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Out-Lab Therapy Approach Based on Elected A Restriction Enzyme to Transfer Target Gene

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Abstract

An important approach of therapy the target gene sequence causes diseases via repair/recombine the mutated gene (gene transfer) using a restriction enzymes in the laboratory. This approach will cause multiple problems happening accompany to biological laboratory if ruled out problems outside of it like the digested DNA ran as a smear on an agarose gel, incomplete restriction enzyme digestion, extra bands in the gel, etc. The paper suggested new approach of therapy via repair/replacement mutated gene caused disease by detecting primers and finding restriction enzymes using bioinformatics tools, software, packages etc. then achieving the repair/ recombine of mutations before going to the biologic lab (out-lab) to avoid the problems associated these laboratories. Implement and apply this a proposed therapy approach on TP53 gene (which caused more than 50% of human cancers) and after confirming there is mutations on P53 tumor protein shows an effective cost, friendly therapy methodology and comprehensive.

Keywords — Therapy, Gene Transfer, Target Sequence, Polymerase Chain Reaction, Primers, Restriction Enzymes, P53 Tumor Protein.

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1. INTRODUCTION

THERAPY of mutated gene caused diseases may be done by replacing the damaged gene with another non-damaged using restriction enzymes and that needed to scan along a DNA looking for a particular sequence of bases in general from 4-6 base pairs in length within laboratory that will take approximately 3 days. The restriction digestion in the lab takes place overnight and can be kept in the freezer until the next class period when it will be used for gel electrophoresis, the gels may be stained overnight prior to
photographing or recording results.

n this pargraph will explain briefly some terminologies like DNA template is sample DNA contains the target sequence (means the gene sequence which needed therapy). DNA polymerase a type of enzyme, which is synthesizes new strands of DNA complementary to the target sequence. PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the offered template strand. Primers - short pieces of single-stranded DNA that are complementary to the target sequence [1].

Process of gene transfer explained in following steps:

- Isolation of gene and vector (by PCR): Fig. 1 shows this task.

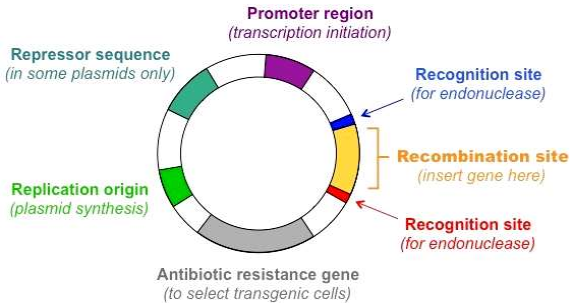

Fig. 1. Reveals Isolation of gene and vector.

Digestion of gene and vector (by restriction endonuclease): Both must be cut with restriction enzymes at specific recognition sites as show in Fig. 2

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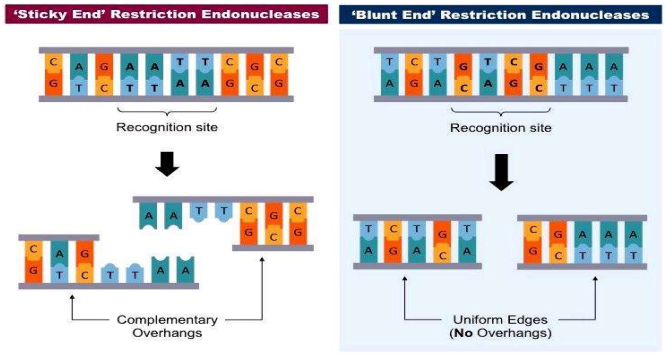

Fig. 2. Shows Digestion of gene and vector.

- Ligation of Vector and Insert (by DNA ligase): Fig. 3 shows a format of a recombinant construct.

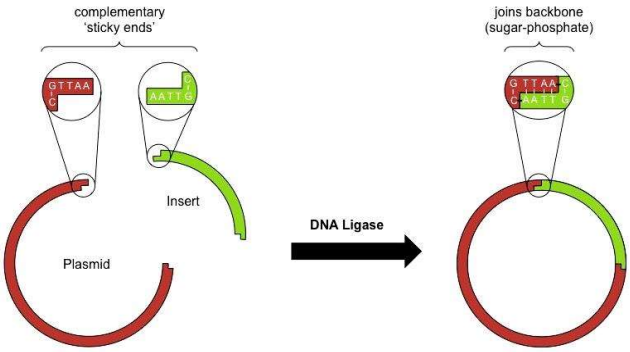

Fig. 3. Reveals Ligation of Vector and Insert.

Many of problems happening accompany to biological laboratory if ruled out problems outside of it like finding genes, primers, diagnosis and classify mutations, etc. Some of important problems are [2]:

1) Extra bands in the gel that may cause if larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate and partial restriction enzyme digest.
2) No transforming that causes restriction enzyme(s) didn't cleave completely.
3) Partial restriction enzyme digestion will cause cleavage is blocked by methylation, Inhibition by PCR components, too few units of enzyme used, addition may lead to presence of slow sites, DNA is contaminated with an inhibitor, etc.

4) Digested DNA ran as a smear on an agarose gel this causes the restriction enzyme(s) is bound to the substrate DNA.

So needed to integrate new approach for replacement a mutated gene (which caused diseases) via candidate restriction enzymes that done by employing programs, online took and software (supported bioinformatics tools) can produce integrated therapy approach outside the laboratory to avoid the problems related.

2. RELATED WORK

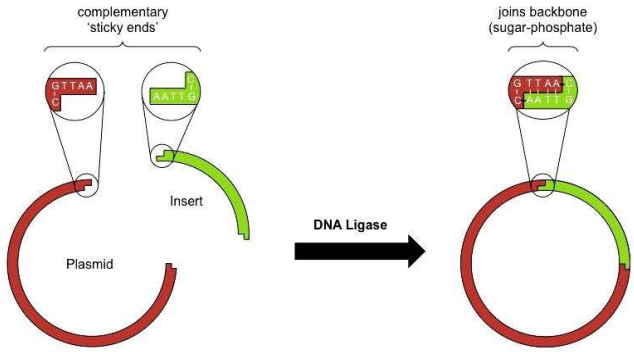

Fig. 3. Reveals ligation of Vector and Insert.

Colosimo A, Guida V, Antonucci, and others [2007], reveals gene therapy has been proposed as a definitive cure for □-thalassemia applied a gene targeting approach, based on the introduction of small DNA fragments (SDF) into erythroid progenitor cells, to specifically modify the □-globin gene sequence at codon 39. The strategy was first tested in normal individuals by delivering mutant SDF that were able to produce the □39 (C=>T) mutation. Secondly, wild-type SDF were electroporated into target cells of □39/□39. □-thalassemic patients to correct the endogenous mutation in both cases, gene modification was assayed by allele-specific polymerase chain reaction of DNA and mRNA, by restriction fragment length polymorphism analysis and by direct sequencing. Unfortunately, the number of corrected cells remaining from each experiment was insufficient to carry out protein studies by MALDI-TOF analysis. More importantly, improvements in delivery approaches

and/or design of SDF are clearly required to yield sufficient quantities of corrected cells for a significant therapeutic benefit. [3].

Owen T. M. Chan, MD, PhD, Kenneth D. Westover, and others [2010], suggested methods that assay hemoglobin β-globin chain variants can have limited clinical sensitivity when applied techniques identify only a predefined panel of mutations. Even sequence-based assays may be limited depending on which gene regions are investigated. They sought to develop a clinically practical yet inclusive molecular assay to identify β-globin mutations in multicultural populations. The paper highlights the β-globin mutation detection assay (β-GMDA), an extensive gene sequencing assay. The polymerase chain reaction (PCR) primers are located to encompass virtually all hemoglobin β locus (HBB) mutations. In addition, this assay is able to detect, by gap PCR, a common large deletion (Δ619 base pair), which would be missed by sequencing alone. We describe our 5-year experience with the β-GMDA and indicate its capability for detecting homozygous, heterozygous, and compound heterozygous sequence changes, including previously unknown HBB variants. The β-GMDA offers superior sensitivity and ease of use with comprehensive detection of HBB mutations that result in β-globin chain variants. [4].

The drawbacks of these methods (If you do not take into account the determinants of their own), implementing the therapy of mutation gene caused diseases done within laboratory and as referring to in section (1) there are multiple problems associated to the lab. The motivation overcome those drawbacks in previous techniques to reach a new therapy methodology, can replacement a mutated gene by predict the restriction enzymes based on bioinformatics tools and software then later can go to biological laboratory to implement the plan of replacement mutated gene via CAT methodology after classifying mutations at protein (not only in its gene) too because “two sequences may have big differences in DNA sequence but have similar protein” [5, 6].

3. PROPOSED THERAPY APPROACH

The proposed therapy approach started with find the gene (Homology normal gene) using NCBI (DNA template). after that diagnoses and classify there is mutation, If GC% content excepted then continue, else return from start to find another DNA Diagnosis and

Classifying is there mutations at Person's gene and at protein too using ClustalW within BioEdit. The algorithm for therapy approach as PCR application shows as follow:

4. EXPERIMENTAL RESULTS

The implement and applied of proposed therapy approach (shown in subsection 3) on mutated TP53 gene and confirm stile there is mutation on the tumor protein P53 (which caused more than 50% types of human cancers) explained as follow:

Algorithm of Proposed Therapy Approach

Input: DNA template includes sequence of gene target, and sequence of mutated gene for the Person holds disease.
Output: Recombine gene of the patient holds disease via predicting of restriction enzymes.

BEGIN
Step 1: Determine the primers of normal gene via package, program, etc to avoid them when used the restriction enzymes.

Step 2: Predicting restriction enzymes using program of Analyze Sequence.

Step 3: While (there is mutation) do:

i. Selecting the adjacent enzymes (around the mutilation which needed replacement).
ii. Obtaining the foreign (Homology Normal) gene sequence to replace a mutated gene for Person.
iii. Expected replacement mutated gene using restriction enzymes which predicted in (a) above.

End While-do
END

Sequence.

A. Tools and Software are Required

The tools and software needed to implement and applied the proposed therapy approach for replacement/ recombine is summarised in:

1) Searching a NCBI for nucleotide (DNA sequence) via the gene name to obtain the DNA template (TP53 gene) then saved as FASTA format.
2) Determine unmuted TP53 Gene (Homology Normal Gene) using COSMIC/GENSCAN Web Server at MIT within the gene sequence which obtained at (1) above, will paste as shown in Fig. 4, A; this package can be extracted the normal TP53 gene (target sequence) and save it (in FASTA file) as shown in Fig. 4, B [8].

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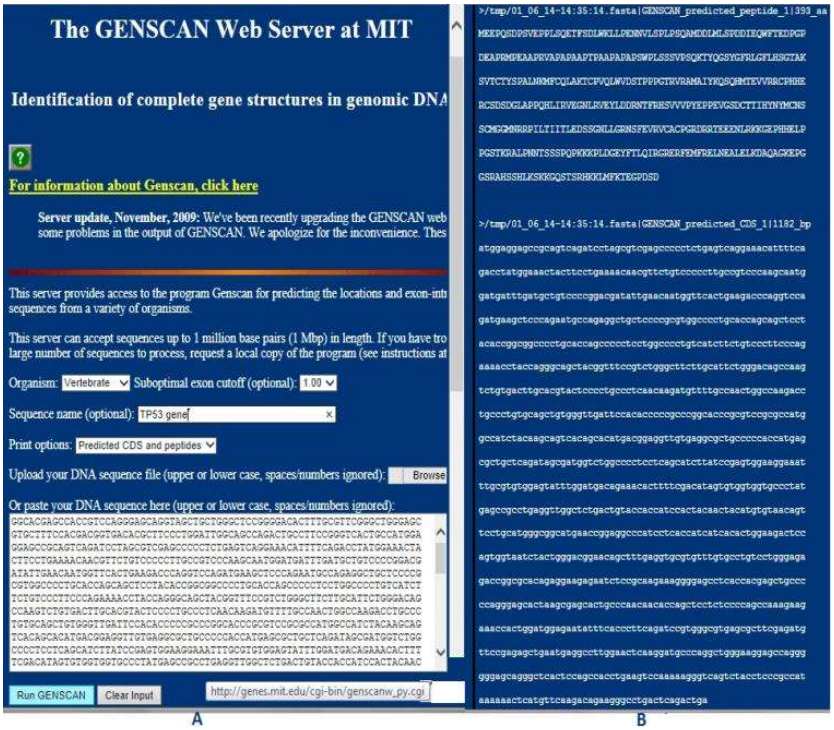

Fig.4. Shows extract target TP53 sequence via GENSCAN

3) The FASTA file which obtained in steps (1 and 2) will use in ClustalW to display result of alignment, i.e. diagnosis there is malignant mutations by comparing the normal TP53 gene sequence with one sequence (or more than one) TP53 gene sequences for persons at the same time. That is done using BioEdit by selecting Accessory Application→ ClustalW Multiple Alignment→ Run ClustalW, then obtained the result whether there is malignant mutation or not, and as example will suppose mutation at codon 349 in TP53 gene for certain Person comparing with normal TP53 gene shown mutation on that codon.

This classification on (3) above not enough as referring to in section (2) [5, 6], so needed to transform the normal homology TP53 gene to tumor protein P53 as well as TP53 gene for the Person transform to the tumor protein P53 then using the same tool ClustalW via BioEdit package for diagnosis still there is mutation (in level of proteins) or not. In this experiment shows the mutation at codon 349 (GAA→ TAA) when transform to tumor P53 will transformation from (E) to (stop) at Person's P53 gene. This confirm will classify

in protein level in this mutation (position, which discovered) may cause lung cancer, head or neck cancers based on the database (UMD_Cell_line_2010) of TP53 website as modern and comprehensive database (URL: http://p53.free.fr/Database/p53_MUT_MAT.html [9]).

B. Applied the Algorithm of Therapy Approach

This subsection applies the algorithm of therapy approach as following using the mutated TP53 gene (of Person) and the normal TP53 gene as referring to them in (A) above:

1) Step 1: determine the primers at normal TP53 gene (target sequence) that is done using BioEdit by selecting normal TP53 gene→ BLAST NCBI→ then select Primer-BLAST→ paste the FASTA sequence of normal TP53 gene, finally will obtain the primers determines in (10), some of them shown in Fig. 5.

2) Step 2: Predict restriction enzymes via program of Analyze Sequence to select adjacent enzymes (around each mutation of mutations at TP53 gene), can obtain by open Analyze Sequence → paste the FASTA sequence.

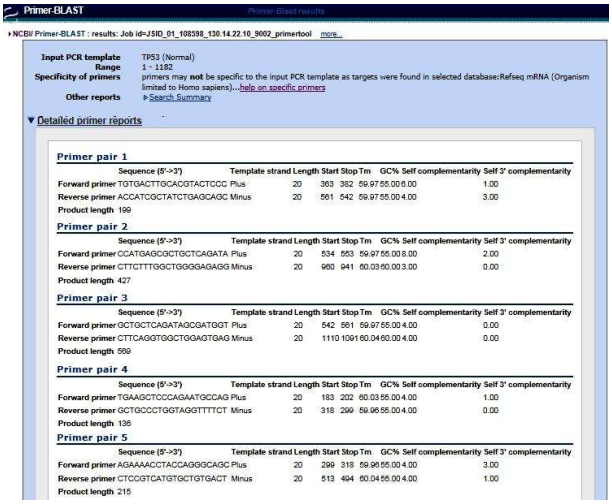

Fig. 5. Reveals part of extract primers using NCBI-BLAST

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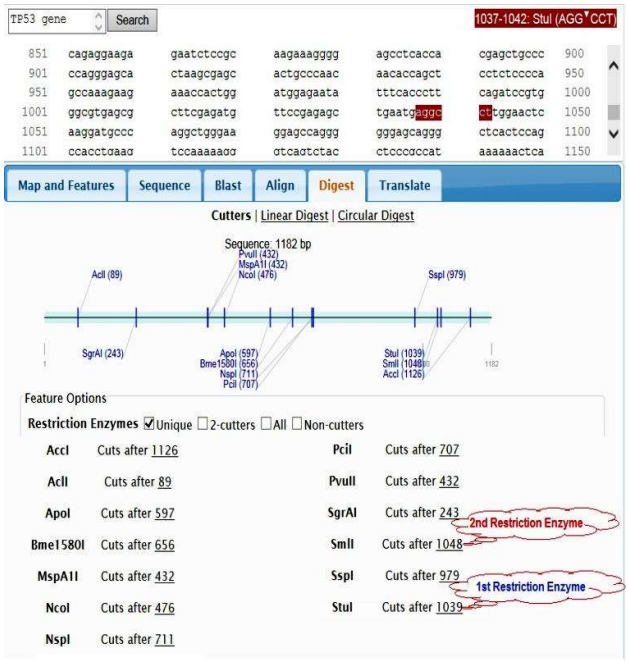

Fig. 6. Shows part of restriction enzymes at normal TP53 sequence

2) Step 3: Multiple steps required to implement continuity while there is mutation in TP53 gene as follow:

i. Selected the restriction enzymes (using program of analyze sequence), which are around (adjacent) the mutation at codon 349 in TP53 gene are the Eco147I (StuI) restriction enzyme recognizes AGG^CCT sites (Blund end) and cuts best at 37°C in B buffer (Isoschizomers: AatI, PceI, SseBI, StuI). While the second restriction enzyme recognizes is SmoI (SmlI), where C^TYRAG sites (overhang) and cuts best at 55°C in Tango buffer (Isoschizomers: SmlI) [10, 11].

ii. Obtained the foreign TP53 sequence (without mutation and not primer) (AGGCCTTGGAACTCAAG) will use for replacement the mutated TP53 sequence of Person (AGGCCTTGTAACTCAAG) as shown the mutation at codon 349 (in red), while restriction enzymes (in blue).

iii. Fig. 7 reveals an expected procedure in biologic lab (before going to the laboratory) to replace PT53 gene of Person which has mutation at codon 349 with foreign TP53 sequence (Normal gene).

C. Discussion the Results

3) Table 1 shows comparing the results of proposed therapy approach of replacement mutated gene with other methods.

5. CONCLUSIONS

The proposed therapy approach of replacement mutated genes via predicting the restriction enzymes out-lab shows the following conclusions:

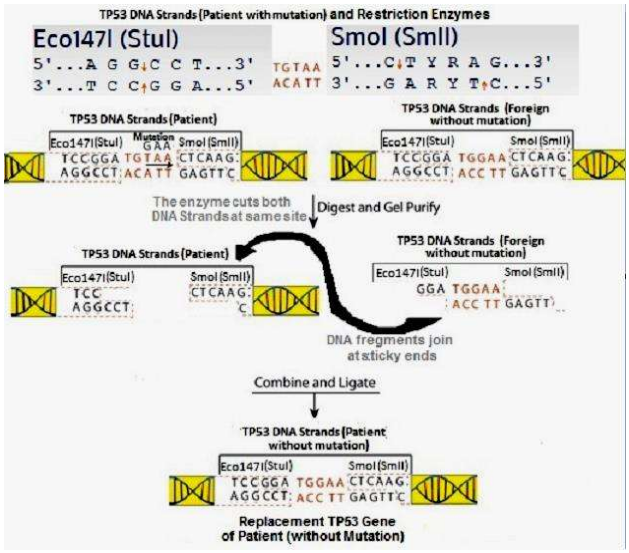

Fig. 7. Reveals an expected procedure of replacement TP53 gene by foreign sequence in biologic laboratory.

TABLE I
SHOWS THE COMPARISON OF PROPOSED THERAPY APPROACH WITH OTHER TECHNIQUES/METHODS

Proposed Therapy Approach	Method of Colosimo A, and et al. [3]	Method of Owen T. M. Chan, and et al. [4]
Integrity the proposed therapy approach out the lab starting with finding DNA template, target gene, mutations in gene and its protein, primers; finally replacement mutated Person's gene causes disease.	**Focus on doing these operations within lab**	**Focus on doing these operations within lab**
Predict the restriction enzymes via bioinformatics tools, software, programs, etc.	Not applicable	Not applicable
Effective cost for analysis, predict mutating gene and restriction enzymes, etc may reach about $3000 [6].	**Doesn't has this effective-cost**	**Doesn't has this effective-cost**

The following conclusions:

a) Proposed therapy approach will classify the mutations at Person's TP53 gene and confirm with its P53 tumor protein, and then start the process of replacement/correction the mutated gene.

b) The proposed therapy approach offers friendly diagnosis for mutations at gene causes disease and predicting restriction enzymes, and finally replacement mutated gene for Person has diseases (e.g. cancer) as referring to in Table 1 Can use this therapy approach by researcher or any other person interested, who needed to therapy malignant mutations gene and its' protein caused diseases.

c) That therapy approach reveals the finding of target gene from DNA template, detecting primers, predict restriction enzymes, etc. before going to biologic lab make reduce to the problems associative to laboratory which referring to them in section 1.

d) As future works:

- Using the Cas-Analyzer as tool in CRISPR RGEN [12] to simulate the implementation of the replacement/recombine of mutated gene (as referring to it in subsection 4; B; 3; iii and shown in Fig. 7) instead of keeping it manually.
- Create database based on the proposed therapy approach for all mutations (in codons) of TP53 gene and their TP53 tumor protein caused diseases (50% of human cancers) addition to any other important genes like breast cancer (BRCA1 and BRCA2 genes). This database will allow mining technique and flexible retrieving the results related in replacement a mutated gene using predicting restriction enzymes.

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