# Genomical Environments Characterization by means of Novel Fingerprints Features

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## Abstract

Bla bla bla

## Introduction

The metatranscriptomics and metagenomics are new molecular approaches developed with the aim to explore the genetic potential of the ecosystems, bringing an unprecedented understanding of the relationships between microbial communities without the need of previews knowledge of them or their environment. This approaches allowed the first large-scale insight into the ecology of the environments since both taxonomical and functional diversity outlook.

Since an ecology perspective a key step to characterize one ecosystem is studding its biodiversity, that implies to discover, to describe and to analyse the organization of all elements involved in, classifying both by evolutionary (phylogenetic) and ecological (functional) criteria [1]. In this sense, a metagenomic and metatranscriptomic analysis is aiming to decipher the microbial community structure by characterizing some microbes residing therein and quantifying their diversity in terms of some level of biological population like specie, genera, families, order or even patterns of evolutionary diversification.

In this context, a frequently employed diversity metric is the richness, describing the number of distinct microbial taxa within a given unit area inhabiting a particular ecosystem, measured by the relative abundance, that refers to the quantity of rarity and commonness among taxonomical or functional individuals in the sample or community [1, 2].

Currently high-throughput sequencing (HTS) technologies have offered the opportunity to obtain genomic and/or transcriptomic information at increasingly high throughput and low price. Consequently, studies aiming to investigate taxonomical and functional diversity use an approach referred to as shotgun sequencing, in which genomic or transcriptomic fragments originated from organisms constituing microbiome are extracted and massively sequenced [3]. Habitually, the HTS technologies, the most commonly used Illumina and Roche454 platforms, generates millions of sequences, referred to as ”reads”, that could be considered to represent the compositional propierties of their source genomes and/or transcripts.

Subsequent analysis of the reads involve the process referred to as ”binning”, where the reads derived from a mixture of different organisms are assigned to phylogenetic groups according to their traxonomic origins, analogous to machine learning process, where the reads are clustered into specific bins using reference sequences with known taxonomic origin like a supervised learning method.

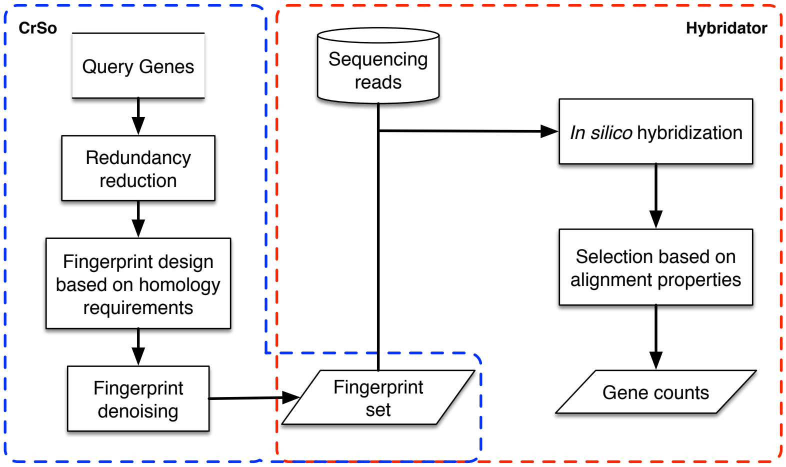
In the binning process typically we attempt to classify the reads through two strategies: composition-based or similarity-based. The composition base strategy involves compare the information related with GC content [4], codon usage [5] or k-mer frequency [6] from reads with those calculates from reference sequences. The similarity-based strategy relies on homology information obtained from string comparison through sequence alignment methods between reads and reference sequences. This strategy can be sub-diveded in two general methods: those to use Hidden Markov Models (HMM) [7] or BLAST-based homology searches [8, 9].

Despite of all efforts, the bioinformatic tools able to read binning are unable to make good accurate assignments for short fragments ($< 400$pb) [10, 11]. Therefore we present an enhancing of the HISS pipeline [12], an integrated approach that combines bioinformatic algorithms to fingerprint design and *in silico* hybridization using BLAST algorithm in order to characterize the ecosystems assessing key genes involved in a functional context.

## Materials and Methods

Succeeding, we briefly describe the HISS pipeline and then present the algorithmic improvements implemented to fingerprint design and *in silico* hibridization. The HISS pipeline consist in 2 main stages (Figure 1). The first stage is the fingerprint designer, this take interest genes sequences and design the best non-redundant fingerprints from each of them. Later, the hybridator perform an *in silico* hybridization between designed probes and the reads from metagenome or metatranscriptome, subsequently the hybridization is evaluated with the aim to count the significant ones.

**Figure 1. Overview of HISS pipeline stages.** CrSo: Fingerprint designer. Hybridator: *In silico* hybridization step.


CrSo
Query Genes
Redundancy reduction
Fingerprint design based on homology requirements
Fingerprint denoising
Hybridator
Sequencing reads
*In silico* hybridization
Selection based on alignment properties
Fingerprint set
Gene counts

### Fingerprints design (CrSo).

CrSo split up the fingerprint design in three general steps (Figure 1): 1) redundancy reduction 2) homology score calculation and best fingerprint compilation and 3) fingerprint denoising. At the first stage, CrSo performs a clusterization process using

UCLUST [13] program, at the end of this step several sequences that covering the same biological sequence, and sequence fragments of one biological sequence that are globally alignable will be eliminated from initial input data set.

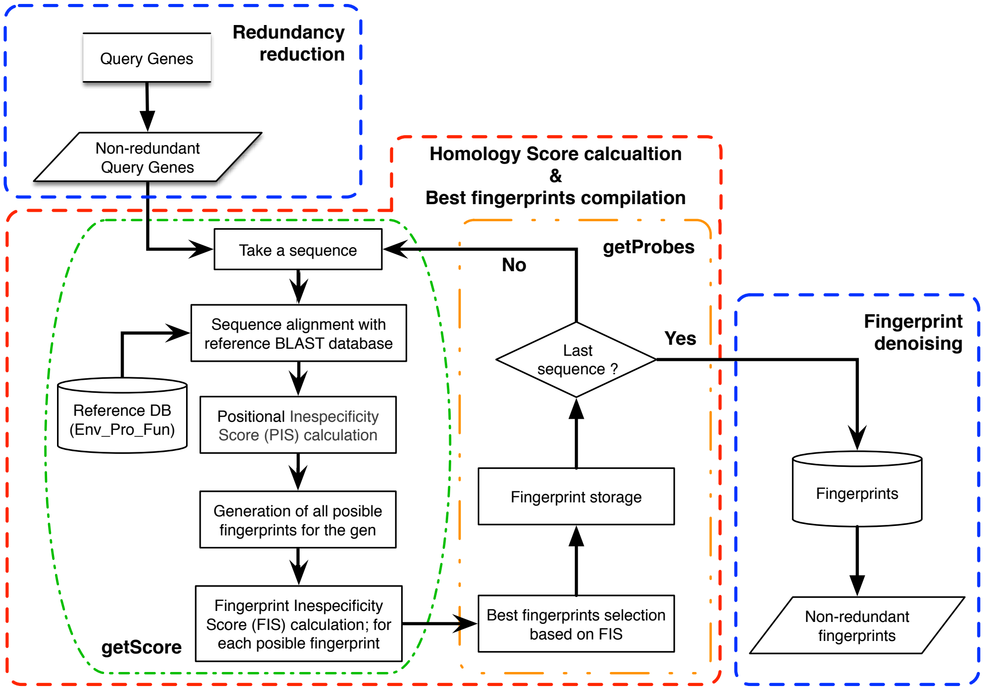
For fingerprint metrics calculation, the previous version of CrSo used the program OligoWiz 2.0 [14], however this software implement some algorithms to satisfy microarray experimental condition, such as melting temperature ($T_m$) and GC content, not relevant by *in silico* hybridization, therefore here we will focus on specificity constraint of the fingerprints. A fingerprint of given gene $g_t$ by definition will be any sub-sequence of gene $g_t$ that is not sub-sequence of any $g_i \in G, i \neq t$, where $G$ is a reference database of genes $G = \{g_1, g_2, g_3, ..., g_N\}$ consisting of $N$ sequences.

In order to prevent hybridization to unintended targets (cross-hybridization), CrSo estimates this cross-hybridization evaluating the similarity of the target gene with other transcripts using BLAST. CrSo calculates a homology score for each possible oligonucleotide (FIS), based on BLAST search of the taget gene against a reference database constituted by prokaryote, fungi and environmental sequences reported in EMBL-GeneBank-DDBJ [15] database. Each BLAST hit resulting is evaluated along the target sequence, where $M$ is the number of BLAST hits regarded by $j$ position of the target gene and $H = \{h_1 j, ..., h_M j\}$ be the BLAST hits identity in position $j$.

(Figure 2).

the homology score for the probes the fingerprint was consider a sub-string of the genes, where they were a strigs with

**Figure 2. Overview of CrSo pipeline.**


Redundancy reduction
Query Genes
Non-redundant Query Genes
Homology Score calcualtion & Best fingerprints compilation
getProbes
Take a sequence
No
Yes
Last sequence ?
Sequence alignment with reference BLAST database
Reference DB (Env_Pro_Fun)
Positional Inespecificity Score (PIS) calculation
Generation of all posible fingerprints for the gen
Fingerprint Inespecificity Score (FIS) calculation; for each posible fingerprint
getScore
Fingerprint storage
Best fingerprints selection based on FIS
Fingerprint denoising
Fingerprints
Non-redundant fingerprints

In the second stage, HISS uses the fingerprints designed to make an *in silico* hibridization with the reads from metagenomic or metatrasncriptomic sample following a general rule: each read aligned significantly with a fingerprint should be considered originated or homologous of the enzyme gene that fingerprint represents.

$$D_{coll} = \frac{D_f + \frac{[S]^2}{K_D S_T} D_S}{1 + \frac{[S]^2}{K_D S_T}}, D_{sm} = \frac{D_f + \frac{[S]}{K_D} D_S}{1 + \frac{[S]}{K_D}}, \qquad (1)$$

**Figure 3. Figure Title first bold** Figure A: Lorem. B: Consectetur.

### *In Silico* Hibridization

1. react
2. diffuse free particles
3. increment time by dt and go to 1

## Results

Nulla Table 1 volutpat.

**Table 1. Table caption title.**

| **Heading1** | | | | **Heading2** | | | |
|---|---|---|---|---|---|---|---|
| $cell1row1$ | cell2 row 1 | cell3 row 1 | cell4 row 1 | cell5 row 1 | cell6 row 1 | cell7 row 1 | cell8 row 1 |
| $cell1row2$ | cell2 row 2 | cell3 row 2 | cell4 row 2 | cell5 row 2 | cell6 row 2 | cell7 row 2 | cell8 row 2 |
| $cell1row3$ | cell2 row 3 | cell3 row 3 | cell4 row 3 | cell5 row 3 | cell6 row 3 | cell7 row 3 | cell8 row 3 |

Table notes.

### Probe Design.

Maecenas.

### Annotation.

Nulla

**Taxonomical annotation** Nulla.

**Functional annotation** Nulla.

## Discussion

Nulla Table 1 volutpat.

### LOREM and IPSUM Nunc.

$CO_2$ Maecenas. For more information, see S1 Text.

## Supporting Information

### S1 Video

**Bold the first sentence.** Maecenas.

## S1 Text

**Lorem Ipsum.** Maecenas.

## S1 Fig

**Lorem Ipsum.** Maecenas.

## S1 Table

**Lorem Ipsum.** Maecenas.

# Acknowledgments

Cras.

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