Visualisation of genomic data with the Hilbert curve

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ABSTRACT
In many genomic studies, one works with genome-position-dependent data, e.g., ChIP-chip or ChIP-Seq scores. Using conventional tools, it can be difficult to get a good feel for the data, especially the distribution of features. This article argues that the so-called Hilbert-curve visualisation can complement genome browsers and help to get further insights into the structure of one’s data. This is demonstrated with examples from different use cases. An open-source application, called HilbertVis, is presented that allows the user to produce and interactively explore such plots.

1 INTRODUCTION
A feature of modern experimental techniques such as ChIP-Seq is that the data produced by a single experiment now routinely covers, say, a whole vertebrate genome while still reaching base-pair resolution. Typically, each base-pair is assigned a numerical value. For example, in the case of ChIP-Seq, this score would be the number of reads aligned to this position. Another example is base-by-base conservation scores, which are calculated using programs such as phastCons (Siepel et al., 2005). All the scores of a given chromosome can be thought of as forming one extremely long vector. Good tools to visualise such a vector are essential to explore the data, assess its quality, note peculiarities and form hypotheses. A common approach is to load the data into a genome browser such as Affymetrix’s IGB, navigate to one’s “favourite” genes and check whether the data meets one’s expectations there.

This can be very time-consuming as one has to study many loci to get an overview from representative samples. Furthermore, one might easily miss noteworthy aspects of the arrangement and spacing of the features when one looks at only a single feature at a time. In order to get an overview, one might try to plot large portions of a chromosome, but then features typically blend into each other, giving rise to an uninformative plot.

In the present paper, a visualisation technique is described that complements genome browsers by presenting the whole chromosome at once but still giving access to details. This is achieved by mapping the data from its one-dimensional arrangement along the chromosome onto a two-dimensional shape in order to make more efficient use of the plotting space. This so-called Hilbert curve visualisation (HCV) was first suggested by Keim (1996) to display stock market prices. While it has already been used in genomics before (Wong et al., 2003; Deng et al., 2008), these applications have not caught on. There are already too many features to be reasons for this: on the one hand, there is a lack of flexible and easy-to-use software to produce such plots, and on the other hand, the Hilbert curve visualisation might have not been that appropriate for the use cases discussed in these articles.

The remainder of this paper is organised in four sections: First, in Section 2, an example of a Hilbert plot is discussed to motivate why HCV is useful. Then, Section 3 explains how HCV is done and how these plots should be interpreted. Section 4 discusses for which genomics applications HCV is suitable. Finally, Section 5 introduces HilbertVis, an open-source application to explore data with the help of HCV.

2 INTRODUCTORY EXAMPLE
Fig. 1 shows some fictive data to demonstrate the purpose of Hilbert curve visualisation. The data, a vector of length 1.8 millions, is plotted in Fig. 1a with its whole length condensed to the width of the plot. One cannot infer much from this: it is neither possible to see whether the peaks are all similar nor how they are spaced. One cannot even judge the number or density of peaks as all the needles merge with each other. The standard solution is to zoom in, creating many plots like the one in Fig. 1b.

Fig. 1c is the Hilbert plot for the vector. Each of its $256 \times 256$ pixels represents a “bin” of $1.8 \text{ mio}/256^2 \approx 27$ consecutive vector elements. The shade of a pixel represents the maximum value within the bin (from white for 0 to black for 320). As explained in more detail in the next section, the pixels are arranged such that bins that are close to each other on the data vector are represented by pixels that are close to each other in the plot. Especially, adjacent bins are mapped to adjacent pixels. Hence, each of the many dark spots in the figure is a peak; the area of the spot in the 2D plot is proportional to the width of the peak in the 1D data, and the darkness of the spot corresponds to the height of the peak.

Thus, one can infer the following facts from Fig. 1c, which are not easily noticed otherwise: There are two populations of peaks. The small spots that consist of only a single or occasionally two pixels correspond to narrow peaks with a width of typically at most the bin width (27 bp). These peaks are numerous and occur in clusters: there are usually two to five peaks in close proximity to each other. On the other hand, there are larger patches, with an area, i.e., peak width, 10 to 30 times larger than the narrow peaks. These are lower in peak height (lighter in colour) and do not appear in clusters. Furthermore, their width depends on the position within the data vector: those in the bottom left-hand quadrant of the plot (which corresponds to the second quarter of the data vector’s length, see below) are much wider.

Even though this example data was constructed to show these properties, it is the kind of properties one might want to check.
first, before forming any specific hypotheses during the analysis of, e.g., ChIP-chip or ChIP-Seq experiments: are there wide or narrow peaks, tall or low ones? Are they all the same or are there sub-populations? Are they equally spaced or do they cluster? HCV allows one to judge such questions at a glance because, by spreading the vector out in a two-dimensional square, it provides enough detail to be able to distinguish individual peaks but still allows for an overview of the whole data.

The next section explains the manner in which the pixels are arranged in the square.

3 PRINCIPLE OF HCV

Space-filling curves caused significant excitement among mathematicians when Peano noticed their existence in 1890 (Peano, 1890). These curves are continuous and bijective mappings of the unit interval onto the unit square, i.e., a one-dimensional line is “folded up” (infinitely often) such that it passes through every point of the square. Their existence blurs the distinction between one- and two-dimensional objects, which was a starting point towards the theory of fractal geometry. For our purposes, we take out a specific example of such a curve, the one proposed by Hilbert (1891) shortly after Peano’s discovery. The Hilbert curve is constructed in a recursive way (Fig. 2): In the first iteration, the curve is divided into four parts, which are mapped to the four quadrants of the square. In the next iteration, each quadrant is divided up into four sub-quadrants, which, in a similar way, each hold 1/16 of the curve, and the quadrants of these sub-quadrants each hold 1/256, etc.

The square in the Hilbert plot in Fig. 1c consists of $2^8 \times 2^8$ pixels, i.e., the data vector is folded into the square according to the 8th iteration of the Hilbert curve.

Due to this highly symmetric construction principle, a regularly or randomly spaced arrangement of peaks results in a pattern that appears regular or random, allowing us to infer properties of the spacing in the data from the appearance in the plot. As the curve is continuous, bins which are in close distance to each other on the vector will stay close in the square. Hence, a peak in the data gives
rise to a small connected patch of dark (or coloured) pixels that is
easily perceived. (One might expect that simply filling the square
line by line with the data serves the same purpose but, in fact, the
patches would then be horizontal streaks that are harder to perceive
as distinct entities.) It is unavoidable that some pairs of pixels
in close proximity correspond to distant loci on the vector (that
happens whenever the curve “folds back” towards already covered
regions). However, compared to simple line-by-line filling or other
space-filling curves such as Peano’s original curve or Morton’s Z
curve, the Hilbert curve keeps these distortions to a minimum. They
are also easily spotted, as they give rise to typical “chequerboard-
like” patterns (e.g., in Fig. 3b). Furthermore, if one is interested in
small distinct peaks, it happens only rarely that two such peaks meet
due to a distortion and mislead the viewer into perceiving a single,
wider peak.

Apart from the discussed distortions, the obvious disadvantage of
HCV is that it is rather hard to relate a position on the plot back
to a position on the vector. This limits applicability when absolute
positions are of interest but is not an issue if one is interested in
judging relative positions, i.e., spacing, homogeneity, etc. Note also
that the HilbertVis tool discussed below allows the user to move
the mouse cursor to any position in a Hilbert plot and read off the
corresponding absolute position from a gauge.

4 USE CASES

To demonstrate HCV with real data, I have re-analysed the ChIP-
Seq data for histone methylation marks H3K4me1 and H3K4me3
reported by Barski et al. (2007). After remapping the reads (taken
from the NCBI Short Read Archive, accession number SRA000206)
to the human genome with MAQ (Li et al., 2008), Figure 3 was
produced with HilbertVis. The colour saturation shows the coverage
of the bins with aligned reads. H3K4me3 is known to mark the
transcription start sites of active genes and hence has narrow, sharp
and strong peaks. H3K4me1 is believed to be associated with active
genes in a more general sense and the peaks are much more diffuse
and less clearly localised. Thus, HCV is shown to be helpful in
quickly noticing the stated facts and forming hypotheses for further
tests. Furthermore, when one knows roughly what to expect, such a
plot helps to do a first quality assessment of newly acquired data.

Another application is the comparison of data vectors: One
might be especially interested whether me1 and me3 marks usually
coincide and whether they are restricted to exonic regions. Fig. 4
addresses these questions by overlaying the two plots in red and
green and marking exonic regions in blue. Note how the additive
colour mixing allows to assess co-location of features.

Overlay plots are useful again to assess the quality of raw data.
In a Chip-Seq experiment, one might, for example, be interested in
the uniformity of the coverage of the reads from the input control.
In a HCV, one could display coverage in one colour and mark out
in another colour the parts of the chromosome that are repetitive on
the length scale of the reads and hence cannot be aligned to.

Application is not limited to ChIP-Seq. Experiments involving
tiling arrays (e.g., ChIP-chip and array-CGH) gives rise to similar
data vectors. Results from computational genomics can also be
visualised, e.g., base-by-base conservation scores, gene density and
repetitive element density. Please see Fig. 4 and the Gallery section
of the HilbertVis web site (www.ebi.ac.uk/~anders/hilbert)
for examples.

The common characteristic of the suggested applications is that
the visualised vectors contain from hundreds up to tens of thousands
of features, which can be seen as distinct entities in the plot.
Fig. 4. Three-colour overlay of the plots from Fig. 3: H3K4me1 is displayed in red, H3K4me3 in green and exonic regions in blue. The colours are additive, i.e., bins with both me1 and me3 marks appear in yellow. The sparsity of yellow pixels indicate that the two marks do not tend to occur close to each other. Furthermore, the blue colours for exons mixes with the red for me1 to purple. Comparing purple and red shows that me1 marks spread well into non-coding areas. (Colours are optimised for display on screen, not on paper.)

HCVs of a quantity that varies in a very smooth manner, without pronounced localised features, will not be very informative. This may apply to the previous appearances of HCV in genomics literature (Wong et al., 2003; Deng et al., 2008), which were restricted to visualising the base composition of chromosomes, an application which, I believe, does not demonstrate the strengths of HCV well.

5 HILBERTVIS

When one finds an interesting feature in a Hilbert plot, one may want to explore it in detail, i.e., zoom in, learn its genomic coordinates, or obtain an ordinary linear plot of it. Hence, I have developed an application, named “HilbertVis”, that not only allows to generate Hilbert plots but also to explore them in an interactive manner with the help of a graphical user interface (Fig. 4).

Two variants are offered, a stand-alone version, and a package for the statistical environment “R” (R Development Core Team, 2008). Both are available as open-source software under the GNU General Public License, version 3, with binaries being provided for Mac OS X, Linux, and Microsoft Windows.

The stand-alone version allows one to read in data in the formats GFF, BED and Wiggle, and the “map” output format of the MAQ alignment program (Li et al., 2008). In order to facilitate displaying data in other formats or pre-processing the data, the R packages “HilbertVis” (for batch processing) and “HilbertVisGUI” (for interactive exploration) are available as part of the Bioconductor project (Gentleman et al., 2004). With these, the functionality of R and Bioconductor can be used to produce the data vectors as R variables, which can then be displayed with HilbertVis. For more information, see the package vignette (manual) supplied with the HilbertVis package.

A few special features of HilbertVis should be pointed out: If the mouse is moved over the plot, a pointer in the ruler on the right-hand
side of the GUI (Fig. 4) shows the position of the mouse cursor within the displayed part of the sequence, giving visual feedback that helps the user to keep oriented in the Hilbert curve. Another important feature are the buttons labelled “Lighter” and “Darker”. They allow the user to change the palette in order to explore either low-intensity or high-intensity features. This is important because the limited dynamic range of human colour perception makes it difficult otherwise to analyse data with different intensity scales.

If one wishes to compare many data sets corresponding to the same chromosome, e.g., ChIP data for related transcription factors, one can load them simultaneously and flip back and forth between them. A caching mechanism makes this smooth and fast such that it is easy to look for differences between the vectors.

Finally, a call-back facility to R is provided that allows one to use custom routines to inspect or analyse features selected with the mouse. For example, one may want to use the GenomeGraphs package (Durinck et al., 2009) to automatically load gene annotation data and display it along with the feature.

For source code, binaries, documentation, and further example images, please see the following web page:
http://www.ebi.ac.uk/huber-srv/hilbert/

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