Supplemental Information

Oligo DNAs

Name of oligo	DNA Sequence (5′ to 3′)
RPB2tag5FW	CCGCTCTAGATGCCTCCGAACTTCACACTTATGG
RPB2tag5RV	GTCGACACTAGTGGATCCAACATACTTTTTACTAGTGACATCAAC
RPB2tag3FW	GGATCCACTAGTGTCGACTGGTCTTAGTCCATCTATCC
RPB2tag3RV	GGCGCTCGAGCATTTAGTAGGTCTGCCTC
BamHI-mcherry_Fw1	GGATCCTATCCTTATGATGTTCCTGATTATGCTTGAAC
HindIII-Neo4_Rv2	AAGCTTGATATCGAATTCAGATCCCCCGGGCTGCATTTTTCCAGT
5Am-Pdd1_Fw	GCTGATGGCGATGAATGAACACTGATGTCTCTTAGCCATAATGGGTAGATGTTG
Pdd1-mCherry_Rv	ATCTTCTTCTCCTTTTGAAACCATGGATCCATGAGTAAGTTATTAATTAGCTTGTC
Neo4-Pdd1_Fw	CCCGGGGGATCTGAATTCGATATCAAGCTTTCACATTTTAATTTATGAAAATTTATTC
3Am-Pdd1_Rv	GCGAGCACAGAATTAATACGACTTCTGAGGAAGTCTTGTAATAAGGTGAAAGC
DCL1GFP5FW	GCTGATGGCGATGAATGAACACTGTCTAATTTATAGAGGATTGCAACC
DCL1GFP5RV	AAGTTCTTCACCCTTAGAAACCATGGATCCTACTTACTTTCCTTAGAATATTAATCCTAG
DCL1GFP3FW	CCCGGGGGATCTGAATTCGATATCAAGCTTTTTAAATATTGGTTTCAGCC
DCL1GFP3RV	GCGAGCACAGAATTAATACGACTCTTATACACAAAGAGGAAGTGCCC
NUP4HA5FW	GCTGATGGCGATGAATGAACACTGCTACATATATTGACACTG
NUP4HA5RV	GCATAATCAGGAACATCATAAGGATAGGATCCACAACTCCAGTCAAATACTC
NUP4HA3FW	CCCGGGGGATCTGAATTCGATATCAAGCTTATAGATTTTCCTTTAATTAATTTTGCG
NUP4HA3RV	GCGAGCACAGAATTAATACGACTGAGACCTGAACCTATTTTGACTGG
5′RACE-Outer	GCTGATGGCGATGAATGAACACTG
3′RACE-Outer	GCGAGCACAGAATTAATACGACT
T7	CGCGTAATACGACTCACTATAG
T3	GCGCAATTAACCCTCACTAAAG

Protocol for Preparation of C-terminal tagging construct by PCR

1. Materials required

- Genomic DNA of a wild-type strain, RNaseA treated, ~0.5 μg/μl
- Plasmid DNA containing C-terminal tagging module prepared by Qiagen Mini-prep kit
- Primers to amplify ~0.5-1.0 kb of C-terminal coding and 3' flanking of your target gene
  5'FW primer should have 5'RACE-Outer sequence for second PCR
  5'RV primer should have overlapping sequences with tag
  3'FW primer should have overlapping sequences with neo3/neo4
  3'RV primer should have 3'RACE-Outer sequence for second PCR
  - For EGFP-neo4:
    - 5'FW: 5'-GCTGATGGCGATGAATGAACACTG-Your gene-3'
    - 5'RV: 5'-AAGTTCTTCACCCTTAGAAACCATGGATCC-Your gene-3'
    - 3'FW: 5'-CCCGGGGGATCTGAATTCGATATCAAGCTT-Your gene-3'
    - 3'RV: 5'-GCGAGCACAGAATTAATACGACT-Your gene-3'
  - For HA--neo3/4:
    - 5'FW: 5'-GCTGATGGCGATGAATGAACACTG-Your gene-3'
    - 5'RV: 5'-GCATAATCAGGAACATCATAAGGATAGGATCC-Your gene-3'
    - 3'FW: 5'-CCCGGGGGATCTGAATTCGATATCAAGCTT-Your gene-3'
    - 3'RV: 5'-GCGAGCACAGAATTAATACGACT-Your gene-3'
  - For mCherry--neo4:
    - 5'FW: 5'-GCTGATGGCGATGAATGAACACTG-Your gene-3'
    - 5'RV: 5'-ATCTTCTTCTCCTTTTGAAACCATGGATCC-Your gene-3'
    - 3'FW: 5'-CCCGGGGGATCTGAATTCGATATCAAGCTT-Your gene-3'
    - 3'RV: 5'-GCGAGCACAGAATTAATACGACT-Your gene-3'
- T7 and T3 Primers (or primers to amplify EGFP-neo4 or mCherry-neo4 cassette)
  T7: 5'-CGCGTAATACGACTCACTATAG-3'
  T3: 5'-GCGCAATTAACCCTCACTAAAG-3'
  BamHI-EGFP-Fw2: 5'-GGATCCATGGTTTCTAAGGGTGAAGAACTTTTCACTGGTGTTGTT-3'
  BamHI-mcherry_Fw1: 5'-GGATCCTATCCTTATGATGTTCCTGATTATGCTTGAAC-3'
  HindIII-Neo4-RV2: 5'-AAGCTTGATATCGAATTCAGATCCCCCGGGCTGCATTTTTCCAGT-3'
- Primers for 2nd PCR
  5'RACE-Outer: 5'-GCTGATGGCGATGAATGAACACTG-3'
  3'RACE-Outer: 5'-GCGAGCACAGAATTAATACGACT-3'
  (These primers are complementary to 5'FW or 3'RV. We are just using primers of Ambion LIM-RACE kit. You can use other primers but you also have to change underlined sequences in 5'FW and 3'RV.)
- PrimeStar HS DNA polymerase (Lonza/Takara)
- PCR Clean-up kit (Qiagen, Promega, etc)

2. Preparation of Tag-*neo* cassette for overlapping PCR

1) Mix following materials
   - 14.8 µl water
   - 5 µl 5X PrimeStar buffer
   - 2 µl 2.5 mM dNTPs
   - 1 µl 10 µM T7 primer (or BamHI-EGFP-Fw2 for EGFP, BamHI-mcherry_Fw1 for mCherry)
   - 1 µl 10 µM T3 primer (or HindIII-Neo4-Rv2 for EGFP and mCherry)
   - 1 µl ~1 ng/µl (or ~1/200 diluted Qiagen miniprep) plasmid containing C-terminal tagging module
   - 0.25 µl PrimeStar HS DNA polymerase
2) Run PCR cycle:
   - 98ºC-3 min
   - 35 cycles of 98ºC-10 sec/55ºC-15 sec/72ºC-3 min
   - 72ºC-6 min
3) Check PCR products by running 3 µl of reaction in agarose gel[*1]

3. Preparation of 5' and 3' flanking DNA

1) Mix following materials
   - 14.8 µl water
   - 5 µl 5X PrimeStar buffer
   - 2 µl 2.5 mM dNTPs
   - 1 µl 10 µM 5'FW or 3'FW primer
   - 1 µl 10 µM 5'RV or 3'RV primer
   - 1 µl ~0.5 µg/µl *Tetrahymena* genomic DNA
   - 0.25 µl PrimeStar HS DNA polymerase
2) Run PCR cycle:
   - 98ºC-3 min
   - 35 cycles of 98ºC-10 sec/55ºC-15 sec/72ºC-1 min
   - 72ºC-3 min
3) Check PCR products by running 3 µl of reaction in agarose gel[*2, 3]

4. Overlapping PCR

1) Mix following materials
   - 28.5 µl water
   - 10 µl 5X PrimeStar buffer
   - 4 µl 2.5 mM dNTPs
   - 1 µl 1/10 diluted 5' PCR reaction
   - 1 µl 1/10 diluted 3' PCR reaction
   - 1 µl 1/100 diluted Tag-*neo* cassette PCR reaction
   - 2 µl 10 µM 5'RACE-Outer primer
   - 2 µl 10 µM 3'RACE-Outer primer
   - 0.5 µl PrimeStar HS DNA polymerase
2) Run PCR cycle:
   - 98ºC-3 min
   - 35 cycles of 98ºC-10 sec/55ºC-15 sec/72ºC-4.5 min
   - 72ºC-10 min
3) Check PCR products by running 3 µl of reaction in agarose gel[*2]
4) Purify PCR product using a PCR clean-up kit[*4]

*1) Amplification of the mCherry-neo4 cassette with BamHI-mcherry_Fw1 and HindIII-Neo4-RV2 provides a few extra bands besides the mCherry-neo4 cassette, and it is necessary to gel-purify the mCherry-neo4 cassette.
*2) If PCR is not successful, try PCR with annealing at 50ºC and/or 60ºC.
*3) If you see byproducts and cannot eliminate them by changing annealing temperature, separate PCR reaction in an agarose gel, cut/elute expected size of product, and use 1 µl out of the 50 µl elution in 4.1 without dilution.
*4) If byproducts longer than ~2 kb is visible in 4.3, run whole PCR product in an agarose gel, cut expected size of product, and elute in 50 µl water.