Application of Genetic Engineering & Bioinformatics in Gene Therapy

B.Sc. Biotechnology -II year
Subject-Computational Biology & Bioinformatics

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Introduction

- Gene therapy involves repair or replacement of mutated genes.

- Genes expression can also be altered in order to regulate the immune system.

Gene therapy requires,

- Characterization of defective gene, cloning and their availability to be used in clinical study.

- Use of vector system for insertion of gene into the correct site in the patient body. Vector is used to carry DNA sequence into the target cells by physical delivery like inhalation, injection.

- Recombination between defective gene and inserted functional copy is necessary for expression.

Approaches for gene therapy

- Introduction of transgene into somatic cells of diseased tissue.

- Introduction into the reproductive cells – involve ethical implications

Ex vivo gene therapy

- Ex vivo gene therapy require removal of diseased tissues from patient body and insertion of correct gene outside the patient body.

- A mutation in LDL receptor gene on chromosome 19 is responsible for a genetic disease hypercholesterolemia which was treated by altering the expression of LDL gene by beta actin promoter from chickens and Cytomegalovius.

ldl receptor gene

- Normal ldl receptor gene regulates the cholesterol metabolism in blood, LDL receptors are abundantly occur at the surface of liver cells.

- A mutation in LDL receptor gene on chromosome 19, raise the level of LDL (low-densitylipoprotein) which is responsible for a genetic disease hypercholesterolemia. This makes slow cholesterol metabolism.

- Second case of application of gene therapy was Severe combined immune deficiency (immunodeficiency),a genetic disorder which was treated by ex vivo gene therapy

Figure 1: Mutations associated with ldlr gene are depicted; annotation is retrieved from NCBI dbSNP database.

Figure 2: Full annotation of LDRL gene retrieved from gene database.
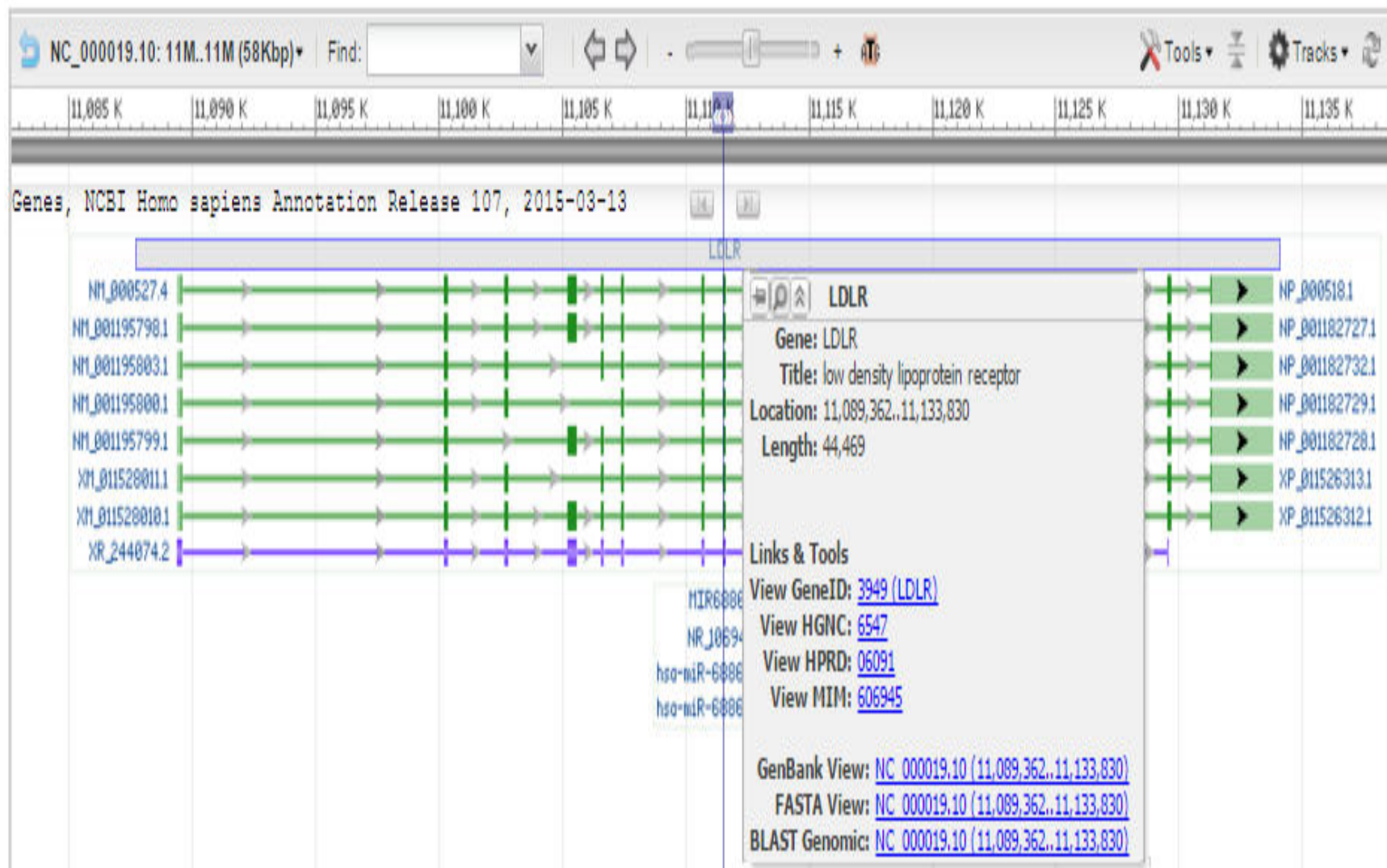
Adenosine deaminase deficiency (ADA)

- A mutation in a gene on chromosome X causes Adenosine deaminase deficiency (ADA) which reduces T and B lymphocytes significantly (Thrasher AJ, Gaspar HB, 2006).

- The gene was introduced into the LASN vector under the control of LTR(long terminal repeat) promoter. Vector also contains neo gene b expressed by promoter Simian virus promoter(SMV).

- A recombinant breed was generated from samples of two patients and introduced into retroviral vector by transfection, then modified cells were delivered into patient body(Gaspar HB, Bjorkegren E,2006.

In vivo gene therapy

- In vivo gene therapy requires the treatment of tissue at the location of affected organ.

- Duchenne Muscular Distrophy and Cystic Fibrosis are treated by in vivo gene therapy where lung, pancreas and skeletal muscles are affected.

Cont…

- In vitro therapy require the replacement of cells and tissues from body with new gene containing cells using transfection method.
- Both types of therapy require different vectors to introduce new gene into cells which depend on the gene and site of disease.

Dmd gene

- Expression of dmd gene using active CMV promoter (REF) and delivery of transgene is done using rAAV vector with ITR sequence .[135]

- non-viral plasmid DNA and CFTR gene-liposome complex is used to deliver Cftr transgene.

viral, non-viral and naked DNA vector

- Various viral, non-viral and naked DNA vector and vehicle system are used to deliver transgene into the patient body.

- Liposomes, Lipoplexes and polyplexes are example of non-viral vector system.

- Mutation greatly affects the expression of gene and produces disease; expression of these defective genes may be interrupted by RNA interference.

A. Bioinformatics Applications: Databases and Information resources

- **Genetics Home References https** (//ghr.nlm.nih.gov/gene): a Genetics home reference provides useful information in the form about all the gene and product which are involved in disease. Gene orthologs, Genomic regions, transcript, SNPs, organism and Mutations

- **Gene (http**//www.ncbi.nlm.nih.gov/gene/: Gene database provide information about function of gene, integrated from Reference sequences, Maps, Pathways, Variations, Phenotypes, links to Genome , phenotype

Databases and Information resources

- **OMIM (Online Mendelian Inheritance in Man)**(www.omim.org):All the abnormal traits listed in this database with information about Genetic disorder, mutation, Mapping, Gene-Phenotypic, allelic variants

- **dbSNP (Single Nucleotide Polymorphism)**http://www.ncbi.nlm.nih.gov/snp/: Single Nucleotide Polymorphism, insertions/deletions, microsatellites, and non-polymorphic variants.

Databases and Information resources

- **UniGene:** http://www.ncbi.nlm.nih.gov/unigene/: Analyses expression by tissue, age and health status and provide the information about this.

- **Geo** (gene expression profile)http://www.ncbi.nlm.nih.gov/geoprofiles/ : Contain information about specific gene Profile derived from microarray data. Microarray data can also be downloaded from these resources.

B. Micro Array data analysis

- Gene expression profiling using microarrays is used to identify the sequence variants synthesised by splicing.

- Gene expression profile contains the expression of individual gene in different condition whereas sample profile contains the expression of the entire gene in a particular environmental condition.

- Supervised and unsupervised methods are used to analyse the microarray data.

Unsupervised learning

- In unsupervised learning, similar patterns are searched in gene expression profile while supervised method uses the previous data to classify the genes.

- Unsupervised method include Hierarchical clustering principal component analysis(Hastie T, Tibshirani R,2003), self-organizing maps (Sultan M, Wigle DA,2002), multidimensional scaling (Bittner M et. al.2000, Mischel PS,2003), and singular value decomposition (Kluger Y,2003).

Supervised learning

- Supervised learning use predefined class and build the model, first training set is prepared using predefined class to predict gene classifier , this classifier is then used to classify the test data(Simon R,2003)

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