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DNA Fingerprinting and CRISPR cas9 System

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Abstract

Leicester university geneticist Alec Jeffrey's develops a technique called DNA finger printing in 1985 it allows the DNA sample from different people to be compared look for similarities and differences. It used the solving crime and can confirm if the people are related to each other like paternity testing. There is section of chromosomes where an instead of gene consisting of a long sequence of bases, they are usually (15-100) base pairs long that are repeated in many times these repeated sequences called Variable Number of Tandem Repeat. We can know the VNTR sequence of any person than we can design a gRNA. Let's say you have a DNA sample with fluorescent labeled from victim and you want to make sure that VNTR sequence you are interested is in match with victim. We can design a CRISPR to scan through DNA or find specific VNTR. The CRISPR scan the DNA if the CRISPR does not find targeted VNTR it does not bind to it its means that no fluorescence color appears under UV-light but its scan and find its target and this binding create a fluorescence signal its means that VNTR can be occur in a DNA..

Keywords: DNA finger printing; CRISPR cas9; UV-light; VNTR sequence; Fluorescence color

Introduction

DNA Fingerprinting System Parts

CAS9-Protein

Cas9 protein is also called destructive protein. Its main function is to cut DNA and thereby alter a cell's genome [1-5].

Guide-RNA (VNTR)

The main part of our technique is gRNA or VNTR the guide RNA is a specific that recognizes the target DNA area of interest and directs the Cas nuclease there for editing (Figure 1).

Template DNA

The template DNA is our target DNA that we want to check either Specific STR is present or not.

DNA Fingerprinting and CRISPR Cas9 Protocol

The protocol contains two steps:

- Labeling of Template DNA
- CRISPR cas9 working protocol

Labeling of Template DNA

Reagent requires

- DNA dye 70 µl
- Buffer 1:275 µl
- Buffer 2:255 µl
- AT rich DNA:400 µl
- GC rich DNA:400 µl
- 50:50 at and GC:500 µl
- 100 NAOH 650 µl

Procedure

- Label the tubes 1,2,3 and 4
- Add 10 µl buffer to each tube
- Add 10 µl dye to each tube
- Add 5 µl of DNA sample to tube 1,2 and 3
- Do not add an any DNA to tube 4 because tube 4 is serve as negative control
- Mix the regent by pipetting up and down 3-4 time
- Enter the heat block parameter such as 95°C for 2 min
- Run a PCR
- View under UV-light

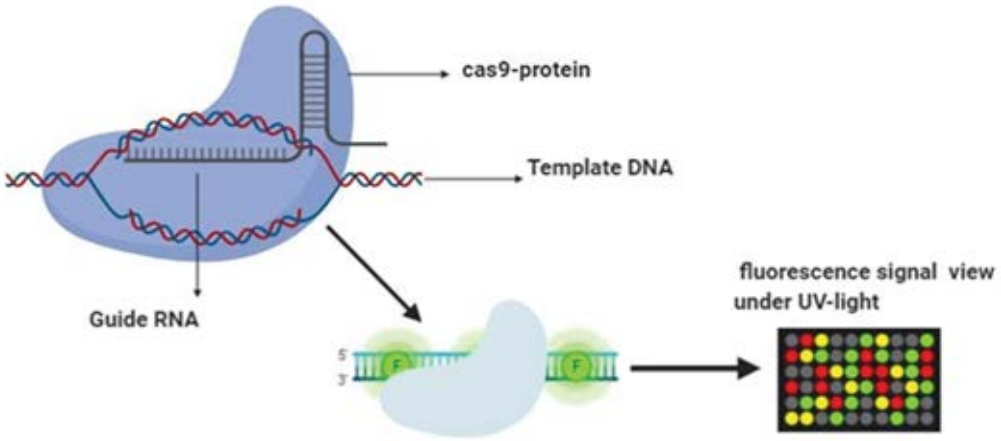

Figure 1 Cs9 bind and cut the desire sequence and emit a fluorescence signal.

CRISPR Cas9 Working Protocol

Procedure

Table 1: a): Position a 30 µl reaction along micro centrifuge tube on ice with the following sequence; b): Softly mix the reaction mixture and centrifuge it; c): Incubate at 37°C for 20min; d): View under UV-light

Component	20 µl Reaction
Template DNA	x µl (~100 ng)
Guide RNA	x µl (~4000 ng)
10 X Cas9 Reaction Buffer	3.0 µl
Cas9 Nuclease	1.0 µl
water	30.0 µl

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