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 COLLEGE OF

 VETERINARY MEDICINE




Filamentous Phage as a Platform for Development of Contraceptive Vaccines for Animals

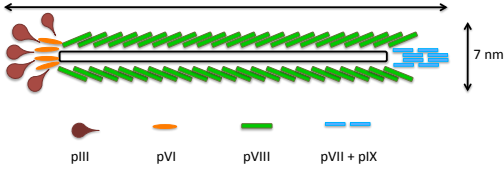
5th International Symposium on Non-Surgical Methods of Pet Population Control
 Portland, Oregon, U.S.
 June 21, 2013
 Presenter: Tatiana Samoylova

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Filamentous phage structure



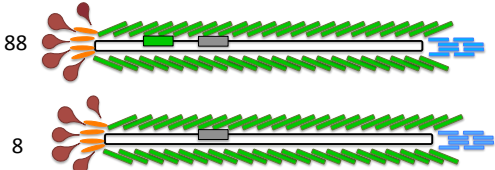
← 900 nm →





pIII pVI pVIII pVII + pIX

Phage particle can be genetically modified to carry antigenic peptides

Phage vectors

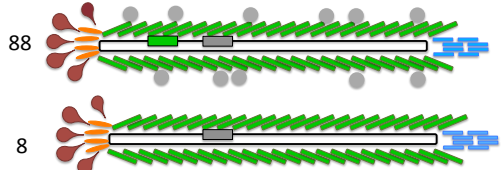


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

 

 wild type gene 8 recombinant gene 8

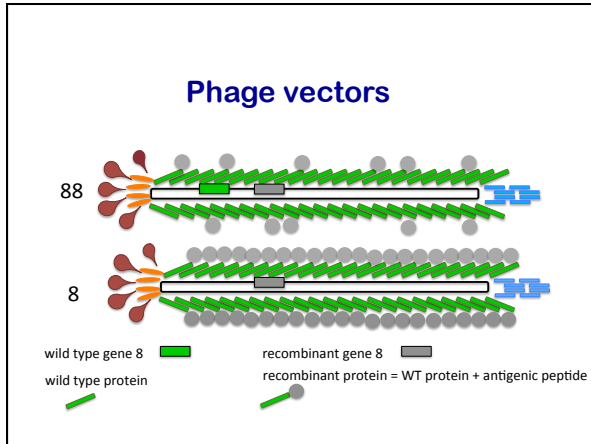
Phage vectors



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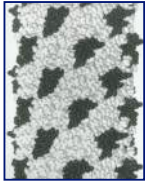
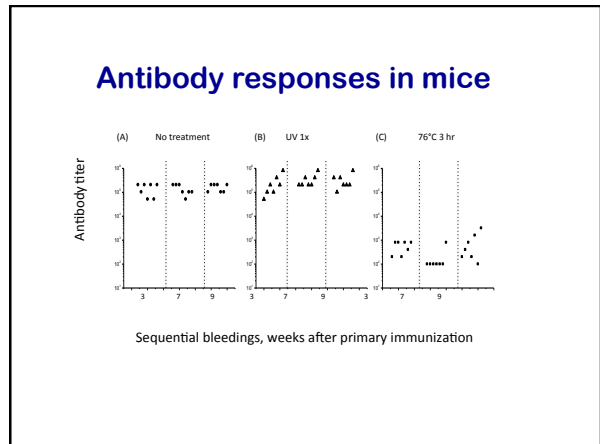
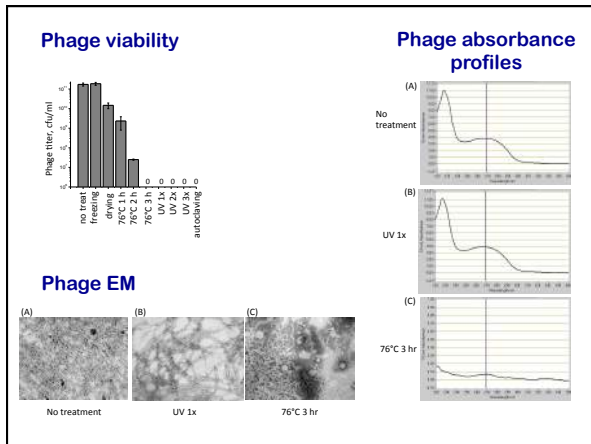
 

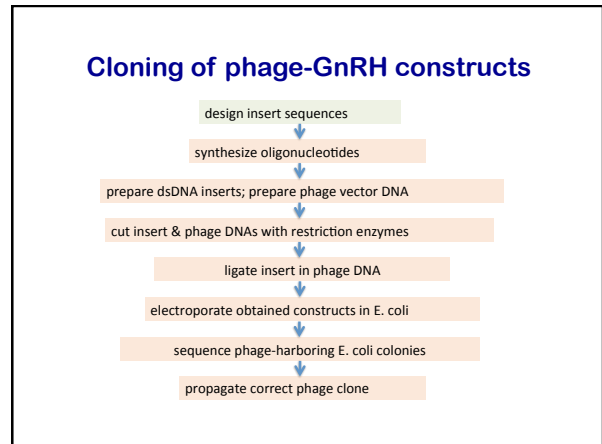
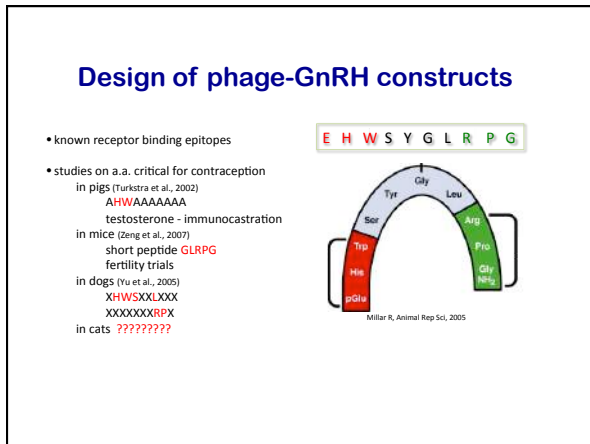
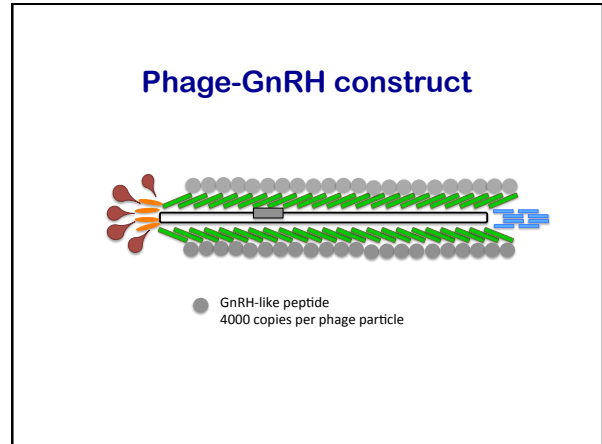
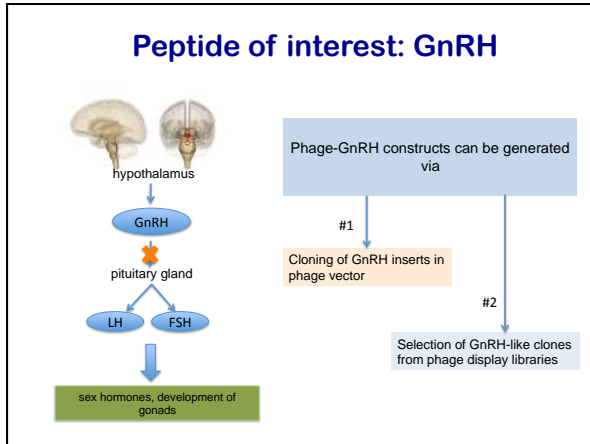
 wild type gene 8 recombinant gene 8



Why use landscape phage

- effective immunogen**
 - structure of Ag surface display
 - highly organized
 - repetitive, high density
 - particulate antigen, size and shape attractive for APCs
 - stimulate strong T helper cell responses
- low cost to produce**
 - 1L of ON culture yields ~10 mg phage, 50-100 doses
- stable to transport, store and deliver**
 - years if refrigerated
 - six months at room temperature
 - six weeks at 63°C
 - hours at 76°C
- easily administered via parenteral routes, no adverse reactions**
 - tested in mice, dogs, pigs
- viable pathway to regulatory approval**
 - can be used in inactivated form
 - retains immunogenicity (Samoylova et al, Virological Methods, 2012)

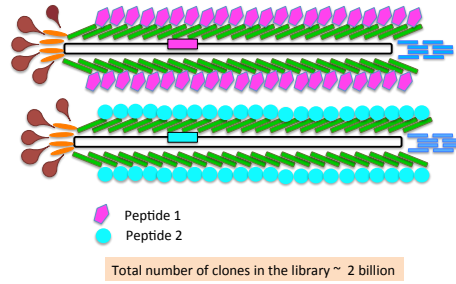


Phage-GnRH constructs obtained via cloning: example

Insert sequence	Titer, cfu/ml
GRPGGS	4×10^{11}
EHWSYGL	low
GGEHWGGS	1.4×10^{12}
GGRPGGGS	1.2×10^{11}
EHWGRPGGS	low
EHWGGRPG	low

Constructs obtained = 61
 Constructs that propagate relatively well = 10
 Constructs that propagate well and show good binding to GnRH Ab - several
 Stable?

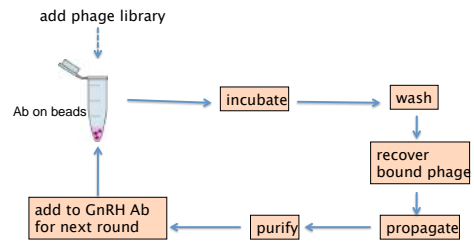
Phage display library is multibillion mixture of phage clones



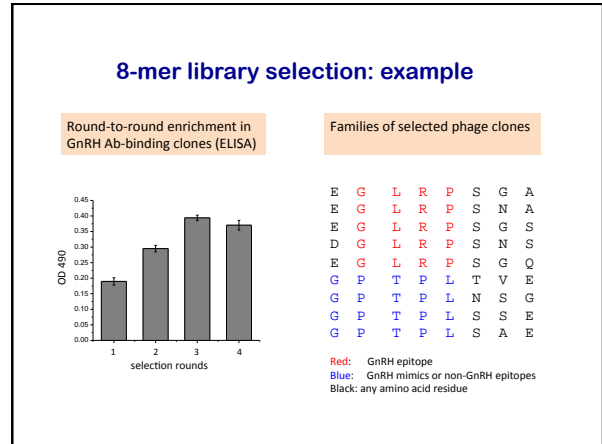
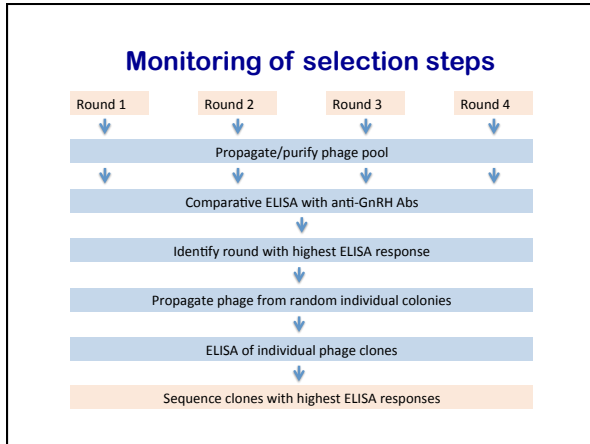
How phage display works...



Selection from phage library



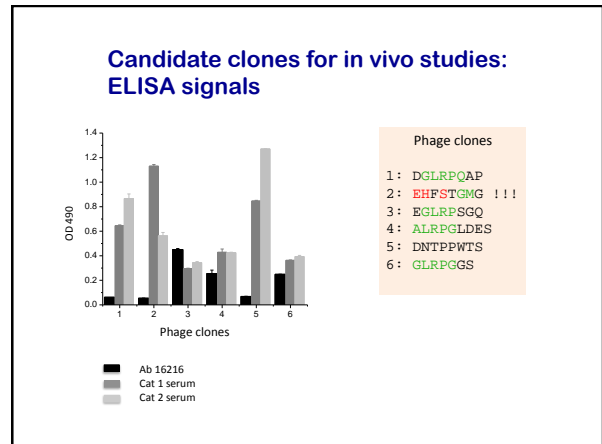
Ab = anti-GnRH antibodies
 3-4 rounds required

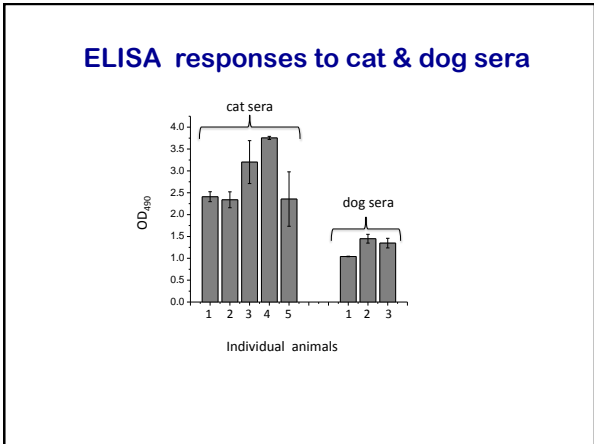
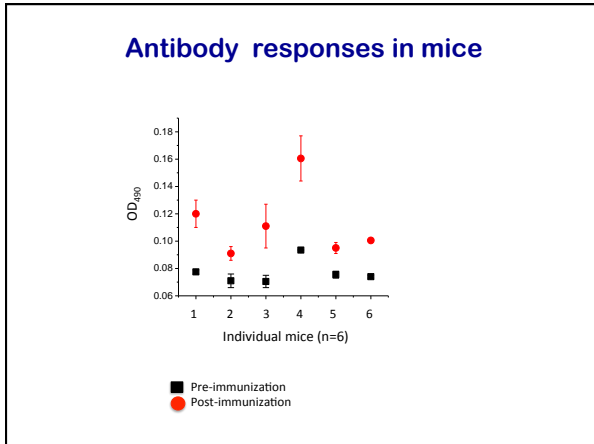
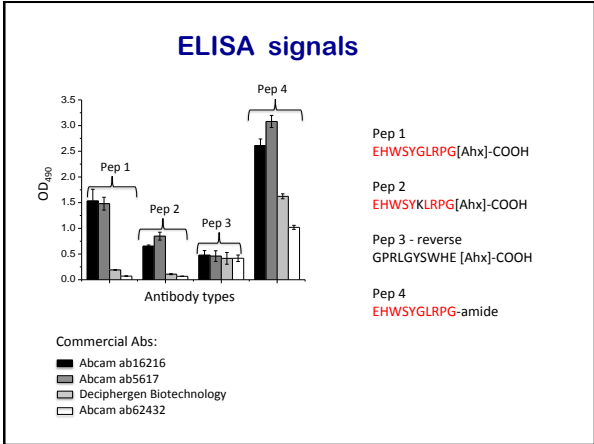
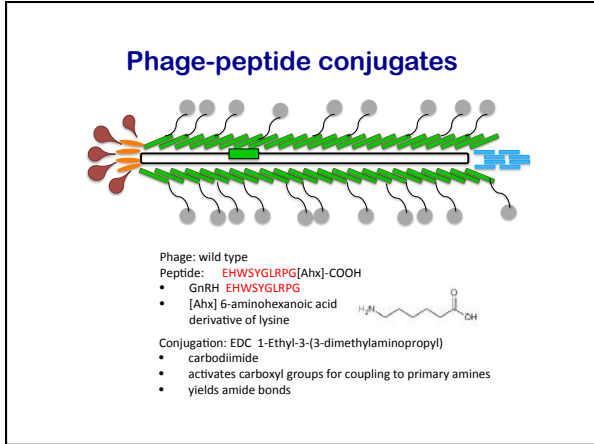


Phage libraries selection using different Abs

8-mer on Ab 16216	9-mer on Ab 16216	8-mer on cat Ab Protein A purified	8-mer on cat Ab GnRH pep purified
XGLRPXXX GPTPXXXX XLXPPGGXX APLXXXXXX GTPMXXXX	ALRPGLDES XLXPPGGXX APLXXXXXX GTPMXXXX	DQGGNXXX GANXXXXX	DGLRPQAP EHFSTGMG

Green: GnRH epitope C terminus
 Red: GnRH epitope N terminus
 Others colors: GnRH mimics or non-GnRH epitopes
 Black: any amino acid residue



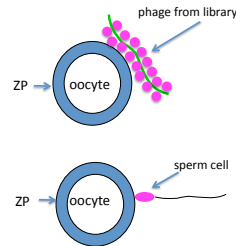


Another target – ZP on oocytes

- ZP- zona pellucida
- protective membrane surrounding oocyte
- 3 or 4 glycoproteins depending on the species
- play critical role in sperm-oocyte binding and induction of acrosome reaction



ZP-binding epitopes on sperm and on phage



Incubation:
phage-ZP binding

Fertilization:
sperm-ZP binding

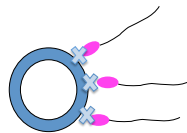
Hypothesis: phage epitopes mimic sperm epitopes

Potential contraceptive mechanisms

inject ZP-binding phage



anti-sperm antibodies



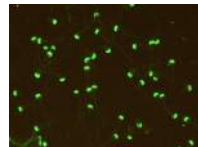
Mechanisms of action:

- inhibition of sperm capacitation, acrosome reaction
- decreasing sperm motility
- interference with sperm-ZP interaction/penetration/fusion

Anti-sperm antibodies in humans:

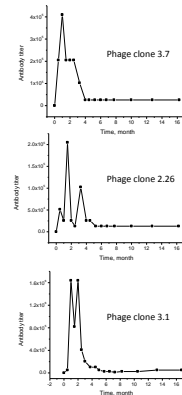
- cause infertility, 30%
- long lasting condition
- difficult to treat

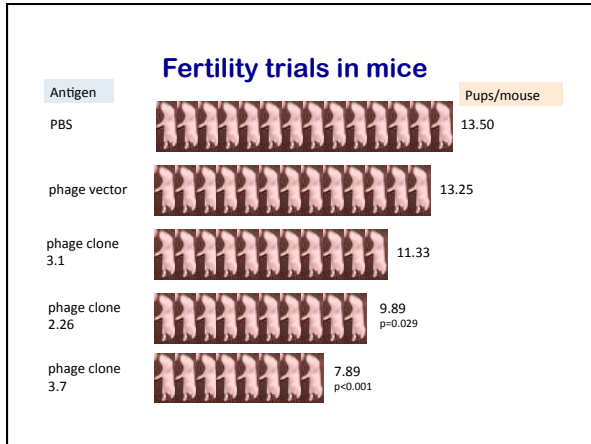
Antibody responses in dogs



Sperm cells reacted with serum from immunized dog

- anti-sperm antibodies
- high, long-lasting Ab responses
- phage is safe (7 dogs, dose 500ug)





Thank you!!!

Scott-Ritchey Research Center
AUCVM

Nancy Cox
Anna Cochran
Alexandre Samoylov
Nancy Morrison
India Napier
Henry Baker
Adam Breitenreicher
Anita Patel
Mandy Norris

Oregon State University
Michelle Kutzler
Caitlin Donovan

Department of Pathobiology
AUCVM
Valery Petrenko

Department of Lab animal Health
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Department of Anatomy, Physiology, & Pharmacology, AUCVM
Karen Wolfe



Thank you!!!



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