Psychiatry and Mental Health 2021: In vitro site specific DNA editing via restriction enzyme- Umair Masood- COMSATS University, Islamabad

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Abstract

The most important gift your mother and father ever gave you was the two sets of 3 billion letter of DNA but like anything 3billion components that gift is fragile. Sunlight, Smoking, unhealthy eating even spontaneous mistake by your cell all cause changes to your genome. There are different types of technique to recover to your genome one of the immortal technique is CRISPR cas9 is a tool for cutting DNA research. There are two main parts of CRISPR cas9 system a DNA cutting protein called cas9 protein and guideRNA bound together and form a complex that can identify and cut the specific section of DNA. Restriction digestion is a process in which DNA is a cut at a specific site the main and most important powerful property of Restriction enzyme is they can bind and cut only the double stranded DNA.If we have a DNA having particular site or gene is cover with short fragment of the DNA the particular restriction enzyme according to the gene sequence act only the short fragment of the DNA and created a break at the place of interest. There are two components of in-vitro site specific DNA editing system a DNA cutting enzyme called Restriction enzyme and short fragment of the DNA know as guided that can identify the double stranded DNA and cut specific section of the DNA Steps of DNA editing via restriction enzyme and protocol : • DNA denature is a process in which DNA is heated at 90 °C and double stranded DNA is unwind and the hydrogen bonds between the two strand is weak and broken • Restriction digestion is a more important or key step of this technique because they can find a short fragment of DNA which is attach to the Template DNA and cleave only the double stranded DNA and rest of the sequence could not be cleave because rest of the sequence is single stranded. Restriction digestion protocol: Material: • Centrifuge • Incubator • Water • Buffer • BSA • DNA template • Restriction enzyme • Incubator Procedure: 1. Defrost all reagents on ice. 2. collect reaction mix into 50 µL volume in a micro-centrifuge tube 3. Put-on reagents in following sequence: water, buffer, BSA, DNA template, restriction enzyme 4. slowly mix by tapping the tube. in a short centrifuge to settle tube contents. 5. Produce negative control reaction without template DNA 6. Produce positive control reaction with template of known cutting site corresponding to The restriction enzyme of option 7. Representative Incubation time and temperature is 37°C for 1h 8. Incubation time temperature is 65°C for 20m • Short fragment of DNA is most commonly 50 base pair long and by altering the 6- base pair mostly. There is different standard method for guided DNA synthesis like: oligonucleotide synthesis, endonuclease-mediated assembly, annealing base connection of oligonucleotide. Oligonucleotide synthesis is a method of relatively short fragment of nucleotide with defines sequence. The production of oligonucleotide the necessary bases adenine, thymine, guanine, and cytosine is added the synthesis is a computer control process that repeats several steps until full length product. Finally, the concentration of each oligonucleotide adjusted. The mixture of oligonucleotide is than

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provided gene synthesis lab. Oligo lab is assembled into the gene of your choice assembly by PCR (Polymerase chain reaction) Result in Snap gene tool: • First, we can recognize the restriction site by using snap gene. The gene is MSTN gene we want to knockout or edit this gene. • Then design a short fragment of the DNA for the both end according to the restriction site in the givengene we want to select a SWAI and DraIII restriction enzyme. We can used both restriction site or one restriction site the choice is yours. • For SWAI short fragment: TAAATTACAATAAGAGTTGTGTGAGGATTAGTAA GATTTAAGTACAGTTT • For DraIII short fragment sequence: CGTGCAGGTTTGTTACATATGTATACATGTGCCA TGTTGGTGTGCTGCAC • we can perform an In Silicon gel electrophoresis we can cut the gene by two enzyme SwaI and DraIII and the result we can get two fragment of the DNA 345 and 291 bp and the total base pair of the given sequence is 8230 which mean that we can knock out the gene successfully.

Bottom Note: *This work is partly presented at 34th World Neuroscience and Neurology Conference Feb 27 2021*