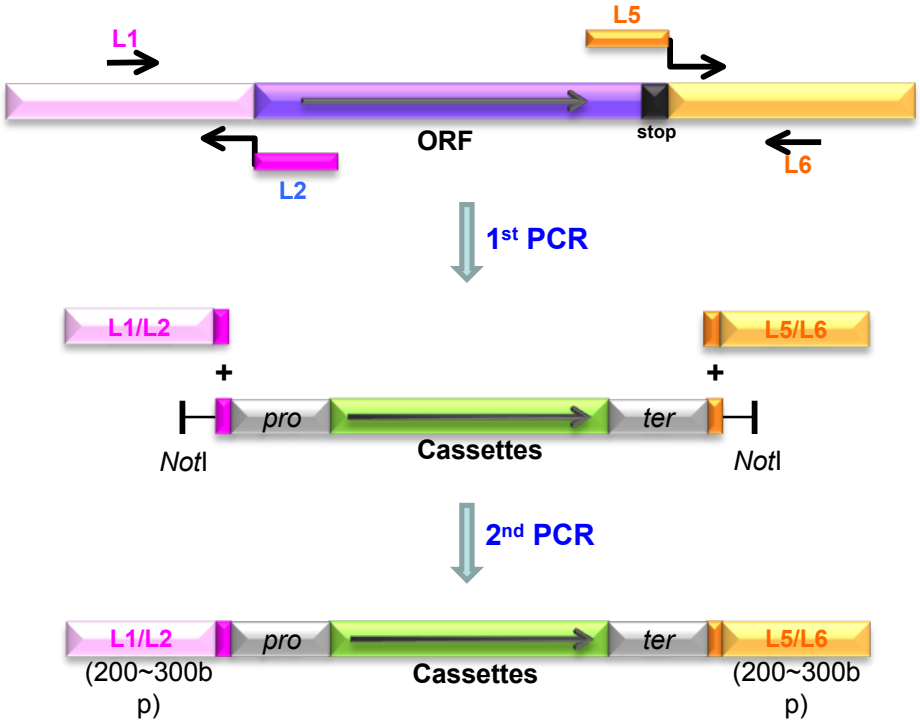
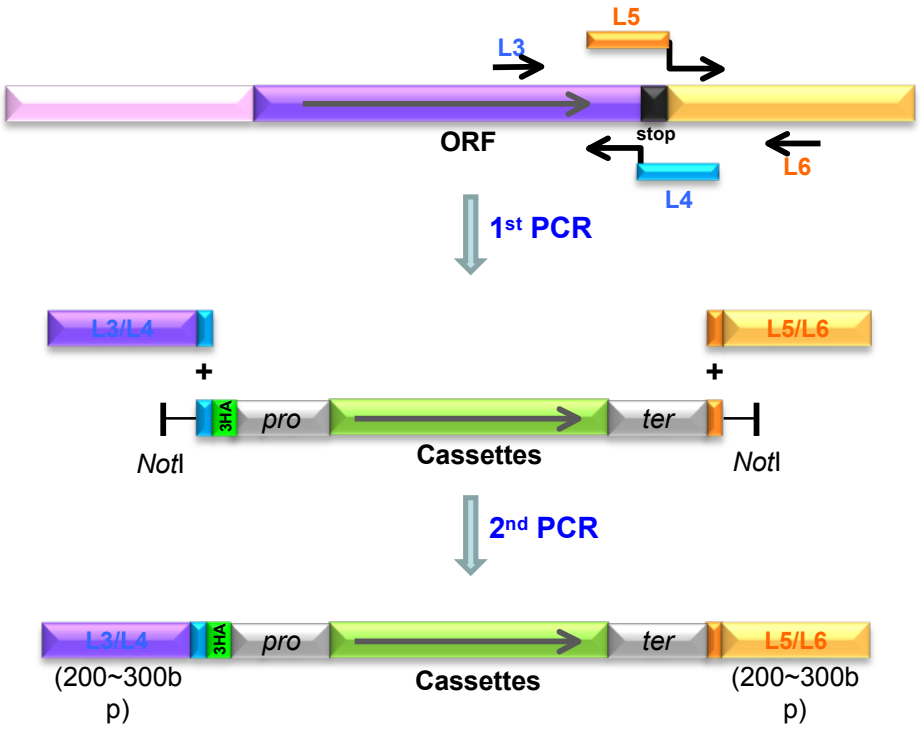
Construction of deletion cassette with KanMX6 (Nat, Hph, Pat)



Construction of C-terminus Tagging cassette with KanMX6



PCR conditions for 2-step Deletion/TAP method in *S. pombe*

1. Not1 Digestion of template cassettes

pFA6a series vectors	(2~4ug)
NEBuffer 3	3ul
10X BSA	3ul
Not1	3ul
DW	to 30ul

→37°C for O/N → purification with PCR purification kit → 0.7% Gel running & EtBr Staining

2. 1st PCR (Deletion & C-terminal Tagging Cassette)

Genomic DNA (WT)	3ul	<Pombe 1 / HSK>
10X buffer	5ul	
dNTP mix (2mM each)	5ul (final 0.2mM each)	94/4m
L1* (or L3, L5) (10uM)	1.5ul	94/30s 50***/30s 72/1m (X30)
L2* (or L4, L6) (10uM)	1.5ul	72/5m
Taq (Sure-Pol**)	2ul	
DW	32ul (to 50ul)	

* L1/L2, L3/L4, L5/L6 pair
** Pfu version taq polymerase
*** Try 40℃ or 45℃ if there is no product (usually L5/L6 product has problem because of high AT-content of L5)
→ purification with PCR purification kit → 0.7% Gel running & EtBr Staining→ measure DNA concentration

3. 2nd PCR (Deletion & C-terminal Tagging Cassette)

Template cassette	50ng	<Pombe 2 / HSK>
10X buffer	5ul	
dNTP mix (2mM each)	5ul (final 0.2mM each)	94/4m
L1/L2 product (or L3/L4)	50ng	94/15s 40/30s 72/3m (X5)
L5/L6 product	50ng	94/15s 55/30s 72/3m (X25)
L1 (or L3) (10uM)	1ul	72/5m
L6 (10uM)	1ul	
Taq (Sure-Pol)	2ul	
(DMSO* for NAT cassette)	2.5ul (5%)	
DW	to 50ul	

* because of high GC-content in NAT cassette (other cassettes are fine)
→purification with PCR purification kit → 0.7% Gel running & EtBr Staining→ measure DNA concentration
→Transformation to *S. pombe* cell (500ng-1ug, up to 10ul per rxn)

4. PCR screening

Genomic DNA	3ul	<Pombe 3 / HSK>
10X buffer	3ul	
$MgCl_2$ (50mM)	1.5ul (2.5mM)	94/4m
dNTP mix (2mM each)	3ul (0.2mM each)	94/30s 50/30s 72/3m (X35)
A1* (or A2) (10uM)	0.6ul	72/5m
A3 (10uM)	0.6ul	(* you can also use A1/KanMX6-5'-Rev (#505) or KanMX6-3'-For(#1134)/A3)
Taq (Taq-pro)	0.3ul	
DW	18ul (To 30 ul)	

→ Control sample must be included !!

Oligomers for Deletion & C-terminal tagging (forward)

spSwc6-L1(20mer):

spSwc6-L2(45mer): taattaacccgggatccgtcgacctatgagctactcaaaaattg

spSwc6-L3(20mer):

spSwc6-L4(45mer): taattaacccgggatccgtcgaccggcatatactttcatacacc

spSwc6-L5(46mer): aaacgagctcgaattcatcgatgataaagttgacctttgcttact

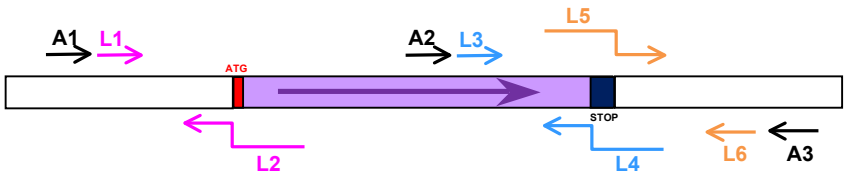
spSwc6-L6(20mer):

spSwc6-A1(20mer):

spSwc6-A2(20mer):

spSwc6-A3(20mer):

"add gene-specific sequences to colored bold sequences"



(L1-L2, L3-L4, L5-L6: 200~300bp)

caatttttgagtagctcataatgtttgtcacccccattgaaca
gttaaaaactcatcgagtattacaaacagtgggggtaacttgt
ATG
L2
ccagctgcctagggcccaattaat

aaacgagctcgaattcatcgatgata
L5
ggtgtatgaaagtatatgcctaaaagttgacctttgcttact
ccacatactttcatatacggatttttcaactggaaaacgaatga
STOP
L4
ccagctgcctagggcccaattaat

Oligomer for Deletion & C-termial tagging (Reverse)

spAsh2-L1 (20mer):

spAsh2-L2 (45mer): taattaacccgggatccgtcgaccggctgattaatattgaatcc

spAsh2-L3 (20mer):

spAsh2-L4 (45mer): taattaacccgggatccgtcgaccgtttaaatagccacgacatg

spAsh2-L5 (46mer): aaacgagctcgaattcatcgatgataacaatgatgctcttggagaa

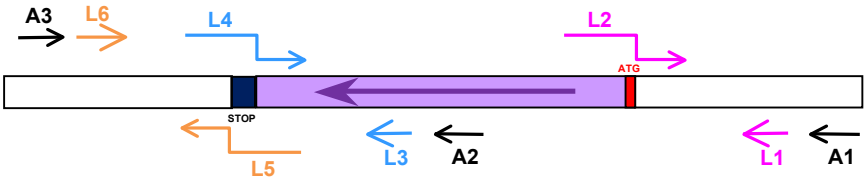
spAsh2-L6 (20mer):

spAsh2-A1 (20mer):

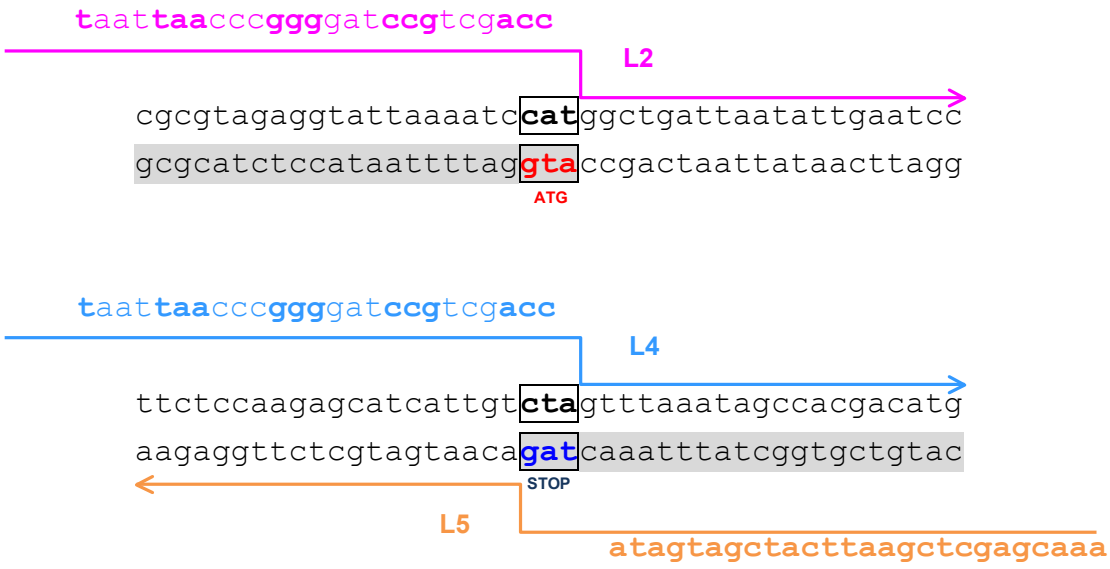
spAsh2-A2 (20mer):

spAsh2-A3 (20mer):

"add gene-specific sequences to colored bold sequences"



(L1-L2, L3-L4, L5-L6: 200~300bp)



pFA6a-KanMX6

(all pFA6a cassettes have same DAN sequences except resistant genes (Kan^R, Nat^R..)

```
   1 gaacgcggcc gccagctgaa gcttcgtacg ctgcaggtcg acggatcccc gggttaatta   multi cloning site
  61 aggcgcgcca gatctgttta gcttgcctcg tccccgccgg gtcacccggc cagcgacatg
 121 gaggcccaga taccctcct tgacagtctt gacgtgcgca gctcaggggc atgatgtgac
 181 tgtcgcccgt acattTAGCC CATACATCCC CATGTATAAT CATTTGCATC CATACATTTT   KanMX6-5'Rev(#505)
 241 gatggccgca cggcgcgaag caaaaattac ggctcctcgc tgcagacctg cgagcaggga   TEF promoter
 301 aacgctcccc tcacagacgc gttgaattgt ccccacgccg cgcccctgta gagaaatata
 361 aaaggttagg atttgccact gaggttcttc tttcatatac ttccttttaa aatcttgcta
 421 ggatacagtt ctcacatcac atccgaacat aaacaaccat ggtaaggaa aagactcacg
 481 tttcgaggcc gcgattaaat tccaacatgg atgctgattt atatgggtat aaatgggctc
 541 gcgataatgt cgggcaatca ggtgcgacaa tctatcgatt gtatgggaag cccgatgcgc
 601 cagagttgtt tctgaaacat ggcaaaggta gcgttgccaa tgatgttaca gatgagatgg
 661 tcagactaaa ctggctgacg gaatttatgc ctcttccgac catcaagcat tttatccgta
 721 ctcctgatga tgcatggtta ctcaccactg cgatccccgg caaaacagca ttccaggtat
 781 tagaagaata tcctgattca ggtgaaaata ttgttgatgc gctggcagtg ttcctgcgcc
 841 ggttgcattc gattcctgtt tgtaattgtc cttttaacag cgatcgcgta tttcgtctcg   Kan^R
 901 ctcaggcgca atcacgaatg aataacggtt tggttgatgc gagtgatttt gatgacgagc
 961 gtaatggctg gcctgttgaa caagtctgga aagaaatgca taagcttttg ccattctcac
1021 cggattcagt cgtcactcat ggtgatttct cacttgataa ccttatttt gacgaggga
1081 aattaatagg ttgtattgat gttggacgag tcggaatcgc agaccgatac caggatcttg
1141 ccatcctatg gaactgcctc ggtgagtttt ctccttcatt acagaaacgg ctttttcaaa
1201 aatatggtat tgataatcct gatatgaata aattgcagtt tcatttgatg ctcgatgagt
1261 ttttctaatc agtactgaca ataaaaagat tcttgttttc aagaacttgt catttgtata
1321 gtttttttat attgtagttg ttctatttta atcaaatgtt agcgtgattt atattttttt   KanMX6-3'For (#1134)
1381 tcgcctcgac atcatctgcc cagatgcgaa gttaagtgcg cagaaagtaa tatcatgcgt   TEF terminater
1441 caatcgtatg tgaatgctgg tcgctatact gctgtcgatt cgatactaac gccgccatcc
1501 agtttaaacg agctcgaatt catcgatgat atcagatcca ctagtggcct atgcggccgc   multi cloning site
1561 ggatctgccg gtctccctat agtgagtcgt attaatttcg ataagccagg ttaacctgca
1621 ttaatgaatc ggccaacgcg cggggagagg cggtttgcgt attgggcgct cttccgcttc
1681 ctcgctcact gactcgctgc gctcggtcgt tcggctgcgg cgagcggtat cagctcactc
1741 aaaggcggta atacggttat ccacagaatc aggggataac gcaggaaaga acatgtgagc
1801 aaaaggccag caaaaggcca ggaaccgtaa aaaggccgcg ttgctggcgt ttttccatag
1861 gctccgcccc cctgacgagc atcacaaaaa tcgacgctca agtcagaggt ggcgaaaccc
```

KanMX6-5'Rev (#505): acatggggatgtatgggcta

KanMX6-3'For(#1134): ttttcgcctcgacatcatct

1921 gacaggacta taaagatacc aggcgtttcc ccctggaagc tccctcgtgc gctctcctgt

1981 tccgaccctg ccgcttaccg gatacctgtc cgcctttctc ccttcgggaa gcgtggcgct

2041 ttctcaatgc tcacgctgta ggtatctcag ttcggtgtag gtcgttcgct ccaagctggg

2101 ctgtgtgcac gaaccccccg ttcagcccga ccgctgcgcc ttatccggta actatcgtct

2161 tgagtccaac ccggtaagac acgacttatc gccactggca gcagccactg gtaacaggat

2221 tagcagagcg aggtatgtag gcggtgctac agagttcttg aagtggtggc ctaactacgg

2281 ctacactaga aggacagtat ttggtatctg cgctctgctg aagccagtta ccttcggaaa

2341 aagagttggt agctcttgat ccggcaaaca aaccaccgct ggtagcggtg gttttttgt

2401 ttgcaagcag cagattacgc gcagaaaaaa aggatctcaa gaagatcctt tgatcttttc

2461 tacggggtct gacgctcagt ggaacgaaaa ctcacgttaa gggattttgg tcatgagatt

2521 atcaaaaagg atcttcacct agatcctttt aaattaaaaa tgaagtttta aatcaatcta

2581 aagtatatat gagtaaactt ggtctgacag **tta**ccaatgc ttaatcagtg aggcacctat

2641 ctcagcgatc tgtctatttc gttcatccat agttgcctga ctccccgtcg tgtagataac

2701 tacgatacgg gagggcttac catctggccc cagtgctgca atgataccgc gagacccacg

2761 ctcaccggct ccagatttat cagcaataaa ccagccagcc ggaagggccg agcgcagaag

2821 tggtcctgca actttatccg cctccatcca gtctattaat tgttgccggg aagctagagt

2881 aagtagttcg ccagttaata gtttgcgcaa cgttgttgcc attgctacag gcatcgtggt

2941 gtcacgctcg tcgtttggta tggcttcatt cagctccggt tcccaacgat caaggcgagt **Beta-lactamase**

3001 tacatgatcc cccatgttgt gcaaaaaagc ggttagctcc ttcggtcctc cgatcgttgt

3061 cagaagtaag ttggccgcag tgttatcact catggttatg gcagcactgc ataattctct

3121 tactgtcatg ccatccgtaa gatgcttttc tgtgactggt gagtactcaa ccaagtcatt

3181 ctgagaatag tgtatgcggc gaccgagttg ctcttgcccg gcgtcaatac gggataatac

3241 cgcgccacat agcagaactt taaaagtgct catcattgga aaacgttctt cggggcgaaa

3301 actctcaagg atcttaccgc tgttgagatc cagttcgatg taacccactc gtgcacccaa

3361 ctgatcttca gcatctttta ctttcaccag cgtttctggg tgagcaaaaa caggaaggca

3421 aaatgccgca aaaaagggaa taagggcgac acggaaatgt tgaatact**ca t**actcttcct

3481 ttttcaatat tattgaagca tttatcaggg ttattgtctc atgagcggat acatatttga

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3601 tgacgtctaa gaaaccatta ttatcatgac attaacctat aaaaataggc gtatcacgag

3661 gccctttcgt ctcgcgcgtt tcggtgatga cggtgaaaac ctctgacaca tgcagctccc

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3781 gtcagcgggt gttggcgggt gtcggggctg gcttaactat gcggcatcag agcagattgt

3841 actgagagtg caccatatgg acatattgtc gttagaacgc ggctacaatt aatacataac

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