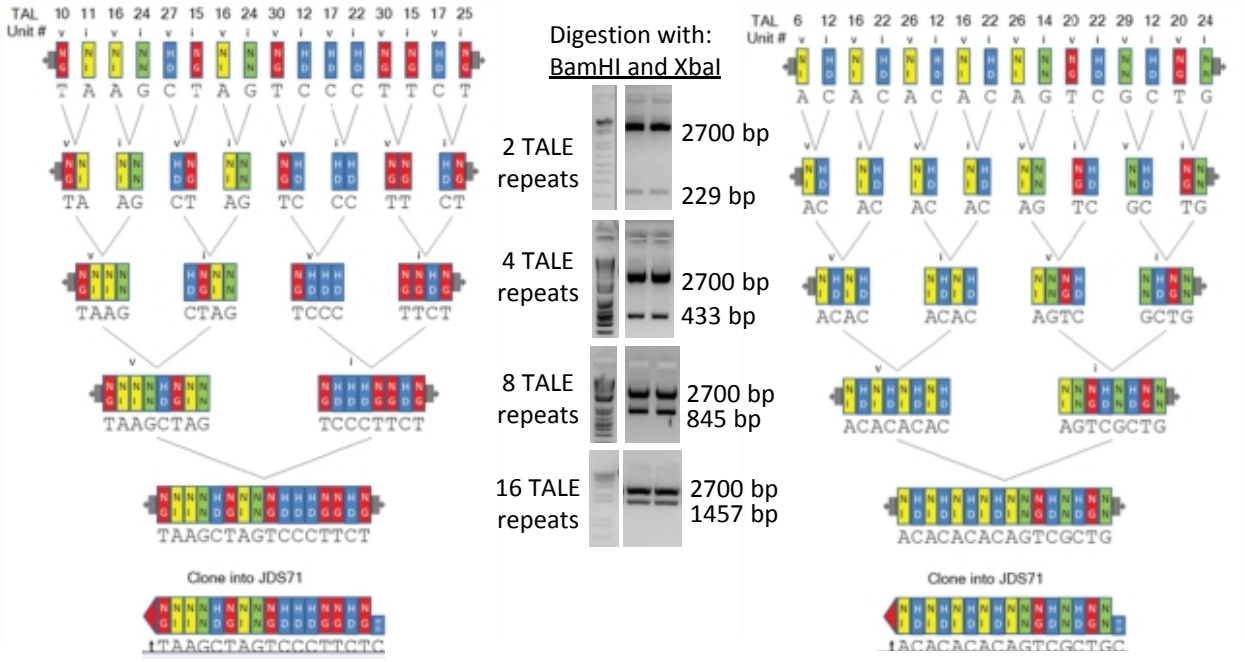
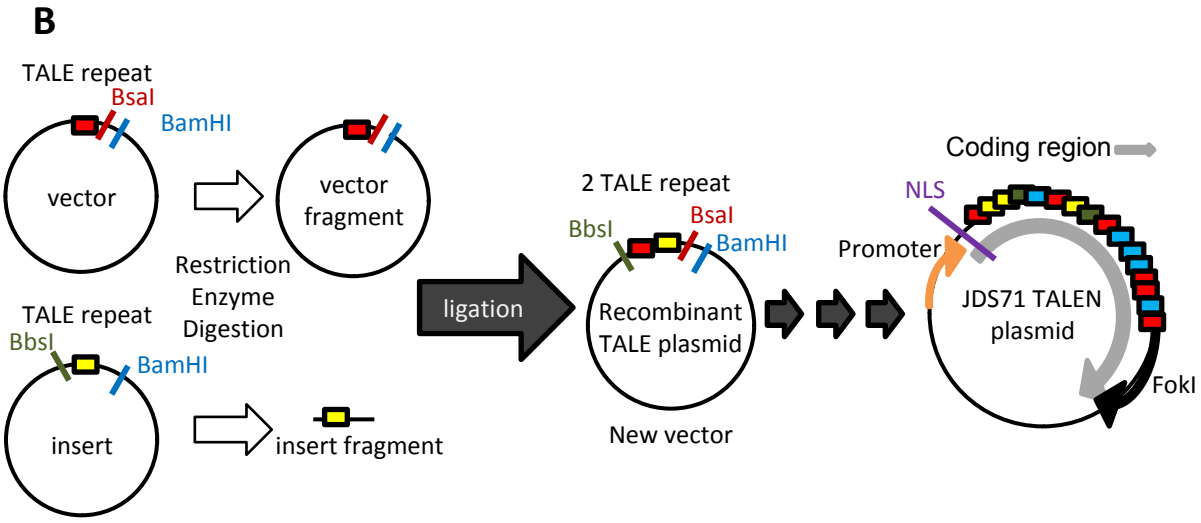
Supplemental Figure 1





Supplemental Figure 1. TALEN sequences were designed using the ZiFiT Targeter software package (http://zifit.partners.org/ZiFiT/) and constructed using the Joung Laboratory TAL Effector Engineering Reagents TALEN kit (www.addgene.org/TALEN/) combined with the REAL (Restriction Enzyme and Ligation) assembly method[1]. The TAL plasmid kit contains 28 TAL plasmids (each encoding a single TALEN repeat) and 4 expression vectors (JDS70, JDS71, JDS74 and JDS78), each of which has the promoter and FOK1 endonuclease domain. Each final TALE repeat (coding for a specific region of DNA) was cloned into the appropriate expression vector. (A) ZiFiT program design for targeting the ATG start site of the gastrin gene. Two TALENs are required to create a double stranded break in DNA. The numbers represent the name of each plasmid and the flow diagram shows the order in which each clone was made using the vector (v) and insert (i). The letter represents the repeat variable residues (RVRs). RVRs specify specific nucleic acid binding. For example, amino acids Asn-Ile (NG) bind to A, His-Asp (HD) bind to C, Asn-Asn (NN) bind to G and Asn-Gly (NG) bind to T[2]. The middle panel shows plasmid size after digestion with BamHI and XbaI restriction enzymes. (B) Illustration of the cloning process for each vector and insert. BsaI and BbsI restriction enzyme sites are lost between each TALE when the plasmid is ligated, resulting in a plasmid that can be used as either vector or insert plasmid in the next step. As each step occurs, the TALE repeat grows until it is the desired length when it is cloned into the expression vector. The grey arrow represents the coding sequence for the TALEN protein, with the nuclear localisation sequence (NLS), highlighted in purple. The TALE repeats of the protein are shown in the colours green, blue, red, and yellow, while the FokI endonuclease is depicted in black. The promoter (orange) drives the expression of the TALEN protein.

Supplemental Table 1. Primers used for cloning and screening.

Primer Name	Primer Sequence 5' to 3'	Site added (lower case)	T_m
A (ML5GasF)	cgaggtaccCATGCCTGGCCTAAAAAGTTCACT	KpnI	60
D (ML5GasRb)	ggcatcttccaTCGTCTGCAAAGGGGAGAAGGGACT	Start of luciferase	60
B (ML5LucFa)	cctttgcagacgaTGGAAGATGCCAAAAACATTAAGAAGGGC	End of 5' arm of gastrin	65
F (ML5LucR)	tgcctcgagTTACACGGCGATCTTGCCGCC	XhoI	65
G (ML3GasF)	ccttctagaTTTGCACTGGCTCTGGCCGCC	XbaI	63
H (ML3GasR)	caggtgccgcggACTCTGGCTATGAACCCTACCATA	SacII	63
P1 (OEKOR)	CAATGTATCTTATCATGTCTGGATC		60.9
P2 (ML3GASR)	CAGGTGCCGCGGACTCTGGCTATGAACCCT		53.8
P3 (G-probe3R)	TGTGGTAGAGAATAACTGGGTC		60.1

References

1. J. D. Sander, L. Cade, C. Khayter, D. Reyon, R. T. Peterson, J. K. Joung and J. R. Yeh, *Nat. Biotechnol.*, 2011, **29**, 697-698.
2. M. J. Moscou and A. J. Bogdanove, *Science*, 2009, **326**, 1501.