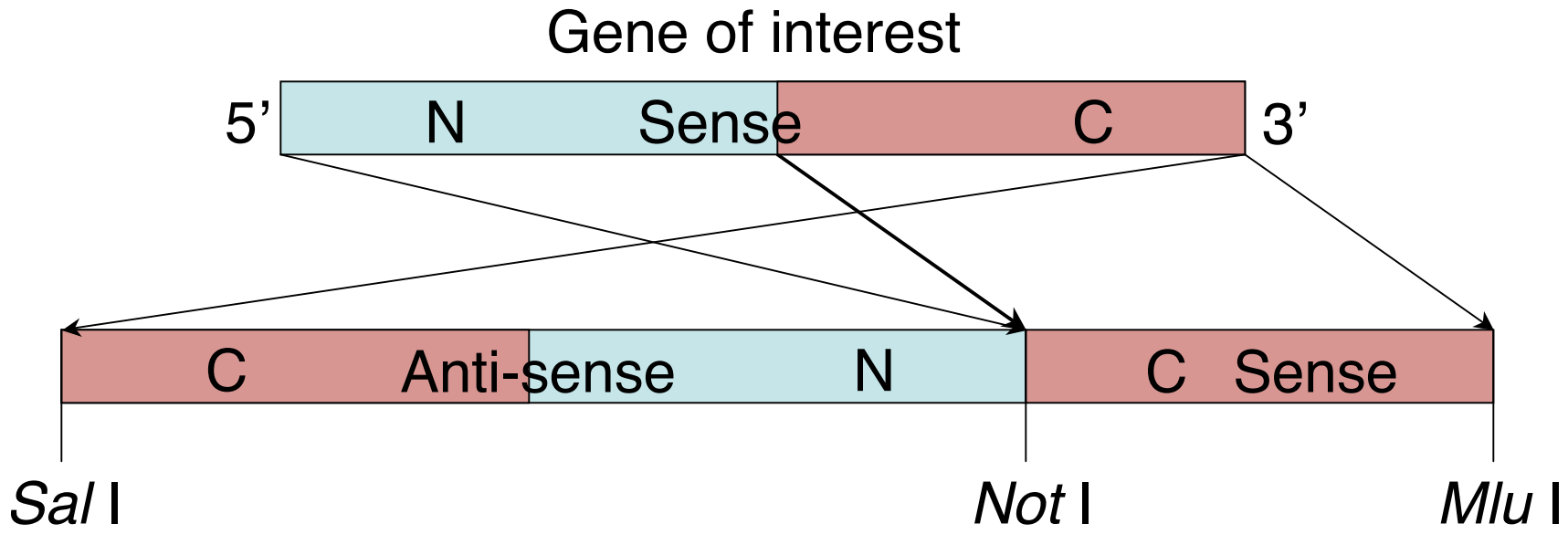
Building hairpin constructs in *Dictyostelium* for RNA interference



PCR gene with primers that introduce a *Sal* I site at 3' end and *Not* I site at 5' end.
Clone into *Sal* I and *Not* I sites of pLD1A15SN (Robinson and Spudich, 2000 J. Cell Biol.), yielding an anti-sense construct.
PCR the 3' end half (300-500 bp if possible though we have not tried to determine the minimal required yet) with a *Not* I site at 5' end and *Mlu* I site at 3' end.
Clone into *Not* I and *Mlu* I sites of anti-sense construct in pLD1A15SN.
In *Dictyostelium*, this yields a single transcript that will fold into a hairpin with a large loop (100-500 nts). We have found that STBL2 (GibcoBRL) bacterial host cells are preferable for stabilizing the inverted repeat that encodes the hairpin stem. The large loop has helped stabilize the construct in *E. coli*. We have not determined how small the loop needs to be and size requirements are probably gene specific. Large loops may not be necessary for RNA interference in *Dictyostelium*.

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