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Silencing the Genes Responsible for Prostate Cancer

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Abstract **- Prostate cancer is one the most common cancer globally. The prostate is a small gland seen in the pelvis of men and hence prostate cancer is common in men. It develops very slowly and hence it is very difficult to see its signs for many years. If treated with chemotherapy, undesired side effects are seen. Hence a novel therapy is followed in this work, the RNA interference (RNAi). Main aim of this work is to silence the double stranded (ds) RNA sequence in prostate cancer gene receptors like ACPT,BRCA1,BRCA2,HOXB13, FGFFR4 and RNASEL using Gene silencing software siDIRECT.**

Keywords**: Prostate cancer, chemotherapy, gene silencing, RNA interference, geneboy**

I. INTRODUCTION

Cancer is the second leading cause of death worldwide [1]. Prostate cancer exhibits tremendous differences in incidence among populations worldwide [1]. The ratio of countries with high and low rates of prostate cancer ranges from 60-fold to 100-fold [1]. Prostate cancer affects the prostate gland, the gland that produces some of the fluid in semen and plays a role in urine control in men [1]. Several genes and chromosomal regions have been found to be associated with prostate cancer in various linkage analyses, case-control studies, genome-wide association studies (GWAS), and ad mixture mapping studies [2]. Pathogenic variants in genes of high and moderate penetrance, such as BRCA1, BRCA2, RNASEL, ACPT, FGFR4 and HOXB13 confer modest to high lifetime risk of prostate cancer [3]. GWAS have identified more than 100 SNPs associated with the development of prostate cancer, but the clinical utility of these findings remains uncertain [4]. Several advancements are made towards treatment and control of cancer progression including chemotherapy but it is seen that undesired side effects occur during chemotherapy [5]. In recent years a novel therapy has emerged, the gene silencing therapy or RNA interference (RNAi) which is derived from nucleic acid-based molecules that is evolving from *in-silico*, *in-vitro* to clinical therapy. This involves double stranded (ds) RNAs mediate sequence-specific gene silencing. This technique finds application basic cancer research, is facilitating the identification and validation of potential therapeutic targets (the dsRNA) for cancer, and this could be further developed into cancer therapeutics by selectively silencing the involved oncogenes [6, 7].

Genes involved in Prostate Cancer

ACPT (Acid Phosphatase, Testicular gene Protein Coding): Acid phosphatases are enzymes capable of hydrolysing ortho-phosphoric acid esters in an acid medium [8, 9]. This gene is up-regulated by androgens and is down-regulated by estrogens in the prostate cancer cell line [8]. This gene exhibits lower level of expression in testicular cancer tissues than in normal tissues [9]. The protein encoded by this gene has structural similarity to prostatic and lysosomal acid phosphatases [8, 9].

BRCA1 (Breast cancer type 1 susceptibility protein coding): This gene encodes a nuclear phosphoprotein that plays a role in maintaining genomic stability, and it also acts as a tumour suppressor. The encoded protein combines with other tumour suppressors, DNA damage sensors, and signal transducers to form a large multi-subunit protein complex known as the BRCA1-associated genome surveillance complex [10].

BRCA2: BRCA2 is considered a tumour suppressor gene, as tumours with BRCA2 mutations generally exhibit loss of heterozygosity (LOH) of the wild-type allele [10].

FGFR4 (Fibroblast Growth Factor Receptor 4 gene protein Coding): The protein encoded by this gene is a member of the fibroblast growth factor receptor family, where amino acid sequence is highly conserved between members and throughout evolution. FGFR family members differ from one

another in their Ligand affinities and tissue distribution [11].

HOXB13 (Homeobox B13 (Gene Protein Coding): This gene encodes a transcription factor that belongs to the Homeobox gene family. Genes of this family are highly conserved among vertebrates and essential for vertebrate embryonic development [12].

RNASEL (Ribonuclease L gene protein coding): Mutations in this gene have been associated with predisposition to prostate cancer and this gene is a candidate for the hereditary prostate cancer 1 (HPC1) allele [13].

II. METHODOLOGY

In this work the following nucleotide sequences related to prostate cancer are used (Table 1).

Table 1: Genes with their NCBI Accesion number.

Gene name	NCBI Accession number
ACPT	NM_033068
BRCA1	BC106745
BRCA2	KJ625203
FGFR4	EU826603
HOXB13	BC070233
RNASEL	BC090934

siDirect 2.0: The siDirect 2.0 is novel web server which is used for providing efficient and target-specific siRNA design for mammalian RNAi and this web server has been extensively updated to eliminate off-target silencing effects and its design algorithm is based on such mechanistic features which is combined with the reliable algorithms [14].

III. RESULTS AND DISCUSSION

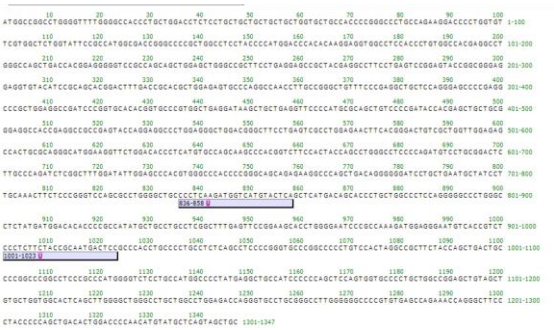
The DNA sequence of the corresponding gene receptors in Table 1 was taken. Using GENEBOY software the DNA sequence was converted to its corresponding RNA sequence. Using software siDIRECT 2.2 the RNA sequence was silenced by microRNA [15].

Using siDIRECT the RNA sequence of the gene receptors in table 1 was silenced (Table 2).

Table 2: Effective siRNA candidates of ACPT

a. ACPT

target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)	functional siRNA selection: Ui-Tei	seed-duplex stabilty (Tm);		
					guide	passenger
836-858	CCCTCAA GATGGTC ATGTACTC A	AGUACAU GACCAUC UUGAGGG CUCAAGA UGGUCAU GUACUCA	U		20.4 °C	20.4 °C
1001-1023	CCCTCTTC TACCGCA ATGACTCC	AGUCAUU GCGGUAG AAGAGGG CUCUUCU ACCGCAA UGACUCC	U		20.5 °C	20.2 °C



b. BRCA1.

target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)	functional siRNA selection: Ui-Tei	seed-duplex stabilty (Tm);		
					guide	passenger
28-50	AAGUUCA UUGGAAC AGAAAGA AA	UCUUUCUGU UCCAAUGAA CUU GUUCAUUGG AACAGAAAG AAA	U		16.6 °C	13.8 °C
31-53	UUCAUUG GAACAGA AAGAAAU GG	AUUUCUUUC UGUUCCAAU GAA CAUUGGAAC AGAAAGAAA UGG	U		5.5 °C	20.1 °C
36-58	UGGAACA GAAAGAA AUGGAUU UA	AAUCCAUUU CUUUCUGUU CCA GAACAGAAA GAAAUGGAU UUA	U		20.1 °C	19.2 °C

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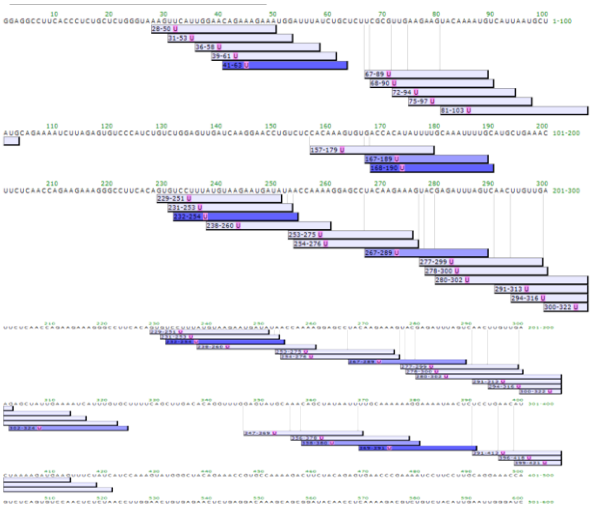
39-61	AACAGAAAGAAAUGGAUUUAUCU	AUAAAUCCAUUUCUUUCUGUUCAGAAAGAAAUGGAUUUAUCU	U		15.5 °C	19.1 °C
41-63	CAGAAAGAAAUGGAUUUAUCUGC	AGAUAAAUCCAUUUCUUUCUGGAAAGAAAUGGAUUUAUCUGC	U		1.8 °C	5.5 °C
67-89	UCGCGUUGAAGAAGUACAAAAUG	UUUUGUACUUCUUCAACGCGACGCGUUGAAGAAGUACAAAAUG	U		14.7 °C	21.1 °C
68-90	CGCGUUGAAGAAGUACAAAAUGU	AUUUUGUACUUCUUCAACGCGCGUUGAAGAAGUACAAAAUGU	U		7.2 °C	19.2 °C
72-94	UUGAAGAAGUACAAAAUGUCAUU	UGACAUUUUGUACUUCUUCAAGAAGAAGUACAAAAUGUCAUU	U		14.8 °C	17.7 °C
75-97	AAGAAGUACAAAAUGUCAUUAAU	UAAUGACAUUUUGUACUUCUUGAAGUACAAAAUGUCAUUAAU	U		20.5 °C	19.0 °C
81-103	UACAAAAUGUCAUUAAUGCUAUG	UAGCAUUAAUGACAUUUUGUACAAAAUGUCAUUAAUGCUAUG	U		19.7 °C	5.3 °C
157-179	CACAAAGUGUGACCACAUAUUUU	AAUAUGUGGUCACACUUUGUGCAAAGUGUGACCACAUAUUUU	U		13.3 °C	17.8 °C
167-189	GACCACAUAUUUUGCAAAUUUUG	AAAUUUGCAAAAUAUGUGGUCCCACAUAUUUUGCAAAUUUUG	U		14.0 °C	13.3 °C
168-190	ACCACAUAUUUUGCAAAUUUUGC	AAAAUUUGCAAAAUAUGUGGUCACAUAUUUUGCAAAUUUUGC	U		-3.3 °C	6.7 °C
229-251	GUGUCCUUUAUGUAAGAAUGAUA	UCAUUCUUACAUAAAGGACACGUCCUUUAUGUAAGAAUGAUA	U		12.0 °C	19.9 °C
231-253	GUCCUUUAUGUAAGAAUGAUAUA	UAUCAUUCUUACAUAAAGGACCCUUUAUGUAAGAAUGAUAUA	U		16.2 °C	3.5 °C
232-254	UCCUUUAUGUAAGAAUGAUAUAA	AUAUCAUUCUUACAUAAAGGACUUUAUGUAAGAAUGAUAUAA	U		8.7 °C	6.9 °C



c. **BRCA2**

starget position	target sequenc 21nt target + 2 overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)	functional siRNA selection: Ui-Tei		seed-duplex stabilty (Tm); guide	passenger
10-32	UAGUGAAGAUUCUAGUAGUUAAU	UAACUACUAGAAUCUUCACUAGUGAAGAUUCUAGUAGUUAAU	U		17.6 °C	20.4 °C
12-34	GUGAAGAUUCUAGUAGUUAAUGA	AUUAACUACUAGAAUCUUCACGAAGAUUCUAGUAGUUAAUGA	U		6.6 °C	14.8 °C
15-37	AAGAUUCUAGUAGUUAAUGAAAA	UUCAUUAACUACUAGAAUCUUGAUUCUAGUAGUUAAUGAAAA	U		8.9 °C	11.6 °C
19-41	UUCUAGUAGUUAAUGAAAAUUUU	AAUUUUCAUUAACUACUAGAACUAGUAGUUAAUGAAAAUUUU	U		7.4 °C	18.8 °C
22-44	UAGUAGUUAAUGAAAAUU	AAAAAUUUUCAUUAACUACUA	U		-12.0 °C	6.6 °C

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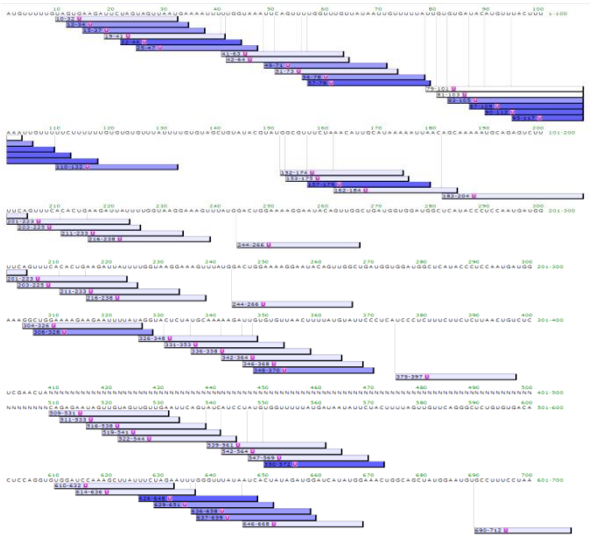
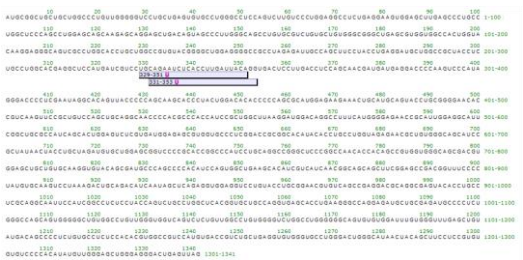
	UUUGG	GUAGUUAAUGAAA AUUUUUGG				



d. **FGFR4**

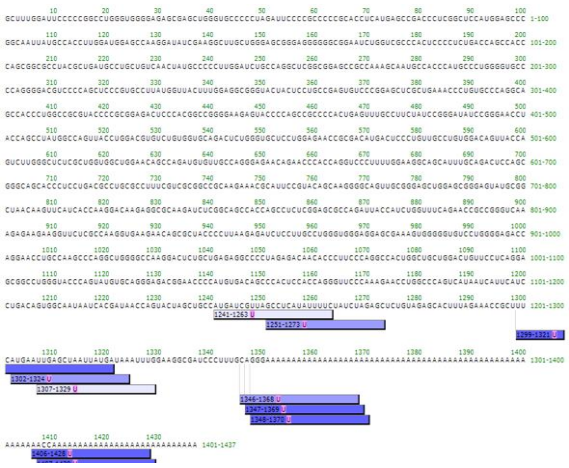
target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)	functional siRNA selection: Ui-Tei		seed-duplex stabilty (Tm);	
					guide	passenger
329-351	UGCAGAAUC UCACCUUGA UUACA	UAAUCAA GGUGAGA UUCUGCA CAGAAUC UCACCUU GAUUACA	U		12.0 °C	19.1 °C
331-353	CAGAAUCUC ACCUUGAUU ACAGG	UGUAAUC AAGGUGA GAUUCUG GAAUCUC ACCUUGA UUACAGG	U		16.1 °C	20.4 °C



e. **HOXB13**

target positio	target sequence	RNA oligo sequences	functional siRNA	seed-duplex stabilty (Tm);

n	21nt target + 2nt overhang	21nt guide (5′→3′) 21nt passenger (5′→3′)	selection: Ui-Tei			
					guide	passenger
1241-1263	AUGAUCG UUAGCCU CAUAUUU UC	AAAUAUG AGGCUAA CGAUCAU GAUCGUU AGCCUCA UAUUUUC	U		8.7 °C	16.5 °C
1251-1273	GCCUCAU AUUUUCU AUCUAGA GC	UCUAGAU AGAAAAU AUGAGGC CUCAUAU UUUCUAU CUAGAGC	U		13.0 °C	8.7 °C
1299-1321	UUCAUGA AUUGAGC UAAUUAU GA	AUAAUUA GCUCAAU UCAUGAA CAUGAAU UGAGCUA AUUAUGA	U		-2.3 °C	7.2 °C
1302-1324	AUGAAUU GAGCUAA UUAUGAU AA	AUCAUAA UUAGCUC AAUUCAU GAAUUGA GCUAAUU AUGAUAA	U		8.7 °C	12.0 °C
1307-1329	UUGAGCU AAUUAUG AUAAAUU UG	AAUUUAU CAUAAUU AGCUCAA GAGCUAA UUAUGAU AAAUUUG	U		1.8 °C	18.3 °C
1346-1368	CAGGGAA AAAAAAA AAAAAAA AA	UUUUUUU UUUUUUU UUCCCUG GGGAAAA AAAAAAA AAAAAAA	U		-11.3 °C	14.3 °C
1347-1369	AGGGAAA AAAAAAA AAAAAAA AA	UUUUUUU UUUUUUU UUUCCCU GGAAAAA AAAAAAA AAAAAAA	U		-11.3 °C	0.7 °C
1348-1370	GGGAAAA AAAAAAA AAAAAAA AA	UUUUUUU UUUUUUU UUUUCCC GAAAAAA AAAAAAA AAAAAAA	U		-11.3 °C	-11.3 °C
1406-1428	AACCAAA AAAAAAA AAAAAAA AA	UUUUUUU UUUUUUU UUUGGUU CCAAAAA AAAAAAA AAAAAAA	U		-11.3 °C	-2.9 °C
1407-1429	ACCAAAA AAAAAAA AAAAAAA AA	UUUUUUU UUUUUUU UUUGGUC CAAAAAA AAAAAAA AAAAAAA	U		-11.3 °C	-11.3 °C



f. RNASEL

target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)	functional siRNA selection: Ui-Tei		seed-duplex stabilty (Tm); guide	seed-duplex stabilty (Tm); passenger
96-118	GAGAACAU UGUUCCUUC UUCAUA	UGAAGAAGG AACAAUGUU CUC GAACAUUGU UCCUUCUUC AUA	U		19.1 °C	12.1 °C
132-154	GCCAGAGA AUCCCAAUU UACACU	UGUAAAUUG GGAUUCUCU GGC CAGAGAAUC CCAAUUUAC ACU	U		-0.3 °C	19.1 °C
149-171	UACACUCAA AGCUUCUU UGAUUA	AUCAAAGAA GCUUUGAGU GUA CACUCAAAG CUUCUUUGA UUA	U		20.4 °C	19.2 °C
177-199	UAGGAGAU AAAUUUGC AUUUUCU	AAAAUGCAA AUUUAUCUC CUA GGAGAUAAA UUUGCAUUU UCU	U		20.0 °C	16.0 °C
198-220	CUCAAGGA AAAGGCUA AAAGUGG	ACUUUUAGC CUUUUCCUU GAG CAAGGAAAA GGCUAAAAG UGG	U		1.3 °C	18.7 °C
314-336	GACAAUCAC UUGCUGAU UAAAGC	UUUAAUCAG CAAGUGAUU GUC CAAUCACUU GCUGAUUAA AGC	U		8.9 °C	19.2 °C
324-346	UGCUGAUU AAAGCUGU UCAAAAC	UUUGAACAG CUUUAAUCA GCA CUGAUUAAA GCUGUUCAA AAC	U		20.5 °C	8.9 °C
334-356	AGCUGUUC AAAACGAA GAUGUUG	ACAUCUUCG UUUUGAACA GCU CUGUUCAAA ACGAAGAUG UUG	U		20.4 °C	20.5 °C
426-448	CUCUGCAUA ACGCAGUAC AAAUG	UUUGUACUG CGUUAUGCA GAG CUGCAUAAC GCAGUACAA AUG	U		19.0 °C	21.1 °C
563-585	CUGAAACU UUUCCUUUC UAAAGG	UUUAGAAAG GAAAAGUUU CAG GAAACUUUU CCUUUCUAA AGG	U		7.1 °C	3.2 °C
593-615	GUCAAUGA GUGUGAUU UUUAUGG	AUAAAAAUC ACACUCAUU GAC CAAUGAGUG UGAUUUUUA UGG	U		-9.7 °C	19.2 °C
658-680	AGCCCUAAA AUUCCUUU AUAAGA	UUAUAAAGG AAUUUUAGG GCU CCCUAAAAU UCCUUUAUA AGA	U		-2.3 °C	11.0 °C
660-682	CCCUAAAAU UCCUUUAU AAGAGA	UCUUAUAAA GGAAUUUUA GGG CUAAAAUUC CUUUAUAAG AGA	U		-2.3 °C	-9.7 °C
692-714	GUGAAUUU GAGGCGAA AGACAAA	UGUCUUUCG CCUCAAAUU CAC GAAUUUGAG GCGAAAGAC AAA	U		21.5 °C	7.4 °C
935-957	GUCAAUGU GAGGGGAG AAAGAGG	UCUUUCUCC CCUCACAUU GAC CAAUGUGAG GGGAGAAAG AGG	U		19.1 °C	20.5 °C
1026-1048	AGCACAUA GAGAUUAA UGACACA	UGUCAUUAA UCUCUAUGU GCU CACAUAGAG AUUAAUGAC ACA	U		16.1 °C	20.3 °C
1078-1100	UGCUGUUG AACUCAAAC UGAAGA	UUCAGUUUG AGUUCAACA GCA CUGUUGAAC UCAAACUGA AGA	U		19.2 °C	20.5 °C

Graphical view of effective siRNA candidates

start-end	Functional, off-target reduced siRNA (seed duplex Tm < 10 °C)
start-end	Functional, off-target reduced siRNA (seed duplex Tm < 15 °C)
start-end	Functional, off-target reduced siRNA (seed duplex Tm < 21.5 °C)
start-end	Functional siRNA

IV. CONCLUSION

The ds RNA sequence of the prostate cancer gene receptors viz. BRCA1, BRCA2, RNASEL, ACPT, FGFR4 and HOXB13 are silenced using siRNA technique.

REFERENCES

1. Taitt HE, 2018, "Global Trends and Prostate Cancer: A Review of Incidence, Detection, and Mortality as Influenced by Race, Ethnicity, and Geographic Location", Am J Mens Health, 12(6):1807–1823.
2. Sebastiani P, Timofeev N, Dworkis DA, Perls DT, and Steinberg MH, 2009, "Genome-wide association studies and the genetic dissection of complex traits", Am J Hematol., 84(8): 504–515.
3. Han MR, Zheng W, Cai Q, Gao YT, Zheng Y, Bolla MK, Michailidou K, Dennis J, Wang Q, Dunning AM, Brennan P, Chen ST, Choi JY, Hartman M, Ito H, Lophatananon A, Matsuo K, Miao H, Muir K, Sangrajrang S, Shen CY, Teo SH, Tseng CC, Wu AH, Yip CH, Kang D, Xiang YB, Easton DF, Shu XO, Long J, 2017, "Evaluating genetic variants associated with breast cancer risk in high and moderate-penetrance genes in Asians", Carcinogenesis, 38(5):511-518.
4. Turner AR, Kader AK, and Xu J, 2012, "Utility of Genome-Wide Association Study findings: prostate cancer as a translational research paradigm", J Intern Med., 271(4): 344–352.
5. Desai AG, Qazi GN, Ganju RK, El-Tamer M, Singh J, Saxena AK, Bedi YS, Taneja SC, Bhat HK, 2008, "Medicinal plants and cancer chemoprevention", Curr Drug Metab., 9(7):581-91.
6. Song CZ. (2007) Gene Silencing Therapy Against Cancer. In: Hunt K.K., Vorburger S.A., Swisher S.G. (eds) Gene Therapy for Cancer. Cancer Drug Discovery and Development. Humana Press.
7. Mansoori B, Shotorbani SS, and Baradaran B, 2014, "RNA Interference and its Role in Cancer Therapy", Adv Pharm Bull., 4(4): 313–321.
8. Igawa M, Kishi H, Ishibe T, 1995, "Acid phosphatase (ACP)", Nihon Rinsho., 53(5):1203-8.
9. Yousef GM, Diamandis M, Jung K, Diamandis EP, 2001, "Molecular cloning of a novel human acid phosphatase gene (ACPT) that is highly expressed in the testis", Genomics., 15; 74(3):385-95.
10. Castro E and Eeles R, 2012, "The role of BRCA1 and BRCA2 in prostate cancer", Asian J Androl., 14(3): 409–414.
11. FitzGerald LM, Karlins E, Karyadi DM, Kwon EM, Koopmeiners JS Stanford JL and Ostrander EA, 2009, "Association of FGFR4 Genetic Polymorphisms with Prostate Cancer Risk and Prognosis", Prostate Cancer Prostatic Dis., 12(2): 192–197.
12. Chandrasekaran G, Hwang EC, Kang TW, Kwon DD, Park K, Lee JJ, Lakshmanan VK, 2017, "Computational Modeling of complete HOXB13 protein for predicting the functional effect of SNPs and the associated role in hereditary prostate cancer", Sci Rep., 7: 43830. Xiang Y, Wang Z, Murakami J, Plummer S, Klein EA, Carpten JD, Trent JM,
13. Isaacs WB, Casey G, and Silverman RH, 2003, "Effects of RNase L Mutations Associated with Prostate Cancer on Apoptosis Induced by 2,5-Oligoadenylates", Cancer Research, 63, 6795–6801.
14. Naito Y, Yamada T, Ui-Tei K, Morishita S and Saigo K, 2004, siDirect: highly effective, target-specific siRNA design software for mammalian RNA interference", Nucleic Acids Res., 32(Web Server issue): W124–W129.
15. http://www.dnai.org/geneboy/