	1	10	20	30	40	50	60	70	80	90	100
			20	30	40		60	70	80	90	
SWEET10-GFP repair product extraction 2				TATATGC IA/	ATAACTGACA.	ATACIGIAA				GAAAACAAGC	GAAGGTTTCCAGT
			171. SWEET	10a g-F							
FWD TA_31large-a-M13F.ab1											
REV TA_31large-a-M13R.ab1											
	10	120	130	140	150	160	170	180	190	200	210
	10	120	130	140	150	160	170	180	190	200	210
SWEET10-GFP repair product extraction 2	CTATTCCA		GCACTATTTA	GTGCTATGC	<mark>ГGТGĠТТ</mark> GТТ	CTATGCAATT	TTTGATGAGG.	ATTCCACCCT	TCTCATCACC	ATTAACTCCT	TCACTTTCTTCAT
FWD TA_31large-a-M13F.ab1											
REV TA_31large-a-M13R.ab1											
	220	230	240	250	260	270	280	290	300	310	320
SWEET10-GFP repair product extraction 2	220 GGAGACTG	230	240	250 TATCTATGC	260	270 GACAGGG <mark>T</mark> AT	280	290 AACACTACTT	300 CCC <mark>TGATTTA</mark>	310 AAGAAC <mark>TTT</mark> G	320 TCAGAGTGGGATA

FWD TA_31large-a-M13F.ab1

REV TA_31large-a-M13R.ab1

	330	340	350	360	370	380	390	400	410	420	430
	330	340	350	360	370	380	390	400	410	420	430
SWEET10-GFP repair product extraction 2	TGTTCA,	A <mark>TTCATACAGAA</mark>	GCAAICIII	GTTTCAGATGT		LHA	CTTCTTCAATA	ATTTTT <mark>GGTT</mark>	T <mark>C</mark> GGAA <mark>T</mark> GATC	GCTATCTTA	

FWD TA_31large-a-M13F.ab1

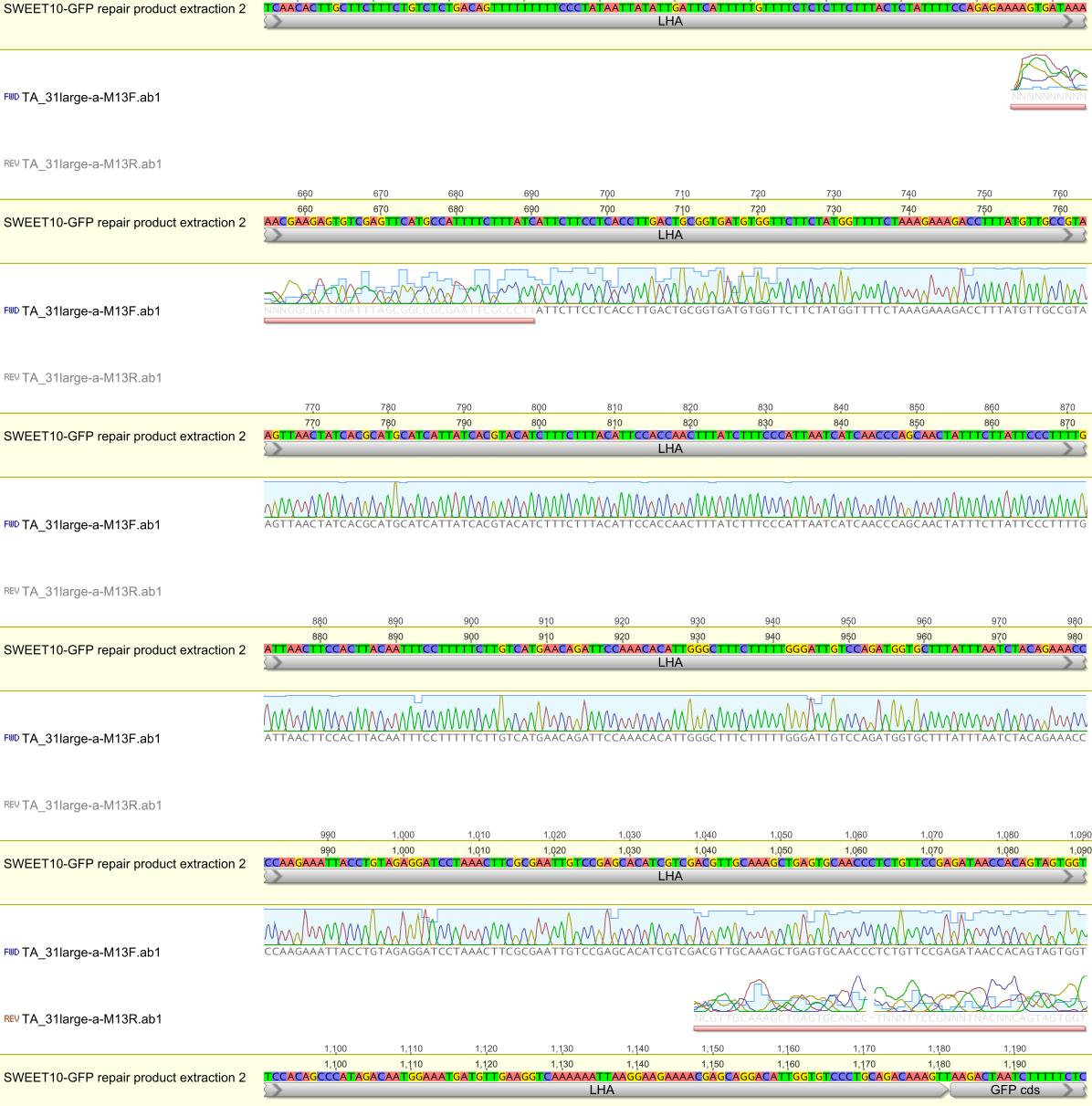
REV TA_31large-a-M13R.ab1

	440	450	460	470	480	490	500	510	520	530	540
	440	450	460	470	480	490	500	510	520	530	540
SWEET10-GFP repair product extraction 2		CGCAAACGCG		I GGA I GGA I I		LHA			GIAICAIGG	IGAGAA IGCGA	AGTCGCAAATT

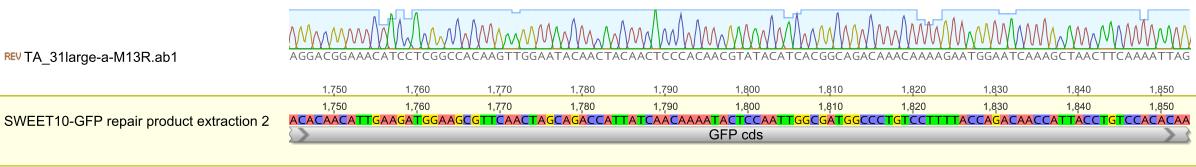
FWD TA_31large-a-M13F.ab1

REV TA_31large-a-M13R.ab1

550	560	570	580	590	600	610	620	630	640	650
550	560	570	580	590	600	610	620	630	640	650



F₩D TA_31large-a-M13F.ab1			TGGAAATGAT	GTTGAAGGT		AGGAAGAAAA		ATTGGTGTCCC	TGCAGACAA	AGTTAAGACT/	
REV TA_31large-a-M13R.ab1								ATTGNTGTCC-	-TGCAGACAA		
		1,210	1,220	1,230	1,240	1,250	1,260	1,270	1,280	1,290	1,300
SWEET10-GFP repair product extraction 2		1,210 TTTTCACTT	1,220 C <mark>TCCAATCAT</mark>	1,230 TATCCTCGG	1,240 CCGAATTCAG	1,250 TAAAGGAGAA GFP cds	1,260 <mark>\GAACTTTTCA</mark> S	1,270 C <mark>TGGAGTTGTC</mark>	1,280 CCC <mark>AATTCTT</mark>	1,290 GTTGAATTAG/	1,300 ATGGTGATGTTA
FWD TA_31large-a-M13F.ab1	СТСТСАТС			TATCCTCGG	CCGAATTCAG		GAACTTTTCA	CTGGAGTTGTC		GTTGAATTAG	
REV TA_31large-a-M13R.ab1					CCGAATTCAG	TAAAGGAGAA		CTGGAGTTGTC		GTTGAATTAG	ATGGTGATGTTA
SWEET10-GFP repair product extraction 2	1,310 1,310 ATGGGCACA	1,320 1,320 \AATTTTCTG	1,330 1,330 <mark>TCAGTGGAGA</mark>	1,340 1,340 . <mark>GGGTGAAGG</mark>	1,350 1,350 <mark>TGATGCAACA</mark>	1,360 1,360 TACGGAAAAC GFP cds		1,380 1,380 <mark>ATTTAITTTGC</mark> A	1,390 1,390 \C <mark>TACTGGAA</mark>	1,400 1,400 AAC <mark>TACCTGT</mark>	1,410 1,410 FCCATGGCCAAC
FWD TA_31large-a-M13F.ab1	ATGGGCACA	AATTTTCTG			TGATGCAACA	TACGGAAAAC		ATTTATTTGCA			
REV TA_31large-a-M13R.ab1	ATGGGCACA					TACGGAAAAC					
SWEET10-GFP repair product extraction 2	1,420 1,420 ACTTGTCAC	1, <u>4</u> 30 1,430 TACTTTCAC	1,440 1,440 TTATGGTGTT	1,450 1,450 CAATGCTTT	1,460 1,460 <mark>TCAAGATACCO</mark>	1,470 1,470 CAGATCATAT GFP cds		1,490 1,490 GACTTCTTCAA	1,500 1,500 AGAGCGCCAT	1,510 1,510 GCCTGAGGGA	1,520 1,520 FACGTGCAGGAG
FWD TA_31large-a-M13F.ab1				CAATGCTTT			GAAGEGGEAE		AGCGCCAT		TACGTGCNNAN-
REV TA_31large-a-M13R.ab1	ACTTGTCAC	1,540	 TTATGGTGTT 1,550	CAATGCTTT 1,560	TCAAGATACCO	CAGATCATAT	GAAGCGGCAC	GACTTCTTCAA 1,600	AGAGCGCCAT 1,610	GCCTGAGGGA	TACGTGCAGGAG
SWEET10-GFP repair product extraction 2	1,530	1,540	1,550	1,560	1,570 CACGTGCTGA	1,580	1,590 GAGGGAGACA	1,600	1,610	1,620	1,630 ICGATTICAAGG
FWD TA_31large-a-M13F.ab1						AGTCAAGTTI					
REV TA_31large-a-M13R.ab1	AGGACCATO		GACGACGGGA		CACGTGCTGA	AGTCAAGTTT	GAGGGAGACA	CCCTCGTCAAC		CTTAAGGGAA	
SWEET10-GFP repair product extraction 2	1,640 1,640 AGGACGGAA	1,650 1,650 AACATCCTCG	1,660 1,660 <mark>GCCACAAGTT</mark>	1,670 1,670 <mark>GGAA<mark>T</mark>ACAA</mark>	1,680 1,680 .CTACAACTCC	1,690 1,690 CACAACGTAT GFP cds		1,710 1,710 <mark>AGACAAACAAA</mark>	1,720 1,720 AAGAA <mark>T</mark> GGAA	1,730 1,730 <mark>TCAAAGCTAA(</mark>	1,740 1,740 CITTCAAAATTAG

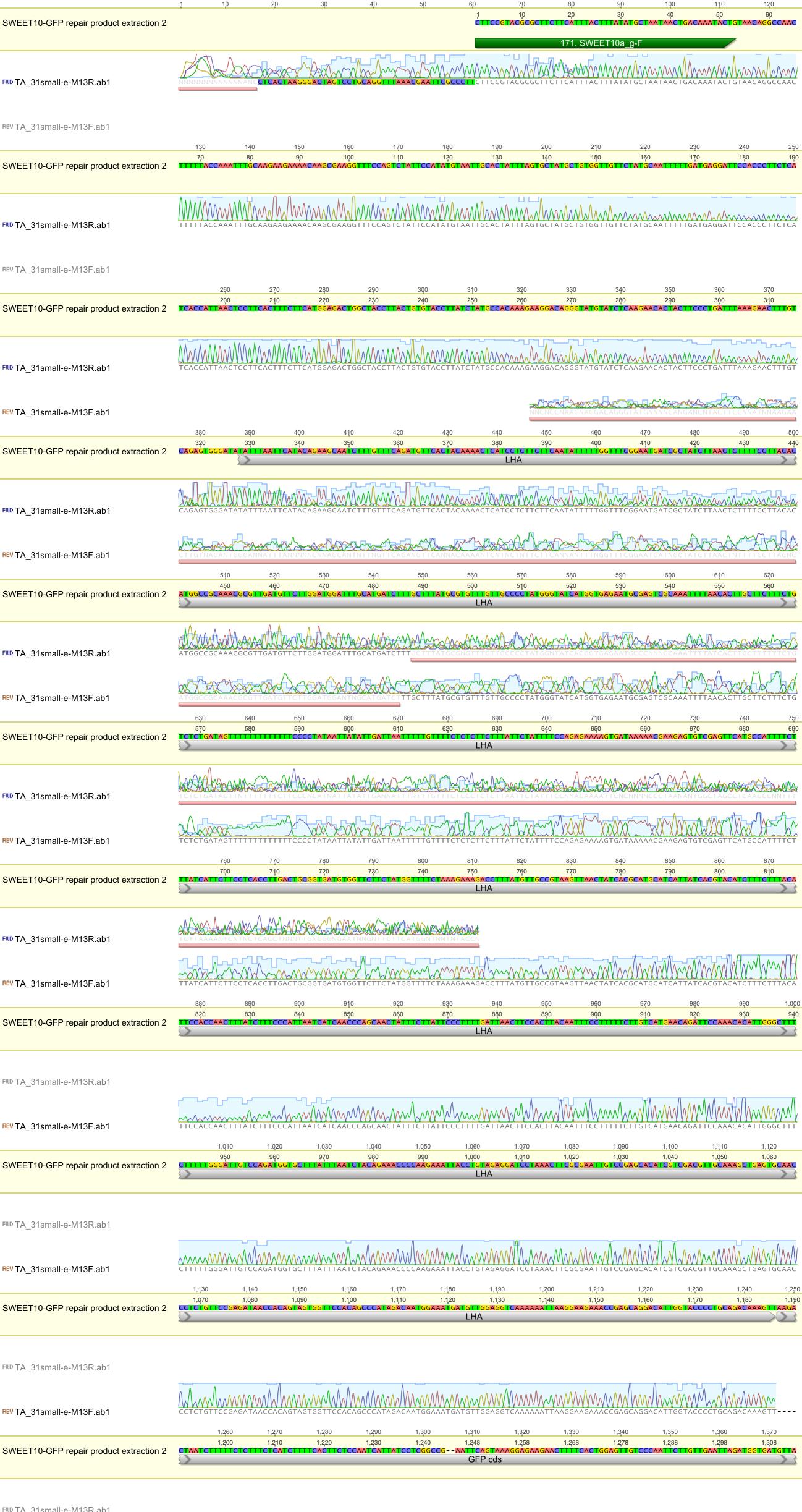


FWD TA_31large-a-M13F.ab1



FWD TA_31large-a-M13F.ab1

AGCTTTGATAACATTAACATTAACGTGTTCTTGGTTATGAAGGGCGAATTCGTTTAAACCTGCAGGACTAGTCCC

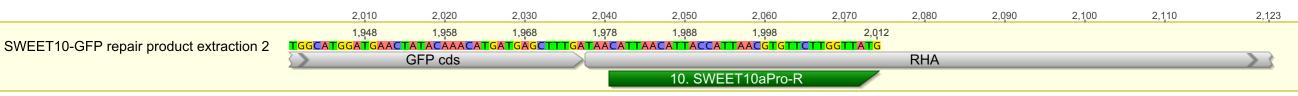


FWD TA_31small-e-M13R.ab1														
REV TA_31small-e-M13F.ab1														
	1,380	1,390	1,4	100	1,410	1,420	1,430	1,4	40 1,450	1,4(60 1,470	1,4	480 1	,490 1,500
	1,318	1,328	1,3	338	1,348	1,358	1,368	1,3	78 1,388	1,3	98 1,408	1,4	418 1	,428 1,438
SWEET10-GFP repair product extraction 2	A <mark>T</mark> GGGCACA	AATTTTC		GAGAGGG	GAAGGTGAT	GCAACA	TAC <mark>GG</mark> AAAA	GFP cds		CACTACTGG	AAAACTACCTG	TTCCATGG	CCAACACTT	TCACTACTTT C
	Σ								5					
FWD TA_31small-e-M13R.ab1														
REV TA_31small-e-M13F.ab1														
	1	510	1,520	1,530	1,540		1,550	1,560	1,570	1,580	1,590	1,600	1,610	1,620
														1,558 CGACGGGAACTA
SWEET10-GFP repair product extraction 2	ACTTATGGT	GTTCAAT	GCTTTTCA	AGA <mark>T</mark> AĊCC	AGATCATAT	GAAGCG	GCACGACTT		CGCCATGCCTG	AGGGA <mark>T</mark> ACG	T GCAGGAGAGG	ACCATCTC	TTTCAAGGA	GACGGGAAC <mark>T</mark> A
	<u>\</u>							GFP cds	5					
FWD TA_31small-e-M13R.ab1														
REV TA_31small-e-M13F.ab1														
	1 620	1 6 4 0	1.6	250	1 660	1 670	1 690	1.6	00 1 700	1 7	10 1 7 20	4 -	720 4	740 1 750
	1,630 <mark>1,568</mark>	1,640 1,578			1,660 1,598	1,670 1,608	1,680 1,618							,740 1,750 ,678 1,688
SWEET10-GFP repair product extraction 2	CAAGACACO	TGCTGAA	GTCAAGTT	GAGGGAG	ACACCCTCG		GGA <mark>T</mark> CGAĠC	TTAAGGGAA	TCGATTTCAAG	GAGGA <mark>C</mark> GGA	AACATCCTCĠG	CCACAAGT	TGGAATACA/	CTACAACTCCC
	<u>\</u>	_				_		GFP cds	5					<u>} }</u>
FWD TA_31small-e-M13R.ab1														
REV TA_31small-e-M13F.ab1														
		760	1,770	1,780	1,790		1,800	1,810	1,820	1,830	1,840	1,850	1,860	1,870
SWEET10-GFP repair product extraction 2	1 ACAACGTAT	698 ACATCAC	1,708 <mark>GGCAGACA/</mark>	1,718 AA <mark>C</mark> AAAAG	1,728 AA <mark>T</mark> GGAA <mark>T</mark> C/	AAAG <mark>CT</mark> A	1,738 AA <mark>CTTC</mark> AAA			1,768 TGGAAGCGT	1,7 ₇ 8 TCAACTAGCAG	1,788 ACCATTAT	1,798 <mark>CAACAAAAT</mark> /	1,808 ACTCCAATTGGC
								GFP cds						

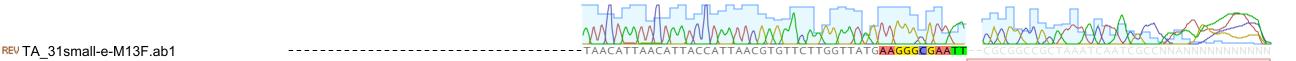
FWD TA_31small-e-M13R.ab1

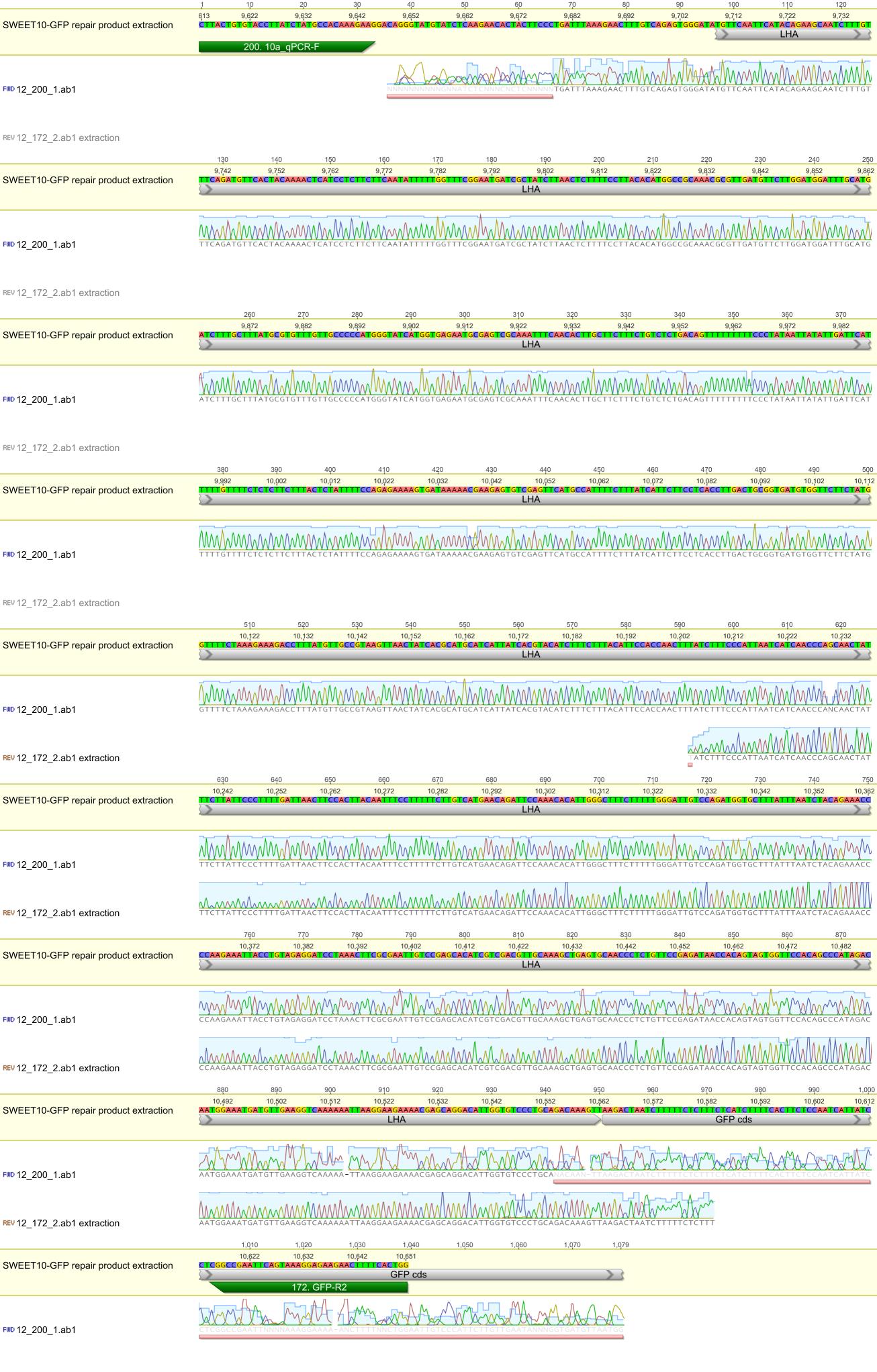
REV TA_31small-e-M13F.ab1													
	1,880	1,890	1,900	1,910	1,920	1,930	1,940	1,950	1,960	1,970	1,980	1,990	2,000
	1,818	1,828	1,838	1,848	1,858	1,868	1,878	1,888	1,898	1,908	1,918	1,928	1,938
SWEET10-GFP repair product extraction 2	GATGGCCCTGTCCTTTTACCAGACÁACCATTACCTGTCCACACAÁTCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACÁTGGTCCTTCTTGAGTTTGTÁACAGCTGCTGGGATTAC												

REV TA_31small-e-M13F.ab1



FWD TA_31small-e-M13R.ab1





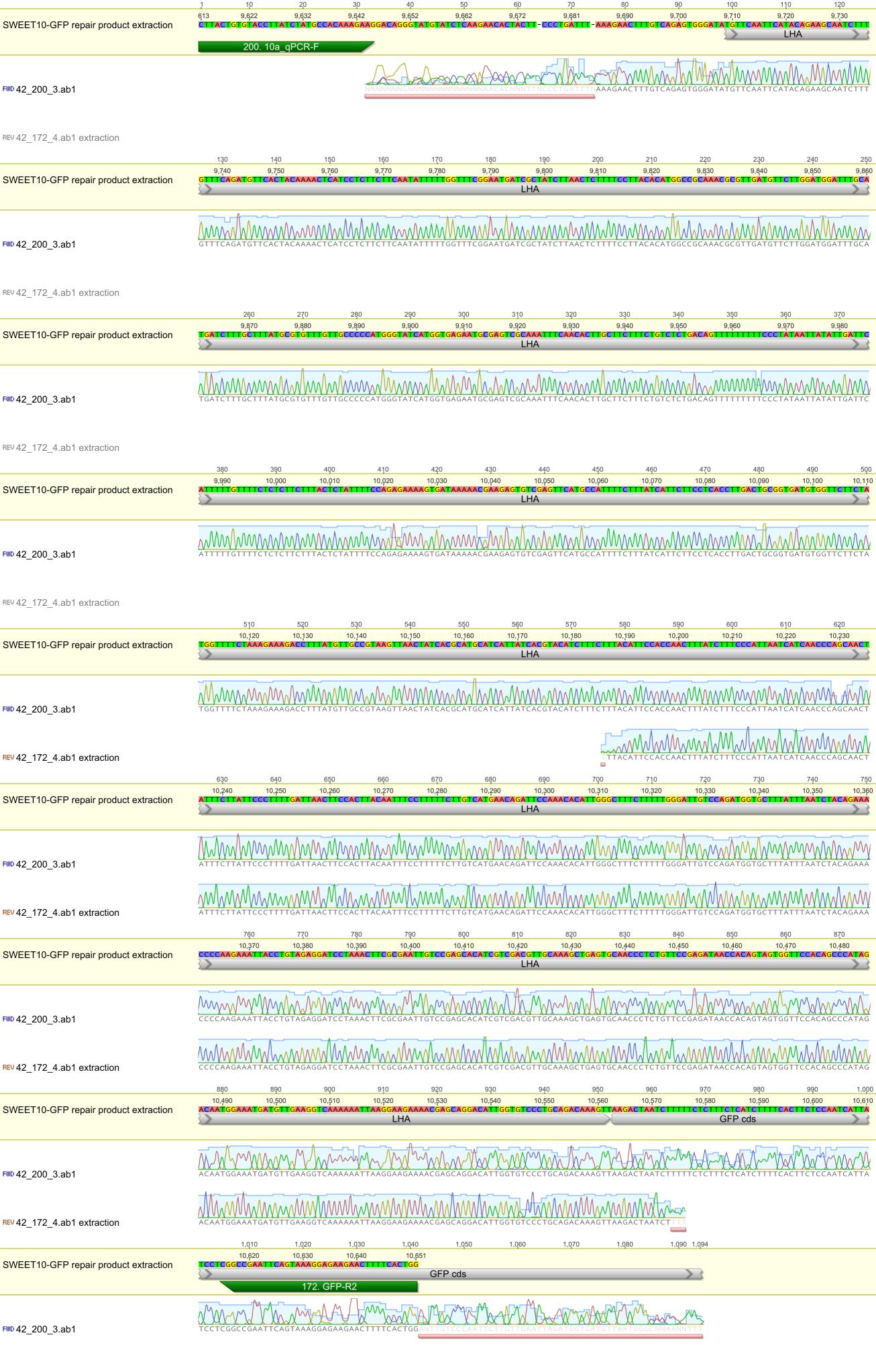


Figure S2. Sanger sequencing of successful knock-in lines. (Supports Figure 2)

Sanger sequencing of successful knock-in lines 31 (the focus of this manuscript), 12 and 42. Shown: raw sequencing data aligned to cultivar TME 419 (WT) reference sequence expected following scarless integration of GFP. The data are wrapped in multiple rows within a single page per sequencing product to aid in viewing. Page 1: sequencing data (base call and quality graphs) for line 31 from the upper (larger) band shown in the gel in Figure 2D (primers 171 and 10, Table S1). Page 2: data from the lower (smaller) band. Heterozygous SNPs are highlighted with colored backgrounds. These PCR products were sequenced using clone-seq with standard M13 sequencing primers. The products that were sequenced for lines 12 (page 3) and 42 (page 4) were generated using primers 171 and 164 (Table S1). Sequencing of these lines was performed directly on the PCR product using primers 200 and 172 (Table S1). Each page shows the sequencing data aligned to the expected product of HDR (highlighted in yellow). The LHA, GFP, and RHA sequence of the repair template are labeled in grey. Consensus sequence is given above the yellow-highlighted reference and sequencing data below. The names of the sequencing runs, which include the line number and either "large" or "small" for the bands sequenced from line 31 or the primer number used for lines 12 and 42, are to the left. Trimmed areas of poor sequence quality are indicated with a pink bar. Relevant primer sequences are shown in green. Analysis was performed using the algorithm built in to Geneious® version 9.1.8.