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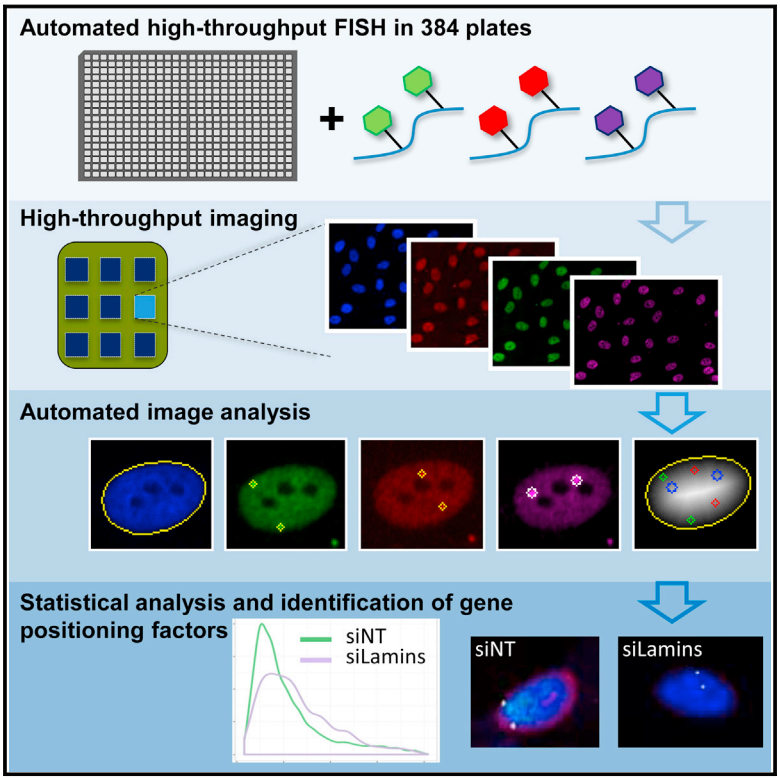
Identification of Gene Positioning Factors Using High-Throughput Imaging Mapping

Graphical Abstract



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In Brief

HIPMap is a FISH-based, high-throughput platform that can be applied to determine the spatial position of a gene in the 3D nuclear space and to discover factors that determine genome organization.

Highlights

- Development of a method for accurate mapping of gene position in the 3D nuclear space at large scale
- siRNA screen identifies nuclear factors involved in genome organization
- Replication is a critical step in determining spatial gene positioning

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Identification of Gene Positioning Factors Using High-Throughput Imaging Mapping

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SUMMARY

Genomes are arranged non-randomly in the 3D space of the cell nucleus. Here, we have developed HIPMap, a high-precision, high-throughput, automated fluorescent in situ hybridization imaging pipeline, for mapping of the spatial location of genome regions at large scale. High-throughput imaging position mapping (HIPMap) enabled an unbiased siRNA screen for factors involved in genome organization in human cells. We identify 50 cellular factors required for proper positioning of a set of functionally diverse genomic loci. Positioning factors include chromatin remodelers, histone modifiers, and nuclear envelope and pore proteins. Components of the replication and post-replication chromatin reassembly machinery are prominently represented among positioning factors, and timely progression of cells through replication, but not mitosis, is required for correct gene positioning. Our results establish a method for the large-scale mapping of genome locations and have led to the identification of a compendium of cellular factors involved in spatial genome organization.

INTRODUCTION

Chromosomes and individual regions of the genome occupy preferential non-random positions inside the 3D space of the cell nucleus (Bickmore, 2013; Misteli, 2007). The position of genomic loci has been linked to numerous nuclear functions, including transcription, replication, DNA repair, and chromosome translocations (Chiolo et al., 2011; Gilbert et al., 2010; Roix et al., 2003; Takizawa et al., 2008). The non-randomness of genome architecture can be measured by the proximity of a gene locus to the nuclear periphery, to nuclear structures such as the nucleolus or transcription centers, or by the proximity of a locus to another genomic region (Branco and Pombo, 2006; Chubb et al., 2002; Roix et al., 2003; Thomson et al., 2004; Zhang et al., 2012).

The spatial position of a genomic locus is routinely determined using fluorescence in situ hybridization (FISH), which allows physical mapping of a genomic region relative to a defined landmark (Speicher and Carter, 2005; Wei et al., 2013). DNA FISH has been used extensively to visualize the position of a locus and to document changes in positioning that occur during physiological and pathological processes (Ferrai et al., 2010; Meaburn et al., 2007b; Takizawa et al., 2008), such as the relocation of genes during differentiation (Hewitt et al., 2004; Kosak et al., 2002; Williams et al., 2006) or the proximity of translocation-prone genome regions in 3D space (Hakim et al., 2012; Mathas et al., 2009; Misteli and Soutoglou, 2009). The development of chromosome conformation capture techniques such as 3C, 4C, and Hi-C, which allow mapping of intra- and inter-chromosomal interactions by biochemical means, has further highlighted the non-randomness of higher genome organization and has revealed several novel principles of organization, including the existence of functionally and structurally defined genomic sub-domains (de Wit and de Laat, 2012; Dixon et al., 2012; Lieberman-Aiden et al., 2009).

Although the notion of non-randomness of genome organization in the cell nucleus is well established and some factors involved in shaping global higher-order chromatin structure such as CTCF, cohesin, and Mediator have been identified (Botta et al., 2010; Ling et al., 2006; Phillips and Corces, 2009; Sofueva et al., 2013; Vogelmann et al., 2011; Zhao et al., 2006); however, the molecular machinery that determines the location of a gene or genome region in the 3D space of the nucleus is largely unknown. Physical mapping methods identified genome regions preferentially associated with the nuclear lamina, pointing toward a role for nuclear lamins in retaining genome regions at the nuclear periphery and thus determining their spatial location (Guelen et al., 2008; Meuleman et al., 2013; Peric-Hupkes et al., 2010; Pickersgill et al., 2006). Furthermore, a genetic screen using a reporter gene in *C. elegans* identified histone methyltransferases and the H3K9me3 modification as determinants of peripheral localization (Towbin et al., 2012).

The systematic identification of molecular determinants of genome positioning has been hampered by the fact that spatial gene mapping by either imaging or chromosome conformation capture technology has not been amenable to implementation at a high-throughput scale and is thus not well suited for use in screening approaches. To overcome this limitation, we describe here the development of HIPMap (high-throughput imaging position mapping), a fully automated FISH-based imaging pipeline to quantitatively determine the position of multiple endogenous loci in the nucleus of mammalian cells with high accuracy and high throughput. We use HIPMap in combination with siRNA screening to discover human genome positioning factors in an unbiased, large-scale fashion. We identify 50 cellular factors,