Hva viser genanalyser av muskulatur hos laks med mørke flekker

Aleksei Krasnov, Hooman Moghadam

Nofima, Ås

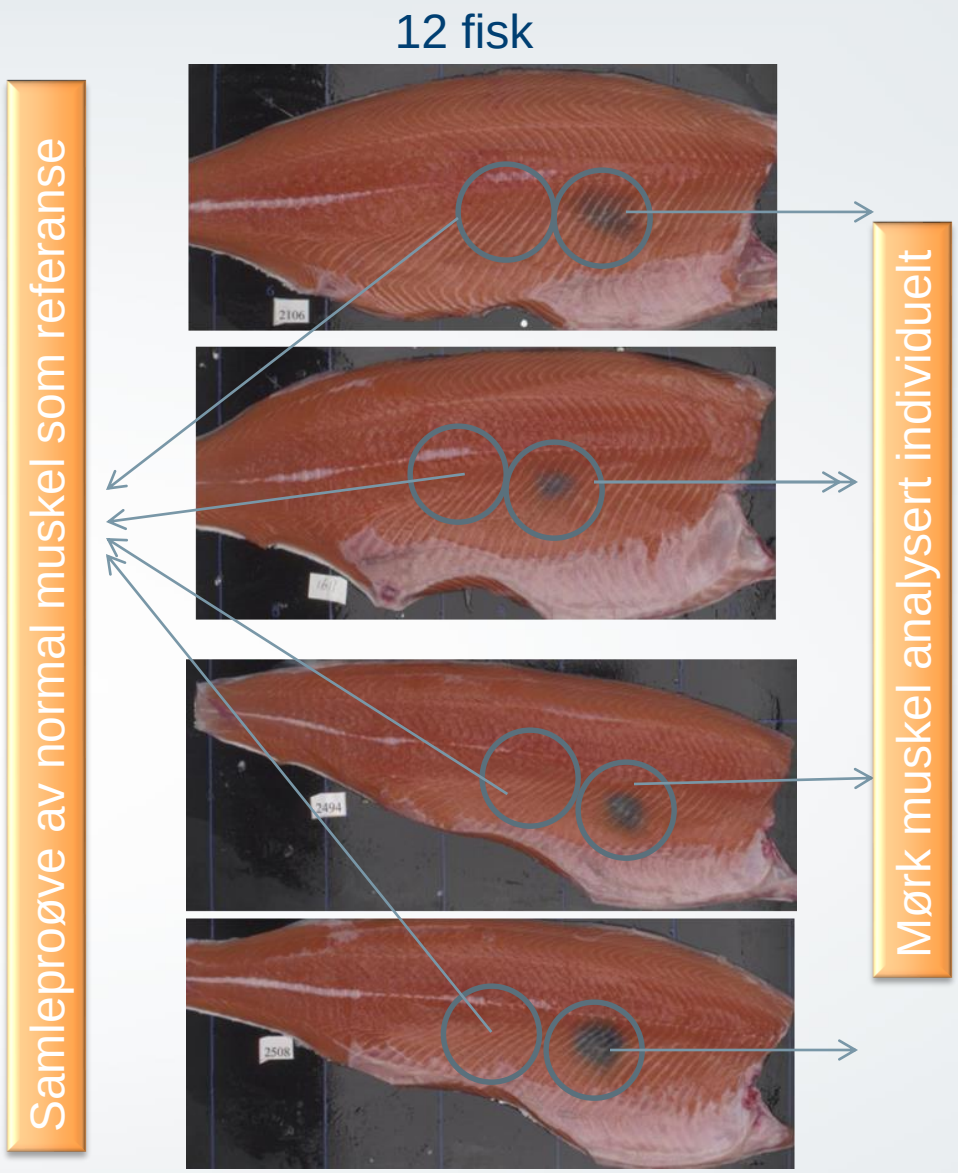
Spørsmål

Har mørke flekker lik eller ulik profil?

Vevsskade og nydannelse av vev?

Type betennelse?

Årsak til mørkpigmentering?



Undersøkt:
Forskjell mellom normal og mørk muskel

Metode:
Nofima's 15 k Atlantic salmon oligo chip,
STARS bioinformatics

Resultater genuttrykk:
1570 DE genes (>2-fold, p<0.01)
946 høyere i mørk muskel
624 lavere i mørk muskel

Genuttrykk viste vesentlig forskjell mellom normal og mørk muskel

Spørsmål

Har mørke flekker lik eller ulik profil?

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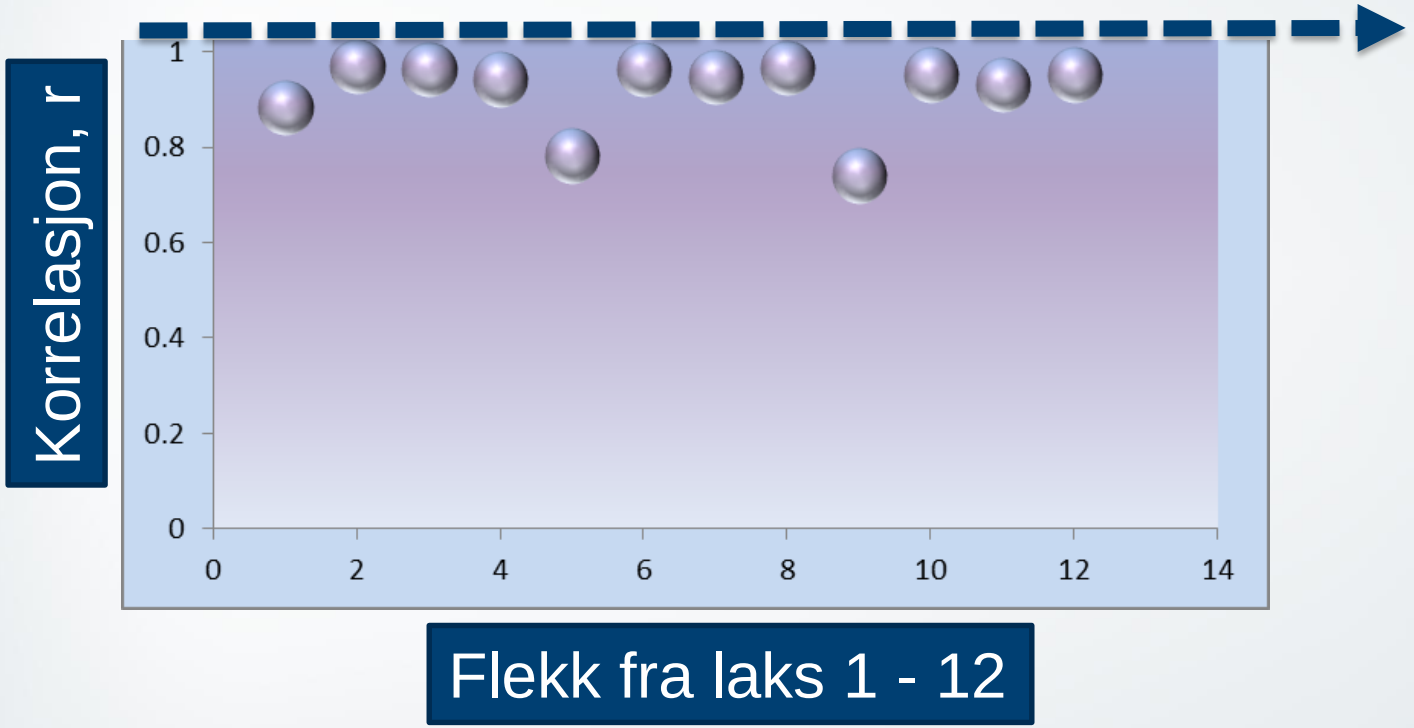
Årsak til mørkpigmentering?

Tilnærmet likt genuttrykk uavhengig av misfarging og størrelse på flekkene



Korrelasjon = 1 betyr at genuttrykket er eksakt det samme. For vårt materiale var korrelasjonen 0,92

Resultatene tyder på at vevsskaden hadde samme årsak

Correlation: Pearson r (mean = 0.917)

Spørsmål

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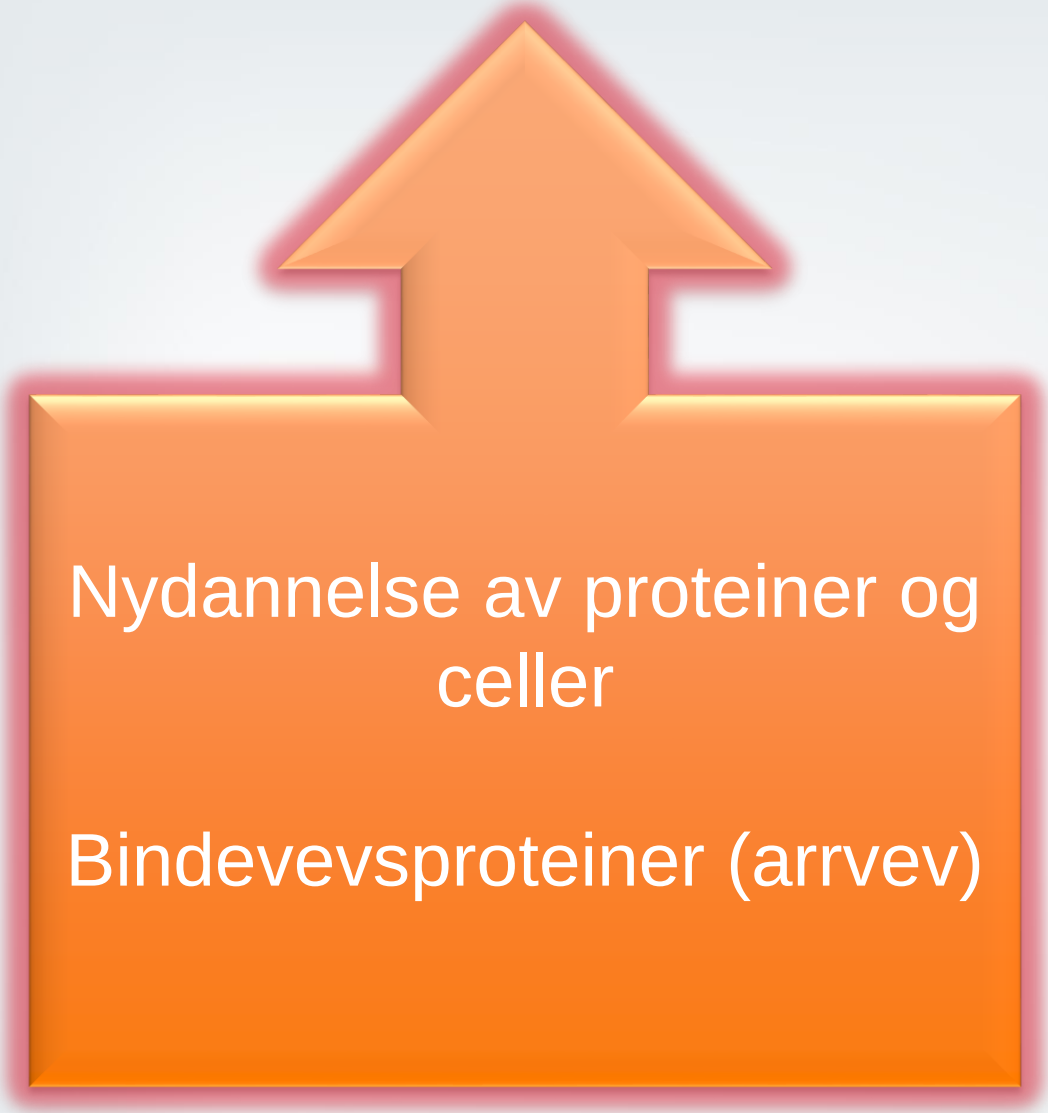
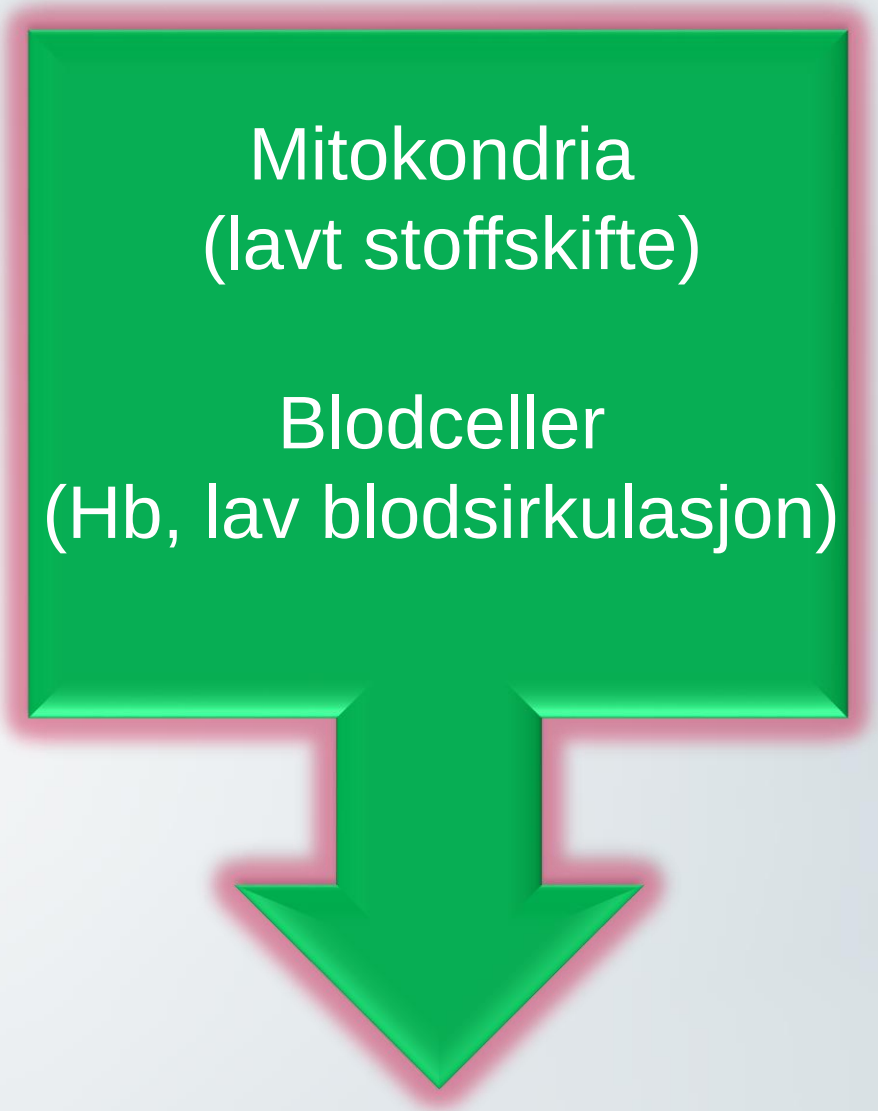
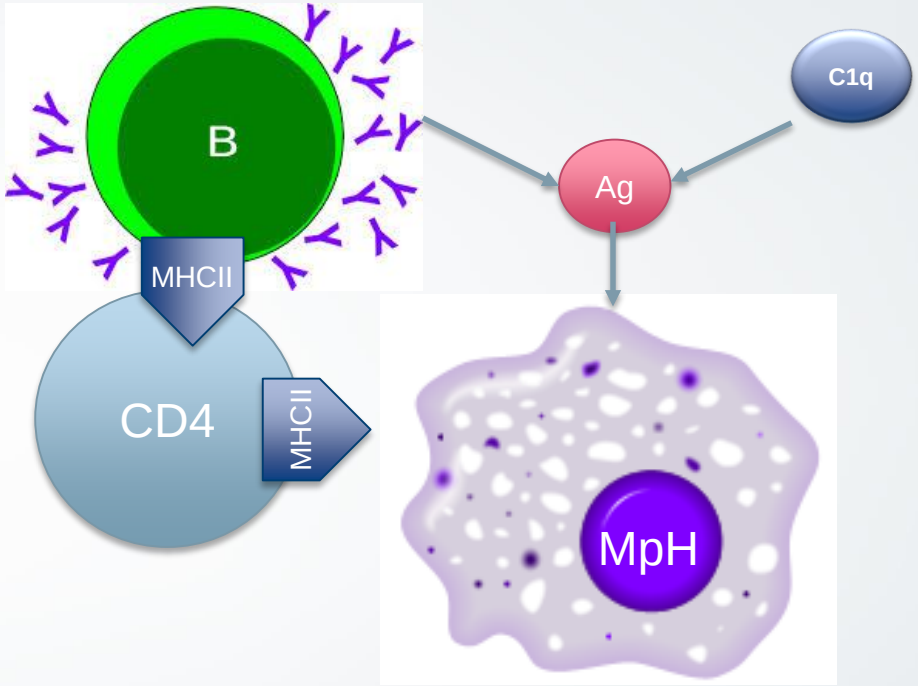




Reparasjon & nydanning, erstatning av muskel med bindevev

VRG

AFP



CD8

Mild kronisk betennelse

Årsak?

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VETERINARY RESEARCH

RESEARCH ARTICLE Open Access

Piscine orthoreovirus (PRV) in red and melanised foci in white muscle of Atlantic salmon (*Salmo salar*)

Håvard Bjørgen[1], Øystein Wessel[2], Per Gunnar Fjelldal[3], Tom Hansen[3], Harald Sveier[4], Håkon Rydland Sæbø[5], Katrine Bones Enger[6], Eirik Monsen[7], Agnar Kvellestad[1], Espen Rimstad[2] and Erling Olaf Koppang[1*]

Concerns:

Relativt lav inteferon respons

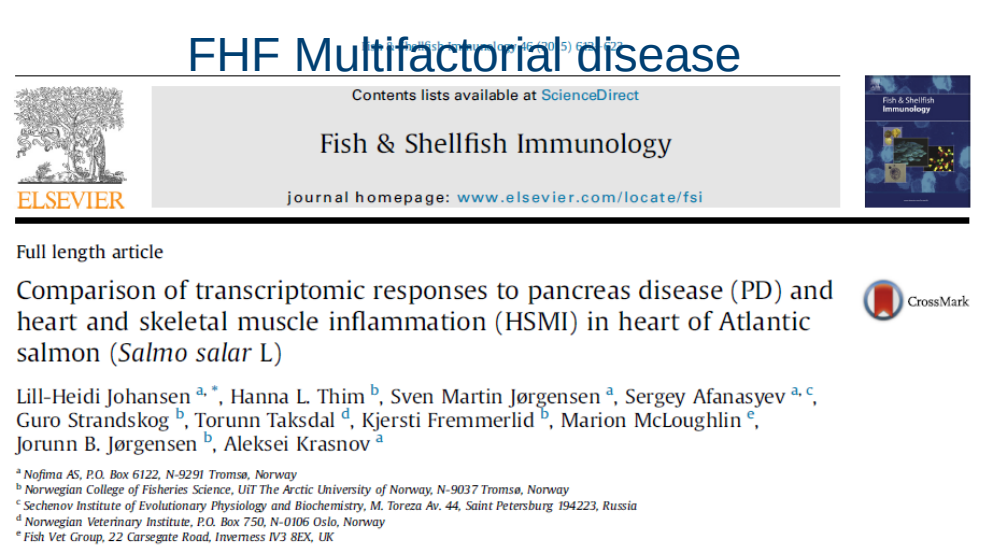
CD4 > CD8

MHCII > MHCI

It's like anything known?

Statistisk Meta Analyse: Sammenligning med andre forsøk

FHF Multifactorial disease



Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Comparison of transcriptomic responses to pancreas disease (PD) and heart and skeletal muscle inflammation (HSMI) in heart of Atlantic salmon (*Salmo salar* L)

Lill-Heidi Johansen [a, *], Hanna L. Thim [b], Sven Martin Jørgensen [a], Sergey Afanasyev [a, c], Guro Strandskog [b], Torunn Taksdal [d], Kjersti Fremmerlid [b], Marion McLoughlin [e], Jorunn B. Jørgensen [b], Aleksei Krasnov [a]

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[d] Norwegian Veterinary Institute, P.O. Box 750, N-0106 Oslo, Norway
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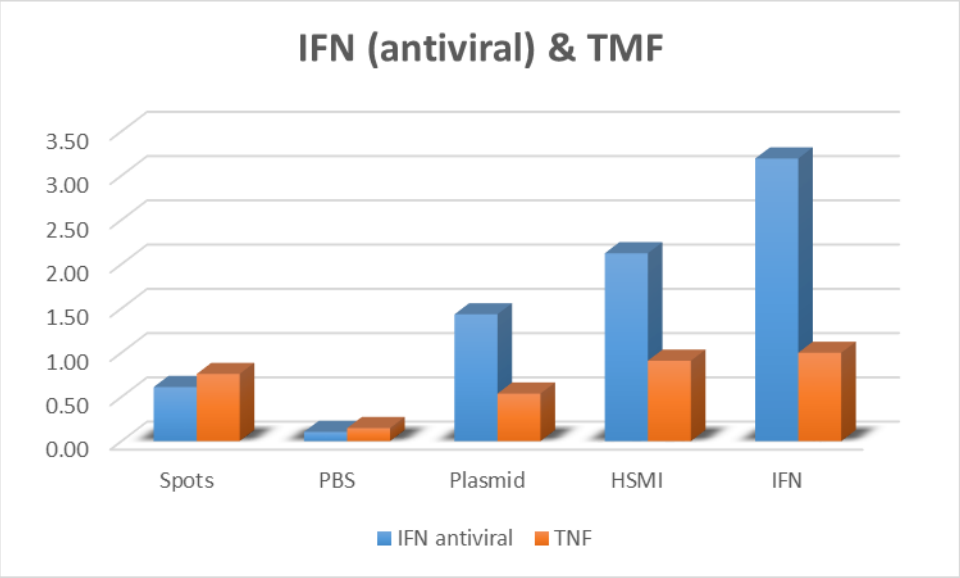
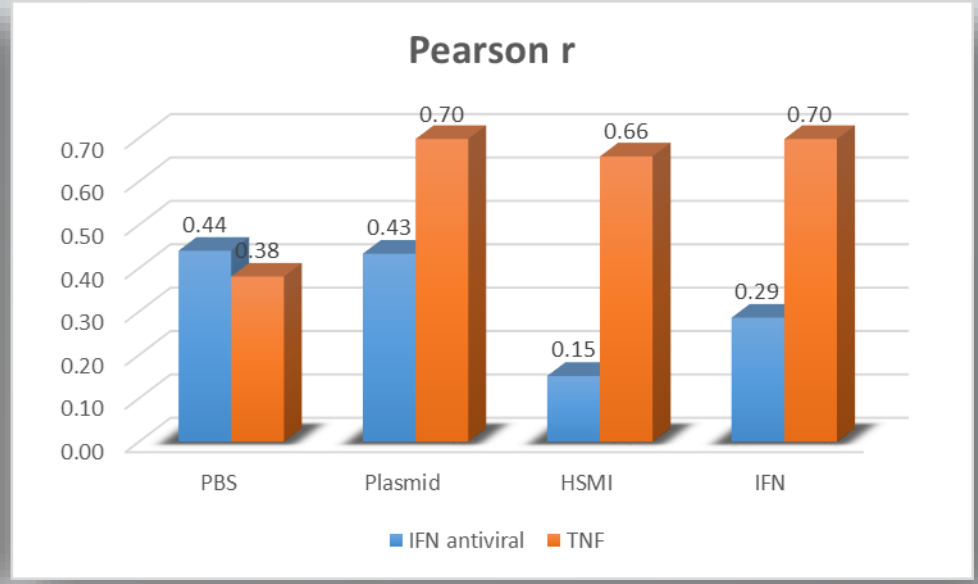
NFR Plattform for Virusvaksiner i fisk, VivaFish
Børre Robertsen (Univ Tromsø)

Muskel injeksjon:

- Placebo (PBS)
- Kontroll plasmid (bakterie DNA)
- Plasmid som produserer IFNa

Condition	Antigen	Trauma	Lesion	Timing
Dark spots	Unknown	Unknown	No	Months?
HSMI	Virus	No	Yes	Weeks
PBS injection	No	Small	No	1-2 weeks
Plasmid	Bacterial DNA	Small	No	1-2 weeks
PLasmid IFNa	Bacterial DNA+IFNa	Small	No	1-2 weeks



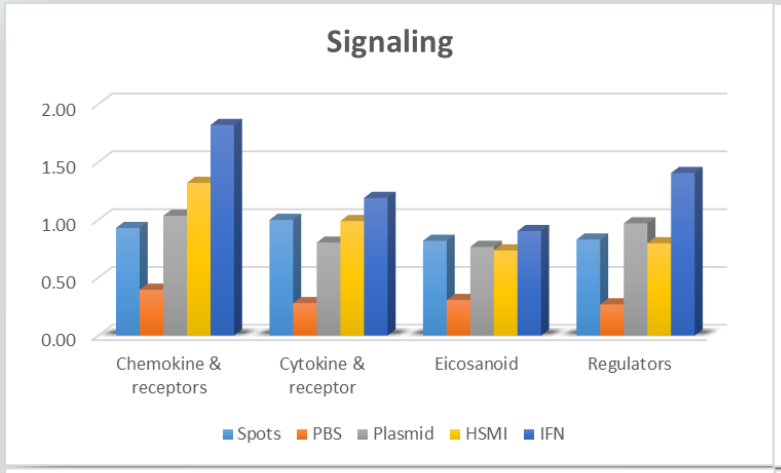


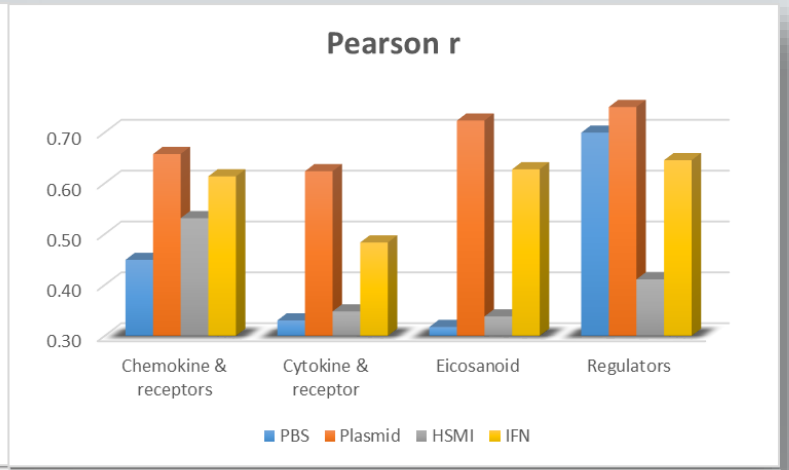
Innate antiviral responses in spots are week
Correlation is low

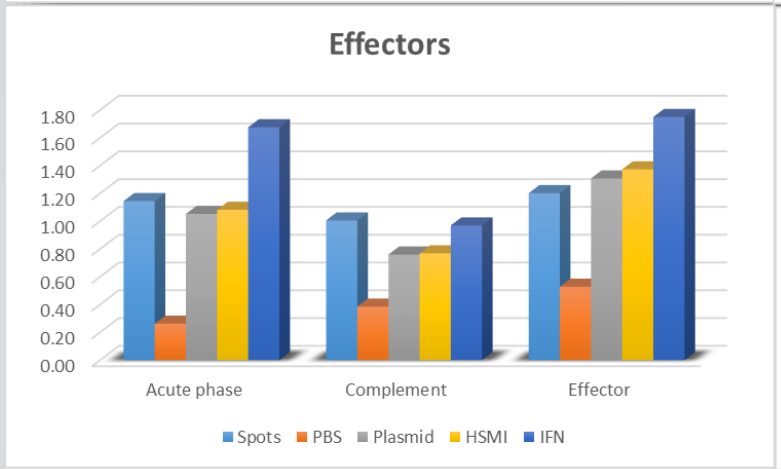
TNFa- related responses to treatments are similar

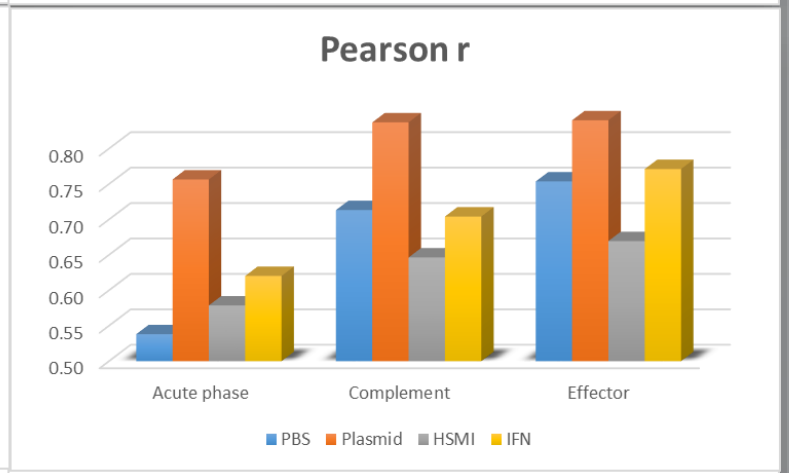
Respons likner ikke muskel med virusinfeksjon

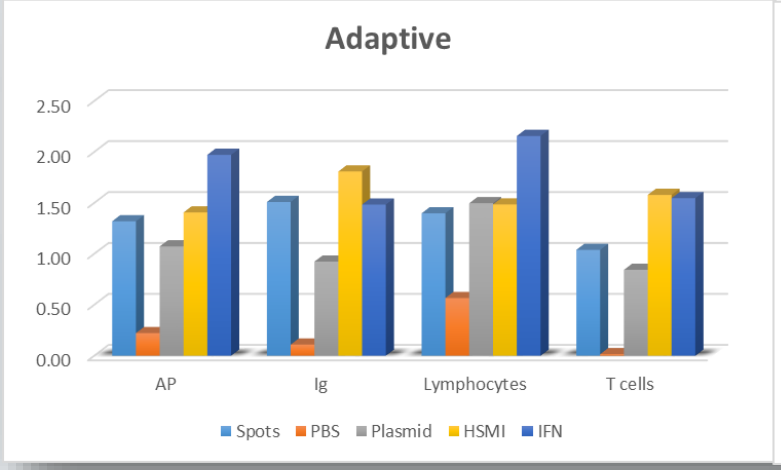
Gene	Spots	HSMI	PBS	Plasmid	IFN
Barrier-to-autointegration factor	1.30	3.14	0.63	3.81	5.64
Receptor transporting protein 3	0.49	4.80	-1.14	1.57	5.49
Interferon-induced protein 44	0.34	4.14	0.74	0.47	5.15
Very large inducible GTPase 1-1	1.12	4.36	-0.05	3.38	6.67
Sacsin	1.29	5.07	-0.28	3.74	6.12
Gig2-3	0.45	2.38	0.80	3.28	5.15
Mx1	0.49	3.91	0.14	3.90	7.09
Viperin	-0.11	3.67	0.15	2.94	6.39
Ubiquitin-like protein-1	-0.29	4.37	0.01	3.97	5.51
VHSV-inducible protein-4	-1.15	3.10	0.40	2.71	4.57

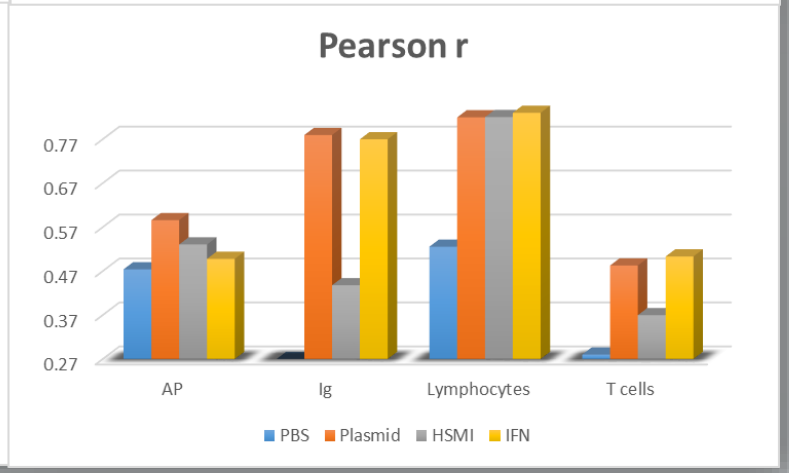












VivaFish fiskemateriale

Immune reactions in spots are similar by magnitude to responses to HSMB and bacterial DNA

Similarity (correlation):

Bacterial DNA > Bacterial DNA + IFNa > HSMI > injection

Least similar: T cells response

Genes	Spots	HSMI	PBS	Plasmid	IFN
CD2	0.69	2.09	1.17	2.19	2.36
CD4 -1	3.24	1.11	0.06	-0.43	1.29
CD4-2	2.98	1.95	1.97	0.71	2.59
CD4-3	3.24	0.06	-0.43	1.11	1.29
CD4-4	0.47	1.05	0.58	0.43	1.46
CD8 alpha	1.73	3.30	1.05	0.43	1.17
CD8 beta	0.94	3.57	0.46	-0.88	0.82
CD8 beta	0.85	2.56	0.53	0.32	1.20
CD8 beta	0.41	2.10	-0.70	-0.60	0.31

Mørke flekker samme type genuttrykk som laks injisert med bakterie DNA

Immungener som var spesifikke for mørke flekker

- Og uttrykk av de samme genene ved kontrollert «smitte»

Genes	Spots	HSMI	PBS	Plasmid	IFN
Transcription factor PU.1	5.60	2.21	-1.23	1.21	-1.17
Transcription factor SOX-4	2.67	1.10	1.13	-1.12	1.33
CD80-like protein	7.16	1.65	1.00	1.64	2.03
C1q TNF-related protein 5	5.32	1.00	1.19	1.61	1.30
Nattectin C-type lectin	44.53	-1.67	-1.28	2.61	0.97
15-hydroxyprostaglandin dehydrogenase	3.95	-1.88	-1.16	1.21	-1.39
Leukotriene b4 12-hydroxydehydrogenase	11.16	1.22	0.95	-1.08	-1.87
72 kDa type IV collagenase	3.90	1.30	-1.41	-1.28	-2.10

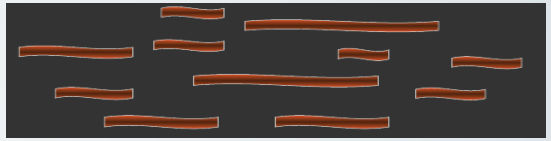
Mulige gen-markører for melanisering

Dual RNA-Seq

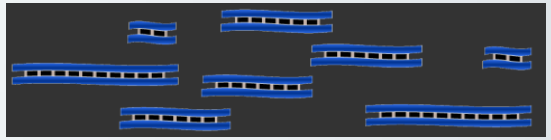
- From 6 individuals total RNA was extracted from both normal and adjacent dark spots;
- RNA sequenced using Illumina platform;
- Sequenced reads aligned against the salmon and all viral and bacterial sequence data;

Total RNA (depleted from salmon rRNA)

Fragment RNA

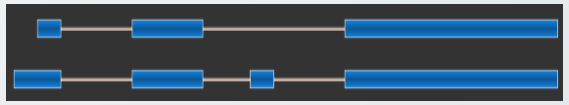


Reverse transcribe into cDNA

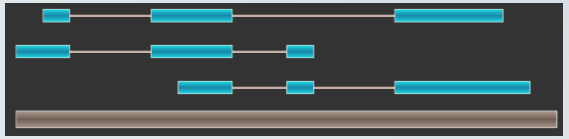


Sequence cDNA

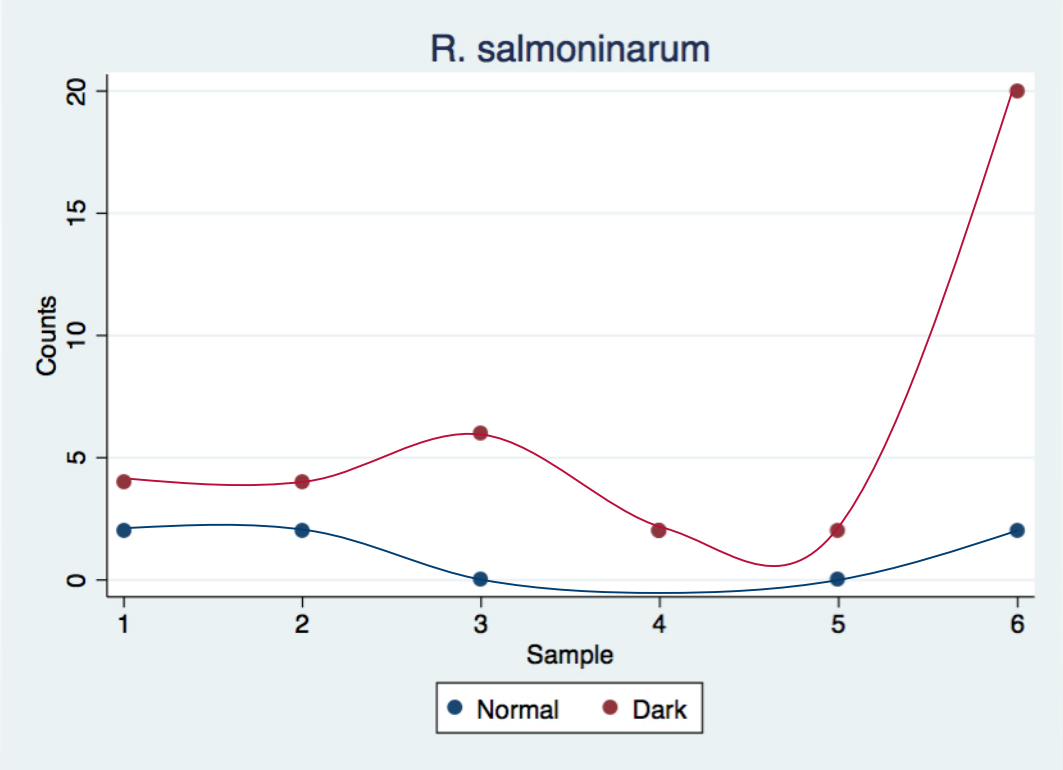
Mapping reads to pathogen genomes

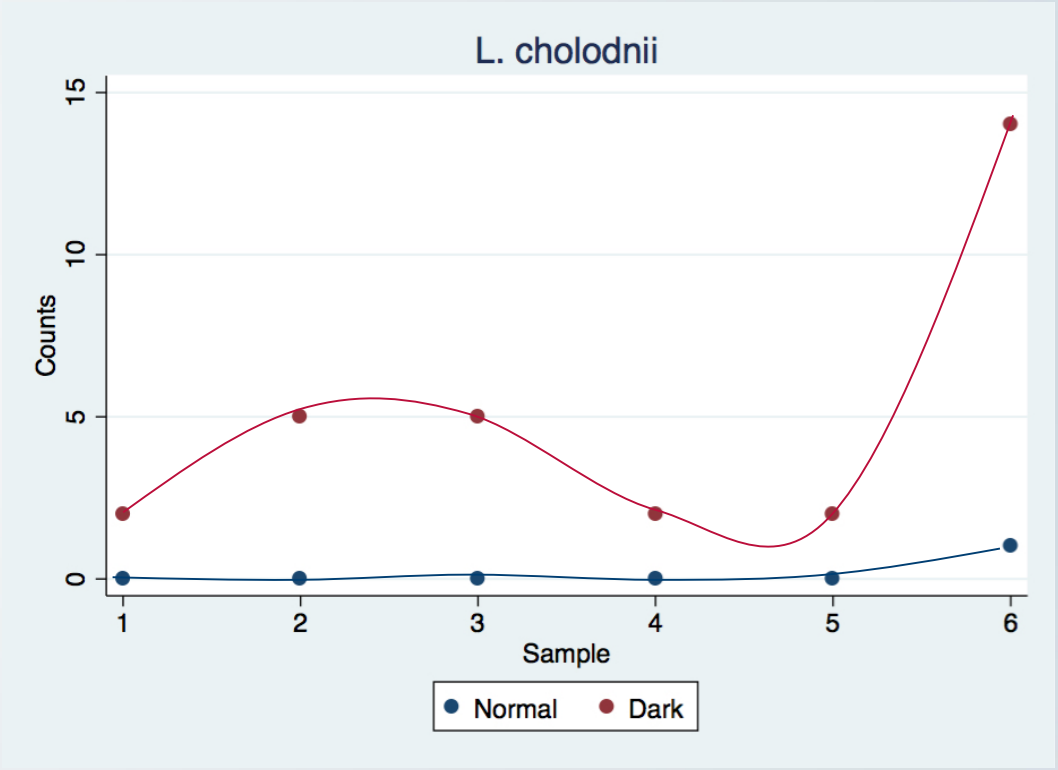


Mapping reads to salmon genome



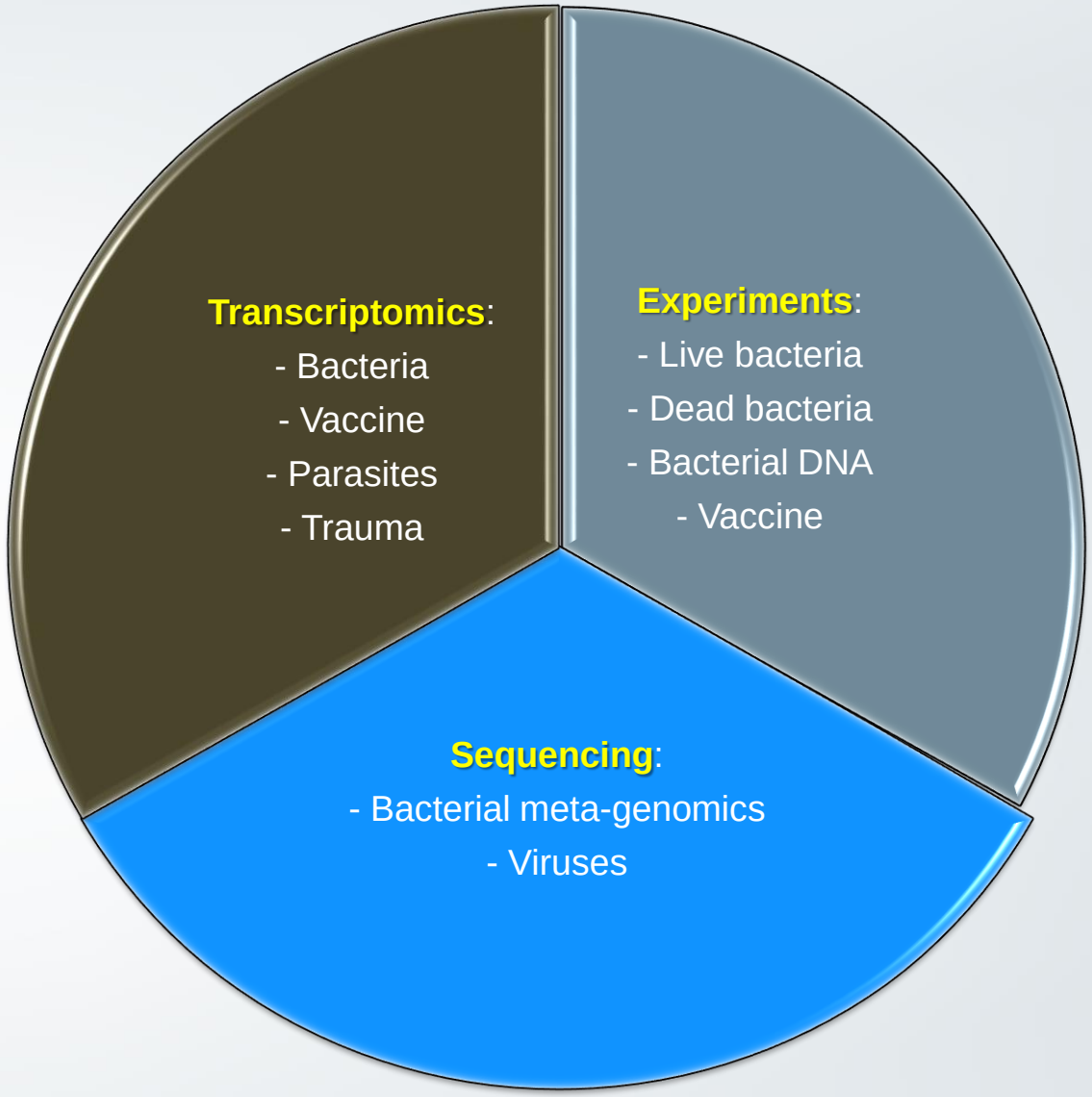
Eksempler på bakterier med ulik konsentrasjon I normal muskel og mørk muskel (loads, rRNA)





Årsak til at flekkene?

Årsak	Sannsynlighet
Virus	Lav
Skader alene	Lav
Patogene bakterier	Lav
Ikke patogene kakterier	Middels høy
Ikke patogene kakterier + skade	Mest sannsynlig
Andre årsaker ???	



Videre forskning

Synes vi bør se litt nærmere på denne på mandag
Bør også highlighte det med genmarkører

Genetic enrichment of functional categories

- Similar to microarry, many immune specific genes are upregulated in dark spots;
- Found evidence many genes involved in muscle development and lipid synthesis and metabolism to also been upregulated;