# Visualization, Analysis, and Design of COMBO-FISH Probes in the *Grid*-Based GLOBE 3D Genome Platform

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**Abstract.** The genome architecture in cell nuclei plays an important role in modern microscopy for the monitoring of medical diagnosis and therapy since changes of function and dynamics of genes are interlinked with changing geometrical parameters. The planning of corresponding diagnostic experiments and their imaging is a complex and often interactive IT intensive challenge and thus makes high-performance grids a necessity. To detect genetic changes we recently developed a new form of fluorescence *in situ* hybridization (FISH) – COMBinatorial Oligonucleotide FISH (COMBO-FISH) – which labels small nucleotide sequences clustering at a desired genomic location. To achieve a unique hybridization spot other side clusters have to be excluded. Therefore, we have designed an interactive pipeline using the *grid*-based *GLOBE 3D Genome Viewer* and *Platform* to design and display different labelling variants of candidate probe sets. Thus, we have created a *grid*-based virtual "paper" tool for easy interactive calculation, analysis, management, and representation for COMBO-FISH probe design with many an advantage: Since all the calculations and analysis run in a grid, one can instantly and with great visual ease locate duplications of gene subsequences to guide the elimination of side clustering sequences during the probe design process, as well as get at least an impression of the 3D architectural embedding of the respective chromosome region, which is of major importance to estimate the hybridization probe dynamics. Beyond, even several people at different locations could work on the same process in a team wise manner. Consequently, we present how a complex interactive process can profit from grid infrastructure technology using our unique *GLOBE 3D Genome Platform* gateway towards a real interactive curative diagnosis planning and therapy monitoring.

**Keywords.** Genome organization, Globe 3D Genome Platform, COMBO-FISH, interactive extreme grid visualization, grid and GPU computing.

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## Introduction

In the last decade diagnosis and therapy monitoring has been more and more based on the investigation of the nanostructure of the cell nucleus in context of its functional dynamics [1-5]. The ability of modern high-resolution microscopy down to the nano level has allowed structural biology and medicine to develop new biochemical methods for visualization of genetic changes. During the development of many diseases, relevant changes of distinguished nuclear and genetic parameters like the number of chromosomes, multiplicities of genes [6], or rearrangements of chromosomes [7] can be relatively easy observed. But also on a finer genetic scale, mutations, loss or gain of parts of genes like exons or introns, and other rearrangements of genetic elements are indicators of malignant changes [8]. These changes can nowadays be quantified: On a global level, geometric descriptors of the architecture of the nucleus like e.g. nuclear diameter, eccentricity of its ellipsoidal form, density of chromatin, or positioning of a gene have been shown to indicate diagnostically relevant changes [9-12]. Also on the local level such changes can be monitored, although appropriate biochemical and nanoscopical imaging and analysis techniques, which are needed to generate experimentally valid quantifications, are still under development.

Especially the planning of diagnostic experiments and their imaging is a complex and often interactive IT intensive challenge requiring high-performance. High-performance grid infrastructures are due to their scalability and variety especially suited for analyses in the life sciences [13]. E.g. the capability of instant access to computing resources makes the grid interesting for online planning of complex experiments. The combination with visualization resources [14, 15] in the grid makes it highly flexible, to run computational expensive calculations in combination with demanding visualizations exported to a laptop computer in the laboratory. A prominent solution for the genomic field from the DNA sequence to the morphologic level is the grid-based *GLOBE 3D Genome Platform* [15]: It is a flexible and easy to use software package, which supports computational aspects of different clinical and molecular biological methods. Beyond, even several people at different locations could work on the same process by a distributed team of scientists in a telemedicine approach.

A prominent technique that has been established for standard diagnosis and treatment monitoring is fluorescence *in situ* hybridization (FISH). Here, long fluorescently labelled polynucleotides are hybridized to their complementary DNA sequence. Thereafter, the targeted loci can be imaged by appropriate microscopic setups. Thus, e.g. their position can be located quantitatively in their geometric nuclear environment. For high-resolution experiments with small regions to be targeted we developed the COMBinatorial Oligonucleotide FISH (COMBO-FISH) method [16]. Here short oligonucleotides of 15 to 25 bp, which uniquely localize at the given sequence of interest (in general a part of a gene), are combined. Due to its specificity COMBO-FISH requires an integrated and interactive analysis and planning to achieve high quality quantitative results. Therefore, we have integrated a sequence of genome-wide acting algorithms [16-18] with the *grid*-based *GLOBE 3D Genome Viewer* and *Platform* [15]: It allows a distributed team of specialists to interactively design and display different labelling variants of candidate probe sets in their 3D architectural embedding [1-5] of the respective chromosome region important to estimate hybridization dynamics. Consequently, we show how a complex interactive process can profit from grid technology using our unique *GLOBE 3D Genome Platform* gateway towards a real interactive curative diagnosis planning and therapy monitoring.

## 1. COMBinatorial Oligonucleotide Fluorescence *in situ* Hybridization

In FISH, fluorescent ligand carrying polynucleotide probes are hybridized to their complementary DNA counterpart. Probes stretch over usually thousands of base pairs sometimes up to the entire chromosome for complete chromosomal labelling. Usually the cells or tissues have to undergo a severe and complex preparation, often modifying nuclear architecture: after chemical fixation of the material, the DNA is denatured so that the single stranded probe DNA can bind by Watson-Crick pairing. Thereafter, the so labelled region can be imaged by e.g. 2D or 3D microscopy – due to the region size in at least one single spot or an entire chromosome part. By subsequent image analysis (geometrical) information as e.g. gene copy number or general topological relations are extracted and used for biological or medical applications. To label very small and non-overlapping targets especially for genomic architecture studies under native or even *in vivo* conditions, COMBinatorial Oligonucleotide fluorescence *in situ* hybridization (COMBO-FISH) was developed [16-18].

### *1.1. The general concept of COMBO-FISH*

In COMBO-FISH, short oligonucleotide probes of 15-25 bp are used in a combinatorial set of 20-40 probes – statistically a single probe has many binding sites throughout the genome – so that they colocalize on the genetic element to be targeted uniquely and thus produce a microscopically nicely to image spot. I.e. that clusters of more than 3 to 6 oligonucleotides colocalizing within 250 kb should not occur. COMBO-FISH probes can be designed for arbitrary sequences but are especially suited for oligopurine and oligopyrimidine probes, which allow binding to the DNA double helix via Hoogsteen pairing in a triple helical manner without any structure/morphology disturbing DNA denaturation. Thus, COMBO-FISH can in principle also be applied to vital cells [19]. Since each oligoprobe carries only one or two fluorescent ligands each colocaliziation spot is detectable well by fluorescence imaging against the background of minor binding (single hybridization or small cluster) events and general background fluorescence which can be easily filtered by appropriate software tools.

### *1.2. Algorithms for COMBO-FISH*

First COMBO-FISH requires that the corresponding genome is completely sequenced to design oligonucleotides combinations colocalizing at the genetic target of interest while excluding further clustering elsewhere (conditions of 1.1.). To ease probe design we either calculate or use a data base of very well binding arbitrary or oligopurine/ oligopyrimidine sequences. Then candidate probes within the genetic region of interest are selected. On average, 30-100 such probes are found for polypurines on a whole gene. Thereafter, the location of the candidate probes is rechecked in the entire genome by an exact search and highly frequent sequences – very often-repetitive ones – are removed from the candidate set. Finally, the candidate set is iteratively (with automatic suggestions) reduced until no further clusters of the specified size exist. This usually results in half of the number of the original probe set. The whole process takes around 15-30 min using the *Globe 3D Genome Viewer* and *Platform* and using either external or grid resources for the calculations. Especially in highly-repetitive genome regions it seems to be an intriguing genetic feature that such uniquely colocalizing probe sets exist (e.g. for the SNRPN-SNURF gene region: Figure 2A).

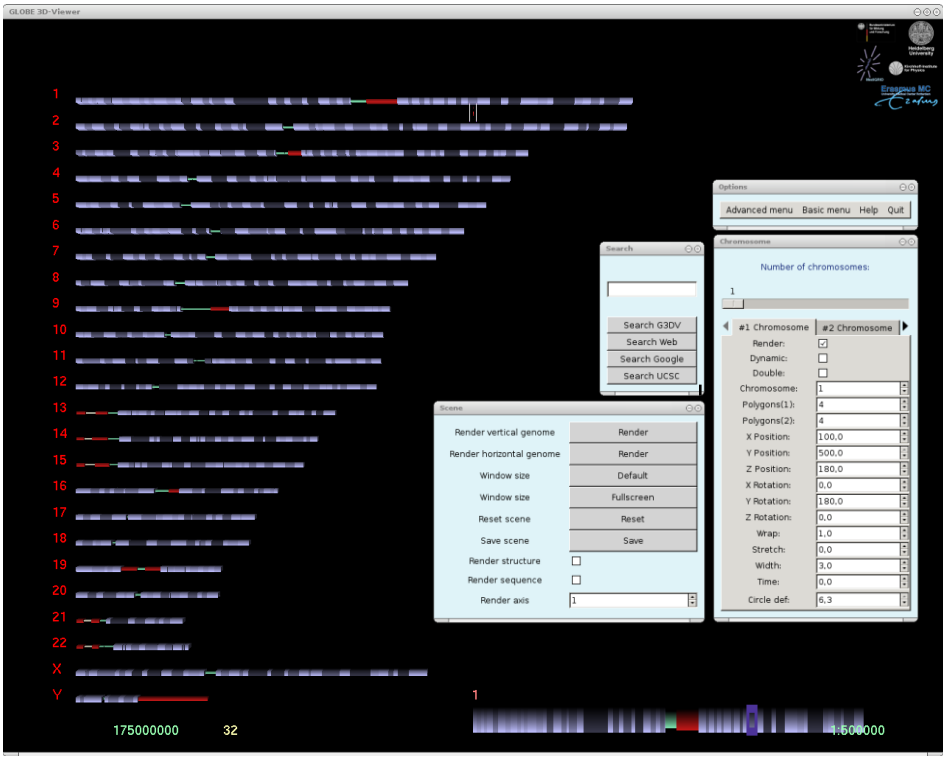


**Figure 1:** Display of all 24 human chromosomes in the *GLOBE 3D Genome Viewer*: chromosome banding (blue tones), centromers (green), and pericentromeric regions (red). At the bottom, the base pair position (green, left) and an overview (right) of the selected chromosome with the selected area (purple box) is displayed next to the frames displayed per second (yellow, left) and the magnification ratio (green, right). Several control panels are displayed, which can be positioned independent of the main viewer frame.

## 2. The grid-based *GLOBE 3D Genome Viewer* and *Platform*

The *GLOBE 3D Genome Platform* is a grid-based integrative virtual "paper" tool, i.e. a three-dimensional virtual desktop environment for genome research and exploitation of genomic information in a service orientated and distributed/teamwork like manner [15]. It integrates three grid wise distributed resources via one single gateway: i) visual data representation and graphical user access using the *GLOBE 3D Genome Viewer* module of the platform, ii) data access and management via a file and web access system, and iii) data analysis and creation on local or high-performance grid infrastructures.

The central part of the platform is the *GLOBE 3D Genome Viewer* module, which is a unique grid and OpenGL 3D environment serving two main functions: i) display of genomic data, and ii) being the graphical user interface for data access and management as well as analysis and creation. It is optimized for the challenges of research and health care to explore the genomic information in a holistic manner: i) the representation of the real genome structure/architecture/organization, ii) the representation of the various genomic experimental data and analysis types, and iii) the extreme technological means to provide a reasonable viewing system to live up to the system-biological/medical quests.

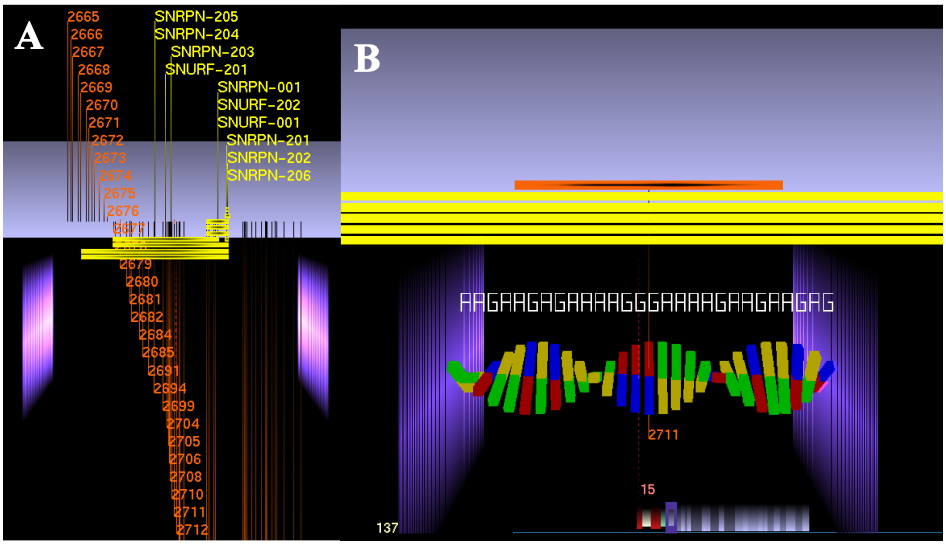


**Figure 2:** (A) Detailed view of the SNRPN-SNURF gene region: the large horizontal bar (blue) represents part of the 15q11.2 chromosomal ideogram band, location of the COMBO-FISH probes (orange), different transcripts of the SNRPN-SNURF gene (yellow). The width of the horizontal yellow boxes is equivalent to the length in base pairs. (B) DNA sequence of the COMBO-FISH probe 2711 (orange bar) of 20 bp length. Since the SNRPN-SNURF gene transcripts are much larger only yellow bars are present. The displayed region is marked by a purple box in the overview (bottom) of the selected chromosome 15.

The *GLOBE 3D Genome Viewer* consists of a main window for display of the data (Figure 1-4). Several control panels can be positioned independently of the main viewer frame. Within the main window three display categories exist: i) the actual data to be visualized, ii) overview navigators concerning the actual data visualized, and iii) supporting viewer status information (Figures 1-4). The actual data is obviously located in the centre of the main frame in contrast to the supporting information, which is displayed at the bottom or eventually at the other sides of the main window. Concerning genomic data, every architectural level of one or several genomes (even of different species) can be visualized simultaneously in a real (Figure 4) and in a symbolic (mostly linear; Figure 1-3) representation and navigated by continuous scale-free zooming from the entire cell nucleus or chromosome down to the base pair level. Other data as e.g. microscopic images can also be displayed (Figure 4). Navigation supports are e.g. 3D spatial orientation, depiction of a selected chromosome and marking of a selected genetic region, zoom level, selected base pair position on a selected chromosome. Status information is e.g. displaying frame rates, memory usage, CPU load, internet connection status. The selected genetic region is marked in the symbolic representation by a box (Figure 1-3) and in the real spatial or image data by high-lighting (Figure 4). Additionally, annotations as e.g. chromosome numbers, ideogram band numberings, gene names and locations are textually placed next to their appearance. Genetic elements can be marked by a combination of different shapes, textures, and colours. They can be correlated by different lines with similar properties based on external or internal correlations. Consequently, for COMBO-FISH like projects, sequential, structural or image data can be combined with external data (as from the ENSEMBL [20] and UCSC [21] data bases) and displayed with annotations and correlations in one single 3D viewing and desktop like working environment.

## 3. Designing COMBO-FISH Probes for the SNRPN-SNURF Region of the Prader-Willi/Angelman Syndrome with the *GLOBE 3D Genome Viewer* and *Platform*

To illustrate how the design pipeline of COMBO-FISH probes benefits from the integration into the *GLOBE 3D Genome Viewer and Platform* – and thus high-performance calculation and visualization using grid infrastructures – we show now in detail the process of designing labelling probes for the SNRPN-SNURF gene region, which plays an important role in the development of the Prader-Willi/Angelman syndrome [4]. PWS/AS is known for the various body deformations, physiological changes, and especially also for the aggressive will to get and eat food. Genetically, it is a prime example for a complex disease where i) DNA mutations and bigger DNA rearrangements, ii) epigenetic modifications, and iii) structural/architectural changes of the chromatin organization, individually or in combination play a role. PWS/AS is a rare disorder (1:10,000-25,000 live birth), but especially the relatively small but nevertheless even more important structural changes and the interplay with the other causes remain unknown. Though progress has been made to unravel the dynamic chromatin architecture on the nano level with sophisticated 3D high-resolution microscopy [4, 5], the use of conventional FISH 16-600kbp probes pose severe limitations. Thus, polypurine/polypyrimidine COMBO-FISH probes for different regions of the SNRPN–SNURF gene are the method of choice for improved resolution and use of Hoogsteen pairing in a triple helical manner avoiding any structure disturbing DNA denaturation with the option of *in vivo* investigations.

The sequence of the SNRPN-SNURF gene has 13 exons according to the NCBI human genome contig analysis file (based on the Human Genome Project [22]), which need to be labelled evenly due to the many a combination of exons (leading to different mRNA transcriptions) to achieve a proper structural investigation:

```
gene 1503593..2099408
      /gene="SNRPN"
      /note="synonyms: SMN, SM-D, HCERN3, SNRNP-N"
      /db_xref="GeneID:6638"
      /db_xref="LocusID:6638"
      /db_xref="MIM:182279"
mRNA join(1503593..1503796,1509744..1509851,
      1566462..1566535,1599957..1600070,1642060..1642155,
      1647878..1648028,1654257..1654402,1655304..1655455,
      1656251..1656362,1656823..1656975,1657724..1657862,
      1658139..1658264,1658353..1658528)
      /gene="SNRPN"
      /product="small nuclear ribonucleoprotein polypeptide N,
      transcript variant 4"
```

Based on this positional information, the COMBO-FISH candidate probe set for SNRPN-SNURF is either calculated or chosen from the polypurine/polypyrimidine database. After visual removal of repetitive and other unfavourable sequences this results in 89 candidate probes (1st #: ordinal number; 2nd #: location within the gene with reference to the contig base numbering; 3rd #: probe length; sequence):

```
2704: 4059501(15): agaggaaaaggagag
2705: 4061047(21): aaaggagaagagagaaaaggg
2706: 4061520(16): gaaggagagagaagaa
2708: 4062351(15): agaagagaggagagg
2710: 4064619(18): gggggggaggggagaggga
2711: 4066761(20): agagaaaagggaaaagaaga
2712: 4069722(16): aggaggaagggaaaag
```

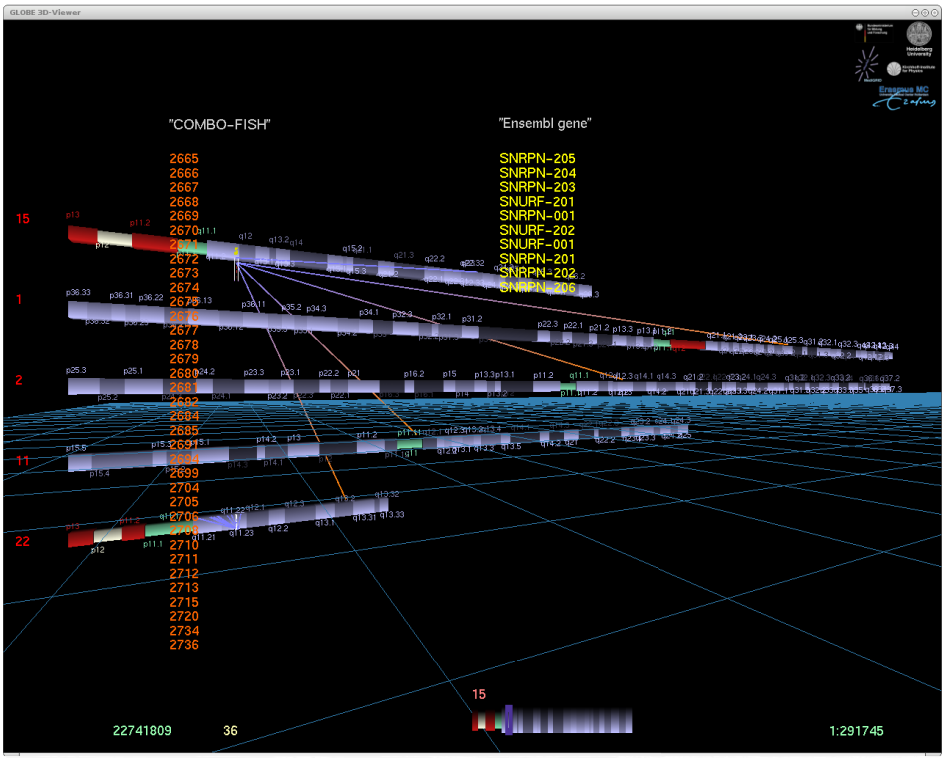


**Figure 3:** Positions of the SNRPN-SNURF transcripts (yellow boxes) on chromosome 15 (top chromosome) with COMBO-FISH probes in this region (orange). The $>10^3$ bp SNRPN-SNURF region has high homologies on chromosomes 1, 2, 11, and 22, indicated by the lines to the corresponding sequence.

After locating all occurrences of each candidate sequence in the whole genome, the real important part of the iterative process of reducing the probe set begins now in the *GLOBE 3D Genome Viewer*. Especially in the case of SNRPN-SNURF in respect to its function in PWS/AS it is important to have a precise visualization of the location of the (candidate) probes within the gene and syndrome region. Though parts of the process of probe set reduction is deterministic in so far as some of the probes have to be eliminated to avoid the build up many a long cluster, there is astonishingly considerable freedom of choice to eliminate clustering probes, especially towards the end of the reduction process, which requires a dedicated and e.g. experiment based individual choice. In that respect, visualizations as in the *GLOBE 3D Genome Viewer* are especially well suited to guide this probe eliminating choice (Figure 2A), since one can easily get an overview on the global level, but nevertheless can move by semantic zooming fast to the base pair level as well. In particular, sequence reappearances with high homologies of the SNRPN-SNURF gene and the PWS/AS region in general on the global level within the same or other chromosomes are of major importance especially during cluster elimination (Figure 3). E.g. one sees immediately that a $10^3$ bp long subsequence is duplicated on four other chromosomes. On the base pair level the binding location and affinity of each single probe can be analyzed in detail (Figure 2B) and choices can be made based on biochemical/physical considerations of the specific binding process. Since here the calculation and analysis go hand in hand online, the

calculation performance can only be delivered via grid resources both in respect to visualization and calculations. After selectively removing probes guided by the above mentioned considerations, a set of 55 probes is obtained with no clusters with a size larger than 4 oligonucleotides. The distribution is nicely covering the SNRPN-SNURF gene region (Figure 2A). It is also clear that different subsets of the probe set can be used to label different transcript regions or regions neighbouring the SNRPN-SNURF gene region. With the *GLOBE 3D Genome Viewer* it is now very easy to find the sequence of a specific region, e.g. probe number 2711 (Figure 2B).

Beyond, the mere selection of probes, which could be done with much more effort and not as intuitively also by using (paper) lists or with a 1D or 2D display system, the *GLOBE 3D Genome Viewer* and *Platform* also allows investigation of a DNA sequence in its spatial context (Figure 4), i.e. not only its spatial position within a structural local or global embedding can be investigated (since the 3D structure of chromatin plays an important role in gene regulation [1-5]), but more importantly estimates about probe diffusion towards the target and chromatin dynamics obstructing binding can be made just by visually inspecting the genomic architecture. Furthermore, also the relations of e.g. the reappearing subregions can be reinvestigated in spatial terms and also other spatial contact information within the same or to other chromosomes can be made. Either structural predictions resulting from chromatin simulations or real experimental data can be used and also directly compared. Relations to multi-dimensional microscopy images (Figure 4) or to contact/interaction frequency maps [2] can also be displayed and analysed. Again the enormous amount of data involved here (chromatin approximation cell nucleus: $10^6$-$10^8$ particles; image stack size: $10^2$-$10^3$ MByte; interaction maps: $10^{12}$ genome wide interactions) can only be analysed and displayed using high-performance grid infrastructures and dedicated systems as the *GLOBE 3D Genome Viewer* and *Platform*.

## Conclusion

The function of genomes is closely connected to its organization and plays an important role in modern microscopy for the monitoring of medical diagnosis and therapy. The planning of corresponding diagnostic experiments and their imaging is a complex and often interactive IT intensive challenge and thus makes high-performance grids a necessity. To take full advantage of a recently by us developed innovative form of cell or tissue labelling – COMBO-FISH – we have designed an interactive pipeline for the *grid*-based *GLOBE 3D Genome Viewer* and *Platform* to design and display different labelling variants of candidate probe sets. Thus, we have created a *grid*-based virtual "paper" tool for easy interactive calculation, analysis, management, and representation for COMBO-FISH probe design with many an advantage: Since all the calculations, analyses and visualizations run in the grid, one can instantly and with great visual ease locate duplications of gene subsequences to guide the elimination of side clustering sequences during the probe design process, as well as get at least an impression of the 3D architectural embedding of the respective chromosome region, which is of major importance to estimate e.g. the probe dynamics. Beyond, even several people at different locations could work on the same process in a team wise manner. Consequently, we show how a complex interactive process can profit from grid infrastructure technology using our unique GLOBE 3D Genome Platform gateway towards a real interactive curative diagnosis planning and therapy monitoring.

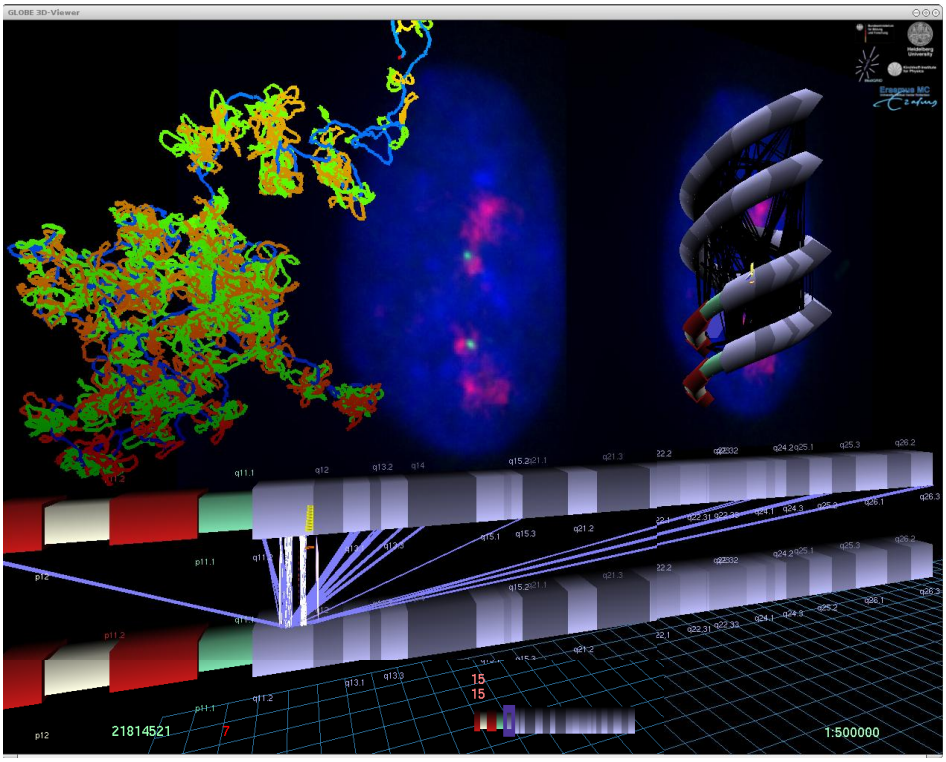


**Figure 4:** Different representations of chromosome 15 in the *GLOBE 3D Genome Viewer*: It is possible to locate any region of interest as e.g. here SNRPN-SNURF transcripts positions in a linear (bottom), twisted (right), and even the location within the embedding into the genomic 3D architecture (here a simulated chromosome [3-5]; left) of eventually the complete cell nucleus, as well as microscopic images (fibroblast nucleus: DAPI Blue; chromosome 15 territory: Texas Red; SNRPN-SNURF region: Oregon Green).

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