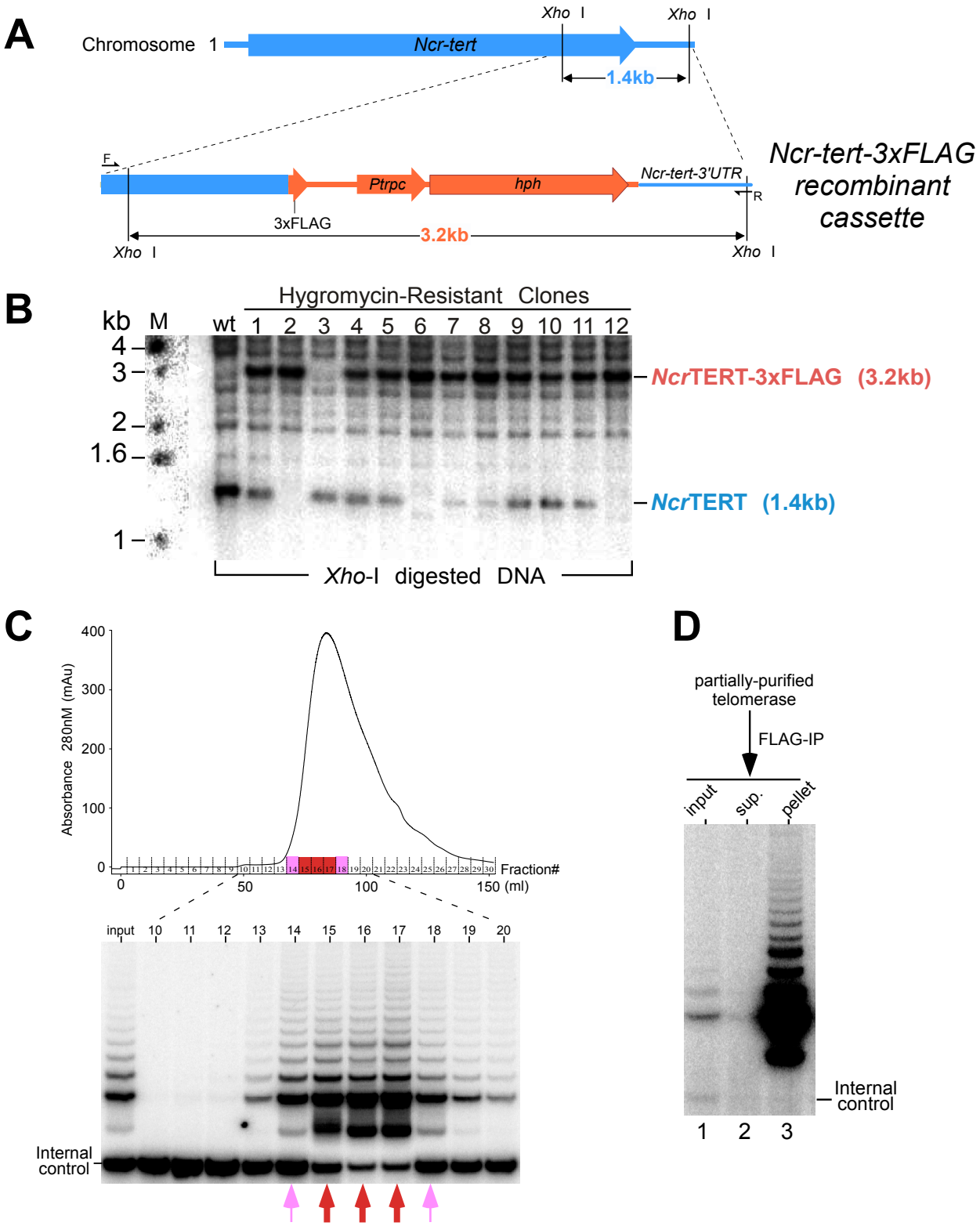
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Chromosome 1
Ncr-tert
Xho I
Xho I
1.4kb
F
Ptrpc
hph
Ncr-tert-3'UTR
R
3xFLAG
3.2kb
Xho I
Xho I
Ncr-tert-3xFLAG recombinant cassette
B
Hygromycin-Resistant Clones
kb M
wt 1 2 3 4 5 6 7 8 9 10 11 12
4
3
2
1.6
1
NcrTERT-3xFLAG (3.2kb)
NcrTERT (1.4kb)
Xho-I digested DNA
C
Absorbance 280nM (mAu)
400
300
200
100
0
0
50
100
150 (ml)
Fraction#
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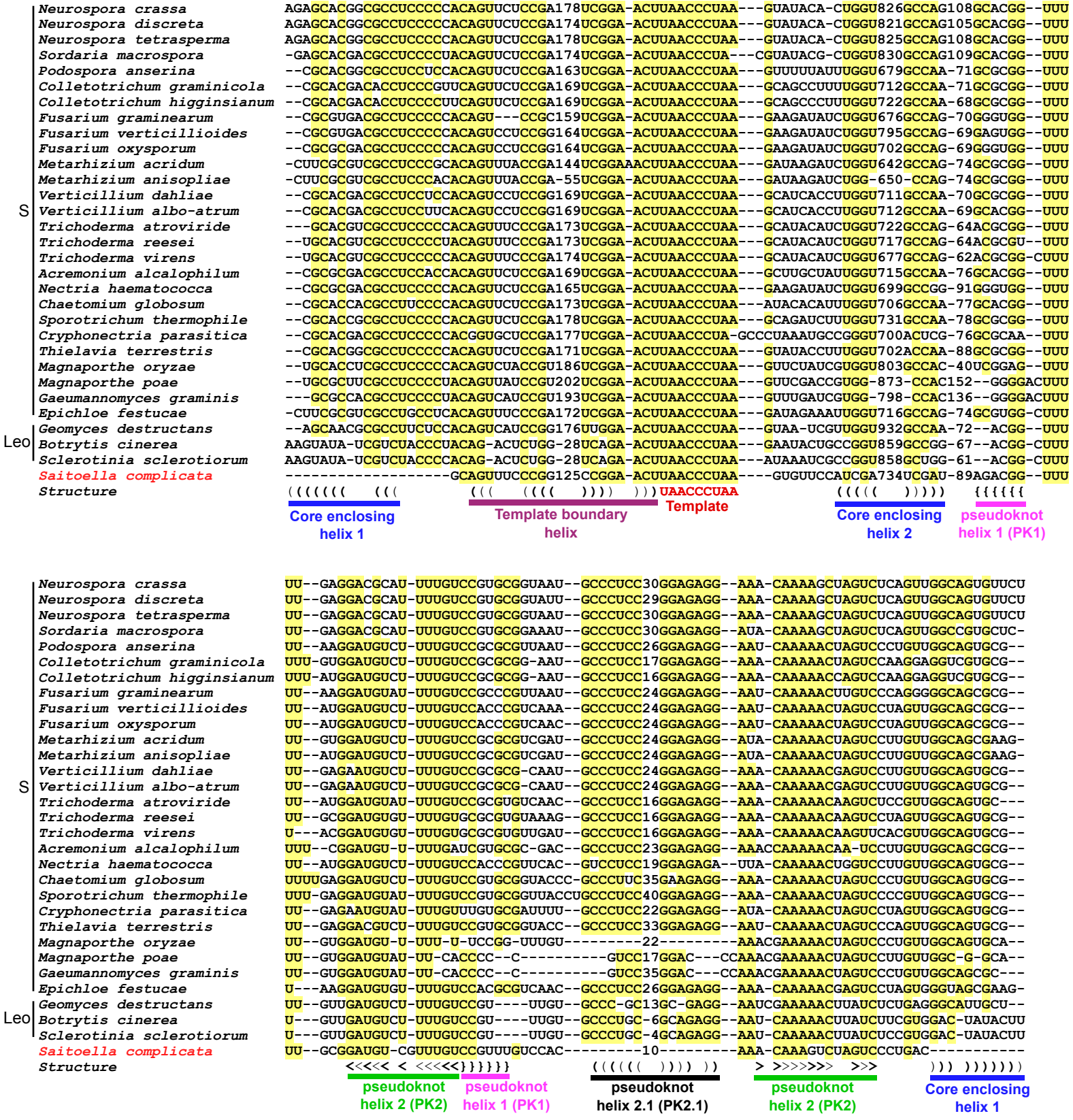
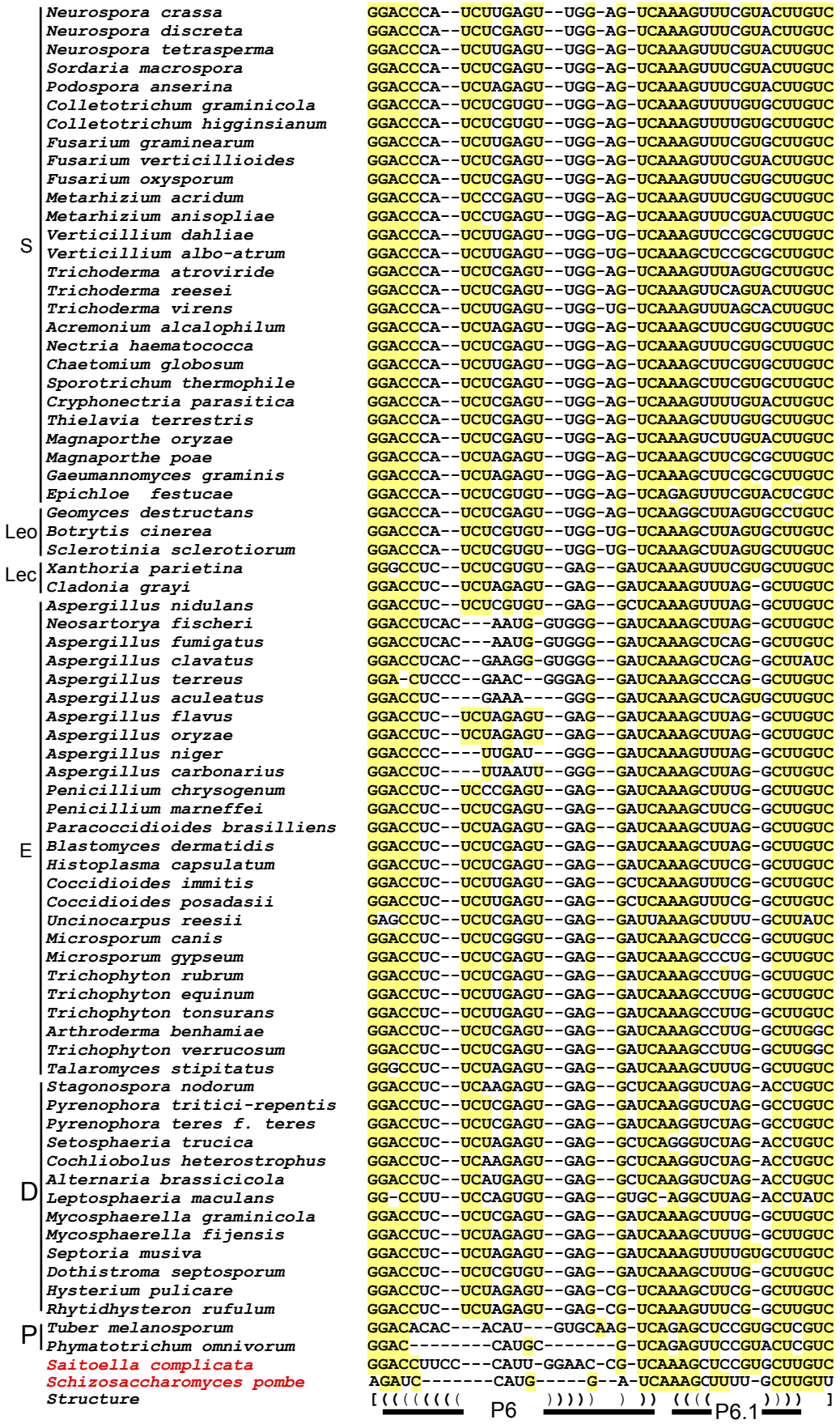
Internal control
D
partially-purified telomerase
FLAG-IP
input
sup.
pellet
Internal control
1 2 3


S
Leo
Neurospora crassa
Neurospora discreta
Neurospora tetrasperma
Sordaria macrospora
Podospora anserina
Colletotrichum graminicola
Colletotrichum higginsianum
Fusarium graminearum
Fusarium verticillioides
Fusarium oxysporum
Metarhizium acridum
Metarhizium anisopliae
Verticillium dahliae
Verticillium albo-atrum
Trichoderma atroviride
Trichoderma reesei
Trichoderma virens
Acremonium alcalophilum
Nectria haematococca
Chaetomium globosum
Sporotrichum thermophile
Cryphonectria parasitica
Thielavia terrestris
Magnaporthe oryzae
Magnaporthe poae
Gaeumannomyces graminis
Epichloe festucae
Geomyces destructans
Botrytis cinerea
Sclerotinia sclerotiorum
Saitoella complicata
Structure
Core enclosing helix 1
Template boundary helix
Template
Core enclosing helix 2
pseudoknot helix 1 (PK1)
pseudoknot helix 2 (PK2)
pseudoknot helix 1 (PK1)
pseudoknot helix 2.1 (PK2.1)
pseudoknot helix 2 (PK2)
Core enclosing helix 1


S
Leo
Lec
E
D
P
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Neurospora tetrasperma GGACCCA--UCUUGAGU--UGG-AG-UCAAAGUUUCGUACUUGUC
Sordaria macrospora GGACCCA--UCUCGAGU--UGG-AG-UCAAAGUUUCGUACUUGUC
Podospora anserina GGACCCA--UCUAGAGU--UGG-AG-UCAAAGUUUCGUACUUGUC
Colletotrichum graminicola GGACCCA--UCUCGUGU--UGG-AG-UCAAAGUUUUGUGCUUGUC
Colletotrichum higginsianum GGACCCA--UCUCGUGU--UGG-AG-UCAAAGUUUUGUGCUUGUC
Fusarium graminearum GGACCCA--UCUUGAGU--UGG-AG-UCAAAGUUUCGUGCUUGUC
Fusarium verticillioides GGACCCA--UCUCGAGU--UGG-AG-UCAAAGUUUCGUACUUGUC
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Metarhizium acridum GGACCCA--UCCCGAGU--UGG-AG-UCAAAGUUUCGUGCUUGUC
Metarhizium anisopliae GGACCCA--UCCUGAGU--UGG-AG-UCAAAGUUUCGUACUUGUC
Verticillium dahliae GGACCCA--UCUUGAGU--UGG-UG-UCAAAGUUCCGCGCUUGUC
Verticillium albo-atrum GGACCCA--UCUUGAGU--UGG-UG-UCAAAGCUCCGCGCUUGUC
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Trichoderma reesei GGACCCA--UCUCGAGU--UGG-AG-UCAAAGUUCAGUACUUGUC
Trichoderma virens GGACCCA--UCUUGAGU--UGG-UG-UCAAAGUUUAGCACUUGUC
Acremonium alcalophilum GGACCCA--UCUAGAGU--UGG-AG-UCAAAGCUUCGUGCUUGUC
Nectria haematococca GGACCCA--UCUCGAGU--UGG-AG-UCAAAGUUUCGUGCUUGUC
Chaetomium globosum GGACCCA--UCUUGAGU--UGG-AG-UCAAAGCUUCGUGCUUGUC
Sporotrichum thermophile GGACCCA--UCUCGAGU--UGG-AG-UCAAAGCUUCGUGCUUGUC
Cryphonectria parasitica GGACCCA--UCUCGAGU--UGG-AG-UCAAAGUUUUGUACUUGUC
Thielavia terrestris GGACCCA--UCUCGAGU--UGG-AG-UCAAAGCUUUGUGCUUGUC
Magnaporthe oryzae GGACCCA--UCUCGAGU--UGG-AG-UCAAAGUCUUGUACUUGUC
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Gaeumannomyces graminis GGACCCA--UCUAGAGU--UGG-AG-UCAAAGCUUCGCGCUUGUC
Epichloe festucae GGACCCA--UCUCGUGU--UGG-AG-UCAGAGUUUCGUACUCGUC
Geomyces destructans GGACCCA--UCUCGAGU--UGG-AG-UCAAGGCUUAGUGCCUGUC
Botrytis cinerea GGACCCA--UCUCGUGU--UGG-UG-UCAAAGCUUAGUGCUUGUC
Sclerotinia sclerotiorum GGACCCA--UCUCGUGU--UGG-UG-UCAAAGCUUAGUGCUUGUC
Xanthoria parietina GGGCCUC--UCUCGUGU--GAG--GAUCAAAGUUUCGUGCUUGUC
Cladonia grayi GGACCUC--UCUAGAGU--GAG--GAUCAAAGUUUAG-GCUUGUC
Aspergillus nidulans GGACCUC--UCUCGUGU--GAG--GCUCAAAGUUUAG-GCUUGUC
Neosartorya fischeri GGACCUCAC---AAUG-GUGGG--GAUCAAAGCUUAG-GCUUGUC
Aspergillus fumigatus GGACCUCAC---AAUG-GUGGG--GAUCAAAGCUCAG-GCUUGUC
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Aspergillus terreus GGA-CUCCC--GAAC--GGGAG--GAUCAAAGCCCAG-GCUUGUC
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Aspergillus flavus GGACCUC--UCUAGAGU--GAG--GAUCAAAGCUUAG-GCUUGUC
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Aspergillus carbonarius GGACCUC----UUAAUU--GGG--GAUCAAAGCUUAG-GCUUGUC
Penicillium chrysogenum GGACCUC--UCCCGAGU--GAG--GAUCAAAGCUUUG-GCUUGUC
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Blastomyces dermatidis GGACCUC--UCUCGAGU--GAG--GAUCAAAGCUUAG-GCUUGUC
Histoplasma capsulatum GGACCUC--UCUCGAGU--GAG--GAUCAAAGCUUCG-GCUUGUC
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Coccidioides posadasii GGACCUC--UCUUGAGU--GAG--GCUCAAAGUUUCG-GCUUGUC
Uncinocarpus reesii GAGCCUC--UCUCGAGU--GAG--GAUUAAAGCUUUU-GCUUAUC
Microsporum canis GGACCUC--UCUCGGGU--GAG--GAUCAAAGCUCCG-GCUUGUC
Microsporum gypseum GGACCUC--UCUCGAGU--GAG--GAUCAAAGCCCUG-GCUUGUC
Trichophyton rubrum GGACCUC--UCUCGAGU--GAG--GAUCAAAGCCUUG-GCUUGUC
Trichophyton equinum GGACCUC--UCUUGAGU--GAG--GAUCAAAGCCUUG-GCUUGUC
Trichophyton tonsurans GGACCUC--UCUUGAGU--GAG--GAUCAAAGCCUUG-GCUUGUC
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Talaromyces stipitatus GGGCCUC--UCUAGAGU--GAG--GAUCAAAGCUUUG-GCUUGUC
Stagonospora nodorum GGACCUC--UCAAGAGU--GAG--GCUCAAGGUCUAG-ACCUGUC
Pyrenophora tritici-repentis GGACCUC--UCUCGAGU--GAG--GAUCAAGGUCUAG-GCCUGUC
Pyrenophora teres f. teres GGACCUC--UCUCGAGU--GAG--GAUCAAGGUCUAG-GCCUGUC
Setosphaeria trucica GGACCUC--UCUAGAGU--GAG--GCUCAGGGUCUAG-ACCUGUC
Cochliobolus heterostrophus GGACCUC--UCAAGAGU--GAG--GCUCAAGGUCUAG-ACCUGUC
Alternaria brassicicola GGACCUC--UCAUGAGU--GAG--GCUCAAGGUCUAG-ACCUGUC
Leptosphaeria maculans GG-CCUU--UCCAGUGU--GAG--GUGC-AGGCUUAG-ACCUAUC
Mycosphaerella graminicola GGACCUC--UCUCGAGU--GAG--GAUCAAAGCUUUG-GCUUGUC
Mycosphaerella fijensis GGACCUC--UCUAGAGU--GAG--GAUCAAAGCUUUG-GCUUGUC
Septoria musiva GGACCUC--UCUAGAGU--GAG--GAUCAAAGUUUUGUGCUUGUC
Dothistroma septosporum GGACCUC--UCUCGUGU--GAG--GAUCAAAGCUUUG-GCUUGUC
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Tuber melanosporum GGACACAC---ACAU---GUGCAAG-UCAGAGCUCCGUGCUCGUC
Phymatotrichum omnivorum GGAC--------CAUGC-------G-UCAGAGUUCCGUACUCGUC
Saitoella complicata GGACCUUCC---CAUU-GGAAC-CG-UCAAAGCUCCGUGCUUGUC
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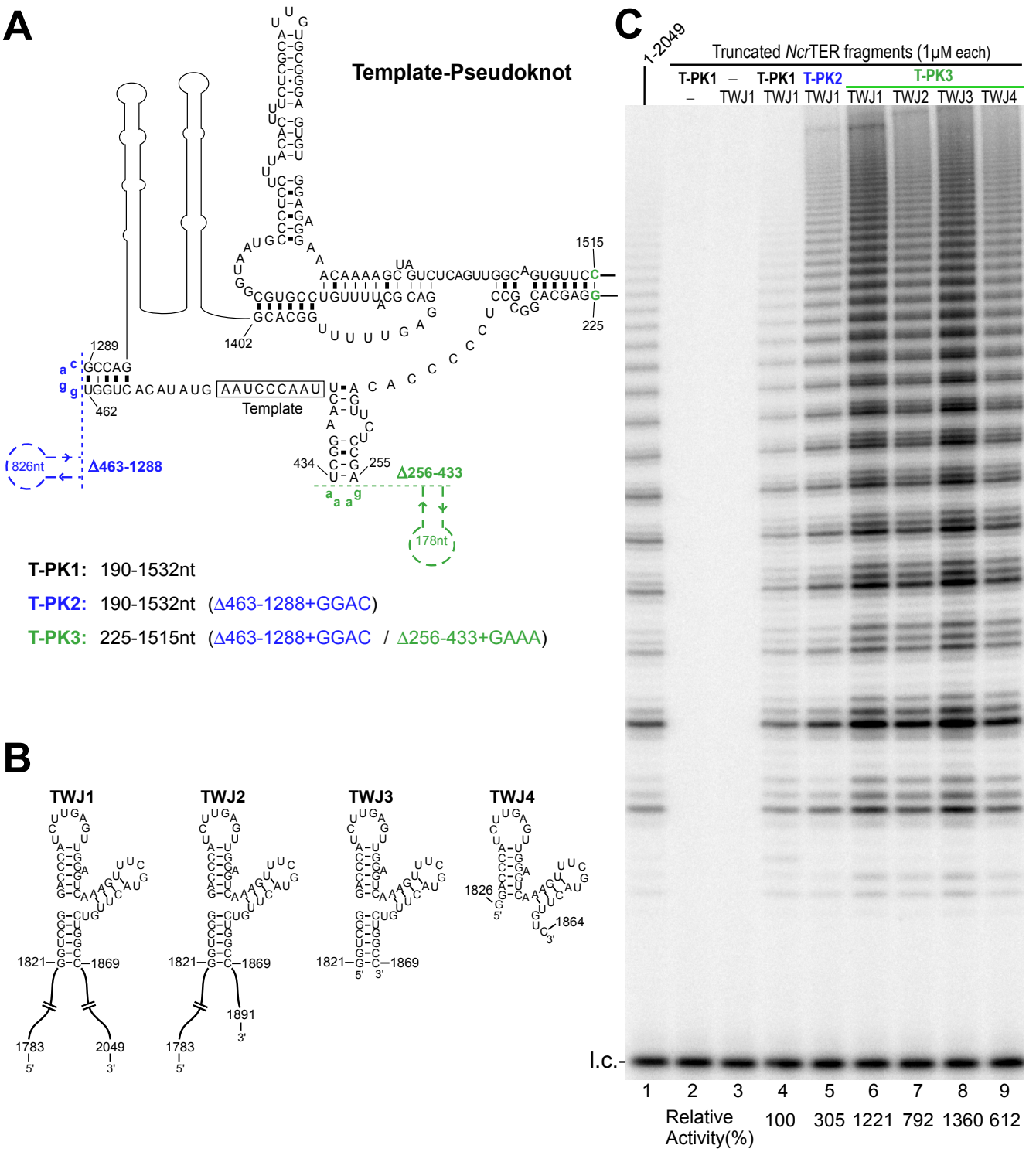
Structure [((((((((P6)))))))) ((((P6.1))))]


A
Template-Pseudoknot
1515
225
1289
462
1402
Template
826nt
Δ463-1288
434
255
Δ256-433
178nt
T-PK1: 190-1532nt
T-PK2: 190-1532nt (Δ463-1288+GGAC)
T-PK3: 225-1515nt (Δ463-1288+GGAC / Δ256-433+GAAA)
B
TWJ1
TWJ2
TWJ3
TWJ4
1821
1869
1783
2049
1891
1826
1864
C
1-2049
Truncated NcrTER fragments (1μM each)
T-PK1 – T-PK1 T-PK2 T-PK3
– TWJ1 TWJ1 TWJ1 TWJ1 TWJ2 TWJ3 TWJ4
l.c.-
1 2 3 4 5 6 7 8 9
Relative Activity(%) 100 305 1221 792 1360 612

Supplementary Table S1. Recombinant *Neurospora crassa* strains used in this study

Strain	Genotype	Reference
NC1	*mat A his-3 Δmus-52::bar*$^{+}$	Honda & Selker (2009)
NC2	*mat A his-3 Δmus-52::bar*$^{+}$*, tert-3xFLAG::hph::loxP*	this study
NC3	*mat a, tert-3xFLAG::hph::loxP*	this study
NC4	*mat A his-3*$^{+}$*::Pccg-1::ncrTER(template:5'-UAA<u>C</u>CCCUAA-3'), Δmus-52::bar*$^{+}$	this study
NC5	*mat A his-3*$^{+}$*::Pccg-1::ncrTER(template:5'-UAA<u>G</u>CCCUAA-3'), Δmus-52::bar*$^{+}$	this study
NC6	*mat A his-3*$^{+}$*::Pccg-1::ncrTER(template:5'-U<u>A</u>AACCCU<u>A</u>AA-3'), Δmus-52::bar*$^{+}$	this study

Honda, S., and Selker, E.U. (2009). Genetics *182*, 11-23.

Supplementary Table S2. Species with TER identified in this study

Class	Species	Template to TWJ (nt)	Full length (nt)	Source
Sordariomycetes	*Neurospora crassa*	1423 (442-1864)	2049[a]	JGI, Broad
	Neurospora discreta	1417	~2032[b]	JGI
	Neurospora tetrasperma	1442	~2065[b]	JGI
	Sordaria macrospora	1458		NCBI
	Podospora anserina	1176		NCBI
	Colletotrichum graminicola	1120		Broad
	Colletotrichum higginsianum	1134		Broad
	Fusarium graminearum	1113		Broad
	Fusarium verticillioides	1132		Broad
	Fusarium oxysporum	1137		Broad
	Metarhizium acridum	1033		NCBI
	Metarhizium anisopliae	1061		NCBI
	Verticillium albo-atrum	1131		Broad
	Verticillium dahilae	1130		Broad
	Trichoderma atroviride	1143		JGI
	Trichoderma reesei	1124		JGI
	Trichoderma virens	1083		JGI
	Acremonium alcalophilum	1157		JGI
	Nectria haematococca	1150		JGI
	Chaetomium globosum	1210		JGI, Broad
	Sporotrichum thermophile	1243		JGI
	Cryphonectria parasitica	1189		JGI
	Thielavia terrestris	1211		JGI
	Magnaporthe oryzae	1274		Broad
	Magnaporthe poae	1520		Broad
	Gaeumannomyces graminis	1407		Broad
	Epichloe festucae	1159		OU
Leotiomycetes	*Geomyces destructans*	1427		Broad
	Botrytis cinerea	1343		Broad
	Sclerotina sclerotiorum	1335		Broad
Lecanoromycetes	*Cladonia grayi*	1478		JGI
	Xanthoria parietina	1834		JGI
Eurotiomycetes	*Aspergillus nidulans*	1162 (270-1431)	1584[a]	JGI, Broad
	Neosartorya fischeri	1494		Broad
	Aspergillus fumigatus	1498		Broad
	Aspergillus clavatus	1491		Broad
	Aspergillus terreus	1513		Broad
	Aspergillus aculeatus	1472		JGI
	Aspergillus flavus	1477		Broad
	Aspergillus oryzae	1479		Broad
	Aspergillus niger	1476		JGI, Broad
	Aspergillus carbonarius	1476		JGI
	Penicillium chrysogenum	1509		NCBI
	Penicillium marneffei	1327		NCBI
	Paracoccidioides brasiliensis	1703		NCBI

	Blastomyces dermatidis	1717		Broad
	Histoplasma capsulatum	1687		Broad
	Coccidioides immitis	1642		Broad
	Coccidioides posadasii	1640		Broad
	Uncinocarpus reesii	1605		Broad
	Microsporum gypseum	1558		Broad
	Microsporum canis	1609		Broad
	Trichophyton rubrum	1559		Broad
	Trichophyton equinum	1561		Broad
	Trichophyton tonsurans	1561		Broad
	Arthroderma benhamiae	1557		NCBI
	Trichophyton verrucosum	1557		NCBI
	Talaromyces stipitatus	1343		NCBI
Dothidiomycetes	*Stagonospora nodorum*	1470		JGI
	Pyrenophora tritici-repentis	1471		JGI
	Pyrenophora teres f. teres	1468		NCBI
	Setosphaeria trucica	1471		JGI
	Cochliobolus heterostrophus	1476		JGI
	Alternaria brassicicola	1466		JGI
	Leptosphaeria maculans	1481		INRA
	Mycosphaerella graminicola	1772 (376-2147)	2425[a]	JGI
	Mycosphaerella fijiensis	1634		JGI
	Septoria musiva	1742		JGI
	Dothistroma septosporum	1632		JGI
	Hysterium pulicare	1616		JGI
	Rhytidhysteron rufulum	1548		JGI
Pezizomycetes	*Tuber melanosporum*	1275		INRA
	Phymatotrichum omnivorum	1190[c]		OU
Saitoella	*Saitoella complicata*	1313 (380-1692)	1893[a]	JGI

a: Full-length mature TER confirmed by 5'- and 3'-RACE and deposited in the GenBank (*N. crassa* TER=JQ793886, *A. nidulans* TER=JQ793887, *M. graminicola* TER=JQ793888 and *S. complicata* TER=JX173807)

b: Size of the full-length TER was predicted by alignment with *Ncr*TER

c: The genomic sequence of TER was determined using the Universal GenomeWalker Kit (ClonTech) and the genomic DNA was obtained from Dr. Stephen Marek.

JGI: DOE Joint Genome Institute, URL: www.jgi.doe.gov

Broad: Broad institute of MIT and Harvard, URL: www.broadinstitute.org

NCBI: National center for Biotechnology Information, URL: www.ncbi.nlm.nih.gov

INRA: Institut national de la recherché agronomique, URL: www.inra.fr

OU: Oklahoma University, URL: www.genome.ou.edu/fungi.html

Supplementary Figure Legends

Supplementary Fig. S1. Purification of *Neurospora crassa* telomerase holoenzyme. **(A)** Generation of the *Ncr*TERT-3xFLAG strain. Schematic drawing shows *Xho*I sites at the *N. crassa tert* locus before and after insertion of the recombinant *Ncr*TERT-3xFLAG DNA cassette. The blue color indicates the endogenous *tert* locus, whereas the orange color shows the inserted DNA cassette. Digestion of the genomic DNA with *Xho*I results in a 1.4 kb band from the endogenous *tert* locus which increases to 3.2 kb with the inserted cassette. Positions of the forward (F) and reverse (R) PCR primers for generating the recombination DNA cassette are shown. The DNA cassette includes a Hygromycin B phosphotransferase gene (*hph*) driven by a TrpC promoter (Ptrpc). **(B)** Southern blot analysis of *Xho*I digested genomic DNA from wild-type (wt) and 12 transformants (lanes 1 to 12) with a radiolabeled probe against *tert* 3' region. **(C)** Gel filtration of *N. crassa Ncr*TERT-3xFLAG strain nuclear extracts. Five mililiter fractions were collected as indicated. Fractions 10-20 were assayed for telomerase activity together with the original nuclear extract. The PCR internal control is shown. Fractions with peak activities (#15-17) were combined for IP. **(D)** Telomerase activity detected by TRAP assay after IP with anti-FLAG antibody conjugated beads. One microliter of input and supernatant after IP together with 5% of the beads (pellet) were subjected to the telomerase TRAP assay.

Supplementary Fig. S2. Sequence alignment of Pezizomycotina and *S. complicata* TERs. (**A**) Sequence alignment of the template-pseudoknot domain. The TER sequences from Sordariomycetes (S), Leotiomycetes (Leo) and *Saitoella complicata* are included. Nucleotides with ≥80% identity are shaded yellow. Numbers within the sequences indicate nucleotides omitted. Base-pairings are denoted below the sequences. Structural features are labeled under the sequence alignment and colored the same as in Fig 3B. (**B**) Sequence alignment of the P6/6.1 region. The TER sequences from Sordariomycetes (S), Leotiomycetes (Leo), Lecanoromycetes (Lec), Eurotiomycetes (E), Dothidiomycetes (D), Pezizomycetes (P) and *Saitoella complicata* (shown in red) are included. Nucleotides with ≥80% identity are shaded yellow. Base-pairings are denoted below the sequences.

Supplementary Fig. S3. The minimal functional core of *Ncr*TER. **(A)** The template-pseudoknot fragments, T-PK1, T-PK2 and T-PK3, contained the template-pseudoknot domain with deletions Δ256-433 and Δ463-1288. For the Δ256-433 and Δ463-1288 deletions, the truncated core-closing helix 2 and template-boundary helix were capped with GGAC or GAAA, respectively, for structural stability. The minimal fragment T-PK3 contained both deletions, while the T-PK2 fragment contained only the Δ463-1288 deletion. Residue numbers are based on the mature *Ncr*TER (1-2049). Predicted *Ncr*TER

structure of nucleotides 225-1515 is presented, with truncations indicated in blue and green. **(B)** TER fragments TWJ1, TWJ2, TWJ3 and TWJ4, contained the 3' portion of *Ncr*TER with various truncations removing regions flanking the P6/6.1 element. *Ncr*TER TWJ3 and TWJ4 secondary structure predictions are shown. **(C)** Telomerase activity assay using the longer fragments T-PK1 and TWJ1 showed that both fragments were required to reconstitute telomerase activity in trans (lanes 2-4). The full-length *Ncr*TER activity is shown in lane 1. Single fragment pseudoknot T-PK1 and TWJ1 reconstituted with *Ncr*TERT in RRL respectively were shown in lanes 2 and 3. Various two-fragment reconstitutions with *Ncr*TERT *in trans* were shown from lane 4 to lane 9. A 15 mer ^{32}P end-labeled oligonucleotide served as the loading control (l.c.). Relative activities (%) are shown under the gel.