Supporting Information

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SI Materials and Methods

Construction of Strain NA1062 Containing FilP Fused to a Yellow Fluorescent Protein Ypet and FilP. First, *filP* was replaced by filP fused to a yellow fluorescent protein ypet (*filP-ypet*) in cosmid 8F4 using λ-RED mutagenesis (1). *Ypet* linked to a chloramphenicol resistance gene (*cat*) was PCR amplified with primers containing sequences homologous to the regions flanking the desired site of insertion in *filP* sequence. The forward primer also contained the sequence encoding for a flexible linker LPGPELPGPE (2). After insertion of the cassette containing *ypet-cat* into cosmid 8F4, the *cat* gene was excised from the recombinant cosmid by the restriction enzyme AflII using sites engineered in the primers, resulting in a seamless replacement of *filP* by *filP-ypet* in the native chromosomal context in 8F4. 8F4::*filP-ypet* was introduced by protoplast transformation into strain NA335 in which *filP* had been replaced by an apramycin-resistance cassette *aac(3)IV* (3). Transformants were selected first for incoming cosmid (kanamycin resistance) and in the next step screened for loss of both resistance markers. Plasmid pNA937 was introduced into the chosen transformant strain after verification of the presence of *filP-ypet* only in the native *filP* locus by diagnostic PCR. pNA937 contains the ORF of *filP* cloned into plasmid pIJ6902 under the thiostreptone-inducible promoter *tipAp*.

Construction of Strains NA1089 and NA1102 for Heterologous Expression of FilP and DivIVA in *E. coli*. For the arabinose-inducible constructs, pBAD-FilP and pBAD-D4A-EGFP, *filP*, or *divIVA-GFP*, respectively, were amplified by PCR and cloned into pBAD/*Myc*-His vector (Invitrogen). Plasmid pKT-FilP, which enables IPTG-inducible production of FilP was obtained by using PCR to replace the *T25* fragment by a stop codon in the construct pKT25-FilP. Plasmid pT-Trx-D4A-EGFP was obtained by cloning PCR-amplified *divIVA-GFP* into vector pT-Trx (4), placing *divIVA-egfp* under the control of the T7 promoter, which can be induced by IPTG in a suitable *Escherichia coli* strain, e.g., BL21. Strain NA1089 was obtained by transformation of plasmids pBAD-FilP and pT-Trx-D4A-EGFP into BL21. Strain NA1102 was obtained by transformation of plasmids pBAD- D4A-EGFP and pKT-FilP into strain DH5α.

1. Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci USA* 100(4):1541–1546.
2. Imai Y, et al. (2000) Subcellular localization of Dna-initiation proteins of *Bacillus subtilis*: Evidence that chromosome replication begins at either edge of the nucleoids. *Mol Microbiol* 36(5):1037–1048.
3. Bagchi S, Tomenius H, Belova LM, Ausmees N (2008) Intermediate filament-like proteins in bacteria and a cytoskeletal function in *Streptomyces*. *Mol Microbiol* 70(4): 1037–1050.
4. Yasukawa T, et al. (1995) Increase of solubility of foreign proteins in *Escherichia coli* by coproduction of the bacterial thioredoxin. *J Biol Chem* 270(43):25328–25331.

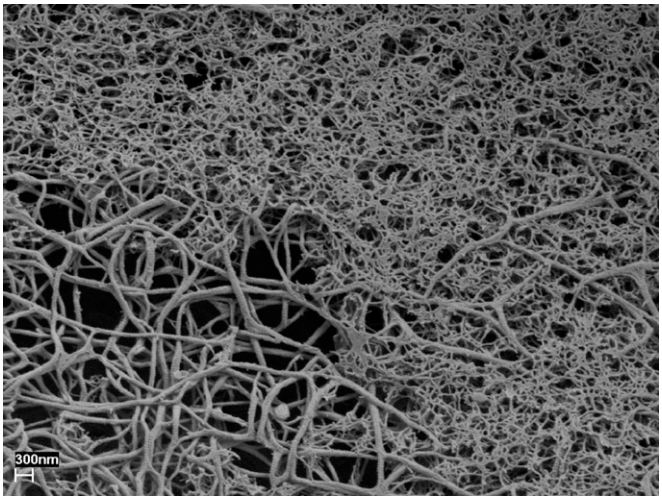

Fig. S1. Scanning electron micrograph of an extensive network of FilP filaments formed in polymix buffer.

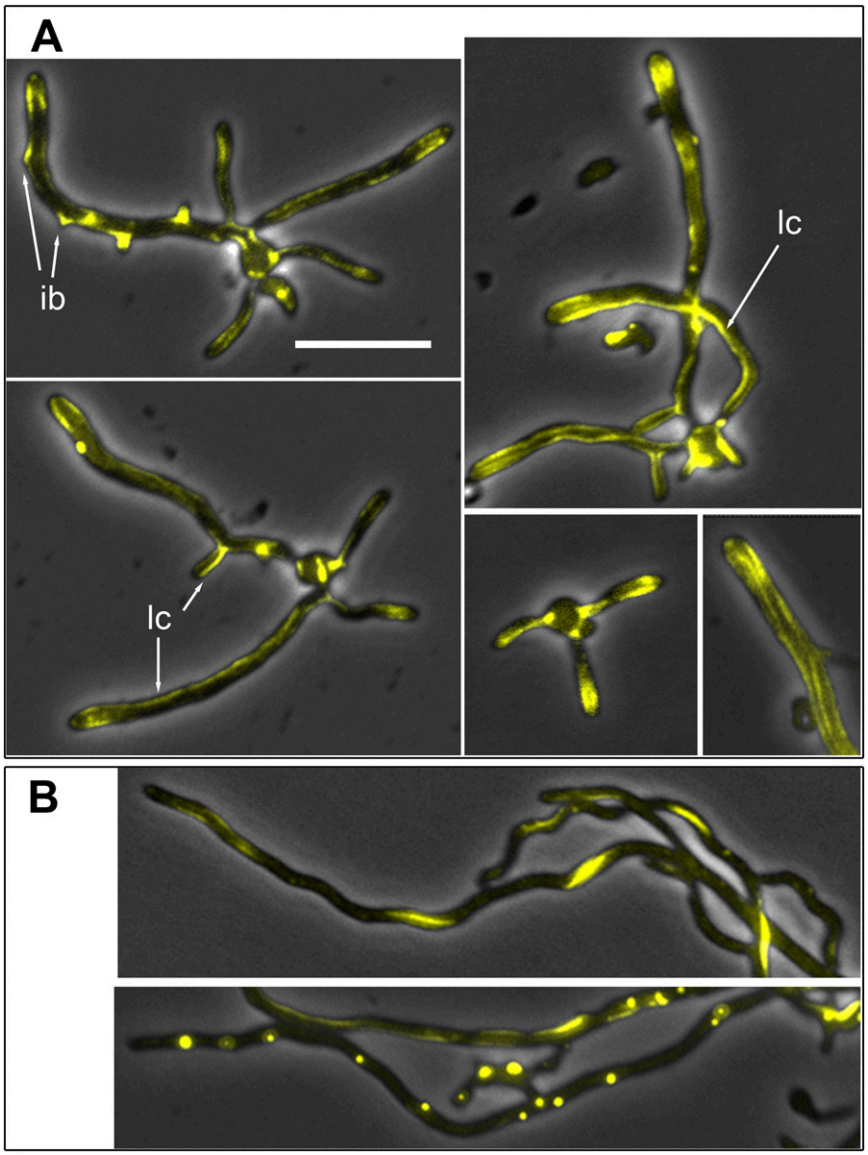

Fig. S2. FilP-YPet in live cells recapitulates the localization pattern of wild-type FilP visualized by immunofluorescence microscopy (IFM). Strain NA1062 (*filP-ypet, tipAp-filP*) was grown in liquid yeast extract-malt extract medium (YEME) without thiostrepton (*B*) or with 20 μg/mL thiostrepton (*A*) for 19 h. The images in *A* and *B* are overlays of phase contrast and FilP-YPet fluorescence (yellow). (Scale bar: 5 μm.) (*B*) Without thiostrepton FilP-YPet is the only source of FilP. The hyphae have a *filP* mutant phenotype (note the "wavy" shape), and FilP-YPet localizes as thick and short cables (*Upper*) or large foci (*Lower*), and no apical gradients of FilP-YPet are formed. (*A*) Upon induction of wild-type FilP, the hyphae gain a wild-type morphology, and the localization of FilP-YPet shows a dramatic change, with 70% of the hyphae containing apical gradients. Other characteristic elements of FilP localization visualized by IFM in *A*, such as lateral cables (lc) and accumulation at incipient branches (ib), are also frequently formed by FilP-YPet. Helical cables present in IFM images were the only structures of FilP not readily visualized by FilP-YPet in strain NA1062.

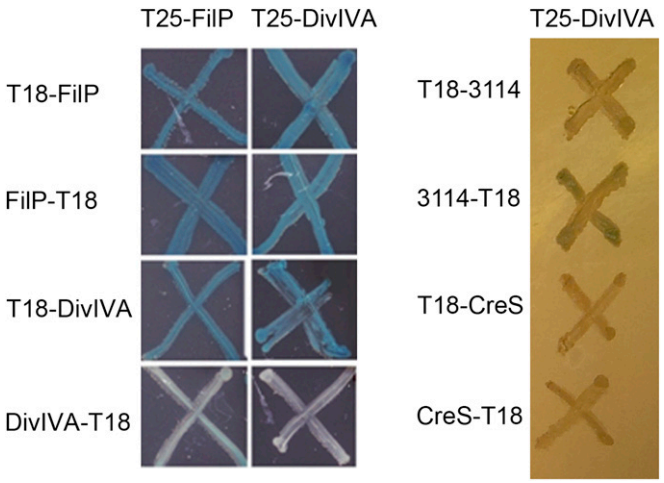

Fig. S3. Bacterial two-hybrid assay shows interaction between FilP and DivIVA. Strains containing pairs of plasmids encoding the bait (T25-FilP, T25-DivIVA, indicated at the top of the figure) and prey (FilP-T18, T18-FilP, DivIVA-T18, T18-DivIVA, indicated to the left) fusion proteins were incubated for 2 d at 30 °C on Luria Agar plates containing X-Gal and photographed (*Left*). Blue color indicates interaction between the bait and prey constructs. To evaluate the specificity of the DivIVA–FilP interaction, the T25-DivIVA bait was coexpressed with pray constructs encoding T18 fusions to coiled-coil proteins crescentin (1) (T18-CreS, CreS-T18) and SCO3114 (T18-3114, 3114-T18). SCO3114 is an uncharacterized protein sharing conserved sequence motifs with FilP. The strains containing bait–pray pairs as indicated in the figure (*Right*) were incubated as described above and photographed. All four strains failed to develop blue color, indicating that DivIVA does not interact with coiled-coil proteins crescentin and SCO3114.

1. Ausmees N, Kuhn JR, Jacobs-Wagner C (2003) The bacterial cytoskeleton: An intermediate filament-like function in cell shape. *Cell* 115(6):705–713.

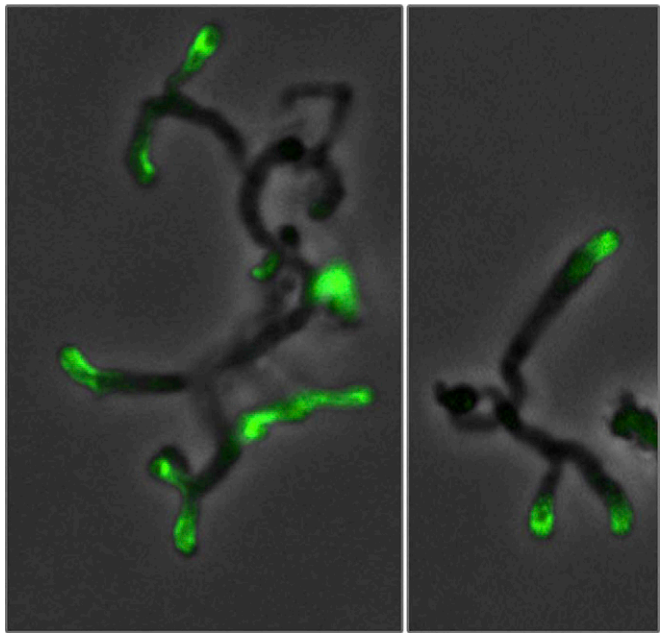

Fig. S4. Formation of apical gradients of FilP is not dependent on the large coiled-coil protein Scy (1). Overlay of phase contrast and anti-FilP immunofluorescence (green) images depicting young hyphae of an *scy* null mutant strain (NA 336).

1. Holmes NA, et al. (2013) Coiled-coil protein Scy is a key component of a multiprotein assembly controlling polarized growth in *Streptomyces*. *Proc Natl Acad Sci USA* 110(5):E397–E406.

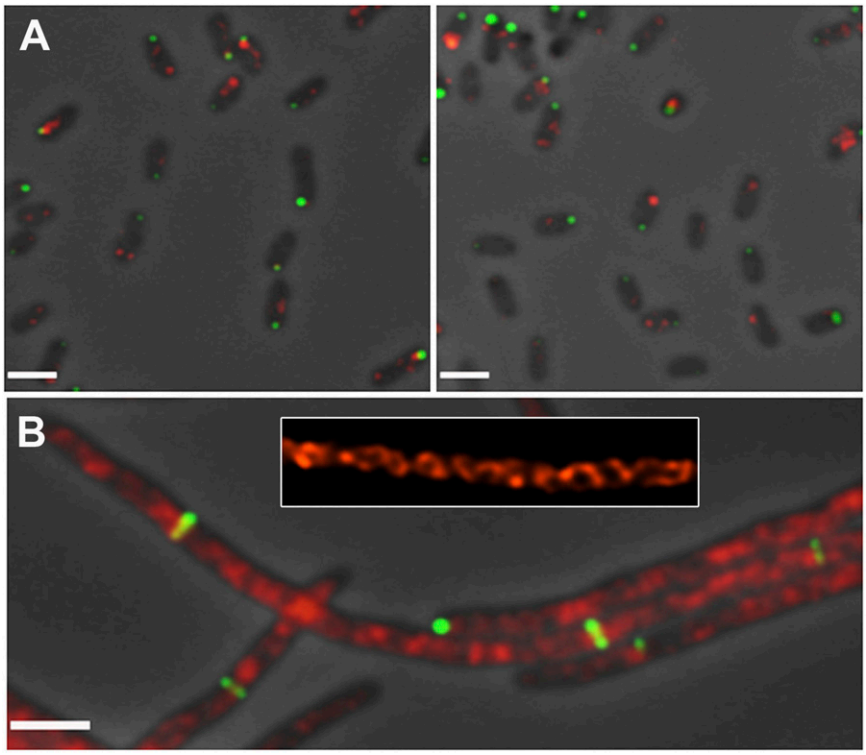

Fig. S5. DivIVA-EGFP does not recruit FilP in *E. coli* cells. To study whether other *Streptomyces*-specific factors are needed for the recruitment of FilP by DivIVA in vivo, *filP* and *divIVA-egfp* were coexpressed from inducible promoters in *E. coli* as a heterologous host. (*A* and *B*) All images are overlays of phase contrast, DivIVA-EGFP fluorescence (green) and anti-FilP immunofluorescence (red). (Scale bars: *A*, 2.20 μm; *B*, 1.60 μm.) (*A*) Cells of strain NA1089 (enables arabinose-induced production of FilP and IPTG-induced production of DivIVA-EGFP) were grown in the presence of 1 mM IPTG and contain polarly localized DivIVA-EGFP (green). Production of FilP has been briefly induced by addition of 0.015% arabinose for 30 min before imaging. Small clusters of newly made FilP (red) are visible in random positions in the cells also harboring polar DivIVA-EGFP clusters, indicating that oligomerization of FilP is not dependent on DivIVA and can be nucleated anywhere in the cytoplasm. (*B*) Cells of strain NA1102 (harbors plasmids pBAD-D4A-EGFP and pKT-FilP) were grown in the presence of 0.15% arabinose to induce DivIVA-EGFP. FilP was expressed in a weak constitutive manner from the lactose-inducible *lac* promoter. Cephalexin was added to the growth medium (40 μg/mL) to inhibit cell division and cause filamentation of the cells to better resolve the spatial distribution of FilP and DivIVA. DivIVA-EGFP was observed at polar and septal sites whereas FilP appeared as a meshwork distributed throughout the cells, visually resembling the structures observed by IFM in stationary phase *Streptomyces coelicolor* hyphae. (*B*, *Inset*) A deconvolved fluorescence image of a cell. We conclude that in the heterologous host *E. coli* FilP can form cytoskeletal structures similar to those observed in *S. coelicolor*. However, because DivIVA was not able to recruit FilP in *E. coli*, formation of FilP polar gradients in *S. coelicolor* must need other *Streptomyces*-specific factors in addition to DivIVA.

Table S1. Sensitivity of wild-type (M145) and *filP* mutant (NA883) strains to vancomycin and lysozyme

Strain	V10*	V20*	L600[†]
M145[‡]	10.0	12.0	8.0
	10.0	12.0	8.0
	9.5	11.5	8.0
Δ*filP*[‡]	10.0	11.5	8.0
	9.5	11.5	8.5
	10.5	12.0	8.0

*V10 and V20 indicate diameters of clearing zones around discs containing 10 μg and 20 μg of vancomycin, respectively. Diameters of the inhibition zones, obtained in three independent experiments, are shown in millimeters. The diameter of the discs is 6.5 mm.

[†]L600 indicates diameters of clearing zones around discs containing 600 μg of lysozyme.

[‡]Approximately 10^8 spores of each strain were spread on TSA plates and grown for 8 h before filter discs containing vancomycin and lysozyme were applied, and results were scored after further overnight growth.

Table S2. Strains and plasmids

Strain/plasmid	Description	Source
Strain		
S. coelicolor		
K112	M145 divIVA::pKF59[Φ(divIVA-egfp)]	(1)
K121	M145 divIVA::pKF59[Φ(divIVA-egfp)] $attB_{pSAM2}$::pKF58(*tipAp-divIVA)*	(2)
K120	M145 $attB_{pSAM2}$::pKF67(*tipAp-FLAG-divIVA)*	(3)
K114	M145 $attB_{pSAM2}$::pKF58(*tipAp-divIVA)*	(1)
NA335	M145 Δ*filP::[aac(3)IV oriT]*	(4)
NA336	M145 Δ*SCO5367::[aac(3)IV oriT]*	This study
NA883	M145 Δ*filP::FRT*	This study
NA956	NA883 $attB_{pSAM2}$::pKF67(*tipAp-FLAG-divIVA)*	This study
NA1062	M145 Δ*filP::filP-ypet* $attP_{\Phi C31}$*pNA937(tipAp-filP)*	This study
E. coli		
DH5a	Cloning strain	
DY380	Δ(*mrr-hsdRMS-mcrBC*) *mcrA recA1* λ *cl857* Δ(*cro-bio*)::*tet*, for PCR-targeted mutagenesis	(5)
ET12567/pUZ8002	*dam-13*::Tn*9 dcm-6 hsdM*, carries RK2 derivative with defective *oriT* for plasmid mobilization	(6)
TOP10	Cloning strain	Invitrogen
BTH101	Bacterial 2-hybrid (BTH) system, F^- *cya-99*, *araD139, galE15, galK16, rpsL1* (Str^r), *hsdR2, mcrA1, mcrB1*	(7)
NA1089	BL21 harboring pBAD-FilP and pRT-Trx-D4A-EGFP	This study
NA1102	DH5α harboring pBAD-D4A and pKT-FilP	This study
Plasmid		
pKT25	BTH system, encodes the T25 fragment of *B. pertussis* adenylate cyclase	(7)
pUT18	BTH system, encodes the T18 fragment of *B. pertussis* adenylate cyclase	(7)
pUT18c	BTH system, encodes the T18 fragment of *B. pertussis* adenylate cyclase	(7)
pT25-FilP	pKT25 containing *filP*	This study
pT25-DivIVA	pKT25 containing *divIVA*	This study
pFilP-T18	pUT18 containing *filP*	This study
pT18-FilP	pUT18c containing *filP*	This study
pDivIVA-T18	pUT18 containing *divIVA*	This study
pT18-DivIVA	pUT18c containing *divIVA*	This study
pCreS-T18	pUT18 containing *creS*	This study
pT18-CreS	pUT18c containing *creS*	This study
p3114-T18	pUT18 containing *SCO3114*	This study
pT18-3114	pUT18c containing *SCO3114*	This study
pNA937	pIJ6902 containing *filP (tipAp-filP)* for thiostrepton-inducible expression of FilP	This study
pBAD-FilP	Vector pBAD/*Myc*-His containing *filP*	This study
pBAD-D4A-EGFP	Vector pBAD/*Myc*-His containing *divIVA-egfp*	This study
pKT-FilP	Derivative of pKT25-FilP with deletion of *T25*	This study
pT-Trx-D4A-EGFP	Vector pT-Trx containing *divIVA-egfp*	This study

1. Flärdh K (2003) Essential role of DivIVA in polar growth and morphogenesis in *Streptomyces coelicolor* A3(2). *Mol Microbiol* 49(6):1523–1536.
2. Hempel AM, Wang SB, Letek M, Gil JA, Flärdh K (2008) Assemblies of DivIVA mark sites for hyphal branching and can establish new zones of cell wall growth in *Streptomyces coelicolor*. *J Bacteriol* 190(22):7579–7583.
3. Wang SB, et al. (2009) Domains involved in the in vivo function and oligomerization of apical growth determinant DivIVA in *Streptomyces coelicolor*. *FEMS Microbiol Lett* 297(1): 101–109.
4. Bagchi S, Tomenius H, Belova LM, Ausmees N (2008) Intermediate filament-like proteins in bacteria and a cytoskeletal function in *Streptomyces*. *Mol Microbiol* 70(4):1037–1050.
5. Lee EC, et al. (2001) A highly efficient *Escherichia coli*-based chromosome engineering system adapted for recombinogenic targeting and subcloning of BAC DNA. *Genomics* 73(1): 56–65.
6. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood D (2000) *Practical Streptomyces Genetics* (The John Innes Foundation, Norwich, UK).
7. Karimova G, Ullmann A, Ladant D (2000) A bacterial two-hybrid system that exploits a cAMP signaling cascade in *Escherichia coli*. *Methods Enzymol* 328:59–73.

Table S3. Primers used for cloning and generation of recombinant strains

Primer	Sequence	Use
N228	ATTTTACATATGGCTAGCAGCGACACTTCCCCCTACGGC	Cloning of pNA937
N231	TAAAATGAATTCGCTCGTCGTAGTGCTTGCCGT	Cloning of pNA937 and pBAD-FilP
N242	ATTTAGGATCCCATGAGACTGCTGTCGAAGAACT	Cloning of pCreS-T18 and pT18-CreS
N243	ATTTAGGTACCGCGGCGCTCGCGGCCACGTCGCCGT	Cloning of pCreS-T18 and pT18-CreS
N482	ATTATATCTAGAGAGCGGTGCATCGGCGTCT	Cloning of p3114-T18 and pT18-3114
N483	TATTAAGGTACCCATTCCACCTCCACCGCACG	Cloning of p3114-T18 and pT18-3114
N486	AATATTTCTAGAGCCGTTGACCCCCGAGGAC	Cloning of pDivIVA-T18, pT18-DivIVA and pT25-DivIVA
N487	TATATTGGTACCCAGTTGTCGTCCTCGTCGA	Cloning of pDivIVA-T18, pT18-DivIVA and pT25-DivIVA
N488	TAATATTCTAGAGAGCGACACTTCCCCCTAC	Cloning of pFilP-T18, pT18-FilP and pT25-FilP
N489	TTATAAGGTACCATGCGGGACTGCTGGGCCGG	Cloning of pFilP-T18, pT18-FilP and pT25-FilP
N516	AGCGACACTTCCCCCTACG	Cloning of pKT-FilP
N517	CATAGCTGTTTCCTGTGTG	Cloning of pKT-FilP
N518	CCGTTGACCCCCGAGGACG	Cloning of pBAD-D4A-EGFP
N519	CAGTGAATAACTGCAGTTACTTGTACAGCTCGTCCATG	Cloning of pBAD-D4A-EGFP
N534	TTCGGCAGGCGTACCCCCCGCGCAGCCGAAACCGCCCG GGCGCAAGGGCGCTTAAGTCTAGAGTCTGCATGCCTGC	Generating NA1062
N535	CGAGTCGGTCTCCCGCGGGGTCCCGGCCCAGCAGTCC CGCCTGCCCGGCCCCGAGCTCCCGGGCCCGGAGA TGGTGAGCAAAGGCGAAGA	Generating NA1062
N562	CCATGGGGAGCGACACTTCCCCCTACG	Cloning of pBAD-FilP
N563	ATTTTACATATGCCGTTGACCCCCGAGG	Cloning of pT-Trx-D4A-EGFP
N564	ATTTTAAAGCTTTTACTTGTACAGCTCGTCCATG	Cloning of pT-Trx-D4A-EGFP