Supplemental Table 1: LNA and ENA® Sequences used in the described experiments.

LNA/ENA	Target	DNA Strand	Sequence 5' to 3'
LNA PR1	-9/+10	(Nontemplate)	gCTgTggaCTggCCagaCa
LNA PR2	-9/+10	(Template)	tGtctGGccAGtccAcAGc
LNA AR1	-24/ -6	(Template)	gTTgcATttGctctcCACc
LNA -Ctrl	N/A	N/A	CTAcgaGaCctCccGggGC
ENA® PR1	-9/+10	(Nontemplate)	gCTgTggaCTggCCagaCa
ENA® PR2	-9/+10	(Template)	tGtctGGccAGtccAcAGc
ENA®-Ctrl	N/A	N/A	CTAcgaGaCctCccGggGC

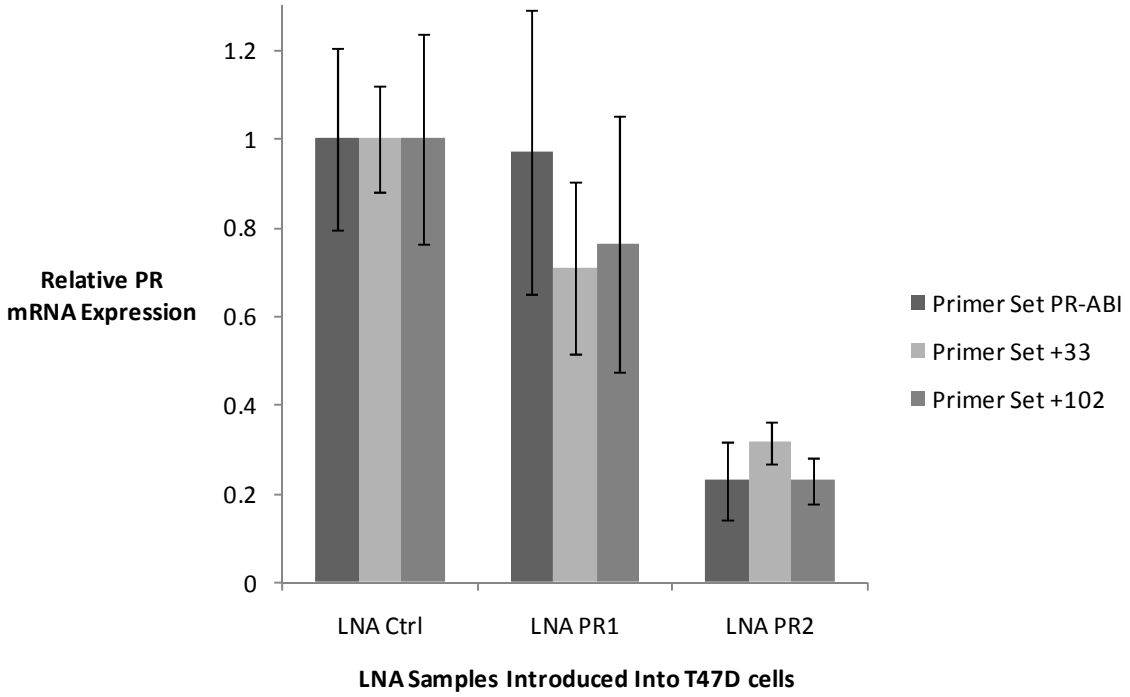
Lower case denotes DNA bases. Upper case denotes LNA bases. 'Target' refers to the sequence targeted by the oligonucleotides and is relative to the major transcription start site for the targeted gene.

Supplemental Table 2: Primer sets used for qPCR

Name	Gene	Experiment	Figure(s)	Sequence 5' to 3'
P1(+33)	PR-B	mRNA Levels	1,S1,S2	CTTGTTGTATTTGCGCGTGT
P1rev	PR-B	mRNA Levels	1,S1,S2	GAAGGGTCGGACTTCTGCT
P2	PR-B	Biotin-Pulldown	3	CCAATTACCGGTAGGATCTGA
P2rev	PR-B	Biotin-Pulldown	3	ATGAGGTTCCATCCCAAAGA
P3	GAPDH	ChIP	2	TACTAGCGGTTTTACGGGCG
P3rev	GAPDH	ChIP	2	TCGAACAGGAGGAGCAGAGAGCGA
P4	PR-B	ChIP	2	CCTAGAGGAGGAGGCGTTGT
P4rev	PR-B	ChIP	2	ATTGAGAATGCCACCCACA
P5	AR	ChIP	2	GGAGCAAGCCCAGAGGCAGA
P5rev	AR	ChIP	2	GCTGCAAGAGGCGTTGGCTG
P6	DHFR	ChIP	2	TCGCCTGCACAAATAGGGAC
P6rev	DHFR	ChIP	2	AGAACGCGCGGTCAAGTTT
PR+102R	PR-B	mRNA Levels	S2	TACAACCCGAGGCGGCTA
PR+102F	PR-B	mRNA Levels	S2	GAAGGGTCGGACTTCTGCT

*AR and GAPDH primers for mRNA levels in Figure 1 were obtained from Applied Biosystems. PR primers labeled PR-ABI in supplemental figure 1a were also obtained from Applied Biosystems.

Supplemental Figure 1: qPCR data depicts the effect of adding LNA oligomers on mRNA levels of the PR gene. Cells were treated with LNA at 50 nM targeting PR-B. Data shown below essentially confirms the results shown in Figure 1 using additional primer sets targeting the PR gene. Error bars represent the standard deviation among ≥3 replicates. Primer sets are provided in Supplemental Table 2. Primer Set PR-ABI was obtained from Applied Biosystems.



Methods:

Oligonucleotides. LNA or ENA® oligonucleotides were synthesized and characterized by MALDI-TOF mass spectrometry at SIGMA-PROLIGO (Paris, France). Upon arrival, each LNA was purified using Microspin G-25 Columns (Amersham Biosciences) according to the manufacturer's instructions. Concentrations were determined by UV spectrophotometry. The absorbance for each LNA or ENA® oligonucleotide at 260 nm was noted. Extinction coefficients for each LNA or ENA® were provided by SIGMA-PROLIGO. ENA® is a registered trademark of Mitsubishi-Kagaku Foods Corporation.

Cell Culture. T47D breast cancer cells were obtained from the American Type Culture Collection (ATCC) and maintained at 37 ℃ and 5% CO_2 in standard media: RPMI-1640 (ATCC) supplemented with 10% heat-inactivated (56 ℃, 1 hour) fetal bovine serum (FBS, Gemini Bioproducts), 0.4 units mL^{-1} of bovine insulin, and 0.5% MEM nonessential amino acids (Sigma).

Lipid-Mediated Transfection. T47D cells were plated at 100,000 cells per six-well plate (Costar) 2 days before transfection. LNA or ENA® oligonucleotides were transfected using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen) according to the manufacturer's instructions. Prior to transfection, LNA/ENA® stocks were heated at 75℃ for 5 minutes to dissolve any aggregates that may have formed. Per well on a 6-well plate, 50 nM (1.8 µL lipid) single-stranded LNA/ENA® oligonucleotides in OptiMEM (Invitrogen) were added to a final volume of 250 µL and were incubated for 20 minutes. OptiMEM was added to the LNA-lipid mixture for a final volume of 1.25 mL and then added to cells in a drop-wise manner. Media were exchanged 24 hours later with RPMI supplemented as described above.

Analysis of PR or AR mRNA Expression by qPCR. Cells were harvested 72-96 hours post-transfection using Trizol (Invitrogen) according to the manufacturer's instructions. We performed qPCR on an ABI7900 real-time PCR (Applied Biosystems) using iTaq SYBR Green Supermix w/ ROX (BIO-RAD). Primers were designed using primer3 software (http://fokker.wi.mit.edu/primer3/input.htm) with the exception of primers for GAPDH and AR, which were obtained from Applied Biosystems (Supplementary Table 2). Only those primer sets that show linear amplification over several orders of magnitude were used. RNA was treated with DNase before reverse transcription. Reactions for qPCR analysis were performed in 20 µL reactions containing 10 µL of SYBR Green Supermix, 100 nM primers, and approximately 100 µg of cDNA using the following thermal profile: 50℃ for 2 minutes, 95℃ for 2 minutes, followed by 45 cycles of 95℃ for 20 seconds, 57℃ for 30 seconds, and 72℃ for 45 seconds. qPCR data was analyzed and reported as relative fold change using the $2^{-(\Delta\Delta Ct)}$ method as described in *Current Protocols in Human Genetics*, unit 11.10. Samples were normalized to a loading control, GAPDH, and then normalized to the negative LNA control. Error for qPCR experiments was shown as the standard deviation as determined by the fold change of least three biological replicates.

Chromatin Immunoprecipitation. Approximately 2 million cells were plated per dish (150 mm, Corning), and 2 dishes were used per final sample. Cells were incubated for 48

hours prior to transfection. Using a 20 µM stock of LNA, 51.2 µL of LNA or ENA® was combined with 1.34 mL of OptiMEM. 28.8 µL of RNAiMAX was combined with 580 µL of OptiMEM. These two solutions were mixed for a total of 2 mL and allowed to incubate for 20 minutes. During this incubation step, media was aspirated from each dish and replaced with 18 mL of OptiMEM. Immediately following the 20 minute incubation, the 2 mL of Lipid/LNA/ENA® solution was added to each dish. Cells were allowed to incubate for 24 hours, and then media was replaced with RPMI supplemented as described above.

ChIP samples were harvested 72-96 days post-transfection. Media was aspirated from each dish and the cells were washed with 1X PBS. PBS was aspirated and 10 mL of 1% formaldehyde (in PBS) was added to each dish for 10 minutes. The reaction was quenched using 1 mL of 1.25 M glycine (in PBS) followed by a five-minute incubation. The formaldehyde and glycine solution was aspirated and the cells were washed once with PBS. PBS was then aspirated. A total of 12 mL of PBS was added to each sample in order to harvest cells. Cells were removed from dishes using a rubber policeman.

Replicates were combined and centrifuged at 500Xg for 5 minutes in a S4180 rotor at 4°C. PBS supernatant was discarded and the pelleted cells were resuspended in hypotonic lysis buffer (10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris-HCl pH 8.0, 0.5% NP-40) and allowed to incubate on ice for 5 minutes. The samples were centrifuged at 500Xg for 5 minutes in a S4180 rotor at 4°C, and the hypotonic lysis was repeated. Following the second hypotonic lysis, samples were centrifuged at 500Xg for 5 minutes in a S4180 rotor at 4°C, and samples were resuspended in buffer B (1%SDS, 10 mM EDTA, 50mM Tris-HCl pH 8.1, 1X Roche Protease inhibitor cocktail, 50 units RNase inhibitor) and flash-frozen using liquid nitrogen.

Nuclear lysate was stored at -80°C for sonication. Lysed nuclei were sonicated, and DNA was sheared into base fragments approximately 500 bases long using a Model 150 V/T ultrasonic homogenizer (Biologics; five 20-second pulses at 40% power, in an ice bath, with 1 minute between each pulse). Aliquots of sheared DNA were analyzed on a 3% agarose gel to ensure proper fragment size. Following sonication, samples were pre-cleared. Samples were centrifuged for 10 minutes at 12,000 rpm at 4°C to pellet cell debris. Prior to continuing, 25 µL of supernatant was transferred to a sterile 1.7 mL tube for use as an input control.

Approximately 40 µL of Protein G Plus/Protein A agarose suspension (Calbiochem, cat. No. IP05) was added per sample into 1 mL of IP buffer. Beads were washed 3 times with 1 mL of IP buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH 8.1, 167 mM NaCl, 1X Roche protease inhibitor, 50 units/mL of RNase inhibitor). Per sample, two tubes were prepared (IgG control and experimental). For each sample, 770 µL of IP Buffer, 30-35 µL of 3X washed beads and 200 µL of each sample were combined. Samples were allowed to incubate for over 1 hour at 4°C while continuously being inverted. Once the samples were pre-cleared, the samples were centrifuged down using a C-1200 microcentrifuge and the supernatant was transferred to a new 1.7 mL tube. Following this step, 4 µg of the antibody of interest was added to the appropriate samples. Samples were rotated at 4°C for over 12 hours.

Beads were blocked prior to immunoprecipitation to decrease nonspecific binding of protein and nucleic acids. Blocking was accomplished by adding approximately 50

µL of Protein G Plus/Protein A agarose suspension (Calbiochem) per sample into 1 mL of IP buffer containing 15 µL of sheared salmon sperm DNA (Ambion). Samples were rotated at 4°C for a minimum of 1 hour. Following this incubation, samples were centrifuged (using a C-1200) for 10 seconds to pellet the beads and the supernatant was discarded. Beads were washed with 1 mL of IP buffer a total of three times.

Immunocomplexes were isolated by adding the immunoprecipitated samples to the beads blocked in the manner described above. The beads were resuspended and rotated at 4°C for 2 hours. After this incubation, the beads were pelleted using centrifugation (C-1200 microcentrifuge). Beads were resuspended and washed for a total of five times, which consisted of a five minute incubation period with each wash, centrifugation to pellet the beads, and the removal of the supernatant. The washes were performed in the following order: (i) Low-salt wash (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 150 mM NaCl); (ii) High-salt wash (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl pH 8.1, 500 mM NaCl); (iii) LiCl wash; (iv) TE pH 8.0; (v) TE pH 8. After the final wash, samples were prepared for elution.

Complexes were eluted from beads eluted by adding 250 µL of freshly prepared elution buffer (1% SDS, 0.1 M $NaHCO_3$, and 50 units/mL of RNAse inhibitor) to the washed beads. Samples were placed on a thermomixer (setting 5-6) at 25-28°C for 15 minutes. Following this incubation, samples were centrifuged to pellet the beads, and the supernatant was transferred to an sterile tube. An additional 250 µL of elution buffer was added to each sample, and this process was repeated for a total of 500 µL. Input samples were diluted to 500 µL using elution buffer. 20 µL of 5M NaCl was added to each sample for a final concentration of 200 mM. Samples were placed at 65°C for a minimum of 4 hours to reverse cross-links.

Following reversal of crosslinks, 1 µL of RNase was added to each sample and then the sample was incubated at 37°C for 30 minutes. Following this incubation, 20 µL of 1M Tris-HCL pH 6.5, 10 µL of 0.5M EDTA, and 20 µg of proteinase K was added to each sample and allowed to incubate at 42°C for 45 minutes. DNA was extracted from each sample using 520 µL of phenol:chloroform:isoamyl alcohol (25:24:1). Samples were briefly vortexed and allowed to incubate on ice for 15 minutes. Samples were centrifuged at 14,000 rpm at 4°C to separate phases. The aqueous phase was transferred to a 2 mL tube. To facilitate precipitation, 2.5 µL of glycogen (Invitrogen) and 25 µL of sodium acetate (3M, pH 5.5; Ambion) were added to each sample, mixed thoroughly, and allowed to incubate for 5 minutes. DNA was precipitated by adding 1.3 mL of 100% ethanol to each sample. Samples were placed at -80°C for a minimum of 2 hours (maximum of overnight) to allow DNA to precipitate.

DNA was pelleted by centrifugation at 14,000 rpm at 4°C. The supernatant was discarded and the pellet was washed with 2 mL of 75% ethanol. Samples were centrifuged at 8,000 rpm for 10 minutes at 4°C to pellet the DNA after the 75% ethanol wash. The supernatant was discarded and samples were resuspended in 30-40 µL of nuclease-free water. DNA sequences were detected using qPCR. Primers were designed to amplify regions of chromosomal DNA near the transcriptional start sites of genes. qPCR was performed in 20 µL reactions containing 10 µL of iTaq SYBR Green Supermix w/ ROX (BIO-RAD), 100 nM primers, and 1-2 µL DNA using the following thermal profile: 50°C for 2 minutes, 95°C for 10 minutes, followed by 52 cycles of 95°C for 20 seconds, 57°C for 30 seconds, and 72°C for 45 seconds.

qPCR data was analyzed as the relative fold change in protein occupancy relative to a negative control sample. Each sample was normalized to loading controls (GAPDH for RNA Polymerase II, DHFR for SP1 ChIP), then normalized to Input C_t values, and then normalized to an IgG control (each sample had its own IgG control). The normalized C_t value for a negative control LNA was then subtracted from experimental samples. Results are shown as fold change using the $2^{-(\Delta\Delta Ct)}$ method. Error for ChIP experiments is shown as the standard deviation as among at least three replicates.

LNA Biotin-Pulldown. T47D cells were plated and transfected as described above. Cells were harvested using trypsin 72-96 hours post-transfection. Cells were washed once with 1 mL PBS buffer, which was then aspirated, and then the cells were treated with a trypsin solution (0.05% trypsin, 0.53 mM EDTA•4Na, Invitrogen) at 37 ℃ for 2 min. Trypsin was inactivated using 800 µL of RPMI media. Replicates (6 individual wells) were pooled for each experimental sample. Nuclei were harvested using 2 consecutive 5 minute incubations in 1 ml of hypotonic lysis buffer (10 mM Tris pH 8.0, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40) and centrifugation at 3500 rpm for 5 minutes each time. Isolated Nuclei were re-suspended in 100 µL of nuclear lysis buffer (150 mM NaCl, 0.5% NP-40, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 20 mM Tris pH 7.5, 1 mM DTT, and 1X Complete-Mini Protease Inhibitor (Roche)) and stored at -80℃.

Per sample, approximately 30 µL of avidin-coated beads (NeutrAvidin Agarose Resin, Thermo Scientific) were blocked using salmon sperm DNA (Ambion) prior to use. Lysed nuclei were thawed on ice and then centrifuged at 12,000 rpm for 10 minutes to pellet cellular debris. The supernatant for each sample was transferred to a sterile 1.5 mL tube. A 10 µL aliquot of each sample was removed and stored at -80℃ for use as an input control. The remaining 90 µL of supernatant was combined with 870 µL of wash buffer (500 mM NaCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 20 mM Tris pH 7.5) containing 3 µL of RNasein (Promega) and 30 µL of pre-blocked avidin-beads. The samples were rotated with the beads at 4℃ for two hours.

Following the incubation, beads were centrifuged for 20 seconds using a C-1200 mini-centrifuge to pellet the avidin-beads. Beads were washed with 1 mL of wash buffer, mixed by flicking, and allowed to sit on ice for 2 minutes. Samples were centrifuged with the C-1200 mini-centrifuge, the supernatant was discarded, and the wash repeated for a total of 5 times. Beads were then incubated with 500 µL of elution buffer (1.5 % Biotin, 4 M guanidine thiocyanate, 25 mM sodium citrate, 0.5 % sodium N-lauroyl sarcosinate) for 2 hours at 45℃ with continuous vortexing using a Thermomixer 5436 (Eppendorf) at a setting of 10.

Samples were centrifuged and the supernatant was transferred to a sterile 1.5 mL tube. DNA was extracted using 500 µL of phenol:chloroform:isoamyl alcohol (25:24:1). Samples were centrifuged at 12,000 rpm at 4℃ for 12 minutes. The aqueous phase was transferred to a sterile 1.5 mL tube. DNA was precipitated using 3 µL of glycogen (Invitrogen), 25 µL of sodium acetate (3M, pH 5.5; Ambion), and 525 µL of isopropyl alcohol. Samples were incubated at -80℃ for 2 hours. The resulting pellet was washed using 1 mL of 75% ethanol. Samples were centrifuged at 7,000 rpm at 4℃, and the supernatant was discarded. Residual alcohol was removed by pipette.

The pellet was resuspended in 24 µL of nuclease-free water and heated to 65°C for 5 minutes.

For the analysis of LNA binding to DNA, PCR reactions were performed in a total volume of 20 µL containing 10 µL of SYBR Green Mastermix (Qiagen), 100 nM primers, and 2-4 µL of resuspended DNA. Each sample was amplified in quadruplicate using the following thermal profile: 50°C for 2 minutes, 95°C for 2 minutes, followed by 50 cycles of 95°C for 20 seconds, 57°C for 30 seconds, and 72°C for 45 seconds. Primers sequences are given in Supplemental Table 2.

PCR products were resolved on a 3% agarose gel and analyzed by the presence or absence of signal. Products were excised from the gels, cloned using the *TOPO TA Cloning Kit for Sequencing* (Invitrogen) and then sequenced by the UTSWMC McDermott Center Sequencing Core. Sequences were verified and aligned to the PR gene using NCBI BLAST and BLAT.