FAQ (Frequently Asked Questions) about custom DNA Synthesis

1. What is scale of synthesis?

Scale of synthesis refers to the amount of starting CPG (controlled-pore glass) support-bound monomer used to initiate the DNA synthesis, not the amount of final material synthesized. As an example, a 20 mer synthesized at a 200 nanomole scale of synthesis will produce approximately 80 nanomoles. The losses occur during synthesis, post-synthetic processing, transfer of material, and quality control.

2. Do I need to have my oligo purified?

It depends on whether or not modifications are requested and what the application will be. Failure sequences may be generated both during the synthesis and post-synthesis processing. We recommend that all modifications be purified either by cartridge or HPLC. For recommended purity and scale (based upon application), please see Table I.

Table I
Recommended Scales of Synthesis and Purification

Application	Scale of Synthesis	Purification
For Non-modified Oligos		
DNA sequencing	50 or 200 umol	OPC OR PAGE
PCR (general mplification)	50 or 200 umol	OPC
PCR (diagnosticapplication)	200 umol	OPC OR PAGE
Subcloning, site-directed mutageneses or cDNA synthesis	200 umol	OPC\PAGE\HPLC
Gene Construction		
<80mer	200 umol	PAGE
>80mer	1.0 umol	PAGE
Antisense	1.0 um or more	PAGE\HPLC
For Modified Oligos		
Modified bases and chemical linkers	200 umol	PAGE\HPLC
Reporter groups (biotin, DIG or fluorescent dyes)	200 umol	PAGE\HPLC

3. How much do I get or what scale of synthesis should I order?

Refer to the Table II

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Tel: +86-21-54460832 Web: www.synthesisgene.com
Fax:+86-21-54460831 E-mail: master@shinegene.org.cn

Table II Scale of Synthesis, Purification and Yields		
Scale of synthesis	Purification	Yield
50 nanomole	Desalt	150 ug: actual yield is sequence and length dependent
	Reverse phase cartridge	Average yield: 50-75 ug
	Reverse phase HPLC	Average yield: 50-75 ug
	Anion-exchange HPLC	Average yield: 50-75 ug
200 nanomole	Desalt	Average yield: 600 ug
	Reverse phase cartridge	Average yield: 300 ug
	Reverse phase HPLC	Average yield: 300 ug
	Anion-exchange HPLC	Average yield: 300 ug
	PAGE: less than 40mer	Average yield: 25-75 ug
	Greater than 40mer	Average yield: 10 ug
1 umole	Desalt	Average yield: 2 mg
	Reverse phase cartridge	Average yield: 1 mg
	Reverse phase HPLC	Average yield: 1 mg
	Anion-exchange HPLC	Average yield: 1 mg

4. What do I re-suspend my oligo in and what concentration should I make it?

Purified water, PBS or any biological buffers are acceptable as diluents. The recommended diluent volume is 100 ul - 1 ml, the concentration depending on the application to be used and the yield of the resulting product. Standard concentration for PCR primers is 0.1 mM.

5. How do I determine my concentration?

Concentration is determined by measuring the OD260 of the diluted oligo. Prepare a dilution of the resuspended oligo and measure the OD260 . Determine the concentration as follows:
(?g or pmoles/ OD260) X dilution factor = final concentration / mL.

6. How stable is my oligo once I have resuspended it?

If sterile diluent is used to resuspend the oligo, it will be stable at 4℃ for about a month. If stored frozen

at -20℃ or -70℃, it will remain stable for 2-3 months. Repeated freeze-thaw should be avoided, as it will denature the oligo. Avoid the use of distilled water, since solution pH may be as low as 4-5.

7. Does my oligo have a phosphate on the 5' end?

Unless requested, oligos are synthesized without either 3’ or 5’phosphate. The 5’ phosphate modification is available, normally as an additional charge.

8. My annealed oligos will not ligate. What is the problem?

Ligation reactions require a 5’ phosphate. If your oligos do not contain a 5’phosphate, ligation will not occur. The problem can be addressed without ordering an additional oligo pair: phosphorylate your oligos enzymatically with kinase before use in ligation reactions.

9. How do you calculate the molecular weight of my oligo?

The molecular weights for oligos is the sum of the component molecular weights of all bases, with mixed bases contributing proportionately. The component molecular weights of the bases vary as to their salt form. Many times desalted oligos are ammonium salts, while cartridge, HPLC and PAGE purified oligos are sodium salts. The molecular weights used in the calculations are listed in the following Table III.

Table III
Molecular Weight Calculations

Molecular Weights of the Bases			
	Sodium Salt		Ammonium Salt
	DNA	Thioate	DNA
WA	313.21	329.27	330.24
WC	289.18	305.25	306.24
WG	329.21	345.27	346.24
WT	304.19	320.26	321.23
WCORR	61.96	61.96	96

Molecular Weight Calculation: (PA * WA) + (PC * WC) + (PG * WG) + (PT * WT) +(Pmod * Wmod) - WCORR
Where PA is the number of As and WA is the component weight of A and Pmod is the number of Modifications, and Wmod is the component weight of the added modification.
Please refer to Table IV for the molecular weights of common modifications.

Table IV

Molecular Weights of Common Modifications			
Modification	Molecular Weight	Modification	Molecular Weight
5'-Biotin	405.45	3'-TAMARA	623.60
5'-(6 FAM)	537.46	3'-Dabsyl	498.49
5'-HEX	744.13	3'-Fluorescein-dT	815.71
5'-TET	675.24	3'-(6 FAM)	569.46
5'-Cy5	533.63	3'-Amino Modifier C3	153.07
5'-Cy3	507.59	3'-Amino Modifier C7	209.18
5'-Dabcyl	430.18	3'-Thiol Modifier C3	154.12

11. What is coupling efficiency?

Coupling efficiency is a measure of the DNA synthesizer's ability to couple each new monomer to the growing chain. If all the monomers coupled completely to the growing chain, the coupling efficiency would be 100%. If 1% of the growing monomer chain fails to react, then the coupling efficiency of that step is only 99%. The coupling efficiency for the complete synthesis of the oligo is usually determined from the yields of full-length sequence after the first and last cycle. Coupling efficiencies greater than 99.0% are essential for good oligo product with minimum purification.

12. How is the coupling efficiency determined?

Following the first coupling step, the amount of Trityl released during deblocking is directly proportional to the amount of full-length oligo made in the previous cycle. When the Trityl is cleaved during the deblocking step, the resulting Trityl cation is orange in color. The intensity of this color can be measure by UV spectrophometry. By comparing the intensities of the Trityl produced after the first and last coupling, one can calculate the average successful base coupling per cycle and hence the coupling efficiencies.

13. I sequenced a clone I prepared with your primer and the sequence for the primer region was different from the one I ordered. Why?

There are number of possible explanations for apparent errors in the sequence of the oligonucleotide used in cloning experiments. There could have been human error during the ordering and synthesis of the oligonucleotides. Human error can be easily checked by looking at the sequence on the specification sheet that accompanies each oligo. Below is a list of problems associated with using the beta-cyanoethyl phosphoramidite chemistry that give the impression that a synthesis error had been introduced.

1. The G base may have been converted to the enol tautomer, 2,6 diaminopurine, which is recognized as A by DNA polymerase. Thus, clones generated from an oligo with this modified base will appear as a G to A transition.

2. The chemical process of synthesis may cause depurination. Depurinated oligos are usually degraded at the deprotection stage, but it is possible for a small percentage to remain. Clones containing an inserted oligo that was depurinated will appear as having an A or G deletion. Oligos that are purine rich have an increased risk of having these artifacts.
3. Usually failed sequences that do not couple to the next incoming base are capped to prevent further synthesis. Unfortunately, sequence failure that are not capped are still capable of further synthesis and will appear as deletions in cloning experiments. These events are usually rare and the effects can be overcome by screening additional clones for those that were created with an oligo with the correct sequence.
4. During the base addition step, a small percentage of the incoming bases may couple with each other prior to coupling with the growing oligo chain. Clones generated using these oligos will appear as an insertion. Again, this event is rare and the effects can be overcome by screening additional clones for those that were created with an oligo with the correct sequence.

It is therefore highly recommended that HPLC or PAGE purified oligos be used for cloning experiments and that more than 1 clone should be collected and screened.

14. Why are some modified oligos so expensive in relation to the cost of the modifying reagent?

The limited reagent stability (most <48 hours) and lower coupling efficiencies of the reagent requires that excess modifying reagent be used to insure adequate quantities of full length product is made. As a result, higher cost incurred in synthesis.

15. Why are the yields lower for modified bases?

Many of the modified amidites are unstable and do not couple as efficiently as the unmodified bases (even though longer coupling procedures may be used), thus failure sequences are more abundant than in normal synthesis. Consequently, all modified oligos should be purified either by cartridge or HPLC to remove the more abundant failure sequences. Yields are reduced as a result of purification.. The end product, although with a lower yield, is much more pure.

16. Why isn't the yield for 1 ?m scale syntheses five times greater than 0.2 um ol scale syntheses?

For 0.2 umol scale, the monomer coupling is done at a 40-50-fold excess. To do so for larger scale syntheses (such as 1.0 umol scale) would be cost-prohibitive. Large-scale syntheses are done at 10-fold mole excess of amidites. However, to increase the yields for these larger scale syntheses, the coupling times are extended to increase coupling efficiencies.

17. What is the longest length an oligo can be synthesized?

The real answers lies in the limit of resolution of the purification method and the coupling efficiency of the DNA synthesizer. It is not unusal to synthesize oligo in excess of 150 bases and to obtain sufficient

quantities by PAGE purification to do successful gene construction. It should be remembered that the longer the oligo, the greater the chance of accumulated sequence errors.

18.What is the best way to calculate the Tm of my oligo?

We use an algorithm to calculate the Tm, rather than the CG ratio as is gives a more accurate result. The values will be given on the oligo data sheet.

The Tm can be calculated online at:-

http://alces.med.umn.edu/rawtm.html
http://www.PremierBiosoft.com/netprimer.html

(Biocolors used a similar method to these)

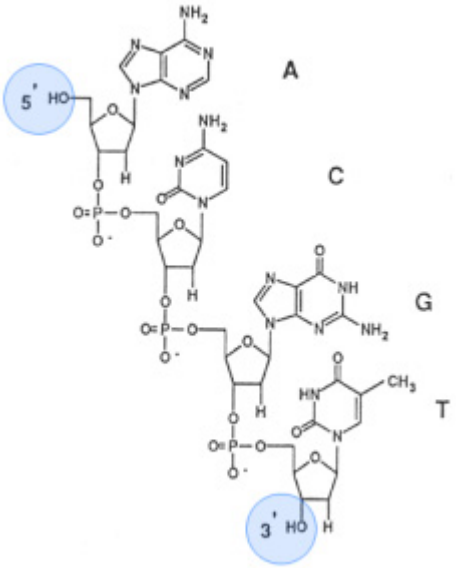
You could also use the equation:
Tm = 64.9 + 0.41 * (%C + G) - 600 / #bases)
not as accurate but it gives a good idea.

19. Codes for the mixed bases

K=G+T M=A+C R=A+G S=C+G W=A+T Y=C+T B=C+G+T D=A+G+T

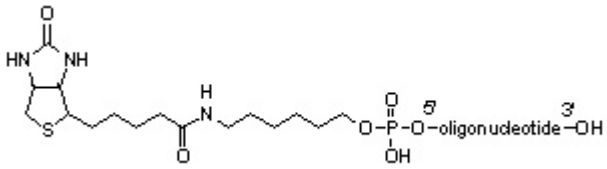
H=A+C+T V=A+C+G N=A+C+G+T

20. Standard Oligo Structure



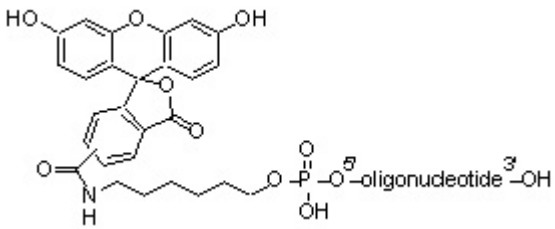
21. Modified Oligo Structure

- 5’-Biotinylated Oligonucleotide



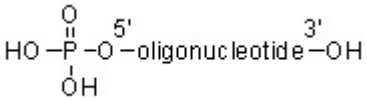
Biotinylated oligonucleotides are used for a number of applications, which include colorimetric detection of DNA and solid phase capture by Streptavidin coated magnetic beads for use in restriction mapping, genomic walking and differential display.

- 5’-Fluorescein Labeled Oligonucleotide



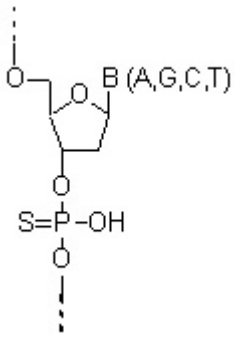
Cy3, Cy5 and TAMRA are also available as labeling dyes for oligonucleotides.
Fluorescent dye-labeled oligonucleotides have been widely used in automated DNA sequencing, quantitative PCR, and in situ hybridization reactions.

- 5’-Phosphorylated Oligonucleotide



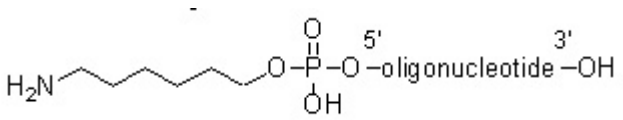
5’-Phosphorylated oligonucleotides are commonly used in site directed mutagenesis and linker insertion.

- Phosphorothioation (S-Oligo)

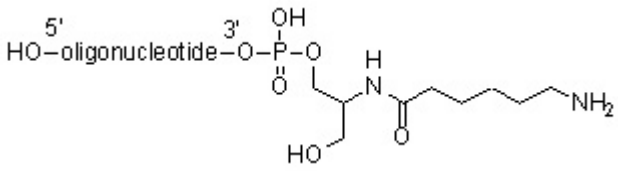


The modified “backbone” of an S-Oligo is resistant to the degradation by most endo- and exonucleases. This property allows increased intracellular effectiveness of antisense oligonucleotides. The replacement of the inter-nucleotide phosphate groups with phosphorothioate groups (substituting one of the oxygen atoms of phosphate group with a sulfur atom) can be made in the entire of partial sequences of oligo depending on the customers’ needs.

- 5’-Amino Modified Oligonucleotide



- 3’-Amino Modified Oligonucleotide



The presence of a primary aliphatic amine group at the terminus of an oligonucleotide allows the post ?synthesis attachment of a number of amine reactive molecules. The amino modified oligonucleotides can also be used for the immobilization of the desired oligonucleotide on the matrix to

make the oligonucleotide-based microarray.

22. Can you do mixed bases?

Yes. There is no charge for any equal molar mixed bases except for the 3' end bases. We can also do "poisoned" bases for an extra charge.

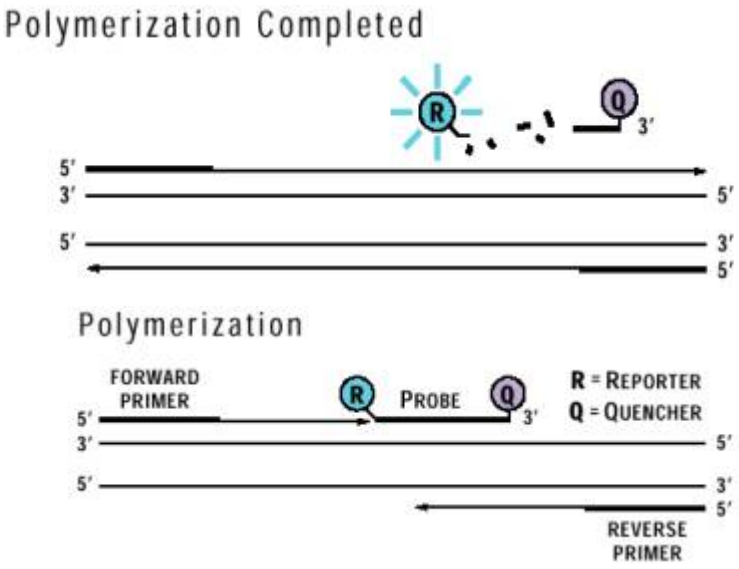
23.Can you do other bases besides A, C, T and G?

If the amidite or NHS-ester of the base of choice is commercially available, we can incorporate it into an oligo.

24. TaqMan Probe &Primer Design guidelines

Probe Design Guidelines

- The melting temperature (Tm) of the probe should be 68-70 ℃
- Keep G-C contents in the 30-80% range.
- Avoid runs of an identical nucleotide. This is true especially for G, where runs of four or more Gs should be avoided.
- Do not put Gs on the 5'end.
- Select the strand that gives the probe more Cs than Gs. Primer express does not automatically screen for this feature. It needs to be done manually.
- If the TaqMan probe is designed for allelic discrimination, the position of the polymorphic site (mismatch) should be approximately in the middle third of the sequence.
- The principle of taqman



- Design primers as close to probe as possible
- Keep G-C contents in the 30-80% range.
- Avoid runs of an identical nucleotide. This is true especially for G, where runs of four or more Gs should be avoided.
- The melting temperature (Tm) of the primer should be 58-60 ℃.
- The total number of Gs and Cs in the last five nucleotides at the 3?end of the primer should not exceed two. The software does this automatically if the option is selected.
- Primers should scan exon-exon junction. Contaminating genomic DNA will not be amplified by these primers.
- Use primers that contain dA nucleotides near the 3'ends so that any primer-dimer generated is efficiently degraded by AmpErase UNG. If primers cannot be selected with dA nucleotides near the ends, the use of primers with 3'terminal dU nucleotide should be considered.
- Of course,you can visit the websites of real time PCR primer design.

https://www.genscript.com/ssl-bin/app/primer

Also you can download the soft from the next websits to design it.

http://www.premierbiosoft.com/molecular_beacons/index.html

If you want to get more information,please visit the next websites.

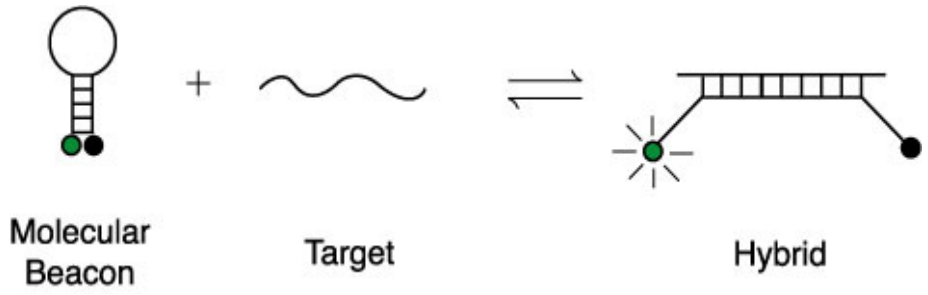
http://www.appliedbiosystems.com.cn/chinese/pe_h2-add1.html

25. Introduction to Molecular Beacons

Molecular beacons are single-stranded oligonucleotide hybridization probes that form a stem-and-loop structure. The loop contains a probe sequence that is complementary to a target sequence, and the stem is formed by the annealing of complementary arm sequences that are located on either side of the probe sequence. A fluorophore is covalently linked to the end of one arm and a quencher is covalently linked to the end of the other arm. Molecular beacons do not fluoresce when they are free in solution. However, when they hybridize to a nucleic acid strand containing a target sequence they undergo a conformational change that enables them to fluoresce brightly.

In the absence of targets, the probe is dark, because the stem places the fluorophore so close to the nonfluorescent quencher that they transiently share electrons, eliminating the ability of the fluorophore to fluoresce. When the probe encounters a target molecule, it forms a probe-target hybrid that is longer and more stable than the stem hybrid. The

rigidity and length of the probe-target hybrid precludes the simultaneous existence of the stem hybrid. Consequently, the molecular beacon undergoes a spontaneous conformational reorganization that forces the stem hybrid to dissociate and the fluorophore and the quencher to move away from each other, restoring fluorescence.



If you want to get more information about it,please visit the next website.

http://www.molecular-beacons.org/default.htm

26.Can you help us to design primers?

Yes.of course,you can visit the next websites to design it online.

http://www.broad.mit.edu/cgi-bin/primer/primer3_www.cgi

27. Fluorescein Labeled

Name	Name	Absorbwavelenth	Emissionwavelenth	colors
6-FAM	6-carboxy-fluorescein	494nm	518nm	Green
TET	5-tetrachloro-fluorescein	521nm	538nm	Orange
HEX	5-hexachloro-fluorescein	535nm	553nm	Pink
TAMRA	tetramethyl-6-carboxyrhodamine	560nm	582nm	Rose
ROX	6-carboxy-x-rhodamine	587nm	607nm	Red
Cy3	Indodicarbocyanine	552nm	570nm	Red
Cy5	Indodicarbocyanine	643nm	667nm	Violet

28. How do I make double-stranded DNA?

It is sometimes necessary to make double-stranded DNA from single-stranded oligos. While the annealing procedure is very simple, attention to a few details can greatly reduce the presence of undesired single stranded material.

Method:

1. Dissolve oligos in STE Buffer (10 mM Tris pH 8.0, 50 mM NaCl, 1 mM EDTA). The presence of some salt is necessary for the oligos to hybridize. Dissolve each oligo at high concentration (1 - 10 OD_{260} units / 100 uL).
2. Mix two stands together in equal molar amounts. If you do not there will always be single stranded material left over.
3. Heat to 94^{o}C and gradually cool. For many oligos this can be as simple as transferring to the bench-top at room temperature. For sequences with significant hairpin potential, a more gradual cooling/annealing step is beneficial; this is easily done by placing the oligos in a water bath or temp block and "unplugging the machine".
4. The resulting product will be in stable, double-stranded form and can be stored at 4^{o}C or frozen.

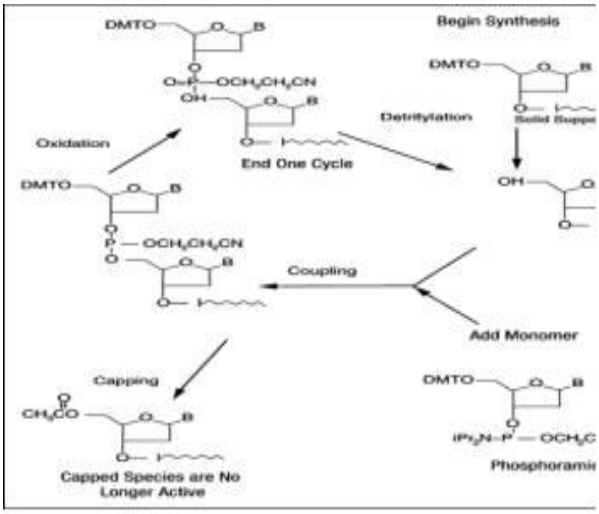
Things to consider: If the product will be used in a ligation reaction, the addition of 5' -phosphate may be needed. This can be done at the time of oligo synthesis (chemical phosphorylation) or at any time thereafter using PNK (enzymatic phosphorylation). If the oligos are relatively long or to be used in cloning, starting with PAGE purified oligos is recommended.

29. How are oligonucleotides synthesized?

Oligonucleotides are produced using an automated DNA synthesizer that adds a base in the 3’ to 5’ direction. The addition of each base is termed a cycle. There are 4 basic steps to a cycle.

1. A new base is added to the 5’ end of the growing chain. Approximately 99% of the available sites will react.
2. The sites that did not react are chemically capped. These capped bases will no longer be used in the synthesis and result in failed sequences.
3. The new base-to-base bond is stabilized by oxidation.
4. The 5’ base has its protecting groups removed and is now ready to bond with the next added base when the first step is repeated.

When the oligonucleotide synthesis has completed the oligo is cleaved form its solid support, which was used to hold it in place during synthesis. Protecting groups are then removed using concentrated ammonium hydroxide. While the oligo is ready for use in some application at this point, the commercial oligo vendors used by the DNA Facility will also remove the reaction salts (a process called “Desalting”). Desalting will not remove the failed sequences.



30.Other data which we have been often asked for:

(although if you don't know this you should be ashamed of yourself)
1 millimole = 1000 micromoles
1 micromole = 1000 nanomoles
1 nanomole = 1000 picomoles
to convert moles to grams:- multiply by the molecular weight
to convert grams to moles:- divide by the molecular weight