Summary

Synthetic Biology is an emerging interdisciplinary field that aims to apply the engineering principles of modularity, abstraction and standardization to genetic engineering. The nascent branch of Synthetic Biology devoted to plants, Plant Synthetic Biology (PSB), offers new breeding possibilities for crops, potentially leading to enhanced resistance, higher yield, or increased nutritional quality. To this end, the molecular tools in the PSB toolbox need to be adapted accordingly, to become modular, standardized and more precise. Thus, the overall objective of this Thesis was to adapt, expand and refine DNA assembly tools for PSB to enable the incorporation of functional specifications to the description of standard genetic elements (phytobricks) and to facilitate the construction of increasingly complex and precise multigenic devices, including genome editing tools.

The starting point of this Thesis was the modular DNA assembly method known as GoldenBraid (GB), based on type IIS restriction enzymes. To further optimize the GB construct-making process and to better catalog the phytobricks collection, a database and a set of software-tools were developed as described in Chapter 1. The final web-based software package, released as GB2.0, was made publicly available at www.gbcloning.upv.es. A detailed description of the functioning of GB2.0, exemplified with the building of a multigene construct for anthocyanin overproduction was also provided in Chapter 1. As the number and complexity of GB constructs increased, the next step forward consisted in the refinement of the standards with the incorporation of experimental information associated to each genetic element (described in Chapter 2). To this end, the GB package was reshaped into an improved version (GB3.0), which is a self-contained, fully traceable assembly system where the experimental data describing the functionality of each DNA element is displayed in the form of a standard datasheet. The utility of the technical specifications to anticipate the behavior of composite devices was exemplified with the combination of a chemical switch with a prototype of an anthocyanin overproduction module equivalent to the one described in Chapter 1, resulting in a dexamethasone-responsive anthocyanin device. Furthermore, Chapter 3 describes the adaptation and functional characterization of CRISPR/Cas9 genome engineering tools to the GB technology. The performance of the adapted tools for gene editing, transcriptional activation and repression was successfully validated by transient expression in *N. benthamiana*. Finally, Chapter 4 presents a practical implementation of GB technology for precision plant breeding. An intragenic construct comprising an intragenic selectable marker and a master regulator of the flavonoid biosynthesis was stably transformed in tomato resulting in fruits enhanced in flavonol content.

All together, this Thesis shows the implementation of increasingly complex and precise genetic designs in plants using standard elements and modular tools following the principles of Synthetic Biology.