Eradication of Invasive Quagga and Zebra Mussels using Engineered Disseminated Neoplasia

Steve Suhr and Marie-Claude Senut

Biomilab LLC

4209 S. Pennsylvania, Lansing MI 48910

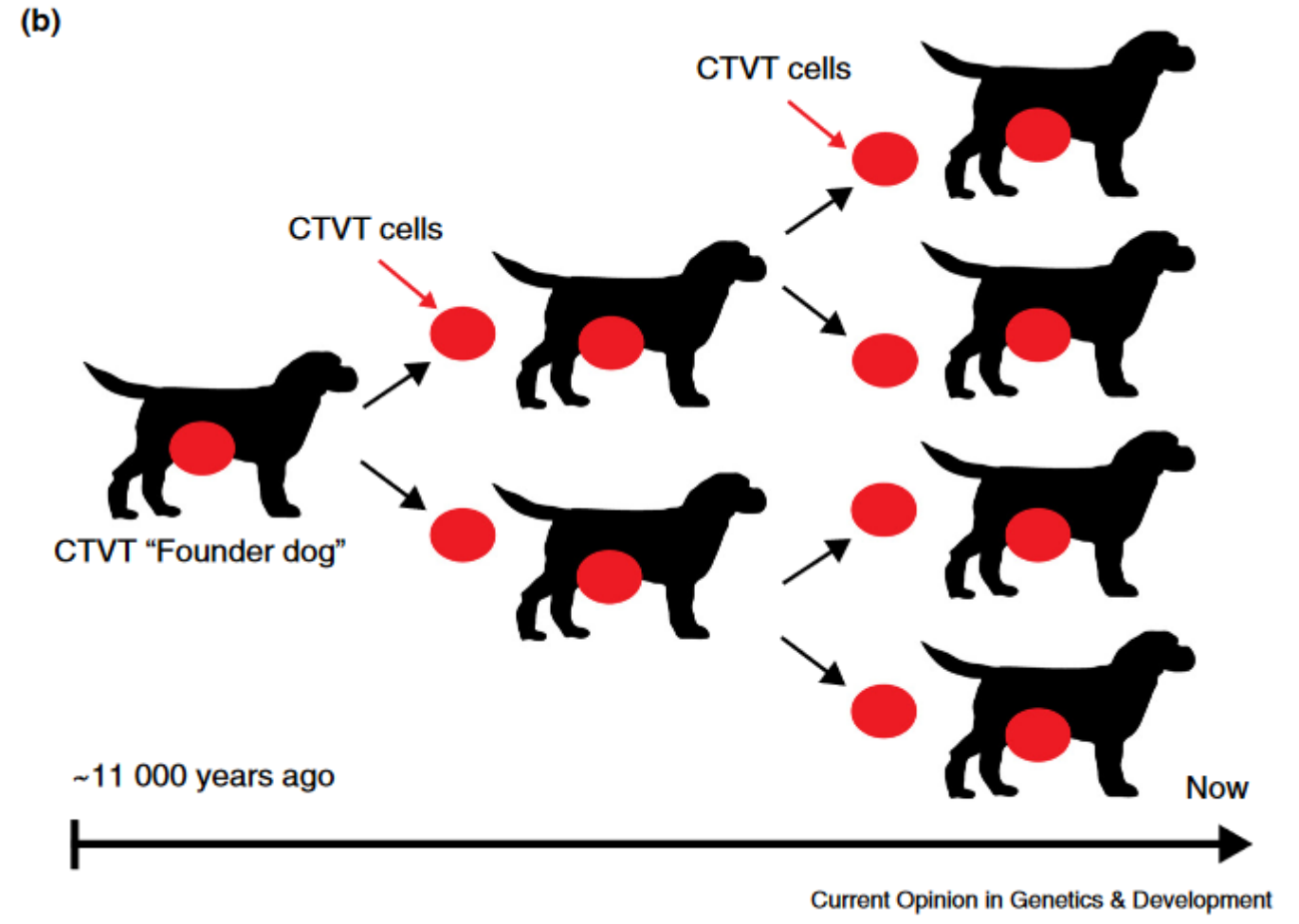
office@biomilab.com

517-492-9900

What is disseminated neoplasia (DN)?

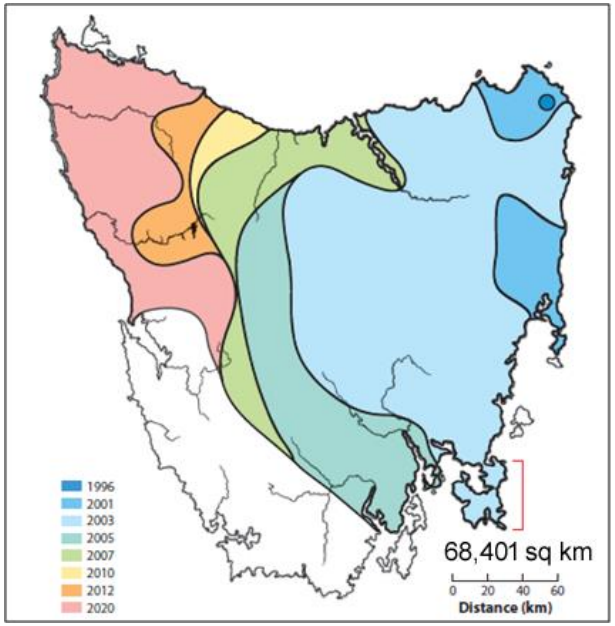
- **Disseminated neoplasia is a very rare form of cancer where neoplastic cells are spread by direct transfer from one individual to another.**

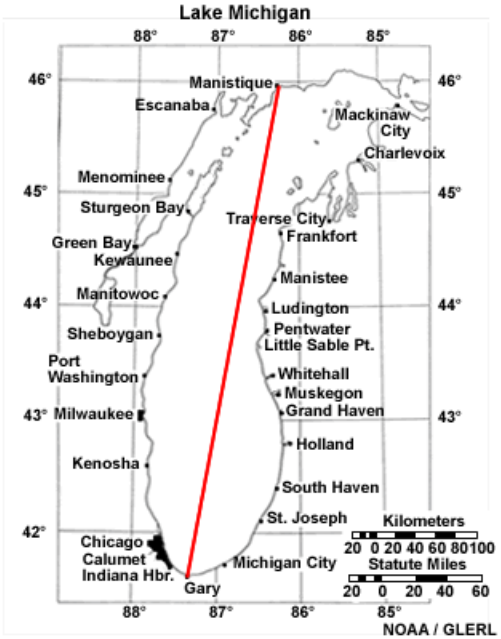
Canine Transmissible Venereal Tumor



Strakova and Murchison, Current Opinion in Genetics & Development (2015),**30**:49–55

Devil Facial Tumor Disease

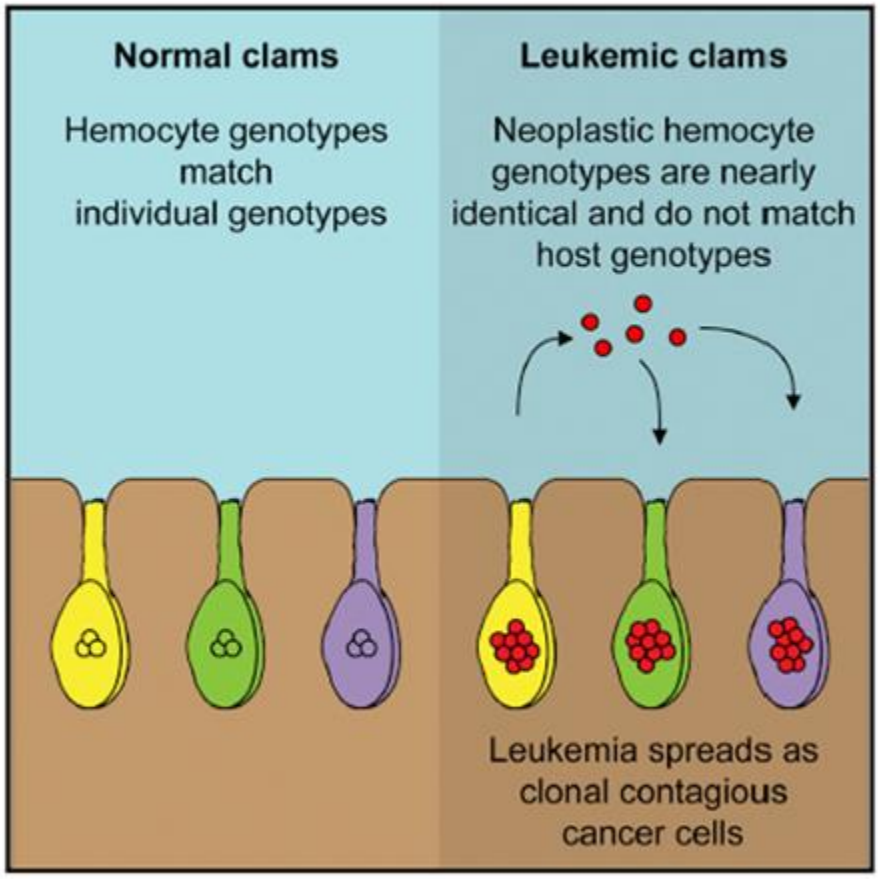




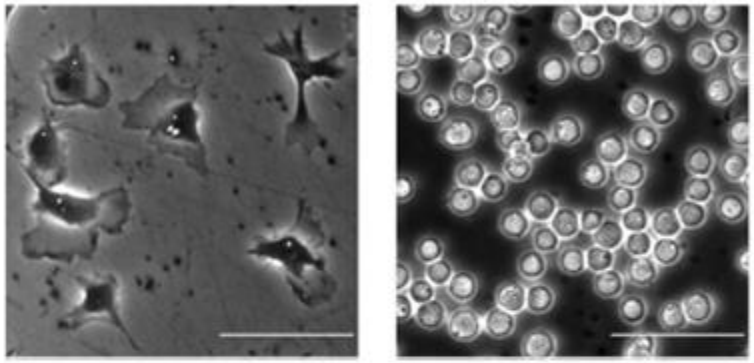
>90% decline in devil population since 1996 and extinction by 2026.

Stammnitz et al. (2018) Cancer Cell 33, 607–619 April 9, 2018
Bender et al. (2014) Ann. Rev. Anim. Biosci. 2014; 2: 165-187.

Bivalve Transmissible Neoplasia



Metzger et al. (2015) Horizontal transmission of clonal cancer cells causes leukemia in soft-shell clams. Cell. 161:255-263



Metzger et al. (2015)

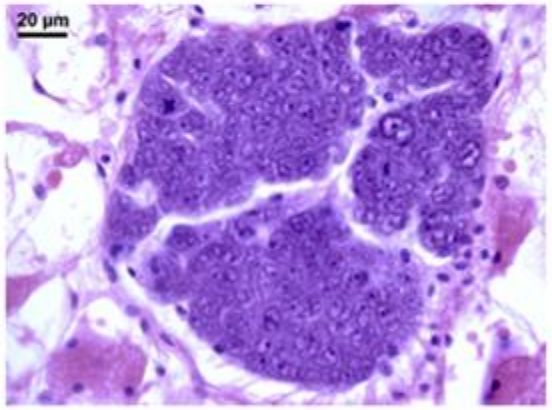
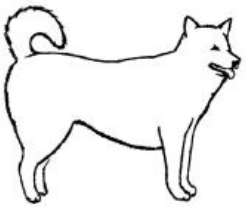


Fig. 6. Light micrograph of a histological section through the mantle of a mussel *Mytilus galloprovincialis* showing a gonad follicle filled with neoplastic cells.

Carballal et al. (2015) Neoplastic diseases of marine bivalves. J. Invertebr. Pathol.; 131:83-106.

Comparison of DNs

PLOS | PATHOGENS



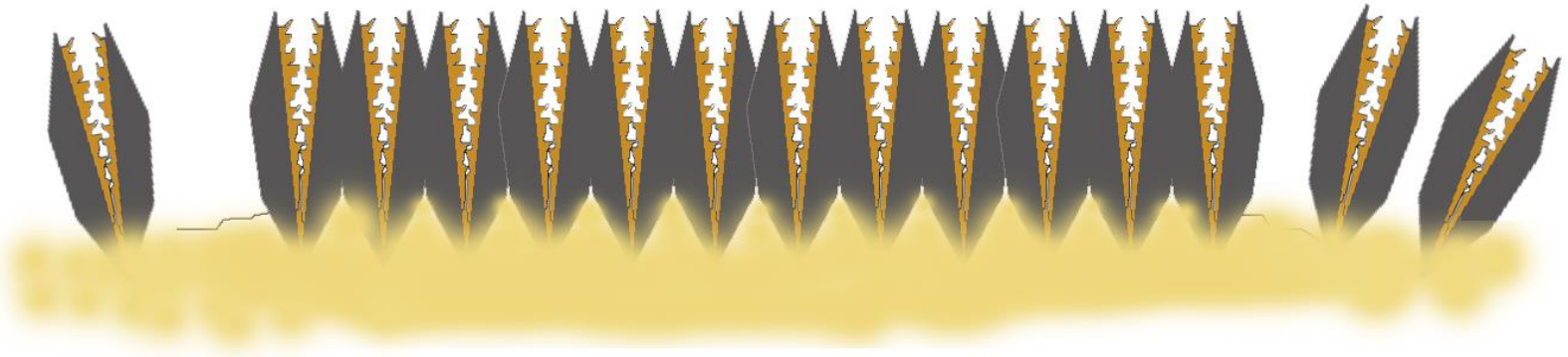
	Canine Transmissible Venereal Tumor	Devil Facial Tumor Disease	Bivalve Transmissible Neoplasia
Host species	Dog	Tasmanian devil	Soft-shell clam, mussel, cockle, golden carpet shell clam, and possibly others
Lineages	1	2	At least 5
Outcome	Spontaneous regression in most experimental and some natural cases	Uniformly fatal	Predominantly fatal
Route of transmission	Sexual contact	Biting	Through seawater (no direct physical contact)
Evasion of MHC	MHC downregulation and mutations in antigen processing	MHC downregulation and minimal diversity	Invertebrates do not have MHC

Fig 1. Comparison of the known lineages of infectious cancers in natural populations.

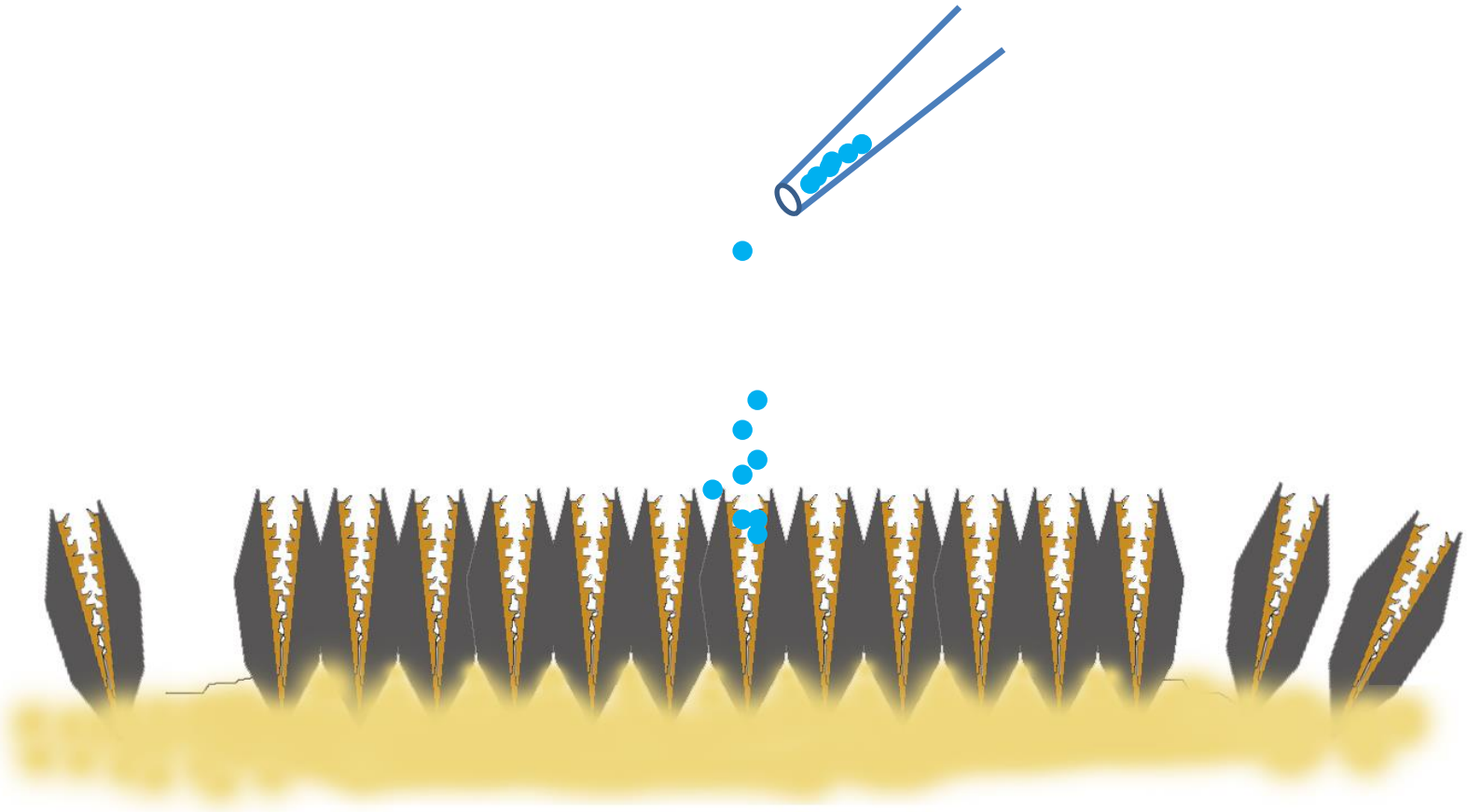
doi:10.1371/journal.ppat.1005904.g001

Metzger MJ, Goff SP (2016) A Sixth Modality of Infectious Disease: Contagious Cancer from Devils to Clams and Beyond. PLOS Pathogens 12(10): e1005904.

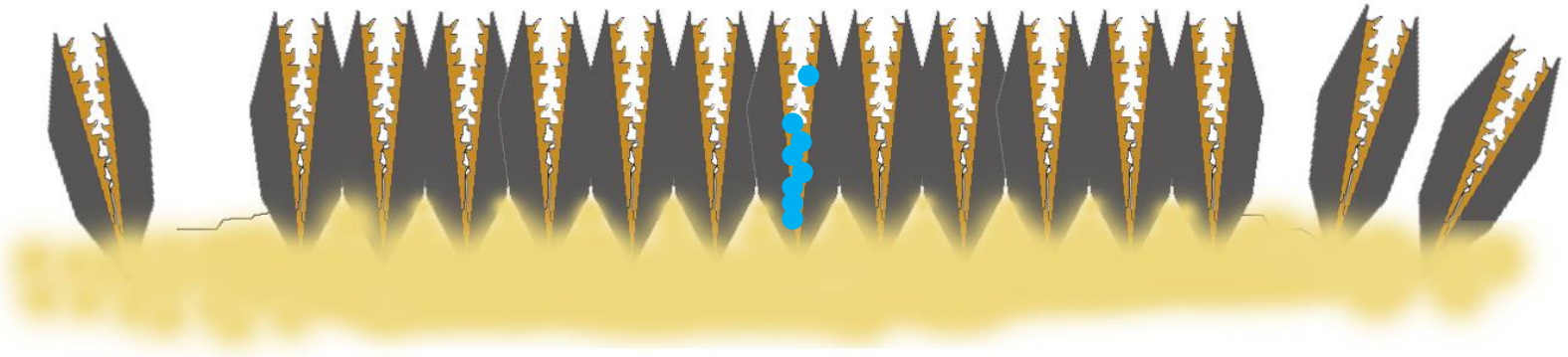
A DN for invasive bivalves



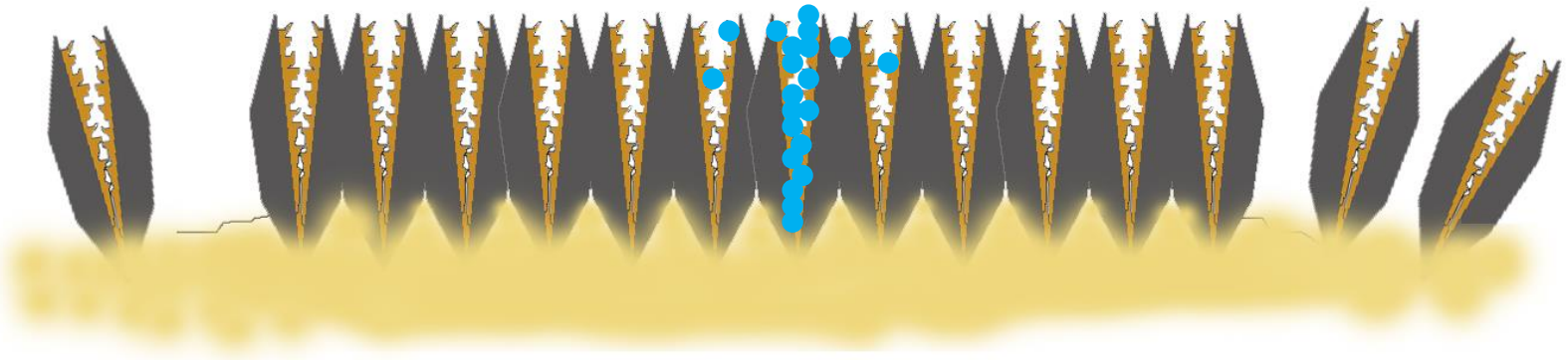
1. Transplantation



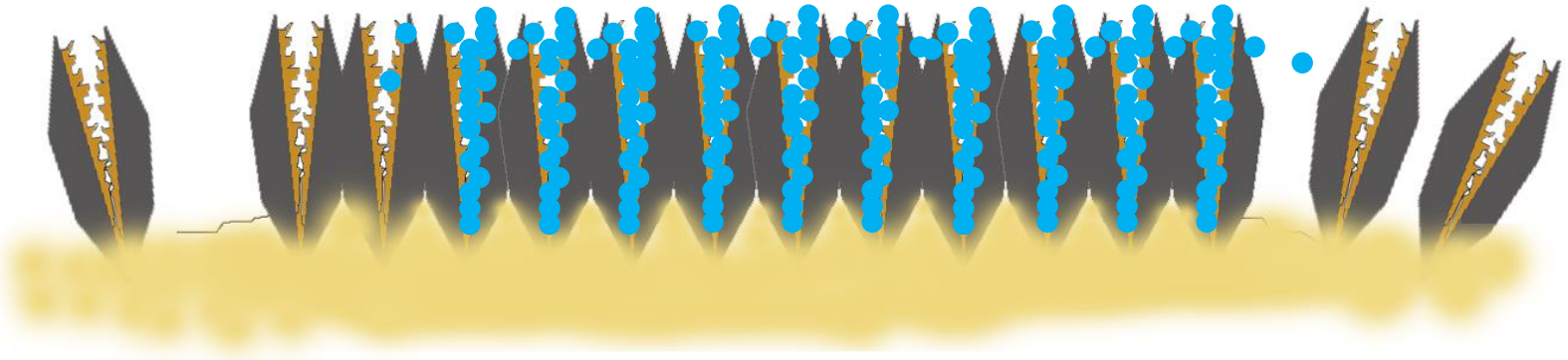
2. Engraftment



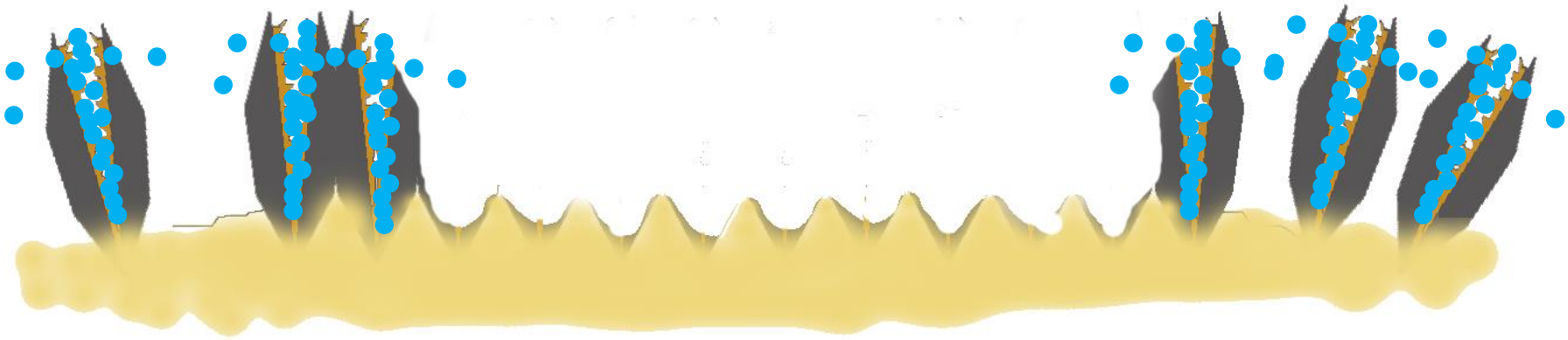
3. Systemic cancer in “Mussel Zero” and early dissemination



4. Widespread infection of the target population



5. Population collapse in target waters.



Some Advantages

- A single inoculum could result in endemic disease within a targeted waterway leading to complete collapse of invasive mussel populations.
- There is no chance of toxicity or injury to aquatic or terrestrial organisms with the possible exception of very closely related species.
- US invasive mussel populations are thought to be highly inbred and good targets for allografts such as DN.

Some Disadvantages

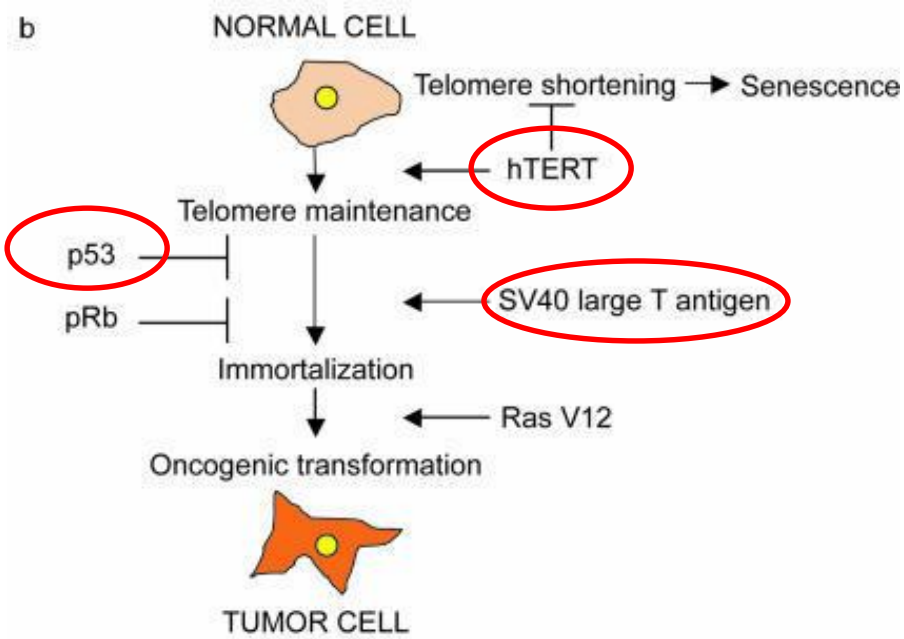
- Much of the molecular and cellular biology of *dreissenid* mussels is unknown.
- Population collapse may take years to complete.
- Closely-related species may be impacted by the DN.

The nuts and bolts of creating a DN for invasive mussels

- Establish a colony of live mussels within Biomilab.
- Work out conditions for isolation, culture and cryopreservation of hemocytes and other cell types.
- Test methods of transduction (transiently or permanently introducing genetic material into mussel cells).
- Explore methods of engrafting cultured cells to live mussels.
- Complete characterization of molecular targets (genes).
- Introduce oncogenic determinants into cultured cells and select for cancer phenotype.
- Engraft neoplastic cells to live mussels.
- Analyze establishment of cancer in engrafted animals.
- Detect and track dissemination to neighboring mussels.
- Quantify disease progression and lethality.
- Test in target and non-target species in the laboratory.
- Move everything outside.

One nut (or bolt): Key cellular events and factors in carcinogenesis

- **Immortalization**
- **Cell cycle deregulation**
- **Apoptosis suppression**
- **Growth factor independence**



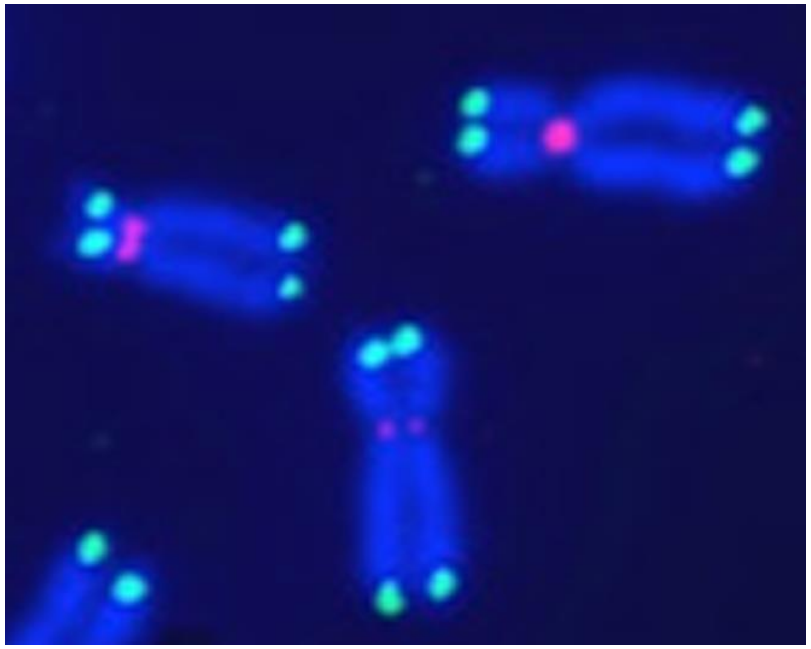
Pedraza-Fariña LG. Yale J Biol Med. 2006 Dec;79(3-4):95-103.

Oncogenic factors

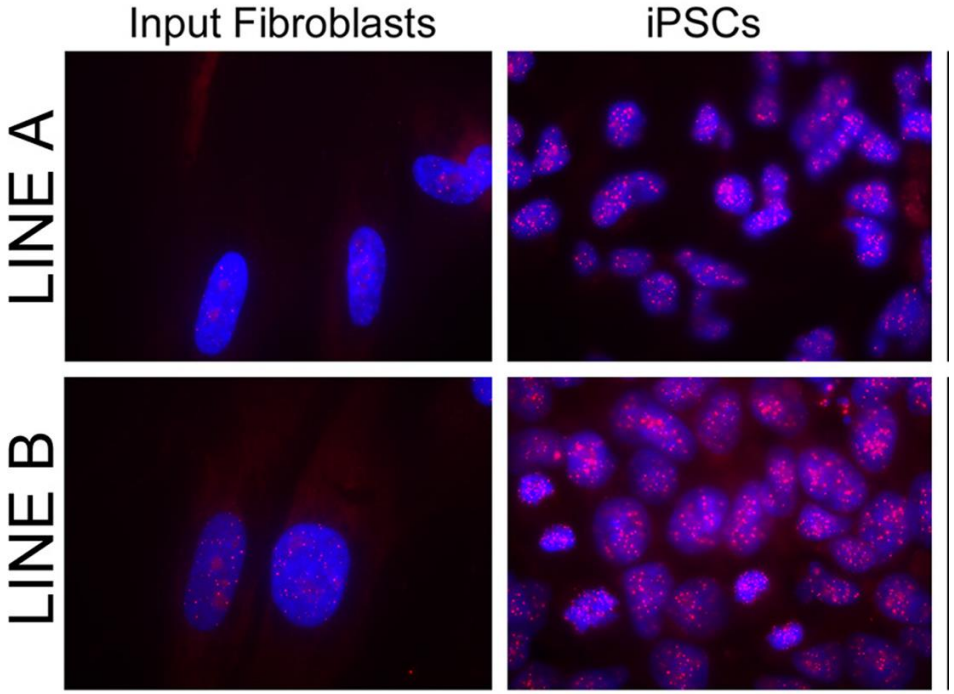
- Telomerase Reverse Transcriptase (TERT) over-expression to promote immortalization.
- Inactivation of p53 to prevent exit of cell cycle and suppress associated cell death.
- Over-expression of SV40 Large T-antigen to activate and suppress multiple oncogenic pathways.

Factor 1: TERT

- Telomerase reverse transcriptase is the protein that puts telomere "bumpers" on chromosomes protecting them from the rigors of replication.
- In mammals and many other organisms, telomeres are created early in development and then degrade with cell division eventually leading to senescence, but protecting against cancer.



https://www.utsouthwestern.edu/labs/shay-wright/research/



Suhr et al. (2010) PLOS ONE 5(3): 10.1371.

TERT and non-mammals

- In many aquatic organisms (i.e. teleost fishes) and other species, TERT expression can continue in some or all tissues indefinitely.

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Scientists accidentally kill world's oldest animal at age 507

Rob Quinn, Newser Published 9:42 a.m. ET Nov. 15, 2013 | Updated 12:00 p.m. ET Nov. 15, 2013

(Photo: Bangor University)

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The oldest animal ever known lived from 1499 until the day researchers cracked its shell open, killing it in the process.

Ming, an ocean quahog from the species Arctica islandica, was initially thought to be a record-setting 402 years old. But the scientists who found it on a seabed near Iceland in 2006 now say further analysis has revealed that it was an incredible 507 years old, reports CBS.

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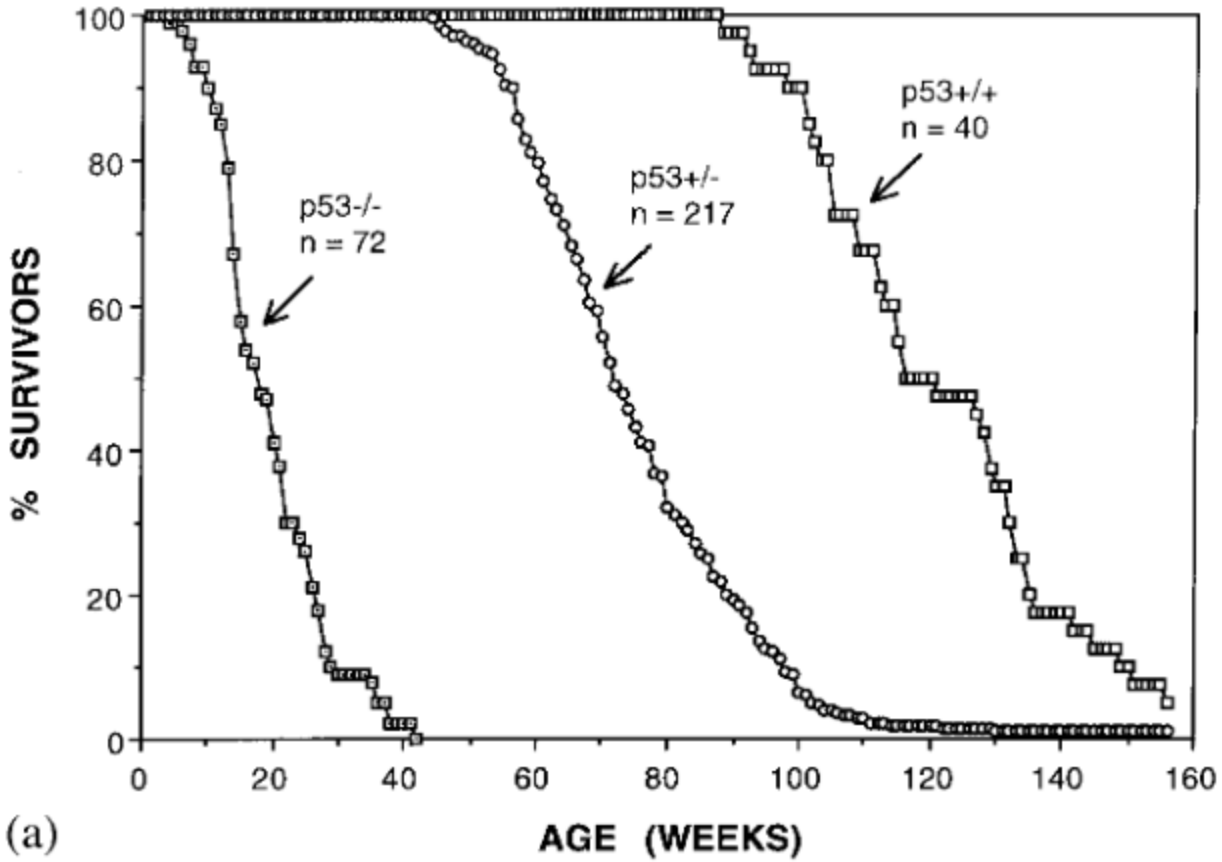
Factor 2: Tumor protein 53 (p53)

- **p53 is a tumor suppressor referred to as "the guardian of the genome"**
- **It is the protein most frequently mutated in human cancers (>50%).**
- **Lack of p53 disables capacity for cell cycle arrest.**
- **Mutation of p53 has been associated with bivalve DN.**

P53 is a potent tumor suppressor as indicated by its loss

CMLS, Cell. Mol. Life Sci. Vol. 55, 1999

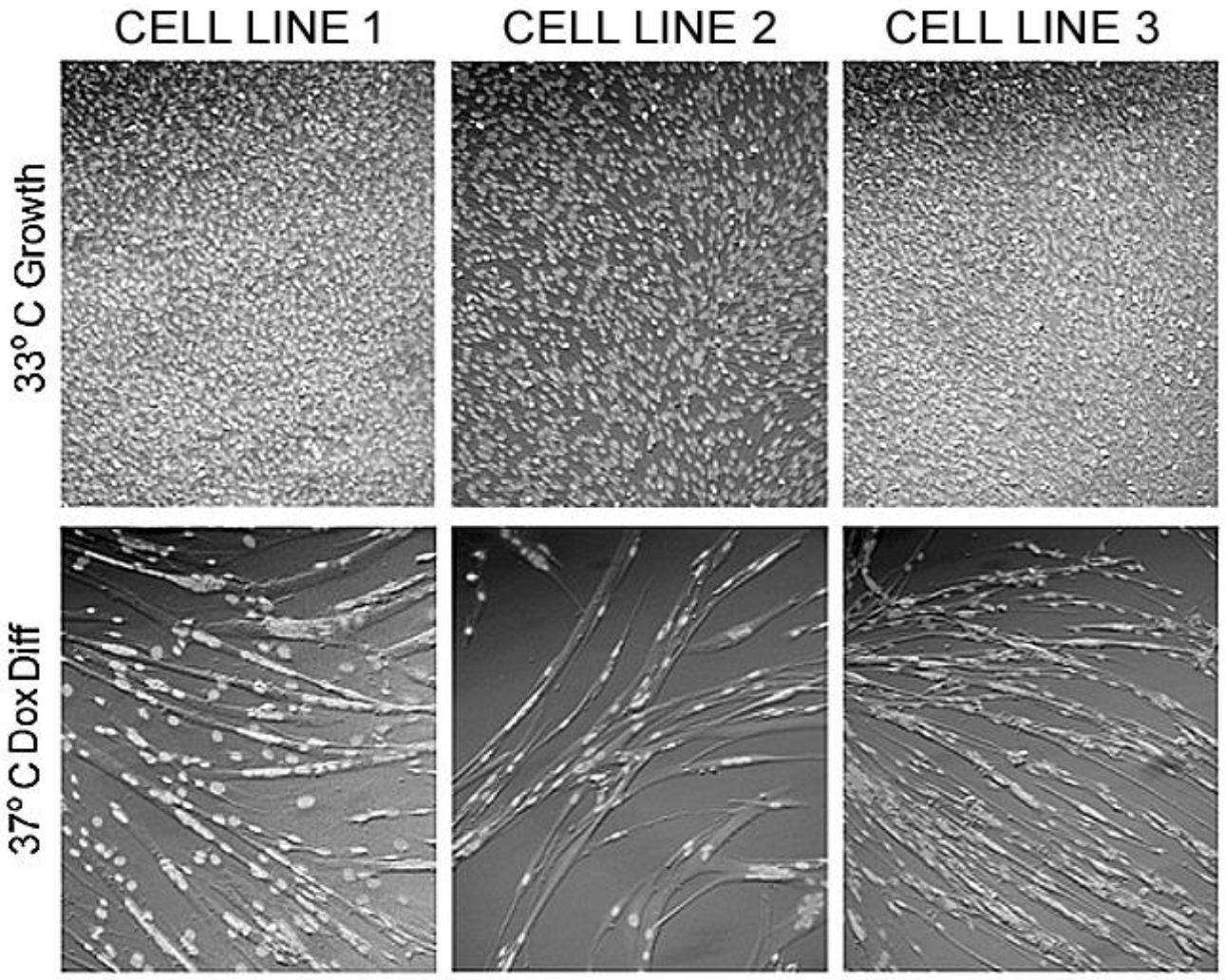
Review by LD Attardi and T. Jacks (Data from LA Donehower)



Factor 3: Large T-Antigen (T-Ag)

- Derived from Simian Virus 40 discovered in the 1960's
- Polyoma virus replicates during cell S phase, so T-Ag interacts with host mitotic factors (p53, Rb) to induce cell cycle.
- A temperature-sensitive form was isolated in 1989 (Jat and Sharp (1989) MCB, Apr.1989, p.1672-1681)

T-Ag control of oncogenesis



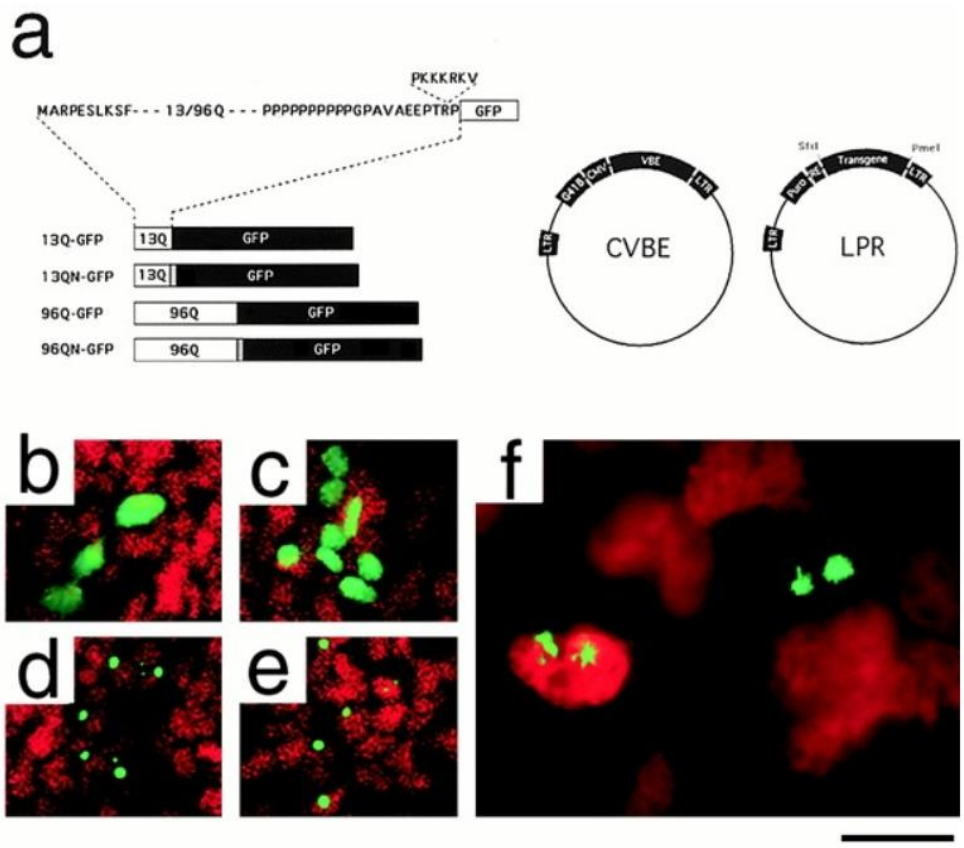
Macpherson et al., J Cell Biochem. 2004 Mar 1;91(4):821-39.

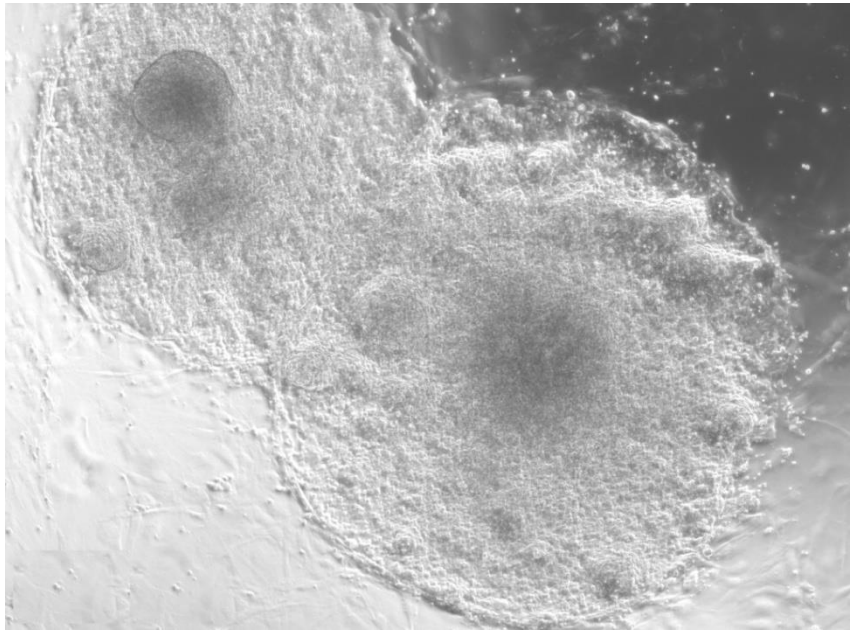
Manipulating the levels of oncogenic factors

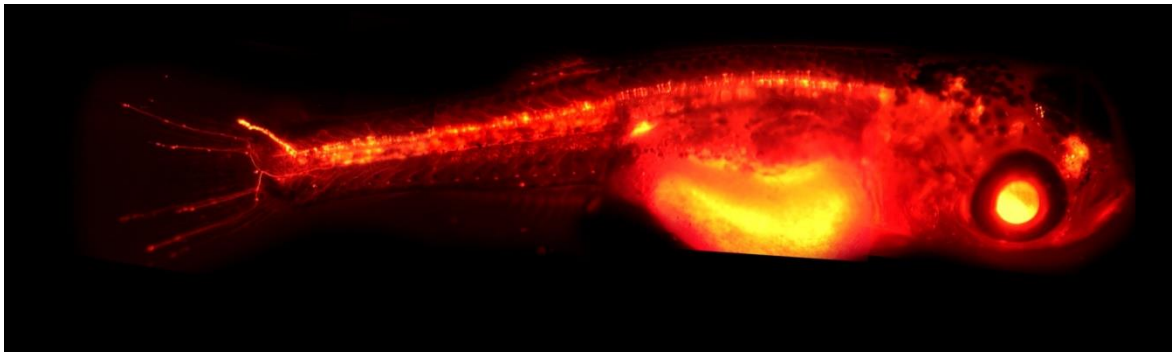
- **Over-expression of TERT and T-Ag via transgenesis.**

- **Knock-out of p53 using CRISPR/Cas9**

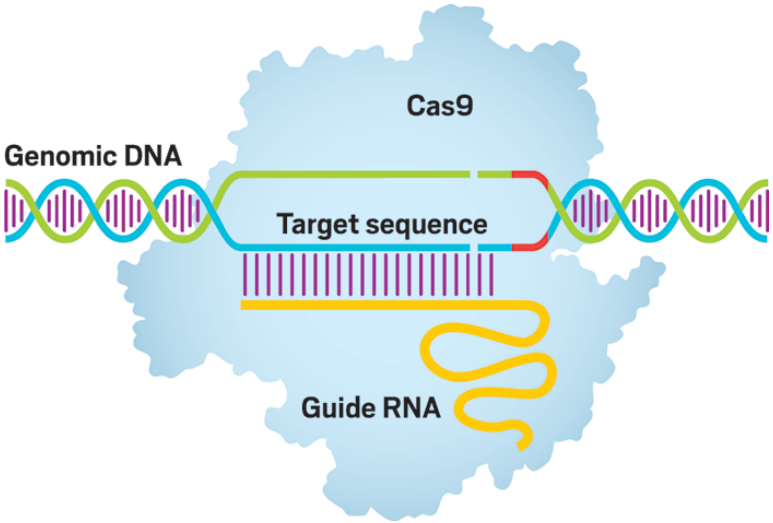
Over-expression by transgenesis



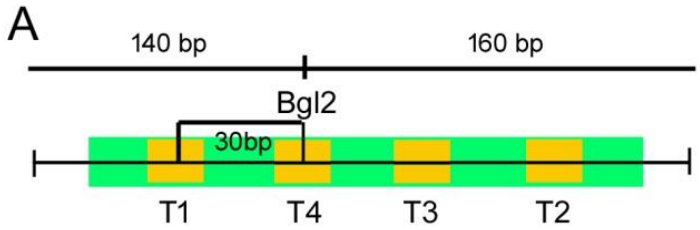


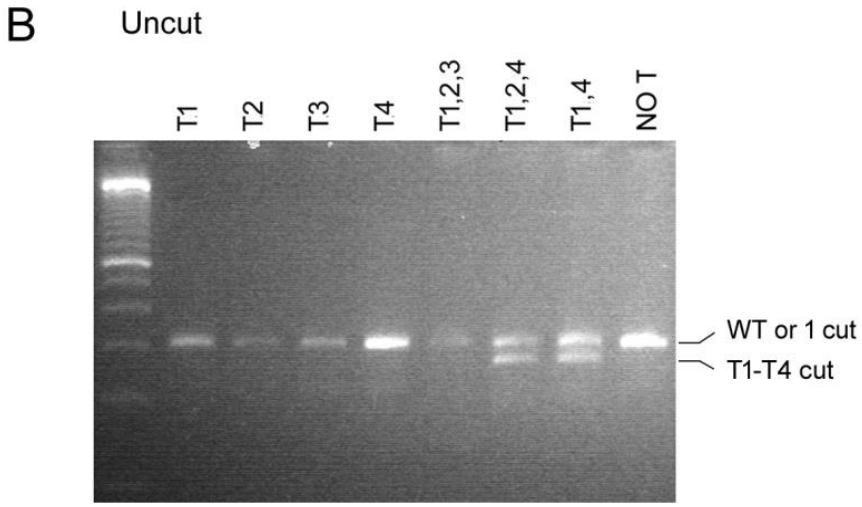


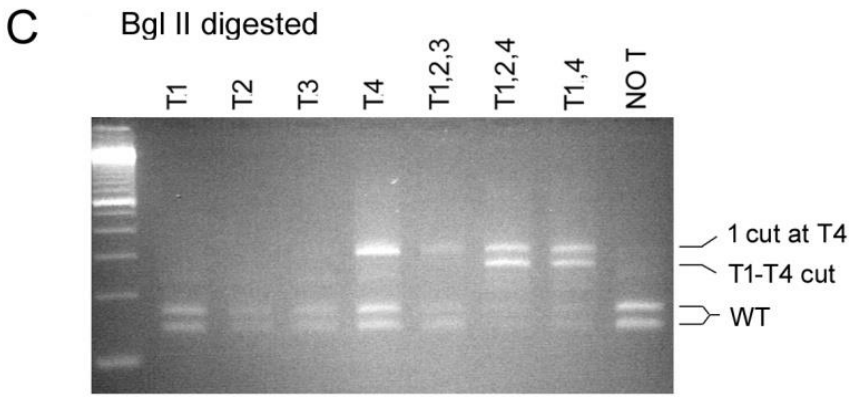
Knock-out by CRISPR/Cas9 gene targeting



https://cen.acs.org/articles/95/i24/CRISPR-new-toolbox-better-crops.html







Conclusion

- DN is a rare cancer transmissible within very closely related species.
- Forms of DN have been found in wild or cultivated bivalve populations.
- The molecular pathways of oncogenesis are well-understood and can be exploited to induce DN in dreissenid mussel cells.
- Additional understanding of dreissenid molecular and cellular biology will allow us to design and refine methods of producing engineered DN cells.

Thanks!

- We are grateful for any ideas or suggestions that you might have. Please contact us at biomilab@gmail.com or office@biomilab.com or come by our laboratory in Lansing MI!
- Many thanks to the USBR and the organizers and reviewers of the Invasive Mussel Challenge.
- Thanks to our scientific colleagues at USBR: Sherri Pucherelli, Jacque Keele, and Yale Passamaneck
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