28S typical amplification

Generate 1.1-1.3 using genomic DNA, then run 2.1-2.2 (using product from step 1.1), 2.3-2.4 (product from step 1.2) (see notes about 2.3a), and 2.5-2.6 (product from step 1.3). 2.5 may be used as positive control to detect the presence of DNA (see the respective note)

Name	1.1. 28SD1-5.P	1.2. 28SD5-10.P	1.3. 28SD11.P	2.1. 28SD1-5P.s1 (=28SIt)	2.2. 28SD1-5P.n1 (=28S3L)	2.3. 28SD5-10P.n1 (=28SIV)	2.3a. 28SD5-10P.n1 (=28S4)	2.4. 28SD5-10P.n2 (=28S5)	2.5. 28SD11P.n1 (=28SD9)	2.6. 28SD11P.sh2 (=28S11a)
approx size				1100	860	810	~1035	~835	650	~800
Primer (F)	28S_f1_4F	28S_NS2F_m	28S_1656F	28S_1F_Arach3_T	28Sa_DD_T	28S_NS2F_i1Fm_T	28S_3677F	28S_NS2_i2Fm_T	28SV (D9-10)	28S_2732F_T
Primer (R)	28S_P1_3R	28S_NS2R	28S_3531R_Arachn	28S_1661R_T	28SIR_acar2_T	28S_NS2_i1Rm_T	28S_NS2_i1R_v3	28S_NS2_i2R_T	28SX (D9-10)	28S_3528R_Arachn_T
dH$_2$0	9.81	9.81	9.81	13.22	13.22	13.22	13.32	13.22	12.93	13.22
PCR buffer	1.5	1.5	1.5	2	2	2	2	2	2	2
MgSO$_4$ (50 mM)	1.05	1.05	1.05	1.4	1.4	1.4	1.4	1.4	1.4	1.4
dNTPs (10 mM each)	1.05	1.05	1.05	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Primers (10uM each)	0.6	0.6	0.6	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Plat Taq (1u=0.2uL p	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.08	0.12	0.08
DNA	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.55	0.3
n Volumes (1 sample):	**15**	**15**	**15**	20	20	20	20	20	20	20

PCR Profile	°C	T	°C	T	°C	T	°C	T	°C	T	°C	T	°C	T	°C	T	°C	T	°C	T
denature	94	0:30	94	0:30	94	0:30	94	0:30	94	0:30	94	0:30	94	0:30	94	0:30	94	0:30	94	0:30
anneal	50	0:30	51	0:35	51	0:35	51	0:20	53	0:25	53	0:25	53	0:25	53	0:25	50	**0:25**	51	0:25
extension	72	2:15+1s/cycle	72	2:20+1s/cycle	72	2:20+1s/cycle	72	1:40	72	1:20	72	1:20	72	1:30	72	1:20	72	1:40	72	1:20
#cycles		**35**		**35**		**35**	**20**		**34**		**34**		**34**		**34**		**38**		**34**	
denature							94	0:30												
anneal							57	0:20												
extension							72	1:40												
#cycles							**15**													

NOTE

Use 2.3a if does not work or Alternative to 2.3 (more specific to astigmatid mites). Use if 2.3 does not work or produces seperimposed sequences
Because these primers are universally conserved, this protocol is used for amplification from genomic DNA as positive control to detect if DNA is present.
Use 34 cycles and dna=0.3 for nested amplification

Primer Name	Sequence (5' to 3')	M13 tails
28S_f1_4F	GACCTCAGATCARGCgAGAH	-
28S_P1_3R	GCTGTTCACATGRAACCCTTC	-
28S_NS2F_m	TCTTGAAACACGGACCAAGG	-
28S_NS2R	AACCTGTCTCACGACGGTCT	-
28S_1656F	TACCVATATCCGCAKCAGGT	-
28S_3531R_Arachn	AAGCYTCARTAGATCGCAGT	-
28S_1F_Arach3_T	TGTAAAACGACGGCCAGTACCCGCTGAATTTAAGCAT	FORW
28S_1661R_T	CAGGAAACAGCTATGACCAGTTCACCATCTTTCGGGTA	REV
28Sa_DD_T	TGTAAAACGACGGCCAGTGACCCGTCTTGAAACACGGA	FORW
28SIR_acar2_T	CAGGAAACAGCTATGACCTGCTACTACCACCAAGATCTGC	REV
28S_NS2F_i1Fm_T	TGTAAAACGACGGCCAGTGGCCATTTTTGGTAAGCAGA	FORW
28S_NS2_i1Rm_T	CAGGAAACAGCTATGACCRTTTTGCCGACTTCCCTTA	REV
28S_NS2_i2Fm_T	TGTAAAACGACGGCCAGTATATCCGCAKCAGGTCTCC	FORW
28S_NS2_i2R_T	CAGGAAACAGCTATGACCGTCTTCTTTCCCCGCTGATT	REV
28SV(D9-10)	GTAGCCAAATGCCTCGTCA	-
28SX(D9-10)	CACAATGATAGGAAGAGCC	-
28S_2732F_T	TGTAAAACGACGGCCAGTTGACTGGGGCGGTACATC	FORW
28S_3528R_Arachn_T	CAGGAAACAGCTATGACCGCYTCARTAGATCGCAGTATGG	REV
28S_3677F	TCAAACTTTRAATGGGTGAGATG	-
28S_NS2_i1R_v3	CCATCCTTCAGAGCCAATCC	-