



# Construction of an Expression Cassette

Submitted for

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II M.Sc., Microbial Gene Technology



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# Dopamine Biosynthesis

## Neurotransmitters

Neurotransmitters are small molecules that are liberated by a presynaptic neuron into the synaptic cleft and cause a change in the postsynaptic membrane potential. This change can be either a direct depolarization or hyperpolarization or the activation of second messengers that eventually lead to changes in firing rate. Neurotransmitters act on receptors, which are transmembrane proteins that have an extracellular site that binds the neurotransmitters and suffers some conformational change.

Neurotransmitters are synthesized in the cell body and are transported to the terminal synaptic buttons of the axon where they are encapsulated into vesicles and stay close to the synaptic region of the button, when the action potential occurs, an influx of calcium ions induces the vesicles to fuse with the presynaptic membrane and its contents are poured into the synaptic cleft.

Each neurotransmitter has a particular biosynthetic pathway, but can be divided into three principal classes, since they share some enzyme or metabolic pathway. The first class is made up of acetylcholine alone, second class are the biogenic amines that are molecules formed by an amino acid losing a hydroxyl or carboxyl group. The third class is made up of amino acids. There is also a specific chain of enzymatic reactions that decompose the transmitter, either for destruction or for recycling. This is important since a synaptic signal does not subside until the transmitter is removed from the synaptic cleft. The transmitter can be degraded in the cleft, or absorbed by the postsynaptic neuron or a glial cell and then decomposed.

Neurotransmitters can act as inhibitor or excitatory signals to the postsynaptic cell, by hyperpolarizing or depolarizing its membrane, although the same molecules can function as an inhibitor or an excitatory. This happens because there are a small number of neurotransmitters but a great variety of their receptors on different types of cells. Acetylcholine, for instance, can act as an excitatory when it binds to one type of receptor, and as an inhibitor when bound on another kind, even if both types of receptors are present in the same cell.

Dopamine acts both an excitatory and inhibitory function. Dopamine comes under the group of amino acid derived neurotransmitter. They derived from tyrosine. The majority of tyrosine that does not get incorporated into proteins is catabolized for energy production. One other significant fate of tyrosine

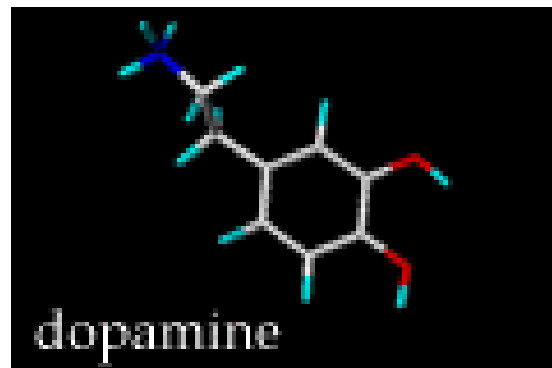
is conversion to the catecholamines. The catecholamine neurotransmitters are Dopamine, Norepinephrine, and Epinephrine.

Norepinephrine is the principal neurotransmitter of sympathetic postganglionic endings. Both norepinephrine and the methylated derivative epinephrine are stored in synaptic knobs of neurons.

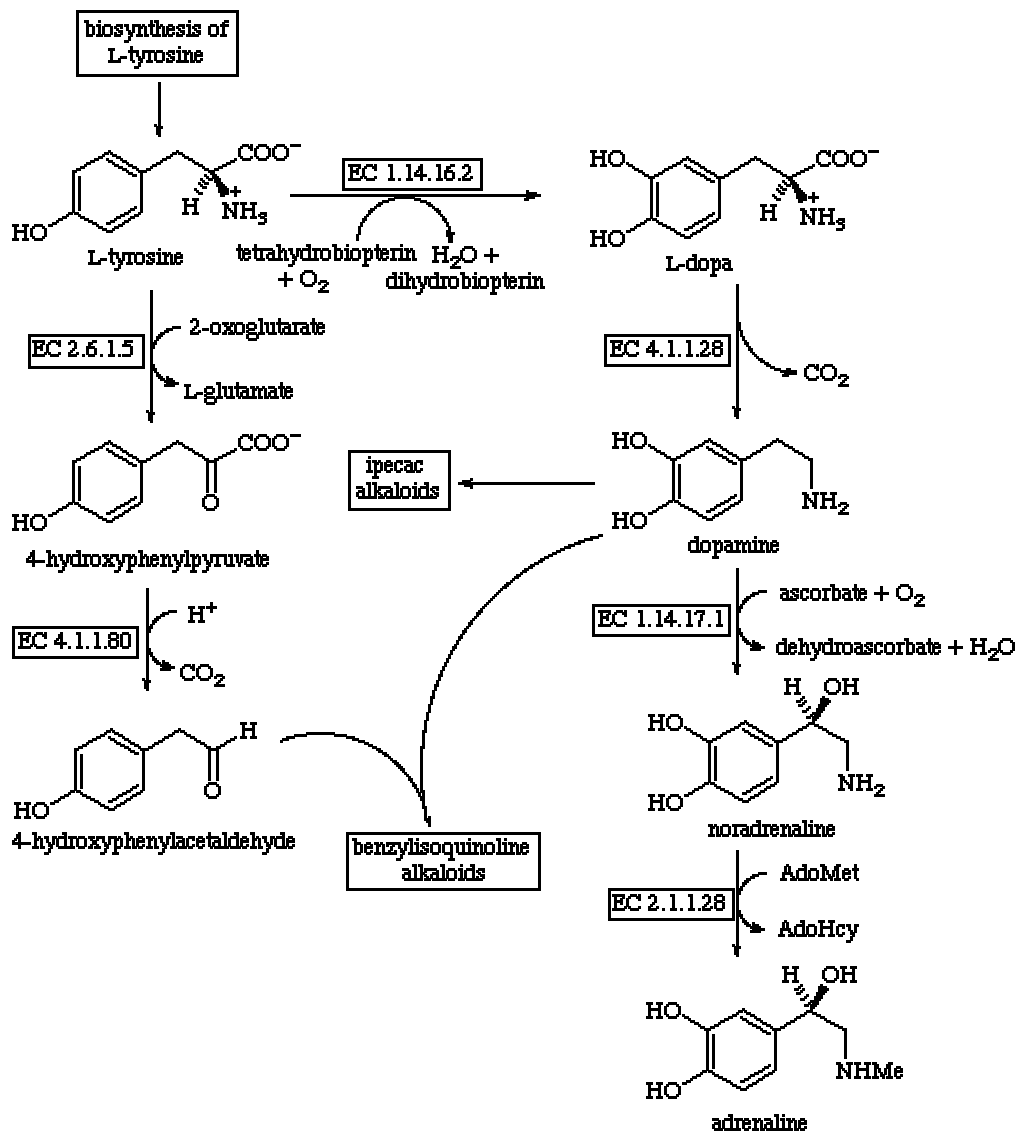
Tyrosine is transported into catecholamine secreting neurons and adrenal medullary cells where catecholamine synthesis takes place. The first step in the process requires Tyrosine hydroxylase requires tetrahydrobiopterin as cofactor. The hydroxylation reaction generates DOPA (3,4-dihydrophenylalanine). DOPA decarboxylase converts DOPA to Dopamine. Within the substantia nigra and some other regions of the brain, synthesis proceeds only to Dopamine.

Regulation of dopamine plays a crucial role in our mental and physical health. Neurons containing the neurotransmitter dopamine are clustered in the midbrain in an area called the **substantia nigra**. In Parkinson's disease, the dopamine-transmitting neurons in this area die. As a result, the brains of people with Parkinson's disease contain almost no dopamine. To help relieve their symptoms, we give these people L-DOPA, a drug that can be converted in the brain to dopamine.

Structure Of Dopamine: -

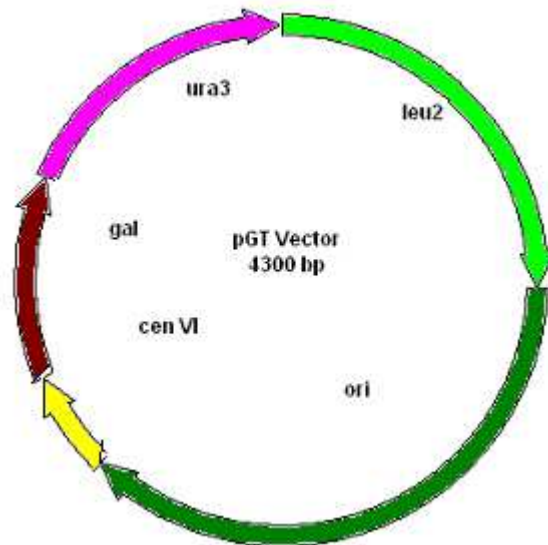


Dopamine Biosynthesis: -

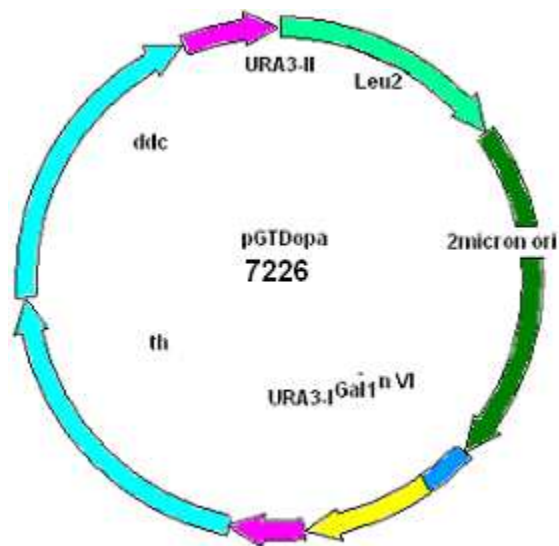


### pGT Yeast expression vector

(Constructed by Mr.Thirupugal.G)



### pGT Yeast expression vector



### pGTDopa Yeast expression Vector

## pGTDopa Yeast expression Vector

## Features: -

1-1095	:	Leu2 beta-isopropylmalate dehydrogenase (pFL36)
1096-2682	:	Rep origin (2 micron plasmid) (Derived from pCM190)
2683-2958	:	Cen VI (pFL36)
2959-3508	:	GAL1 Promotor
3509-3857	:	URA3 –I (pCS19)
3858-5350	:	Tyrosine hydroxylase
5351-6796	:	Dopamine decarboxylase
6802-7226	:	URA3-II

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 7201 agtagcagca cgttccttat atgtag

**Primer Design Criteria**

<b>Size</b>	Ideal size: 20-25 nucleotides long Generally: 18-30 nucleotides long
<b>3' end base</b>	should be a G or a C
<b>Melting temperatures (Tm)</b>	50-65 degrees Celcius
<b>GC content</b>	40-60%
<b>self-complementarity</b>	Should be avoided to minimize primer secondary structure and primer dimer formation
<b>Similarity</b>	Should have 100% match with the template

Sense primer Tyrosine hydroxylase : 5'cctaggatgccaaactccagatg 3'

<b>Size</b>	22 nucleotides long
<b>3' end base</b>	Guanine
<b>Melting temperatures (Tm)</b>	61.17 degrees Celcius
<b>GC content</b>	54.55%
<b>self-complementarity</b>	Dimer 1 (5')
<b>Similarity</b>	81

Antisense primer Tyrosine hydroxylase : 5'cagctcagtaatggttatcgtc 3'

<b>Size</b>	22 nucleotides long
<b>3' end base</b>	Cytosine
<b>Melting temperatures (Tm)</b>	61.17 degrees Celcius
<b>GC content</b>	54.83%
<b>self-complementarity</b>	Dimer 1 (5')
<b>Similarity</b>	82

## Primer Designing

Sense primer Dopamine decarboxylase : 5' ctcgagatgcttctgaatttc 3'

<b>Size</b>	21 nucleotides long
<b>3' end base</b>	Cytosine
<b>Melting temperatures (Tm)</b>	54.39 degrees Celcius
<b>GC content</b>	42.86%
<b>self-complementarity</b>	Dimer 3 (5')
<b>Similarity</b>	82

Antisense Dopamine decarboxylase : 5'ctgcaagtgtgcaagtcgtg 3'

<b>Size</b>	20 nucleotides long
<b>3' end base</b>	Guanine
<b>Melting temperatures (Tm)</b>	55.95 degrees Celcius
<b>GC content</b>	55%
<b>self-complementarity</b>	Dimer 3 (5')
<b>Similarity</b>	80