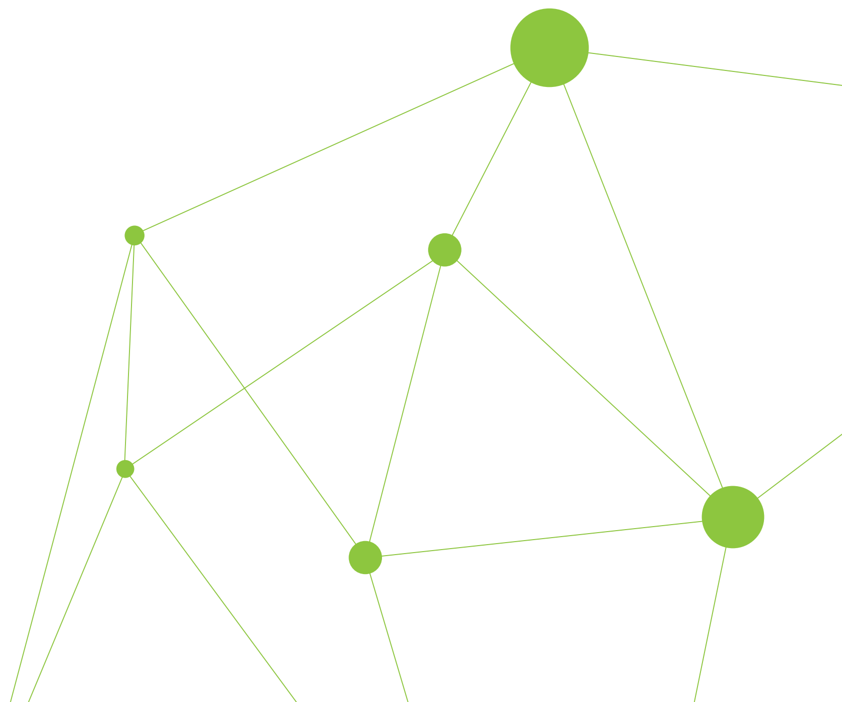
A DNA/RNA Oligonucleotide Synthesis

01. Oligonucleotide Synthesis Service
02. CRISPR



Oligonucleotide FAQs

Handling and Storage

1. How should I store my oligo?

Normally, the oligos should last a year at -20℃.
However, those are subject to be degraded, leading to abnormal quality when repetitive freezing and thawing are done on a place other than on a clean bench due to hydrolysis reactions by nuclease contamination. For a long-term usage after receiving oligos, it is advised to use on a clean bench to prevent infiltration of airborne bio-particles. Thus, we recommend to divide the samples in nuclease-free tubes to store the aliquots and use only the amount of oligos needed for your protocol without having to thaw the whole. This is especially the case of RNA oligos as they are more subject to be degraded by the bio-particles than the DNA oligos. so please open the lid on the clean bench when using them. For your reference, all oligos in Bioneer are manufactured in the clean room, allowing the oligos to be stored for a long time as long as those are not contaminated by the bio-particles.

Additionally, DNA generally undergoes degradation and precipitation gradually in the presence of heavy metal ions such as Fe ions, even if those exist in a small amount. Therefore, it can be stored for a longer period of time by dissolving in TE (10 mM Tri-HCl (pH 8.0), 0.1 mM EDTA) buffer rather than in sterilized, distilled water.

Storage Condition	Shelf Life (Month)
at RT in water	2
at 4℃ in water	9
at -20℃ in water	18
at -20℃ (Dry)	24

2. How can I dissolve my oligos?

Ordered oligos are usually shipped in a dry form.
Dried oligonucleotides have high solubility in water, but some may not due to their customized sequences, resulting in formation of structures. In this case, try incubating the oligos in 60~70℃ water bath for 10~15 minutes, then use after undergoing vortexing and centrifuge.

3. I accidently left my oligo solution over a week at the room temperature. Is it still safe to use?

As mentioned earlier, under normal circumstances, your oligos will still be safe to use, unless those are contaminated by airborne bio-particles. If you suspect the latter case, you can request us to conduct a MALDI-TOF analysis.

4. How should I store the fluorescent dye modified oligos?

Fluorescent dyes gradually degrade when exposed to luminescence. This is also the case for those attached to oligo, making them vulnerable to even under the light of normal laboratories. It is recommended to store them in a shaded container and a dark room capable of completely blocking the light.

Preferred TE Buffer for Reconstitution & Storage pH for Fluorescent Probes	
6-FAM, HEX, TET, ROX, and TAMRA	TE Buffer pH 7.5 or 8.0
Cyanine3, Cyanine3.5, Cyanine5, and Cyanine5.5	TE Buffer pH 7.0 or 7.5
Cyanine dyes rapidly degrade in acidic pH	

Quantity, Concentration, and Tm

1. What does the oligo's O.D. values represent?

The synthesized oligos are quantified by measuring the absorbance of light using the inherent absorption coefficient of oligo according to the sequence, then we mark its value as O.D(Optical density).

2. How do you quantify the amount of synthesized oligos from O.D.?

After measuring the O.D. value at UV light of 260 nm wavelength, use the following formula to obtain the amount of synthesized oligos.

$$O.D. = \varepsilon C$$

In the formula, ε (Extinction Coefficient) is a constant indicating the degree to which a substance absorbs light, a unique value depending on the material, while C represents the concentration of the oligo. The equation to get ε for the oligonucleotide is known, allowing to easily calculate the value. Therefore, by measuring the O.D., concentration of the solution, or the molar concentration of oligos, can be gained by applying the above formula.

The ε value of an oligo can be calculated by adding the extinction coefficient values acquired from each base at 260 nm UV Light (Table 1) or extinction coefficient values (Table 2) considering the difference due to sequence interference between bases.

Table 1. (Unit: L/mole.cm)

dA	15,400
dC	7,400
dG	11,500
dT	8,700

Table 2. (Unit: L/mole.cm)

5' → 3'	dA	dC	dG	dT
dA	27,400	21,200	25,000	22,800
dC	21,200	14,600	18,000	15,200
dG	25,200	17,600	21,600	20,000
dT	23,400	16,200	19,000	16,800

3. If the O.D. value of an 18 mer oligo having a sequence of 3G, 4C, 5A, 6T / GGGCCCCAAAAATTTTTT is 0.7, how much oligo is there?

The amount of oligo is calculated according to the method of obtaining Extinction Coefficient as follows.

- The extinction coefficients according to (Table 1) (Extinction Coefficient)

= Number of bases in G * 11500 + Number of bases in C * 7400 + Number of bases in A * 15400 + Number of bases in T * 8700
= 11500 x 3 + 7400 x 4 + 15400 x 5 + 8700 x 6 = 193.3 (mL/mole)

Therefore O.D. = Calculated by substituting into εC

C = 0.7 /193.3 = 0.003621 (mmole/mL) = 3.6 (nmole/mL)

- The extinction coefficients according to (Table 2) (Extinction Coefficient)

= (GG + GG + GC + CC + CC + CC + CA + AA + AA + AA + AA + AT + TT + TT + TT + TT + TT) - (G + G + C + C + C + C + A + A + A + A + A + T + T + T + T + T)
= (21600 + 21600 + 17600 + 14600 + 14600 + 14600 + 21200 + 27400 + 27400 + 27400 + 27400 + 22800 + 16800 + 16800 + 16800 + 16800 + 16800)
- (11500 + 11500 + 7400 + 7400 + 7400 + 7400 + 15400 + 15400 + 15400 + 15400 + 15400 + 8700 + 8700 + 8700 + 8700 + 8700)
= (342,200) - (173,100)
= 169,100 (mL/mole)
Therefore O.D. = Calculated by substituting into εC
C = 0.7 /169.1 = 0.004139562 (mmole/mL) = 4.14 (nmole/mL)

Our company utilizes the second method (Table 2). We show the result as 'Total nmole' value on Oligo Q.C Report.

4. How can I calculate the molecular weight of the synthetic oligo?

The following formula is used for the calculation.
M.W. = (NA x 249.2) + (NC x 225.2) + (NG x 265.2) + (NT x 240.2) + (oligolength-1) x 63.98) + 2.02

NA = Total number of A
NC = Total number of C
NG = Total number of G
NT = Total number of T

5. How do I convert the μmole units of a synthetic scale into ng units?

Generally, the scale of synthetic oligos will be ordered in the unit of μmole, and the we will express the amount of synthesized oligonucleotides in number of nmole. However, if you wish to use the oligos in ng or ug units, you can simply convert the mole into grams. Refer to your report for the molecular weight of the oligo for the conversion.

Molecular Weight (g) X Number of mole (nmole)
= Amount of oligo (ng)

6. How can I adjust the concentration of the synthesized oligonucleotides?

The QC report will show the 'volume for 100 pmole/μl,' which represents the amount of TE buffer or D.W. required to be added in the dried oligos.
For example
If the value is shown is 189 in your QC report, 189 μl of D.W or TE-Buffer will be required to be added to make 100 pmole/μl. This will also mean that the number of oligo molecules will be 189.0 μl x 100 pmole/μl = 18,900 pmole = 18.9 nmole.
Although the primer concentration must be adjusted appropriately by its use, in general cases, as PCR and sequencing usually uses 100 pmole/μl as the nucleotide concentration for the test protocol, we provide the concentration as the following.

7. I have ordered on a 50 nmole scale, but the oligos I received seemed to be less than that. Why?

50 nmole scale synthesis does not represent the finalized amount, but the loading amount used at the beginning of the synthesis process. Thus, the actual oligonucleotide amounts will always be less than your ordered scale. For instance, when the oligo length of 30 mers and the coupling efficiency for attaching one base is 98%, the yield will be 0.98^{29} = 0.56. However, the final yield be less than that, being 28%, 14 nmole, due to the deprotection reaction and purification process which also reduce the yield by 50%.
Synthesizing oligos with more the base sequences will result in the lower yield for the base coupling, deprotection, and purification. For the long oligos, the final amount will be about 5 nmole or less.

8. How can I get accurate measurements for long oligos?

Long oligos are affected by hyperchromicity, having lower absorption than theoretically calculated values based on the extinction coefficient of the bases, due to their self-hybridization characteristics. This phenomenon can be reduced by increasing the temperature when taking the O.D. measurement. However, readings may be taken less than the actual concentration when strong self-hybridization is taking place. In this case, a temperature scanning spectrophotometer can be used to not only measure the exact concentration, but also the melting temperature. If you wish, we also provide a paid analytical service. Please contact us for more details.

9. How can I convert units of molarity and mole?

We use molarity (mole/L) for the unit of concentration. As gene experiments synthesize and use small amounts of oligos, we also use low density units such as such as μ (micro), n (nano), and p (pico). For the unit conversions, please refer to the following list:

10^{-1} = deci [d]
10^{-2} = centi [c]
10^{-3} = milli [m]

10^{-6} = micro [μ]
10^{-9} = nano [n]
10^{-12} = pico [p]
10^{-15} = femto [f]

1 pmole/μl = 1×10^{-12} mole / 1×10^{-6} L = 1×10^{-6} mole / L
= 1 μmole/ L = 1 μM
Thus, μM and pmole/μl can be considered as the same unit. This allows to consider that the oligo concentration is 100 μM when dissolved as the amount specified in the Oligo QC sheet.

10. What is the difference between the calculated Tm of oligo and the value of Tm provided by Bioneer?

Unlike the traditional Tm calculator which multiplies the number of A, G, C, T by a specific coefficient, Bioneer uses a different method, which changes the Tm value by the sequence arrangement. This is because the hybridization intensity is affected by the bases adjacent to the back and front of the others. Thus, even if the two oligos have same amounts of A, G, C, T, they may have different Tm according to their arrangement. Also, stability of the oligo double helix is influenced by pH and salt concentrations. A temperature scanning spectrophotometer can be used to gain Tm values at the specific pH and salt concentration. As shown mentioned above, we provide a paid analytical service. Please contact us for more details.

11. Can you define these two terms: a degenerate primer and a universal primer?

A degenerate primer is a combination of primers having different base sequences at a specific base position. For instance, let's suppose that a sequence of a gene in species A is 5'-gga ttc ggg ccc gag tct-3 while species B is 5'-ggc ttc ggg ccc gaa tct-3'.The sequence containing all the genes of two species can be predicted as 5"-gg (a/c) ttc ggg ccc ga (g/a) tct-3 ', meaning that the 3rd sequence will be either adenine or cytosine while the 15th sequence will be either guanine or adenine. In this case, the degenerate primer for this gene will be 5'-ggM ttc ggg ccc gaR tct-3 '. Therefore, the degenerate primer can be defined as a mix of primers where two or more possible bases differ at a certain position to cover all the sequence variations. The number of oligo types contained in the synthesized nucleotides having mixed sequences can be gained by multiplying each of the respective mixed base. For the example case above, as there are two different bases at each of the two positions, the degenerate primer will have four different kinds of sequences.
A universal primer is a sequence found in most of the plasmids. Most of plasmids used in the cloning step is made based on pBR322 families with modification on M13 bacteriophage sequences. Examples include T7 promoter, SP6 promoter, T7 terminator, M13 forward/reverse, etc. The universal primers are designed to bind to such sequences and used for sequencing the DNA fragments in a vector.

Modified Oligo

1. What are the structure and type of modified oligos?

For wider applications, modified oligos having different sequences as naturally found DNA and RNA sequences are required. To make them, there are several methods such as modifying the (deoxy) ribose rings, modifying the bases, changing the phosphodiester bonds, and attaching the conjugates at the 5' or 3' end. All those variations can be done through the oligo-synthesizer using the phosphoramidite method.
If modification is needed on the 5' position, labeling of phosphoramidite is carried out by binding after synthesizing the oligo with the requested sequences.
In case of modification on the 3' position, a solid support attached with the label is used during the synthesis process, allowing it to be labeled as it is made.
By using phosphoramidite with the label attached to the 5' position of the deoxyuridine base, the label can be implanted on the desired position of the sequence resulting in synthesis of internal modified siRNA. Please check our homepage for the full lists of modifications that can be ordered along with their structures.

Synthesis Method

1. Does the synthesized oligos have phosphates at the 5' or 3' ends?

We generally provide oligos with their 5' and 3' positions marked with -OH unless otherwise specified. If you wish for 5' phosphate oligos, please order 5' phosphorylation modified oligos.

2. Can I order oligos having high percentage of G-residues?

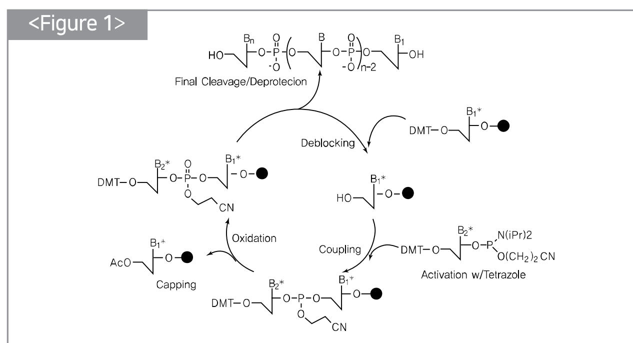
Oligos with repetitive G residues are difficult to be synthesized. This is because if the sequence have more than 4 G-residues repeatedly, the oligo is prone to be aggregated in the form of guanine tetraplex (Poon and MacGregor (198) Biopolymers 45: 427-434). This can be avoided by adding inosines in some places of G. With our rich experiences and expertises, our company have gained technologies to synthesize oligos having up to 10 repetitive G-residues without any difficulties.

Oligonucleotide FAQs

3. How are the oligos synthesized?

The most common method for oligonucleotide synthesis is using the cyanoethyl phosphoramidite to form the backbone structure of DNA and continuing to connect the phosphodiester bonds through "phosphite triester method" (Nucl. Acids Res. 1984, 12, 4539; Tetrahedron Lett. 1983, 24,5843). This makes it possible to synthesize oligonucleotides with high efficiency in a short time (synthesis efficiency > 98%). Long term storage is possible as phosphoramidite monomers are already stabilized before being activated for coupling process. The synthesis process begins with a solid support attached with a nucleoside. Cycles of deblocking, coupling, oxidation, and capping is repeated to attain the desired sequence of oligonucleotides. Refer to the following figure.

<Figure 1>



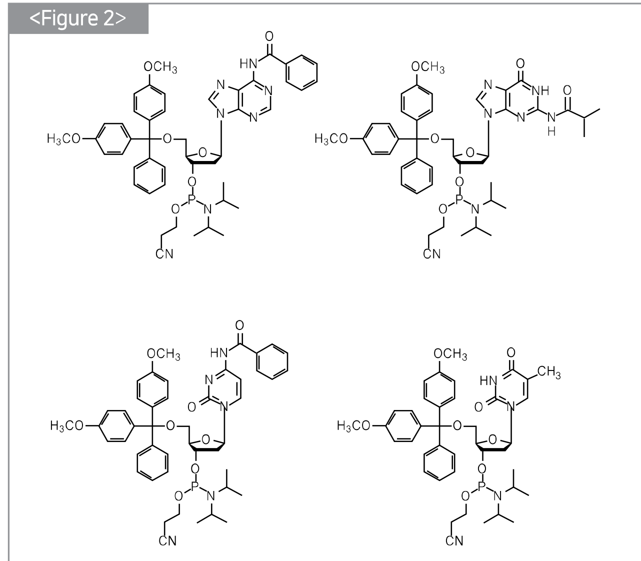
A. Deblocking

The deblocking process, the first step in the synthesis cycle, involves a reaction to remove DMT group, a protecting group for 5'OH base attached to a solid support. For this to happen, an acidic condition is necessary, and 3% trichloroacetic acid is mostly used. However, some reports claim that acidity may cause depurination, which the bonds between a purine base and a sugar ring are broken, especially when the base is adenosine. Furthermore, trichloroacetic acid is a very strong acid (pKa: ~ 1.5). If it is used for the deblocking process, one must be cautious not to leave the reaction for a long time, or else the depurination will occur. To avoid this problem, in some cases, dichloroacetic acid, a weak acid, may be used instead. During deblocking, the DMT positive ion leaving the solid support are dark orange in color, and their absorbance can be used to measure binding efficiency during oligo synthesis.

B. Coupling

The coupling reaction is done with the nucleoside phosphoramidite monomers and 5'-hydroxyl groups produced during the deblocking process on the solid support to synthesize the oligonucleotide having the desired sequence. The amine group of nucleosides phosphoramidite monomers used in this step is protected with benzoyl groups (for adenosine and cytidine) or isobutyryl groups (for guanosine), while 5'-hydroxyl groups of all the monomers are protected with DMT. Those serve as bridges during the coupling process. Refer to the following figure.

<Figure 2>



As the phosphoramidite used in this process is already in stabilized structure, those must first undergo activation process to bind with the 5' hydroxyl group on the solid support. Tetrazole is normally used as an activator to react with phosphoramidite to protonate the nitrogen and convert diisopropyl amino groups into a highly reactive tetrazolide structure.

This results in the formation of phosphite triester bonds from the combination reaction between the tetrazolide and the 5'-hydroxyl group of the solid support. The high reactive nature of the tetrazolide structure will form unwanted structures in the presence of water, even if it exists in a small amount. Thus, having anhydrous conditions are highly important in the coupling process.

C. Oxidation

The phosphite triester structure formed by the coupling of phosphoramidite and 5'-hydroxyl group must be stabilized by converting it to phosphate triester structure. This is done through an oxidation reaction using an iodine.

D. Capping

Since the coupling reaction cannot occur quantitatively (usually > 98%), some 5'-hydroxyl groups remain unreacted on the solid support. If those oligos are carried to the coupling step of the next cycle, those will result in having the sequence of one less amino acid, with those being (N-1) mer long.

Therefore, it is important to remove them after the synthesis process, as they make the purification process to get the desired oligonucleotides difficult, making the 'capping' process necessary on the 5' hydroxyl groups to prevent their further reaction. For this, acetylation must be done using acetic anhydride and N-methylimidazole.

Oligonucleotide synthesis of desired length is accomplished by repeating the above procedures. Afterwards, oligonucleotides are treated with ammonia to purify and isolate the oligonucleotides.