Unfortunately pCS300 was derived from pCS2+ which contains a functional SP6 promoter and a very weak T7 promoter. The six nucleotides after the start of transcription for this promoter are not in "good" context and as a result the promoter is only weakly active. To circumvent this problem we generally make in situ probes from pCS300 clones with a PCR strategy where a T7 promoter sequence is appended to one of the PCR oligomers. In the case of Mixer, you will want to avoid including the homeobox in your probe so this strategy is very helpful in creating probes that represent particular areas of the transcript of interest. We are sending pCS300-Mixer and below describe the basic approach for making the in situ probes. I was unable to find the exact nucleotide sequence of the oligos that I used but think that it should be very straight forward to do what I did in the Science paper. Please let me know if there are any problems.

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