TABLE 14.3 Strong *E. coli* Promoters

Promoter	Induction method	Characteristics[a]
lacUV5	Addition of IPTG	(about 5%)
tac	As above	Induction results in cell death (> 30%)
Ipp-OmpA	As above	Suitable for secreted proteins (20%)
Ipp^p-5	As above	Strongest *E. coli* promoter (47%)
trp	Tryptophan starvation	Relatively weak (around 10%)
λp_L	Growth at 42°C	See text (> 30%)
$\lambda p_L/cl_{trp}$	Addition of tryptophan	Easily inducible in large-scale production (24%)
att-nutL-p-att-N	10-Min incubation at 42°C	No product is synthesized before induction (on/off promoter)
T7 promoter	Addition of IPTG or viral infection	As above (> 35%), low basal levels
T4 promoter	Viral infection	Method of induction inhibits product degradation
phoA	Phosphate starvation	Induction in large-scale production is complicated

[a]Typical values of accumulated product as a percent of the total protein of induced cells are given in parentheses.

With permission, from G. Georgiou, *AIChE J. 34:*1233 (1988).

Another approach to preventing intracellular proteolysis is to develop a secretion vector in which a signal sequence is coupled to the target protein. If the protein is secreted in one host, it will usually be excreted in another, at least if the right signal sequence is used. Replacement of the protein's natural signal sequence (e.g., from an eukaryotic protein) with a host-specific signal sequence can often improve secretion.

The secretion process is complicated, and the fusion of a signal sequence with a normally nonsecreted protein (e.g., cytoplasmic) does not ensure secretion, although several cases of secretion of normally cytoplasmic proteins have been reported. Apparently, the mature form of the protein contains the "information" necessary in the secretion process, but no general rules are available to specify when coupling a signal sequence to a normally cytoplasmic protein will lead to secretion.

To ensure the genetic stability of any construct and to aid in the selection of the desired host–vector combination, the vector should be developed to survive under selective growth conditions. The most common strategy is to include genes for antibiotic resistance. The common cloning plasmid, pBR322, contains both ampicillin and tetracycline resistance. Multiple resistance genes are an aid in selecting for human-designed modifications of the plasmid.

Another strategy for selection is to place on the vector the genes necessary to make an essential metabolite (e.g., an amino acid). If the vector is placed in a host that is auxotrophic for that amino acid, then the vector complements the host.[†] In a medium without that amino acid, only plasmid-containing cells should be able to grow. Because the genes for the synthesis of the auxotrophic factors can be integrated into the

[†]Recall that an auxotrophic mutant would be unable to synthesize an essential compound on its own.