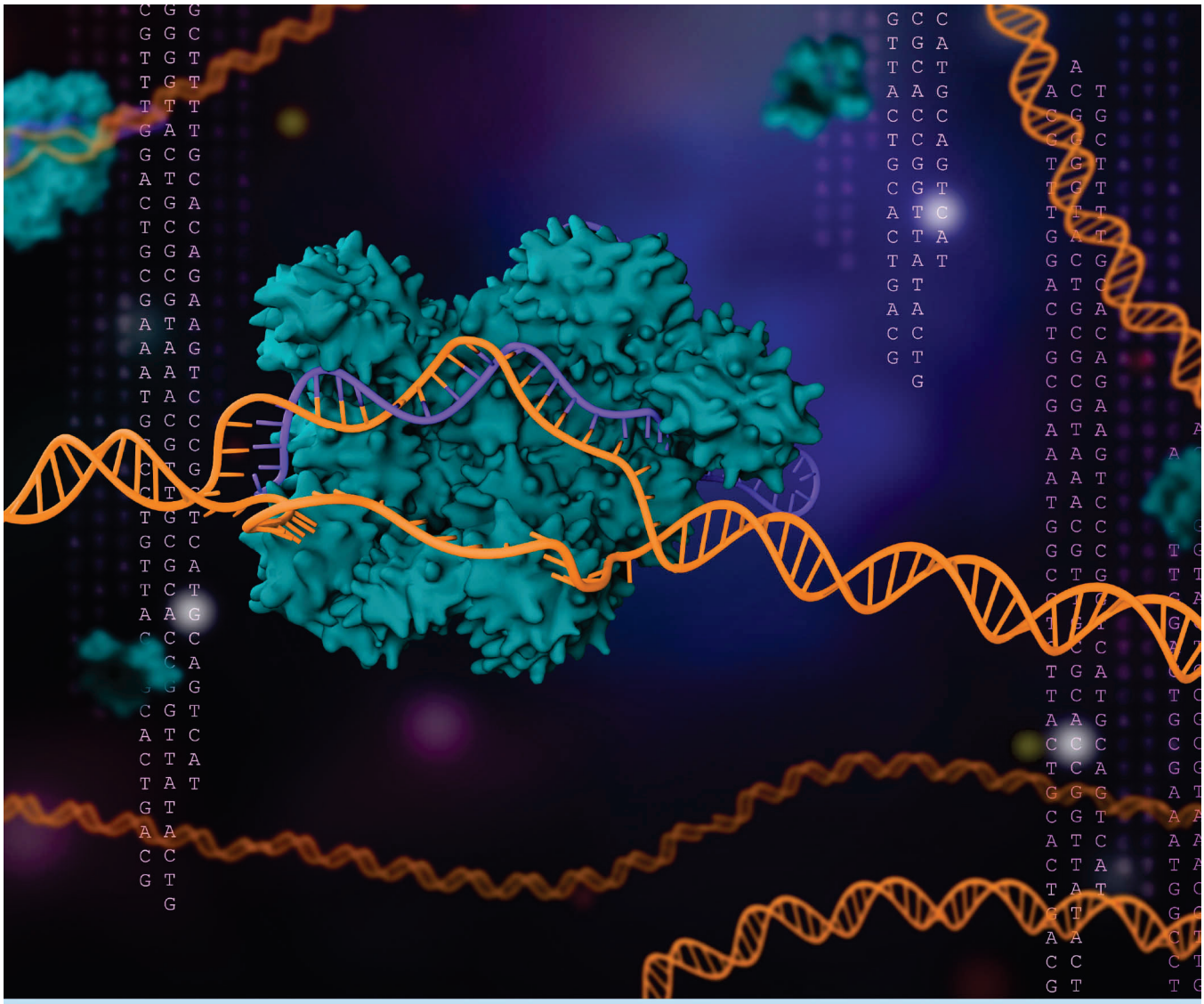


CRISPR AND PLANT FUNCTIONAL GENOMICS

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11 Using CRISPR to Unlock the Full Potential of Plant Immunity

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11.1 INTRODUCTION

Our understanding of plant–pathogen interactions has undergone significant advancements since the establishment of the gene-for-gene theory proposed by Harold Henry Flor [1,2]. Flor's studies on the genetic interactions between host resistance genes and pathogen avirulence genes paved the way for further investigations in plant pathology. Over time, the field has witnessed remarkable progress with the advent of molecular biology techniques, such as RNA interference (RNAi) and transgene technologies, enabling precise manipulation of organism genomes, including plants. Among the notable developments in molecular biology, the emergence of site-specific nucleases has played a pivotal role in unraveling the intricacies of plant–pathogen interactions and facilitating the generation of disease-resistant plants. Particularly, the optimization of CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) technology has revolutionized our ability to manipulate the genomes of model organisms as well as plants, offering a rapid and efficient means to generate mutant lines [3,4]. This powerful tool has opened new avenues for studying the regulatory elements involved in plant–pathogen interactions by creating loss-of-function mutants and expanding our knowledge in this field. In this chapter, we will delve into the utilization of CRISPR-based approaches to advance our understanding of plants and their interactions with phytopathogens, drawing upon the wealth of previous research findings. We will explore how CRISPR technology has enabled precise manipulation of host genes, allowing researchers to dissect the complex molecular mechanisms underlying plant defense responses. Furthermore, we will address the potential of this technology in enhancing plant resistance by precisely modifying host genes and developing commercially viable disease-resistant plants.

11.2 THE SIGNIFICANCE OF PLANT–PATHOGEN INTERACTIONS IN ENHANCING PLANT IMMUNITY

The first step of engineering disease resistance in plants is understanding host immunity within host–pathogen interactions. Throughout evolution, plants have developed sophisticated defense mechanisms to counteract the onslaught of phytopathogens. The arms race between hosts and microbes resembles a biochemical battle, where pathogens employ diverse strategies, including the deployment of novel effectors, to overcome the plant's defense systems. However, most pathogens are intercepted by the plant's robust multilayered immune system, while only a few succeed in overcoming the defense system and infecting the host [5,6].

Plant immunity comprises two branches: Pathogen Triggered Immunity (PTI) and Effector Triggered Immunity (ETI) [7]. PTI is initiated when the host recognizes Microbe/Pathogen-Associated Molecular Patterns (M/PAMPs) through pattern recognition receptors (PRRs) [8]. This recognition triggers a series of defense responses that hinder pathogen growth and survival. These responses include the reinforcement of the cell wall through the production of cellulose,

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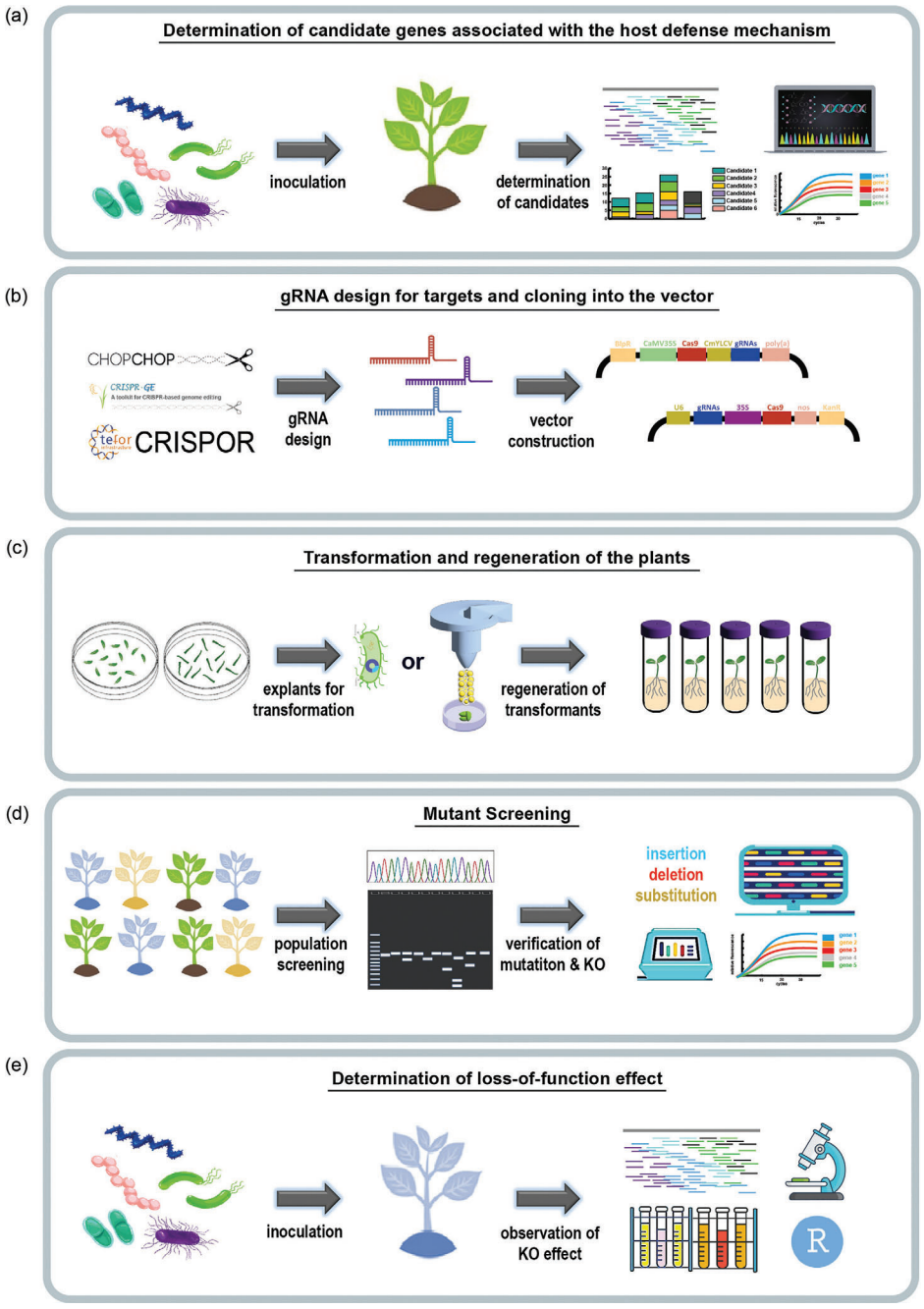


FIGURE 11.1 Workflow for generating loss-of-function mutants using CRISPR/Cas9 to investigate the role of genes in plant defense response against pathogens. (a) Identification of candidate genes through transcriptomic analysis, followed by validation using qPCR. (b) Selection of gRNAs by using different tools [24–26] and transfer into a Cas9 vector for the targeted plant. (c) Transformation of plant explants (cotyledon, hypocotyl) via Agrobacterium or particle bombardment and regeneration of T0 plants. T1 seeds can be harvested after acclimatization and maturation of T0 plants. (d) Screening of the T1 population using PCR-based techniques or sequencing to identify mutant plants; and confirmation of KO at the target site using bioinformatic and transcriptomic analyses. (e) Assessment of loss-of-function effects in T2 plants through advanced microscopy, biochemistry, and bioinformatic approaches.