Molecular Cloning Simulation in SnapGene

By: Deng Luo; Course No: B206

Get the insertion in SnapGene

Google "gateway cloning" for videos gives "SnapGene | Simulate Gateway Cloning". Follow it.

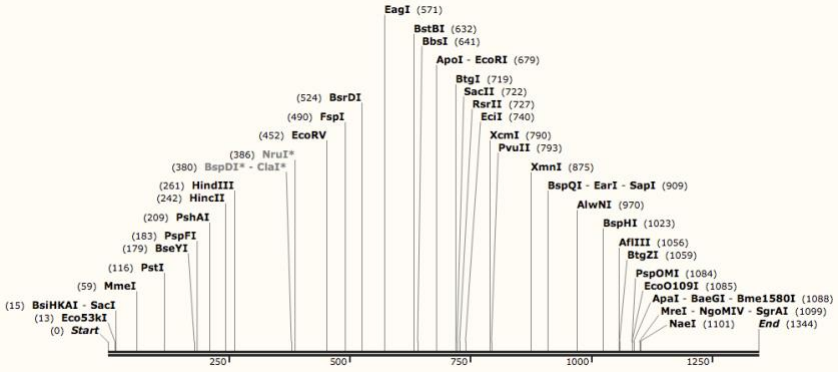
We were using VirD2 as insert. Google "agrobacterium tumefacies vird2 sequence" gives "virD2 - T-DNA border endonuclease VirD2 - Agrobacterium fabrum ...". Under "Sequence", "Sequence databases", "RefSeq", there is "NC_003065.3".

Search (Command+F) "vird2", click on the "CDS", see screenshot below. There will be a tool bar at the bottom of the page. Click on the "FASTA" at the very right of the bar. Then the VirD2's cDNA sequence is here.

```
CDS             196503..197846
                /gene="virD2"
                /locus_tag="Atu6182"
                /old_locus_tag="AGR_pTi_21"
                /codon_start=1
                /transl_table=11
                /product="type IV secretion system T-DNA border
                endonuclease VirD2"
                /protein_id="NP_396503.1"
                /db_xref="GeneID:1137505"
                /translation="MPDRAQVIIRIVPGGGTKTLQQIINQLEYLSRKGRLELQRSARH
                LDIPLPPDQIHELARSWVQETGTYDESQPDEERQQELTTHIIVSFPAGTSQVAAYAAS
                REWAAEMFGSGAGGGRYNYLTAFHIDRDHPHLHVVVNRRELLGHGWLKISRRHPQLNY
                DALRIKMAEISLRHGIALDASRRAERGITERPITYAQYRRLEREQARQIRFEDADLEQ
                SSPQGDHPEFSQPFDTSPFEASAGGPEDMPRPNNRQNESQVHLQEPAGVSNEAGVLVR
                VALETERLAQPFVSETILADDIGSGSSRVAEGRVESANRTPDIPRAATEAATHTTHDR
                QRRAKRPHDDDGGPSGAKRVTLEGIAVGPQANAGEQDGSSGPLVRQAGTSRPSPPTAT
                TRASTATDSLSATAHLQQRRGVLSKRPREDDDGEPSERKRERDERSKDGRGGNRR"
```

Click "Send to" on the upper right of the main body, under "Choose Destination" choose "File", click "Create file" button. Then the sequence should be downloaded as "FASTA" format.

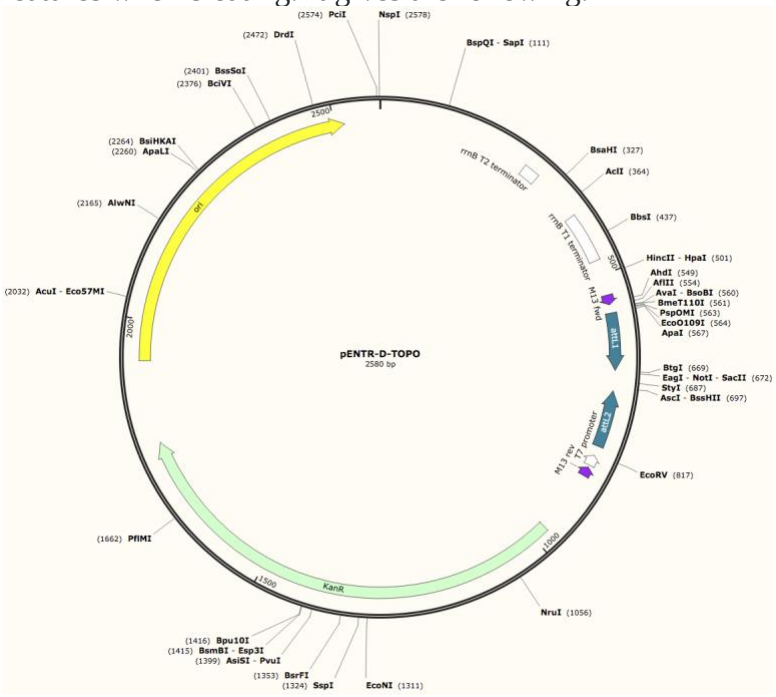
Open the fasta file in SnapGene as linear DNA. It gives you the following.



Get the donor vector in SnapGene

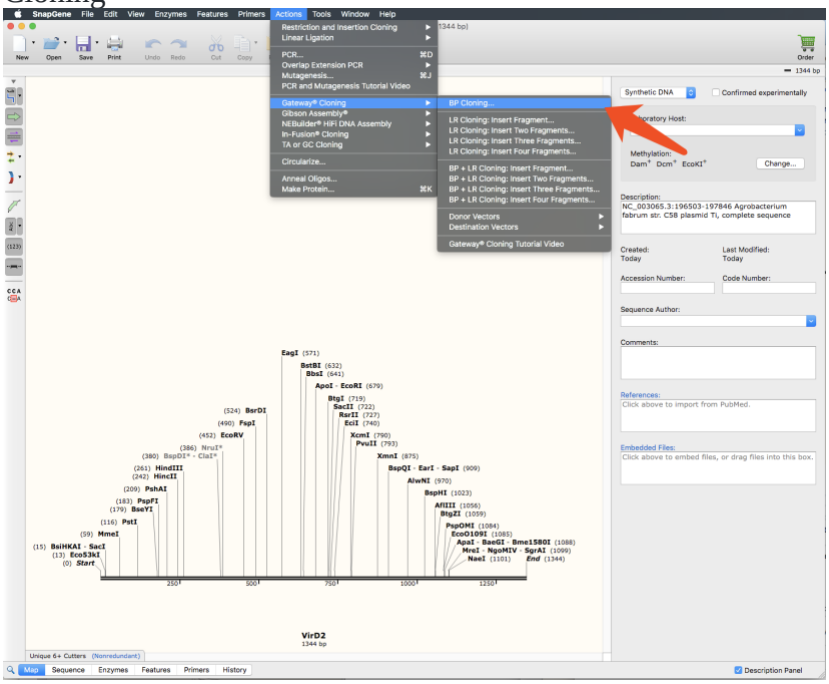
For Entry clon. Google "pENTER-D-topo" gives "[pENTR/D-TOPO Cloning Kit, with One Shot TOP10 Chemically ...]".

Command+F "sequence", and then click the page icon to get the sequence. Command+A to choose all. Command+N in SnapGene and paste the sequence to create a new sequence, add all 9 features when creating. It gives the following.

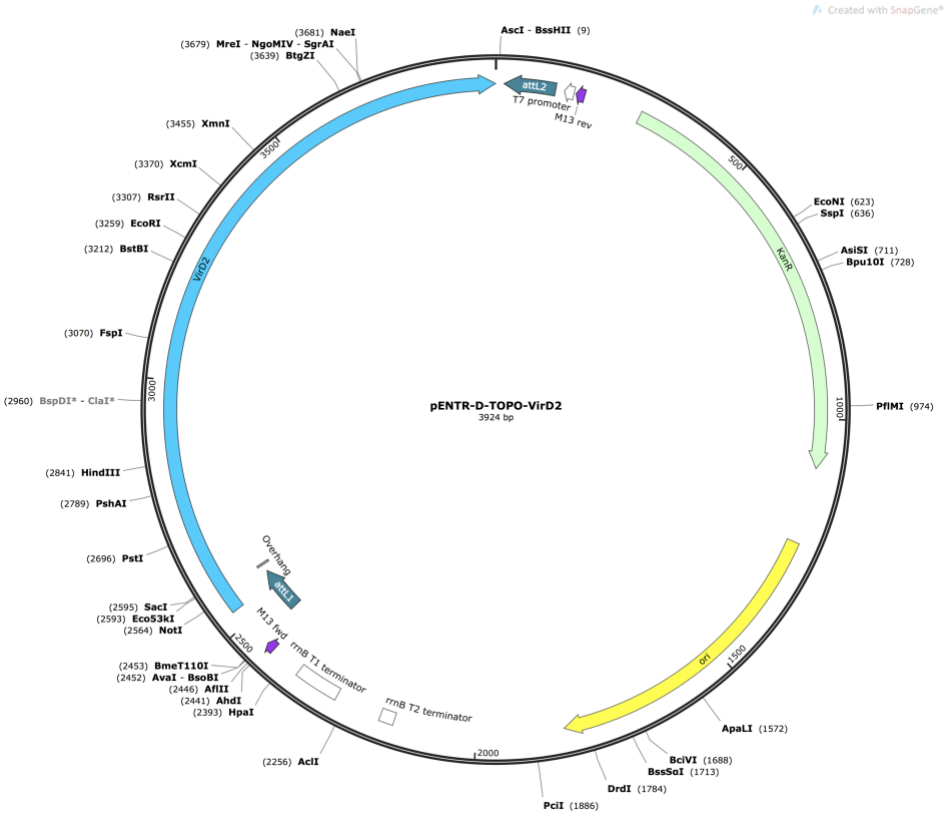


Save it with name "pENTR-D-TOPO.dna".

In the "VirD2.fasta" window inside SnapGene, Click "Actions", "Gateway Cloning", "BP Cloning"



In the new window, click the "Donor Vector" tab, choose the "pENTR-D-TOPO.dna" just created. There is a problem, there is no attB site in our insert, VirD2; and there is no attP site in our donor vector, pENTR-D-TOPO. Later, I figure out that we are actually using directional topo cloning which directly ligates the VirD2 with pENTR-D-TOPO. We are using pENTR™/D-TOPO™ Cloning Kit. First, the 5' overhang, CACC, needs to be added to the insert usually by PCR with designed primer. Then the CACC overhang pairs with the donor vector's 3' overhang, GGTG (5'->3'). The outcome on the sequence level is just like first cut immediately before the GGTG (5'->3') and then insert the gene of interest. However, one thing of note is that on the molecular level it is the ligation between two sticky ends and the chop of the redundant overhangs on the insert. To roughly represent the process in SnapGene, the pENTR-D-TOPO is linearized by cutting immediately before the GGTG (5'->3'), and the gene of interest is then inserted there. The steps in SnapGene are: Actions, Linearize; then in the new linearized sequence window, Actions, Linear Ligation, Ligate Two Fragments, in the Fragment 1 tab, leave as default, in the Fragment 2 tab, choose "VirD2.fasta", check the orientation at the bottom, and click ligate; in the newly generated linear sequence, click Actions, Circularize to generate the final entry clone. Note again that this is just a rough representation of the real process to get the final sequence shown below because there is no Topo cloning in SnapGene. Then the CDS (coding sequence) feature is added for the inserted gene. The sequence is saved as "pENTR-D-TOPO-VirD2.dna".

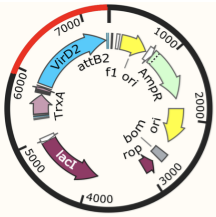


Perform Gateway Cloning

First, download the destination vector. Google "PET32a destination vector" shows "Addgene: pET32a-DEST". This is the closest one (7611bp) to the one in our lab manual (7575bp). I have not found a one named "pET32a-GW".

In the "pENTR-D-TOPO-VirD2.dna" sequence window, click Actions, Gateway Clong, LR Cloning: Insert Fragment. In the new window, leave as default in the Entry Clone tab; in the Destination Vector tab, choose "pET32a-DEST.dna"; then click the third tab, Expression Clone, choose "DH5α" as Bacterial Transformation Strain and click Clone. The final sequence is 7336bp, which is 3bp longer than that from the manual, which I think is the minor difference in the destination vector. The final vector is shown below.

The cloning history

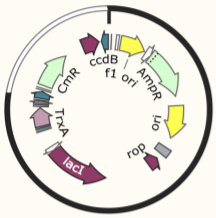


pET32a-VirD2
7336 bp

GATEWAY LR CLONING

Replace
attR1 – attR2

Insert
attL1 – attL2



pET32a-DEST
7611 bp

pENTR-D-TOPO-VirD2
3924 bp

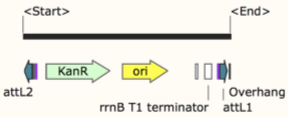
CIRCULARIZE

pENTR-D-TOPO-VirD2
3924 bp

LIGATE FRAGMENTS

Choose
Start (0) – End (2580)

Choose
Start (0) – End (1344)

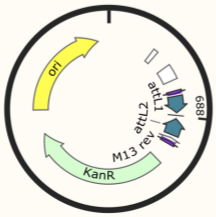


VirD2
1344 bp

Linear-pENTR-D-TOPO
2580 bp

LINEARIZE

Linearize at
base 689



pENTR-D-TOPO
2580 bp

In similar steps in SnapGene, but using Gibson Assembly, the HiFi cloning is simulated. The history of it is shown below.