

OUS AMG Monday seminar

Targeted Amplicon Sequencing

Arvind Sundaram
Aug 31 2020

Illumina sequencing

Fragment of interest

Sequencing adapters (60 bp)

Sequencing adapters (60 bp)

Bridge amplification

read 1

index 1

Flowcell

Reverse chemistry

Flowcell

index 2

read 2

Library preparation

Fragment of interest



Sequencing adapters (60 bp)

Sequencing adapters (60 bp)

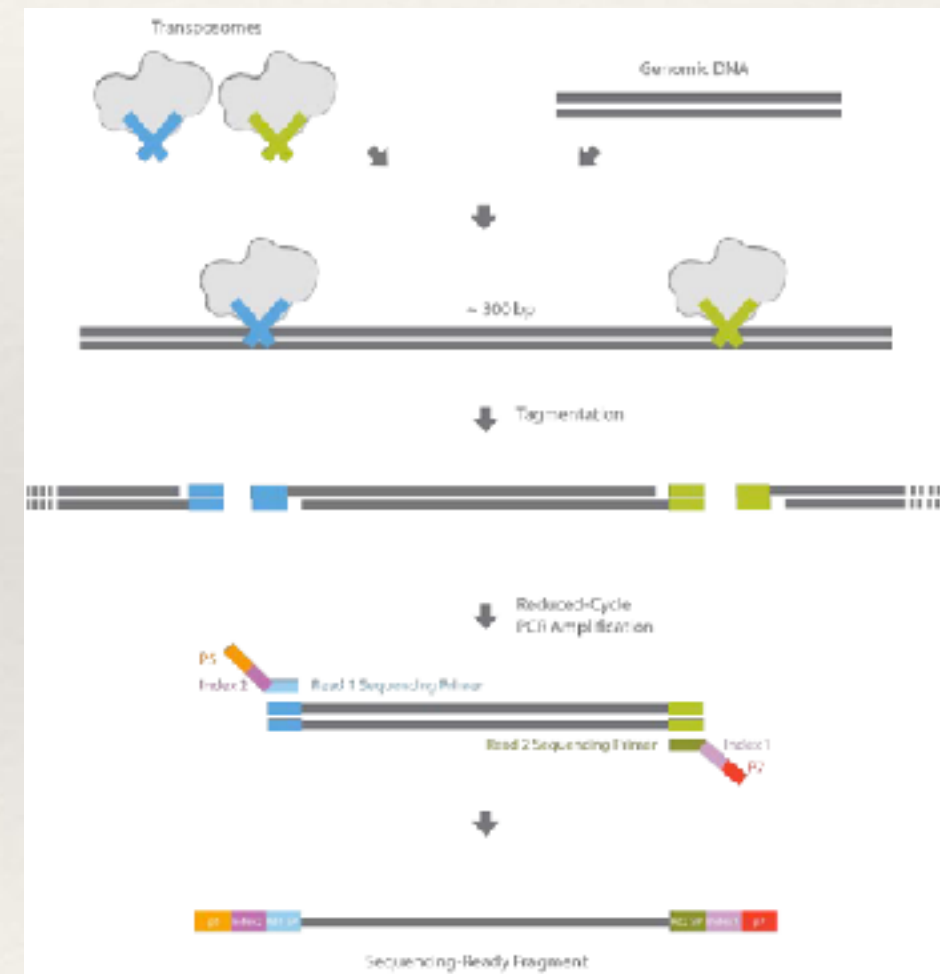
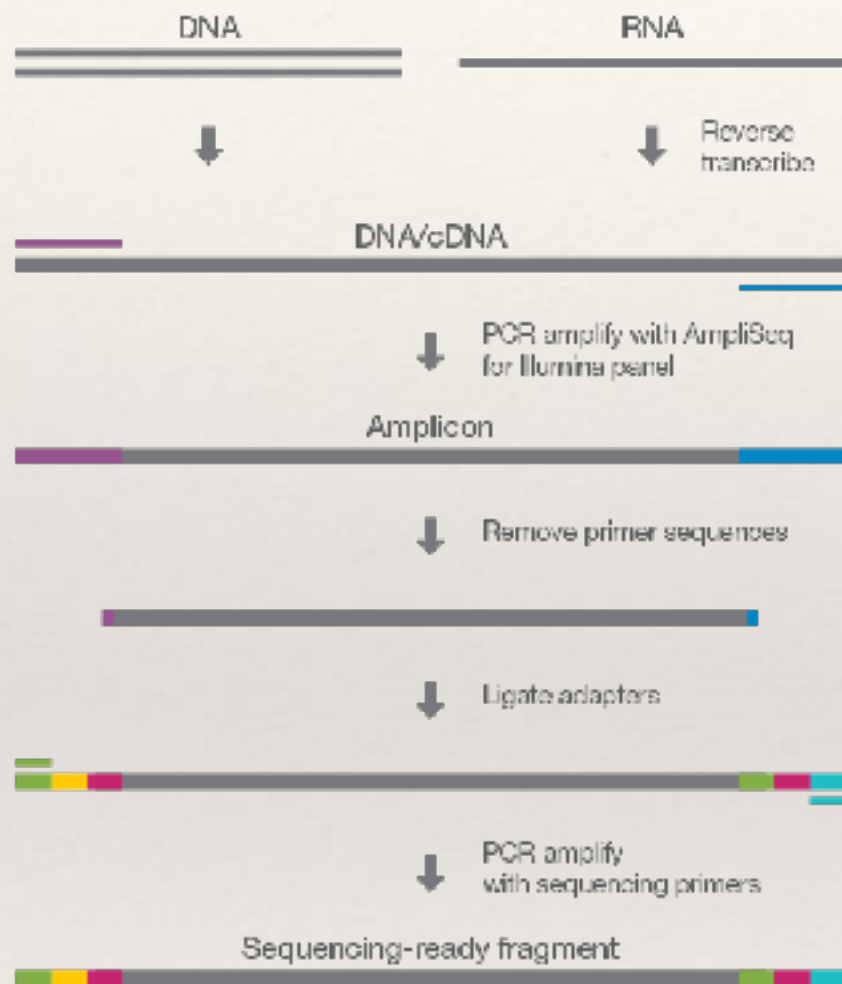
Targeted sequencing ~ 300 - 550 bp

Ampliseq (Illumina): PCR based amplification + adapter ligation

Nextera (Illumina): Transposomes to target + PCR + adapter ligation

Library preparation

Ampliseq (Illumina): PCR based amplification + adapter ligation



Nextera (Illumina): Transposomes to target + PCR + adapter ligation

Library preparation

Fragment of interest



Sequencing adapters (60 bp)

Sequencing adapters (60 bp)

Targeted sequencing ~450-550 bp

Ampliseq (Illumina): PCR based amplification + adapter ligation

Nextera: Transposomes to target + PCR + adapter ligation

Normal library preparation on amplified product (treated as DNA)



Design primers that includes sequencing adapters and amplifies the target.

Might be 80+ nt long primers

Amplicon sequencing issues

Fragment of interest

Sequencing adapters (60 bp)

read 1

Sequencing adapters (60 bp)

read 2

Low complexity problem: solution - Heterogeneity spacers!!!

Sequencing Cycle																																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30											
Sample 1	C	C	T	A	A	A	C	T	A	C	G	G	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C	A	G	C											
Sample 2	G	T	G	G	T	A	T	G	G	G	A	G	T	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C	A	G											
Sample 3	T	G	T	T	G	C	G	T	T	T	C	T	G	T	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C	A											
Sample 4	A	C	A	G	C	C	A	C	C	C	A	T	C	G	A	A	C	T	C	C	T	A	C	G	G	G	A	G	G	C											
Sample 5	G	T	T	A	C	G	T	G	G	T	T	G	A	T	G	A	A	C	T	C	C	T	A	C	G	G	G	A	G	G											
Sample 6	T	A	C	C	G	G	C	T	T	G	C	A	T	G	C	G	A	A	C	T	C	C	T	A	C	G	G	G	A	G											
Sample 7	C	A	C	C	T	T	A	C	C	T	T	A	G	A	G	T	G	G	A	C	T	C	C	T	A	C	G	G	G	A											
Sample 8	T	T	A	A	C	T	G	G	A	A	G	C	C	C	T	G	T	G	G	A	C	T	C	C	T	A	C	G	G	G											
												Index 1										Heterogeneity Spacer										16S rRNA gene sequence									

500 + samples

Fadiosh et al. Microbiome 2014, 2:6
<http://www.microbiomejournal.com/content/2/1/6>



Microbiome

METHODOLOGY

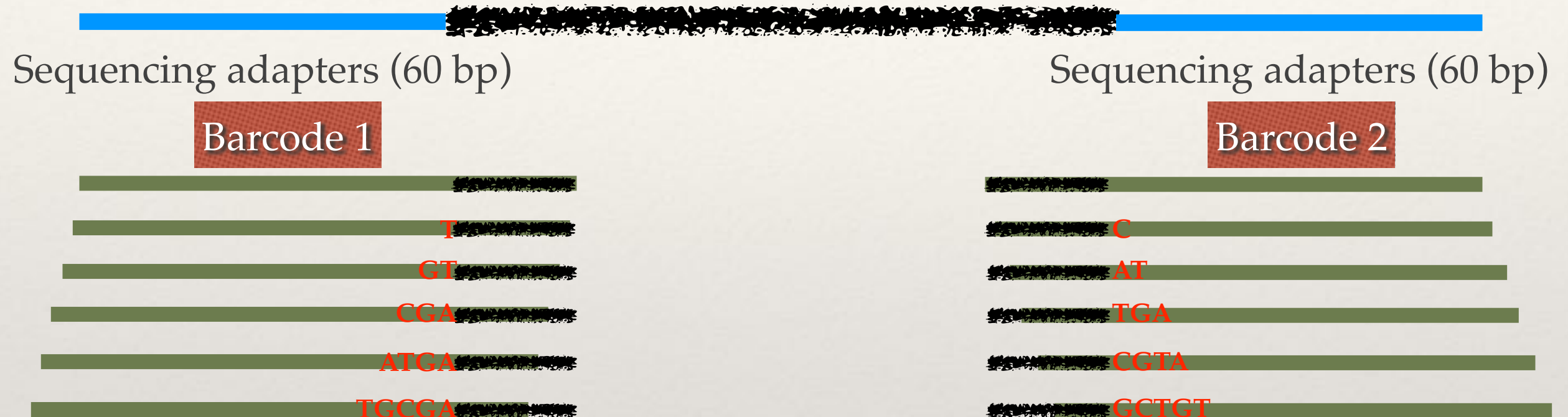
Open Access

An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform

Douglas W Fadiosh^{1*}, Bing Ma^{1†}, Pawel Gajer¹, Naomi Sengamelay¹, Sandra Ott¹, Rebecca M Brotman² and Jacques Ravel^{1*}

Fadrosh et. al. 2014

Fragment of interest



Design primers that includes sequencing adapters and amplifies the target.

Now it includes **Heterogeneity spacers**

500 + samples

24 samples : 7 primers (3 Fwd / 4 Rev)

48 samples: 14 primers (6 Fwd / 8 Rev)

576 samples: 48 primers (24 Fwd / 24 Rev)

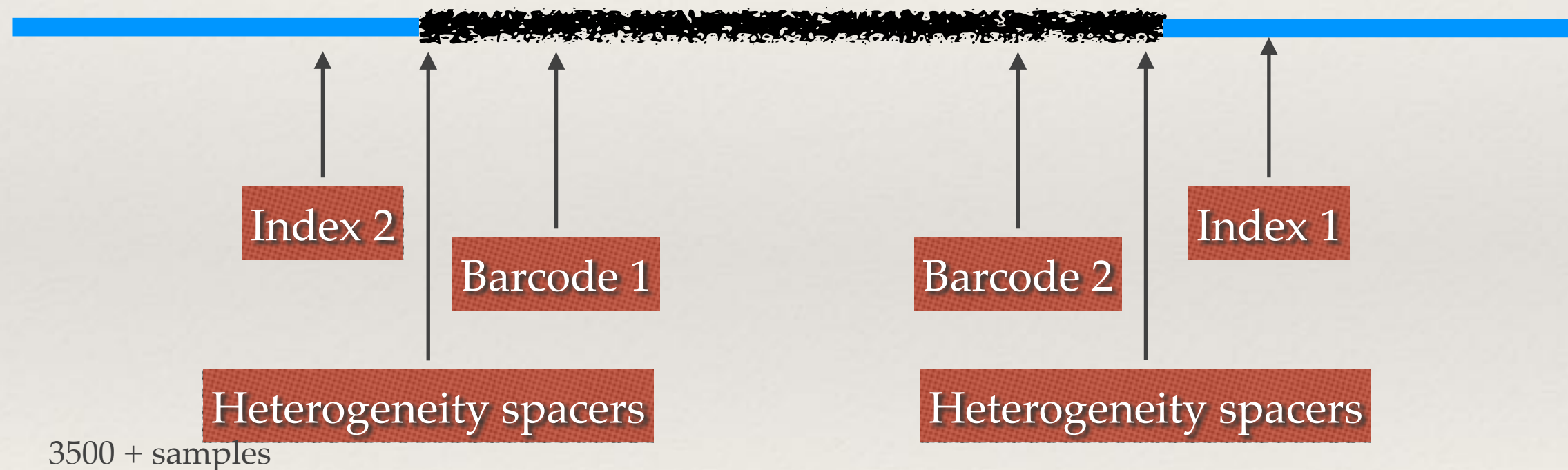
Offered at NSC for 16S V3 / V4 region

de Muinck et. al. 2017 (Designed at NSC)

PCR 1

PCR 2

We can design two sets of primers which will be used in 2 consecutive PCR reactions.
Smaller length primers - cheaper



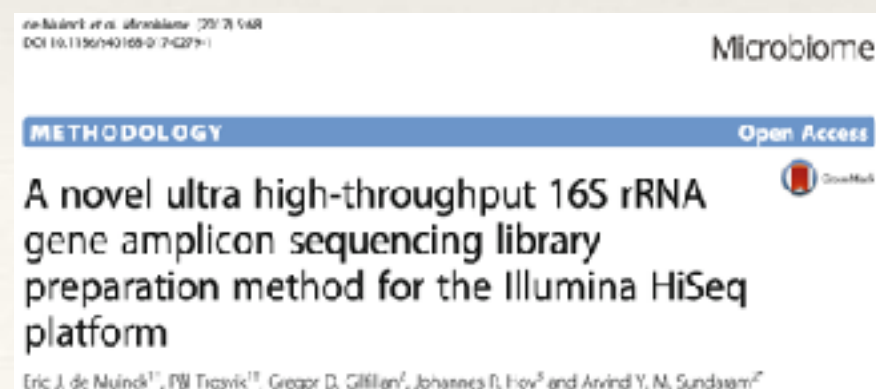
96 samples: 20 primers (12 Fwd / 8 Rev)

864 samples: 26 primers (24 Fwd / 24 Rev) + (3 Fwd / 3 Rev)

1728 samples: 29 primers (24 Fwd / 24 Rev) + (3 Fwd / 6 Rev)

Demultiplexer

https://github.com/nsc-norway/triple_index-demultiplexing







MHC typing in Atlantic salmon

Immunogenetics

<https://doi.org/10.1007/s00251-019-01143-8>

REVIEW

An Illumina approach to MHC typing of Atlantic salmon

Arvind Y. M. Sundaram^{1,2}  · Åse Helen Garseth¹  · Giuseppe Maccari^{3,4}  · Unni Grimholt¹ 

MHC typing in Atlantic salmon



Onmy-UBA	Sasa-UBA	Sasa-DAB	Sasa-DAA	Onmy-DAB	Onmy-DAA
48	48	42	22	22	3
Class I	Class I	Class II	Class II	Class II	Class II



Unni Grimholt
Veterinærinstituttet

MHC typing in Atlantic salmon

Target 5 amplicons (approx. 410 bp) across 9 animals

MHC class I UBA

(2 amplicons, 2 Fwd and 1 shared Rev)

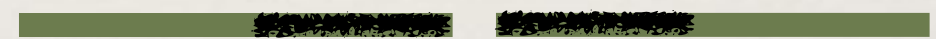
MHC class II DAA

(1 amplicon, 1 Fwd and 1 Rev)

MHC class II DAB

(2 amplicon, 2 Fwd and 1 shared Rev)

PCR 1



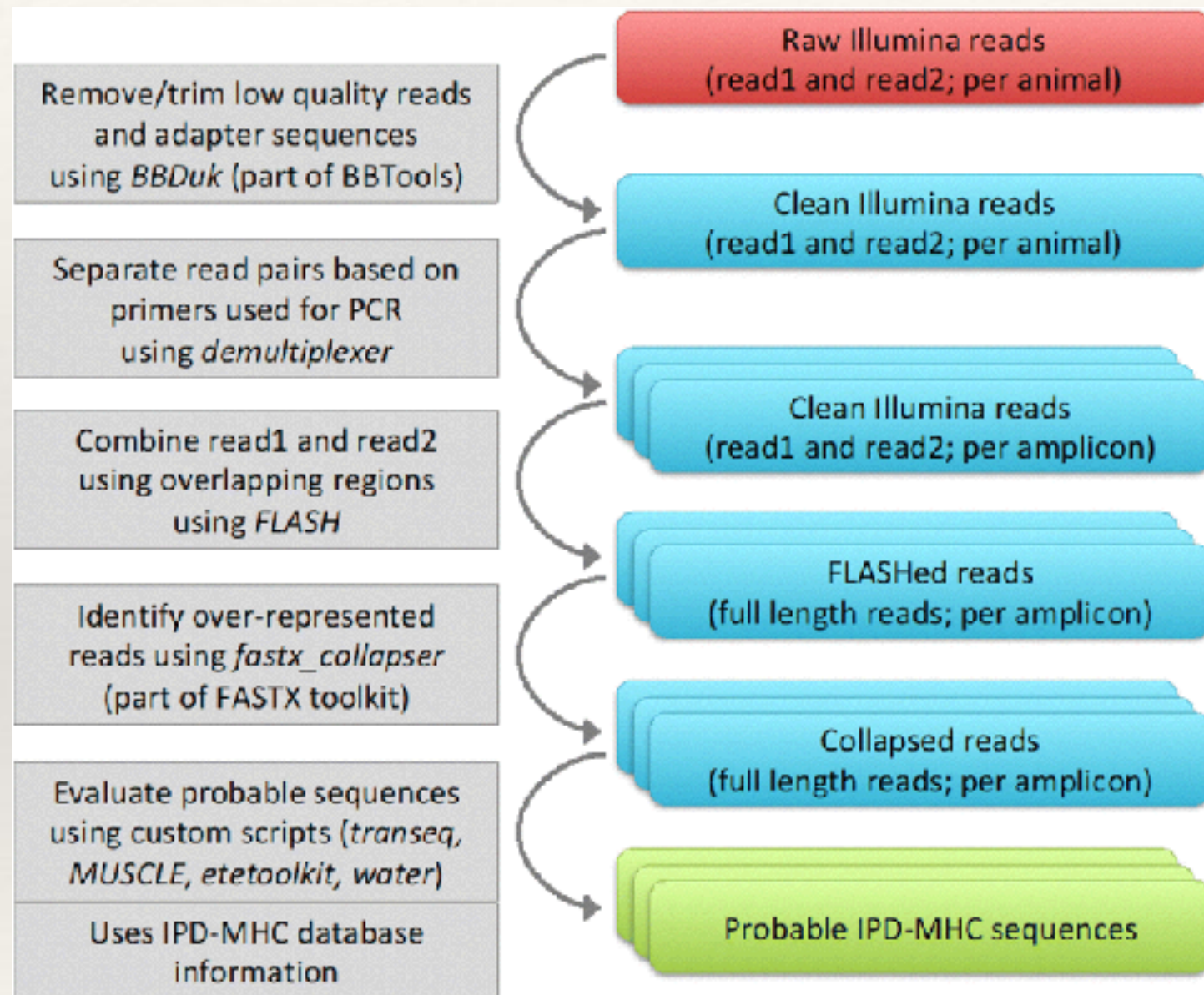
No Heterogeneity spacers !!

PCR 2



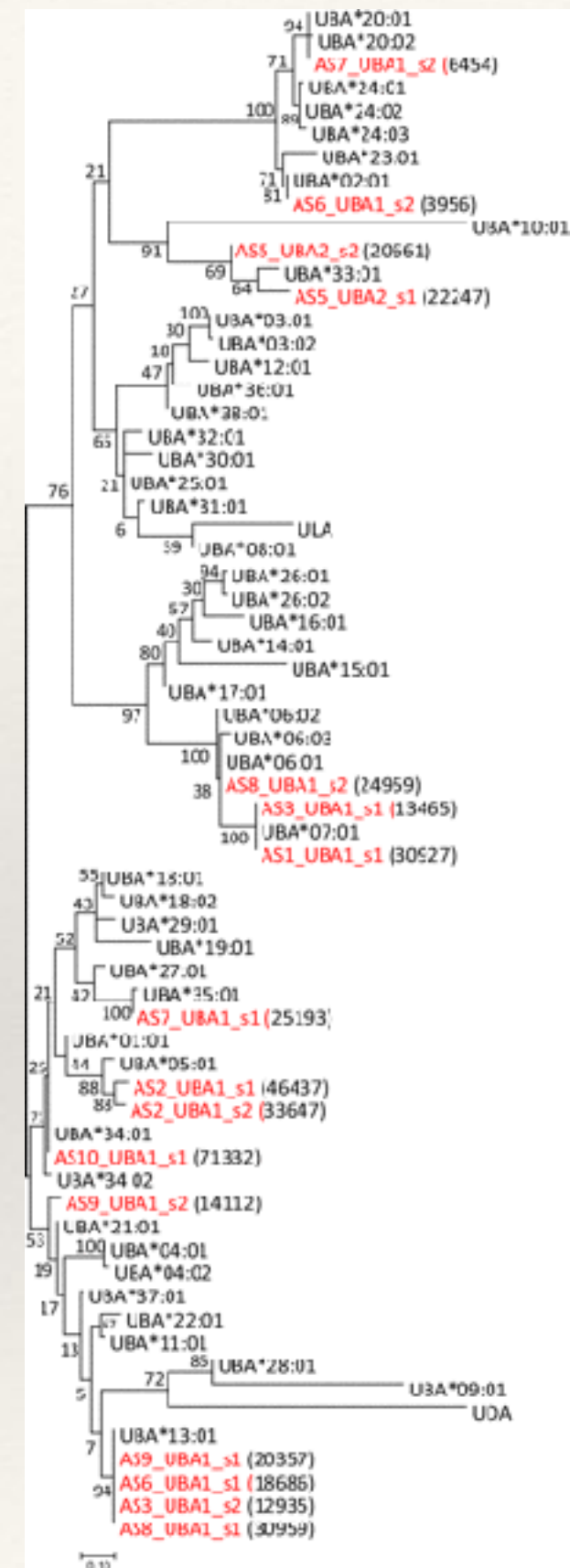
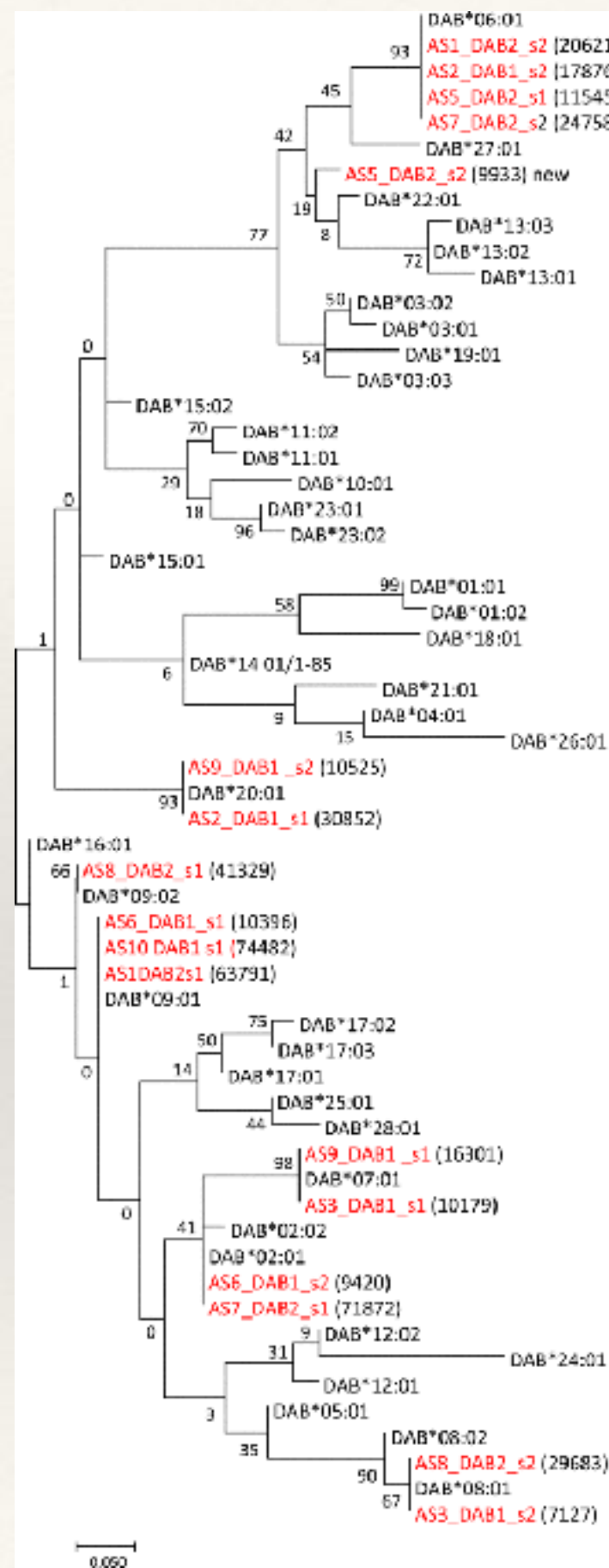
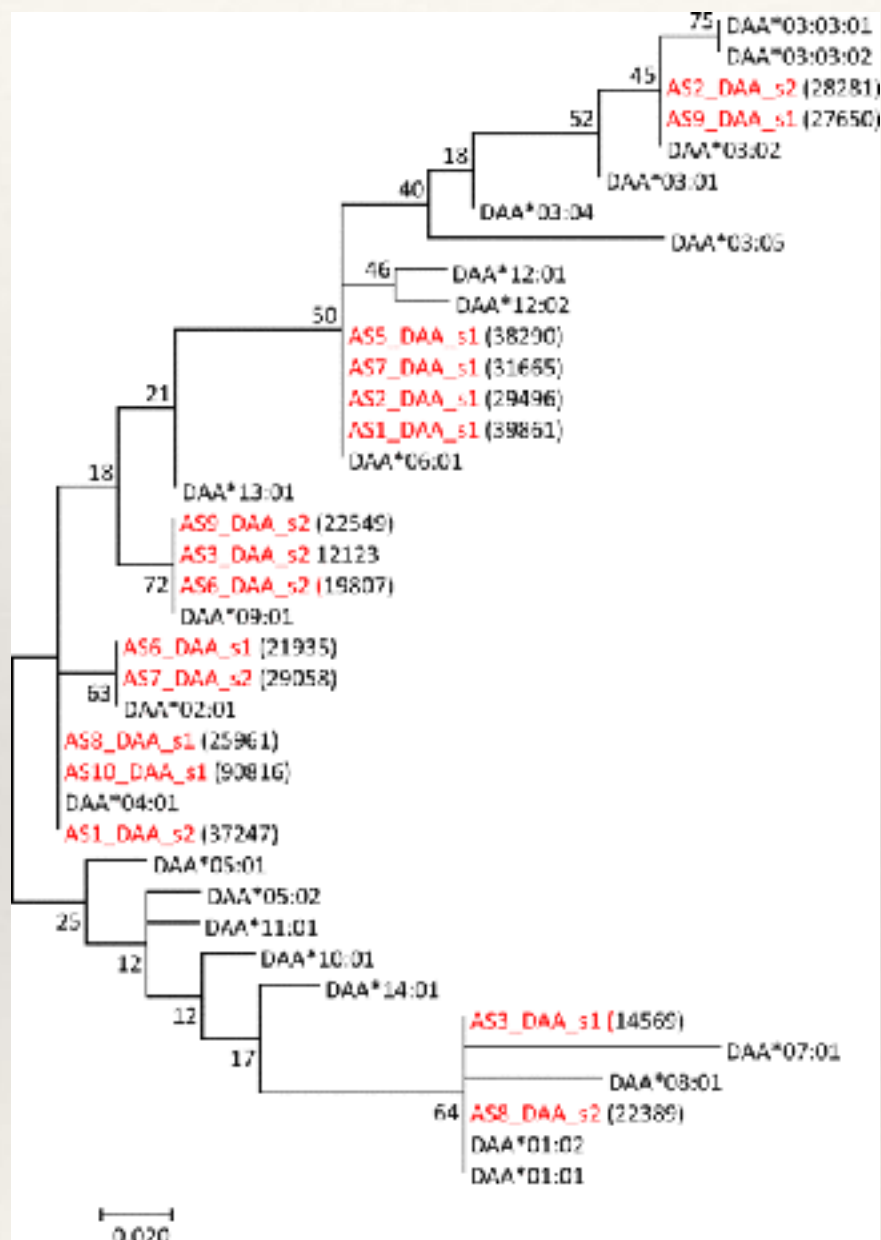
- Sequenced on a MiSeq v3
- 300 paired end

MHC typing in Atlantic salmon



Identified 8 new alleles
 7 Class I UBA
 1 Class II DAB

Onmy-UBA	Sasa-UBA	Sasa-DAB	Sasa-DAA	Onmy-DAB	Onmy-DAA
48	48	42	22	22	3
Class I	Class I	Class II	Class II	Class II	Class II







MHC typing in Atlantic salmon

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Search or jump to...



Pull requests

Issues

Marketplace



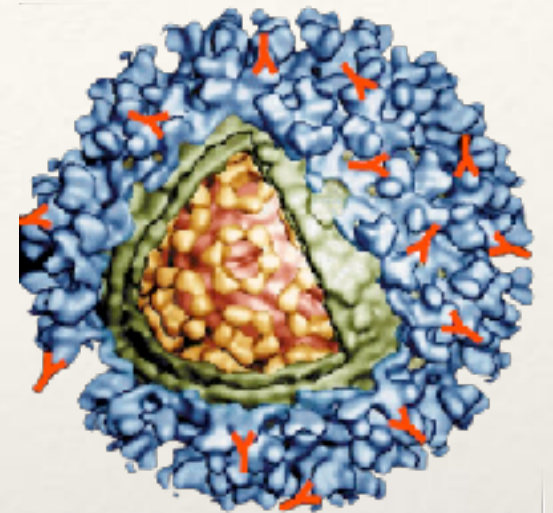
NorwegianVeterinaryInstitute / Salmonid_MHC_classifier

DOI: 10.1007/s00251-019-01143-8

https://github.com/NorwegianVeterinaryInstitute/Salmonid_MHC_classifier

Salmonid Alphavirus (SAV)

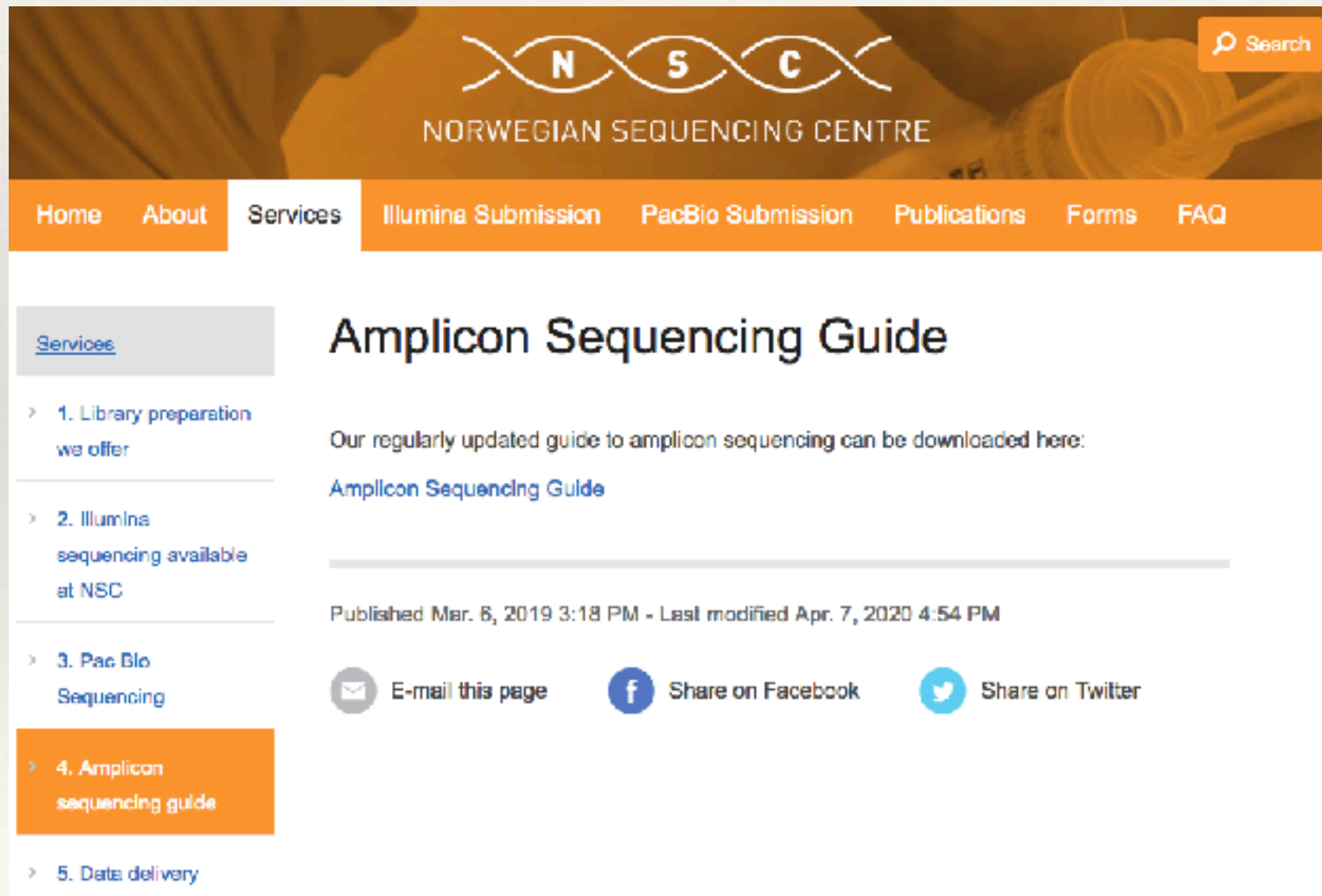
- ❖ Single strand RNA virus ~ 12 kb
- ❖ Affects Salmonids causing pancreas and sleeping disease
- ❖ 6 subtypes (SAV 1-6) of which 2 and 3 are found in Norway
- ❖ SANGER sequencing (~ 1 kb) used to identify the presence of SAV in Atlantic salmon.
- ❖ Replace SANGER with amplicon sequencing for routine diagnosis
- ❖ Plan to amplify shorter amplicons to cover 1 kb region
- ❖ Potentially up to 4 amplicons per fish
- ❖ Also to differentiate SAV 2 and 3



Aderito Luis Monjane
Veterinærinstituttet

Amplicon guide @ NSC

Fadrosh et. al. based 16S rRNA sequencing of V3/ V4 for up to 500+ samples



The screenshot shows the homepage of the Norwegian Sequencing Centre (NSC). The header features the NSC logo (a stylized DNA double helix with 'N', 'S', and 'C' in the loops) and the text 'NORWEGIAN SEQUENCING CENTRE'. A search bar is located in the top right corner. The main navigation menu includes links for Home, About, Services, Illumina Submission, PacBio Submission, Publications, Forms, and FAQ. The 'Services' section is highlighted, and a sidebar on the left lists five services: 1. Library preparation we offer, 2. Illumina sequencing available at NSC, 3. Pac Bio Sequencing, 4. Amplicon sequencing guide (highlighted in orange), and 5. Data delivery. The main content area is titled 'Amplicon Sequencing Guide' and contains the text: 'Our regularly updated guide to amplicon sequencing can be downloaded here: [Amplicon Sequencing Guide](#)'. Below this, it states 'Published Mar. 6, 2019 3:18 PM - Last modified Apr. 7, 2020 4:54 PM'. At the bottom of the main content area, there are three social sharing options: 'E-mail this page' (with an envelope icon), 'Share on Facebook' (with a Facebook 'f' icon), and 'Share on Twitter' (with a Twitter bird icon).

Gregor/Teodora

They have a list of companies (other than NSC) who can help you with primer design, library preparation and sequencing.

Targeted sequencing

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Targeted sequencing ~450-550 bp

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													Index 1										Heterogeneity Spacer								16S rRNA gene sequence									

500 + samples

3500 + samples



Microbiome

METHODOLOGY Open Access

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Microbiome 2014, 2(1):1-11
DOI: 10.1186/1940-1540-2-1

Microbiome

METHODOLOGY Open Access

A novel ultra high-throughput 16S rRNA gene amplicon sequencing library preparation method for the Illumina HiSeq platform

Eric J. de Mulder^{1*}, P.W. Ticevik^{1*}, Gregor D. Gillman², Johannes H. Iov³ and Arvind Y. N. Sundaram^{2*}