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PHYSICAL MAPPING OF GENOME AND GENES

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ABSTRACT

Mapping genome of an organism is an important tool to provide a guide for the sequencing experiments by showing the exact positions of genes and other distinctive features in the chromosomal DNA. Whole genome sequencing of eukaryotic genomes is greatly facilitated by high- density genome maps. The high-density maps serve as foundation during organizing and assembling the nucleotide sequences of genomes. Reference to the map ensures that regions containing repetitive DNA are assembled correctly. Distinctive features on the genome map are used as landmarks to aid assembly of master sequence from huge number of short sequences obtained from genomic library. First step for manipulation of genes for various applications is cloning that relies on mapping close to a convenient marker. A complete physical-genetic map of genome is necessary for comparative genomics studies with other genomes. High-resolution comparative physical maps will reveal regions of colinearity and rearrangement and will have important implications for phylogenetic studies and genome evolution.

Keywords: Whole Genome Sequencing, High-Density Genome Maps, Repetitive DNA, Physical Maps, Marker

Mapping genome of an organism is an important tool to provide a guide for the sequencing experiments by showing the exact positions of genes and other distinctive features in the chromosomal DNA. Whole genome sequencing of eukaryotic genomes is greatly facilitated by high-density genome maps. The high- density maps serve as foundation during organizing and assembling the nucleotide sequences of genomes. Reference to the map ensures that regions containing repetitive DNA are assembled correctly. Distinctive features on the genome map are used as landmarks to aid assembly of master sequence from huge number of short sequences obtained from genomic library. First step for manipulation of genes for various applications is cloning that relies on mapping close to a convenient marker. A complete physical-genetic map of genome is necessary for comparative genomics studies with other genomes. High-resolution comparative physical maps will reveal regions of colinearity and rearrangement and will have important implications for phylogenetic studies and genome evolution.

TYPES OF GENOME MAP

There are different kinds of genome maps, which differ in techniques used to construct them and in the degree of resolution that is, the ability to measure the separation of elements that are close together.

The major types are cytogenetic maps, linkage maps and physical maps.

Cytogenetic Map

A cytogenetic map is produced by direct observation of stained or labeled chromosomes. Map units are given in fraction of a chromosomal arm or centiMcClintocks (cMc). The deletion-based cytogenetic map of an organism is a type of low-resolution physical map. It is constructed using a "genome deletion panel" which consists of a series (or collection) of mutant lines containing sub-chromosomal deletions and a reference wild-type line. In IL mapping a series of mutant lines contain sub-chromosomal substitutions and both reference parental lines.

Genetic Map

Construction of a genetic map requires genetic techniques such as cross-breeding experiments in case of plants or examination of family histories (pedigrees) as in case of humans. The genetic map is a representation of a meiotic-recombination map based on analysis of marker segregation in a population of offspring derived from a bi-parental cross. The resolution of genetic map depends upon the number of crossovers in a plant species that have been scored or large number of progeny in humans and other eukaryotes.

Marker polymorphism between the parents is required to monitor recombination among loci along a chromosome. There are three types of DNA markers that are useful for genetic mapping are RFLPs, SSLPs and SNPs. The QTL map is a type of genetic map, which indicates the approximate location of a quantitative trait locus (QTL) within an interval delineated by two or more markers on a genetic map.

Physical Map:

Unlike genetic map the construction of a physical map requires molecular biology techniques. A physical map of an organism is a representation of its entire genome as a set of overlapping cloned DNA fragments that make up a genome and ordered with respect to a reference map (such as genetic map). The resources for construction of a physical map are a high-density genetic map and large insert genomic library.

High Density Genetic linkage maps

The first RFLP map was constructed by **Donis**-Keller et al. (1987) for the **human** genome [1]. The map positioned one **RFLP** marker per approximately 10 million nucleotides. The map was constructed by studying **RFLP** in 21families over 3 generations. RFLP markers were then ordered on the chromosome following computational analyses of recombination .The various loci were arranged into linkage groups representing 23 human chromosomes. RFLP maps have not been restricted to human genome and have been published for most of major crops. Excellent molecular genetic maps of the tomato genome are available. For example, >1000 restriction fragment length polymorphisms (RFLPs have been located on a map that totals over 1276 cM [2].

Genetic maps based on variations in simple sequence repeats also enable generation of highly detailed genetic maps [3]. Single nucleotide polymorphisms (SNPs) are single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in a population. SNPs are most important sequence markers for mapping of genomes. They have the potential to provide greatest density of markers.

GENETIC MAPS VS PHYSICAL MAP

Genetic mapping allows localization of inherited markers relative to each other. It does not tell the actual distance in base pairs but provide an estimation of distance between loci in genome in terms of recombination frequency represented by cM. The resolution of a genetic map depends on the number of crossovers that can be scored. If genes (or markers) are very close, one cannot resolve their order, because the observed recombination frequencies will be zero. Because linkage map distances are not simply related to physical distances, physical mapping is needed to determine the locations of markers on chromosomes. Physical mapping reflect actual distances in base pairs. Restriction mapping, STS mapping are few of the mapping techniques developed to overcome limitations of genetic mapping. Construction of restriction map depends on comparing the fragment sizes when a DNA molecule is digested with two different restriction enzymes. However

this technique is more applicable to small rather than large molecules. STS is a short region of DNA about 200-300 bases long and unique in being represented only singly in genome. Maps based on sequence-tagged site (STS) landmarks provide greater coverage of the genome [4]. In organisms with large amount of repetitive DNA, the generation of random genomic STS sequence is time consuming. The partial DNA sequences termed expressed sequence tags (ESTs) are easier to generate and serve same purpose as genomic STS. Majority of genetic markers on RGP high-density genetic map represent ESTs [5].

LARGE INSERT GENOMIC LIBRARIES

Large insert genomic library are important in order to get a manageable number of clones covering entire genome**.** Several kinds of vectors are available, which can accommodate large size of DNA fragments. Some of these are as follows:

Yeast artificial chromosome (YACs):
The first major breakthrough in attempts to clone DNA fragments much longer than 50Kb came with the construction of yeast artificial chromosomes [6]. YACs are the vectors based on chromosomes rather than plasmids or viruses. In a YAC centromere, telomeres and origin of replication are linked with selectable markers and unique restriction sites. YAC have the potential to clone 600 Kb to 1.4 Mb sized DNA fragments. However Instability, high chimera frequency, and difficulties in manipulation and purification make YAC clones less than ideal substrates for genome sequencing.

Bacteriophage P1 vectors (Sternberg, 1990):
These are very similar to lambda vectorsand are based on a deleted version of a natural phage genome. The cloning capacity of Pi vertors is upto 125 kb [7].

Bacterial artificial chromosome (BACs; Shizuya et al., 1992):
Bacterial artificial chromosomes or BACs are based on naturally occuring F plasmid of E.coli. The F plasmid is a relatively large and vectors based on it have a higher cloning capacity for more than 300 Kb DNA fragments [8].

P1-derived artificial chromosomes (PACs; Ioannou et al., 1994):
These vectors combine features of P1 vectors and BACs and have capacity of up to 300 Kb [9]. Development of PACs and BACs cloning systems was pivotal to the success of the whole-genome map. They provided larger inserts, more stable clones and better coverage of the genome. Clone-based maps have been important in the sequencing of most large genomes, including those of human, (International Human Genome Mapping Consortium, 2001; International Human Genome Sequencing Consortium, 2001) [10] rice, Saccharomyces cerevisiae[11], Caenorhabditis elegans[12] and Arabidopsis thaliana [13,14]. A clone-based map also contributed to the sequencing of the Drosophila melanogaster genome [15] and a combined mapping and sequencing strategy was applied to the mouse genome [16].

Construction of the whole-genome PAC/BAC map:
Whole-genome PAC/BAC map relies on the identification of a tiling path composed of large-insert clones that spans a given genomic region with minimal overlaps. To construct clone based maps, PAC and BAC clones are screened for markers, the positive clones fingerprinted, integrated into the existing maps, and the largest, intact clones with minimal overlap is selected for sequencing. The general approach for screening genomic BAC and PAC libraries is by PCR or by probe hybridization using over go probes to identify clones corresponding to specific markers [17]. Overgo probes are made by filling in the single-stranded overhangs of two overlapping oligonucleotides using radiolabelled nucleotides and Klenow polymerase. Typically, two 24-mers overlapping by 8 bp are used to generate a radiolabelled double-stranded 40-mer.

Fingerprinting the BAC clones:
BAC clones are generally fingerprinted using the method described by Marra et al. (1997) [18]. Briefly, purified BAC clone DNA is digested to completion with Hind-III, and visualized on a SYBR-green-stained high-resolution agarose gel.

The fingerprint of each clone is formulated with IMAGE software based on the mi-gration distances of restriction fragments with extensive manual editing [19]. The Hind-III fingerprint data is subjected to overlap analysis using the Finger Printed Contig software package FPC version 4.7 [20]. The contigs are then assigned to chromosomes.

PHYSICAL MARKERS CAN BE PLACED ON CYTOGENETIC MAP USING IN SITU HYBRIDIZATION:

FISH is the most versatile and accurate method for the locations of chromosome-specific BAC clones, and the locations of repetitive and single copy DNA sequences FISH-based physical maps play an important role in advanced Genomics research including map-based cloning of agronomically important traits and whole genome sequencing. In this method, the DNA probe is labeled by addition of a reporter molecule. The probe is hybridized to a preparation of metaphase chromosomes in which the DNA has been denatured with formamide. Following hybridization and washing to remove excess probe, the chromosome preparation is incubated in a solution containing a fluorescently labeled affinity molecule which binds to the reporter on the hybridized probe. The preparation is then examined with a fluorescence microscope.

The non-specific binding due to repetitive sequences is eliminated by competitive suppression hybridization. Before the main hybridization, the probe is mixed with an aqueous solution of unlabelled total genomic DNA. This saturates the repetitive elements in the probe so they no longer interfere with in situ hybridization of unique sequences. In interphase nuclei, the chromatin is less condensed than in metaphase chromosome and hence provides a good target for high resolution FISH. The resolution of FISH is further improved by loosening the organization of interphase chromatin using concentrated salt, alkali or detergent treatment of cell preparations. These techniques are referred to as fibre –FISH.

Radiation hybrid mapping:

This method was developed to facilitate mapping of human genome. In this method a high dose of X-rays is used to break human chromosome into fragment. These fragments were recovered in rodent cells using somatic cell hybrids. The rodent –human hybrid clones are then examined for presence or absence of specific human DNA markers. The distances between markers are expressed in CentiRays where 1cR equals 1% frequency of breakage. (The International Radiation Hybrid Mapping Consortium 1999) [21].

Integration of maps generated by different methods:

Integration of genetic linkage map, and physical map or genetic linkage map and cytogenetic map is a prerequisite for characterization of a genome. An Important goal of the human genome project was to assemble fully integrated physical**,** genetic and cytogenetic maps for each human chromosome. For Rice genome sequencing project, both ends of every BAC clone insert in the HindIII and EcoRI libraries were sequenced to generate 110,438 sequence-tagged connectors (STCs) [22]. All 110,438 STCs were used to tentatively anchor contigs based on sequence homology with sequenced restriction fragment length polymorphism (RFLP) markers detected in-silico [23]. By this method, 418 rice genetic markers were associated with BAC end sequences with high confidence. The integration of high-density Bacterial Artificial Chromosome Fluorescence in Situ Hybridization (BAC FISH)-based cytogenetic map and genetic linkage map was done for full characterization of the tomato chromosome 2 [24].

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