

Supplementary information for

**Characterization of *Tt*ALV2, an essential charged repeat motif protein of the
Tetrahymena thermophila membrane skeleton**

by

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Spectrin *Homo sapiens* (EAX00140.1; 2,364 aa)

RKFANSLVG...VQQQL.QAFNTYRTVEK.PPKFTEKGNL
EQLARRFDRkaamRETWL.SENQRLVSQDNFGFDLPVEAA
QKIFQEMLY...IMDWM.DEMKVLVLSQDYGKHLGVEDL
WKFFWEMAE...EEGWI.REKEKILSSDDYGKDLTSVMRL
HQFQADADD...IDAWM.LDILKIVSSSDVGHDEYSTQSL
YKMFSEADA...CELWI.DEKEQWLNMQIPEKLEDLEVI
QNYHLECNE...TKSWIRKTKVIESTQDLGNLAGVMAL
QQFLRDLDLDD...FQSWL.SRTQTATAISEMPNTLTEAEKL
QQFLRDTKQ...AEAFL.NNQEYVLAHTEMPPTLEGAEAA
QKFLQDCQE...LSLWI.N.EKMLTAQDM..SYDEARNL
ELFTQSCAD...LDKWL.HGLESQIQSDDYGKDLTSVNIL
HQFNRDVED...EILWV.GERMPLATSTDHGHNLTQTVQLL
QQYYFDAAE...AEAWM.SEQELYMMSEEKAKDEQSAVSM
FQLNREVDD...LEQWI.AEREVVAGSHELQDYEHVTML
HKFYHDAKE...IFGRI.QDKHKKL.PEELGRDQNTVETL
FRFFSMVRD...LMLWM.EDVIRQIEAQEKPRDVSSVELL
HQFSRDASV...AEAWL.LGQEPYLSREIGOSVDEVEKL

Kinesin light chain *Homo sapiens* (BAB14039.1; 622 aa)

EIPARLRTLHNLVIQyasQGRYEVAVPLCKQALE.DLEKTS
DHPDVATMLNILALVyrDQNKYKEAAHLLNDALA.IREKTLGK
DHPAVAATLNNLAVLygkRGKYKEAEPCKRALE.IREKVLGK
FHPDVAKQLSNLALLcqnQGKAEEVEYYRRALE.IYATRLGP
DDPNVAKTKNNLASCylkQGKYQDAETLYKEILTrAHEKEFGS
DSPTVNTTLRSGLALyrrQGKL.EAAHTLEDC.A.SRNRKQGL

ICAMP1 *Toxoplasma gondii* (EEA99429.1; 1,251 aa)

ERKLQELS.....ESLNAAQKARDRSAQ.ElnAMR
.SKYNILR.....GELEKAEQRNHAEQ.E..AIR
EKEVSELR.....EAKNKIKASLDLAYQKE..QFR
GTDLKVAVQ.....QQLDTVTRQKEQLQT.D..AER
KR.TSELQvqvvedvkaEATQHTE.GLEKRYQ.E..QLR

Myosin heavy chain *Gallus gallus* (AAA48972.1; 1,940 AA)

.KELQAR...IE....ELEEEIEAER.TSRAK.A.EK
RADLSRE...LE....EISERLEEAG.GATAAQI.DM
KARLQTE...SG....EYSRQVEEKD.ALISQ.L.SR
KKKLAQR...LQ....DAEEHVEAVN.SKAS.L.EK
KQRLQNE...VE....DLMIDVERSN.AACAA.L.DK
NKNLQOE...IS....DLTEQIAEGG.KAIHE.L.EK
KKQIEQkseLQ....TA...LEEAE....AS.L.EH
KKNLQQT...VK....DLQHRLDEAE.Q..LA.L.KG
GKKQIQK...LEarvrELEGEVDAEQ.KRSAE.A.VK
RK.YERR...VK....ELTYQSEEDR.KNVLR.LqDL
VDKLVmk...VK....SYKRQAEAEELSNVN.L.SK

Tetrin D *Tetrahymena thermophila* (EAS03720.1; 741 aa)

E...QLRKLRL...NIVEDREKEI..AKLSSQISQA
SlndEINRLmadvDNWKIKYTYLD.NQ.RAQENQE
...EINLK...QLLLAKNQEIDSNRAQHAEIRD
E...DIQRLK...DTIEIKEKEVQ.NHLKHRELQD
T...EINDLK...HLVNLKQDEVH.DMINKYEKLE

Fig. S1.: Examples of charged repeat motifs (CRMs) from different cytoskeletal proteins of eukaryotes. Shown are only the sections of the protein sequences that contain the charged repeat motif as predicted by RADAR at www.ebi.ac.uk/Tools/pfa/radar/. In parentheses the according accession numbers and the total length of the proteins in amino acid (aa).

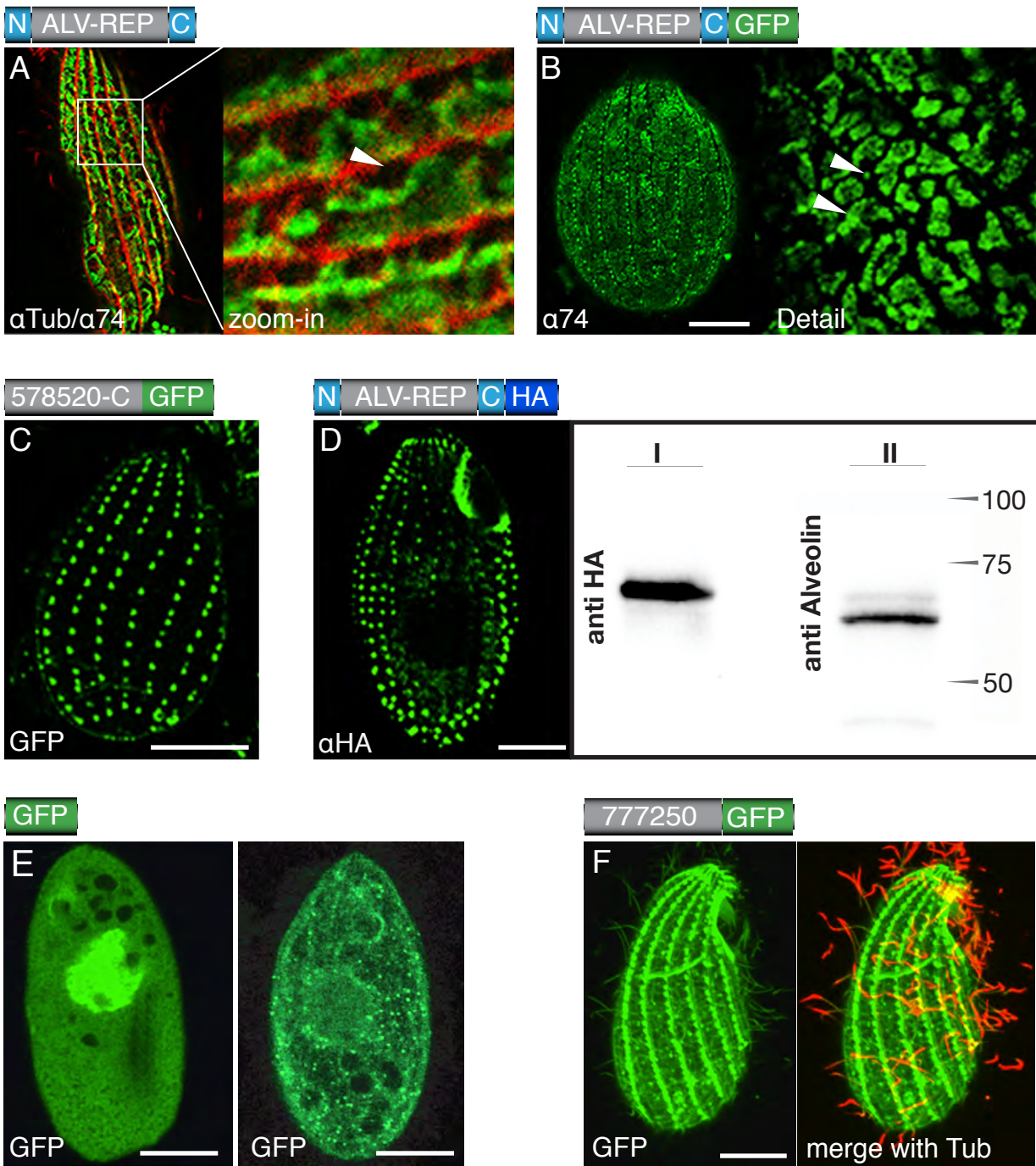


Fig. S2.: Additional immunolocalization. **A)** *TtALV2* is excluded from the circumciliary zone (highlighted by arrow head in the zoom-in; tubulin in red). **B)** Cells expressing *TtALV2::GFP* were fixed and labeled with α -alveolin, which marks both, the patches between the longitudinal microtubules and the basal body association (arrow heads). A close-up of the pattern from another cell is shown on the right. **C)** Localization of the C-terminal (CRM-containing) domain of THERM_00578520. **D)** Localization of *TtALV2* tagged C-terminally with an hemagglutinin-tag. Corresponding Western blots on the right demonstrates the correct, full-length expression of the construct. **E)** Localization of the green fluorescent protein (GFP) reporter expressed alone (single median optical slice on the left; stacked images including cortical optical slices on the right). **F)** Co-localization of THERM_77250 and alpha-tubulin. Scale: 10 μ m.

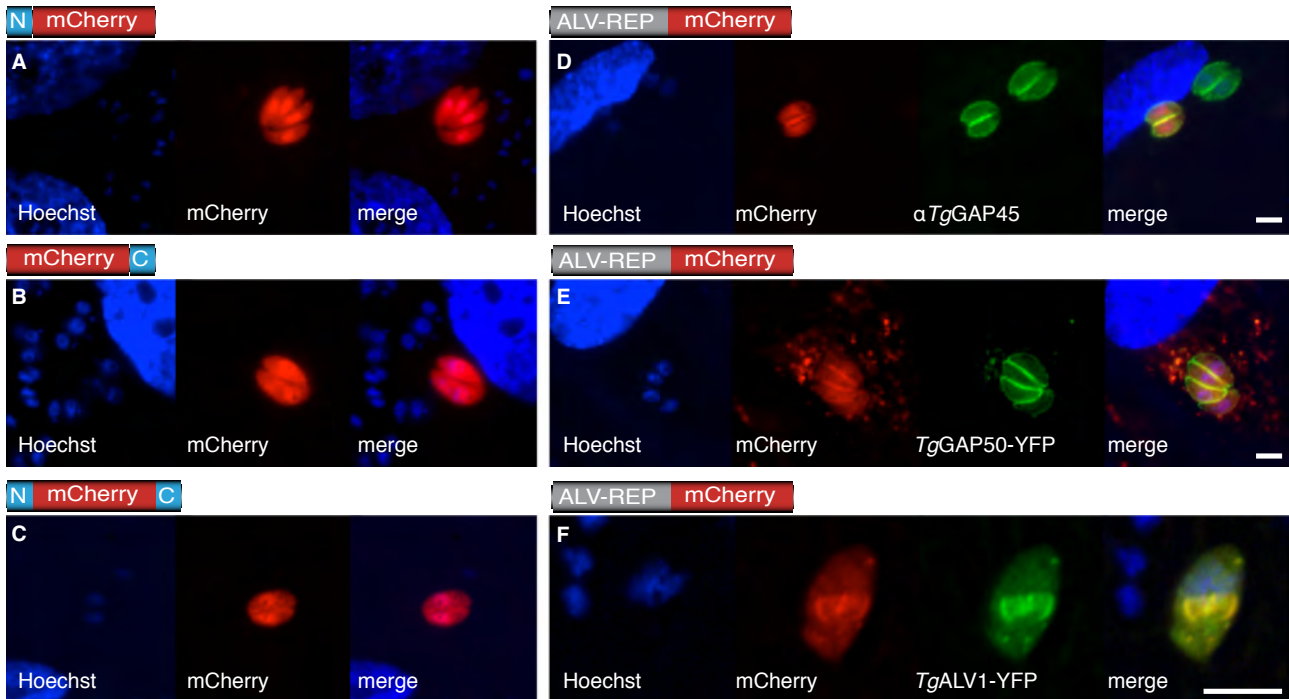


Fig. S3: The repeats, but not the termini of *Toxoplasma gondii* alveolin1 associate with the alveoli. Neither the termini of *TgALV1* alone (A and B), nor both in combination (C) are able to target the marker protein to the alveolar sacs. In contrast, the repeat motifs of *TgALV1* alone are sufficient for the association with alveolar membranes as shown in D to F, also in newly forming daughter cells as seen in F, and as indicated by the co-localization with established alveolar markers (anti-TgGAP45, TgGAP50-YFP and TgALV1-YFP). Scale 5 μ m.

For *Toxoplasma gondii* experiments we generated a new vector, pN-Cherry-C based on the existing TUB8mycGFPMyoATy-HX *Toxoplasma* transfection vector (21) to simplify cloning. mCherry was amplified from plasmid PFA660-mCherry (22) using primers mCherry_E_X_A_F 5' and mCherry_N_TAA_P_R. The resulting PCR product was restricted with *EcoRI/PacI* and inserted into similarly restricted TUB8mycGFPMyoATy-HX. This generated a vector containing the mCherry coding sequence, flanked by a 5' and 3' multiple cloning site.

A full-length cDNA copy of the *TgALV1* coding sequence (TGME49_231640) was amplified from *T. gondii* (RH strain) total RNA using the Invitrogen Superscript III kit and primers Alv_N_F 5' and Alv_C_R. The resulting PCR product was then used for all subsequent amplification steps. A region coding for the N-terminal region of *TgALV1* was amplified using Alv_N_EcoRI_F and Alv_N_AvrII_R, restricted with *EcoRI/AvrII* and cloned into similarly restricted pN-Cherry-C. A region coding for C-terminal region of *TgALV1* was amplified using Alv_C_NsiI_F and Alv_C_PacI_R, restricted with *NsiI/PacI* and inserted into similarly restricted pN-Cherry-C. Additionally, a region coding for the C-terminal region, amplified as detailed above, was cloned into the construct already containing the N-terminal region via *NsiI/PacI*. Finally, a sequence coding for the repeat region of *TgALV1* was amplified using Alv_Rep_EcoRI_F and Alv_Rep_AvrII_R, and

inserted into pN-Cherry-C via *EcoRI*/*AvrII*. All constructs were verified by restriction digest and automated DNA sequencing. TgGAP50-YFP was a gift of M. Meissner, TgALV1-YFP from B. Striepen. *T. gondii* tachyzoites (RH strain) were transiently transfected by electroporation (23).

All parasites were viewed post-fixation (1% paraformaldehyde/PBS) 24-48 hours post-transfection on a Zeiss Cell Observer microscope system using suitable filters. Immuno-staining was carried out using anti-TgGAP45 (a gift of D. Soldati-Favre, 1:5000 dilution in 3% BSA/PBS) for 1 hour at room temperature followed by suitable secondary antibodies (Dianova 1:2000 in 3%BSA/PBS, 30 minutes at room temperature). Fixed parasites were co-stained with Hoechst 33258 (50 ng ml⁻¹) to visualize nuclear DNA. Z-stack images were de-convoluted using Axiovision software, exported to ImageJ and overlaid. Data shown are representative of at least 20 independent observations. No gamma adjustments were applied to any images, and all data are presented in accordance with the recommendations of Rossner and Yamada (24).

Table S1: List of common cytoskeletal proteins. Screened were proteins for different characteristics (such as coiled-coils or the presence of ‘SMC’ and Viral A-like domains), and for which empirical evidence exists for their cytoskeletal association (relevant publications listed in the far right column/bottom).

Organism	Gene	GeneBank	CRMP	Coiled-Coil	SMC	Viral A	Reference
<i>H. sapiens</i>	Vimentin	EAW86215.1	No	Dimeric	Yes	No	(1)
<i>H. sapiens</i>	Lamin	EAW52996.1	Yes	Dimeric	Yes	No	(2)
<i>H. sapiens</i>	Keratin1	EAW96642.1	Yes	Dimeric	Yes	Yes	(3)
<i>T. vaginalis</i>	p477	EAY10879.1	Yes	Dimeric	Yes	Yes	(4)
<i>T. gondii</i>	TgICMAP1	EEA99429.1	Yes	Dimeric	Yes	Yes	(5)
<i>H. sapiens</i>	Spectrin	EAX00140.1	Yes	Dimeric	Yes	No	(6)
<i>H. sapiens</i>	Kinesin light chain 2	BAB14039.1	Yes	Dimeric	Yes	No	(7)
<i>H. sapiens</i>	Golgin 84	EAW81502.1	No	Dimeric	Yes	No	(8)
<i>T. thermophila</i>	TtALV2	EAR92507.1	Yes	Dimeric	No	No	this report
<i>T. thermophila</i>	Epiplasmin C	EAR95236.2	Yes	Dimeric	Yes	Yes	(9)
<i>T. pyriformis</i>	Tetrin C	EAR86044.1	Yes	Trimeric	Yes	Yes	(10)
<i>T. thermophila</i>	TtDFB1	EAR96064.1	Yes	Dimeric	Yes	Yes	(11)
<i>T. gondii</i>	TgSPM1	EEA98311.1	No	No	No	No	(12)
<i>H. sapiens</i>	Tropomyosin alpha chain 1	EAW77635.1	Yes	Dimeric	Yes	No	(13)
<i>H. sapiens</i>	Tektin-1	EAW90284.1	Yes	Dimeric	Yes	No	(14)
<i>R. norvegicus</i>	Spinophilin	AAC05183.1	Yes	Dimeric	Yes	No	(15)
<i>H. sapiens</i>	Gelsolin	EAW87491.1	No	No	No	No	(16)
<i>E. coli</i>	FtsZ	AAC73206.1	No	No	No	No	(17)
<i>H. sapiens</i>	Ankyrin 2	EAX06290.1	Yes	No	No	No	(18)
<i>H. sapiens</i>	Ezrin	EAW47646.1	Yes	Dimeric	No	No	(19)
<i>M. musculus</i>	KRIT-1	EDL14618.1	Yes	No	No	No	(20)
<i>H. sapiens</i>	Desmin	EAW70739.1	No	Dimeric	Yes	No	(21)
<i>G. gallus</i>	Myosin heavy chain 2	AAA48972.1	Yes	Dimeric	Yes	No	(22)
<i>C. reinhardtii</i>	BLD10	EDP06416.1	No	Dimeric	Yes	No	(23)
<i>H. sapiens</i>	MARCKS	EAW48258.1	No	No	No	No	(24)
<i>H. sapiens</i>	Filamin	EAW72745.1	Yes	No	No	No	(25)
<i>H. sapiens</i>	TEM4	EAW74891.1	No	Trimeric	No	No	(26)
<i>H. sapiens</i>	Supervillin	EAW86019.1	Yes	No	No	No	(27)
<i>T. thermophila</i>	Tetrin D	EAS03720.1	Yes	Trimeric	Yes	Yes	(11)
<i>T. gondii</i>	TgIMC 3	EEB03770.1	Yes	No	No	No	(28)
<i>T. gondii</i>	TgIMC 4	EEB00904.1	Yes	No	No	No	(29)
<i>T. gondii</i>	TgIMC 12	EEB00076.1	Yes	No	No	No	(30)
<i>T. gondii</i>	TgIMC 13	EEB02264.1	Yes	No	No	No	(30)
<i>T. gondii</i>	TgIMC 14	EEA98086.1	Yes	No	No	No	(30)
<i>P. falciparum</i>	PfALV1 (PFE1285w)	CAD51621.1	Yes	No	No	No	(31)

<i>P. falciparum</i>	PfALV2 (PF10_0039)	AAN35237.1	No	No	No	No	(31)
<i>P. falciparum</i>	PfGAP45	AAN36304.1	No	No	No	Yes	(32)
<i>T. thermophila</i>	Basal body protein 39	EAS06694.1	Yes	Trimeric	Yes	No	(33)
<i>G. gallus</i>	Titin	AAF35436.1	Yes	No	No	No	(34)

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Table S2: List of primers used in this study.

<i>Tetrahymena thermophila</i>	
Alv2GFP_HindIII_F	5'-ATGAAGCTTTAATCAAGCAATTTTGG-3'
Alv2GFP_XhoI_R	5'-AACTCGAGAACATACTATTTAATGTATG-3'
Alv2_REP_HindIII_F	5'-CAGACACAGCAAAGCTTCTTGGATC-3'
Alv2_REP_XhoI_R	5'-GAACTCGAGTTTTTCAATAACTCTTTC-3'
N_GFP_C1_SphI_F	5'-TAATAAGAAGCATGCGTATATAATC-3'
N_GFP_C1_MluI_R	5'-GCTGTGTCTGTTTGACGCGTTTCTTGC-3'
N_GFP_C2_NsiI_F	5'-GCGTATGCATAGAGTTATTGAAAAACC-3'
N_GFP_C2_BamHI_R	5'-CAAATAAAGGATCCTCAAACATAC-3'
KO1f	5'-AAATTGAAGAAGGGCCCATCAAAG-3'
KO1r	5'-CTTTATATTTAAAATGTCGACTTTTAC-3'
KO2f	5'-CTCACCTGCAGCTAATTATTAGAG-3'
KO2r	5'-CATTTTCGTTCCGCGGAACATTTGTTTATC-3'
tKD1f	5'-CCTGAAAGATTACTGCAGTATAC-3'
tKD1r	5'-GGAAGCCGGTCTTGTGCGATCAAGATG-3'
tKD2f	5'-GATGAATGAGCTATGTTTTGAGCAGC-3'
tKD2r	5'-GAATGGTGAACAGATAGATAAATG-3'
Alv2_qPCR_FOR	5'-TTCCAAATGCGCAGACTATT-3'
Alv2_qPCR_REV	5'-TGAAGTGCTCACTCCTCCTTT-3'
777250_SphI_F	5'-AGTCAAGCATGCATGCAACACCAAGATC-3'
777250_MluI_R	5'-ATTTTATATATTTACGCGTATAATTGCTT-3'
578520-4_SphI_F	5'-ATTAGCATGCATGTAATTAGAAGGTG-3'
578520-4_MluI_R	5'-GATACGCGTTTCTTCAGTTTCAAG-3'
<i>Toxoplasma gondii</i>	
mCherry_E_X_A_F	5'-CCGAATTCCTCGAGCCTAGGATGGTGAGCAAGGGCGAGGAGG-3'
mCherry_N_TAA_P_R	5'-GCTTAATTAAGGCTTAATGCATCTTGTACAGCTCGTCCATG-3'
Alv_N_F	5'-ATGTTTAAGGACTGCGCC G-3'
Alv_C_R	5'-TTAATTAGCACTGGC ATC GGC AC-3'
Alv_N_EcoRI_F	5'-CCGAATTCATG TTT AAG GAC TGC GCC G-3'
TgAlv_N_AvrII_R	5'-CCC CTA GGG GCA ACC CAT TGT CTC TCA G-3'
Alv_C_NsiI_F	5'-GGATGCATCCTTGCGACGTCGAGATC-3'
Alv_C_Pacl_R	5'-GCTTAATTAATTAGCACTGGCATCGGCAC-3'
Alv_Rep_EcoRI_F	5'-CAGAGATCTATGACTGCATACCAGCCTATTGAC-3'
Alv_Rep_AvrII_R	5'-GTCCCTAGGAACTGGCTTGATCACGTCTTGCACC-3'