ZYMO RESEARCH

SIMPLE TIPS TO BOOST YOUR BISULFITE-BASED APPLICATIONS

This piece is brought to you by Zymo Research scientists, Lam Nguyen and Ron Leavitt.

Bisulfite conversion remains a key player in almost every study involving DNA methylation. Since DNA methylation is emerging as a key mechanism in a diverse range regulator processes and disease mechanisms, many researchers new to the field are turning to bisulfite-based analyses that might not be experts in the approach.

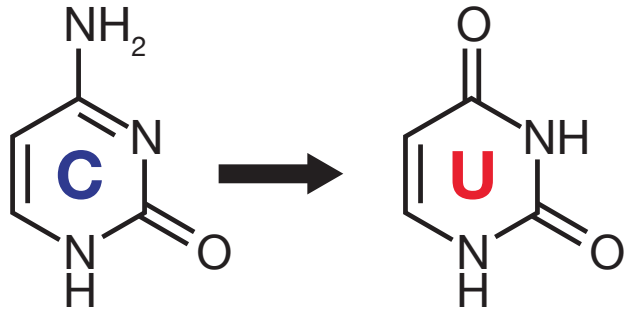
We've been optimizing bisulfite conversion for nearly a decade. We've designed our kits to take a lot of the guesswork out of the process, but in many cases, it's the downstream analysis method (e.g. MS-PCR, bisulfite sequencing etc.,) that can be problematic.

The bisulfite conversion process takes your nice DNA samples complete with 4 bases and reduces the complexity to three bases. On paper this might not seem like a big deal, but when you try to design unique primers for PCR or sequencing armed with one less base, it can be challenging to say the least, so we thought we'd share a few quick tips to minimize the hassle.

FIND YOUR POLYMERASE

When amplifying bisulfite converted DNA, go with a hot start polymerase. They minimize primer dimers better than regular *Taq* polymerase and the specific amplification products that come along with them. Using a hot-start polymerase helps by preventing the polymerase from being active during the PCR setup period, when primers at room temperature are more prone to nonspecific binding.

Not all PCR mixes will perform well though, so be sure to use a hot start mix that has been optimized for bisulfite treated DNA. Most standard PCR mixes have not been optimized for amplifying bisulfite treated DNA templates.



We typically optimize the buffer conditions after conducting many titrations of known PCR boosters and other salt constituents that lower annealing temperature, reduce primer dimerization, and limit non-specific binding from occurring when working with bisulfite treated samples.

Try and screen as many polymerases as you can and see what works best in your hands. We've tested every available hot start polymerase we could find that delivered the most specific and the most robust amplification with bisulfite converted DNA. It's laborious, but it pays off.

THE BASICS OF BISULFITE PRIMER DESIGN

No matter how optimized your polymerase and buffers are, they're not going to be much help without welldesigned primers. When designing the primers from the known DNA sequence, it is a good exercise to 'convert' the DNA first – changing all of the non-CpG cytosines to uracil.

The first primer should be designed to base-pair with this converted sequence (normally this would be thought of as the reverse primer). The second (forward) primer should be designed to base-pair to the extension of the first primer and not the "opposite strand" like in traditional PCR. Primers

for bisulfite PCR normally range from 26-35 bases, with amplicon sizes generally ranging from 200-500 bases.

Higher annealing temperatures are key for good amplification. Longer than normal primers are necessary to aid in specificity to the template and to bring melting temperature up. Bisulfite primers should have Tms greater than 50°C.

It's best to include as many guanines as possible in the primer regions to increase the melting temperature of the primer. Additional bases can also be added to the primer to bring up the melting temperature.

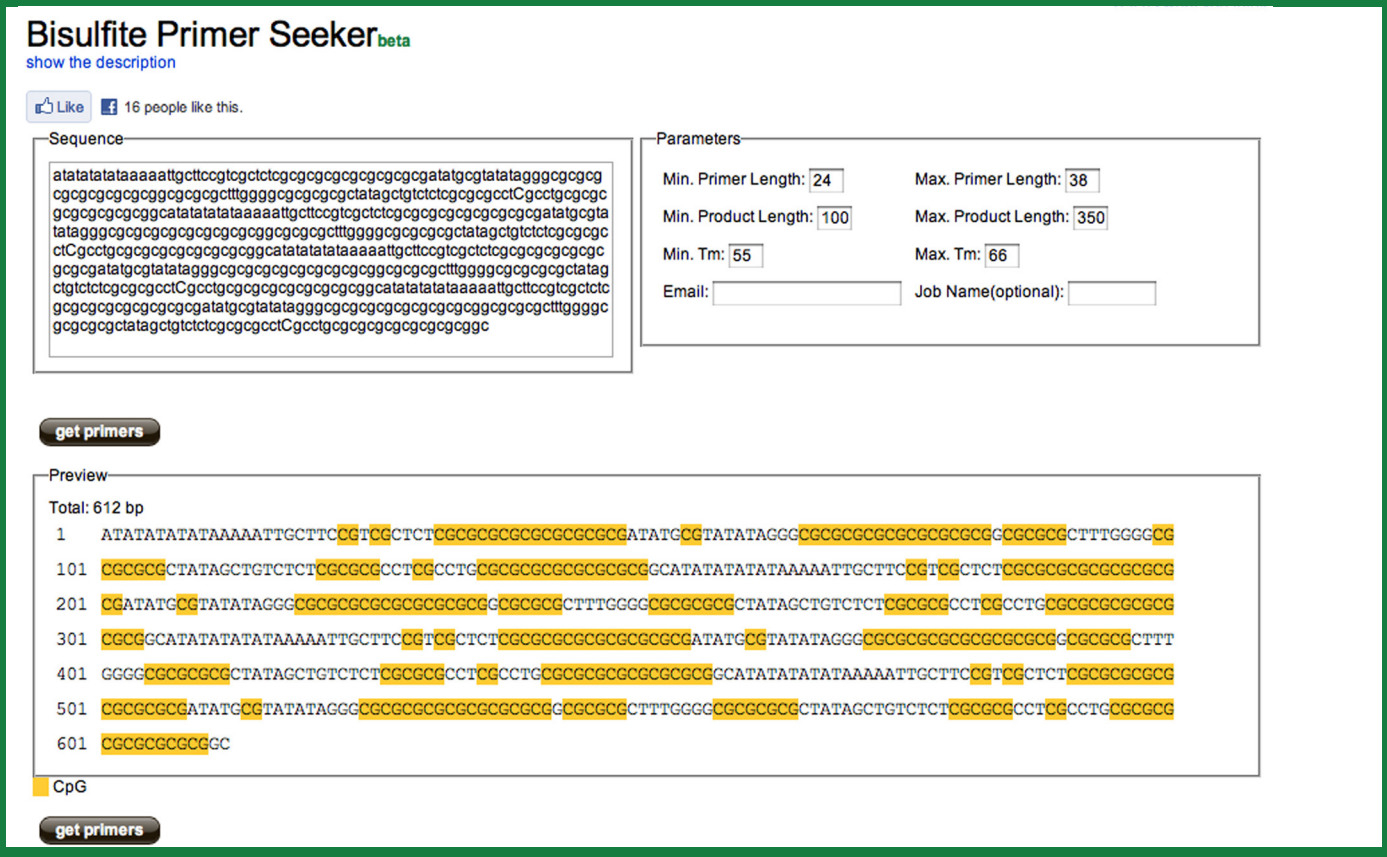
For unbiased amplification, each primer can contain at most one CpG dinucleotide. If the primer does contain a CpG, use the mixed bases purine (A or G, usually abbreviated 'R' and used in the first primer) and pyrimidine (C or T, usually abbreviated 'Y' and used in the second primer) so that both methylated and non-methylated DNAs are amplified.

Also, ending the primer with a converted C (from the original sequence) has been shown to increase the specificity of the reaction. Lastly, when using a new set of primers, we suggest performing an annealing temperature gradient spanning the calculated temperature to determine where the primers are functioning most efficiently.

BISULFITE PRIMER SEEKER

Bisulfite Primer Seeker is a free program that has been optimized to design primers for CGrich sequences where others usually fail. It recommends multiple options for amplicons that span different regions within the sequence where other programs tend to center in on one area.

Try it today at **www.zymoresearch.com/tools/bisulfite-primer-seeker**



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