Kallikrein siRNA: Biomarker for Prostate Cancer

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Abstract

Prostate-specific antigen (PSA) is a protein found in the prostate; it can also be found in the blood of men who may have or may develop prostate cancer. A PSA test can be performed on a male to diagnose the possibility of prostate cancer, depending on the PSA level in the subject's blood.

PSA Kallikrein siRNA has been used to investigate the difference in the acquiring of prostate cancer. For amplification of a certain DNA sequence in Kallikrein siRNA, we performed a polymerase chain reaction followed by a ligation to place the plasmid DNA into a vector. We then amplified this Kallikrein siRNA with the vector by transforming them into E. coli cells. After a purification of the DNA using a Qiagen Miniprep kit, the sample was restriction digested with Xho1 enzyme to ensure that the vector was present. After this, we sequenced the DNA. The sample can now be used for further experimenting and analysis.

Since mice do not get prostate cancer, we had to inject the rodents with the wild-type PSA—PSA found in men. Mice with the Kallikrein siRNA were compared with those injected with the wild-type PSA. The two sets of transgenic mice are going to be analyzed to see how fast and how differently they acquired prostate cancer.

Procedures

Polymerase Chain Reaction

Ligation

Transformation

Miniprep

Restriction Digestion

Gel Purification

Sequencing

Analysis

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is a technique use to amplify an amount of a specific DNA sequence. We only need a relative small amount of double-stranded DNA as template (as little as one molecule!). DNA sequences can be correctly amplified quickly since the amount expands exponentially. Two primers are also required, as well as polymerase.

The two primers used in PCR are regarded as forward and reverse primers. These are essentially made up of nucleotides (bases) which are complementary to a specific DNA sequence.

Starting from the primers, the polymerase synthesized the DNA by adding nucleotides to the primer which are complementary to the DNA template. The polymerase reads the information from the 3' end to the 5' end of a DNA chain and synthesizes the new DNA chain in the 5'-3'direction. It is worth mentioning that DNA is made up of two strands (coding strand and antisense strand) that have a 5'-phospho group at the nucleotide that starts the strand and a 3'-hydroxyl group at the ending nucleotide. The 5' end of the coding strand is attached to the 3'end of the antisense strand and vice versa.

In our case, we used Kallikrein siRNA, as our DNA template, a forward primer, a reverse primer, water, and Hotstart (reaction buffer). The recipe is as follows:

1). mix:
- 12.5 µl Hotstart
- 9.5 µl water
- 1 µl HK2 siRNA
- 1 µl forward primer
- 1 µl reverse primer

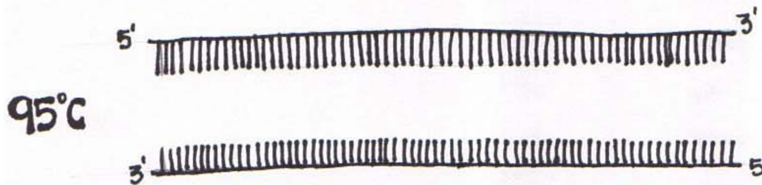
2). Place mixture in PCR machine and set to the following temperatures and times:
a- 95°C for 5 min
b- 95°C for 30 sec ←*the two template DNA strands are separated so the primers can be attached*
c- 55°C for 30 sec ← *the primers anneal (attach) to template*
d- 72°C for 1 min ← *the polymerase comes in and synthesized new chain*
e- 72°C for 10 min ← *stabilization*
f- 4°C for storage ← *to keep PCR products from degrading*

Note: for more PCR products, steps b, c, and d are repeated. In the case of HK2 siRNA, we had 30 cycles.

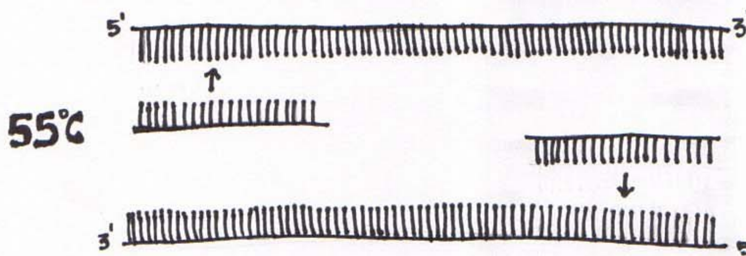
(Illustration shown on next page.)

Polymerase Chain Reaction

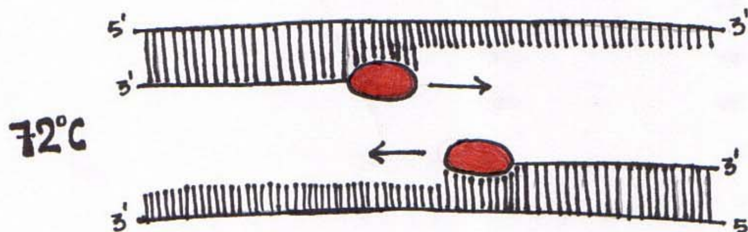
desired DNA fragment (template)



template strands separate



primers attach to strands



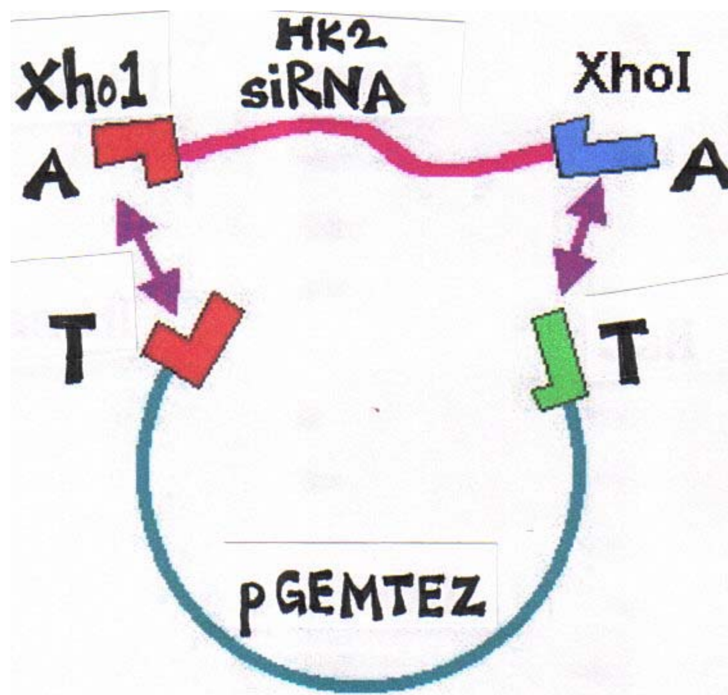
Polymerase attaches and elongates primers

* Repeat 30 times

Ligation

The term ‘ligation’ means “a process of uniting of connecting.” This is precisely what happens in DNA ligation. A linear DNA is introduced into, and connected with, a vector. In our case, we introduced HK2 siRNA (which we now have a large amount of thanks to PCR) into a pGEMTEZ vector. We achieve this in adenine (A) and thymine (T) restriction sites. The bases have an Xho1 restriction enzyme attached to these bases. The Kallikrein siRNA ends have the A bases while the pGEMTEZ vector has the T nucleotides. Since both bases complement each other, they attach. Why do we do this? We ligate the Kallikrein siRNA to a vector so the plasmid’s gene can be expressed. For Kallikrein siRNA, we had a mixture sit at room temperature for one hour. The components of that mixture are as follows:

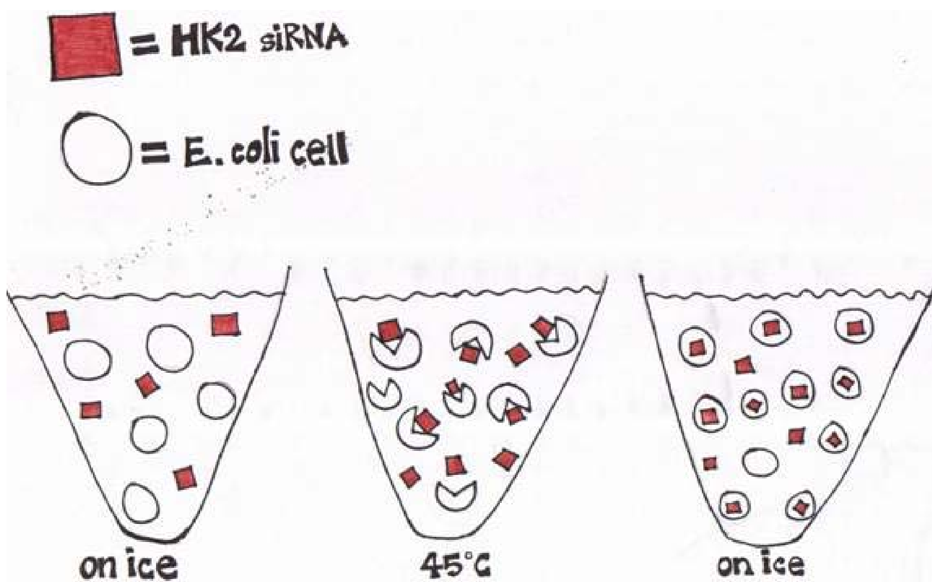
- 3 µl Kallikrein siRNA insert
- 5 µl 2X buffer
- 1 µl pGEMTEZ vector
- 1 µl T4 ligase



Transformation

Now that the Kallikrein siRNA has been ligated into the pGEMTEZ vector, we have a problem: there is just a small amount of this ligated product. This can be easily solved by performing transformation. Transformation is the genetic modification of bacteria by insertion of another DNA. In the case of Kallikrein siRNA, we introduced this DNA into E. coli cells. Just as in PCR, the success of transformation relies on specific temperatures. The steps to transformation are as follows:

- place 2 µl Kallikrein siRNA in 50 µl E. coli
- incubate mixture on ice for 30 min.
- heat shock sample at 40°C for 45 sec ← *E. coli open and plasmid enters*
- place on ice ← *E. coli shrivel and plasmid becomes secured in the bacteria*
- add 950 µl LB media ← *nutrient for cell proliferation*
- shake at 37°C for 1h ← *cells multiply*
- place 200 µl of mixture on culture plate w/ LB agar and ampicillin ← *cells further proliferate but only those which are ampicillin- resistant. Amp- resistance ensures the presence of the HK2 siRNA in the bacteria.*
- incubate o/n (overnight at 37°C) ← *amp-resistant bacteria multiply*

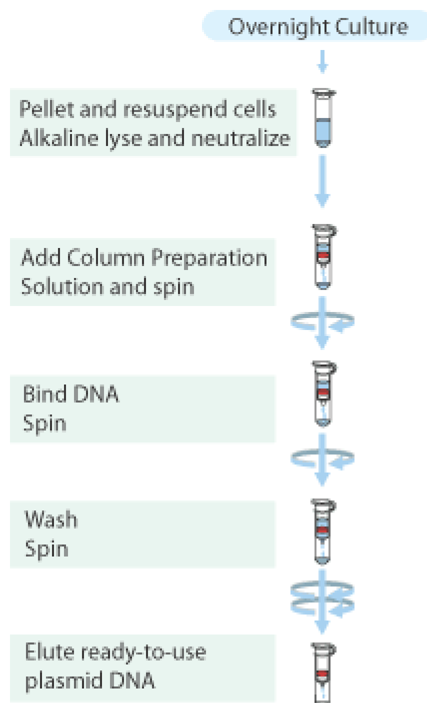


E. coli cells that have undergone a transformation →

Miniprep

The Miniprep technique is used to purify plasmid DNA. In our case, we purified the Kallikrein siRNA (still in the pGEMTEZ vector and in the E. coli) to get rid of the E. coli. We used the bacteria solely for the purpose of proliferating the Kallikrein siRNA. Now that the bacteria did its job, we get rid of the E. coli through Miniprep. We handpicked the transformed colonies from the LB agar and amp plates that were grown overnight. We dumped these colonies into 3 ml of LB and Ampicillin culture and grew them overnight. The steps to Miniprep are:

- spin colonies with the culture on 13,000 rpm for 30min ← *bacterial pellet forms*
- resuspend bacteria in Buffer P1
- add 250 µl Buffer P2 ← *opens the E. coli and releases the plasmid DNA and unwanted proteins*
- add 350 µl Buffer N3 and centrifuge for 10min. at 13,000 rpm ← *N3 will cause the proteins to precipitate out of solution*
- place new pellet in spin column
- wash column with Buffer PE ← *PE contains ethanol which will wash plasmid while still keeping the plasmid stuck to the column*
- elute Kallikrein siRNA with Buffer EB or water ← *EB and water have very low salts which aids in the release of the plasmid from the column*



Restriction Digestion

After the ligation was completed, the Kallikrein siRNA (now purified and in the pGEMTEZ vector) underwent a restriction digestion using Xho1 as a restriction enzyme. A restriction digestion is a technique used to cut DNA molecules into smaller fragments by using restriction enzymes. We undergo this process to ensure that the pGEMTEZ vector has in fact the Kallikrein siRNA insert. The steps were as follows:

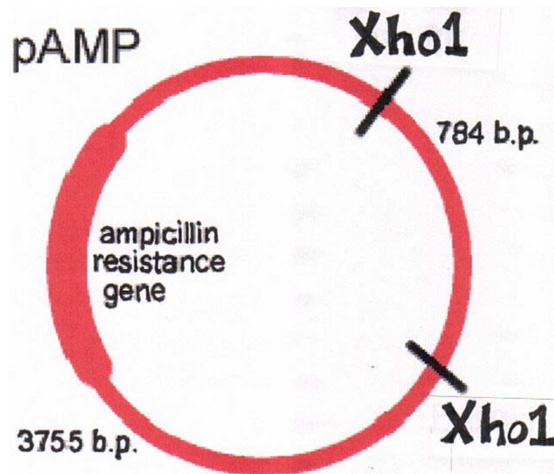
1). mix:
- 23 µl water
- 3 µl 10X Buffer H
- 3µl of Kallikrein siRNA
- 1 µl of Xho1 ← *only small amount needed since it doesn't get digested*

2). Incubate for 1h at 37°C (optimal temperature for Xho1).

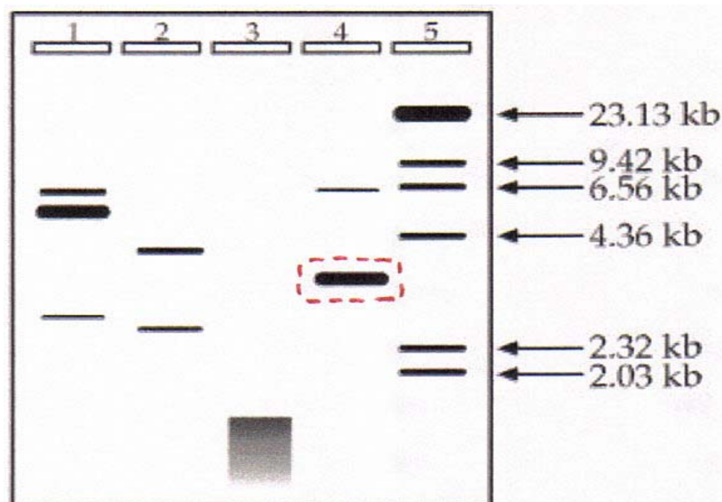
3). We run the mixture on a 1.2% gel to see the results of the aforementioned process. If the Kallikrein siRNA sample was digested properly, we should see two bands in the lane of the sample on the gel since the gel itself separates the fragments according to size. These two bands are the pGEMTEZ vector and Kallikrein siRNA insert. The gel that has the band containing the Kallikrein siRNA can be cut and gel purified to extract the insert. This sample can be used for further experimenting.

(illustrations shown on next page.)

Restriction Digestion Contd.



The Xho1 enzymes cut the DNA at specific sites. The ampicillin resistant gene is the Kallikrein siRNA in our case.



The lanes represent different samples which are spread on the agarose gel based on their sizes; the smallest samples will run the farthest. The sample in lane 4 best depicts the digested sample of the Kallikrein siRNA. We know that the digestion was a success since there are two bars in lane 4. The top bar depicts the vector while the lower bar in lane 4 depicts the Kallikrein siRNA plasmid. The samples in this picture run down towards the positive end of the gel electrophoresis box since the DNA are negatively charged. The Kallikrein siRNA sample is cut directly from the gel (around the red in this picture) and is gel purified by using a Qiagen Gel Purification Kit.

Continuing Research

Now that we have restriction digested the Kallikrein siRNA with the enzyme Xho1 and have purified the gel containing the plasmid, we sequenced the DNA to ensure that we have the precise sequence of nucleotides in the DNA sample. The most popular sequencing method is the dideoxy method in which the dideoxy nucleotides are labeled with a “tag” that fluoresces in color.

Once we know that the plasmid has in fact the right sequence, we inject the DNA in a set of mice. The Kallikrein siRNA protein will be found in every single cell of the transgenic mice but it will only be turned on in the prostate, due to a regulator. We have performed this experiment with other mutated plasmids as well, such as serine 189.

Once the set of mice has the Kallikrein siRNA gene injected, it will be compared to another set of mice which has the normal prostate-specific antigen found in men. The two sets of mice will be analyzed to see how fast one set acquires prostate cancer in comparison to the other one.

Works Cited

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