Argonne National Laboratory
qPCR protocol May 2014
Author: Jason Koval

Bacterial 16S qPCR run conditions:

PCR reaction ingredient list:

SYBR Green Master Mix	10 uL
Caporaso 515F FW primer (10 uM)	1 uL
Caporaso 806R RV primer (10 uM)	1 uL
PCR pure water	7 uL
Template DNA loaded into each well	1 uL

Total reaction volume – **20uL**

Cycling conditions:

94°C	3 min	
94°C	45 sec	
50°C	60 sec	(run this three step cycle 40x)
72°C	90 sec	

Primer sequences:

Caporaso FW primer (5'-GTG YCA GCM GCC GCG GTA A-3')
Caporaso RV primer (5'- GGA CTA CNV GGG TWT CTA AT-3')

Citation: Caporaso, J.G., Lauber, C.L, Walters W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS. March 2011 108: 4516-4522.

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Fungal ITS qPCR run conditions:

PCR reaction ingredient list:

SYBR Green Master Mix	10 uL
Fierer FW primer (10 uM)	1 uL
Fierer RV primer (10 uM)	1 uL
PCR pure water	7 uL
Template DNA loaded into each well	1 uL

Total reaction volume – **20uL**

Cycling conditions:

95°C	15 min	
95°C	60 sec	
53°C	30 sec	(run this three step cycle 40x)
72°C	60 sec	

Primer sequences:

Fierer FW_5.8s (5'-CGC TGC GTT CTT CAT CG -3')
Fierer RV_ITS1f (5'-TCC GTA GGT GAA CCT GCG G -3')

Citation: Fierer, N., Jackson, J.A., Vilglays, R., Jackson, R.B. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology. 2005 (71) 4117-4120.

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Archaeal 16S qPCR run conditions:

PCR reaction ingredient list:

Roche SYBR Green master mix	10 uL
Gantner Arch FW Primer (10 uM)	1 uL
Gantner Arch RV Primer (10 uM)	1 uL
PCR pure water	7 uL
Template DNA loaded into each well	1 uL

Total reaction volume – **20uL**

Cycling conditions:

98°C	2 min	
95°C	30 sec	
57°C	30 sec	(run this three step cycle 50x)
72°C	90 sec	

Primer sequences:

Gantner 340F Archaeal (5'-CCCTAYGGGGYGCASCAG -3')
Gantner 1000R Archaeal (5'-GGCCATGCACYWCYTCTC-3')

Citation: Gantner S, Andersson A, Alonso-Saez L, and S Bertilsson. Novel primers for 16S rRNA-based archaeal community analyses in environmental samples. Journal of Microbiological Methods 84 (2011) 12-18.

** All samples are run in triplicate. The three values are then averaged (+/- std. dev) to give a value representative of the sample. Positive controls are run in duplicate to give a more precise standard curve. This is true for all qPCR analyses.