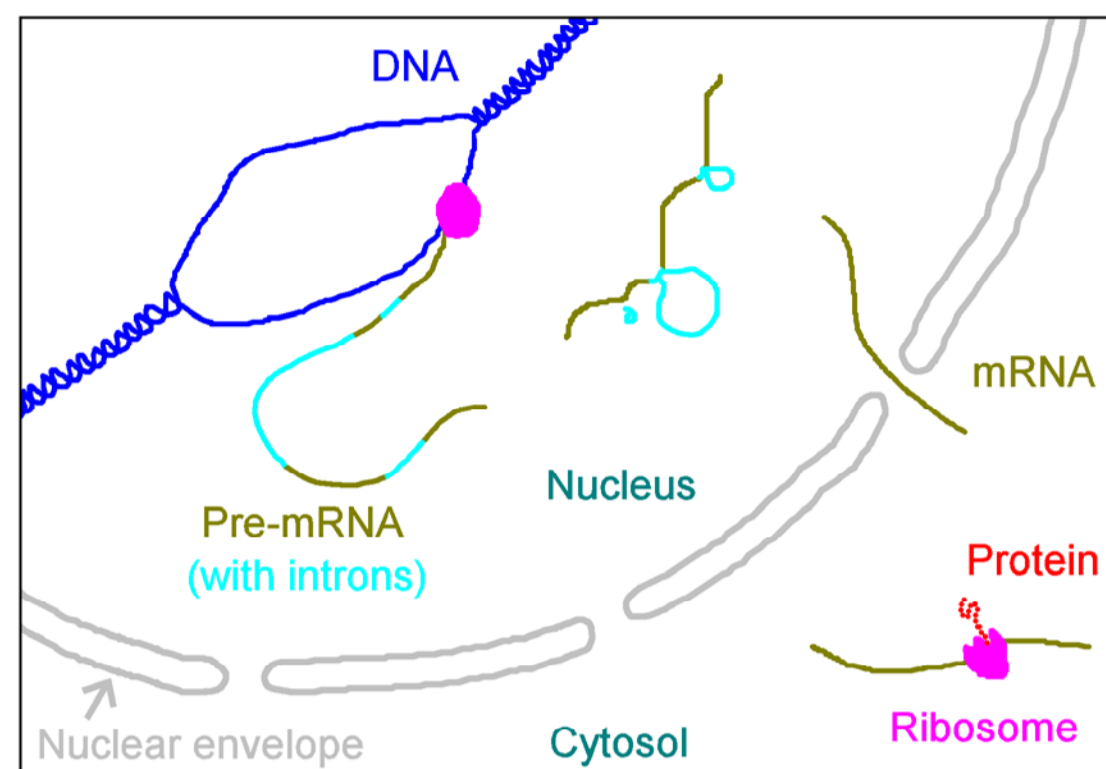


# Morpholino Applications

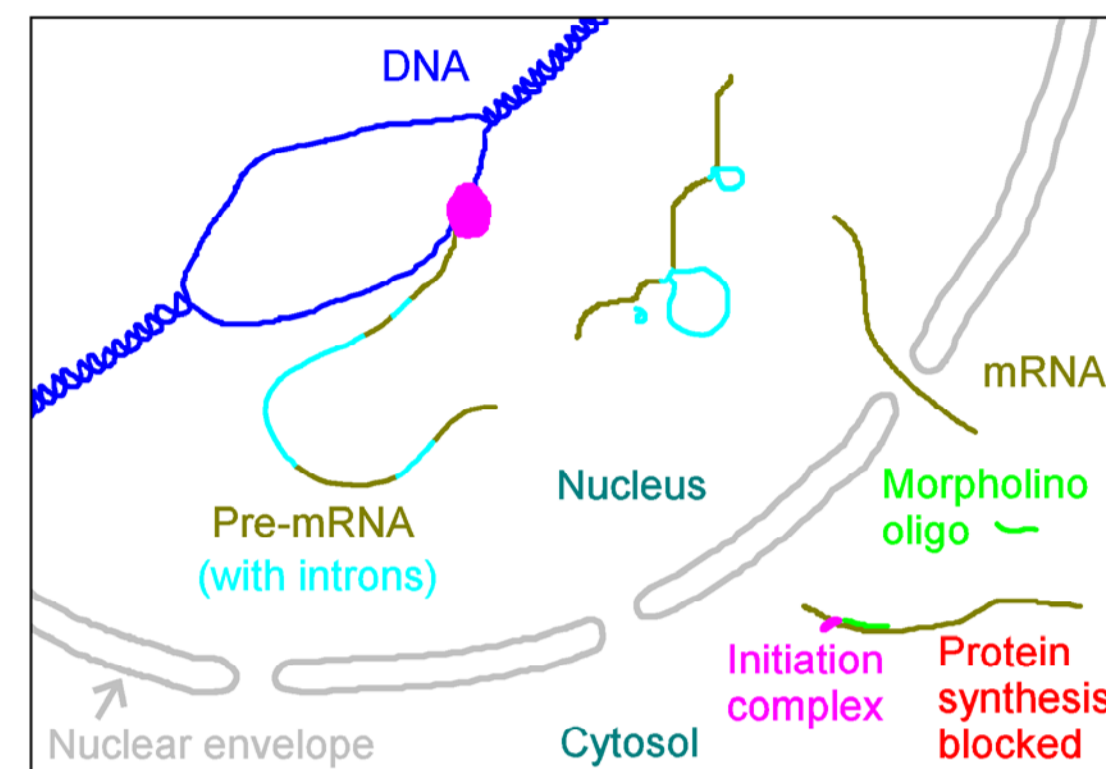
## Morpholino targets

**GENE TOOLS, LLC**

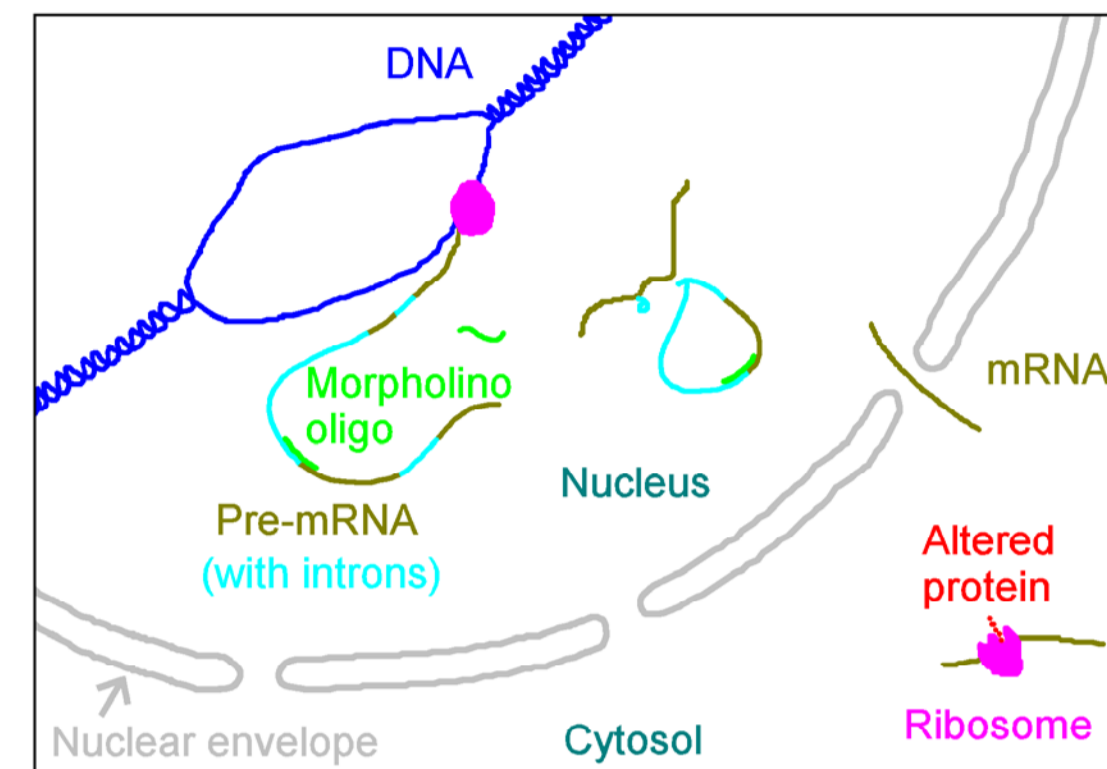
### Normal protein synthesis



### Translation blocked



### Splicing modified



### Also: Blocking ribozymes

Yen L, Svendsen J, Lee JS, Gray JT, Magnier M, Baba T, D'Amato RJ, Mulligan RC. Exogenous control of mammalian gene expression through modulation of RNA self-cleavage. *Nature*. 2004 Sep 23;431(7007):471-6.

### Inducing frameshifts

Howard MT, Gesteland RF, Atkins JF. Efficient stimulation of site-specific ribosome frameshifting by antisense oligonucleotides. *RNA*. 2004 Oct;10(10):1653-1661.

### Blocking miRNA

Kloosterman WP, Wienholds E, Ketting RF, Plasterk RH. Substrate requirements for let-7 function in the developing zebrafish embryo. *Nucleic Acids Res*. 2004 Dec 07;32(21):6284-91.

### Blocking splice regulation

Bruno IG, Jin W, Cote GJ. Correction of aberrant FGFR1 alternative RNA splicing through targeting of intronic regulatory elements. *Hum Mol Genet*. 2004 Oct 15;13(20):2409-20. Epub 2004 Aug 27.

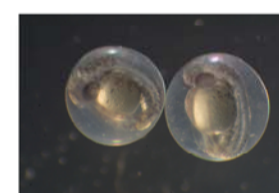
Vetrini F, Tammaro R, Bondanza S, Surace EM, Auricchio A, De Luca M, Ballabio A, Marigo V. Aberrant splicing in the ocular albinism type 1 gene (OA1/GPR143) is corrected in vitro by morpholino antisense oligonucleotides. *Hum Mutat*. 2006 Mar 20; [Epub ahead of print]

### Blocking viral cyclization sequence

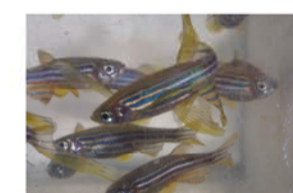
Kinney RM, Huang CY, Rose BC, Kroeker AD, Dreher TW, Iversen PL, Stein DA. Inhibition of dengue virus serotypes 1 to 4 in vero cell cultures with morpholino oligomers. *J Virol*. 2005 Apr;79(8):5116-28.

## Proven organisms and cells

### Zebrafish *Danio rerio*



Two day embryos



Adults

Images courtesy of Steve Baskauf, Vanderbilt Univ.

### Clawed frogs

*Xenopus laevis*



Image courtesy of Enrique Amaya, Univ. of Cambridge

*Xenopus tropicalis*

### Ascidians



*Ciona intestinalis*  
Image courtesy of Dirk Schories

*Ciona savignyi*  
Image courtesy of Broad Institute, MIT

### Cultures

Primary cells  
Immortal cell lines  
Tissue explants

Morpholinos have been shown to work in a broad range of animal cells as well as in bacteria. They are expected to work in any cell into which they can be delivered in sufficient concentration.

### Other organisms

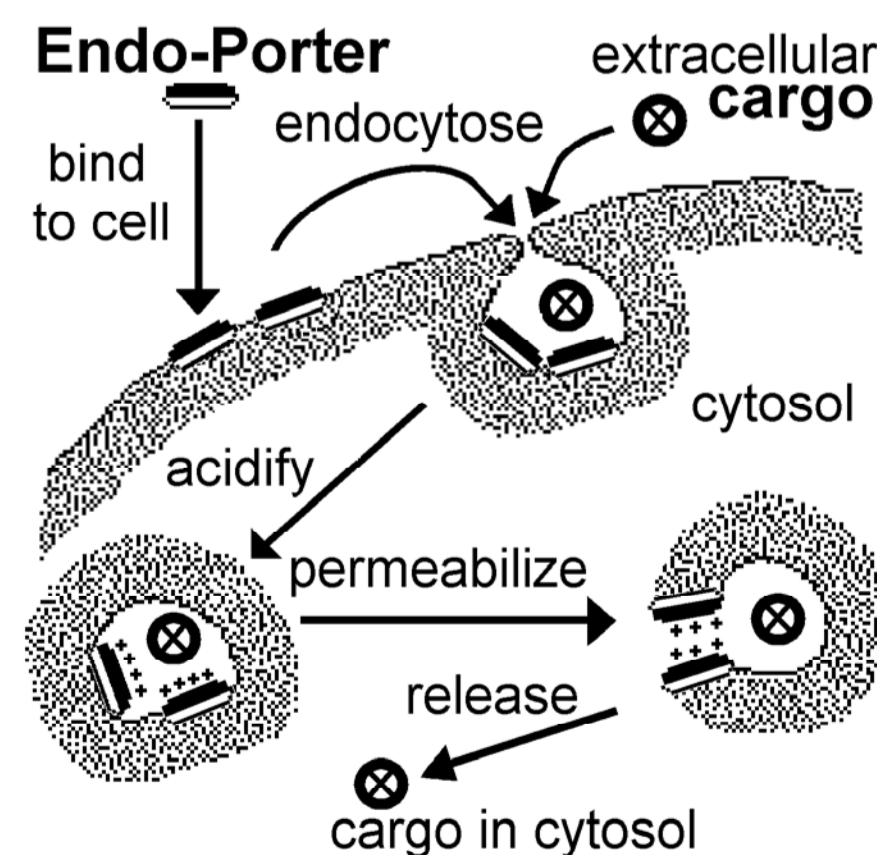
Sea urchins  
Sea stars  
Nematodes  
Beetles  
Fruit flies  
Bacteria

Chicks  
Mice  
Rats  
Medakafish  
Goldfish  
Trout

Pigs  
Sheep  
Monkeys  
Humans  
Placozoans  
Viruses

## Cytosolic delivery methods

### Endo-Porter



### Microinjection

Microinjection into oocytes is the standard method for introducing Morpholino oligos into the cytosol of many embryos including zebrafish, frogs, ascidians and urchins.

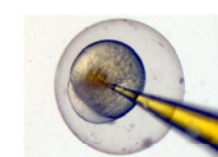


Image courtesy of Ed Devlin, Hampden-Sydney College

### Electroporation

Electroporation is a good method for delivering Morpholino oligos into many kinds of cultured cells and for delivery into some late-stage embryos.

Jubin R. Optimizing electroporation conditions for intracellular delivery of morpholino antisense oligonucleotides directed against the hepatitis C virus internal ribosome entry site. *Methods Mol Med*. 2004;106:309-22.

Kos R, Tucker RP, Hall R, Duong TD, Erickson CA. Methods for introducing morpholinos into the chicken embryo. *Dev Dyn*. 2003 Mar;226(3):470-7.

Takahashi M, Sato K, Nomura T, Osumi N. Manipulating gene expressions by electroporation in the developing brain of mammalian embryos. *Differentiation*. 2002 Jun;70(4-5):155-62.



Image courtesy of Amaxa Biosystems

### Scrape loading

Gently scraping adherent cells from their substrate introduces transient pores through which Morpholinos may diffuse.

Partridge M, Vincent A, Matthews P, Puma J, Stein D, Summerton J. A simple method for delivering morpholino antisense oligos into the cytoplasm of cells. *Antisense Nucleic Acid Drug Dev*. 1996 Fall;6(3):169-75.



### Peptide conjugates



Nelson MH, Stein DA, Kroeker AD, Hatlevig SA, Iversen PL, Moulton HM. Arginine-rich peptide conjugation to morpholino oligomers: effects on antisense activity and specificity. *Bioconjug Chem*. 2005 Jul-Aug;16(4):669-66.

Deas TS, Binduga-Gajewska I, Tilgner M, Ren P, Stein DA, Moulton HM, Iversen PL, Kauffman EB, Kramer LD, Shi PY. Inhibition of flavivirus infections by antisense oligomers specifically suppressing viral translation and RNA replication. *J Virol*. 2005 Apr;79(8):4699-808.

Moulton HM, Moulton JD. Arginine-rich cell-penetrating peptides with uncharged antisense oligomers. *Drug Discov Today*. 2004 Oct 15;9(20):670.

Moulton HM, Nelson MH, Hatlevig SA, Reddy MT, Iversen PL. Cellular uptake of antisense morpholino oligomers conjugated to arginine-rich peptides. *Bioconjug Chem*. 2004 Mar-Apr;15(2):290-9.

Moulton HM, Moulton JD. Peptide-assisted delivery of sterio-blocking antisense oligomers. *Curr Opin Mol Ther*. 2003 Apr;5(2):123-32.

Moulton HM, Hase MC, Smith KM, Iversen PL. HIV Tat peptide enhances cellular delivery of antisense morpholino oligomers. *Antisense Nucleic Acid Drug Dev*. 2003 Feb;13(1):31-43.