

DAX-1 (NROB1) gene expression regulation in prostate cancer cells through activation of Androgen Receptors

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Abstract

The nuclear hormone receptor DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1) is associated with the development and progression of prostate cancer within human males. Previous studies on DAX-1 have suggested a repression of steroidogenesis, indicating an inverse correlation between DAX-1 and steroidogenic gene expressions. To further understand the role of DAX-1 in tumor growth and development, experimentation was performed on Androgen Receptor (AR) sensitive and insensitive prostate cancer cell lines. Analysis of the different gene expression patterns of prostate cancer cell lines (including a CRISPR DAX-1 negative cell line) under separate treatment conditions, provides conclusive data regarding the link between DAX-1 and AR. The utilization of the DAX-1 negative cell line has also permitted the opportunity to develop a better understanding of advanced stage prostate cancer by simulating its inability to express the DAX-1 gene. The collected data on the relationship between DAX-1 and AR may aid in further studies to identify additional targets for the treatment of prostate cancer.

Background

Prostate cancer is the second most common type of cancer found in men. The mechanisms behind the growth and metastasis of prostate cancer is still not fully understood leaving us to rely on findings from other cancers. Previous studies on breast cancer have revealed that Androgen Receptors (AR) when activated can induce the expression of DAX-1 which then can repress the expression and activity of aromatase in the steroidogenesis pathway. Additionally, the studies have indicated that high expression of DAX-1 can promote cell apoptosis. [1]

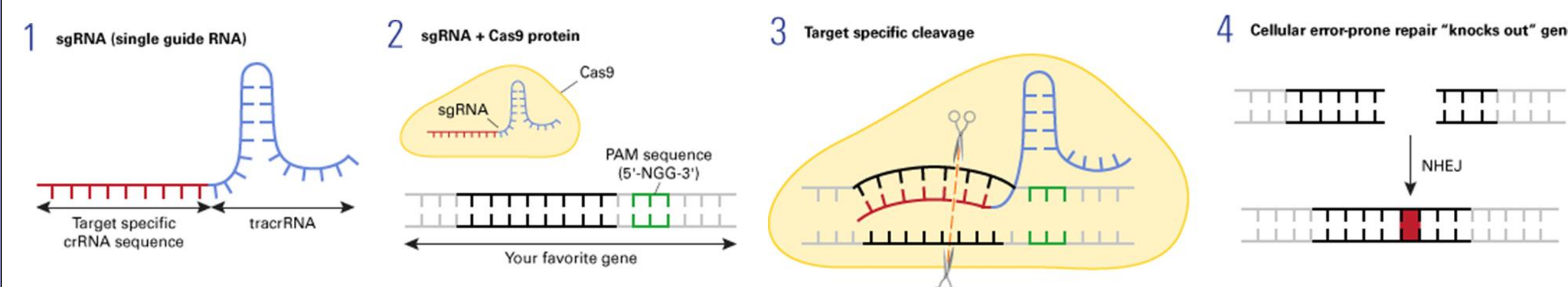
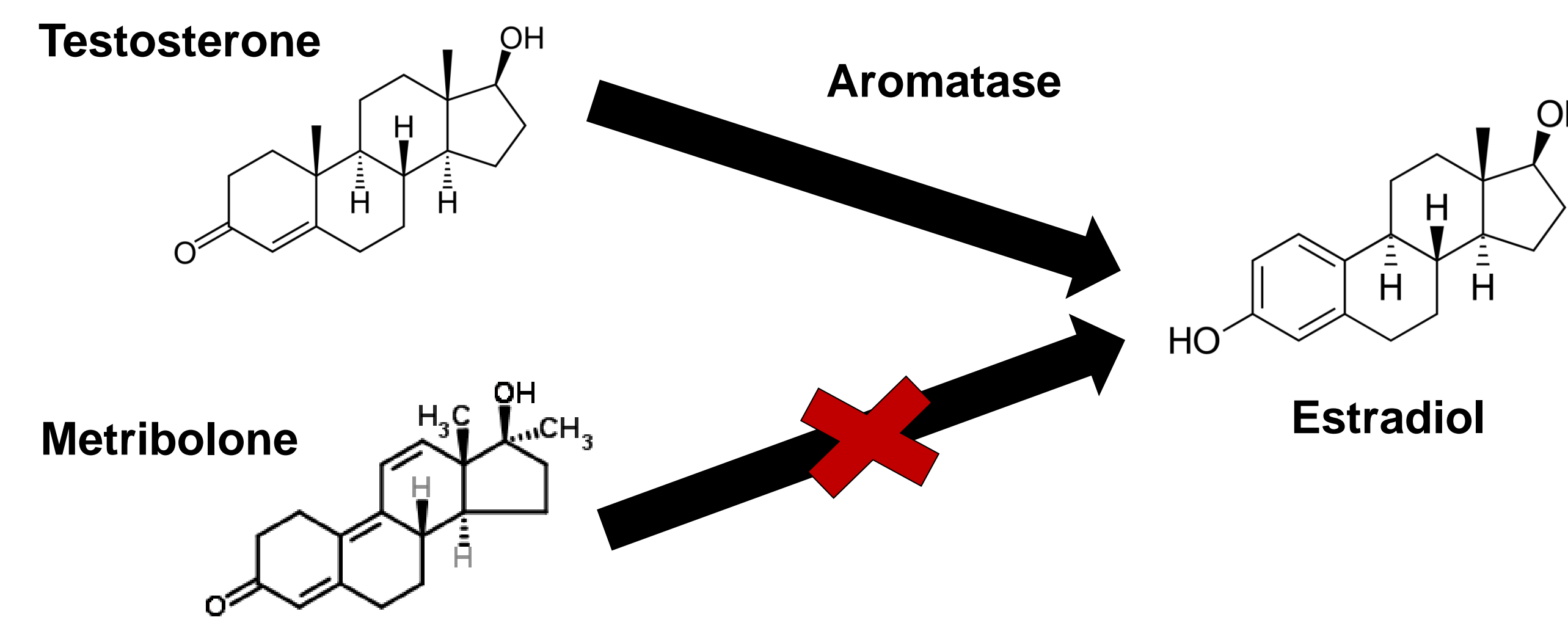


Figure 1. CRISPR/Cas9 gene knockout sequence
Diagram produced by Takara Bio Company © 2017

In some circumstances such as prostatic hyperplasia, DAX-1 expression is reduced drastically. [2] To simulate this behavior CRISPR/Cas9, a new age gene editing technique, was used to knock out the DAX-1 gene from LnCap cells to produce a new cell line known as LCR1. To assess the different cell lines, Metribolone (Mb), a nonaromatizable drug, was used to activate the Androgen Receptors (AR) in order to identify different responses with increasing dosage which the expectation of a change in DAX-1 expression. [3]



DAX-1 Upregulation with AR activation

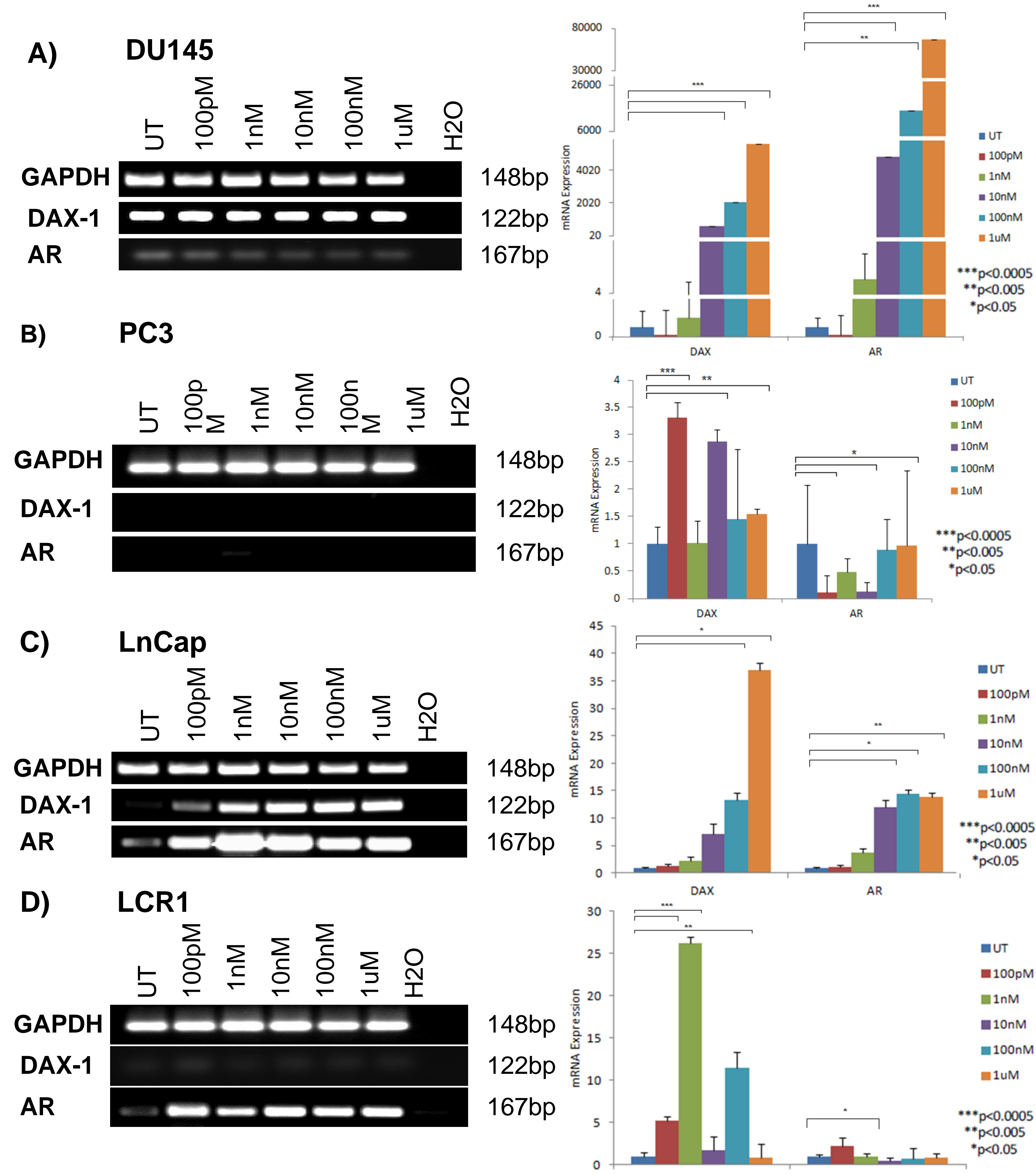


Figure 2. PCR and qPCR analysis of gene expression within prostate cancer cell lines in response to increasing Mb dosage. A) DU145: an AR negative cell line B) PC3: an AR and DAX-1 negative cell line C) LnCap: prostate cancer line known to express both genes D) LCR1: a successful CRISPR DAX-1 gene knock out cell line

Chromatin Immunoprecipitation (ChIP)

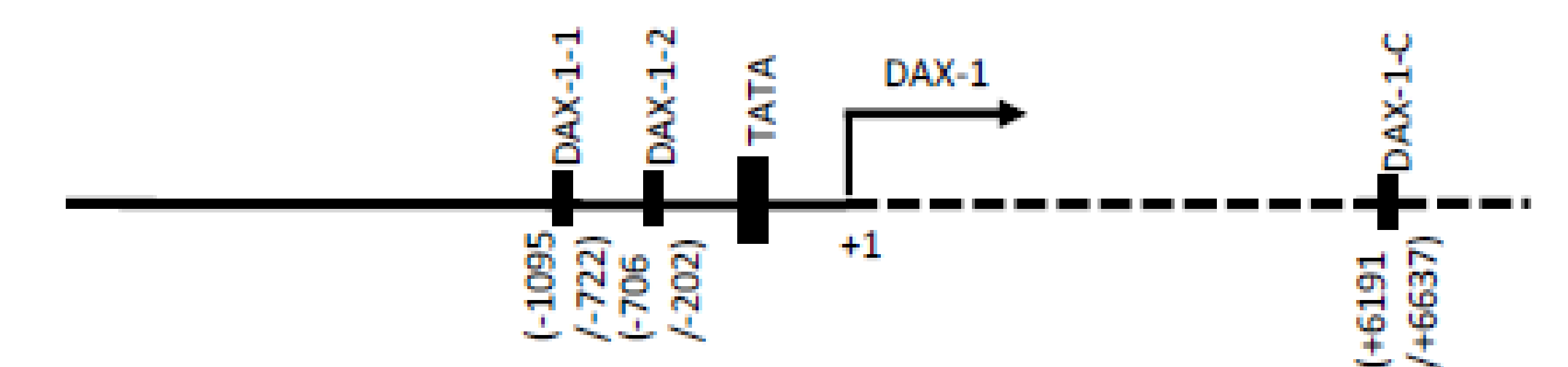


Figure 3. Potential targets on the DAX-1 gene used for ChIP assay. Three target regions within the DAX-1 gene were selected based on prior studies in breast cancer.

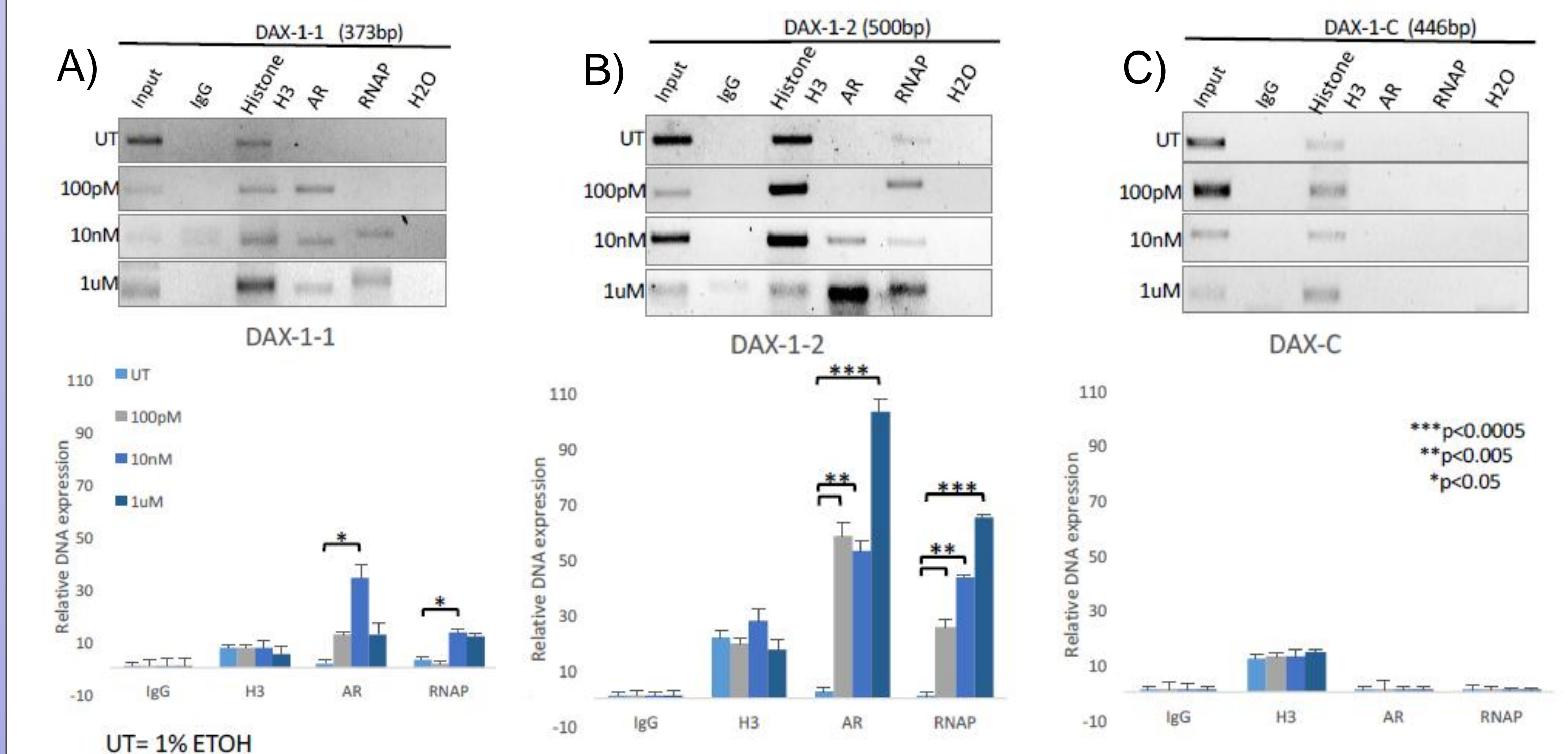


Figure 4. Chromatin Immunoprecipitation analysis of DAX-1 targets. A & B) significant increase in DNA expression resulted from treatment with Mb, indicating a linkage between DNA and protein. C) target marker downstream the promoter region

Future Direction & Conclusions

- The activation of androgen receptors with Metribolone, appears to promote the expression of the DAX-1 gene
- Exposure to androgen agonists, leads to an elevated protein expression in DAX-1-1 and DAX-1-2 which to act as transcription factors, down regulating the expression in DAX-1-C
- Perform western blot analysis to determine protein synthesis patterns between the different prostate cancer lines

References

- 1) Lanzino, M., P. Maris, and R. Sirianni. "DAX-1, as an Androgen-target Gene, Inhibits Aromatase Expression: A Novel Mechanism Blocking Estrogen-dependent Breast Cancer Cell Proliferation." *Cell Death and Disease* 4.7 (2013): *The FASEB Journal*. Web.
- 2) Tindall, Donald, and James Mohler. *Androgen Action in Prostate Cancer*. New York: Springer, 2009. p 325
- 3) Lalli, Enzo. "Role of Orphan Nuclear Receptor DAX-1/NROB1 in Development, Physiology, and Disease." *Advances in Biology* 2014 (2014): 1-19. *Hindawi*. Web.

Figure 1.: http://www.clontech.com/US/Products/Genome_Editing/CRISPR_Cas9/Resources/About_CRISPR_Cas9