

# ■ Index

Summary .....	XI
Resumen .....	XIII
Resum .....	XV
Abbreviations .....	XVI
Index .....	XIX
Figure Index .....	XXII
Table Index .....	XXIII
Supplemental Figure Index .....	XXIV
Supplemental Table Index .....	XXV
<b>Introduction</b> .....	<b>1</b>
1. Multigene assembly is out of necessity and not on a whim .....	3
1.1. Adapt or die. Multigene engineering is possible with traditional Type II cloning enzymes .....	5
1.2. New in town (for Plant Biotech): don't let them out of your sight! .....	7
1.3. Revisiting site-specific recombination tools for multigene engineering ....	7
1.4. The use of Zinc Finger Nucleases and Homing Endonucleases .....	11
1.5. Building multigenic constructs using Type IIs restriction enzymes .....	13
2. The fine-control of the genetic expression .....	15
2.1. Logic gates .....	17
<b>Objectives</b> .....	<b>21</b>
<b>Chapter 1. GoldenBraid: an iterative cloning system for standardized assembly of reusable genetic modules</b> .....	<b>25</b>
1. Introduction .....	27
2. Results .....	29

2.1. Part standardization and multipartite assembly of simple devices .....	29
2.2. The Double Loop design of the GoldenBraid system .....	31
2.3. Multigenic constructs for Plant Biology .....	35
2.4. Construction and combination of therapeutic and biosafety gene modules by GoldenBraid .....	39
3. Discussion .....	41
4. Materials and methods .....	46
4.1. Strains and growth conditions .....	46
4.2. Cloning and assembly of modular pieces .....	46
4.3. GB-Domestication of destination plasmids for plant biology .....	47
4.4. Plant transient transformation .....	47
4.5. Western Blot and ELISA analysis .....	47
<b>Chapter 2. GoldenBraid2.0: a comprehensive DNA assembly framework for Plant Synthetic Biology .....</b>	<b>51</b>
1. Introduction .....	53
2. Results .....	55
2.1. The GB2.0 cloning strategy .....	55
2.2. GBparts: words and phrases. Definition of the GB2.0 grammar .....	56
2.3. GBpart domestication: creating words and phrases .....	58
2.4. The GB2.0 destination plasmids kit .....	58
2.5. The composing strategy: from single words to full compositions .....	60
2.6. Innovative features in the GB2.0 cloning strategy .....	61
2.7. GB collection and software tools .....	68
3. Discussion .....	69
4. Material and methods .....	72
4.1. Strains and growth conditions .....	72
4.2. Restriction-ligation assembly reactions .....	73
4.3. GBpart domestication .....	73
4.4. pDGB construction .....	73
4.5. <i>N. bentamiana</i> transient transformation .....	74
4.6. <i>A. thaliana</i> stable transformation .....	74

4.7. Luciferase/Renilla expression assays .....	74
4.8. Glucocorticoid Receptor induction and Heat shock treatments .....	75
<b>Chapter 3. Engineering modular and orthogonal logic gates for Plant Synthetic Biology .....</b>	<b>77</b>
1. Introduction .....	79
2. Results .....	80
2.1. Simple operations .....	80
2.2. Binary operations .....	89
3. Discussion .....	93
4. Materials and methods .....	94
4.1. Bacterial strains and growth conditions .....	94
4.2. Restriction-ligation assembly and domestication reactions .....	95
4.3. <i>N. bentamiana</i> transient transformation .....	95
4.4. <i>A. thaliana</i> stable transformation and growth conditions .....	95
4.5. Glucocorticoid and estradiol receptor induction .....	96
4.6. Heat-shock treatments .....	96
4.7. Luciferase/Renilla expression assays .....	96
<b>General Discussion .....</b>	<b>99</b>
1. The Goldenbraid Framework: an insight look .....	101
1.1. A binary loop fed by a multipartite standard .....	101
1.2. The generation, selection and characterization of the GBparts .....	103
1.3. Size and speed considerations .....	104
1.4. The software tools .....	104
2. GoldenBraid perspectives: an outlook .....	106
2.1. Compatibility between different multigene assembly methods .....	106
2.2. The creation of a community of users .....	108
3. The development of Logic Gates adapted to Plant Biotechnology .....	108
<b>Conclusions .....</b>	<b>113</b>
References .....	117
Supplemental Figures .....	137
Supplemental Tables .....	152

# ■ Figure Index

Figure 1. Simple graphical overview of the multigene assembly methods described in this chapter .....	6
Figure 2. Detailed diagram of MultiRound Gateway (MRG) .....	9
Figure 3. Detailed diagram of MISSA .....	10
Figure 4. Detailed diagram of pSAT+ZFNs vectors .....	12
Figure 5. Functioning of the Golden Gate Assembly System .....	14
Figure 6. Detailed diagram of MoClo .....	16
Figure 7. Truth tables of the Boolean Logic Gates .....	17
Figure 8. Part standardization and multipartite assembly of single devices .....	30
Figure 9. Structure of the LacZ cassettes in the GoldenBraid system .....	32
Figure 10. The mechanism of GoldenBraid system .....	34
Figure 11. GoldenBraid-assisted co-transformation of fluorescent devices .....	37
Figure 12. GoldenBraid-assisted selection of plant-made IgA isotypes .....	38
Figure 13. New multigene assemblies using reusable composite parts .....	40
Figure 14. Comparison of the topology of MoClo and GoldenBraid .....	44
Figure 15. Analogies between GB2.0 and English grammar .....	56
Figure 16. The complete GB2.0 grammar and its most frequently used structures .....	57
Figure 17. Standardized domestication of GBparts .....	59
Figure 18. GB2.0 cassettes and assembly rules .....	62
Figure 19. Characterization of regulatory regions for basic expression cassettes .....	65
Figure 20. GoldenBraid versus GB2.0 .....	71
Figure 21. Truth tables for the one-input one-output models .....	80
Figure 22. Tautology and contradiction .....	83
Figure 23. Propositions .....	84
Figure 24. Transcriptional strength of the engineered versions of the CaMV 35S promoter and effect of the synthetic transcriptional repressors .....	86
Figure 25. NOT logic gate .....	88
Figure 26. Logical Disjunction .....	91
Figure 27. Logical Conjunction .....	92
Figure 28. Pathways the generation of a 5-gene construct using three different assembly systems .....	105
Figure 29. Wiring options for the development of Boolean Logic gates .....	109

# ■ Table Index

Table 1. Logic Operations for Plant Synthetic Biology .....	81
Table 2. Bacterial Resistances and Type IIS Enzymes used in MoClo and GoldenBraid for the different restriction-ligation reactions .....	103
Table 3. Multigene Assembly methods for Plant Biotechnology .....	107

## ■ Supplemental Figure Index

Supplemental Figure 1. GB Domestication with the removal of internal restriction sites ..	138
Supplemental Figure 2. Combinatorial antibody engineering using the GBpatch assembly level .....	139
Supplemental Figure 3. Theoretical and experimental transcriptional activity of different promoter/terminator combinations .....	140
Supplemental Figure 4. Functional characterization of heat-shock promoters .....	141
Supplemental Figure 5. Functional characterization of transactivation constructs .....	142
Supplemental Figure 6. Frequently Used Structures (FUS) for the protein-protein interaction analysis .....	143
Supplemental Figure 7. The Frequently Used Structures (FUS) for endogenous gene silencing .....	144
Supplemental Figure 8. Adapting Gateway (GW) technology to GB2.0 .....	145
Supplemental Figure 9. GoldenBraid Building pathways for the (A) $\neg$ LACI::GAL4AD and (B) $\perp$ UAS gates .....	146
Supplemental Figure 10. GoldenBraid Building pathways for the (A) P GR (B) P ER and (C) P HS logic gates .....	147
Supplemental Figure 11. GoldenBraid Building pathway for the $\neg$ GR.LexA::SRDX version of the logic gate .....	148
Supplemental Figure 12. GoldenBraid Building pathways for the (A) GRVHS (B) GRVER gates .....	149
Supplemental Figure 13. GoldenBraid Building pathways and results for the AND tests constructs (I) .....	150
Supplemental Figure 14. GoldenBraid Building pathways and results for the AND tests constructs (II) .....	151

## ■ Supplemental Table Index

Supplemental Table 1 GBparts used in Chapter 2 .....	152
Supplemental Table 2 GBparts used in Chapter 3 .....	154