HHS Public Access

Author manuscript

Methods Mol Biol. Author manuscript; available in PMC 2018 March 13.

Published in final edited form as:

Methods Mol Biol. 2014 ; 1114: 103–113. doi:10.1007/978-1-62703-761-7_7.

Preparation and Application of Triple Helix Forming Oligonucleotides and Single Strand Oligonucleotide Donors for Gene Correction

Md. Rowshon Alam, **Arun Kalliat Thazhathveetil**, **Hong Li**, and **Michael M. Seidman**

Abstract

Strategies for site-specific modulation of genomic sequences in mammalian cells require two components. One must be capable of recognizing and activating a specific target sequence in vivo, driving that site into an exploitable repair pathway. Information is transferred to the site via participation in the pathway by the second component, a donor nucleic acid, resulting in a permanent change in the target sequence. We have developed biologically active triple helix forming oligonucleotides (TFOs) as site-specific gene targeting reagents. These TFOs, linked to DNA reactive compounds (such as a cross-linking agent), activate pathways that can engage informational donors. We have used the combination of a psoralen-TFO and single strand oligonucleotide donors to generate novel cell lines with directed sequence changes at the target site.

Here we describe the synthesis and purification of bioactive psoralen-linked TFOs, their co-introduction into mammalian cells with donor nucleic acids, and the identification of cells with sequence conversion of the target site. We have emphasized details in the synthesis and purification of the oligonucleotides that are essential for preparation of reagents with optimal activity.

Keywords

Triple helix forming oligonucleotide; TFO; Gene targeting; Sequence conversion; Oligonucleotide modification; Oligonucleotide synthesis

1 Introduction

DNA triple helices were first described in 1957 [1] and are formed by a third strand of DNA in the major groove of an intact duplex. They are composed of polypyrimidine or polypurine third strands in complex with polypurine:polypyrimidine duplexes and are stabilized by sequence-specific hydrogen bonds between the third strand bases and the purines of the duplex. The recognition of a “triplex” binding code (T·A:T, C^+·G:C for pyrimidine motif triplexes; A·A:T, G·G:C for purine motif triplexes), and the development of procedures and instruments for facile oligonucleotide synthesis, was the basis for the concept of triplex forming oligonucleotides (TFOs) as gene targeting reagents [2, 3]. These could introduce damage into specific sequences in the genome of living cells, either as carriers of DNA

reactive moieties [4–7] or because the triplex structure might be recognized by cellular activities that would introduce nicks and breaks in an effort to “repair” the triplex [8]. Both scenarios could yield mutations at the site or, if accompanied by an informational donor that could enter an appropriate repair pathway, direct a change in the genomic sequence.

Bioactive TFOs must be resistant to nucleases and functional in a physiological environment. These issues have been addressed by introduction of base and sugar modifications in the oligonucleotides, usually in pyrimidine motif third strands [9–11]. For example, triplex stability is enhanced by RNA analogue sugars in the TFO [12]. The most extensively studied ribose derivatives are the 2′-O-methyl; the “locked (LNA)” or bridged (BNA) 2′-O-methyl-4′; and the positively charged 2′-O-aminoethyl (AE) [13–16]. Biochemical and biophysical characterization of triplexes formed by TFOs with these modifications demonstrated enhanced triplex stability and, particularly with the latter two, improved resistance to nucleases (reviewed in ref. 6). Our most active TFOs contain four contiguous 2′-aminoethoxy ribose residues, all other sugars being 2′-O-methyl, with 5-methylcytosine in place of cytosine [17].

In contrast to some purine motif third strands, the modified pyrimidine motif TFOs do not provoke mutagenesis at specific target sites in living cells. However, based on work with psoralen-linked TFOs by the Helene and Glazer groups [4, 5], we find that modified TFOs linked to psoralen can, dependent on photoactivation, induce mutations at genomic target sites [18]. The mutations are base substitutions and deletions, the latter identical to those formed by repair of double strand breaks by the nonhomologous end joining (NHEJ) pathway [19]. Double strand breaks are well-established inducers of recombinational repair, and the psoralen-linked TFOs can stimulate targeted sequence modulation by co-introduced duplex donors, thousand of bases long [20]. Single strand oligonucleotides are also effective informational donors. These can introduce small deletions and base substitutions at the target site at frequencies that are much higher than obtained with the duplex donors. Furthermore, the sequence conversion activity of the single strand oligonucleotide donors is not dependent on recombinational functions. Instead these donors enter an NHEJ pathway, indicating, contrary to conventional wisdom, that NHEJ can be templated [21].

2 Materials

2.1 Oligonucleotide Synthesis (See Note 1)

1. Controlled Pore Glass supports (500 Å).
2. Detritylation: 3 % trichloroacetic acid in dichloromethane.
3. {5′-*O*-(4, 4′-dimethoxytrityl)-5-methyluridine-2′-*O*-methyl-3′-*O*-(*β*-cyanoethyl-*N, N*-diisopropyl)} phosphoramidite (2′-OMe-5Me U) was dissolved in 50 % THF/acetonitrile at a concentration of 0.1 M. Dry the solution over molecular sieves (4 Å) for at least 4 h prior to use (*see* Note 2).

[1]We synthesize our oligonucleotides on an Expedite 8909 synthesizer. Most labs will take advantage of commercial services for oligonucleotide synthesis. However, since it is likely that many suppliers will be unfamiliar with the variations in procedure required for the psoralen-linked TFOs with nonconventional base and sugar analogues, we have included specific details as to synthesis, deprotection, purification, etc.

4. Other amidites: 2′-OMe-5Me C, 2′-AE-5Me-U, 2′-AE-5ME-C, and psoralen are dissolved in 100 % anhydrous acetonitrile at a concentration of 0.1 M. Dry over molecular sieves as above.
5. Coupling: 0.45 M sublimed 1H-tetrazole in anhydrous acetonitrile.
6. Oxidation: 0.02 M iodine in THF/pyridine/H_2O (89.6/.4/10).
7. Capping mix A: 10 % acetic anhydride in tetrahydrofuran.
8. Capping mix B: 10 % *N*-methyl-imidazole in tetrahydrofuran/ pyridine (8:1, v/v).
9. Deprotection: AMA, 28 % concentrated ammonium hydroxide and 40 % aqueous methyl amine (1:1, v/v).

2.2 TFO Purification and Characterization

1. Mobile phase A: 100 mM Tris–HCl (pH 7.8) containing 10 % acetonitrile.
2. Mobile phase B: 1 M NaCl in 100 mM Tris–HCl (pH 7.8) and 10 % acetonitrile.
3. Dionex DNAPac PA-100 column: 4.0 mm × 250 mm (analytical) and 9.0 mm × 250 mm (preparative).
4. Sep-Pak Plus C18 cartridge (Waters Corp).
5. 0.45 μm acrodisc filter.
6. 3-Hydroxypicolinic acid (50 mg/ml in 50 % aqueous acetonitrile, HPLC grade water).
7. Ammonium citrate (50 mg/ml, HPLC grade water).

2.3 Cell Culture and Electroporation Reagents

1. Culture medium: Dulbecco’s modified Eagle medium supplemented with penicillin and streptomycin and 10 % fetal bovine serum.
2. PBS: phosphate buffered saline, 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4, adjusted to pH 7.4.
3. Nucleofection apparatus, cuvettes (Amaxa).
4. Nucleofection cell suspension solution (Amaxa).
5. UVA lamp.

2.4 DNA Purification and Analysis

1. Proteinase K/SDS: 100 μg/ml proteinase K, 0.5 % sodium dodecyl sulfate, in 10 mM Tris–HCl, pH 7.5, 1 mM EDTA.

[2]Solutions of this amidite in 100 % acetonitrile usually precipitate at the concentration of 0.1 M over 24 h. To avoid precipitation the 2′-OMe-5Me U amidite is dissolved in 50 % THF/acetonitrile. The solution should be used within 5 days of preparation. All amidite solutions must be dry. We observe reduced yields, particularly during humid summer months, if these precautions are not taken.

2. NaCl–EtOH: 5 ml of 5 M NaCl mixed with 45 ml of freezer cold EtOH (will appear cloudy).
3. TE buffer: 10 mM Tris–HCl, pH 8.0, 1 mM EDTA.

2.5 PCR

1. 10× buffer: 200 mM Tris–HCl, pH 8.4; 500 mM NaCl.
2. 15 mM $MgCl_2$.
3. 2 mM each dNTP.
4. 5 μM forward and reverse primer.
5. Taq polymerase (5 U/μl).
6. PCR master mix: for 1 reaction (scale as appropriate). 2 μl each 10× buffer, 15 mM $MgCl_2$, 2 mM dNTP, Taq polymerase, and 7 μl H_2O.

3 Methods

3.1 Synthesis of Psoralen-Linked TFO

1. Detritylation: 5′-Detritylation of the DMTr group on the support bound oligonucleotide by treatment with 3 % trichloroacetic acid in dichloromethane for 1.5 min.
2. Coupling: 6 min are allocated for the 0.45 M tetrazole- activated reaction of the 2′-OMe-5Me-U and 2′-OMe-5Me-C phosphoramidites with the free 5′-hydroxyl group of the growing chain. The coupling time for the other amidites (2′-AE-5Me-U, 2′-AE-5ME-C, psoralen) is 15 min (*see* Note 3).
3. Oxidation: The phosphite triester linkage is oxidized to the corresponding phosphate linkage in 0.02 M iodine in THF/pyridine/H_2O for 30 s.
4. Capping: Residual uncoupled 5′-hydroxyl groups (typically <2 %) are blocked with Cap Mix A and Cap Mix B for 25 s to prevent formation of deletion sequences.

3.2 Deprotection of Oligonucleotides

1. After synthesis, dry the oligonucleotide bound supports by flushing with argon gas. Transfer the dry supports to a 3 ml glass vial and suspend in 1 ml of AMA solution, and tightly cap the vial. Shake the suspension for 90 min at room temperature (*see* Note 4). Remove the supports by filtration through a 0.45 μm acrodisc filter and wash the supports with HPLC grade water (0.5 ml ×2). Collect the filtrates and combine them with the deprotection filtrate. Immediately

[3]Coupling efficiencies should be in excess of 98 %. If not, reagents may not be dry or may need to be replaced with fresh solutions.
[4]Psoralen is inactivated by alkali. Thus psoralen-linked oligonucleotides cannot be deprotected using harsh alkaline conditions at elevated temperatures. Oligonucleotides with inactivated psoralen will compete for binding sites with those with active psoralen. Consequently, it is extremely important that the integrity of the psoralen be maintained. Many commercial oligonucleotide synthesis suppliers are unfamiliar with psoralen chemistry and must be carefully instructed.

evaporate the combined solution to dryness in a speed vac. Resuspend the crude mass in HPLC grade water for anion exchange HPLC purification.

3.3 Purification of Oligonucleotides

1. The oligonucleotides are purified by ion exchange on a Dionex DNAPac PA-100 column on a Shimadzu HPLC system (LC-10ADvp) with a dual wavelength detector (SPD-10AVvp) and an autoinjector (SIL-10ADvp). Preparative (9.0 mm × 250 mm) columns are eluted with a linear gradient 0–50 % Buffer B in Buffer A for 45 min, followed by 100 % Buffer B for 60 min, at 4 ml/min. UV monitors are 254 and 315 nm (λ max for psoralen) (*see* Note 5). Lyophilize the peak fractions and desalt two times using a Sep-Pak Plus C18 cartridge (Waters Corp.).

3.4 Desalting of the Purified TFOs

1. The flow rate of the solvents through the cartridge should be regulated at a rate of about 2 drops/s. Connect a 10 ml disposable syringe to the female end of the cartridge and have the male port terminate in a waste vessel. Flush the cartridge sequentially with 10 ml of acetonitrile, 10 ml of H_2O:acetonitrile (1:1), and 10–20 ml of 0.1 M Na Acetate in 2 % acetonitrile in water.
2. Resuspend the lyophilized oligonucleotides containing sodium salts in HPLC grade water (15–20 ml). Load the oligonucleotide solution onto the Sep-PAK cartridge. Discard the pass through. Flush the cartridge subsequently with 0.1 M NaCl solution (10 ml) and water (10 ml ×2). These can also be discarded. Elute the cartridge-bound desalted oligonucleotides by flushing the cartridge with 50 % aqueous acetonitrile (1 ml ×3). Collect all the eluates (*see* Note 6).

3.5 Lyophilization of the TFO

1. After desalting, lyophilize the TFO solution containing 50 % aqueous acetonitrile to dryness. Dissolve the dry oligonucleotide in water and lyophilize to dryness three times to eliminate any trace of acetonitrile (*see* Note 7). Redissolve the TFO in sterile water, measure the OD_{260} and aliquot the oligonucleotides in stock concentrations (300–500 μM), and store at −80 °C.

3.6 Characterization of the TFO

1. Determine the chemical structure of the TFO by positive ion mode MALDI-TOF (matrix-assisted laser desorption ionization time of flight) mass spectroscopy on a Voyager Applied Biosystem instrument. The matrix for preparing the MALDI-TOF mass samples is a mixture of 3-hydroxypicolinic acid (50 mg/ml in 50 %

[5]When very high purity oligonucleotides are desired (these have the greatest biological activity), they are purified on an analytical column (4.0 mm × 250 mm). A volume of 25 μl of the oligonucleotide is injected in multiple runs. The column is eluted with a gradient of 2 min 10–50 % B, 20 min 50–90 % B, and 21 min 90–100 % B, in Buffer A, with a flow rate of 1.5 ml/min.
[6]A single cycle of desalting is adequate for oligonucleotides intended for biochemical experiments. Those employed in biological experiments are desalted twice.
[7]We have found that acetonitrile is extremely toxic to cells. Thus, we employ multiple cycles of lyophilization out of water. It may be necessary to similarly treat donor oligonucleotides obtained from commercial sources if toxicity problems are observed.

aqueous acetonitrile) and ammonium citrate (50 mg/ml in HPLC grade water). The TFO (0.1 OD) is passed through a Millipore ZipTipC18 to remove any trace of salts in the sample. Then mix the oligonucleotide with matrix solution (5 μl) and place 1 μl of the mix solution on the plates for MALDI-TOF mass analysis.

3.7 Single Strand Oligonucleotide Donors

1. The 100 mers contain sequences with homology centered around the cross-link site in the genomic target sequence. They also contain variable nonhomologous sequence designed to modify the target site. They are obtained as HPLC-purified oligonucleotides from commercial suppliers and are used without further purification (but *see* Note 7).

3.8 Cell Culture

1. We have used a variety of rodent and human cell lines in targeting experiments. The procedure for Chinese hamster cells will be described here, although elements that must be tailored to the specific cell type are noted. Cells are grown in Dulbecco's modified Eagle medium supplemented with penicillin, streptomycin, glutamine, and 10 % fetal calf serum (this will vary according to the cell line or cell type). The best results are obtained if the cells are split the day before use. Cells should be plated so as to be subconfluent at the time of harvest. Cells are removed from the plates with trypsin and washed with PBS (*see* Note 8).

3.9 Electroporation

1. Suspend the cells in the nucleofection medium as specified by Amaxa/Lonza (solution T for CHO cells, this will vary depending on cell line or cell type) at a concentration of approximately 2×10^6/95 μl. Add the pso-TFO to a final concentration of 5 μM and 5 μg donor oligonucleotide (the combined volume of both oligonucleotide additions is no more than 5 μl), place the mixture in a cuvette (total volume 100 μl), and electroporate at settings described by the manufacturer for the cell line or cell type. Remove the cell suspension and place in a transparent 1.5 ml microfuge tube containing 0.5 ml complete medium. Cap the tube and hold at room temperature shielded from light for 3 h (*see* Note 9).

3.10 Photoactivation by Long-Wave UV Light (UVA)

1. Psoralen is photoactivated by 365 nm light. Place the cells, still in the tube, behind a glass shield and expose to UVA light for 3 min at 1.8 J/cm^2. Remove the cells and plate in a T75 flask. Incubate in a 37 °C/CO_2 incubator.

---

[8]Cell density has an effect on the efficiency of targeting. If the cells are too dense, or have been on the plates too long, they do not trypsinize cleanly and cannot be suspended as single cells in electroporation media. They clump and electroporation is inefficient.

[9]The psoralen, linked to the TFO, must be photoactivated by 365 nm UVA. However, a few hours of incubation are required to permit equilibration of TFO binding at the target site. We have found that holding in medium in a transparent capped microfuge tube at room temperature keeps the cells at appropriate pH. Incubation at 37 °C results in lower targeting frequencies. We place the capped tubes on their sides as this eliminates cell packing during the incubation. They are shielded from light during this time.

3.11 Determination of Targeting Frequencies by Small Pool PCR

1. The donor oligonucleotide contains information for the desired change in the target sequence, generally including the generation of a novel restriction site or the loss of an existing site. The frequency of the event can be ascertained relatively quickly by small pool PCR analysis of DNA harvested from treated cells. We allow cells to proceed through eight to ten population doublings, by which time both TFO and donor oligonucleotide are lost through dilution and degradation. No positive signals above background were detected in control experiments in which cells were treated with both donor and TFO without photoactivation. This is an important control as persistent donor oligonucleotides can enter the PCR reactions, giving rise to false positives. Extract DNA from cells using SDS/proteinase K/phenol extraction/ethanol precipitation procedures, and dissolve in TE buffer. There is no particular requirement for high-molecular weight DNA as the products of the PCR reactions will be less than a thousand nucleotides long. Adjust the genomic DNA concentration to 10 ng/ml (*see* Note 10).

2. Small pool PCR is performed on 50 pg aliquots of DNA (5 diploid genome equivalents). We find that this is more successful, and more efficient, for identifying positive signals than when single genome equivalents of DNA are examined. Our standard reactions contain 50 pg DNA, 0.2 μM primers, 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 0.5 U Taq polymerase, in a final volume of 20 μl. Our analyses are performed in 96 well plates, but this can be adjusted according to the anticipated conversion frequency.

3. Prepare a reaction master mix scaled to the number of samples (*see* Subheading 2).

4. Place 5 μl of DNA solution in each well of a multiwall plate. Add 15 μl of master mix and mix thoroughly (*see* Note 11). Amplify in a thermal cycler (cycling conditions will vary with the specific primer set).

5. After amplification, remove 10 μl aliquots from each well and analyze by restriction digestion to identify wells in which a site was gained or lost, per the change directed by the donor oligonucleotide. From positive wells remove 1 μl and dilute 200-fold and perform nested PCR, with internal primers, in the reaction mix described above. Analyze 5 μl aliquots of these reactions by restriction digestion to verify the initial identification. Confirm identification of the PCR product by sequence analysis. Conversion frequencies are calculated by dividing the number of reactions with the desired change by the total number of successful amplification reactions multiplied by 5 (since each initial PCR reaction contains five genome equivalents) (*see* Note 12).

---

[10]Care must be taken to ensure that the DNA is completely dissolved prior to dilution. This is essential to avoid preparing a dilution with much less DNA than intended. Because there is no need for very high-molecular weight DNA, the solution can be pipetted which will both shear the DNA and aid solubilization.

[11]Mix the DNA and master mix solutions extensively in each well, while taking care to avoid cross-well contamination. Thorough mixing is essential; otherwise many wells will fail to support a reaction.

[12]Even with thorough mixing, not all wells will support amplification. These must be ignored in the frequency calculations.

3.12 Isolation of Clones with Targeted Changes

1. Assuming that the small pool PCR analysis is encouraging (0.5–1 % conversion, at minimum), individual clones with the desired change can be isolated. Electroporate cells with the psoralen-TFO and donor oligonucleotide, followed by photoactivation as above. Plate the cells and culture for a few days to allow targeted conversion and clearance of the oligonucleotides. Then trypsinize and plate in multiple 100 mm petri dishes at 200 cells/plate. Allow colony formation and transfer the colonies to individual wells in 96 well culture plates, preloaded with 100 µl medium/well.

3.13 Colony Transfer

1. Place the tip of a 20 µl pipetting device on a colony and draw a small amount (5 µl) of medium and colony into the tip. Transfer to a well (*see* Note 13). When the cells reach near confluence, replicate the plates by drawing 10 µl medium up and down several times in a 20 µl pipet tip (we do this a row at a time with a multichannel pipetting device). Then transfer the aliquot, which contains cells and medium, to another multiwall plate. Cells in the newly inoculated plate are allowed to grow. This plate serves as a reference plate. The first plate serves as a source of DNA for PCR analysis.

3.14 Purification of DNA from Multiwell Cultures

1. Remove medium from the wells, and wash 2× with PBS. Then add 50 µl of proteinase K/SDS solution. Wrap the plate in parafilm and enclose in a heat sealed bag and submerge the plate in a 50 °C water bath (*see* Note 14).

After an overnight incubation, allow the plates to equilibrate at room temperature, then spin to collect all liquid in the wells, and add 150 µl NaCl/EtOH (Subheading 2). Mix by rocking and spin at 1156 × *g* in a refrigerated centrifuge. Carefully remove the supernatant and wash 2× with 70 % EtOH and air-dry. Then dissolve the DNA in each well in 100 µl TE buffer and remove 5 µl for amplification as above and analysis by restriction digestion.

Acknowledgments

This research was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging.

References

1. Felsenfeld G, Davies DR, Rich A. Formation of a three stranded polynucleotide molecule. J Am Chem Soc. 1957; 79:2023–2024.
2. Thuong NT, Helene C. Sequence specific recognition and modification of double helical DNA by oligonucleotides. Angew Chem Int Ed Engl. 1993; 32:666–690.

[13]If the cells form colonies, then these can be transferred as described. We find it helpful to illuminate the plates from below with a lamp. When held at the right angle, the colonies will be visible to the eye. Cultures of cells that do not form colonies may have to be screened by analysis of wells plated by limiting dilution.

[14]Evaporation of sample during incubation at 50 °C can be a major problem. Submerging the sealed plates avoids the problem of having all the water in the sample condense on the surface of the plate cover above the well. The plates must be weighted down during the incubation.

3. Sun JS, Garestier T, Helene C. Oligonucleotide directed triple helix formation. Curr Opin Struct Biol. 1996; 6:327–333. [PubMed: 8804836]
4. Takasugi M, Guendouz A, Chassignol M, Decout JL, Lhomme J, Thuong NT, Helene C. Sequence-specific photo-induced cross- linking of the two strands of double-helical DNA by a psoralen covalently linked to a triple helix-forming oligonucleotide. Proc Natl Acad Sci U S A. 1991; 88:5602–5606. [PubMed: 2062839]
5. Havre PA, Gunther EJ, Gasparro FP, Glazer PM. Targeted mutagenesis of DNA using triple helix-forming oligonucleotides linked to psoralen. Proc Natl Acad Sci U S A. 1993; 90:7879–7883. [PubMed: 8356097]
6. Seidman MM, Glazer PM. The potential for gene repair via triple helix formation. J Clin Invest. 2003; 112:487–494. [PubMed: 12925687]
7. Campbell MA, Miller PS. Transplatin- conjugated triplex-forming oligonucleotides form adducts with both strands of DNA. Bioconjug Chem. 2009; 20:2222–2230. [PubMed: 19950917]
8. Wang G, Seidman MM, Glazer PM. Mutagenesis in mammalian cells induced by triple helix formation and transcription- coupled repair. Science. 1996; 271:802–805. [PubMed: 8628995]
9. Lee JS, Woodsworth ML, Latimer LJ, Morgan AR. Poly(pyrimidine).poly(purine) synthetic DNAs containing 5-methylcytosine form stable triplexes at neutral pH. Nucleic Acids Res. 1984; 12:6603–6614. [PubMed: 6473110]
10. Ono A, Ts'o PO, Kan L. Triple helix formation of oligonucleotides containing 2′-O-methylpseudoisocytidine in substitution for 2′-deoxycytidine. J Am Chem Soc. 1991; 113:4032–4033.
11. Shahid KA, Majumdar A, Alam R, Liu ST, Kuan JY, Sui X, Cuenoud B, Glazer PM, Miller PS, Seidman MM. Targeted cross- linking of the human beta-globin gene in living cells mediated by a triple helix forming oligonucleotide. Biochemistry. 2006; 45:1970–1978. [PubMed: 16460044]
12. Roberts RW, Crothers DM. Stability and properties of double and triple helices: dramatic effects of RNA or DNA backbone composition. Science. 1992; 258:1463–1466. [PubMed: 1279808]
13. Escude C, Sun JS, Rougee M, Garestier T, Helene C. Stable triple helices are formed upon binding of RNA oligonucleotides and their 2′-O-methyl derivatives to double-helical DNA. C R Acad Sci III. 1992; 315:521–525. [PubMed: 1300234]
14. Kumar R, Singh SK, Koshkin AA, Rajwanshi VK, Meldgaard M, Wengel J. The first analogues of LNA (locked nucleic acids): phosphorothioate-LNA and 2′-thio-LNA. Bioorg Med Chem Lett. 1998; 8:2219–2222. [PubMed: 9873516]
15. Torigoe H, Obika S, Imanishi T. Promotion of triplex formation by a fixed N-form sugar puckering: thermodynamic and kinetic studies. Nucleic Acids Symp Ser. 2000:241–242. [PubMed: 12903358]
16. Blommers MJ, Natt F, Jahnke W, Cuenoud B. Dual recognition of double-stranded DNA by 2′-aminoethoxy-modified oligonucleotides: the solution structure of an intramolecular triplex obtained by NMR spectroscopy. Biochemistry. 1998; 37:17714–17725. [PubMed: 9922137]
17. Puri N, Majumdar A, Cuenoud B, Miller PS, Seidman MM. Importance of clustered 2′-O-(2-aminoethyl) residues for the gene targeting activity of triple helix-forming oligonucleotides. Biochemistry. 2004; 43:1343–1351. [PubMed: 14756571]
18. Majumdar A, Khorlin A, Dyatkina N, Lin FL, Powell J, Liu J, Fei Z, Khripine Y, Watanabe KA, George J, Glazer PM, Seidman MM. Targeted gene knockout mediated by triple helix forming oligonucleotides. Nat Genet. 1998; 20:212–214. [PubMed: 9771719]
19. Puri N, Majumdar A, Cuenoud B, Natt F, Martin P, Boyd A, Miller PS, Seidman MM. Minimum number of 2′-O-(2- aminoethyl) residues required for gene knockout activity by triple helix forming oligonucleotides. Biochemistry. 2002; 41:7716–7724. [PubMed: 12056903]
20. Majumdar A, Muniandy PA, Liu J, Liu JL, Liu ST, Cuenoud B, Seidman MM. Targeted gene knock in and sequence modulation mediated by a psoralen-linked triplex-forming oligonucleotide. J Biol Chem. 2008; 283:11244–11252. [PubMed: 18303025]
21. Liu J, Majumdar A, Liu J, Thompson LH, Seidman MM. Sequence conversion by single strand oligonucleotide donors via non- homologous end joining in mammalian cells. J Biol Chem. 2010; 285:23198–23207. [PubMed: 20489199]