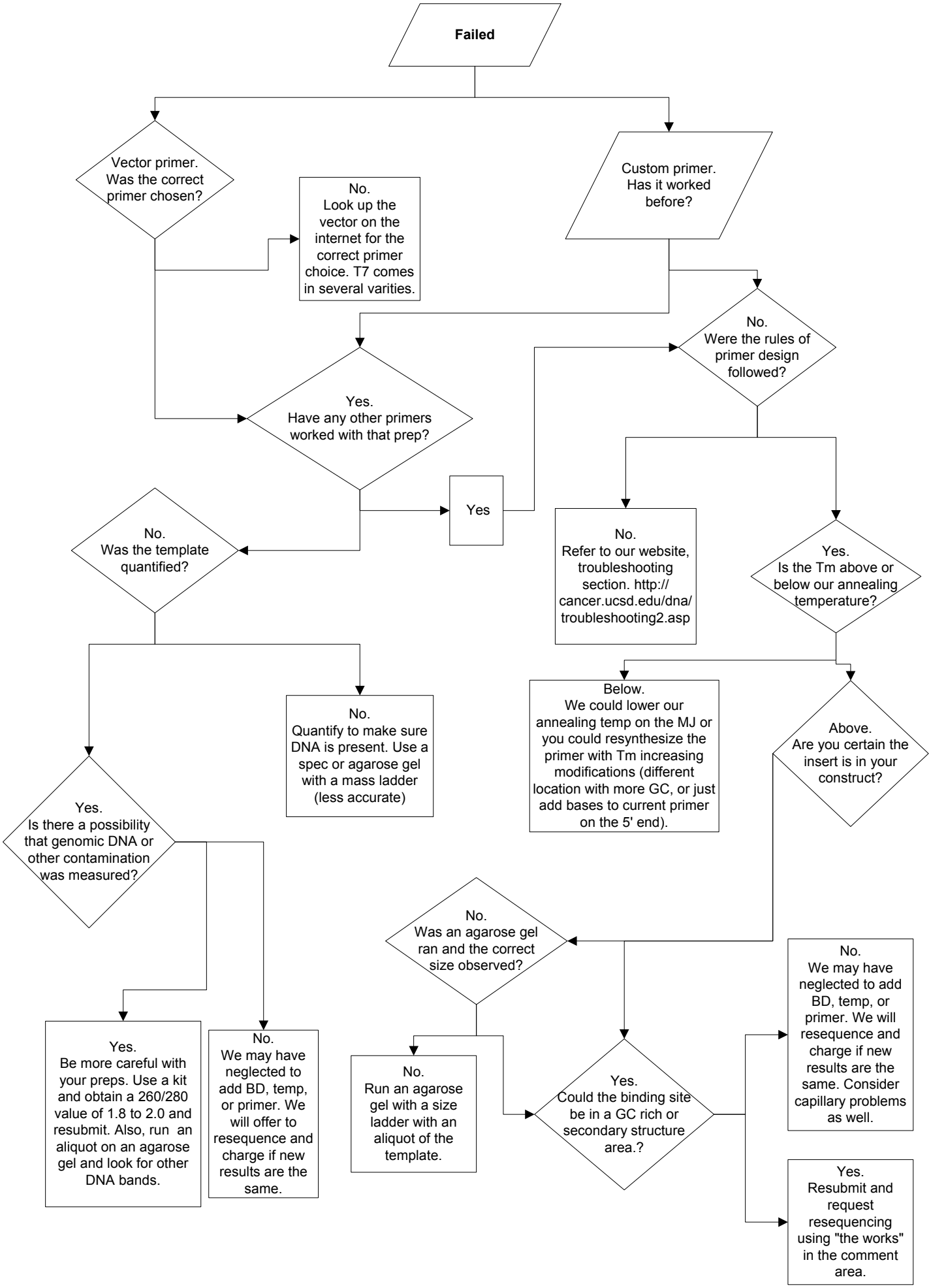
Failed
Vector primer. Was the correct primer chosen?
No. Look up the vector on the internet for the correct primer choice. T7 comes in several varities.
Custom primer. Has it worked before?
Yes. Have any other primers worked with that prep?
No. Were the rules of primer design followed?
Yes
No. Was the template quantified?
No. Refer to our website, troubleshooting section. http://cancer.ucsd.edu/dna/troubleshooting2.asp
Yes. Is the Tm above or below our annealing temperature?
No. Quantify to make sure DNA is present. Use a spec or agarose gel with a mass ladder (less accurate)
Below. We could lower our annealing temp on the MJ or you could resynthesize the primer with Tm increasing modifications (different location with more GC, or just add bases to current primer on the 5' end).
Above. Are you certain the insert is in your construct?
Yes. Is there a possibility that genomic DNA or other contamination was measured?
No. Was an agarose gel ran and the correct size observed?
No. We may have neglected to add BD, temp, or primer. We will resequence and charge if new results are the same. Consider capillary problems as well.
Yes. Be more careful with your preps. Use a kit and obtain a 260/280 value of 1.8 to 2.0 and resubmit. Also, run an aliquot on an agarose gel and look for other DNA bands.
No. We may have neglected to add BD, temp, or primer. We will offer to resequence and charge if new results are the same.
No. Run an agarose gel with a size ladder with an aliquot of the template.
Yes. Could the binding site be in a GC rich or secondary structure area.?
Yes. Resubmit and request resequencing using "the works" in the comment area.