Transfer-to-Excellence (TTE) Program

Synthetic spike-in standards for 16S rRNA gene iTags

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Microbial communities are very complex both in their abundance and composition. As most microbes are yet-to-be cultured, most of the knowledge of the microbial diversity was produced through PCR amplification, cloning and Sanger sequencing of conserved molecules, usually the 16S ribosomal RNA gene. Those traditional methods can be laborious and costly and may not be sufficient to fully characterize all these existing complex communities. Next-generation sequencing platforms such as the Illumina 16S amplicon analysis (iTags) overcome previous limitations such as the number of samples and sequences generated. One limitation for iTags is the fact that results are given as relative abundances. The production of synthetic spike-in standards for the 16S rRNA gene iTags will allow us to determine the actual copy number and sequence of the 16 S rRNA in our samples which will allow us to better analyze changes in 16S rRNA gene expression and better understand the biodiversity in environmental samples. This work describes the design and validation of these internal controls.

Introduction/Background

Biological Soil Crusts (BSC)

Comprises cyanobacteria, lichens, mosses, bacteria, and fungi.
Boundary between the soil and the atmosphere.
Functions
Stability and fertility, nutrient-rich dust trapping, fixation of Nitrogen and Carbon. [1]

16S ribosomal RNA gene

Understanding the complexity and diversity of microbial communities

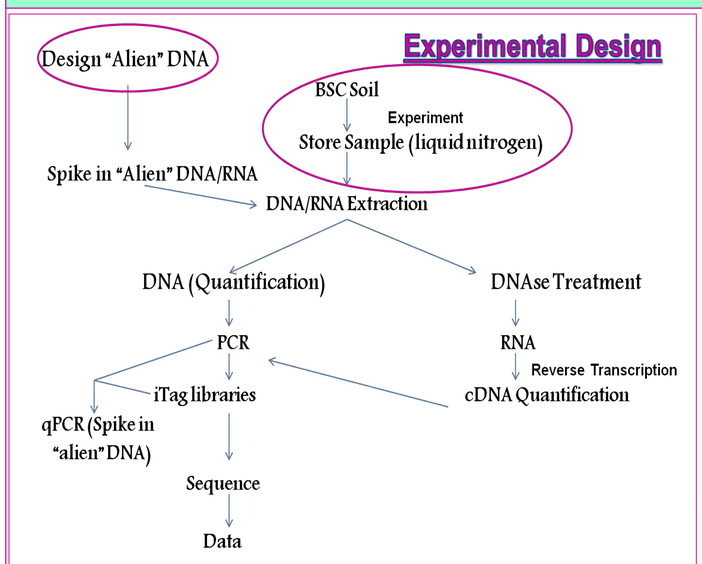
Approaches

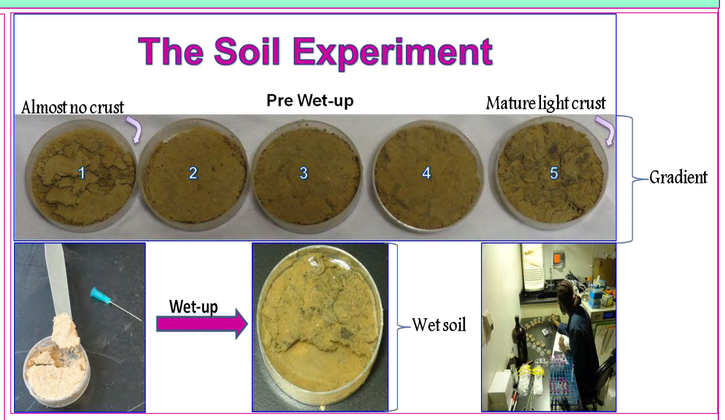
PCR, cloning & Sanger sequencing of the 16S,
x
16S rRNA amplicon next-generation sequencing (iTags) [2]

Goal

Construct iTags internal controls that will be used to determine not only phylogeny but actual copy numbers for bacterial communities.

Methods





Time points: Day: 9h full light, 2h low light, then the Night Time pt and partial day of full light. Sampled 2h into the partial day. **Night:** 9h darkness, 2h low light. Samples 6.5 into the 9h

Designing the "alien" internal controls

- Generation of 250 bp random sequences
- Designed the16S rDNA gene primers compatible with iTags (515F & 806R)
- Chose the right promoter sites for RNA production (SP6 & T7)
- Designed Re-amplification primers (CPF & CPR)

Validating the Controls

- Dilutions
  - Internal controls
- Polymerase Chain Reaction (PCR)
  - Amplifying the internal controls.
- Agarose Electrophoresis Gel

Results/Data Analysis

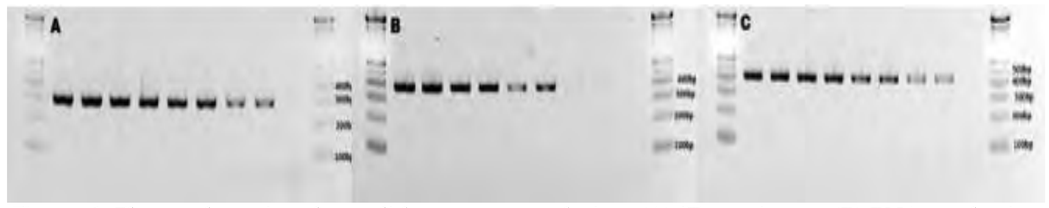


Figure 1: Electrophoresis gel to validate construct design: (A) iTag primers; (B) RNA synthesis site; (C) Re-amplification site.

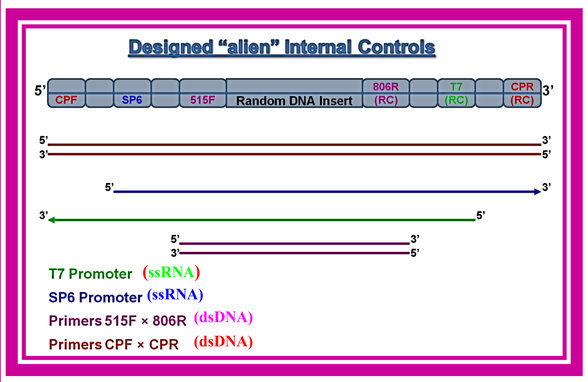


Table 1 – Water content average of the different Biological soil crust gradients in the 3 sample times.

BSC Gradient [a]	Pre wet-up[b]	Night time	Day time
1	6.1(4.9)	42.1(4.4)	32.8(3.5)
2	4.3(4.4)	37.4(3.1)	30.4(5.8)
3	1.4(0.4)	32.6(4.6)	23.8(1.4)
4	1.7(1.0)	28.3(2.3)	25.5(1.9)
5	1.3(0.1)	32.5(3.7)	28.7(2.5)

[a] Numbers from 1 to 5 represent the different gradient (see Fig. 1).
[b] Number in between parenthesis represent the standard deviation of the average of 3 biological replicates.

- Out of forty 250 bp random sequences, 13 had a 50-55 % GC content.
- 6 of these 13 constitute the internal controls because they had variable pair ends.
- The different primers, iTags, RNA production sites and re-amplification, showed the expected size.
- The internal Controls showed no dimer and hairpin at temperatures > 40° C.

Discussion/Conclusion

- Not having a representation of the diversity of microbial communities but knowing in great their actual composition.
- Understanding relations between microbes themselves and between biodiversity and ecosystem functions
  - Driving forces behind the environment
- Developed and validated internal controls
- Performed a soil experiment to validate the method in an 'actual' experiment

Perspectives

- DNA/RNA Extraction
- DNA/RNA Purification
  - DNA quantification
  - DNase treatment
- Spike-in internal controls
- PCR
- iTag Libraries
- Sequencing

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References

[1] J. Belnap, " The world at your feet: desert biological soil crusts," *Frontiers in Ecology and the Environment*, Vol. 1, no. 5, 181-189, May 1, 2003.
[2] P. Degnan, H. Ochman, "Illumina-based analysis of microbial community diversity," *The ISME Journal*, Vol. 6, 183-194, June 16, 2011.

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