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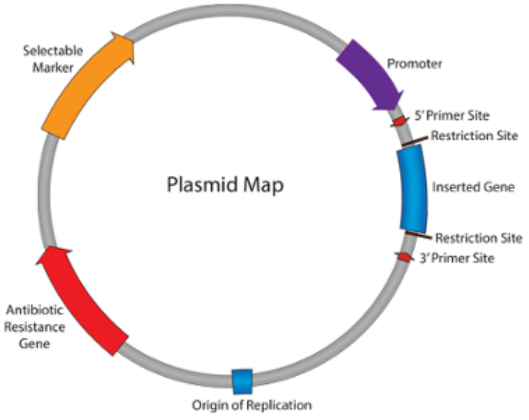
# Plasmids 101: The Promoter Region – Let's Go!

Posted by Kendall Morgan on Apr 3, 2014 4:05:00 PM






Thus far in our Plasmids 101 series we've worked our way through the plasmid map: antibiotic resistance, origin of replication, and so on. Up to this point we can replicate our plasmid and make sure cells maintain it; the next step is getting the plasmid to express our gene of interest. Enter the promoter-- the element responsible for initiating the transcription of your insert into RNA.

In practice, the term "promoter" describes the combination of the promoter (RNA polymerase binding site) and operators (response elements). Promoters are about 100 to 1000 base pairs long and found upstream of their target genes. The sequence of the promoter region controls the binding of the RNA polymerase and transcription factors, therefore promoters play a large role in determining where and when your gene of interest will be expressed.

## The RNA polymerase(s)

RNA is transcribed from DNA using an RNA polymerase (RNAP). In bacteria this is done by a single enzyme; however, eukaryotes have muliple polymerases which are each responsible for a specific subset of RNAs. To gain this specificity, the eukaryotic RNAP can recognize and bind to specific promoter elements. This means that the promoter present in your plasmid backbone must to be compatible with the type of RNA that needs to be made: if you want mRNA (for gene expression) you need to use an RNAP II promoter, whereas small RNAs (such as shRNA) are transcribed from the RNAP III promoters. This post features promoters for general RNAP II and RNAP III transcription; however, using viral LTRs as RNAP II promoters is commonly employed in lentiviral and retroviral constructs and we will discuss these in a future post on viral vector parts.

# Promoter specificity

Aside from choosing a promoter based on type of RNA transcript, you will also need to make sure your plasmid has a promoter suited to working in your host organism. Because transcription machinery differs between cell types or organisms, promoters must be similarly variable. Bacterial promoters only work in prokaryotic cells and typically only in the same or closely related species from which they were derived. Similarly, the various eukaryotic cell types (mammalian, yeast, plants, etc) require unique promoters and there is very little crossover. Generally speaking, promoters in bacteria are less diverse and complex, having fewer parts than those in eukaryotic cells. Some promoters are constitutively active and on all the time while others are more carefully controlled. Regulated promoters might act only in certain tissues or at certain times in development or there may be ways to turn them on or off at will with a chemical, heat, or light. In the cell, promoters themselves are controlled by still other regulatory factors: enhancers, boundary elements, insulators, and silencers; however, some "leakiness" of transcription may occur. This is normally not a big issue for cells, but it may confound research results or even kill your cells if your gene of interest is toxic. To combat this, scientists have created synthetic promoters, which typically include some combination of other promoter elements, and tend to be more tightly regulated.

# Common promoters for eukaryotes and prokaryotes

We have included two reference tables below listing some of the most common bacterial and mammalian promoters. These lists is by no means exhaustive, but should be a good place to start when trying to pick your perfect promoter!

### Eukaryotic Promoters

| Promoter | Primarily used for | RNA transcript | Description | Expression | Additional considerations |
|---|---|---|---|---|---|
| CMV | General expression | mRNA | Stong mammalian expression promoter from the human cytomegalovirus | Constitutive | May contain an enhancer region. Can be silenced in some cell types. |
| EF1a | General expression | mRNA | Stong mammalian expression from human elongation factor 1 alpha | Constitutive | Tends to give consistent expression regardless of cell type or physiology. |
| SV40 | General expression | mRNA | Mammalian expression promoter from the simian vacuolating virus 40 | Constitutive | May include an enhancer. |
| PGK1 (human or mouse) | General expression | mRNA | Mammalian promoter from phosphoglycerate kinase gene. | Constitutive | Widespread expression, but may vary by cell type. Tends to resist promoter down regulation due to methylation or deacetylation. |
| Ubc | General expression | mRNA | Mammalian promoter from the human ubiquitin C gene | Constitutive | As the name implies, this promoter is ubiquitous. |
| human | General | | Mammalian promoter | | Ubiquitous. Chicken |

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|---|---|---|---|---|---|
| beta actin | expression | mRNA | from beta actin gene | Constitutive | version is commonly used in promoter hybrids. |
| CAG | General expression | mRNA | Strong hybrid mammalian promoter | Constitutive | Contains CMV enhancer, chicken beta actin promoter, and rabbit beta-globin splice acceptor. |
| TRE | General expression | mRNA | Tetracycline response element promoter | Inducible with Tetracyline or its derivatives. | Typically contains a minimal promoter with low basal activity and several tetracycline operators. Transcription can be turned on or off depending on what tet transactivator is used. |
| UAS | General expression | mRNA | Drosophila promoter conaining Gal4 binding sites | Specific | Requires the presence of Gal4 gene to activate promoter. |
| Ac5 | General expression | mRNA | Strong insect promoter from Drosophila Actin 5c gene | Constitutive | Commonly used in expression systems for Drosophila. |
| Polyhedrin | General expression | mRNA | Strong insect promoter from baculovirus | Constitutive | Commonly used in expression systems for insect cells. |
| CaMKIIa | Gene expression for optogenetics | mRNA | Ca2+/calmodulin-dependent protein kinase II promoter | Specific | Used for neuronal/CNS expression. Modulated by calcium and calmodulin. |
| GAL1, 10 | General expression | mRNA | Yeast adjacent, divergently transcribed promoters | Inducible with galactose; repressible with glucose | Can be used independently or together. Regulated by GAL4 and GAL 80. |
| TEF1 | General expression | mRNA | Yeast transcription elongation factor promoter | Constitutive | Analogous to mammalian EF1a promoter. |
| GDS | General expression | mRNA | Strong yeast expression promoter from glyceraldehyde 3-phosphage dehydrogenase | Constitutive | Very strong, also called TDH3 or GAPDH. |
| ADH1 | General expression | mRNA | Yeast promoter for alcohol dehydrogenase I | Repressed by ethanol | Full length version is strong with high expression. Truncated promoters are constitutive with lower expression. |

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|---|---|---|---|---|---|
| CaMV35S | General expression | mRNA | Strong plant promoter from the Cauliflower Mosaic Virus | Constitutive | Active in dicots, less active in monocots, with some activity in animal cells. |
| Ubi | General expression | mRNA | Plant promoter from maize ubiquitin gene | Constitutive | Gives high expression in plants. |
| H1 | small RNA expression | shRNA | From the human polymerase III RNA promoter | Constitutive | May have slightly lower expression than U6. May have better expression in neuronal cells. |
| U6 | small RNA expression | shRNA | From the human U6 small nuclear promoter | Constitutive | Murine U6 is also used, but may be less efficient. |

## Prokaryotic promoters

| Promoter | Primarily used for | Description | Expression | Additional considerations |
|---|---|---|---|---|
| T7 | in vitro transcription/ general expression | Promoter from T7 bacteriophage | Constitutive, but requires T7 RNA polymerase. | When used for in vitro transcription, the promoter drives either the sense OR antisense transcript depending on its orientation to your gene. |
| T7lac | High levels of gene expression | Promoter from T7 bacteriophage plus lac operators | Negligible basal expression when not induced. Requires T7 RNA polymerase, which is also controlled by lac operator. Can be induced by IPTG. | Commonly found in pET vectors. Very tightly regulated by the lac operators. Good for modulating gene expression through varied inducer concentrations. |
| Sp6 | in vitro transcription/ general expression | Promoter from Sp6 bacteriophage | Constitutive, but requires SP6 RNA polymerase. | SP6 polymerase has a high processivity. When used for in vitro transcription, the promoter drives either the sense OR antisense transcript depending on its orientation to your gene. |
| araBAD | General expression | Promoter of the arabinose metabolic operon | Inducible by arabinose and repressed catabolite repression in the presence of glucose or by competitive binding of the anti-inducer fucose | Weaker. Commonly found in pBAD vectors. Good for rapid regulation and low basal expression; however, not well-suited for modulating gene expression through varied inducer concentrations. |
| trp | High levels of gene expression | Promoter from E. coli tryptophan operon | Repressible | Gets turned off with high levels of cellular tryptophan. |

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|---|---|---|---|---|
| lac | General expression | Promoter from lac operon | Constitutive in the absense of lac repressor (lacI or lacIq). Can be induced by IPTG or lactose. | Leaky promoter with somewhat weak expression. lacIq mutation increases expression of the repressor 10x, thus tightening regulation of lac promoter. Good for modulating gene expression through varied inducer concentrations. |
| Ptac | General expression | Hybrid promoter of lac and trp | Regulated like the lac promoter | Contains -35 region from trpB and -10 region from lac. Very tight regulation. Good for modulating gene expression through varied inducer concentrations. Generally better expression than lac alone. |
| pL | High levels of gene expression | Promoter from bacteriophage lambda | Can be temperature regulatable | Often paired with the temperature sensitive cI857 repressor. |

Although this list is a great place to start, the tables above do not delve into the tissue or development-specific promoters available to scienitsts. Plasmids are oftentimes put to therapeutic uses, and in those cases it's important to identify the right tissue-specific promoters as described by researchers at the NIH here.

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*Note: A. Max Juchheim contributed to the writing of this article.*

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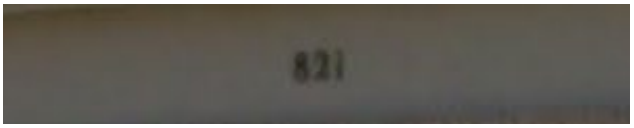

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