



# Open Source Wnt Project



**Gateway Vectors:** You have received all 19 human Wnt clones in two Gateway vector backbones. The first set of clones are in “Entry” vectors, and the second set are in “Destination” vectors. It is the Destination vectors you will use for experiments. There are many types of Destination vectors for applications including Lentiviral expression, bacterial expression, mammalian expression, expression in yeast, fusions with protein open reading frames, regulated expression etc. We are giving you Wnt clones in a Destination vector appropriate for expression in mammalian cells, but you are not limited to this. You can use the Entry clones to shuttle Wnt coding sequences into other Destination vectors (in an afternoon!). For further information, please see: (<http://www.invitrogen.com/gateway>).

## There are two flavors of Entry Clones:

**STOP:** These Entry clones encode a translation **TGA** STOP codon in the natural position at the end of each of the Wnt Open Reading Frames. These clones are ready for shuttling into Destination vectors and once transferred, will make unadulterated Wnt proteins from whatever expression vector is used.

**NONSTOP:** These Entry clones are different from the STOP clones by a single nucleotide which destroys the STOP codon by changing **TGA** to **TGC**. NONSTOP Entry clones allow the user to recombine into Destination vectors for fusion with another open reading frame (such as epitope tags, GFP, etc).

## Destination Clones:

The STOP and NONSTOP clones have been recombined into Destination vector **pcDNA3.2/V5-DEST**. This plasmid contains sequences that code for the V5 epitope tag downstream of open reading frames that have been shuttled into the vector. These clones are ready for expression in mammalian cells.

**STOP** clones will be expressed as untagged “pure” Wnts because the **TGA** stop codon is present in between the WNT ORF and the plasmid-provided V5 coding sequences.

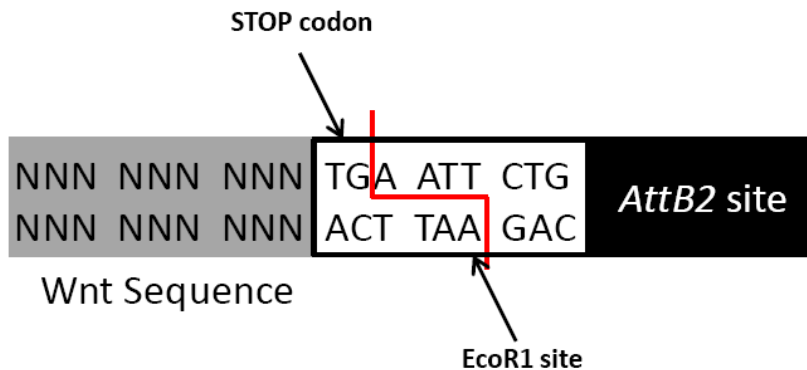
**NONSTOP** clones will be expressed as C-terminal V5-tagged Wnts because the **TGA** stop codon has been changed to **TGC** (see above). The stop codon for this fusion protein occurs downstream of the V5 coding sequences (TAG). The C-terminal V5-tagged Wnt is not very active but still informative when used in parallel with its untagged cousin (which is expressed from a plasmid that differs by only one nucleotide). The V5-tagged Wnt can therefore yield valuable information about expression levels and secretion/localization.

Note: Western blot experiments were performed to determine whether any translational read-through of **TGA** results in any amount of V5 tagging of the “untagged” Wnt. We are unable to detect any amount of V5 tag, even with overexposure of the blot. Therefore, we are concluding that translational read-through of the **TGA** STOP codon is minimal or does not happen.



### Additional notes:

1. All the clones provided in this Open Source Set are in the Gateway system, but users are not married to use of this system. For example, users do not have to buy another Gateway plasmid to use a different tag on the C-terminus. All Wnt STOP clones can be linearized at the translation stop codon by EcoR1 restriction, this both destroys the translation stop codon and enables insertion of user-designed sequences (see sequence diagram below).



This flexibility is built into **ALL** Wnt STOP clones (i.e. internal EcoR1 sites have been eliminated in several Wnts via silent codon changes). Furthermore, most Wnt STOP clones can be linearized with Not1 at the 5' end, enabling the entire coding sequence to be excised with a Not1/EcoR1 digestion (a couple of Wnts such as Wnt3a are exceptions – they have an internal Not1 site).

2. All Wnt clones have been cloned and sequence-verified at multiple stages. Nevertheless, if there are any issues with the identity or sequence authenticity of the ORFs, please contact us.
3. If you develop a second generation Wnt clone or set of clones, we ask that you:
  - a. post a description of these clones and their availability on the Wnt Homepage Forum (<http://www.stanford.edu/group/nusselab/cgi-bin/wnt/forum/1>)
  - b. Freely share this clone or hand over to Addgene for sharing with the Wnt community!

For questions and further information including all plasmid sequences, please contact Rani Najdi from the Waterman Lab at [rnajdi@uci.edu](mailto:rnajdi@uci.edu).