

**Morpholino studies** containing only a “control” morpholino to define non-specific effects will not be considered.

Controls should ideally include

1. rescue of the effect by expressing mRNA that is not targeted by the MO.
2. use of a second, non-overlapping MO to show the same effect. (these MOs may even synergize, which is taken as support for specificity)
3. Species specific targeting of the MO sequence to show an effect in one species but not a related species.
4. use of a four or five mismatch MO. In the case of the four mismatches, the MO is expected to have some of the same effect, though with lower specific activity.

It is also useful to be able to show directly that the MO has an effect on its intended target, though by itself, this does not demonstrate that there are no off-target effects. In the case of splice blocking MOs, RT-PCR can be used to show the effectiveness of the manipulation. The MO can be shown to block production of the endogenous protein

The MO can be shown to block expression of a tagged form of the protein, expressed in the same physiological context.