



**Features:**

roe = recombination cloning, over-expression

1. Vermilion is the selectable marker
2. attB/P site specific integration
3. Amp resistant
4. For over-expression
  - 10X UAS
  - Hsp70 promoter
  - Two insulators
  - One ftz intron
5. Simplified cloning
  - Recombination method
  - Two step cloning:
    - 1st - clone PCR fragment into Entry vector (pENTR/DTOPO or mENTRY)
    - 2nd - recombination

664-2543,	vermillion
2568-2935,	attB
2947-3377,	gypsy
3395-3428,	loxp
3435-3544,	5xUAS
3551-3584,	loxp
3591-3700,	5xUAS
3707-3966,	Hsp70 promoter
3994-5705,	attR cassette 1
5763-5909,	ftz intron
5933-6632,	SV40 polyA
6639-7069,	gypsy

The detailed cloning method can be found on the TRiP website: <http://flyrnai.org/TRiP-HOME.html>

Accession number: GU931386  
Jian-Quan Ni (jni@genetics.med.harvard.edu)

VALIUM10-roe: 9273bp

CACCTAAATTGTAAGCGTTAATATTTTGTAAAAATTCGCGTTAAATTTTTGTAAATCAGCTCATTTTTT  
AACCAATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAGAATAGACCGAGATAGGGTTGAGTGTG  
TTCCAGTTTGGAAACAAGAGTCCACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTA  
TCAGGGCGATGGCCCACTACGTGAACCATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCA  
CTAAATCGGAACCTAAAGGGAGCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAA  
AGGAAGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACAGCTGCGCGTAAC  
CACCACACCCGCCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCCGCCATTCAGGCTGCGCAACTGT  
TGGGAAGGGCGATCGGTGCGGGCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGC  
GATTAAGTTGGGTAACGCCAGGGTTTTCCAGTACGACGTTGTAAAACGACGGCCAGTGAATTGTAATA  
CGACTCACTATAGGGCGAATTGGGTACAAGCTT**ATTTATTTTGTATGTTATATGTATTATATGTCAGAC**  
**ATAAAGAAAAGGAACACATCAAATGTGATAACAAAGACTAAACAAGTAATTTTATTACACAAAACGACA**  
**AAACAGTAGGCAGAACAAACAACGCATAGCCAAACATTGACGAATTGGATACCTTGCCGATTGTCAGACA**  
**CTTTTGTGATCAGTTTCTTGCGAATGGTCTCGTCCAGCGGTGGAATCGCCTCGCGGGGAATCAGAAAAG**  
**TGGACAGATTGAACAGATCCAGAAACACCTTGTACCGATCACTGAAACCAAAAAAAAAACAAAGGGGAGAAC**  
**AGTTTGAGTTCATTGATCCCCGATATAATCACATCTGCGATGATCACCTGAGAGTGGAGCGCAGATATTG**  
**ATATCCAGACGAGCCACAGTGCCCAACTGTTGGGATCCAATCATGCGTTGCACCATGATCACGTGATTG**  
**TCTGCGGCGGGAATAGAAAGTATTTGGTTAGGAAAACAGTCTTAAACATAAGATATATTTATAAAAAGAG**  
**TATCAAAGAATGCAATACTTACATCTCCACTTGGTTATTAACGAGTCGATGTCCATGAGCAGGGTGAGCA**  
**ACTGGTGTGGTTGGCTGAACCTGGGTTCATCCCTATAGAAGGTGATCATGATGGCTCCCTGAAGGGCACG**  
**ATGGCTAAACCGGCGATCCCCACGACGCACAGTGATCGTGCAGTCCCGGATCAAAGATGGAGCGATAC**  
**ACCTCGCGTCGCTTCTCAATGTCCATGAGGCGGTAGTTTTTTCGCTTCTCCACGGGCTCCTCCATGGCGC**  
**TCTGTACCTGCGCCTCCAGGAATCGATCGACGCTCTCCTGAAACTTGGCCCAGAAGTTGAAGCCACTCTC**  
**CTCCAGTCCGGGCGTCTCTCCAGCCATCGCTGCAGTCCAGTAGCGAGGGATCTTTCTCCGAGTTG**  
**CGAATCGAGTTCGCGCCTCCTCGTCGCTAAAGACATCCGAGTACTTCTGGTTGTATCTCACCCGCTGCT**  
**CTGTCAGAACTCCCAGCTTGTCTCGATCAAACGGAAGTGCAGCGACTGAAAACAGATGCGGGTGCCAG**  
**GTACTTGCGGAAGTCCATGAAGTCTAGCGGGTTCATGGTCTCCAGAATGGGCAGTGGTCCACCAGGAGC**  
**TGTACAAAGGAAGTTATAAACGGATTTTGGTAAGAGATTCAGAAAGCACTCACTTTTAGAATCAGAACCA**  
**CTCGGTTGAGTCGCTTGAACATCTCCAGCGTCTTGGTTTTCATCGATGACCTCTGCATCCAACATGTCTCG**  
**TATGGAGTCGAACTCAAAGATGATCTGCTTGAACCAAAGCTCGTAGGCTGTGGCGAAGGTACTTAAATGC**  
**CATTGAGTGTGTCATCAAAGTTGTAACCTACTCACCTGGTGCCTGATGATGAACAGATGCTCATCGT**  
**GCACGGTTCGCTTGTCTCCTCGGACAGCATACTGGGCATCCAGCAGTTTGTCCAGCATCAGATACTC**  
**TCCATAGATTTTGCCACTTCCGTGGTTAATGGCACCGCCGAATCATCGTATCGTTTCTGTATGGGTTT**  
**GAATTGAATCGCAGAAGTGAAGATCGATTGGCATTCCCTGGACAGCACGTGCTGGTGTCCACCCGTTTCT**  
**GCATAGGGACAGCTCATGGTGCACAGCTCAGATCAGATCGTGACTCCTCGAGCGGCGGATGCTGGCGAAC**  
**TGATCTCCGCCAGCGGACCGGAGATGAGACCCAGCGAACCAGATAACAGAGCGAGAGAGCTCCAGTCCG**  
**ACTGATTGCACAGTCGGTATCTGGGCGATGGGCACTGCCAGATAGGCTGGGAATTATCAATCACTTGAG**  
**GTGAAAGTGCGGCGCACACAAATAAGCTTGATATCATCGATCTCGAGGCTGCATCCAACGCGTTGGGAGC**  
**TCTCCGGATCAATTCGGCTTCAGGTACCGTGCAGATGTAGGTACGGTCTCGAAGCCGCGGTGCGGGTG**  
**CCAGGGCGTGCCCTTGGGCTCCCCGGGCGGTACTCCACCTCACCCATCTGGTCCATCATGATGAACGGG**  
**TCGAGGTGGCGGTAGTTGATCCCGGCGAACGCGCGGCGCACCGGGAAGCCCTCGCCCTCGAAACCGCTGG**  
**GCGCGGTGGTACGGTGAGCACGGGACGTGCGACGGCGTGGCGGGTGGCGGATACGCGGGGACGCTCAG**  
**CGGGTCTCGACGGTACGGCGGGCATGTCGACAAGCCGAATTGATCCACTAGAAGGCCTAATTCGGTAC**  
**ACTAGTTGGCCACGTAATAAGTGTGCGTTGAATTTATTCGCAAAAACATTGCATATTTTCGGCAAAGTAA**  
**AATTTTGTGTCATACCTTATCAAAAATAAGTGTGCTGCATACTTTTTAGAGAAAACAAATAATTTTTTATT**  
**GCATACCCGTTTTTAATAAAAATACATTGCATACCTCTTTTAATAAAAATATTGCATACTTTGACGAAA**  
**CAAATTTTCGTTGCATACCCAATAAAAGATTATTATATTGCATACCCGTTTTTAATAAAAATACATTGCAT**  
**ACCTCTTTTAATAAAAATATTGCATACGTTGACGAAACAAATTTTCGTTGCATACCCAATAAAAAGATT**  
**ATTATATTGCATACCTTTTCTTGCATACCATTTAGCCGATCAATTGTGCTCGGCAACAGTATATTGTG**  
**GTGTGCCAACCAACAACACTAGTAGTACCAGCT**ATAACTTCGTATAATGTATGCTATACGAAGTTAT**CT**  
**GCAGGCAGGTTCGGAGTACTGTCTCCGAGCGGAGTACTGTCTCCGAGCGGAGTACTGTCTCCGAGCGG**  
**AGTACTGTCTCCGAGCGGAGTACTGTCTCCGAGCGGAGACTCCATGG**ATAACTTCGTATAATGTATG****  
**CTATACGAAGTTATGGATCCGCAGGTTCGGAGTACTGTCTCCGAGCGGAGTACTGTCTCCGAGCGGAGT**  
**ACTGTCTCCGAGCGGAGTACTGTCTCCGAGCGGAGTACTGTCTCCGAGCGGAGTACTGTCTCGAC**AGCG****  
**AGCGCCGGAGTATAAATAGAGGGCGCTTCGTCTACGGAGCGACAATTCAATTCAAAACAAGCAAGTGAACA**

**CGTCGCTAAGCGAAAGCTAAGCAAATAAACAAAGCGCAGCTGAACAAGCTAAACAATCTGCAGTAAAGTGC**  
**AAGTTAAAGTGAATCAATTAAGTAACCAGCAACCAAGTAAATCAACTGCAACTACTGAAATCTGCCAA**  
**GAAGTAATTATTGAATACAAGAAGAGAACTCTGAATAGGGAATTGG**GAATTCAGATCTGCGGCCGCGGC  
 TCGAATCACAAAGTTTGTACAAAAAGCTGAACGAGAAACGTAAAATGATATAAATATCAATATATTAAT  
 TAGATTTTGCATAAAAAACAGACTACATAACTGTAAAACACAACATATCCAGTCACTATGGCGGCCGC  
 ATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGATTTTGAGTTAGGATCCG  
 GCGAGATTTTTCAGGAGCTAAGGAAGCTAAAATGGAGAAAAAATCACTGGATATAACCACCGTTGATATAT  
 CCCAATGGCATCGTAAAGAACATTTTGAGGCATTTTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGT  
 TCAGCTGGATATTACGGCCTTTTAAAGACCGTAAAGAAAAATAAGCACAAAGTTTATCCGGCCTTTATT  
 CACATTCTTGCCCGCCTGATGAATGCTCATCCGGAATTCGGTATGGCAATGAAAGACGGTGAGCTGGTGA  
 TATGGGATAGTGTTTACCCTTGTACACCGTTTTCCATGAGCAAACCTGAAACGTTTTTCATCGCTCTGGAG  
 TGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTACGGTGAAAAC  
 CTGGCCTATTTCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCA  
 CCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCGTTTTTACCATGGGCAAAATATTA  
 TACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTTCAGTTTCATCATGCCGTCTGTGATGGCTTCCAT  
 GTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAAACGCGTGGAT  
 CCGGCTTACTAAAAGCCAGATAACAGTATGCGTATTTGCGCGCTGATTTTTGCGGTATAAGAATATATAC  
 TGATATGTATACCCGAAGTATGTCAAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTTGACA  
 GCGACAGCTATCAGTTGCTCAAGGCATATATGATGTCAATATCTCCGGTCTGGTAAGCACAAACCATGCAG  
 AATGAAGCCCGTCTGCTGCGTGCCGAACGCTGGAAAGCGGAAAAATCAGGAAGGGATGGCTGAGGTGCCCC  
 GGTTTATTGAAATGAACGGCTCTTTTGTGCTGACGAGAACAGGGACTGGTGAATGCAGTTTAAAGGTTTACA  
 CCTATAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCCGGGCG  
 ACGGATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTGATATAAAGTCTCCCGTGAACTTTACCCGGTG  
 GTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCGATATGGCCAGTGTGCCGGTCTCCGTTATCG  
 GGAAGAAGTGGCTGATCTCAGCCACCGCGAAAATGACATCAAAAACGCCATTAACCTGATGTTCTGGGG  
 AATATAAATGTGAGGCTCCCTTATACACAGCCAGTCTGCAGGTGACCATAGTGACTGGATATGTTGTGT  
 TTTACAGTATTATGTAGTCTGTTTTTTATGCAAAATCTAATTTAATATATTGATATTTATATCATTTTAC  
 GTTTTCTCGTTTTCAGCTTTCTTGTACAAAGTGGTGATTCGAGGGTACCTCTAGAGCAAACCTAGTTCTGATCT  
 GCTAGACAATTGTTGGCATCAG**GTTAGGCATCACACACGATTAACAACCCCTAAAAATACACTTTGAAAT**  
**ATTGAAAATATGTTTTTGTATACATTTTTGATATTTTCAAACAATACGCAGTTATAAACTCATTAGCTA**  
**ACCCATTTTTTCTTTGCTTATGCTTACAG**ATTGCAAAGAACTAGAGCCGCGGG**GATCTTTGTGAAGGAACC**  
**TTACTTCTGTGGTGTGACATAATTGGACAAACTACCTACAGAGATTTAAAGCTCTAAGGTAATATAAAA**  
**TTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTGTGTATTTTAGATTCCAACCTATGGAA**  
**CTGATGAATGGGAGCAGTGGTGAATGCCTTTAATGAGGAAAACCTGTTTTGCTCAGAAGAAATGCCATC**  
**TAGTGATGATGAGGCTACTGCTGACTCTCAACATTCTACTCCTCCAAAAAAGAAGAGAAAGGTAGAAGAC**  
**CCCAAGGACTTTCCTCAGAATTGCTAAGTTTTTTGAGTCATGCTGTGTTTAGTAATAGAACTCTTGCTT**  
**GCTTTGCTATTTACACCACAAAGGAAAAAGCTGCACCTGCTATAACAAGAAAATTATGGAAAAATATTTGAT**  
**GTATAGTGCCTTGACTAGAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCTTTAAAAA**  
**CCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGGAATTGTTGTTGTTAACTTGTTTATGCA**  
**GCTTATAATGGTTACAAATAAAGCAATAGCATACAAATTTACAAATAAAGCATTTTTTTCACTGCATT**  
**CTAGTTGTGGTTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGTTCCAGAGCTCTGGCCACGTAAT**  
**AAGTGTGCGTTGAATTTATTCGCAAAAACATTGCATATTTTCGGCAAAGTAAAATTTTGTTCATACCTT**  
**ATCAAAAATAAGTGTGCATACTTTTTAGAGAAACCAATAATTTTTTATTGCATACCCGTTTTTAATA**  
**AAATACATTGCATACCTCTTTTTAATAAAAAATATTGCATACTTTGACGAAACAAATTTTCGTTGCATAC**  
**CCAATAAAAGATTATTATATTGCATACCCGTTTTTAATAAAATACATTGCATACCTCTTTTTAATAAAAA**  
**ATATTGCATACGTTGACGAAACAAATTTTCGTTGCATACCCAATAAAAGATTATTATATTGCATACCTTT**  
**TCTTGCCATACCATTTAGCCGATCAATTGTGCTCGGCAACAGTATATTTGTGGTGTGCCAACCAACAACG**  
 AGCTCCAGCTTTTGTCCCTTTAGTGAGGGTTAATTTTCAGCTTGGCGTAATCATGGTCATAGCTGTTTC  
 CTGTGTGAAATTGTTATCCGCTCACAAATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTG  
 GGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTCCAGTCGGGAAAC  
 CTGTGCTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCCTATTGGGCGCTCTT  
 CCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTCTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA  
 GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAGGCCAGCAA  
 AAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATC  
 AAAAAATCGACGCTCAAGTCAGAGGTGGCGAAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCC  
 TGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCT

TCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCA  
AGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGA  
GTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGG  
TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTG  
GTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAAC  
CACCGCTGGTAGCGGTGGTTTTTTTTGTTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAA  
GATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCA  
TGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAG  
TATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGT  
CTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCAT  
CTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCA  
GCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGT  
TGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTTCGCAACGTTGTTGCCATTGCTACAGGCA  
TCGTGGTGTACGCTCGTCGTTTTGGTATGGCTTCATTCAGCTCCGGTCCCAACGATCAAGGCGAGTTAC  
ATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCCGATCGTTGTCAGAAGTAAGTTG  
GCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTGTCATGCCATCCGTAAGAT  
GCTTTTCTGTGACTGGTGTGACTCAACCAAGTCATTCGAGAATAGTGTATGCGGCGACCGAGTTGCTC  
TTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGAAAA  
CGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCGATGTAACCCACTCGTG  
CACCCAACCTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTTCTGGGTGAGCAAAAACAGGAAGGCAAAA  
TGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCTTTTCAATATTAT  
TGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAA