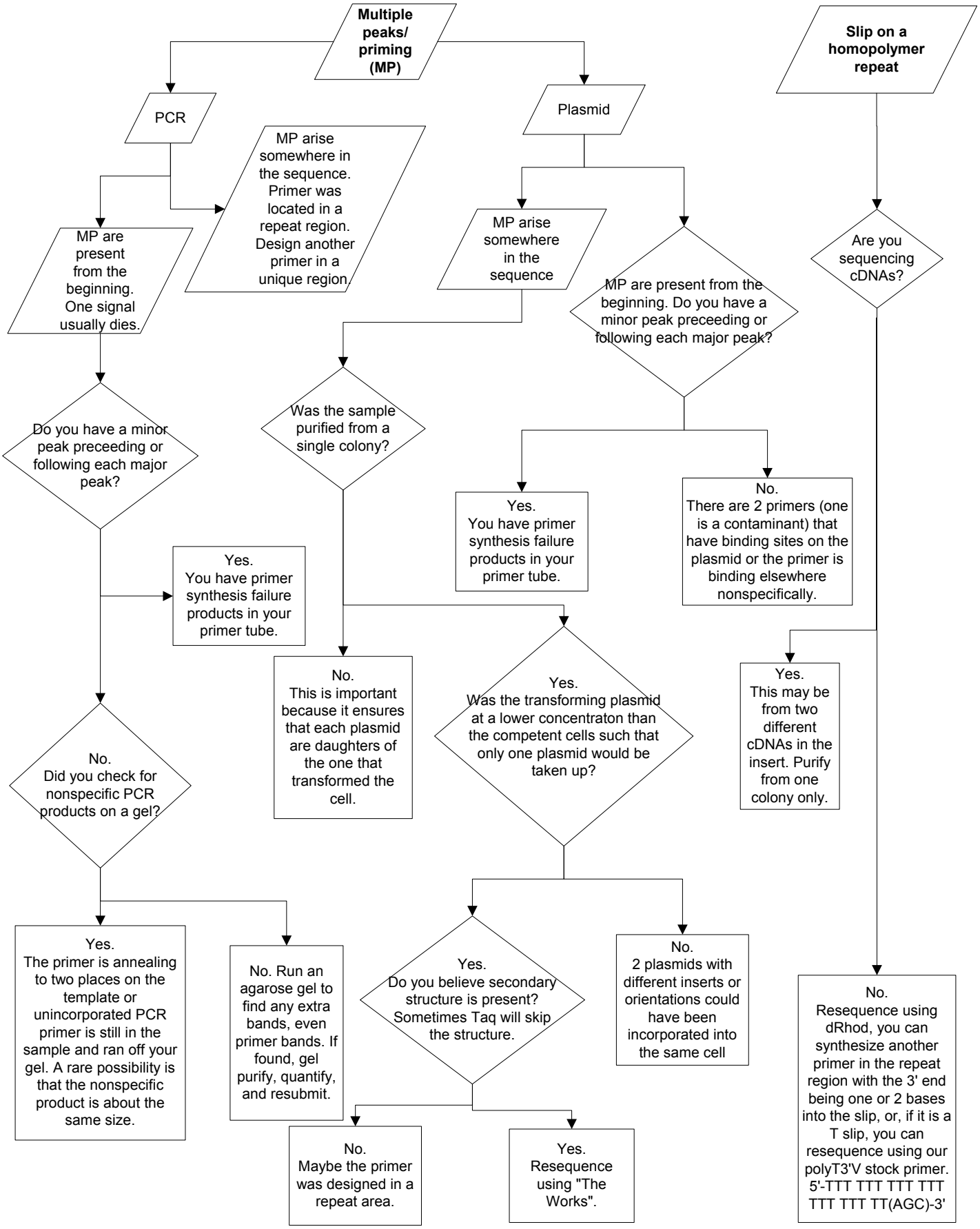
Multiple peaks/ priming (MP)
Slip on a homopolymer repeat
PCR
Plasmid
MP arise somewhere in the sequence. Primer was located in a repeat region. Design another primer in a unique region.
MP are present from the beginning. One signal usually dies.
MP arise somewhere in the sequence
MP are present from the beginning. Do you have a minor peak preceeding or following each major peak?
Are you sequencing cDNAs?
Do you have a minor peak preceeding or following each major peak?
Was the sample purified from a single colony?
Yes. You have primer synthesis failure products in your primer tube.
No. There are 2 primers (one is a contaminant) that have binding sites on the plasmid or the primer is binding elsewhere nonspecifically.
Yes. You have primer synthesis failure products in your primer tube.
No. This is important because it ensures that each plasmid are daughters of the one that transformed the cell.
Yes. Was the transforming plasmid at a lower concentraton than the competent cells such that only one plasmid would be taken up?
Yes. This may be from two different cDNAs in the insert. Purify from one colony only.
No. Did you check for nonspecific PCR products on a gel?
Yes. The primer is annealing to two places on the template or unincorporated PCR primer is still in the sample and ran off your gel. A rare possibility is that the nonspecific product is about the same size.
No. Run an agarose gel to find any extra bands, even primer bands. If found, gel purify, quantify, and resubmit.
Yes. Do you believe secondary structure is present? Sometimes Taq will skip the structure.
No. 2 plasmids with different inserts or orientations could have been incorporated into the same cell
No. Maybe the primer was designed in a repeat area.
Yes. Resequence using "The Works".
No. Resequence using dRhod, you can synthesize another primer in the repeat region with the 3' end being one or 2 bases into the slip, or, if it is a T slip, you can resequence using our polyT3'V stock primer. 5'-TTT TTT TTT TTT TTT TTT TT(AGC)-3'