DESIGNING DEGENERATE PRIMERS

- Degenerate primers may be used to amplify DNA in situations where only the protein sequence of a gene is known, or where the aim is to isolate similar genes from a variety of species.

- A six or seven residue peptide sequence should be selected, corresponding to an oligo of about 20 nucleotides.

- If the oligo is designed to amplify several similar protein sequences, then the most conserved regions of the proteins need to be selected. If some of the residues are not completely conserved, then the oligo sequence will need to accommodate all possible codons of all amino acid residues at that site (e.g. if one protein has an E at a particular site, while all the others have a D, then the corresponding oligo sequence will be GAN – see table overleaf).

- The peptide sequence should avoid amino acids that have a lot of codons, such as leucine (L), arginine (R), and serine (S). Instead, aim for regions that are rich in amino acids that have only one or two possible codons (i.e. M, W, C, D, E, F, H, K, N, Q, Y – see table overleaf).

- Inosine residues can pair with any nucleotide, and so can be used at sites where there is complete degeneracy (i.e. substitute I for N in the table below). This reduces the number of oligos that have to be synthesised. Note that only some manufacturers use inosine, however.

- Try and avoid degeneracy at the 3' end of the oligo (note that it is not necessary to have whole codons), and especially avoid ending in inosine.

- The table overleaf may be used to help with primer design:

amino acid	amino acid symbol	nucleotide sequence (with degeneracy)	complement (for designing reverse primers)
methionine	M	ATG	TAC
tryptophan	W	TGG	ACC
cysteine	C	TGY	ACR
aspartic acid	D	GAY	CTR
glutamic acid	E	GAR	CTY
phenylalanine	F	TTY	AAR
histidine	H	CAY	GTR
lysine	K	AAR	TTY
asparagine	N	AAY	TTR
glutamine	Q	CAR	GTY
tyrosine	Y	TAY	ATR
isoleucine	I	ATH	TAD
alanine	A	GCN	CGN
glycine	G	GGN	CCN
proline	P	CCN	GGN
threonine	T	ACN	TGN
valine	V	GTN	CAN
leucine	L	YTN	RAN
arginine	R	MGN	KCN
serine	S	WSN	WSN

Key to symbols:

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