

## Primer design and dilution

### Primer design-general

- 1) You want to make your primers ~ 20mers; sometimes you may need them to be shorter, other times you will want longer ones.
- 2) Always check your primers in GeneRunner for secondary structure problems, like hairpin loops, and primer dimers.
- 3) Try to end your primers with a GC clamp. Basically you want your primers to end with 2 strong bases (C and G). Even a single C or G is preferable to an A or T. You do not, however, want to make the GC clamp too long or you will get non-specific priming.

### Degenerate primer design.

- 1) Always work from an amino acid alignment.
- 2) Find a, 8-10 amino acid region of the protein where all sequences in your alignment are identical (most proteins have some highly conserved regions that are suitable). Remember, you want to make degenerate primers as long as possible.
- 3) Look for regions that do not have arginine, leucine and serine; these are 6-fold degenerate codons, and are not suitable for primers. Try to find regions with methionine and tryptophane since they only have a single codon.
- 4) Write down the amino acid sequence

ex. F F Y M W A V P A

- 5) Next, translate the amino acid sequence to nucleotides, based on their respective codons. (Note the use of IUPAC symbols for degeneracy)

ex. F F Y M W A V P A  
5' TTY TTY TAY ATG TGG GCN GTN CCN GCN 3'

- 6) Next you want to refine your primer, the first thing to do is remove the terminal nucleotide from the alanine codon, this not only removes degeneracy, but leaves you with a nice 2 nucleotide GC clamp. This gives us

5' TTY TTY TAY ATG TGG GCN GTN CCN GC 3'

but the primer is still 512-fold degenerate (meaning that there will eventually be 512 different primers). There are a couple of ways to reduce degeneracy.

a) you can shorten the primer from either end, eliminating codons with high degeneracy. For example if we go ahead and eliminate the last 2 codons, we would end up with a primer that looks like:

5' TTY TTY TAY ATG TGG GCN GT 3'

although the primer is now shorter it is still a 20mer and it is only 16-fold degenerate!

b) you can go ahead and call the 3<sup>rd</sup> position at the 5' end of the primer, where tight annealing is less important. You can make an informed decision based on the codon usage of your organisms (ie it prefers TTA over TTC for phenylalanine 75% of the time) and use the most common codon, if we do that for the first 3 codons of our primer we would get a primer that now looks like:

5' TTA TTA TAC ATG TGG GCN GTN CCN GC 3'

which is now 64-fold degenerate, and is still quite long (a 26mer).


7) Remember, when you are designing degenerate primers you are looking to do 2 things, 1-get a primer that is as long as possible and 2-reduce degeneracy as much as you possibly can. All refinements of your primers will involve a trade-off between those 2 aspects, it is rare to find a very long, highly conserved region without some 6-fold or a lot of 4-fold degenerate codons.

### Primer Dilution.

When you order primers, they are shipped dry, which means you have to resuspend them. In order to get your primers at the proper stock concentration, the company will give you information about your primer, and how much of it they made. You then have to do some simple calculations to determine how much buffer to add to your dry primer. First, let's look at the information the company gives you. When your primers arrive in the mail, they will be accompanied by an information sheet like this:

9574  
# 53698  
Y VISA

**Data Sheet**



Seq #	Seq Name	Seq 5' to 3'	OD	pmol	Len	MW	µg	E260	Tm	Scale	Purif.
6079	SAC208F	TAGGATGTTTGTCTGGGGC	14.9	83014.69	19	5905.86	490.27	179485.3	60.16	50 nmole	Salt-Free
6080	SAC341R	AACCCTTATGCCCTTCTCC	17.87	113208.46	19	5634.66	637.89	157850.4	60.16	50 nmole	Salt-Free

This information sheet contains everything you need to know to dilute your primer. The 2 critical pieces of information are molecular weight (MW); 7<sup>th</sup>

column) and the optical density (OD 4<sup>th</sup> column). The first, and most difficult calculation we have to do is calculate the dilution factor; X.

$$X = \frac{MW/10}{(OD)(33)}$$

So solving for primer SAC208F (from the data sheet) we get:

$$X = \frac{5905.88/10}{(14.9)(33)} = \frac{590.588}{491.7} = 1.20$$

Then for a 100  $\mu$ M sample we simply divide 1000  $\mu$ L by the dilution factor (X).

$$\frac{1000\mu\text{l}}{1.20} = 833.3 \mu\text{L}$$

This is the amount (in  $\mu$ l) of sterile TE buffer you add to the tube of dried primer in order to get a 100  $\mu$ M solution of your primer.

After you add you sterile TE buffer, vortex the tube to resuspend the primer well, and store at  $-20^{\circ}\text{C}$ .

The working concentration for PCR primers is 10  $\mu$ M, and as long as you will use it within a month or two, the 10  $\mu$ M aliquots can be stored at  $4^{\circ}\text{C}$ .