

# Morpholinos

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25-mer antisense oligonucleotide with modified bases containing morpholine ring, making them very stable.

How do they work?

- Translation blocking: Designed to 5' UTR of mRNA or in coding region close to ATG.  
Monitor loss of protein with Ab or epitope tag.
- Splice blocking: Designed to splice site junction.  
\*\*\*Monitor loss of splicing by RT-PCR, then sequence to determine if non-functional mRNA.  
(Beware: can block translation too!)

Great loss of function technique, if no mutant available.

# The Problems: controls are needed

Control for specificity of knock down

- Rescue with mRNA **NOT** containing MO site.  
Do double injection: 1st MO, 2nd mRNA.
- Second MO, non-overlapping with first, produces same phenotype.
- 5 bp mismatch MO--expect no effect.
- Control MO offered by Gene Tools **NOT** a control.

Nonspecific effect: delay in development. Age-matched controls not appropriate. Need to developmentally stage controls to morphants (stage-matched).

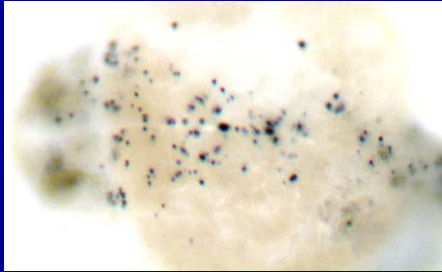
Nonspecific effect: widespread cell death.  $\geq 4$  ng/embryo, but depends on the particular MO.

See also Eisen and Smith, Development, 2008.

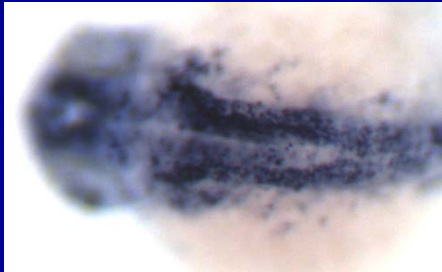
# Combined specific and non-specific MO effects

## Cell death assay

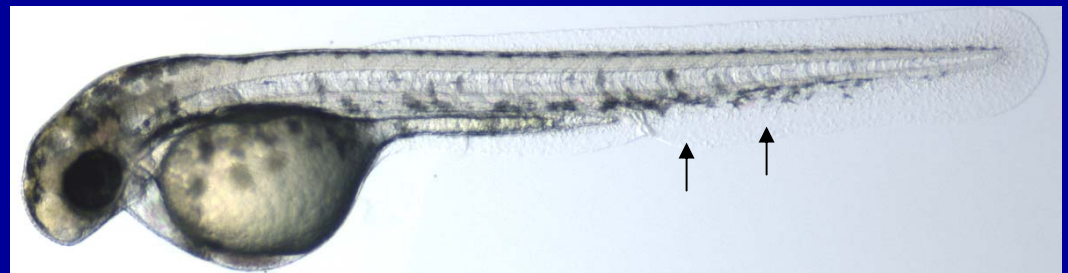
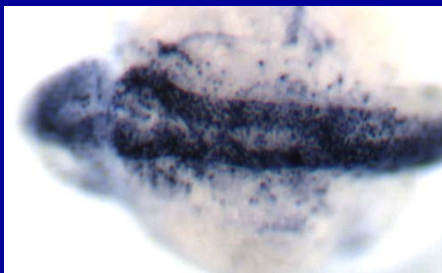
uninjected  
WT



+ 8ng *tsg* MO



+ 8ng MO  
+ 96 pg *tsg*  
mRNA



Reduced head size is non-specific *tsg* MO defect.

Note extensive cell death, BUT mild head reduction.

Vein edema is specific *tsg* MO defect.

Use p53 mutant to block cell death.

# Today

MOs

*smad5*

*smad5* mismatch

mRNA for rescue of MO

mouse *smad5*

*bmp7*

*bmp7* mismatch

Xenopus *bmp7*

MOs can be fluorescently tagged, so can assess where injected/located. More expensive than non-tagged.

Will demonstrate calibration of needle this morning.

## Then start MO injections

Also.....

check injected embryos from yesterday:

Overexpression *chordin*, *bmp4*

Rescue of *somitabun* (*smad5* mutant)