Inserts in Prokaryote Genomes

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Abstract*— The Nucleotide Genomic Signal (NuGS) methodology is based on the conversion of symbolic nucleotide sequences into genomic signals, allowing the use of signal processing methods to investigate genetic information. The approach is adequate for both the large scale analysis of genomes and for the monitoring of local variations in genomic sequences, such as those caused by pathogen variability or by genomic inserts. The inserts can comprise retro-viruses, individual genes, or non-coding segments. Even when the inserts are not fully active viruses, they can still retain a significant pathogenicity when encoding viral enzymes that can facilitate the dissemination of some viruses, generating an increased susceptibility to the contamination with these pathogens. Inserts occur usually in complementary pairs, with the tendency to restore the original regularities of the host genome. The paper illustrates the NuGS methodology for two typical cases: the insert of the *SPBc2* bacillusphage complete genome in the genome of *Bacillus subtilis* and the inserts of genes from the *PE-PGRS* family in the genome of *Mycobacterium tuberculosis.

I. Introduction

THE Nucleotide Genomic Signal (NuGS) methodology is based on the conversion of symbolic nucleotide sequences into digital signals, which allows the efficient signal processing of genetic data [1,2,3]. There have been several other attempts to represent nucleotides in a sequence by numbers [4,5], using some properties of the nucleotides considered essential for the specific application at hand. Such representations are biased and have a limited scope. The approach we are using is general and proved to be adequate for both the analysis of large scale nucleotide genomic sequences [6] and for the comparative study of local features. This last aspect becomes important in real life applications, like the study of pathogen variability [7,8], especially related to the development of their resistance to treatment [9,10,11,12], or for the analysis of genomic inserts [13].

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The striking regularities of the genomic signals reveal restrictions in the distribution of nucleotides and pairs of nucleotides along a nucleotide sequence. Structurally, a chromosome appears to be more than a simple text, as it also satisfies well defined symmetry restrictions [1]. These regularities make also genetic signals convenient for the detection of inserts in the genomes, because the inserts obey usually different regularities than the host sequence [1,13]. The inserted sequence can sometimes be the whole genome of some pathogen, or just a juxtaposition of several coding and non-coding segments. It is remarkable that the combination of an inserted sequence with the endogenous material, or with additional inserts, usually tends to restore the global nucleotide signal regularities.

The paper presents instances of inserts found in the genomes of *Bacillus subtilis* [14,15,16] and of *Mycobacterium tuberculosis* [12,16]. Many other such cases have been identified in the genomes of other prokaryotes downloaded from GenBank [16]. It is interesting to note that even when the inserts are not fully active viruses, they can nevertheless retain a specific pathogenicity, especially when they are genes of some key viral enzymes. This is the case of protease, which is essential in the multiplication of HIV. An inserted protease gene might cause an increased susceptibility to the contamination with the virus [8].

II. Conversion of Nucleotide Sequences to Nucleotide Signals

Two signatures of the nucleotide distribution and of the nucleotide pair distribution along a sequence have been used to reveal genome regularity and the way it is perturbed by the insertion of exogenous genetic material [1,2,3]:

- the *nucleotide imbalance*

$$N = 3*(g - c) + (a - t), \tag{1}$$

where *a*, *c*, *g* and *t* are the numbers of A - adenine, *C* – cyanine, *G* – guanine, and *T* – thymine nitrogenous bases occurring in the sequence from its start to the current locus, and

- the *nucleotide pair imbalance:*

$$P = n_+ - n_-, \tag{2}$$

where n_+ is the number of positive pairs (A→G, G→C, C→T, T→A) and n_- is the number of negative pairs (A→T, T→C, C→G, G→A) occurring along the

sequence from the start to the current locus.

The nucleotide imbalance N is proportional to the cumulative phase θ_c of the complex genomic signal, whereas the nucleotide pair imbalance P is proportional to the unwrapped phase θ_u of the signal [1,2,3]. Due to their direct statistical meaning, the use of N and P is preferred to the use of θ_c and θ_u, respectively.

N can be used to describe transversal interactions between nucleic acid and protein molecules. These interactions determine processes such as replication, transcription, translation and crossover. The regularity and specificity of the N signal could be the effect of an evolutionary pressure linked to species separation. Specific patterns of the genomic signals could contribute to control the exchange of genetic material between species, by allowing or forbidding interactions between the molecules involved in carrying the genetic information. N is highly regular for *Escherichia coli*, *Bacillus subtilis* and many other prokaryotes. However, the structure of the N signal is less restricted for *Mycobacterium tuberculosis*, which has a highly resistant protective mycolic acid coating. The regularity is almost absent for *Aeropyrum pernix*, which has an almost undisputed hot water habitat [1].

III. Insert of the *SPBc2* Bacillusphage Complete Genome in the *Bacillus subtilis* Genome

A good example of how to use genomic signals to detect an insert of exogenous genetic material in the genome of a host organism is provided by the case of the *Bacillusphage* SPBc2 genome (accession AF020713 [15,16]) inserted in the genome of *Bacillus subtilis* (*Bs*, accession AL009126 [14,16]).

Bs is an ubiquitous soil bacterium, one of the best understood prokaryotes, a model organism for the molecular and cellular bacterial biology.

The line marked 'wild' in Fig. 1 gives the nucleotide imbalance N for the genome of an *Bs* bacterium attacked by the SPBc2 bacillusphage. The total sequence, comprising the two genomes, has 4,214,814 base pairs (bp) and 4101 coding segments.

The nucleotide imbalance N of this genome has two approximately linear branches, delimited by the origin of replication (i.e. the origin superposed with the end of the *Bs* circular genome), and the terminus of replication (the maximum of N at 1,941,693 bp). The two branches have a quite low normalized root mean square error $NRMSE \approx 0.010$, measuring the signal nonlinearity. The ascending branch has the average slope $s_N \approx +0.124$ and the descending one $s_N = -0.128$. A distortion of the linearity, which suggests a possible insert, can easily be seen in the interval 2,151,273-2,264,102 bp of the descending branch. Actually the insert covers the larger interval 2,151,273-2,285,688 bp.

A 'hidden' ancestral structure of the genome is revealed by re-orienting all coding segments in the same positive direction [1,17]. The re-orientation of the coding segments can be done in two ways. The first procedure is to 're-frame' the sequence, by keeping in the sequence both the coding and non coding segments,

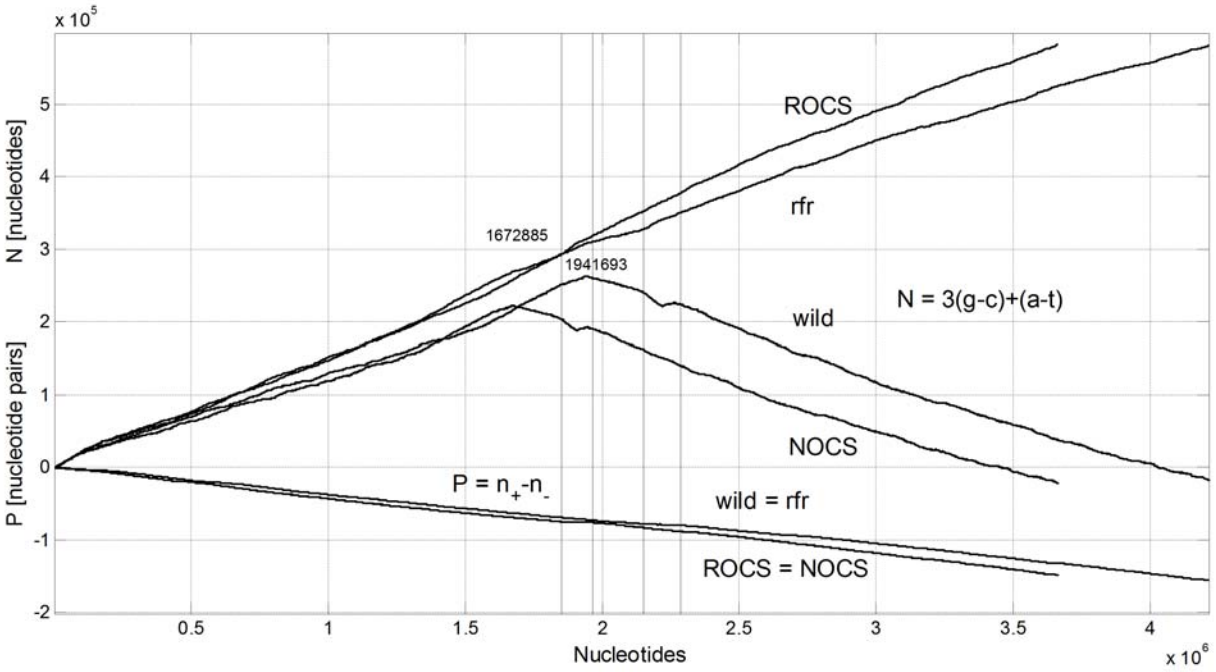

Fig. 1. *Bacillus subtilis* (AL009126 [14,16]): Nucleotide imbalance $N=3(g-c)+(a-t)$ – upper four lines: *wild* – complete genome (4214814 bp, natural orientation), *rfr* – reframed genome (re-oriented coding segments, unchanged non-coding segments), *NOCS* – non-oriented coding segments (3,663,297 bp, 4,101 concatenated coding segments in the natural orientation), *ROCS* – re-oriented coding segments (all coding segments in the positive direction). Nucleotide pair imbalance $P= n_+ - n_-$ – lower two lines: the re-orientation of coding segments does not change P at this scale, so that the curves form two superposed pairs of lines.

and re-orienting only the coding segments for which we have the necessary direction information. The straightened line marked 'rfr' in Fig. 1 is obtained. A second procedure is to keep only the concatenated coding segments and delete the non coding segments, for which we do not have the direction information. A shorter (3,663,297 bp) piece-wise linear curve, similar to the initial ''*wild'* line, marked with NOCS (Non-oriented Coding Segments) in Fig. 1, is obtained. The maximum is shifted to 1,672,885 bp. When aligning inverse coding segments in the positive direction, the NOCS curve becomes the almost straight line denoted ROCS (Re-oriented Coding Segments) in Fig. 1. The ROCS line has an increased average slope and an improved linearity (*NRMSE* ≈ 0.000548).

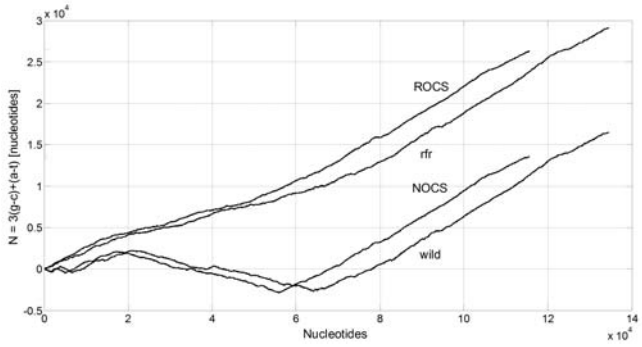

Fig. 2. *N* - Nucleotide imbalance for *Bacillus phage SPBc2* (AF020713 [15,16]): *wild* - complete genome (134,416 bp, natural orientation), *rfr* – reframed genome (re-oriented coding segments, 54 inverted, out of 187, unchanged non coding segments), *NOCS* – non-oriented coding segments (187 concatenated coding segments with the natural orientation, 115583 bp), *ROCS* – re-oriented coding segments (54 coding segments inverted to the positive direction).

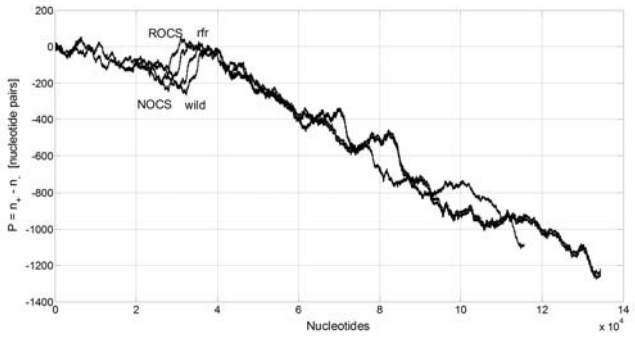

Fig. 3. *P* - Nucleotide pair imbalance for *Bacillus phage SPBc2* (AF020713 [15,16]).

Fig. 1 also gives the lines for the nucleotide pair imbalance *P*. At the scale of the representation, the curves do not change when re-orienting the coding segments [16], so that the curves form two superposed pairs.

It is remarkable that the near-linearity of *N* after coding segments re-orientation is observed for all eukaryotes, pointing to a putative ancestral highly ordered common structure [16]. The invariance of *P* at segment re-orientation is a property shared by all eukaryotes and prokaryotes [17].

The *N* and *P* signals for the *Bacillus phage SPBc2* complete genome (AF020713 [14,15]) are represented in Fig. 2 and Fig.3, respectively. The largest three segments for signal N have $s_N \approx +0.1812$, $NRMSE \approx 0.0118$, $s_N \approx -0.1061$, $NRMSE \approx 0.0883$, $s_N \approx +0.2939$, $NRMSE \approx 0.00471$, respectively. The *P* signal is less regular, with an average slope $s_P \approx -0.0100$, and $NRMSE \approx 0.0166$. After proper re-orientation and alignment, the *N* and *P* signals for the viral genome are superposed exactly over the domain 2151273-285688 bp of the *Bs* genome, as shown in Fig. 4 and Fig. 5. The line superposition is accurate at the level of each nucleotide.

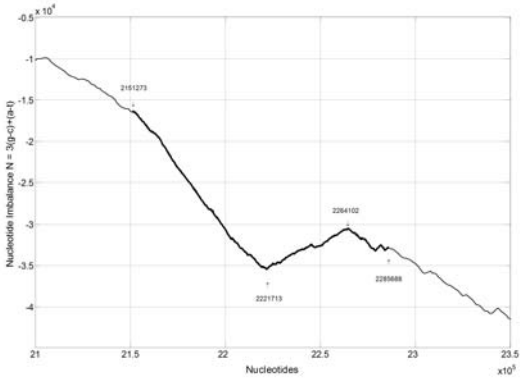

Fig. 4. Superposition of nucleotide imbalance signals of *Bacillus subtilis*, segment 2,151,273-2,285,688 bp (AL009126 [14,16]), and *Bacillus phage SPBc2* (AF020713 [15,16]), inversed and aligned whole genome (134416 bp). The superposition is nucleotide-wise exact.

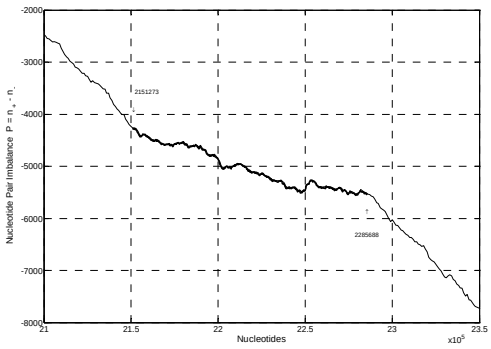

Fig. 5. Superposition of nucleotide pair imbalance signals, P, of *Bacillus subtilis*, segment 2,151,273-2,285,688 bp (AL009126 [14,16), and *Bacillus phage SPBc2* (AF020713 [15,16]).

IV. Inserts in the Genome of *Mycobacterium tuberculosis (MT)*

The complete genome of *MT*, the H37Rv strain (NC0009621 [12,16]), comprises 3989 coding segments, relatively well conserved across the "tuberculosis complex". Figures 6 and 7 present the nucleotide imbalance *N* and the nucleotide-pair imbalance *P* for the complete genome (4,411,532 bp) in the natural order (wild) and after re-orienting all the coding segments in the same positive direction (rfr), leaving non-coding segments unchanged. The

signals for the concatenated coding segments (4,020,531 bp), both non-oriented (NOCS) and after re-orienting all the coding segments in the same positive direction (ROCS), are also given.

The overall shape of the *N* signal is similar to that for *Bs*, but looks less regular because of the many large inserts. Figures 6 and 7 also show the inserts 2,795,301-2,806,236 (2,547,156-2,557,938) and 3,926,569-3,950,263 (3,580,353-3,603,102) that will be analyzed in some detail. The position of the *rpoB* gene (759,807-763,325) is shown because of the importance of mutations in this tiny segment for the development of *MT* resistance to rifampin. The ascending branch of the *N* signal for the complete genome has an average slope $s_N \approx +0.0428$ and a normalized root mean square error *NRMSE* ≈ 0.000975, while the descending branch has $s_N = -0.0465$ and *NRMSE* ≈ 0.00294. It is interesting to notice that the measured linearity of these signals is better than that of *Bs*, contrary to the subjective visual evaluation. This is the result of opposite inserts, the variations tending to mutually compensate so that the regularity of the *N* signal is maintained at the scale of the entire genome. The *N* signal for the NOCS, i.e., for the concatenated coding segments in their natural order and orientation, has the same piece-wise linear shape like the signal for the complete genome (the 'wild' signal). The ascending branch of the *N* signal for the NOCS sequence has $s_N \approx +0.0412$, *NRMSE* ≈ 0.0011, while the descending branch has $s_N \approx -0.0457$, *NRMSE* ≈ 0.00322.

It is noteworthy that, like for *Bs*, the non coding segments contribute to increasing the linearity of the 'wild' genome *N* curves.

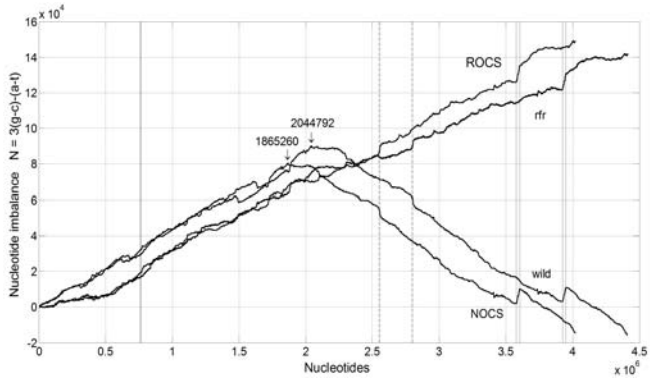

Fig.6 Nucleotide imbalance, N, of *M.tuberculosis* H37Rv strain (NC0009621 [12,16]): *wild* - complete genome (4,411,532 bp) in natural order, *rfr* – reframed genome (coding segments in positive direction, unchanged non coding segments), *NOCS* – non-oriented coding segments (3989 concatenated coding segments, 4,020,531 bp), *ROCS* – re-oriented coding segments (coding segments in positive direction).

The ROCS signal has only one domain for the whole genome, with a slope decreased to $s_N \approx +0.0393$ and, again, with a better linearity (*NRMSE* ≈ 0.00083). However, in this case, the inserts do not tend to become smoother, introducing variations similar to those observed in the NOCS signal. The re-framed signal (rfr) is similar, but with a lower slope ($s_N \approx +0.0334$) and less linear (*NRMSE* ≈ 0.01194).

Quite surprising, the slope for the ROCS and rfr *N* signals are smaller that those for the NOCS and the wild signals, unlike the cases of other prokaryotes, including *Bs* (Fig. 1).

The nucleotide pair imbalance, *P*, for the *MT* complete genome (wild), for the re-framed sequence (rfr), as well as for the non-oriented (NOCS) and re-oriented (ROCS) coding segments are given in Fig. 7.

Again, at this scale, the differences are very small and the curves are overlapping in two pairs. What is remarkable, even among prokaryotes, is the high linearity of the *P* signal for *MT*. For the complete genome, in the native (wild) and reframed (rfr) order, the slope is $s_P \approx -0.0676$, and the normalized root mean square error is only *NRMSE* ≈ 8.075×10^{-5}. This surprising linearity denotes a very high regularity in the distribution of the pairs of nucleotides along the *MT* genome. The *P* signal for the 3989 concatenated coding segments in the *MT* genome, both non-oriented (NOCS) and re-oriented (ROCS), has a high value of the slope ($s_P \approx -0.0676$), and also a remarkably low nonlinearity (*NRMSE* ≈ 8.074×10^{-5}).

Figures 8, 9, 10 and 11 detail the structure of the two insert intervals. Only the 'wild' and 'rfr' sequences are shown, because of the similarity of the signals.

The coding segments in the two inserts are given in Tables 1 and 2. The specific features of the inserts are determined by the coding segments from the *PE-PGRS* family, which encode *MT* typical proteins including some *gly*-rich proteins related to *MT* pathogeneicity.

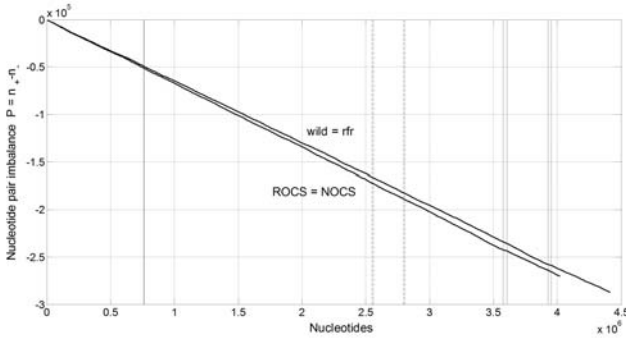

Fig.7. Nucleotide pair imbalance P of *M.tuberculosis* H37Rv strain (NC0009621 [12,16]): *wild* & *rfr* – complete and reframed genome, *NOCS* & *ROCS* – non-oriented and re-oriented coding segments. The four lines superpose in two almost identical pairs.

Coding segments belonging to the *PE-PGRS* family can be found all along the *MT* genome, but such coding segments cluster in the way that distorts locally the signal regularity only in the two studied areas, which compensate each other. A natural hypothesis is that these coding segments are a later acquisition of *MT* involved in its transition from a soil bacterium to a human and mammalian pathogen.

TABLE. 1 CODING SEGMENTS IN THE INSERT 2795301 - 2806236

Coding segment	Start	End	Gene	Length	Cumulated length	Direction
2528	2795301	2797385	*PE-PGRS42*	2085	2549241	-1
2529	2797467	2800880		3414	2552655	-1
2530	2800846	2801145		300	2552955	-1
2531	2801254	2806236	*PE-PGRS43*	4983	2557938	-1

TABLE. 2 CODING SEGMENTS IN THE INSERT 3926569 - 3950263

Coding segment	Start	End	Gene	Length	Cum.length	Direction
3566	3926569	3930714	*PE-PGRS53*	4146	3584499	1
3567	3931005	3936710	*PE-PGRS54*	5706	3590205	1
3568	3936877	3938424	*ilvX*	1548	3591753	-1
3569	3938421	3939257		837	3592590	-1
3570	3939617	3941761	*PE-PGRS55*	2145	3594735	1
3571	3941724	3944963	*PE-PGRS56*	3240	3597975	1
3572	3945092	3945748	*fadD18*	657	3598632	-1
3573	3945794	3950263	*PE-PGRS57*	4470	3603102	1

It is interesting that the *N* signal for the reframed sequences is always influenced by the *PE-PGRS* genes in the same way, namely by producing a strong increase of the signal. This corresponds to the basic structure of the *PE-PGRS* genes. The influence of the *PE-PGRS* genes on the 'wild' signal can be in either direction, depending on the way it is inserted in the sequence. The interceding of direct and inverse direction coding segments in the *MT* genome compensates the local deviations from linearity and ensures the global regularity of the genome.

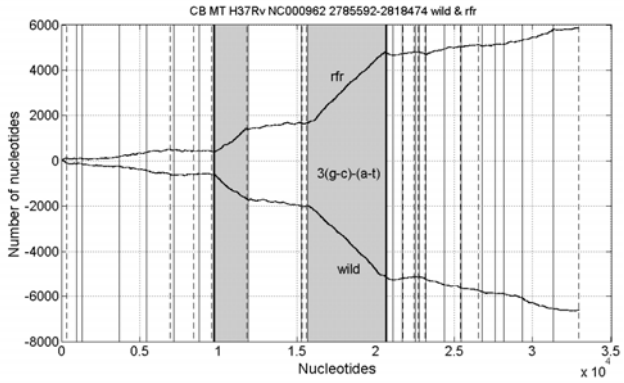

Fig.8. Aligned *N* signals of the 2785592-2818474 segment of *M.tuberculosis* H37Rv strain (NC0009621 [16] *wild* & *rfr*, coding segments 2521-2543) showing the 2795301-2806236 insert. Coding segments 2528 and 2531, which have different features (*PE-PGRS42* and *PE-PGRS43*), are shadowed.

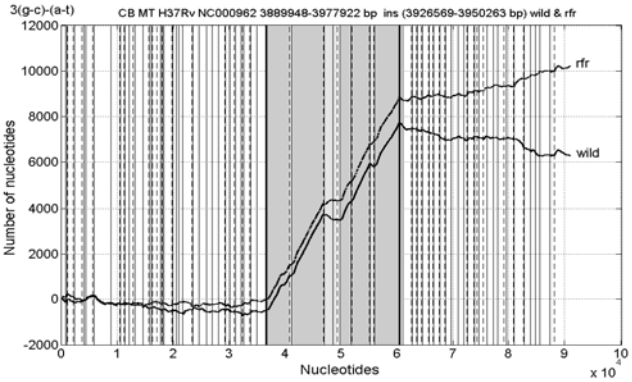

Fig.10. Aligned *N* signals of the 3889948-3977922 segment of *M. tuberculosis* H37Rv strain (NC0009621,16, *wild* & *rfr*, coding segments 3532-3597) showing the 3926569-3950263 insert.

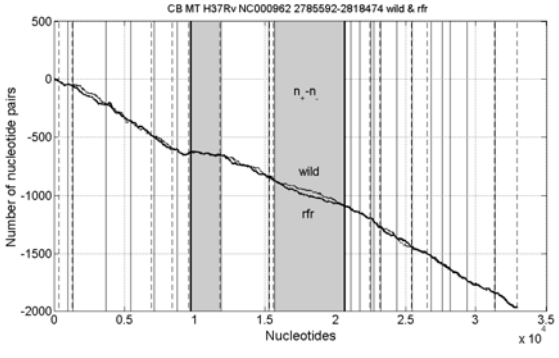

Fig.9. Aligned *P* signals of the segments in Fig. 15. Slight differences between native (wild) and reframed (rfr) signals are noticeable. The coding segment *PE-PGRS43* behaves closer to neighboring ones.

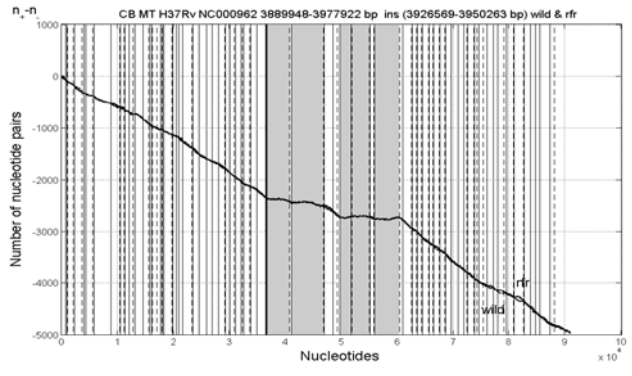

Fig.11. Aligned *P* signals of the segments in Fig. 17. Slight differences between native (wild) and reframed (rfr) signals are noticeable.

V. Conclusion

The most conspicuous features of prokaryote genomic signals are:

- the linearity of the nucleotide pair imbalance *P*,
- the approximately piece-wise linearity of the nucleotide imbalance *N*; with the extremes corresponding to the origin and terminus of replication,
- the invariance of *P* when re-orienting all coding segments in the same positive direction,
- the approximately linear shape of *N* after the re-orientation of all coding segments in the positive direction.

These long range regularities [1,6,17] show that, from the structural point of view, a genome resembles less to a *plain text* and more to a *poem*, which must also obey additional rules of symmetry, giving the "rhythm" and "rhyme". The structural restrictions of genomic sequences are reflected by the regularities observed in the corresponding genomic signals.

The invariance of the nucleotide pair imbalance results from the conservation of the number of positive and negative pairs when reversing a segment of a double helix while simultaneously switching the helix strands to maintain the 5'$\rightarrow$ 3' growth direction [1,17].

The 'hidden' pseudo-linearity of the nucleotide imbalance *N*, which is revealed when all coding segments are re-orientated in the positive direction, suggests the existence of an ancestral genomic structure from which the current structures have derived. The selective pressure could be linked to the selection of allowed transversal intermolecular interactions. These features are essential in processes like replication, transcription and recombination, with a definite role in species separation.

The insertion of exogenous genetic material in prokaryote genomes, either entire retroviruses or individual genes, disturbs only locally the regularity of the nucleotide and nucleotide pair distribution along the sequence, but the global regularity is conserved. This is the result of the compensation produced by complementary local variations. A similar phenomenon has been identified for point mutations (SNPs), which tend to be conserved when produced in groups with effects that mutually compensate to globally satisfy the conditions at the scale of the entire genome [1]. Even if the NuGS analysis method is able to detect inserts based on the local disturbance they produce in the global regularity of the genomic signals of the host nucleotide sequence, it is not possible to precisely determine the localization of the inserts only from the signal analysis. Iterative BLAST search and other procedures have been used to this end. In fact, the extremities of an inserted sequence do obey the local regularities of the framework nucleotide sequence and are practically non-distinguishable from it. This "fitting of the signals" could be a condition for a successful insert.

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