNA\_15 PBS 14 RT\_TagBFP | This study | Targeting RNF2 genomic target |
| 12412\_FANCF pegRNA\_8 PBS 17 RT\_TagBFP | This study | Targeting FANCF genomic target |
| 12413\_HEK3 pegRNA13 PBS 10 RT\_TagBFP | This study | Targeting HEK3 genomic target |
| 12414\_HEK4 pegRNA\_8 PBS 10 RT\_TagBFP | This study | Targeting HEK4 genomic target |
| 12454\_HEK3 +1 CTT ins pegRNA\_BFP | This study | Targeting HEK3 genomic target |
| 12455\_EMX1 +5G del pegRNA\_BFP | This study | Targeting EMX1 genomic target |
| 12456\_RUNX1 +1 ATG ins pegRNA\_BFP | This study | Targeting RUNX1 genomic target |
| 12457\_RUNX1 +2 G del pegRNA\_BFP | This study | Targeting RUNX1 genomic target |
| 12460\_DNMT +3-5 AAG del pegRNA\_BFP | This study | Targeting DNMT genomic target |
| 12462\_FANCF +2C del\_+5GtoT pegRNA\_BFP | This study | Targeting FANCF genomic target |
| 12474\_HBB\_+4 A to T\_pegRNA\_BFP | This study | Targeting HBB genomic target |
| 12475\_HEXA\_+1 TATC ins\_pegRNA\_BFP | This study | Targeting HEXA genomic target |
| 12476\_PRNP\_+6 G to T\_pegRNA\_BFP | This study | Targeting PRNP genomic target |
|  |  |  |
| 12449\_PEAR-GFP pegRNA\_G-GT-TGCG\_10 PBS 24 RT |  |  |
| 12450\_PEAR-GFP pegRNA\_A-GT-AAGT\_10 PBS 24 RT |  |  |
| 12452\_PEAR-GFP pegRNA\_G-GT\_10 PBS 24 RT |  |  |
|  |  |  |
| **sgRNA plasmids** |  |
| 12136\_U6-sgRNA-PEAR-GFP\_nick(+89)\_mCherry | This study | Secondary nick on the PEAR-GFP plasmid |
| 12137\_U6-sgRNA-PEAR-GFP\_nick(+17)\_mCherry | This study | Secondary nick on the PEAR-GFP plasmid |
| 12138\_U6-sgRNA-PEAR-GFP\_nick(-59)\_mCherry | This study | Secondary nick on the PEAR-GFP plasmid |
| 12139\_U6-sgRNA-PEAR-GFP\_nick(-100)\_mCherry | This study | Secondary nick on the PEAR-GFP plasmid |
| 12140\_U6-sgRNA-PEAR-GFP\_nick(-162)\_mCherry | This study | Secondary nick on the PEAR-GFP plasmid |
|  |  |  |
| 12198\_U6-sgRNA-PEAR-GFP-2\_nick(+103)\_mCherry | This study | Secondary nick on the PEAR-GFP-2 plasmid, and in the PEAR-GFP cell line |
| 12199\_U6-sgRNA-PEAR-GFP-2\_nick(+126)\_mCherry | This study | Secondary nick on the PEAR-GFP-2 plasmid, and in the PEAR-GFP cell line |
| 12200\_U6-sgRNA-PEAR-GFP-2\_nick(+ 17)\_mCherry | This study | Secondary nick on the PEAR-GFP-2 plasmid, and in the PEAR-GFP cell line |
|  |  |  |
| 12210\_U6-sgRNA-PEAR-mScarlet\_nick(+103)\_BFP | This study | Secondary nick on the PEAR-mScarlet plasmid and in the PEAR-mScarlet cell line |
| 12211\_U6-sgRNA-PEAR-mScarlet\_nick(+126)\_BFP | This study | Secondary nick on the PEAR-mScarlet plasmid and in the PEAR-mScarlet cell line |
| 12212\_U6-sgRNA-PEAR-mScarlet\_nick(+ 17)\_BFP | This study | Secondary nick on the PEAR-mScarlet plasmid and in the PEAR-mScarlet cell line |
|  |  |  |
| 12415\_U6-sgRNA-EMX1-nick(-57)\_BFP | This study | Secondary nick in the genome |
| 12416\_U6-sgRNA-RNF2 +41 nick\_BFP | This study | Secondary nick in the genome |
| 12417\_U6-sgRNA-FANCF +48 nick\_BFP | This study | Secondary nick in the genome |
| 12418\_U6-sgRNA-HEK3 +90 nick\_BFP | This study | Secondary nick in the genome |
| 12419\_U6-sgRNA-HEK4 -95 nick\_BFP | This study | Secondary nick in the genome |
| 12463\_U6-sgRNA-EMX1 +53 nick\_BFP | This study | Secondary nick in the genome |
| 12464\_U6-sgRNA-RUNX1 +38 nick\_BFP | This study | Secondary nick in the genome |
| 12466\_U6-sgRNA-DNMT +49 nick\_BFP | This study | Secondary nick in the genome |
| 12479\_U6-sgRNA-HBB nick\_BFP | This study | Secondary nick in the genome |
| 12480\_U6-sgRNA-HEXA nick\_BFP | This study | Secondary nick in the genome |
| 12481\_U6-sgRNA-PRNP nick\_BFP | This study | Secondary nick in the genome |
|  |  |  |
| pAT9922\_U6-sgRNA-mock-mCherry | This study | mock sgRNA used in when not nicking the plasmid/genome |
| 9762\_U6-sgRNA-mock-TagBFP | This study | mock sgRNA used in when not nicking the plasmid/genome |