Supplemental Figures for:

# Identification of TOEFAZ1-interacting proteins reveals key regulators of *Trypanosoma brucei* cytokinesis

**Authors:** Nicholas A. Hilton[1¶], Thomas E. Sladewski[1], Jenna A. Perry[1], Zemplen Pataki[1†], Amy N. Sinclair-Davis[1], Richard S. Muniz[1], Holly L. Tran[1], Jenna I. Wurster[1], Jiwon Seo[2], Christopher L. de Graffenried[1‡]

**Affiliations:** [1] Department of Molecular Microbiology and Immunology, Brown University, Providence, RI, 02912. [2] Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, Providence, RI, 02912

# Figure S1

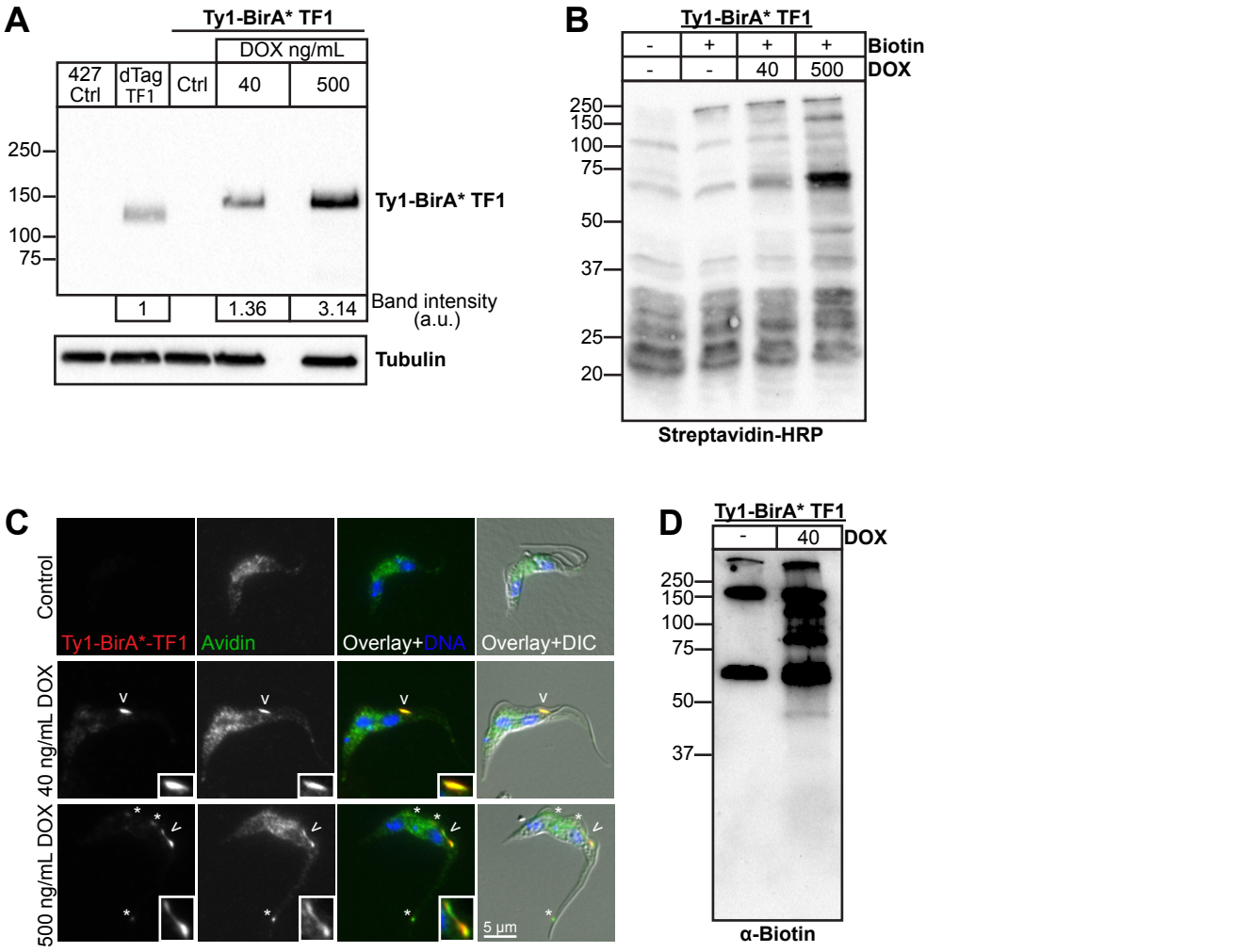



**Figure S1** Matching Ty1-BirA* TOEFAZ1 expression to endogenous TOEFAZ1 levels. **A)** Western blot comparing the expression levels between a cell line in which both alleles of TOEFAZ1 are tagged with a Ty1 epitope tag (dTag TF1) and the Ty1-BirA* cell line induced overnight with either 40 ng/mL or 500 ng/mL doxycycline. **B)** The Ty1-BirA* cell line was induced as above, followed by incubation with 50 µM biotin. Lysates from the induced Ty1-BirA* TOEFAZ1 cells were collected and separated by SDS-PAGE followed by transfer to nitrocellulose for western blotting with streptavidin conjugated to HRP. **C)** The Ty1-BirA* cell line was induced and incubated with biotin as above. Cells were methanol-fixed and stained with anti-Ty1 antibody and streptavidin. Arrowheads: Ty1-BirA* TOEFAZ1 localization. Asterisks indicate spurious localization. DAPI was used to stain DNA. **D)** The Ty1-BirA* TOEFAZ1 cell line was induced as in B) with 40 ng/mL doxycycline, followed by lysis and capture of biotinylated proteins, which were eluted and blotted using anti-biotin antibody.

# Figure S2

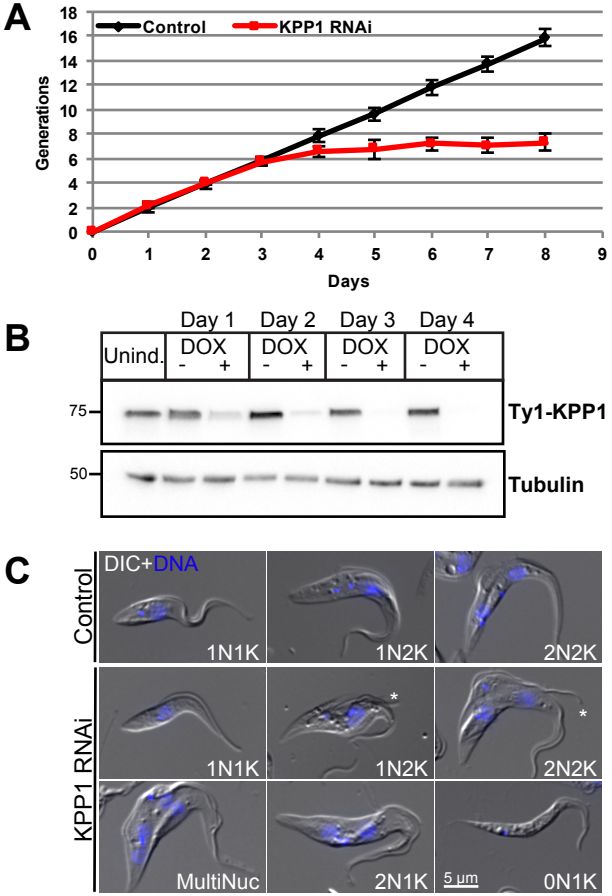



**Figure S2** KPP1 is required for cell division. **A)** KPP1 RNAi was induced for 8 d using 1 µg/mL doxycycline, and cell concentration was counted every 24 h. **B)** Cell lysates were collected every 24 h from the RNAi timecourse in A), western blotted, and labeled with anti-Ty1 antibody. **C)** Cells carrying a KPP1 RNAi construct were treated with either vehicle control or 1 µg/mL doxycycline to induce RNAi for four days. Cells were PFA-fixed and stained with DAPI for DNA. Asterisk indicates a detached flagellar tip. **D)** Quantification of detached new flagella tips in control and KPP1 RNAi cells in which KPP1 has been depleted for 4 d.

# Figure S3

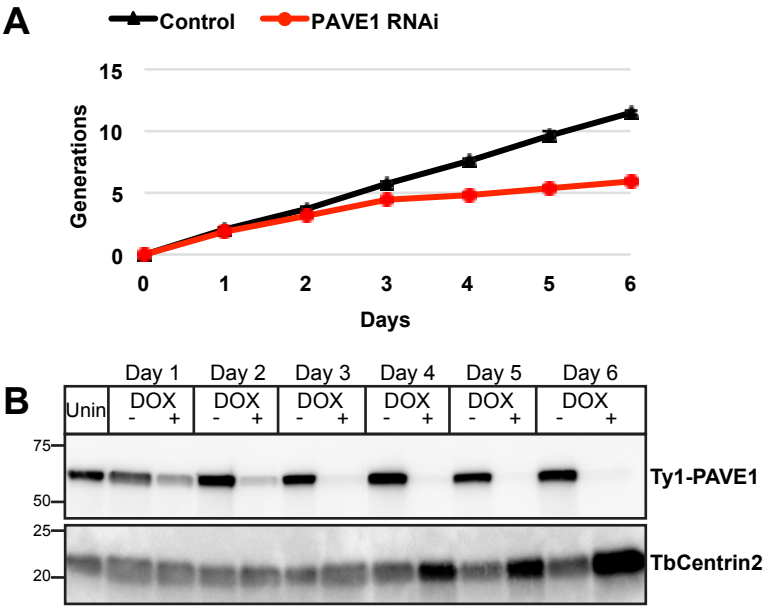



**Figure S3** PAVE1 is necessary for cell division. **A)** PAVE1 RNAi cells were treated with either vehicle control or 1 µg/mL doxycycline for 6 days and cell number was monitored every 24 h. **B)** Cell lysates were collected every 24 h from the time course in A). Lysates were western blotted and stained with anti-Ty1 antibody.

# Figure S4

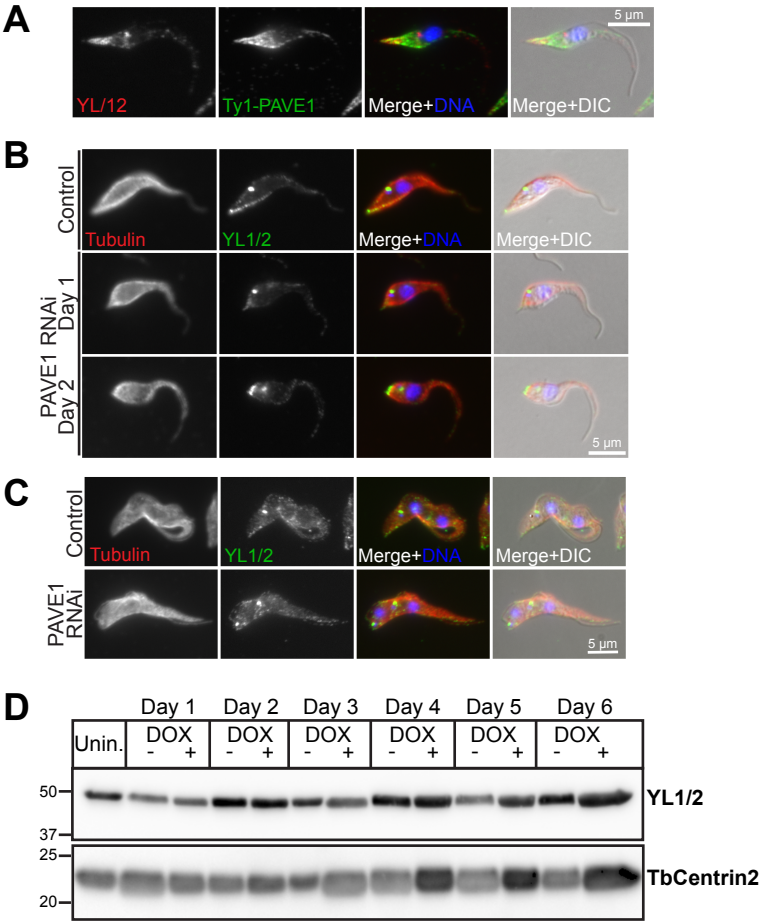



**Figure S4** YL1/2 staining is not altered in PAVE1 RNAi cells. **A)** Immunofluorescence of methanol-fixed wherein PAVE1 was tagged at its endogenous locus with a Ty1 tag. Cells were stained for YL1/2 to detect tyrosinated tubulin, an indicator of growing microtubules, and Ty1 to label PAVE1. **B)** PAVE1 RNAi was induced with 1 µg/mL doxycycline for 1 and 2 d. Cells were methanol fixed and stained with anti-tubulin antibody and YL1/2. DAPI was used to stain DNA in all immunofluorescence panels. **C)** Dividing 2N2K cells from the experiment described in B). **D)** Lysates from the RNAi time course in Fig. S3A were western blotted and labeled with YL1/2 antibody.

# Figure S5

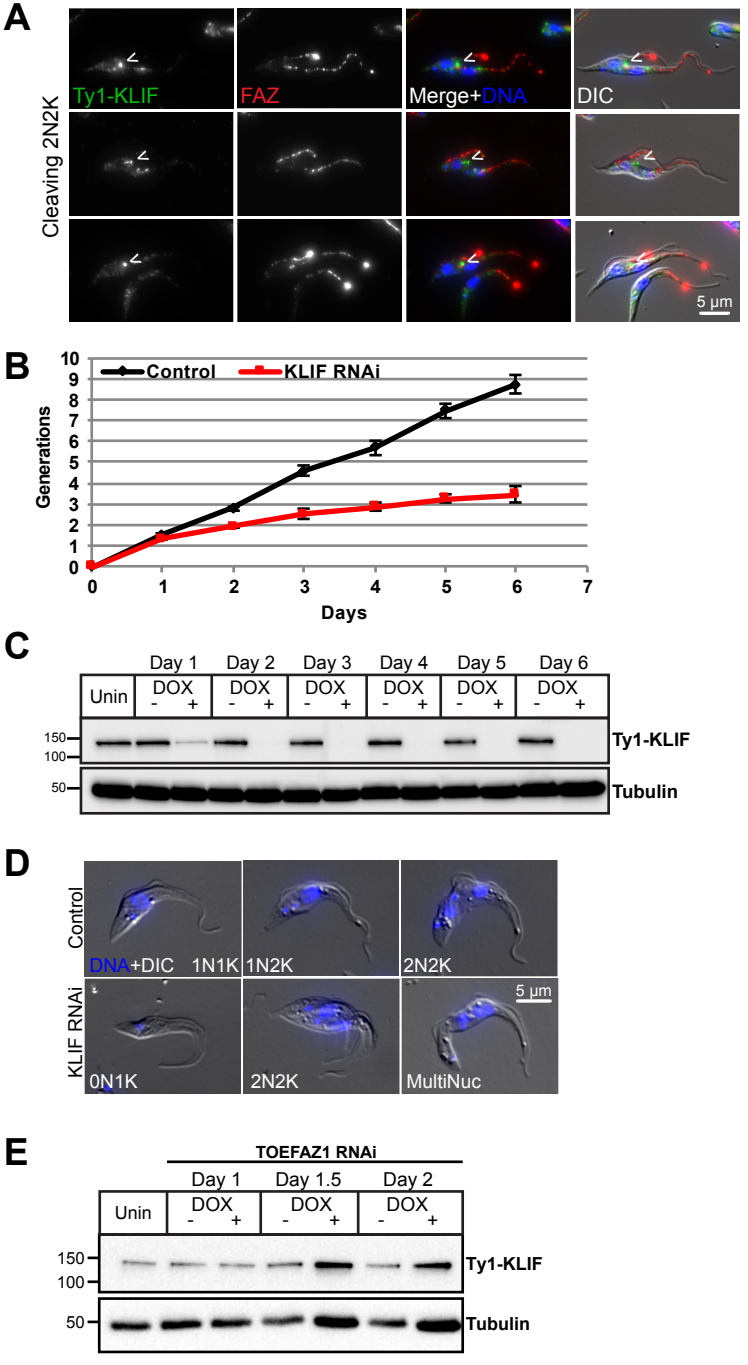



**Figure S5** KLIF is required for cell division. **A)** Cells in which KLIF was tagged at its endogenous locus with a Ty1 epitope tag were methanol-fixed and stained with anti-Ty1 antibody and 1B41 to label the FAZ. DAPI was used to label DNA. Arrowheads indicate Ty1-KLIF localization at the cleavage furrow. **B)** Cells containing the KLIF RNAi construct were treated with either vehicle control or 1 µg/mL doxycycline. Cell concentration was counted every 24 h. **C)** Lysates were collected every 24 h from the RNAi timecourse in B), western blotted, and stained with anti-Ty1 antibody. **D)** Cells containing a KLIF RNAi construct were treated with either vehicle control or 1 µg/mL doxycycline for 3 d. Cells were collected and PFA-fixed for morphological analysis. DAPI was used to stain DNA. **E)** TOEFAZ1 RNAi was induced in a cell line containing KLIF tagged at its endogenous locus with a Ty1 epitope tag. Lysates were collected every 12 h, western blotted, and stained with anti-Ty1 antibody.

# Figure S6

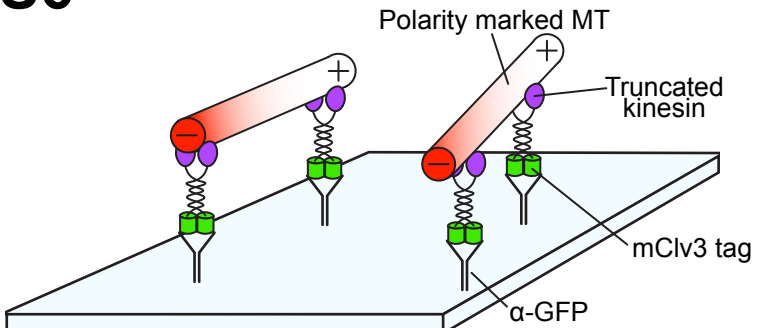
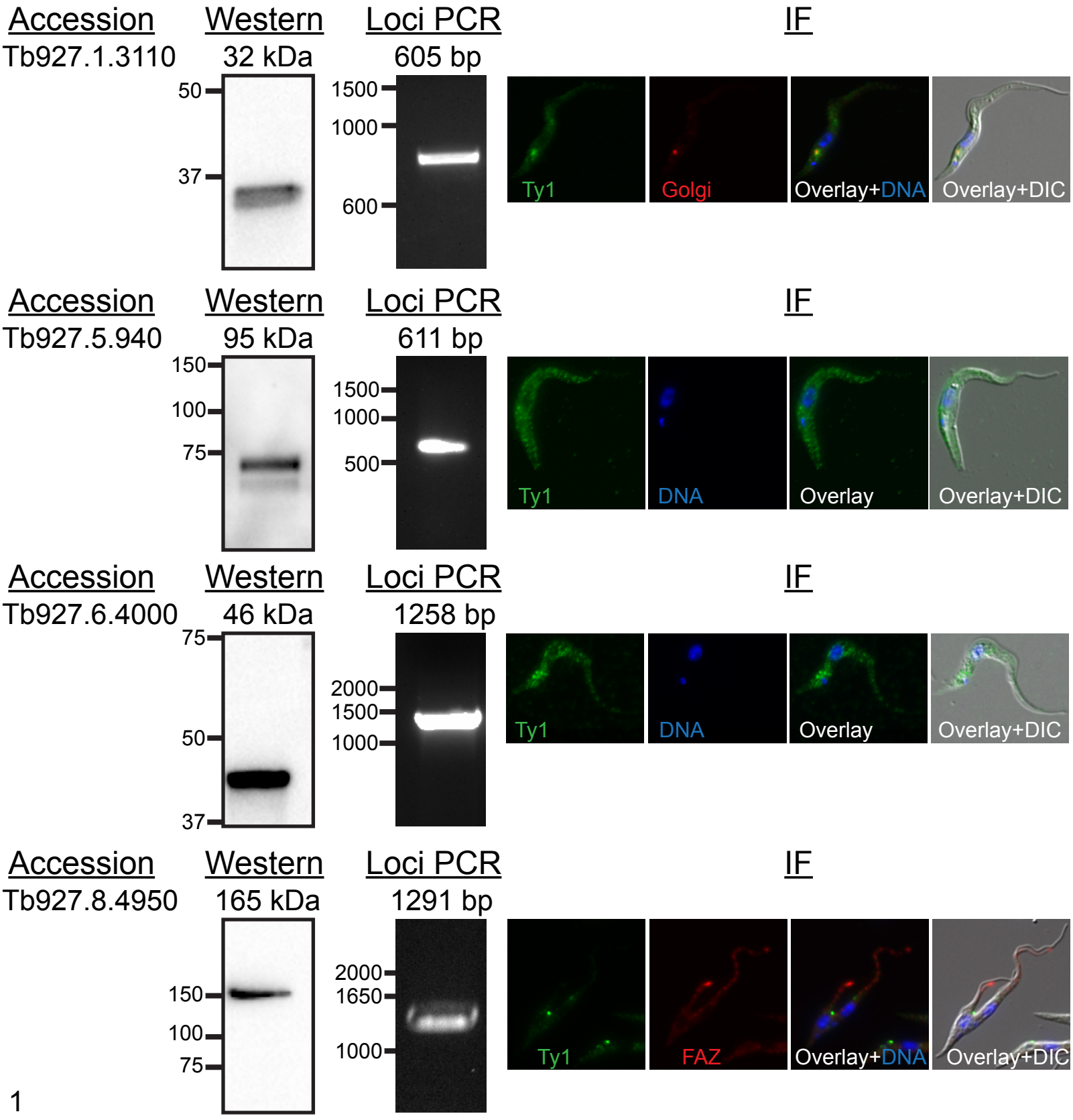
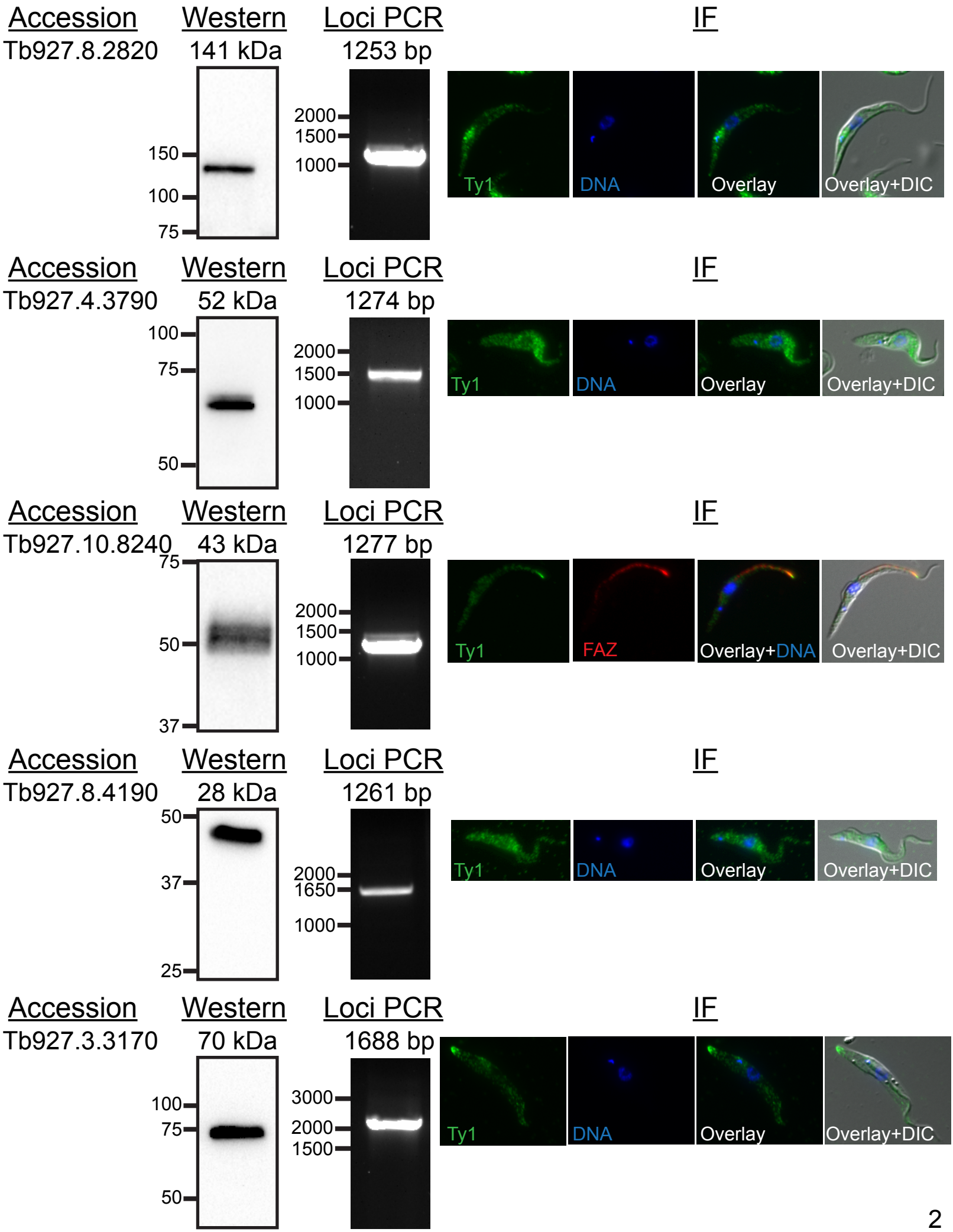



**Figure S6** Schematic of the *in vitro* motility assay. KLIF (purple) containing a mClover3 tag (mClv3, green) is bound to an α-GFP coated flow chamber (light blue) and blocked. Then, rhodamine-labeled polarity marked microtubules (red) containing enhanced fluorescent signal at their minus-ends are introduced to the flow chamber to interact with KLIF in the presence of MgATP.

# Figure S7

**Figure S7** Characterization of curated TOEFAZ1 BioID hits. A Ty1 epitope tag was integrated at either the N- or C-terminus of the endogenous locus of each hit from the TOEFAZ1 BioID screen. The size of each protein was determined by anti-Ty1 western blotting, and the correct integration of the Ty1 tag at the endogenous locus was established by loci PCR. Immunofluorescence analysis was conducted on each protein using anti-Ty1 antibody and, where applicable, using antibodies against known organelles for co-localization.

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Overlay+DIC
Accession
Western
Loci PCR
IF
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Ty1
DNA
Overlay
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Accession
Western
Loci PCR
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37
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Overlay+DIC
Accession
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Loci PCR
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Western
Loci PCR
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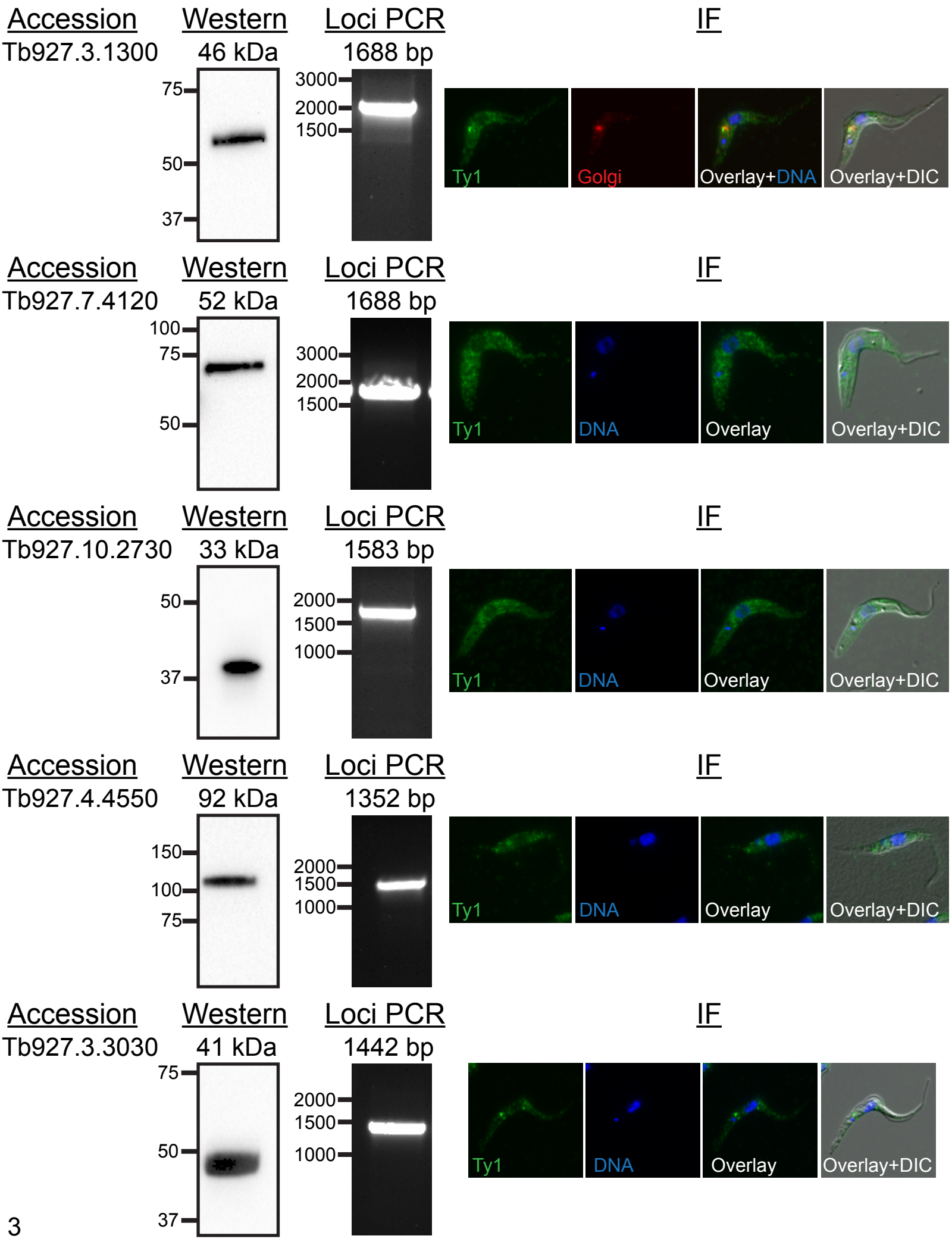
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Ty1
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Accession
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Accession Western Loci PCR IF

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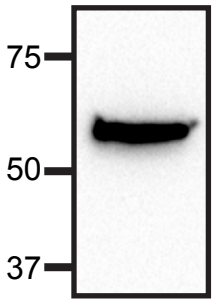


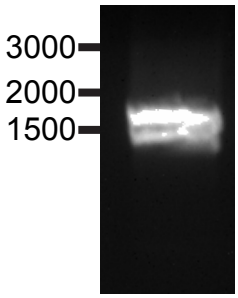


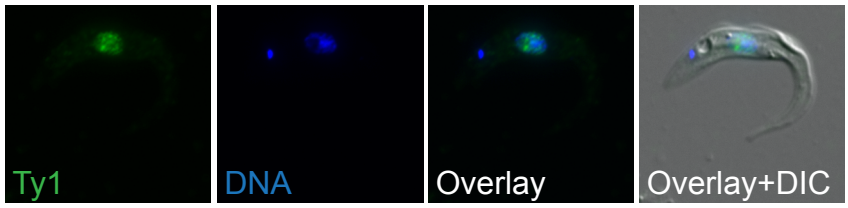


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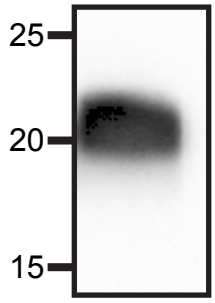


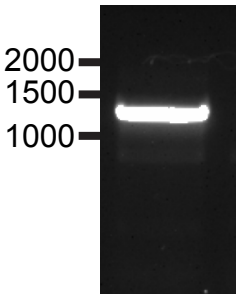


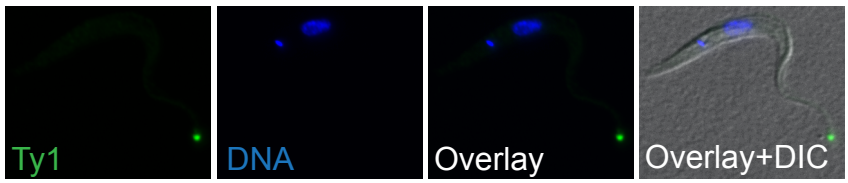


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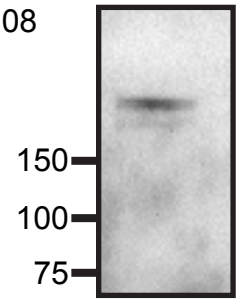


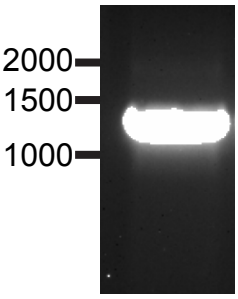


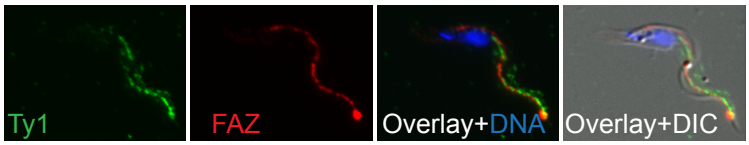


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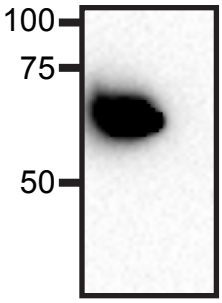


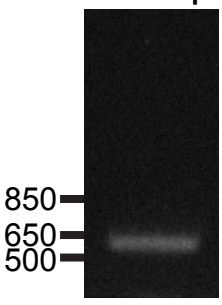


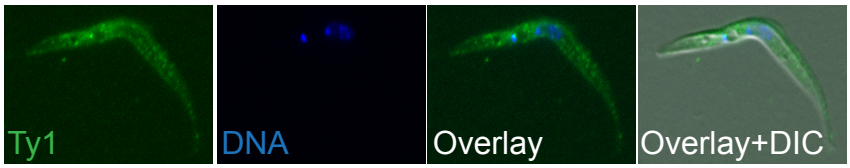


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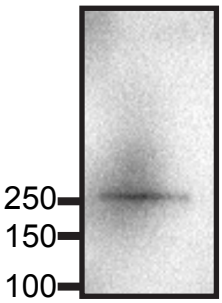


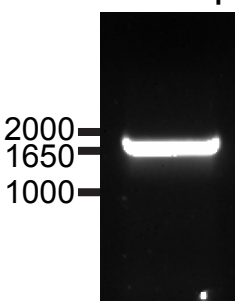


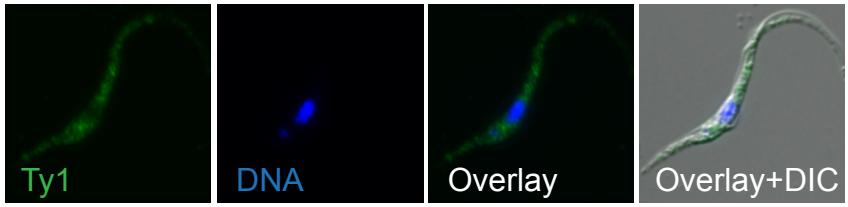

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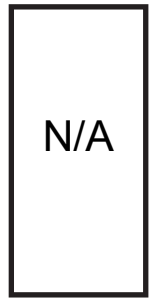


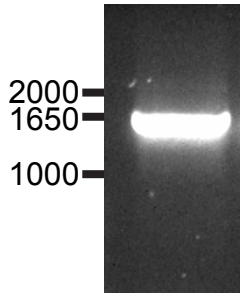


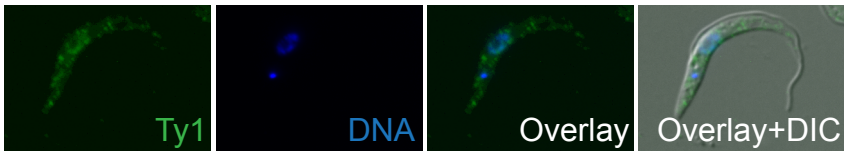


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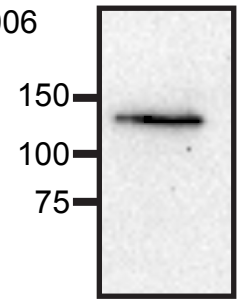


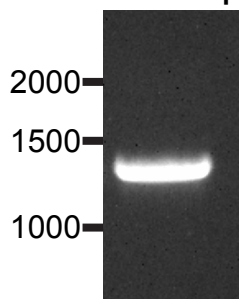


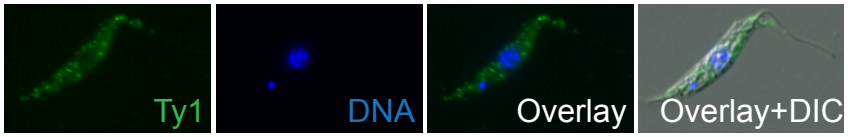


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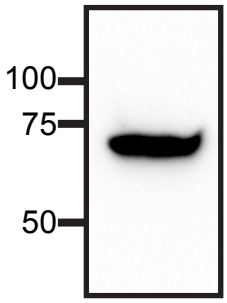


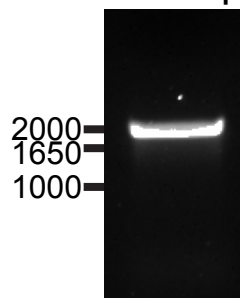


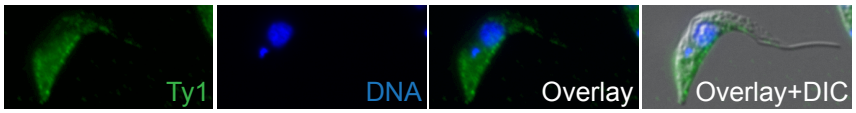


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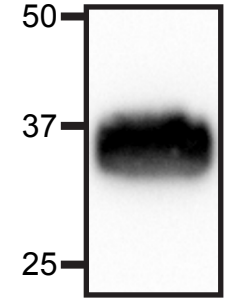


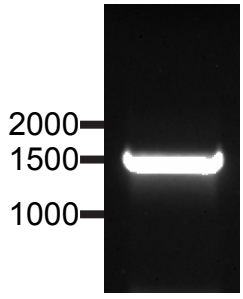


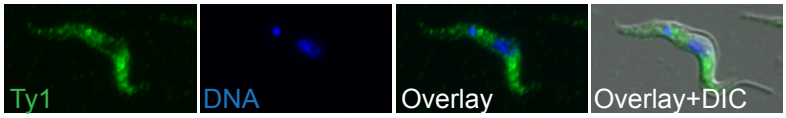


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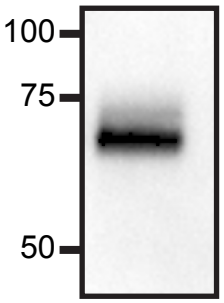


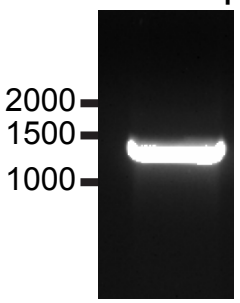


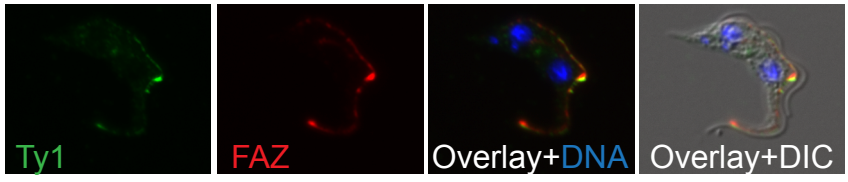

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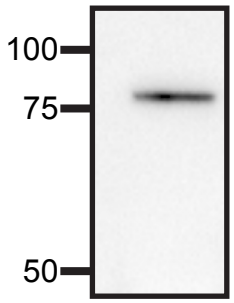


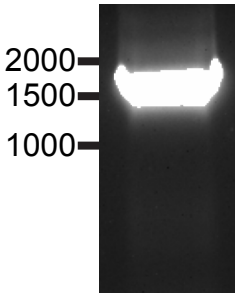


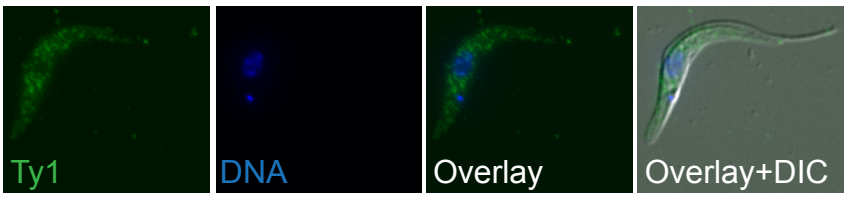


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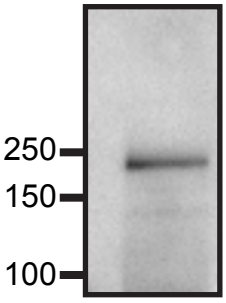
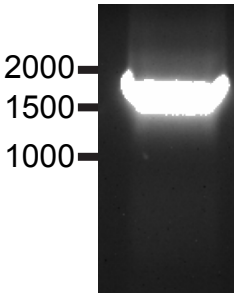
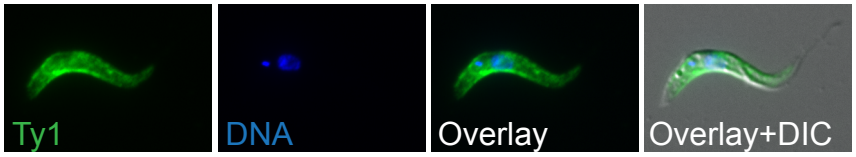
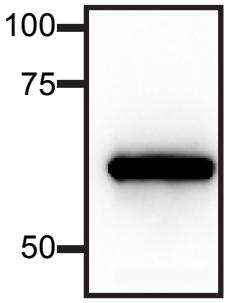
|---|---|---|---|
| Tb927.7.3550 | 138 kDa | 1278 bp | |

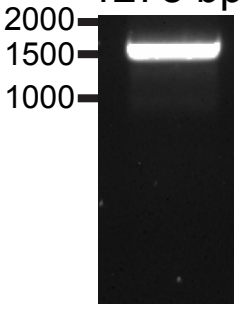


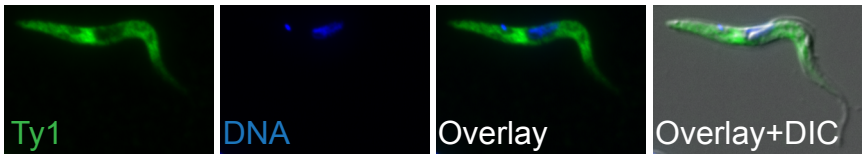



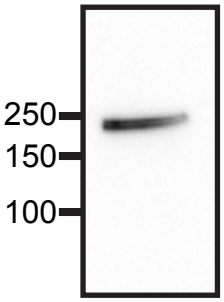

| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.11.1900 | 58 kDa | 1278 bp | |

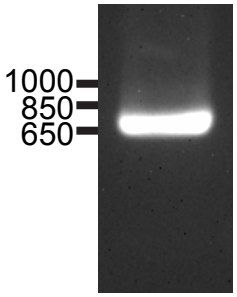


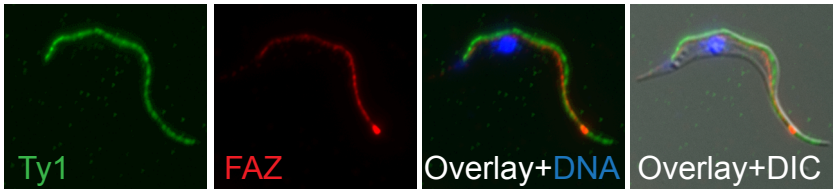



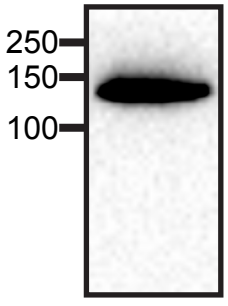

| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.3.930 | 532 kDa | 624 bp | |

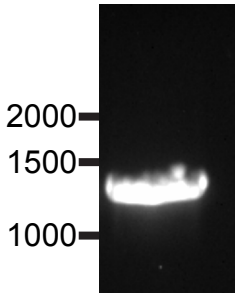


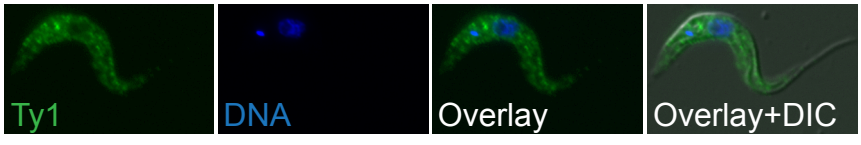



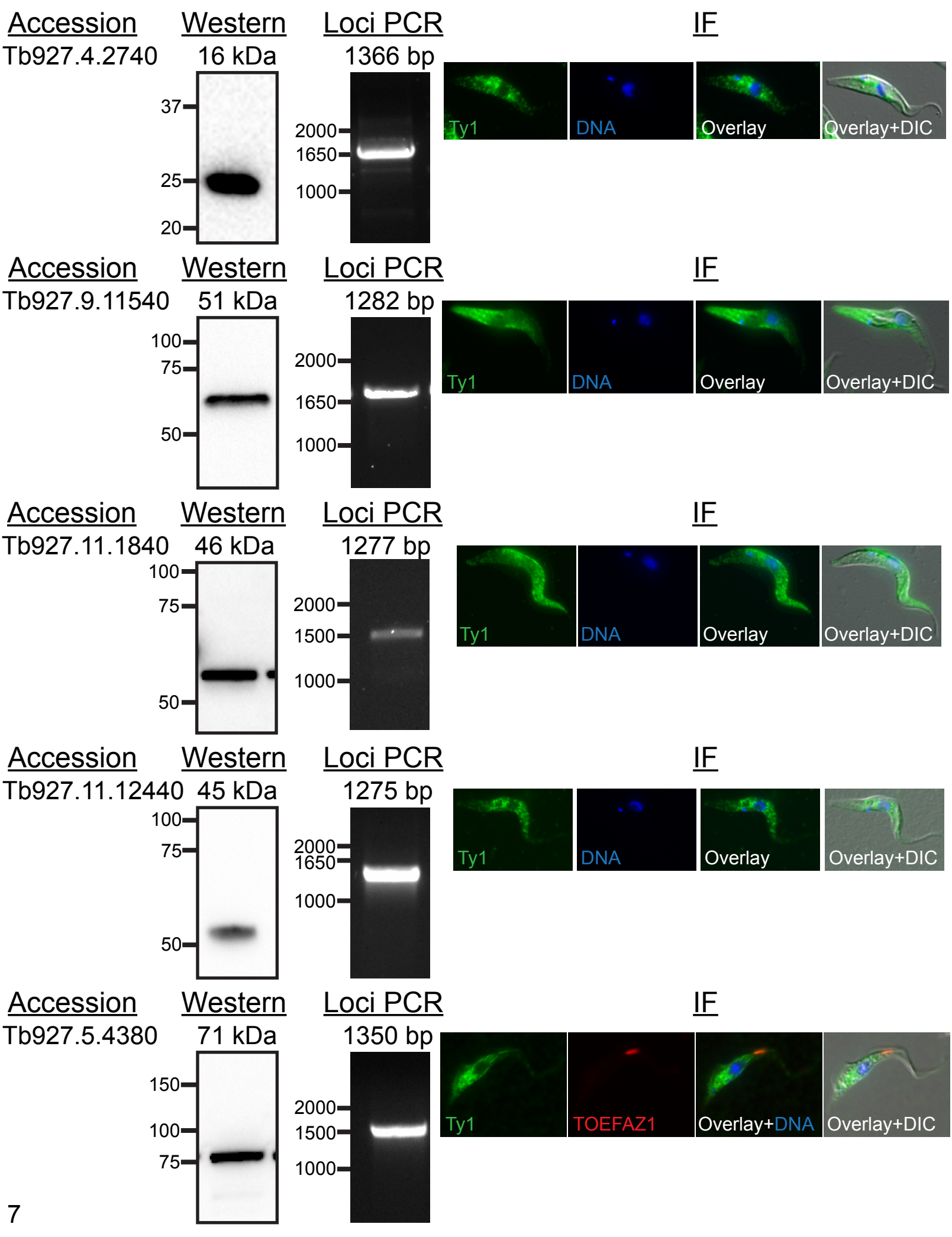




| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.3.2490 | 110 kDa | 1274 bp | |



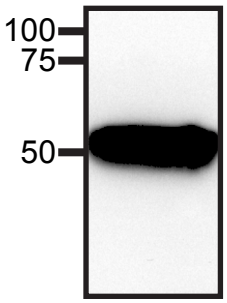



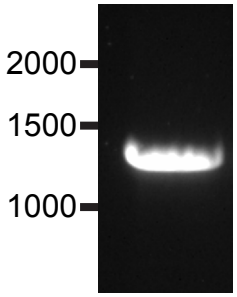

Accession
Western
Loci PCR
IF
Tb927.4.2740
16 kDa
1366 bp
37
25
20
2000
1650
1000
Ty1
DNA
Overlay
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.9.11540
51 kDa
1282 bp
100
75
50
2000
1650
1000
Ty1
DNA
Overlay
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.11.1840
46 kDa
1277 bp
100
75
50
2000
1500
1000
Ty1
DNA
Overlay
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.11.12440
45 kDa
1275 bp
100
75
50
2000
1650
1000
Ty1
DNA
Overlay
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.5.4380
71 kDa
1350 bp
150
100
75
2000
1500
1000
Ty1
TOEFAZ1
Overlay+DNA
Overlay+DIC

Accession
Tb927.5.590

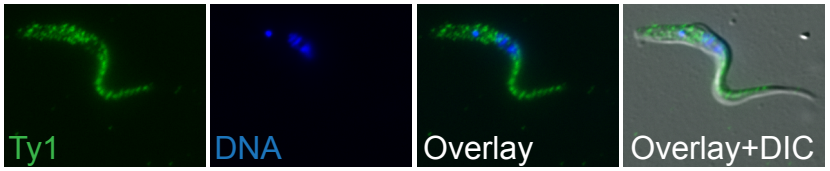
Western
46 kDa




Loci PCR
1260 bp

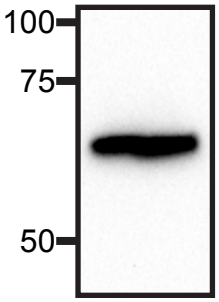



IF

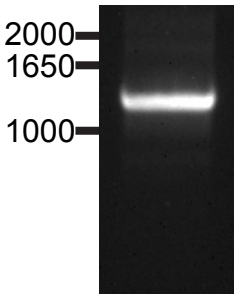



Accession
Tb927.11.4420

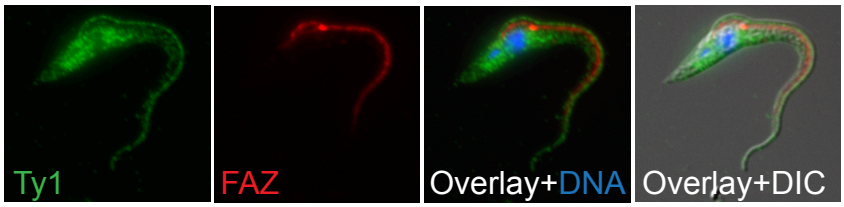
Western
59 kDa




Loci PCR
1271 bp

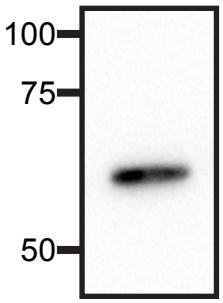



IF

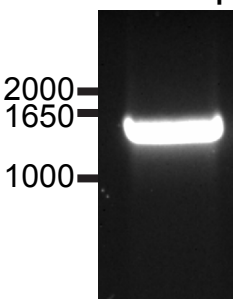



Accession
Tb927.7.660

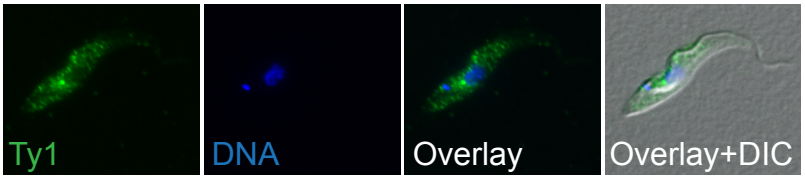
Western
53 kDa




Loci PCR
1284 bp

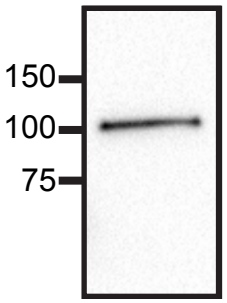



IF

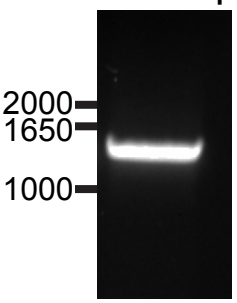



Accession
Tb927.7.650

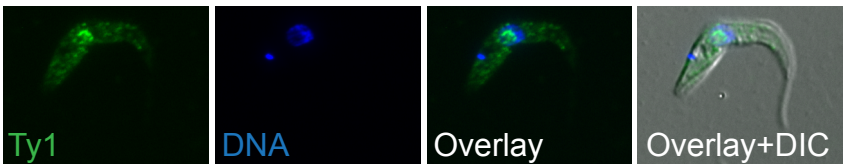
Western
106 kDa




Loci PCR
1359 bp

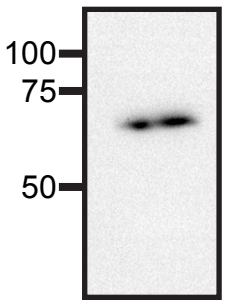



IF

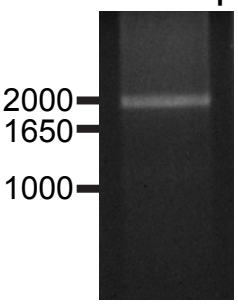



Accession
Tb927.7.3980

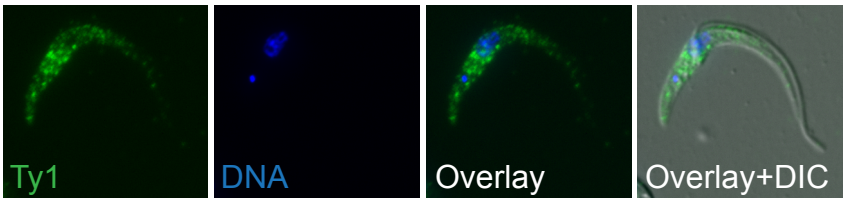
Western
97 kDa

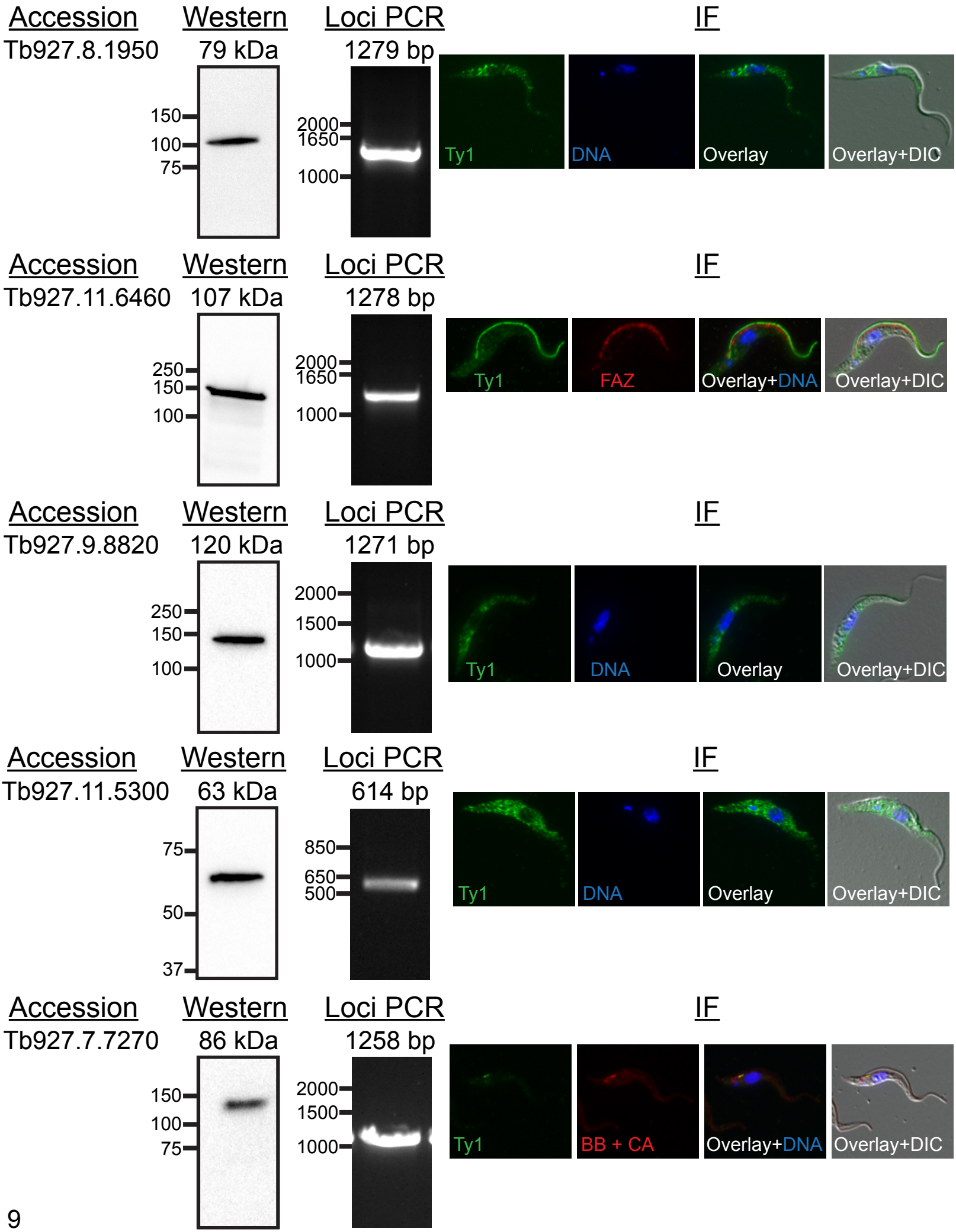



Loci PCR
1484 bp



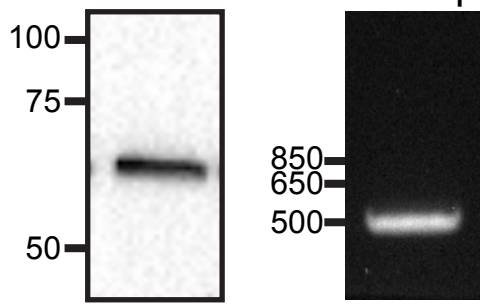

IF

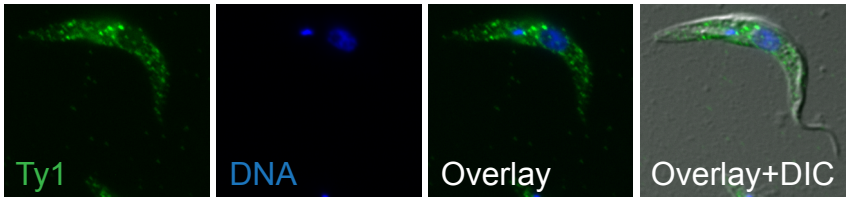



Accession
Western
Loci PCR
IF
Tb927.8.1950
79 kDa
1279 bp
150
100
75
2000
1650
1000
Ty1
DNA
Overlay
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.11.6460
107 kDa
1278 bp
250
150
100
2000
1650
1000
Ty1
FAZ
Overlay+DNA
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.9.8820
120 kDa
1271 bp
250
150
100
2000
1500
1000
Ty1
DNA
Overlay
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.11.5300
63 kDa
614 bp
75
50
37
850
650
500
Ty1
DNA
Overlay
Overlay+DIC
Accession
Western
Loci PCR
IF
Tb927.7.7270
86 kDa
1258 bp
150
100
75
2000
1500
1000
Ty1
BB + CA
Overlay+DNA
Overlay+DIC

Accession Western Loci PCR IF

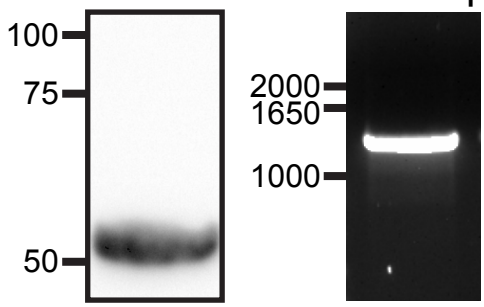
Tb927.6.4750 68 kDa 613 bp

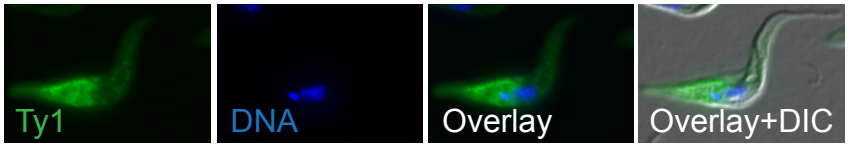



Accession Western Loci PCR IF

Tb927.9.6920 41 kDa 1224 bp

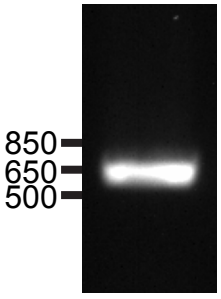


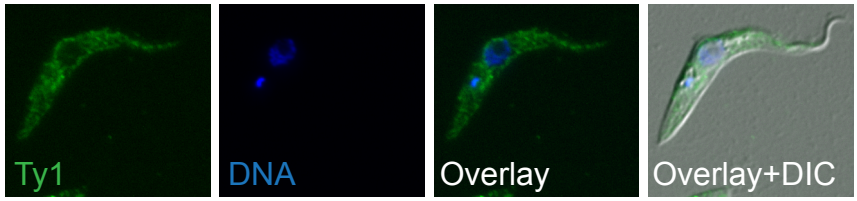




Accession Western Loci PCR IF

Tb927.10.10280 269 kDa 581 bp

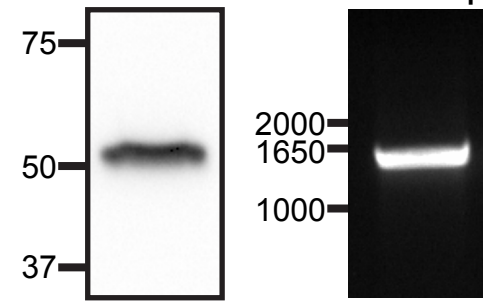
N/A

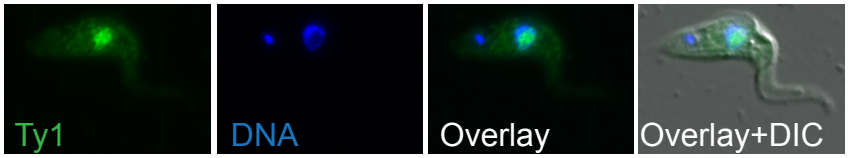



Accession Western Loci PCR IF

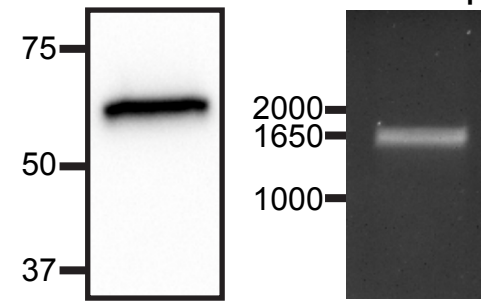
Tb927.10.11210 50 kDa 1251 bp

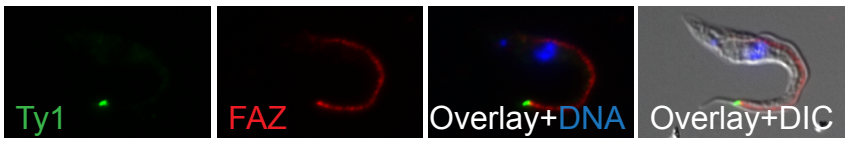



Accession Western Loci PCR IF

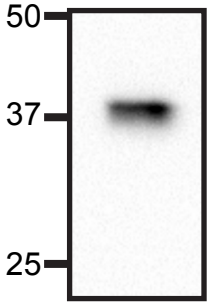
Tb927.7.5240 53 kDa 1214 bp



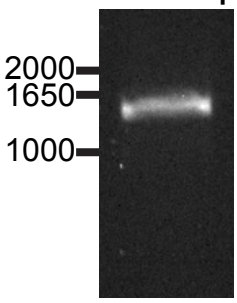



Accession
Tb927.11.12020

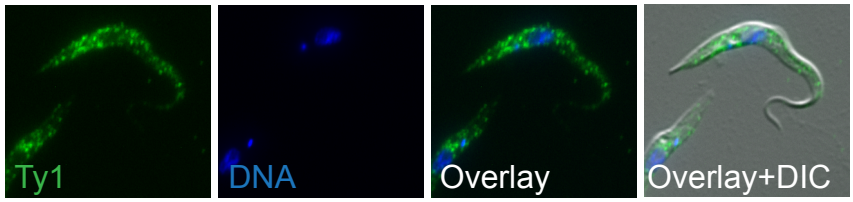
Western
25 kDa




Loci PCR
1238 bp

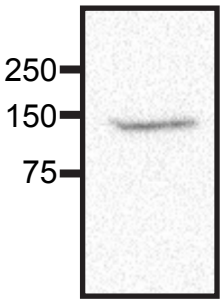



IF

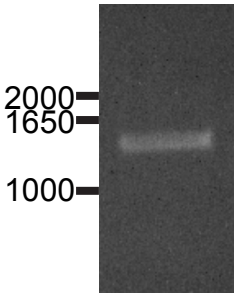



Accession
Tb927.3.4510

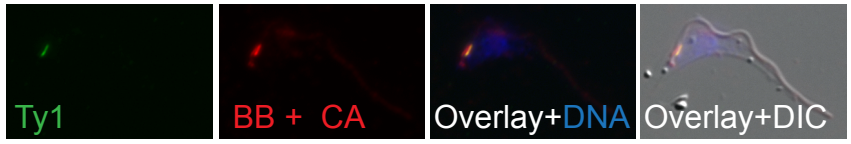
Western
151 kDa




Loci PCR
1250 bp

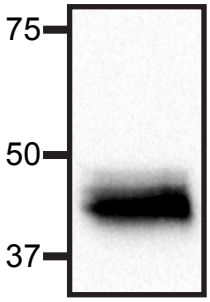



IF

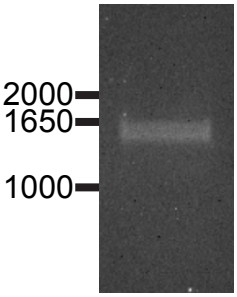



Accession
Tb927.11.7140

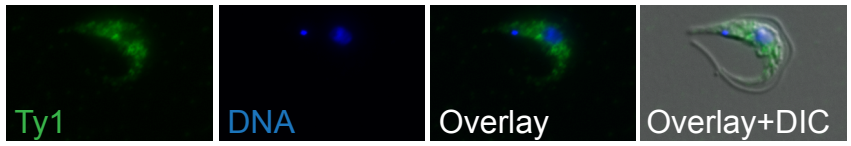
Western
35 kDa




Loci PCR
1269 bp

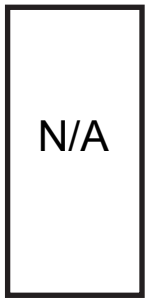



IF

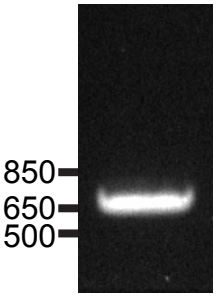



Accession
Tb927.2.5270

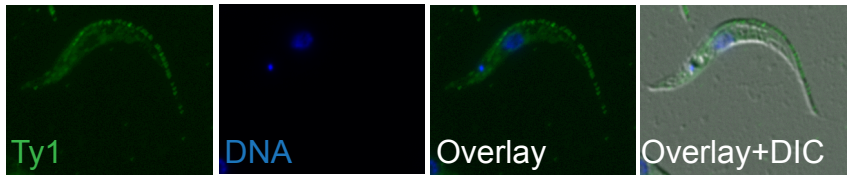
Western
486 kDa




Loci PCR
574 bp

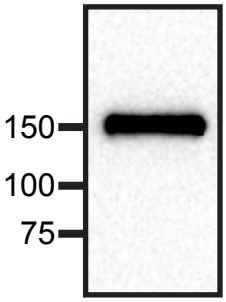



IF

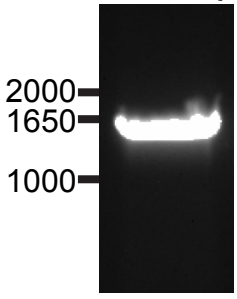



Accession
Tb927.8.2860

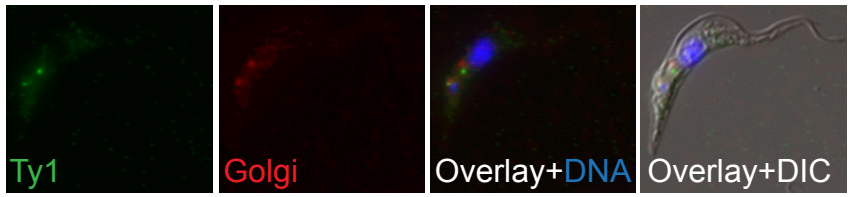
Western
99 kDa



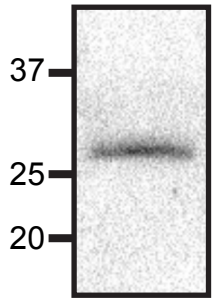

Loci PCR
1287 bp

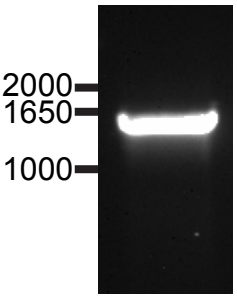


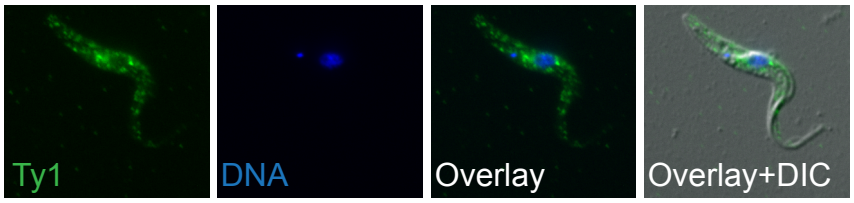

IF



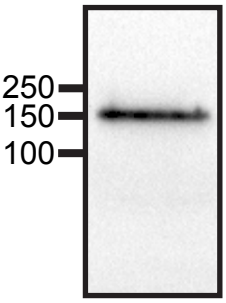
| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.3.3620 | 18 kDa | 1259 bp | |

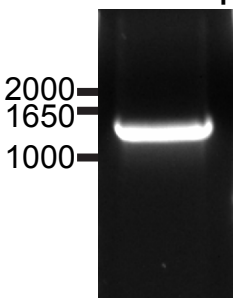


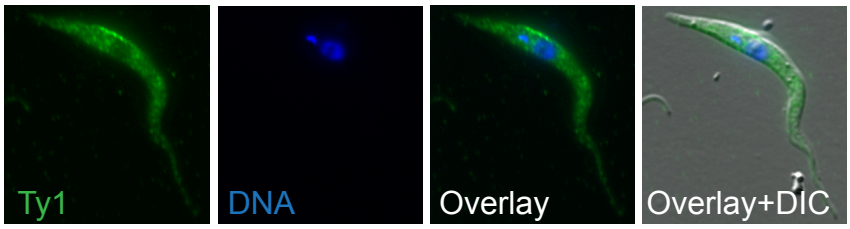



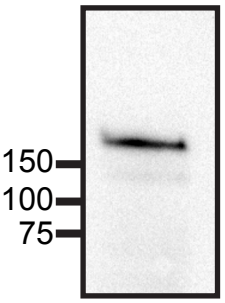

| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.10.3780 | 111 kDa | 1278 bp | |

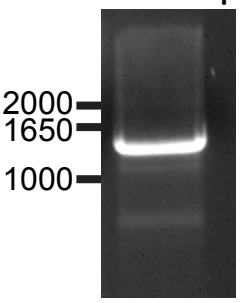


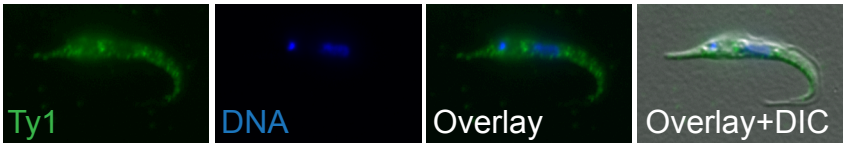



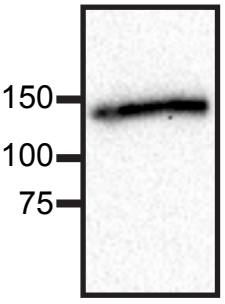

| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.9.2730 | 147 kDa | 1287 bp | |

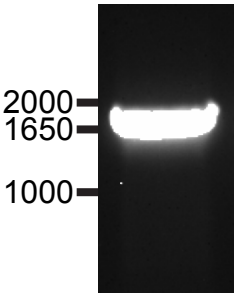


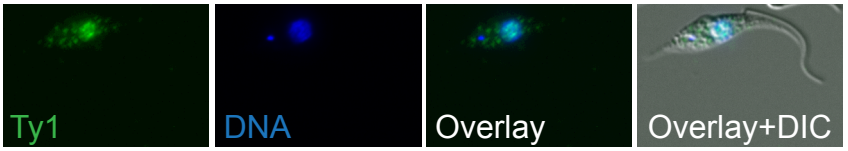




| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.11.6810 | 108 kDa | 1254 bp | |




| Accession | Western | Loci PCR | IF |
|---|---|---|---|
| Tb927.7.4450 | 113 kDa | 1244 bp | |