plasmid that contains an origin of replication, virulence genes that facilitate transfer of the T-DNA region into the plant genome, and the T-DNA region itself (Gelvin, 2003; Lee and Gelvin, 2008).

There are two difficulties that involve the use of wild-type *A. tumefaciens* pTi plasmids for inserting the genes of therapeutic proteins into host plants: cloning these genes into the T-DNA region would be laborious due to the difficulties of working with such a large, low copy-number plasmid; and the presence of oncogenes within wild-type T-DNA regions would hinder growth and affect the morphology of transformed plants. These difficulties have been overcome by the development of the binary vector system, which separated virulence genes and the T-DNA region on two plasmids. The larger pTi plasmid was disarmed by deletion of oncogenes that cause the formation of galls on infected plants while keeping the virulence genes necessary for T-DNA transfer; smaller T-DNA plasmids were developed that contain the T-DNA region itself, delineated by flanking 25 bp left border (LB) and right border (RB) sequences, selectable marker genes, and other genetic elements required for plasmid manipulation in both *A. tumefaciens* and *E. coli* (Gelvin, 2003; Lee and Gelvin, 2008). Binary plasmid systems allow researchers to simply insert genes for therapeutic proteins into T-DNA plasmids, using *E. coli* as an intermediate plasmid host, and then transfer purified T-DNA plasmids to an *A. tumefaciens* strain that contains a disarmed pTi plasmid.

Figure 17.1 illustrates the developmental stages involved in stable transgenic plant production. Sterile leaf discs called explants are cocultivated with an *A. tumefaciens* strain harboring both a disarmed pTi plasmid and a T-DNA plasmid-containing gene(s) encoding therapeutic proteins of interest plus a selectable marker gene. Subsequent development of primary transgenic plants typically requires a 4-month time investment. During this period, the *A. tumefaciens* cells transfer only the T-DNA flanked by the 25 bp LB and RB sequences to the plant genome, and expression of the transferred genes follows. Explants are first treated with medium containing plant growth hormones that direct the formation of callus, which is a mass of unorganized plant cells, and with drug(s) that select for growth of only those cells expressing the selectable marker gene. After adventitious buds have matured to a sufficient size, shoots are excised from the callus and placed on medium lacking growth hormones that induce the formation of roots. Once roots have developed, primary transgenic plantlets can be removed from sterile culture and transplanted into soil for development to mature plants.

Production of a stable transgenic plant line that has a high-level expression level of recombinant therapeutic protein requires many primary transgenic plants to be screened for target gene expression. Screening can be performed when primary transgenic plantlets are judged to be of sufficient size that sampling of tissues will not compromise health and development to mature plants. PCR-based assays can be used for detection of T-DNA incorporation or transcription of the genes of interest (reverse transcription PCR; RT-PCR). However, most useful screening procedures involve assays that measure expression of the therapeutic protein itself. Such assays are usually Western immunoblotting or quantitative enzyme-linked immunosorbent assays (ELISAs) that use an antibody specific for detection of the therapeutic protein. A specific activity assay could also be used for detection of an enzyme intended for use in enzyme replacement therapy (ERT).