E COLI SEQUENCE

gtgccag cagccgcggt aatacggagg

gtgcaagcgt taatcggaat tactgggcgt aaagcgcacg caggcggttt gttaagtcag

atgtgaaatc cccgggctca acctgggaac tgcatctgat actggcaagc ttgagtctcg

tagagggggg tagaattcca ggtgtagcgg tgaaatgcgt agagatctgg aggaataccg

gtggcgaagg cggccccctg gacgaagact gacgctcagg tgcgaaagcg tggggagcaa

acaggattag ataccctggt agtcc

292 bp

BD SEQUENCE (from boyle)

CCTTGATATA ATACAGTGTG CCATATGTCA CGAGTCGAAC AAAATTTATT TATTTTTTCG ACAAATTAAT TGGAAATTGA ATAATTTAAT TGAAAAAAAT TGAAAATAAA TATTAAAAAC AACTTTTGAC AACGGATCTC TTGGCT

BD SEQUENCE (from Schloegel)

ORIGIN

1 tttgattttg ggtgggggag ttttattgat gtgtaaatgt tgatggaatg accagttgtt

61 ttttcaaaaa acac**ccttga** **tataatacag tgtgccatat gtcacgagtc** gaacaaaatt

121 tatttatttt ttcgacaaat taattggaaa tgattttaat ttaattgaaa aaaattgaaa

181 ataaatatta aaacaactt**t tgacaacgga tctcttggct** ctcgcaacga tgaagaacgc

241 agcgaaatgc gatacgtaat gtgaattgca aacctttgtg aatcattaaa tctttgaacg

301 cacattgcac tcgtaaaaga gtatacatgt ttgagaatta taaaaataca ttgtccgaat

361 tgactggaca gatatgaacc atgtcaaaaa tatttgacag gttttaaaag tagtagtaaa

421 tgagtgatac aaaaagtagt gggtctaaac aaccccgtcc atcacaccat aca

MERGED—added sequences at end to “pad” (30nt is optimal. First pad is from E. coli; second pad is from Bd. Intermediate spacer is a “random” spacer supplied by IDT. I deleted a few nt from each spacer so that it comes out under the 500bp

LONG VERSION-511bp

cagaagaagc accggctaac tccgtgccag cagccgcggt aatacggagg

gtgcaagcgt taatcggaat tactgggcgt aaagcgcacg caggcggttt gttaagtcag

atgtgaaatc cccgggctca acctgggaac tgcatctgat actggcaagc ttgagtctcg

tagagggggg tagaattcca ggtgtagcgg tgaaatgcgt agagatctgg aggaataccg

gtggcgaagg cggccccctg gacgaagact gacgctcagg tgcgaaagcg tggggagcaa

acaggattag ataccctggt agtcc Accgagttgcccgttaaagt CCTTGATATA ATACAGTGTG CCATATGTCA CGAGTCGAAC AAAATTTATT TATTTTTTCG ACAAATTAAT TGGAAATTGA ATAATTTAAT TGAAAAAAAT TGAAAATAAA TATTAAAAAC AACTTTTGAC AACGGATCTC TTGGCT

ctcgcaacga tgaagaacgc agcgaaatgc

SHORTER VERSION-500bp

cagaagaagc accggctaac tccgtgccag cagccgcggt aatacggagg

gtgcaagcgt taatcggaat tactgggcgt aaagcgcacg caggcggttt gttaagtcag

atgtgaaatc cccgggctca acctgggaac tgcatctgat actggcaagc ttgagtctcg

tagagggggg tagaattcca ggtgtagcgg tgaaatgcgt agagatctgg aggaataccg

gtggcgaagg cggccccctg gacgaagact gacgctcagg tgcgaaagcg tggggagcaa

acaggattag ataccctggt agtcc Accgagttgcccgt CCTTGATATA ATACAGTGTG CCATATGTCA CGAGTCGAAC AAAATTTATT TATTTTTTCG ACAAATTAAT TGGAAATTGA ATAATTTAAT TGAAAAAAAT TGAAAATAAA TATTAAAAAC AACTTTTGAC AACGGATCTC TTGGCT

ctcgcaacga tgaagaacgc agcga

NICER VERSION OF SHORT ONE (passes initial screening):

CAGAAGAAGCACCGGCTAACTCC**GTGCCAGCAGCCGCGGTAA**TACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGG**ATTAGATACCCTGGTAGTCC**ACCGAGTTGCCCGT**CCTTGATATAATACAGTGTGCCATATGTC**ACGAGTCGAACAAAATTTATTTATTTTTTCGACAAATTAATTGGAAATTGAATAATTTAATTGAAAAAAATTGAAAATAAATATTAAAAACAACT**TTTGACAACGGATCTCTTGGCT**CTCGCAACGATGAAGAACGCAGCGA

NICE VERSION OF LONG ONE (passes initial screening):

CAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACCGAGTTGCCCGTTAAAGTCCTTGATATAATACAGTGTGCCATATGTCACGAGTCGAACAAAATTTATTTATTTTTTCGACAAATTAATTGGAAATTGAATAATTTAATTGAAAAAAATTGAAAATAAATATTAAAAACAACTTTTGACAACGGATCTCTTGGCTCTCGCAACGATGAAGAACGCAGCGAAATGC

ALT: (only e coli

cagaagaagc accggctaac tccgtgccag cagccgcggt aatacggagg

gtgcaagcgt taatcggaat tactgggcgt aaagcgcacg caggcggttt gttaagtcag

atgtgaaatc cccgggctca acctgggaac tgcatctgat actggcaagc ttgagtctcg

tagagggggg tagaattcca ggtgtagcgg tgaaatgcgt agagatctgg aggaataccg

gtggcgaagg cggccccctg gacgaagact gacgctcagg tgcgaaagcg tggggagcaa

acaggattag ataccctggt agtcc acgcc gtaaacgatg tcgacttgga ggtt

NICE VERSION:

CAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTT

|  |
| --- |
| 16S\_515F\_qPCR 5’ –GTG CCA GCM GCC GCG GTA A -3’ |
| 16S\_806R\_qPCR 5’ –GGA CTA CHV GGG TWT CTA AT-3’ |
| ITS1-3\_Chytr 5’- CCTTGATATAATACAGTGTGCCATATGTC -3’ |
| 5.8S\_Chytr 5’- AGCCAAGAGATCCGTTGTCAAA- 3’ |

Bd end pad is from JQ582941 on ncbi; Batrachochytrium dendrobatidis.

AUTHORS Schloegel,L.M., Toledo,L.F., Longcore,J.E., Greenspan,S.E.,

Vieira,C.A., Lee,M., Zhao,S., Wangen,C., Ferreira,C.M.,

Hipolito,M., Davies,A.J., Cuomo,C.A., Daszak,P. and James,T.Y.

TITLE Novel, panzootic and hybrid genotypes of amphibian chytridiomycosis

associated with the bullfrog trade

JOURNAL Mol. Ecol. 21 (21), 5162-5177 (2012)

Main Bd sequence is from “Rapid quantitative detection of chytridiomycosis (Batrachochutrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay” by Boyle et al, Diseases of Aquatic Organisms

@article{boyle2004rapid,

title={Rapid quantitative detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay},

author={Boyle, D G{\=e} and Boyle, DB and Olsen, V and Morgan, JAT and Hyatt, AD},

journal={Diseases of aquatic organisms},

volume={60},

number={2},

pages={141--148},

year={2004}

}

E coli is from GenBank J01859.1 RNA complete sequence

AUTHORS Ehresmann,C., Stiegler,P., Fellner,P. and Ebel,J.P.

TITLE The determination of the primary structure of the 16S ribosomal RNA

of Escherichia coli. 2. Nucleotide sequences of products from

partial enzymatic hydrolysis

JOURNAL Biochimie 54 (7), 901-967 (1972)