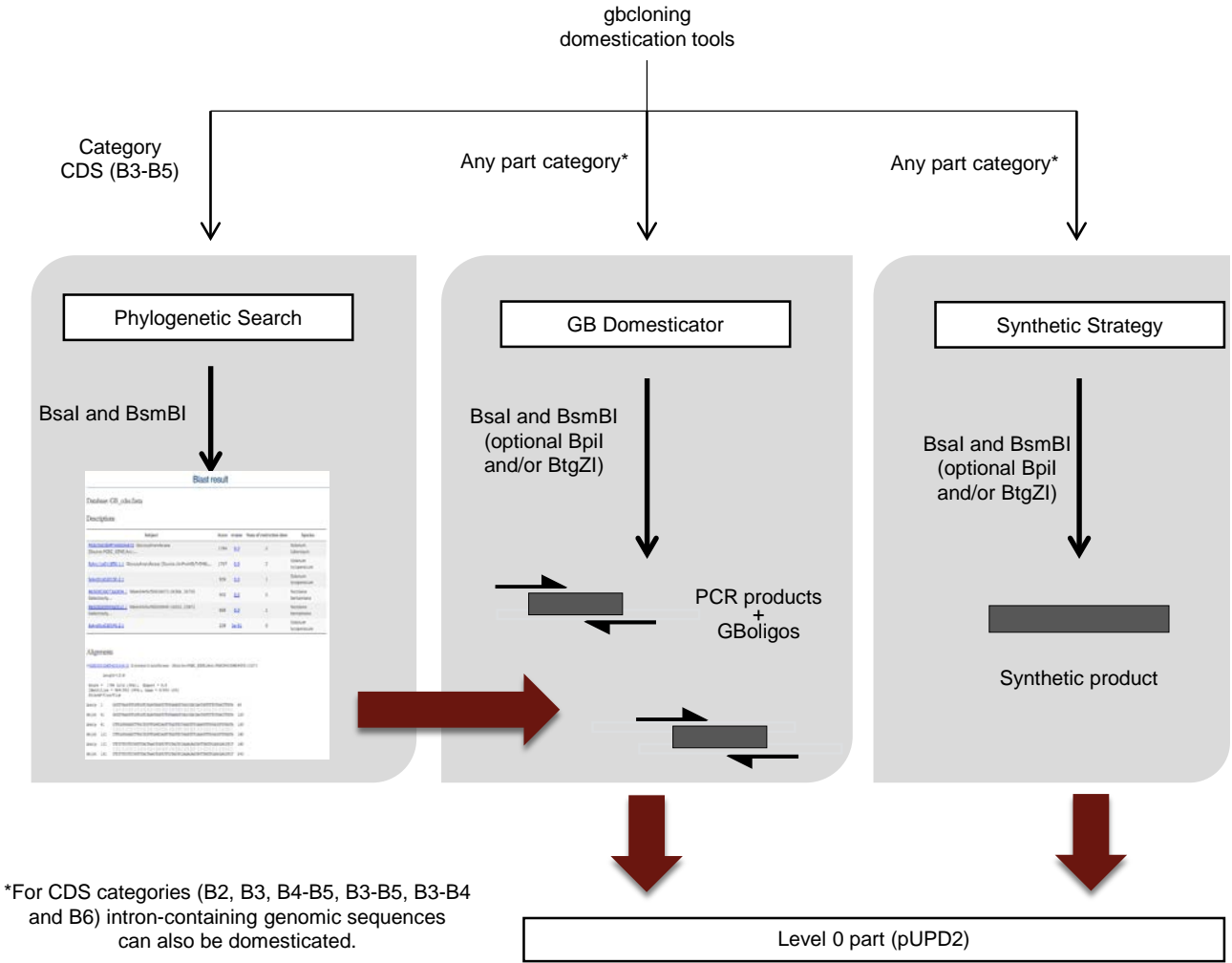


Supplementary Figure 1

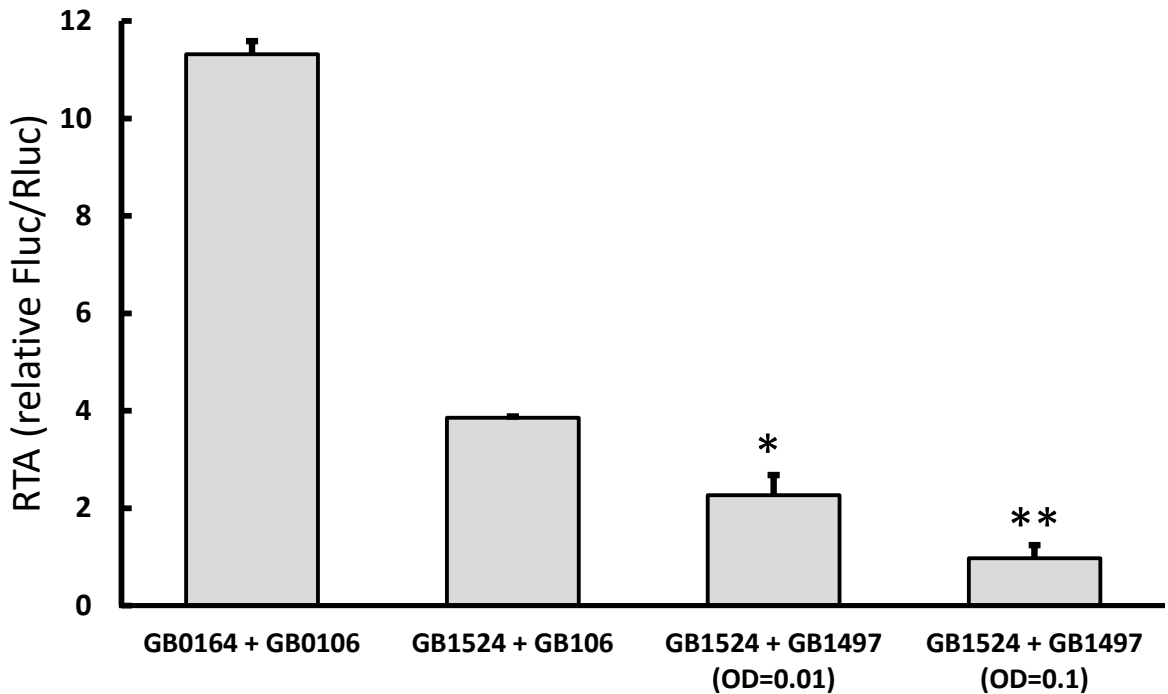


*For CDS categories (B2, B3, B4-B5, B3-B5, B3-B4 and B6) intron-containing genomic sequences can also be domesticated.

Supplementary Figure 1. GB3.0 domestication tools workflow.

The Phylogenetic Search Tool offers sequence retrieval of the best hits with minimal internal restriction sites from a Blast search in a coding sequences database. Upon sequence election, it offers connection with the GBDomesticator. The GB Domesticator provides the list of the PCR products and GB oligos required for part domestication while the Synthetic Strategy Tool provides directly the synthetic product to be ordered. The GB Domesticator and the Synthetic Strategy Tool offer in this new version the possibility of domesticating intron containing genomic sequences when the introns are written in lowercase in the input file. They also offer the choice to domesticate the sequences not only for Bsal and BsmBI but also for Bpil and/or BtgZI. All domestication tools generate a GenBank file with the level 0 part cloned into the pUPD2.

Supplementary Figure 2



Supplementary Figure 2. Repression modulation related to optical density (OD_{600}) in transient expression assays.

Increasing OD_{600} levels of *Agrobacterium* cultures expressing phiC31 recombinase have a direct repression effect on the reporter construct GB1524 comprising the CaMV 35S promoter with an *attR* site inserted immediately downstream of the TSS, followed by Luciferase/Renilla reporter system. GB0164 is a control construct comprised of the CaMV 35S promoter, without inserted *attR* sequence, followed by the Luc/Ren reporter system. GB0106 represents a stuffer fragment used to equalize *Agrobacterium* culture concentrations. GB1497 contains a constitutive transcriptional unit for phiC31 integrase expression. All relative transcriptional activities are expressed as relative promoter units calculated normalizing Luciferase/Renilla (L/R) ratios to GB1116. Error bars represent standard deviation of L/R ratios determined on 3 independent samples (* P-value < 0.05, ** P-value < 0.01)

Table S1. Experimental conditions.

Experiment type	Mandatory experimental conditions	Recommended experimental conditions	Quantitative output
SE_001 Promoter strength tested in discs	<ul style="list-style-type: none"> - Plant species: <i>N.benthamiana</i>. - <u>Chassis</u>: Agroinfiltrated leaves of 5-6 weeks old <i>N.benthamiana</i> plants. - <u>Constructs</u>: tested constructs must include the Luc gene as reporter (GB0096) and the 35s:Renilla:Tnos transcriptional unit in cis. The silencing suppressor P19 must be coinfiltrated either in cis or in trans. - <u>Sampling</u>: collect discs (0.8 cm in diameter) at 3 days post infiltration. - <u>Analysis</u>: keep the discs on plates with/without chemical inductor and take samples at different times. - <u>Data normalization</u>: normalize the data with the values obtained for GB1116 in the same experimental conditions. 	<ul style="list-style-type: none"> - <u>Plant growth conditions</u>: 24°C/20°C 16h light / 8h darkness. - <u>Sampling</u>: collect replicas from 3 independent agroinfiltrated leaves. - <u>Analysis</u>: use the Dual-Glo® Luciferase Assay System (Promega) for the luminiscence assay. 	<ul style="list-style-type: none"> - RTA at 0h. - RTA at 4h. - RTA at 8h. - RTA at 12h. - RTA at 18h. - RTA at 24h. - RTA at 36h. - RTA at 48h. <p>All relative transcriptional activities (RTA) have to be expressed in rpu (relative promoter units to GB1116).</p>
SE_002 Promoter strength tested in leaves	<ul style="list-style-type: none"> - Plant species: <i>N.benthamiana</i>. - <u>Chassis</u>: Agroinfiltrated leaves of 5-6 weeks old <i>N.benthamiana</i> plants. - <u>Constructs</u>: tested constructs must include the Luc gene as reporter (GB0096) and the 35s:Renilla:Tnos transcriptional unit in cis. The silencing suppressor P19 must be coinfiltrated either in cis or in trans. - <u>Sampling</u>: collect discs (0.8 cm in diameter) at 4 days post infiltration for analysis. - <u>Data normalization</u>: normalize the data with the values obtained for GB1116 in the same experimental conditions. 	<ul style="list-style-type: none"> - <u>Plant growth conditions</u>: 24°C/20°C 16h light / 8h darkness. - <u>Sampling</u>: collect replicas from 3 independent agroinfiltrated leaves. - <u>Analysis</u>: use the Dual-Glo® Luciferase Assay System (Promega) for the luminiscence assay. 	<ul style="list-style-type: none"> - RTA (in rpu) <p>Relative transcriptional activity (RTA) has to be expressed in rpu (relative promoter units to GB1116).</p>
SE_003 Transformation efficiency	<ul style="list-style-type: none"> - <u>Analysis</u>: calculate the transformation efficiency by dividing the number of obtained transgenic plants by the number of inoculated explants. 	Not defined	<ul style="list-style-type: none"> - % transformants
SE_004 Recombinant protein production	<ul style="list-style-type: none"> - Plant species: <i>N.benthamiana</i>. - <u>Chassis</u>: Agroinfiltrated leaves of 6-7 weeks old <i>N.benthamiana</i> plants. - <u>Sampling and analysis</u>: extract your protein, purify it and determine the amount of recombinant protein relative to the total amount of protein or weight of plant tissue. 	<ul style="list-style-type: none"> - <u>Plant growth conditions</u>: 24°C/20°C 16h light / 8h darkness. 	<ul style="list-style-type: none"> - µg/gDW (dry weight) - µg/gFW (fresh weight) - %TSP (total soluble protein)
SE_005 CRISPR target efficiency	<ul style="list-style-type: none"> - <u>Constructs</u>: tested constructs must include a Cas gene together with at least one single guideRNA (sgRNA). - <u>Analysis</u>: determine the efficiency calculating the percentage of mutated genomic DNA (for transient expression) or the number of plants with mutations in reference to the total number of transgenic plants (for stable transformation). 	Not defined	<ul style="list-style-type: none"> - Overall mutations efficiency (%)
NS_000 Non-standard experiment	Not defined	Not defined	Not defined

Table S2. Non-exhaustive list of GBexperiments.

*All listed experiments can be consulted at <https://gbcloning.upv.es/search/experiment/> by introducing the 'Experiment IDs'. Extra experiments can be searched on the same link by using different search criteria.

Title	Short description	Experiment type	GB elements	Experiment IDs
Dexamethasone dose-response	Study of the inducibility factor of a genetic element inducible by dexamethasone at different dexamethasone concentrations.	SE_001	GB0162	GB_EXP_3F, GB_EXP_40, GB_EXP_41, GB_EXP_42, GB_EXP_43, GB_EXP_45, GB_EXP_47, GB_EXP_49, GB_EXP_4A, GB_EXP_4B
Dexamethasone time-course	Determination of the induction profile of a genetic element inducible by dexamethasone.	SE_001	GB1254	GB_EXP_7A, GB_EXP_7B
Estradiol dose-response	Study of the inducibility factor of a genetic element inducible by estradiol with different β -estradiol concentrations	SE_001	GB1132	GB_EXP_36, GB_EXP_37, GB_EXP_38, GB_EXP_3A, GB_EXP_3B, GB_EXP_3C, GB_EXP_3D, GB_EXP_3E
Transactivation with a synthetic transcription factor time-course	Determination of the levels of activation of a regulated promoter with either the constitutive or self-regulated expression of a synthetic transcription factor	SE_001	GB1118, GB1121, GB1122, GB1124	GB_EXP_1A, GB_EXP_17, GB_EXP_18, GB_EXP_19
Regulated transactivation of the SIDFR promoter time-course	Determination of the transcriptional activity induced by the <i>Solanum lycopersicum</i> DFR promoter when it is coexpressed with a MYB and a bHLH transcription factors (Rosea1 and Delila) either constitutively expressed or regulated.	SE_001	GB1160, GB0129, GB1156, GB1157	GB_EXP_87, GB_EXP_90, GB_EXP_8C, GB_EXP_8D, GB_EXP_8E, GB_EXP_8F
Expression of the 35s constitutive promoter	Determination of the transcriptional activity induced by the 35s promoter over different experiments to test the stability of its expression and the reproducibility of the employed experimental method	SE_001	GB0164	GB_EXP_2D, GB_EXP_2E, GB_EXP_2F, GB_EXP_33, GB_EXP_26
Expression of the 35s constitutive promoter	Determination of the transcriptional activity induced by the 35s promoter over different experiments to test the stability of its expression and the reproducibility of the employed experimental method	SE_001	GB1119	GB_EXP_34, GB_EXP_35, GB_EXP_16
Transactivation induced by the TEV protease	Test of the ability of the TEV protease constitutively expressed to release a synthetic transcription factor from a transmembrane protein.	SE_002	GB0588 GB0594	GB_EXP_24 GB_EXP_25
Protein-protein interaction determined with the split-TEV system	Test of the interaction of two proteins fused to the N-term and C-term domains of the TEV protease by measuring expression from a promoter that is activated by a synthetic transcription factor released from a transmembrane protein upon reconstitution of the TEV protease.	SE_002	GB0592 GB0593	GB_EXP_22, GB_EXP_23

Table S2 (continuation). Non-exhaustive list of GBexperiments.

Title	Short description	Experiment type	GB elements	Experiment IDs
Transactivation of two DFR promoters with plant transcription factors	Determination of the transcriptional activity induced by the <i>Solanum lycopersicum</i> and the <i>Antirrhinum majus</i> DFR promoters when they are coexpressed with two MYB transcription factors (Rosea1 and Ant1) either alone or in combination with two bHLH transcription factors (Delila and Jaf13).	SE_002	GB1160, GB1161, GB0125, GB0126, GB0127, GB0128, GB0129, GB0130	GB_EXP_4D, GB_EXP_51, GB_EXP_54, GB_EXP_55, GB_EXP_57, GB_EXP_58, GB_EXP_59, GB_EXP_5B, GB_EXP_5C, GB_EXP_5D, GB_EXP_5E, GB_EXP_60, GB_EXP_61, GB_EXP_62
Transcriptional activation using the CRISPR/Cas9 technology	Comparison of the transcriptional activation of the nopaline synthase promoter by targeting to it the dCas9 fused to the EDLL or to the VP64 activation domains with different sgRNAs either alone or combined.	SE_002	GB1116, GB1189, GB1190, GB1221, GB1192, GB1197, GB1195, GB1220	GB_EXP_A3, GB_EXP_A5, GB_EXP_A6, GB_EXP_A7, GB_EXP_A8, GB_EXP_A9, GB_EXP_AB, GB_EXP_AF, GB_EXP_B0, GB_EXP_B1
Transcriptional repression using the CRISPR/Cas9 technology	Transcriptional repression of the nopaline synthase promoter by targeting to it the dCas9 fused to the BRD or to the SRDX repressor domains with with different sgRNAs either alone or combined.	SE_002	GB1116, GB1188, GB1172, GB1221, GB1192, GB1197, GB1195, GB1220	GB_EXP_B2, GB_EXP_B3, GB_EXP_B4, GB_EXP_B5, GB_EXP_B6, GB_EXP_B8, GB_EXP_B9, GB_EXP_BA, GB_EXP_BB, GB_EXP_BC
Transcriptional activation using PhiC31 integrase-based transcription factors	Comparison of the transcriptional activation driven by PhiC31 and RDF either alone or in combination fused to the Gal4 or the VP64 activation domains.	SE_002	GB1580, GB1563, GB1567, GB1576, GB1577, GB1497, GB0106	GB_EXP_DB, GB_EXP_DA, GB_EXP_D9, GB_EXP_D8, GB_EXP_D7, GB_EXP_D6, GB_EXP_D4, GB_EXP_D3, GB_EXP_D2, GB_EXP_D1
Transcriptional repression using PhiC31 integrase-based transcription factors	Comparison of the transcriptional repression driven by PhiC31 and RDF either alone or in combination fused to the BRD repressor domain.	SE_002	GB1524, GB1497, GB1562, GB1568, GB0106	GB_EXP_EB, GB_EXP_EC, GB_EXP_EE, GB_EXP_EF, GB_EXP_F0

Table S2 (continuation). Non-exhaustive list of GBexperiments.

Title	Short description	Experiment type	GB elements	Experiment IDs
Tomato transformation with a intragenic selection marker	Determination of the transformation efficiency obtained using a mutated version of the tomato acetolactate synthase as selection marker.	SE_003	GB0830	GB_EXP_BD
Recombinant antibody production	Comparison of the expression levels of three monoclonal antibody formats against the human tumor necrosis factor.	SE_004	GB_UA_BD1, GB_UA_C27, GB_UA_C29	GB_EXP_95, GB_EXP_98, GB_EXP_99
Gene editing with the CRISPR/Cas9 technology	Mutagenesis efficiency of the Cas9 in combination with two sgRNAs targeting each of them a different locus of the <i>N.benthamiana</i> xylosyltransferase gene.	SE_005	GB0639, GB1108	GB_EXP_83, GB_EXP_85, GB_EXP_86
Anthocyanins production	Quantification of anthocyanins produced in transient expression by the expression of two MYB transcription factors (Rosea1 and Ant1) either alone or in combination with two bHLH transcription factors (Delila and Jaf13).	NS_000	GB0125, GB0126, GB0127, GB0128, GB0129, GB0130	GB_EXP_BF, GB_EXP_C0, GB_EXP_C1, GB_EXP_C2, GB_EXP_C3, GB_EXP_C4

Table S3. List of GBelements tested under standard experimental conditions.

*Datasheets of all listed GBelements can be consulted at <https://gbcloning.upv.es/search/features/> by introducing their GB IDs.

Protein-protein interaction	
GB0592	Device for testing the interaction of Ros1 and SOC1 using the Split TEV system.
GB0593	Device for testing the interaction of FUL and SOC1 using the Split TEV system.
Reporter devices for transcriptional regulation studies	
GB1160	Reporter device including a transcriptional unit for the expression of the Luciferase gene driven by the <i>Solanum lycopersicum</i> DFR promoter.
GB1161	Reporter device including a transcriptional unit for the expression of the Luciferase gene driven by the <i>Antirrhinum majus</i> DFR promoter.
GB0178	Reporter device including a transcriptional unit for the expression of the Luciferase gene driven by a synthetic promoter including the LacI operon and the minimal 35s promoter.
GB1130	Transcriptional unit for the expression of the Luciferase gene driven by a synthetic promoter including the LexA operon and the minimal 35s promoter.
GB1116	Reporter device including a transcriptional unit for the expression of the Luciferase gene driven by the nopaline synthase promoter.
GB1580	Reporter device including a transcriptional unit for the expression of the Luciferase gene driven by 2xattB sites next to a minimal 35s. Inducible expression takes place upon co-expression with a TU encoding PhiC31 integrase fused to an activation domain.
GB1524	Reporter device including a transcriptional unit for the expression of the Luciferase gene driven by the 35s promoter next to an attR site. Inducible repression takes place upon co-expression with a TU encoding PhiC31 integrase fused to a repressor domain.
Constitutive transcriptional regulation	
GB1120	Transcriptional unit for the constitutive expression of a synthetic transcription factor conformed by the LacI DNA binding domain and the Gal4 activation domain.
GB0129	Module for the constitutive expression of the MYB transcription factor Rosea1 and the bHLH transcription factor Delila.
GB0130	Module for the constitutive expression of the MYB transcription factor Ant1 and the bHLH transcription factor Jaf13.
GB1497	Transcriptional unit for the constitutive expression of the PhiC31 integrase.
GB1563	Transcriptional unit for the constitutive expression of an orthogonal transcription factor conformed by the phiC31 integrase and the VP64 activation domain.
GB1577	Transcriptional unit for the constitutive expression of an orthogonal transcription factor conformed by the phiC31 integrase and the Gal4 activation domain.
GB1576	Transcriptional unit for the constitutive expression of an orthogonal transcription factor conformed by the phiC31 Recombination Directionality Factor (RDF) and the Gal4 activation domain.
GB1567	Transcriptional unit for the constitutive expression of an orthogonal transcription factor conformed by the phiC31 Recombination Directionality Factor (RDF) and the VP64 activation domain.
GB1562	Transcriptional unit for the constitutive expression of an orthogonal transcription factor conformed by the phiC31 integrase and the BRD repressor domain.
GB1568	Transcriptional unit for the constitutive expression of an orthogonal transcription factor conformed by the phiC31 Recombination Directionality Factor (RDF) and the BRD repressor domain.

Table S3 (continuation). List of GBElements tested under standard experimental conditions.

Conditional transcriptional regulation	
GB1111	Transcriptional unit for the regulated expression of a synthetic transcription factor conformed by the LacI DNA binding domain and the Gal4 activation domain.
GB1156	Module for the conditional expression of the MYB transcription factor Rosea1 and the constitutive expression of the bHLH transcription factor Delila including a synthetic transcription factor responsive to dexamethasone constitutively expressed.
GB1157	Module for the conditional expression of the bHLH transcription factor Delila and the constitutive expression of the MYB transcription factor Rosea1 including a synthetic transcription factor responsive to dexamethasone constitutively expressed.
GB0157	Transcriptional unit for the constitutive expression of a synthetic transcription factor conformed by the GR glucocorticoid receptor domain fused to the LacI DNA binding domain and the Gal4 activation domain.
GB1129	Transcriptional unit for the constitutive expression of a synthetic transcription factor conformed by the ER estradiol receptor domain fused to the LexA DNA binding domain and the Gal4 activation domain.
Metabolic engineering	
GB0830	Device for tomato transformation expressing the <i>S.lycopersicum</i> MYB12 under the E8 fruit promoter. MYB12 is a master regulator of the flavonoids biosynthetic pathway.
GB0130	Device for overproduction of anthocyanins in transient expression in <i>N.benthamiana</i> leaves comprising the <i>S.lycopersicum</i> MYB and bHLH transcription factors Ant1 and Jaf13.
CRISPR/Cas9 based gene editing	
GB1108	Device including two monocistroninc sgRNAs targeting each of them one locus of the <i>N.benthamiana</i> xylosyltransferase gene and the constitutively expressed Cas9.
GB1222	Device including two polycistroninc sgRNAs, one with two targets for the two genes of the <i>N.benthamiana</i> xylosyltransferase and the second one with three sgRNAs targeting five <i>N.benthamiana</i> fucosyltransferase genes. It also includes the TU for the constitutive expression of the Cas9.
Recombinant antibody production	
GB_UA_BD1	Transcriptional unit for the constitutive expression of the human scFv-Fcgamma1 antibody format against the human TNF-alpha.
GB_UA_C27	Transcriptional unit for the constitutive expression of a monoclonal antibody with the gamma1 heavy chain and the lambda light chain against the human TNF-alpha.
GB_UA_C29	Transcriptional unit for the constitutive expression of a monoclonal antibody with the gamma1 heavy chain and the kappa light chain against the human TNF-alpha.