

S1 Table. DNA fragments, templates, and primers for the construction of pGFP, 2,609 bp

nF	OL nt	No	Template	Primer pair sequence	DNA	Size bp	
4F	18	F1	pTite N-His Kan	AACGAATTCAAGCTTGAT CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	Ori	791	
		F2	pTite N-His Kan	TTAGAAAACTCATCGAGC GACGAATTCTCTAGATATCG	KanR	1003	
		F3	pUC19	ATATCTAGAGAATTCTGCTTAATGTGAGTTAGCTCACTC TTCTCCTTTGCTAGCCATAGCTGTTTCTGTGTG	P _{Lac}	146	
		F4	pGLO	ATGGCTAGCAAAGGAGAA ATCAAGCTTGAATTCGTTTCATTATTTGTAGAGCTCATC	GFP	741	
2F	0	F1	pGFP	TATGGTGTTC AATGCTTTTCC CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	hGF-Ori	1316	
		F2	pGFP	CATCAAGTGA AACTGCAATT AGAGAAAGTAGTGACAAGTGT	KanR-P _{Lac} -hGF	1293	
	6	F1	pGFP	ACTTTC TCTTATGGTGTTC AATGCTTTTCC CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	hGF-Ori	1325	
		F2	pGFP	ATCGAGCATCAAGTGA AAC GAAAGTAGTGACAAGTGTG	KanR-P _{Lac} -hGF	1296	
	9	F1	pGFP	ACTTTC TCTTATGGTGTTC AATGCTTTTCC CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	hGF-Ori	1325	
		F2	pGFP	CTCATCGAGCATCAAGT AGAGAAAGTAGTGACAAGTGT	KanR-P _{Lac} -hGF	1302	
	12	F1	pGFP	ACTACTTTCTCTTATGGTGTTC AATGCTTTTCC CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	hGF-Ori	1328	
		F2	pGFP	AAACTCATCGAGCATCA AGAGAAAGTAGTGACAAGTGT	KanR-P _{Lac} -hGF	1305	
	15	F1	pGFP	ACTACTTTCTCTTATGGTGTTC AATGCTTTTCC CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	hGF-Ori	1328	
		F2	pGFP	GAAAACTCATCGAGCAT ATAAGAGAAAGTAGTGACAAGT	KanR-P _{Lac} -hGF	1311	
	18	F1	pGFP	ACTACTTTCTCTTATGGTGTTC AATGCTTTTCC CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	hGF-Ori	1328	
		F2	pGFP	TTAGAAAACTCATCGAGC ACCATAAGAGAAAGTAGTGAC	KanR-P _{Lac} -hGF	1317	
	25	F1	pGFP	ACTACTTTCTCTTATGGTGTTC AATGCTTTTCC CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	hGF-Ori	1328	
		F2	pGFP	GCCCTCATTAGAAAACTCATCGAG ATTGAACACCATAAGAGAAAGTAGTGACAAGTG	KanR-P _{Lac} -hGF	1331	
	3F	18	F1	pGFP	ACTACTTTCTCTTATGGTGTTC AATGCTTTTCC ATCAAGCTTGAATTCGTTTCATTATTTGTAGAGCTCATC	hGF	555
			F2	pGFP	AACGAATTCAAGCTTGAT CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	Ori	791
F3			pGFP	TTAGAAAACTCATCGAGC ACCATAAGAGAAAGTAGTGAC	KanR-P _{Lac} -hGF	1317	
25		F1	pGFP	ACTACTTTCTCTTATGGTGTTC AATGCTTTTCC ATCAAGCTTGAATTCGTTTCATTATTTGTAGAGCTCATC	hGF	555	
		F2	pGFP	ATAATGAAACGAATTC AAGCTTGAT CTCGATGAGTTTTTCTAATGAGGGCCCAAATG	Ori	798	
		F3	pGFP	GCCCTCATTAGAAAACTCATCGAG ATTGAACACCATAAGAGAAAGTAGTGACAAGTG	KanR-P _{Lac} -hGF	1331	

4F	18	F1	pGFP	ACTACTTTCTCTTATGGT GTTCAATGCTTTTCC ATCAAGCTTGAATTCGTTTCATTATTTGTAGAGCTCATC	hGF	555
		F2	pGFP	AACGAATTCAAGCTTGAT CTCGATGAGTTTTTCTAAATGAGGGCCCAAATG	Ori	791
		F3	pGFP	TTAGAAAACTCATCGAGC GACGAATTCCTCTAGATATCG	KanR	1003
		F4	pGFP	ATATCTAGAGAATTCGTCTAATGTGAGTTAGCTCACTC ACCATAAGAGAAAGTAGTGAC	P _{Lac} -hGF	332
	25	F1	pGFP	ACTACTTTCTCTTATGGTGTTCAAATGCTTTTCC ATCAAGCTTGAATTCGTTTCATTATTTGTAGAGCTCATC	hGF	555
		F2	pGFP	ATAATGAAACGAATTCAGCTTGAT CTCGATGAGTTTTTCTAAATGAGGGCCCAAATG	Ori	798
		F3	pGFP	GCCCTCATTAGAAAACTCATCGAG CACATTAGACGAATTCCTCTAGATAT	Kan	1017
		F4	pGFP	ATATCTAGAGAATTCGTCTAATGTGAGTTAGCTCACTC ATTGAACACCATAAGAGAAAGTAGTGACAAGTG	P _{Lac} -hGF	339
5F	18	F1	pGFP	ACTACTTTCTCTTATGGT GTTCAATGCTTTTCC ATCAAGCTTGAATTCGTTTCATTATTTGTAGAGCTCATC	hGF	555
		F2	pGFP	AACGAATTCAAGCTTGAT CTCGATGAGTTTTTCTAAATGAGGGCCCAAATG	Ori	791
		F3	pGFP	TTAGAAAACTCATCGAGC GACGAATTCCTCTAGATATCG	Kan	1003
		F4	pGFP	ATATCTAGAGAATTCGTCTAATGTGAGTTAGCTCACTC TTCTCCTTTGCTAGCCATAGCTGTTTCCTGTGTG	P _{Lac}	146
		F5	pGFP	ATGGCTAGCAAAGGAGAA ACCATAAGAGAAAGTAGTGAC	hGF	204
	25	F1	pGFP	ACTACTTTCTCTTATGGTGTTCAAATGCTTTTCC ATCAAGCTTGAATTCGTTTCATTATTTGTAGAGCTCATC	hGF	555
		F2	pGFP	ATAATGAAACGAATTCAGCTTGAT CTCGATGAGTTTTTCTAAATGAGGGCCCAAATG	Ori	798
		F3	pGFP	GCCCTCATTAGAAAACTCATCGAG CACATTAGACGAATTCCTCTAGATAT	Kan	1017
		F4	pGFP	ATATCTAGAGAATTCGTCTAATGTGAGTTAGCTCACTC TTCTCCTTTGCTAGCCATAGCTGTTTCCTGTGTG	P _{Lac}	146
		F5	pGFP	AACAGCTATGGCTAGCAAAGGAGAA ATTGAACACCATAAGAGAAAGTAGTGACAAGTG	hGF	218

Notes:

1. **nF** = the number of DNA fragments used for plasmid assembly, e.g. **4F** = 4 fragments
2. OL (nt) = the number of overlapping nucleotides at the ends of DNA fragments
3. P_{Lac} = Lac promoter
4. Kan = kanamycin resistance gene
5. hGF = half GFP sequence
6. Complementary primer ends are color-coded among the multiple primers used to generate DNA fragments for the construction of pGFP. For example, the **5F**–25 nt OL system uses 10 primers to produce 5 DNA fragments with 5 pairs of matching ends. The 5 pairs of complementary primer ends with 25 nt are coded by 5 different colors. The same color cannot appear in the primer pair used to prepare a DNA fragment by PCR. Instead, the same-colored primer sequences must reside within the 5' ends of the forward and reverse primers for neighboring DNA fragments.