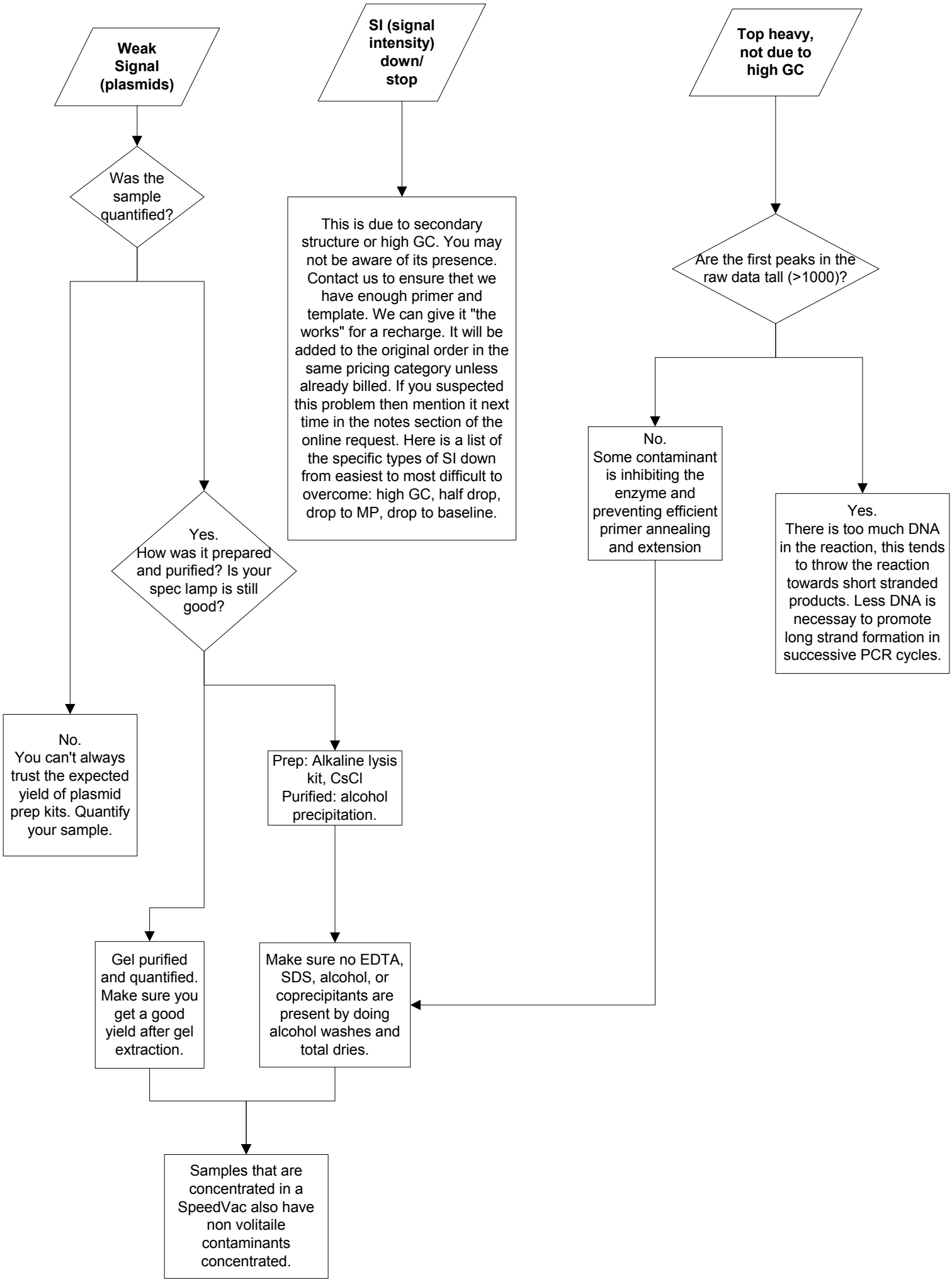
Weak Signal (plasmids)
Was the sample quantified?
No. You can't always trust the expected yield of plasmid prep kits. Quantify your sample.
Yes. How was it prepared and purified? Is your spec lamp is still good?
Gel purified and quantified. Make sure you get a good yield after gel extraction.
Prep: Alkaline lysis kit, CsCl Purified: alcohol precipitation.
Make sure no EDTA, SDS, alcohol, or coprecipitants are present by doing alcohol washes and total dries.
Samples that are concentrated in a SpeedVac also have non volitaile contaminants concentrated.
SI (signal intensity) down/ stop
This is due to secondary structure or high GC. You may not be aware of its presence. Contact us to ensure thet we have enough primer and template. We can give it "the works" for a recharge. It will be added to the original order in the same pricing category unless already billed. If you suspected this problem then mention it next time in the notes section of the online request. Here is a list of the specific types of SI down from easiest to most difficult to overcome: high GC, half drop, drop to MP, drop to baseline.
Top heavy, not due to high GC
Are the first peaks in the raw data tall (>1000)?
No. Some contaminant is inhibiting the enzyme and preventing efficient primer annealing and extension
Yes. There is too much DNA in the reaction, this tends to throw the reaction towards short stranded products. Less DNA is necessay to promote long strand formation in successive PCR cycles.