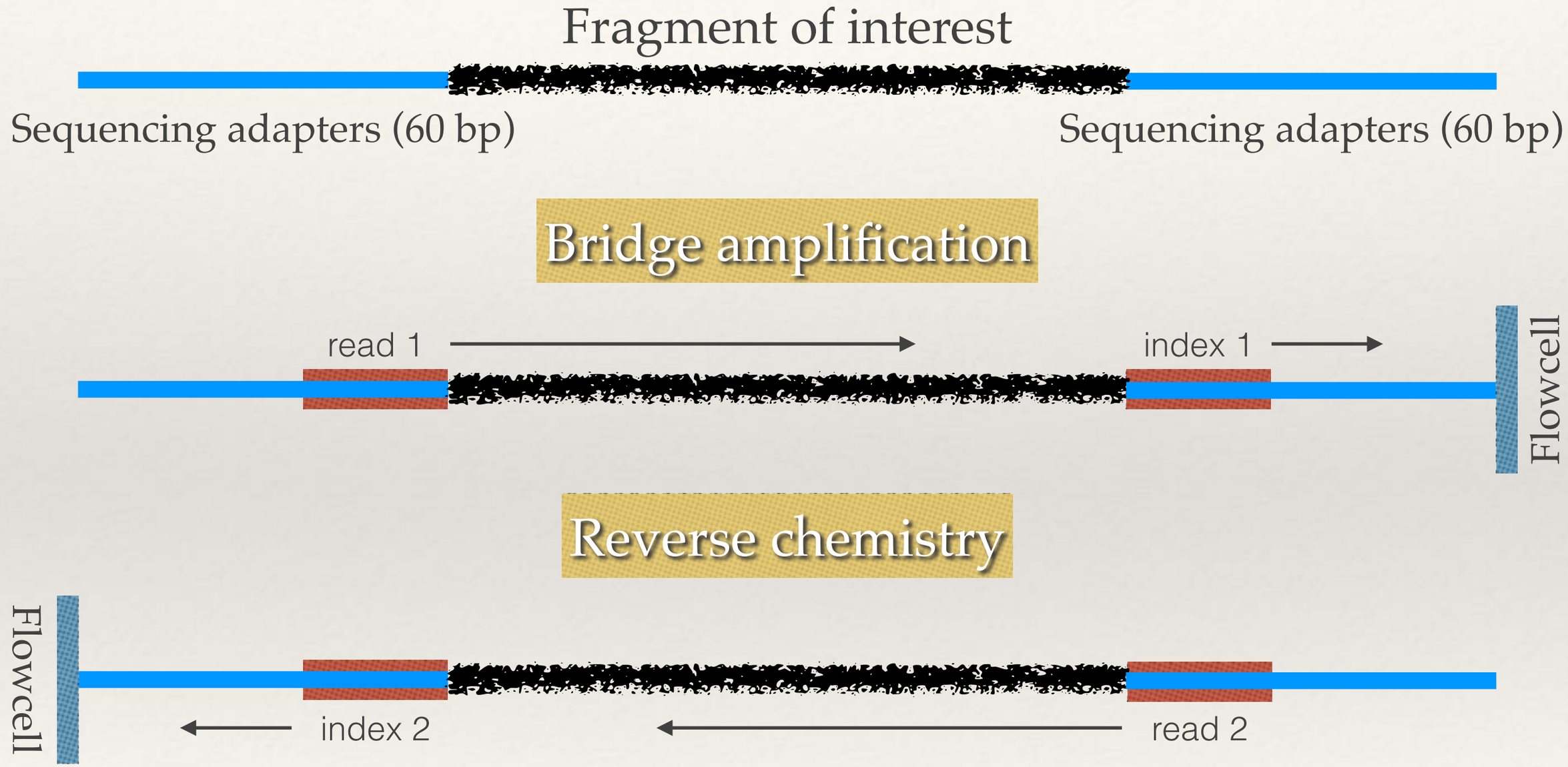
OUS AMG Monday seminar

Targeted Amplicon Sequencing

Arvind Sundaram

Aug 31 2020

Illumina sequencing



Library preparation

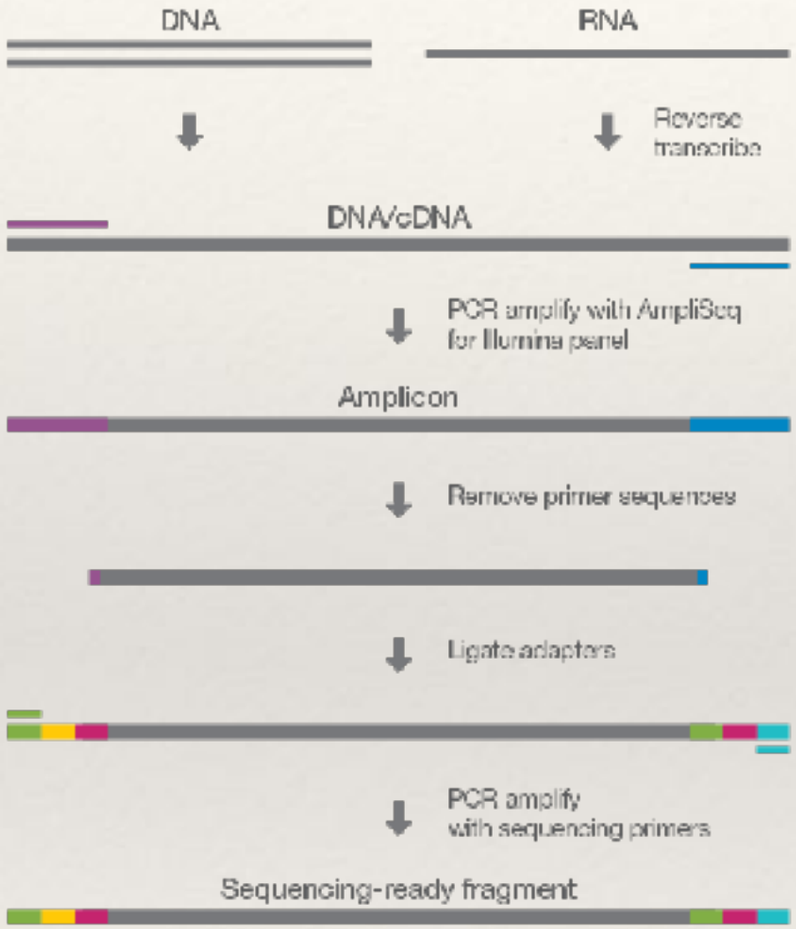
Targeted sequencing ~ 300 - 550 bp

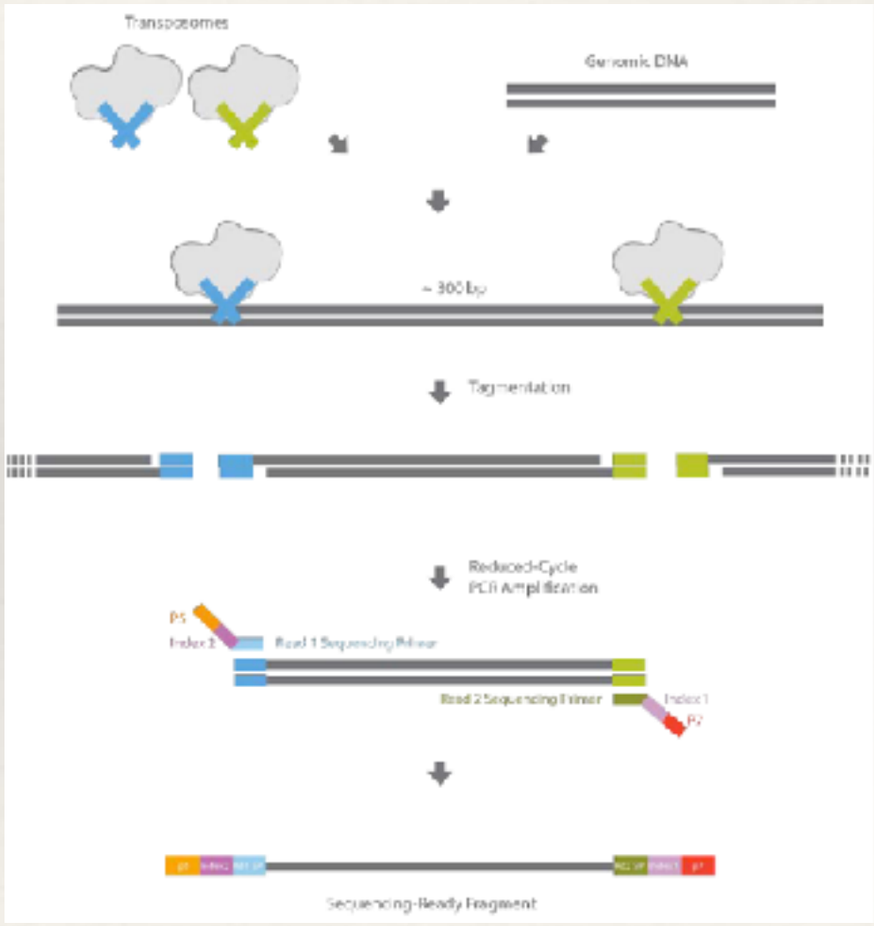
Ampliseq (Illumina): PCR based amplification + adapter ligation

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

Source: Illumina

Library preparation

Fragment of interest

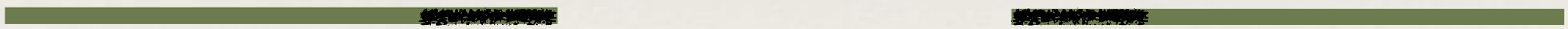
Sequencing adapters (60 bp) Sequencing adapters (60 bp)

Targeted sequencing ~450-550 bp

Ampliseq (Illumina): PCR based amplification + adapter ligation

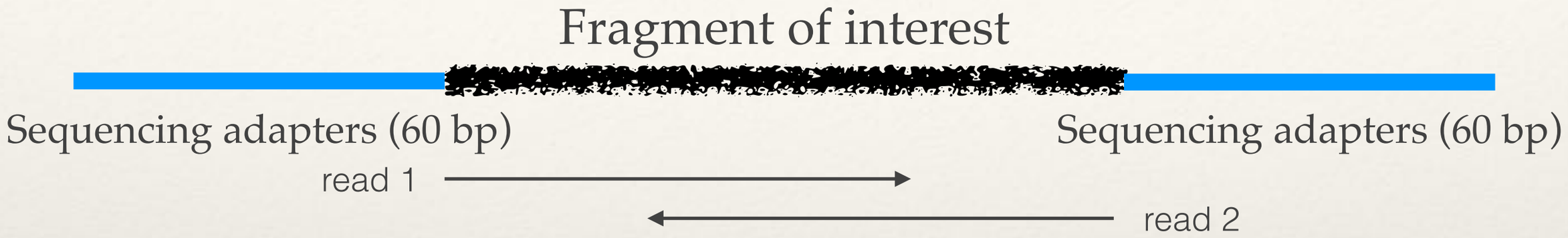
Nextera: Transposomes to target + PCR + adapter ligation

Normal library preparation on amplified product (treated as DNA)

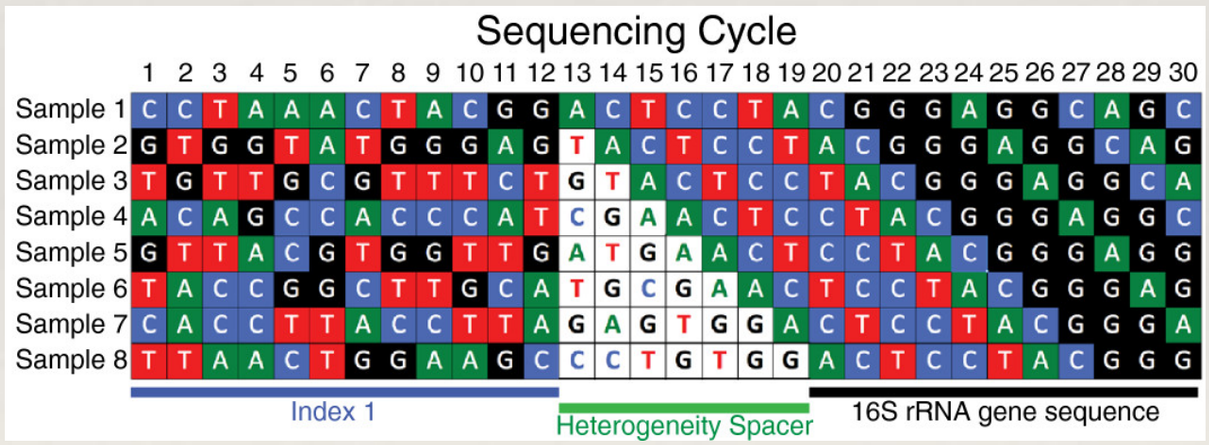


Design primers that includes sequencing adapters and amplifies the target.
Might be 80+ nt long primers

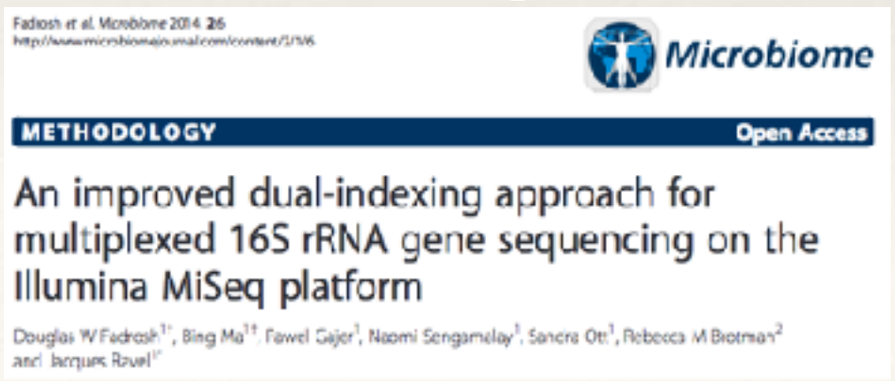
Amplicon sequencing issues



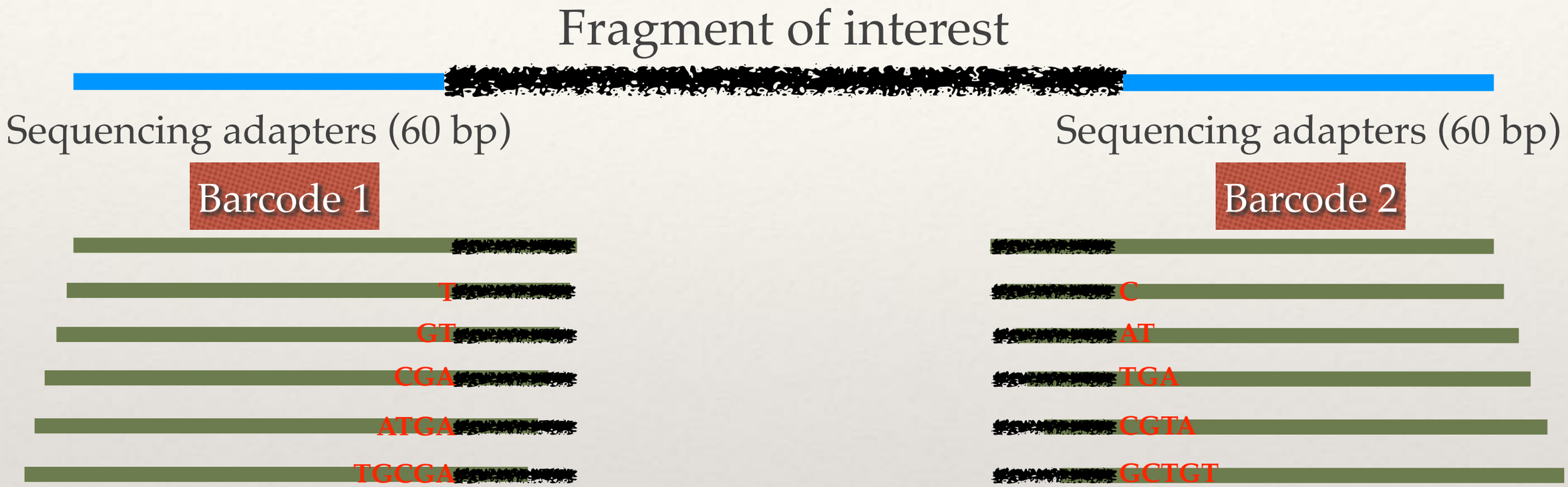
Low complexity problem: solution - Hetergeneity spacers!!!



500 + samples

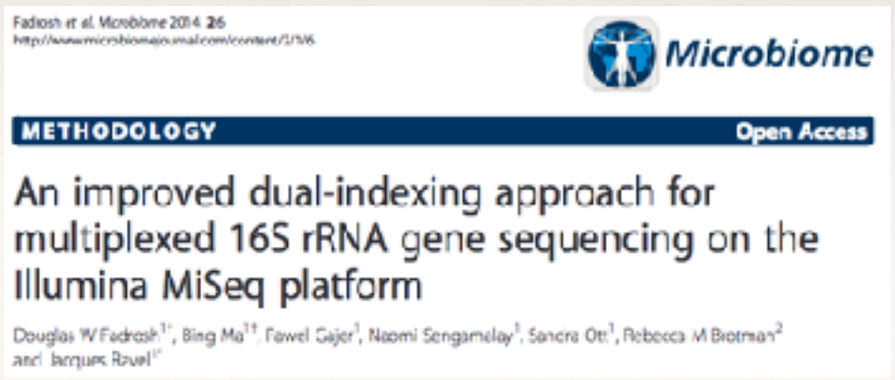


Fadrosh et. al. 2014



Design primers that includes sequencing adapters and amplifies the target.
Now it includes **Heterogeneity spacers**

500 + samples


Fadrosh 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 Fadrosh[1*], Bing Ma[1†], Pawel Gajer[1], Naomi Sengamalay[1], Sandra Ott[1], Rebecca M Brotman[2] and Jacques Ravel[1*]

24 samples : 7 primers (3 Fwd / 4 Rev)
48 samples: 14 primers (6 Fwd/8 Rev)
576 samples: 48 primers (24 Fwd/24 Rev)

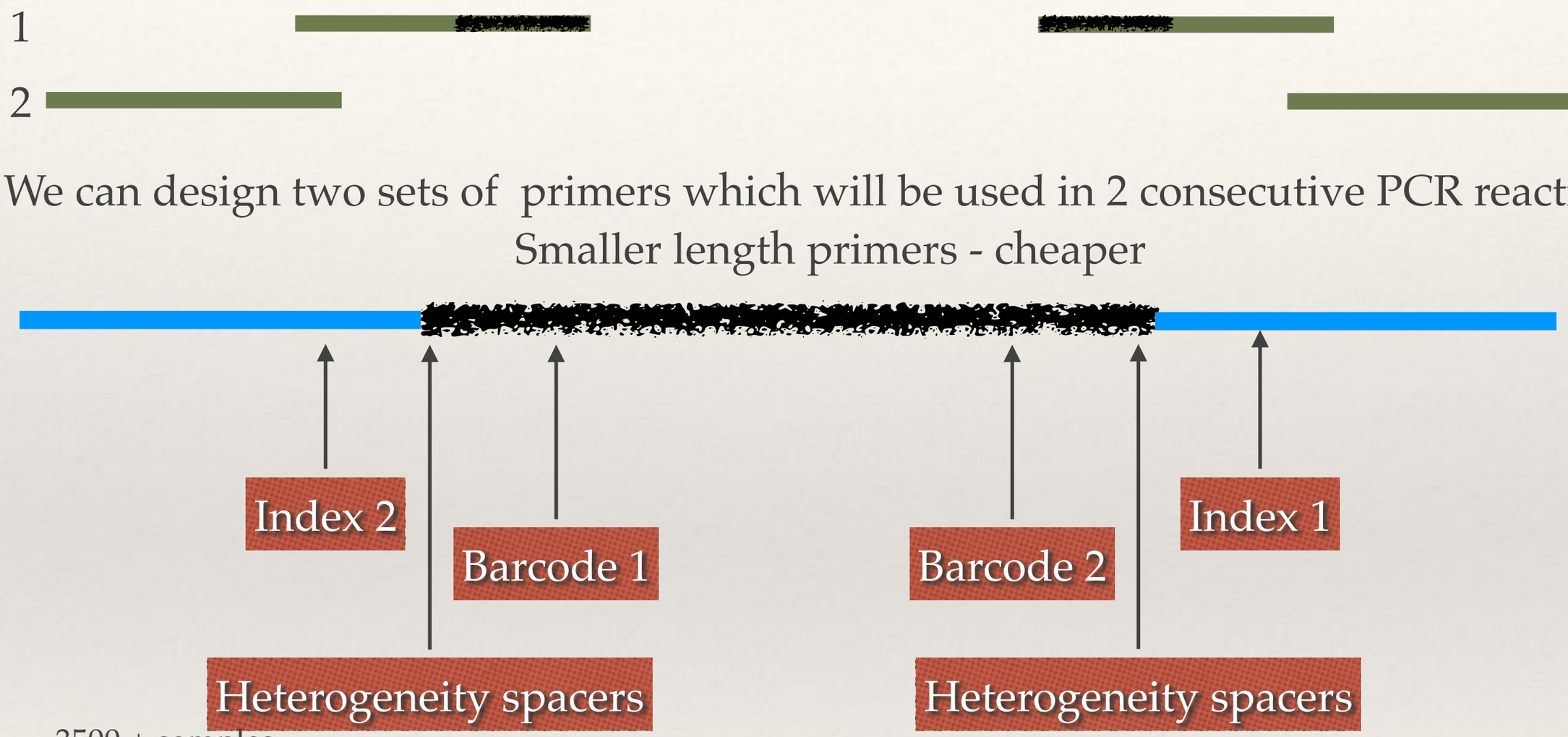
Offered at NSC for 16S V3/V4 region

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

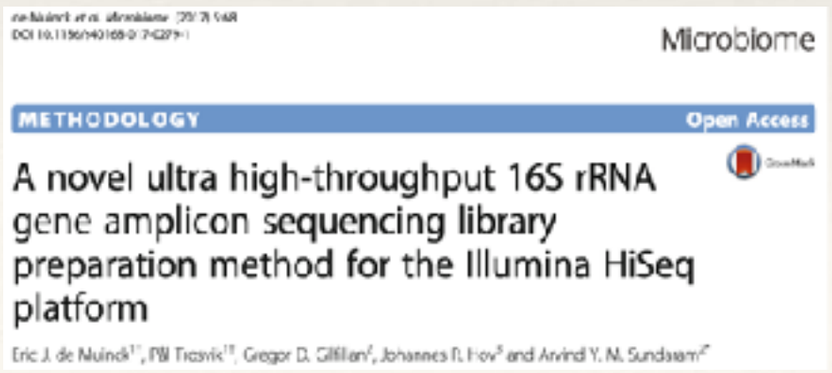
PCR 1

PCR 2

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



3500 + samples


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 Muinck[1*], Pål Trosvik[1†], Gregor D. Gilfillan[2], Johannes R. Hov[3] and Arvind Y. M. Sundaram[4*]

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 Garseth [1] · Giuseppe Maccari [3,4] · Unni Grimholt [1]

DOI: 10.1007/s00251-019-01143-8

MHC typing in Atlantic salmon

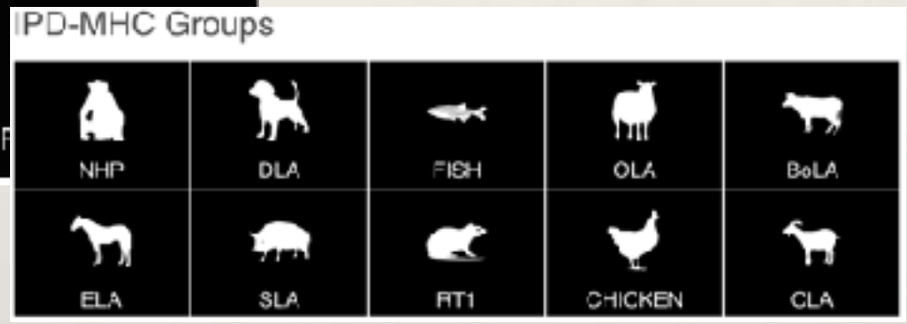
Immuno Polymorphism Database

ANTHONY NOLAN

EMBL-EBI

IPD - MHC

Overview | IMGT/HLA | KIR | MHC | NHKIR | HI



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

https://www.ebi.ac.uk/ipd/mhc/

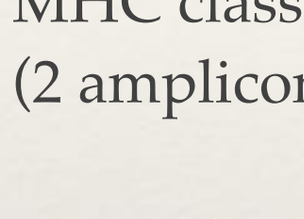
MHC typing in Atlantic salmon

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

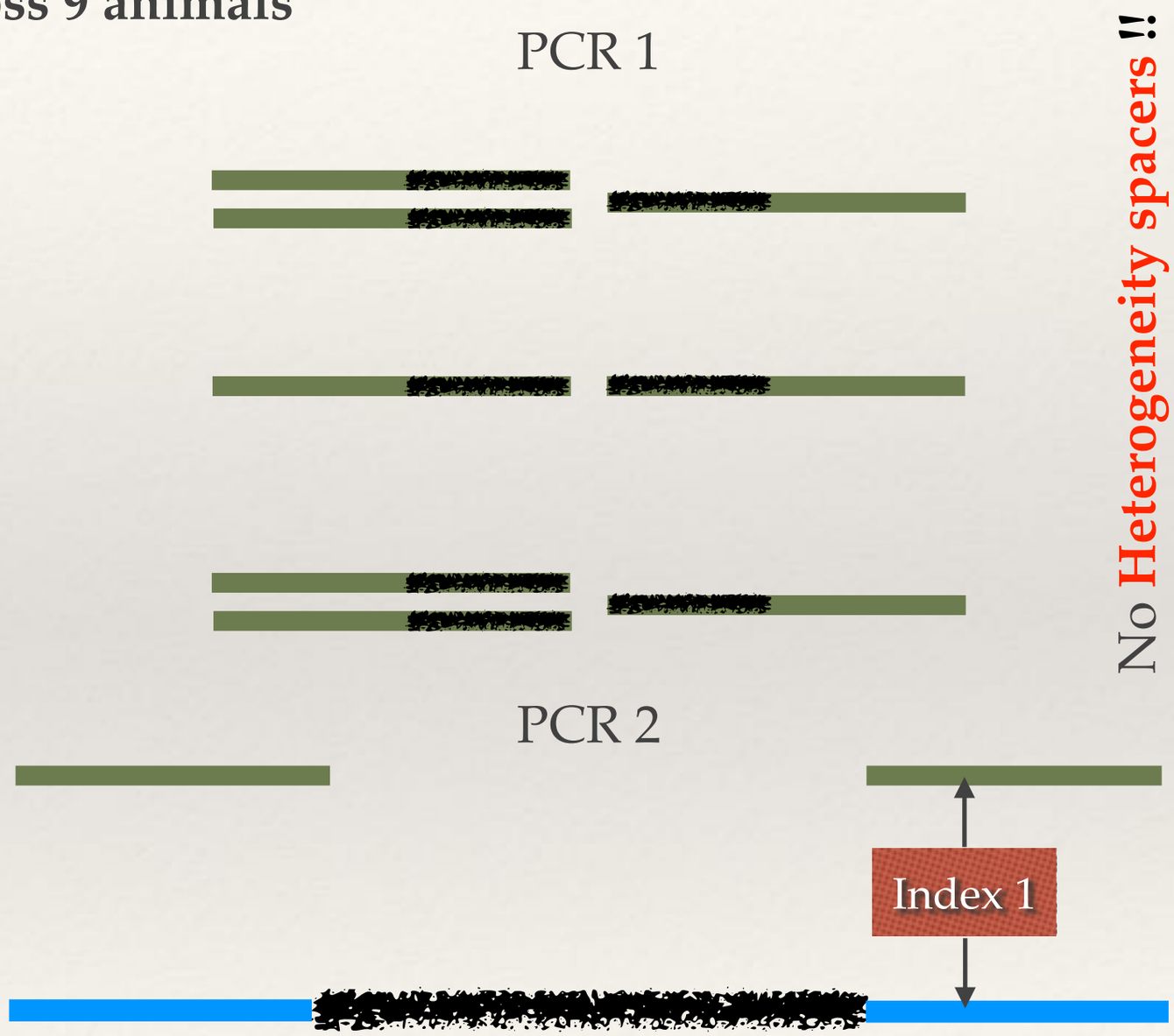
MHC class I UBA
(2 amplicons, 2 Fwd and 1 shared Rev)

MHC class II DAA
(1 amplicon, 1 Fwd and 1 Rev)

MHC class II DAB
(2 amplicon, 2 Fwd and 1 shared Rev)



- Sequenced on a MiSeq v3
- 300 paired end



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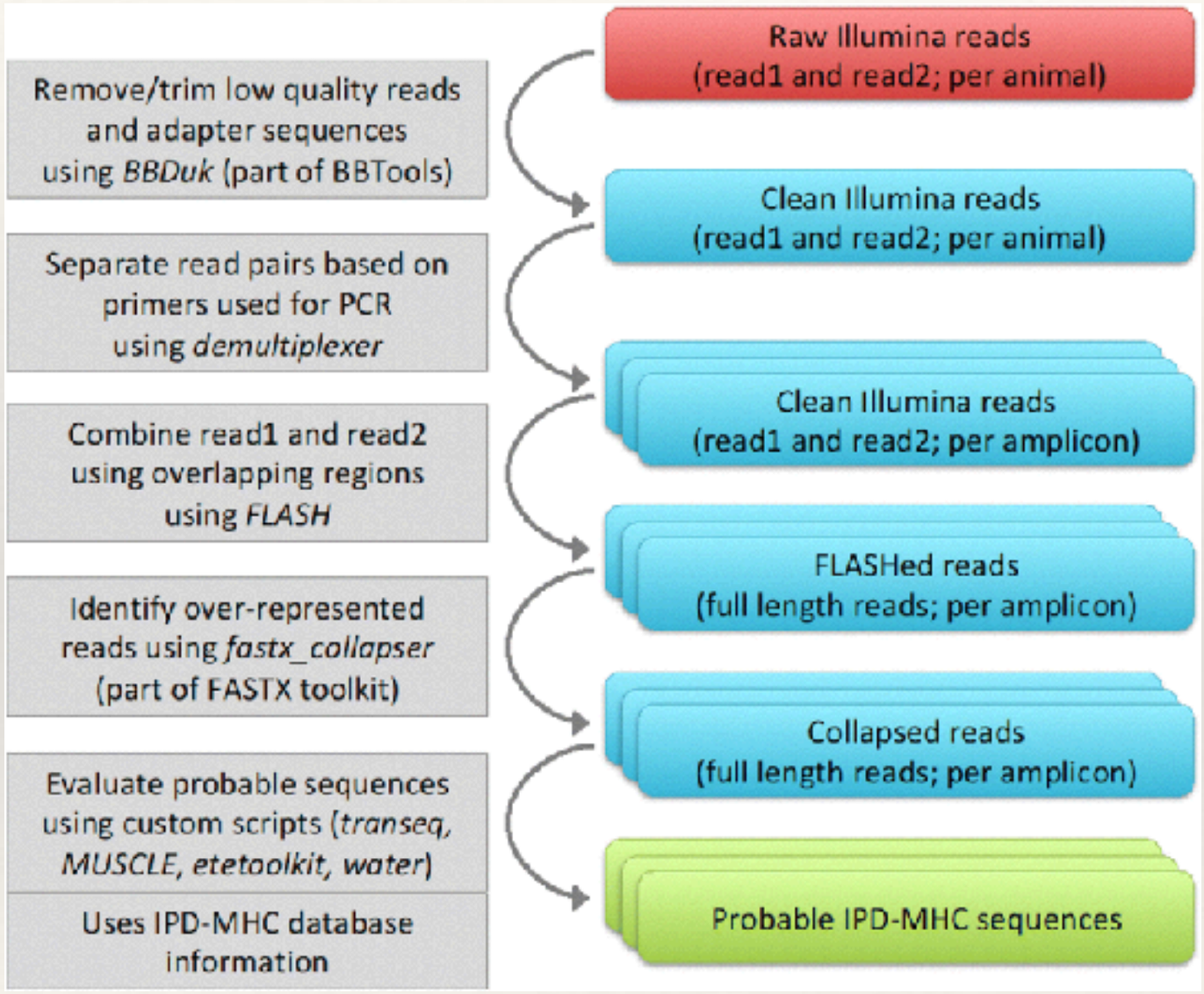

Identified 8 new alleles
7 Class I UBA
1 Class II DAB

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

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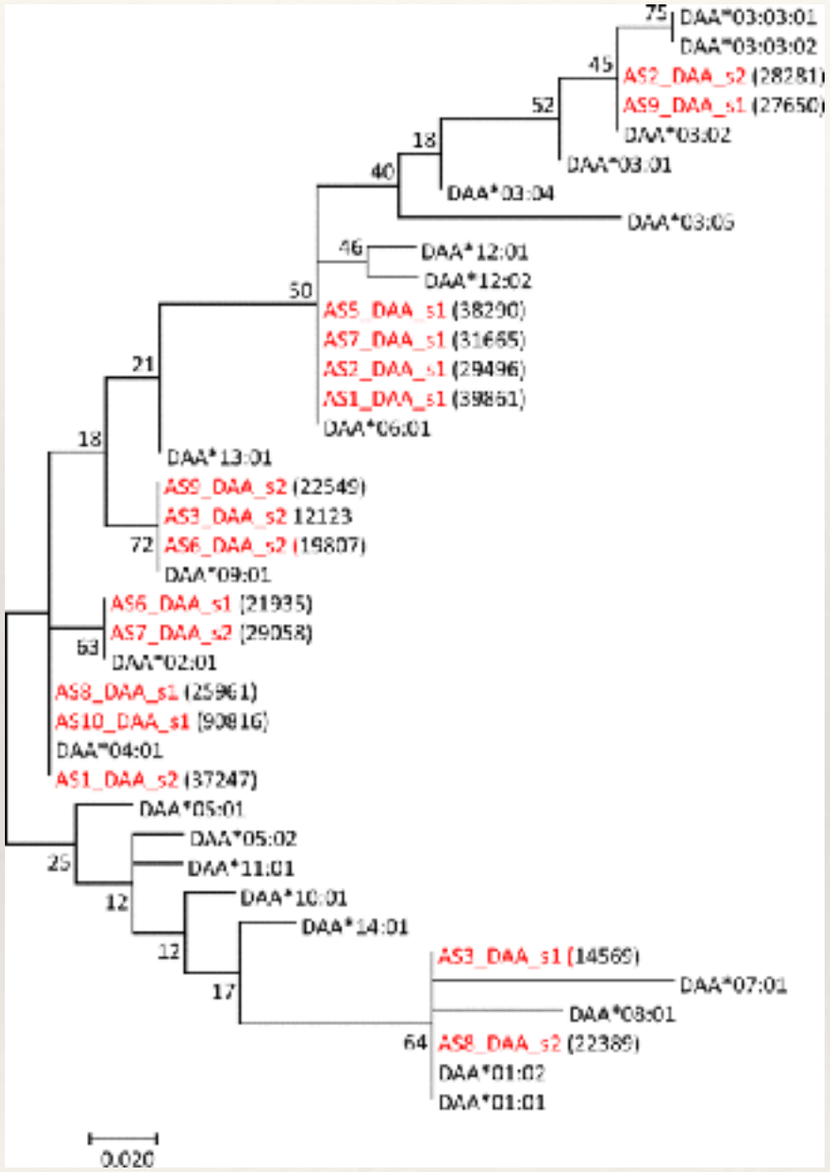

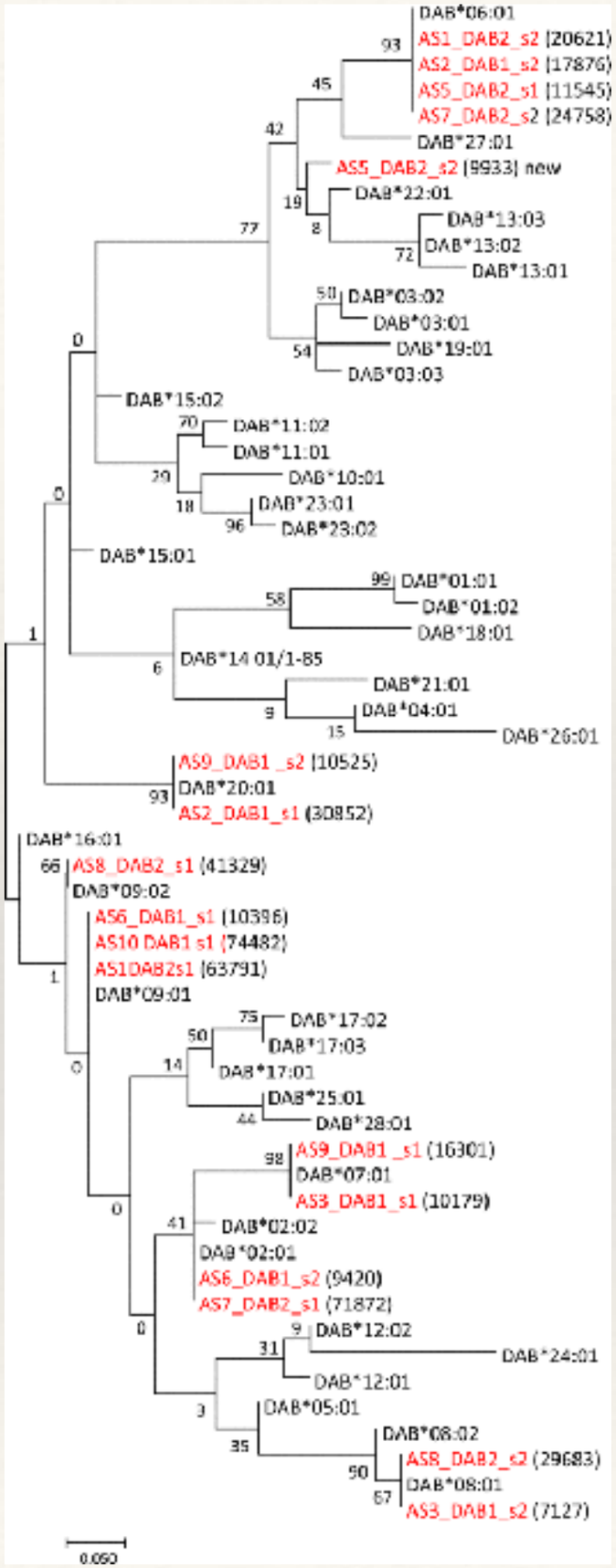

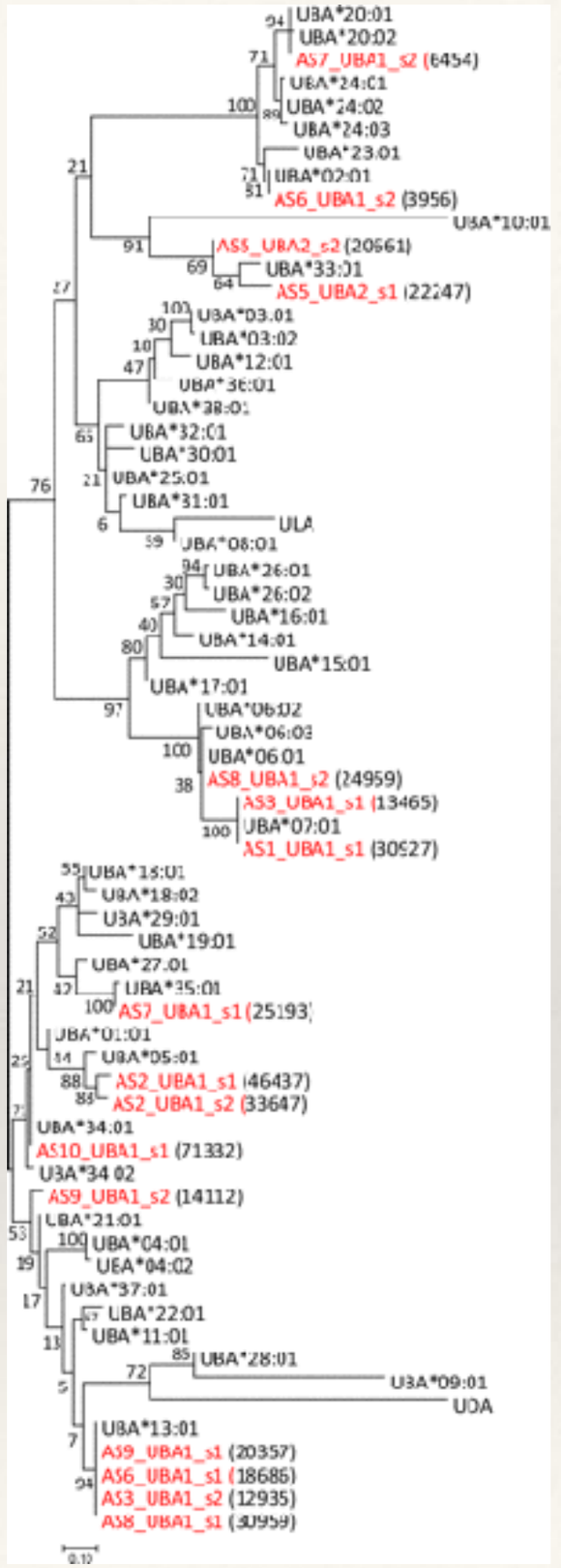

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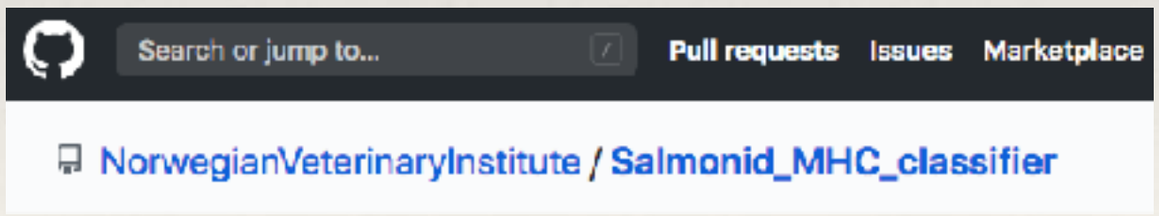

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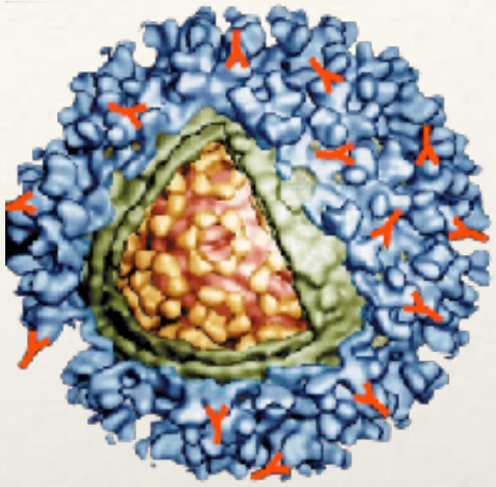
https://github.com/NorwegianVeterinaryInstitute/Salmonid_MHC_classifier

Salmonid Alphavirus (SAV)



- Single strange 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.

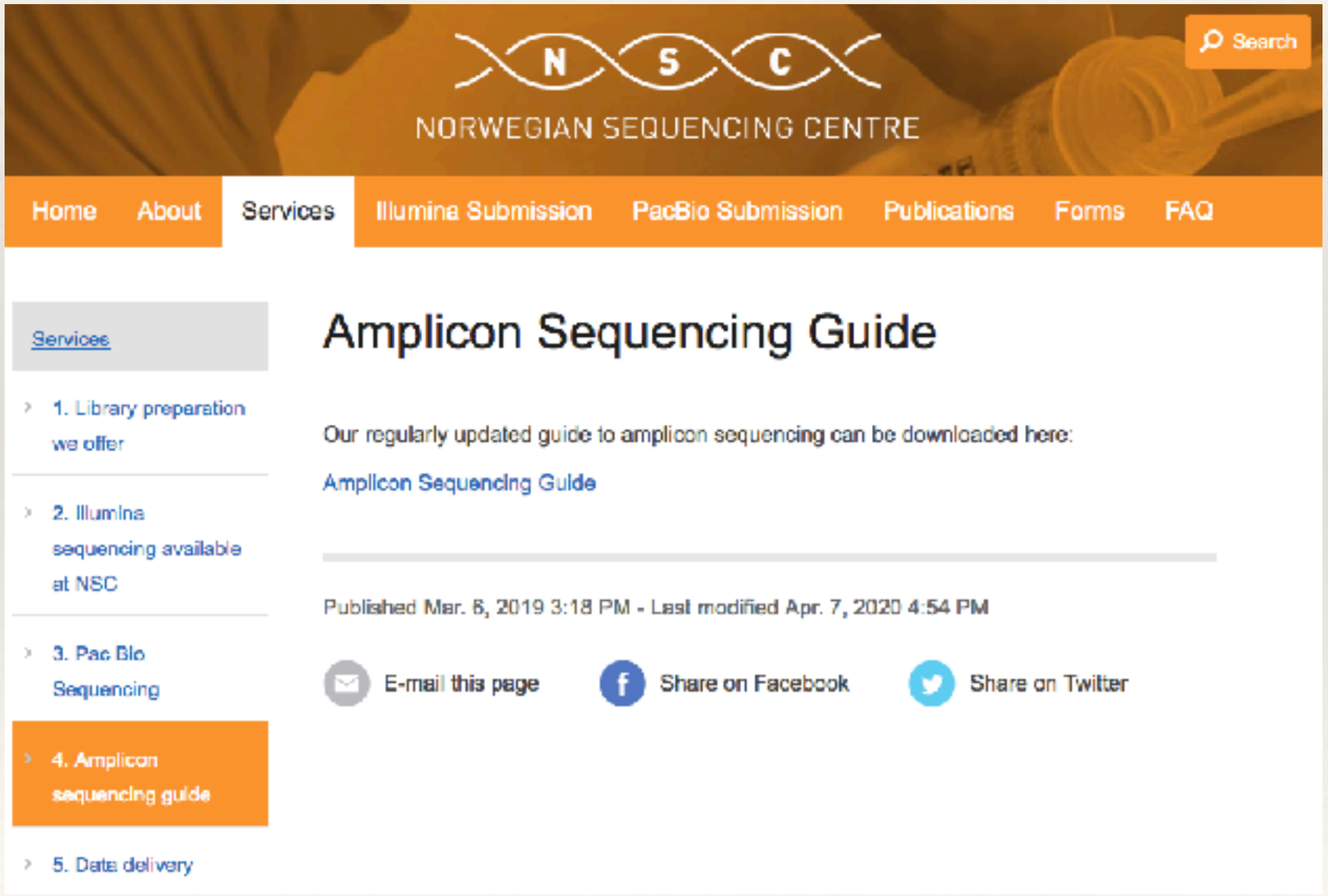
- Replace SANGER with amplicon sequencing for routine diagnosis
- Plan to amplify shorter amplicons to cover 1 kb region
- Potentially up to 4 amplicons per fish
- Also to differentiate SAV 2 and 3

Aderito Luis Monjane
Veterinærinstituttet

https://ilaks.no/pd-vaksine-med-ufortjent-kritikk/

Amplicon guide @ NSC

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



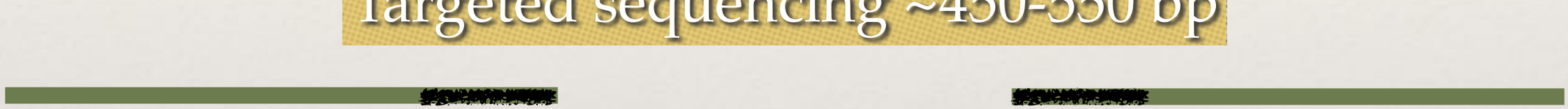
Gregor/Teodora

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

https://www.sequencing.uio.no/

Targeted sequencing

Targeted sequencing ~450-550 bp



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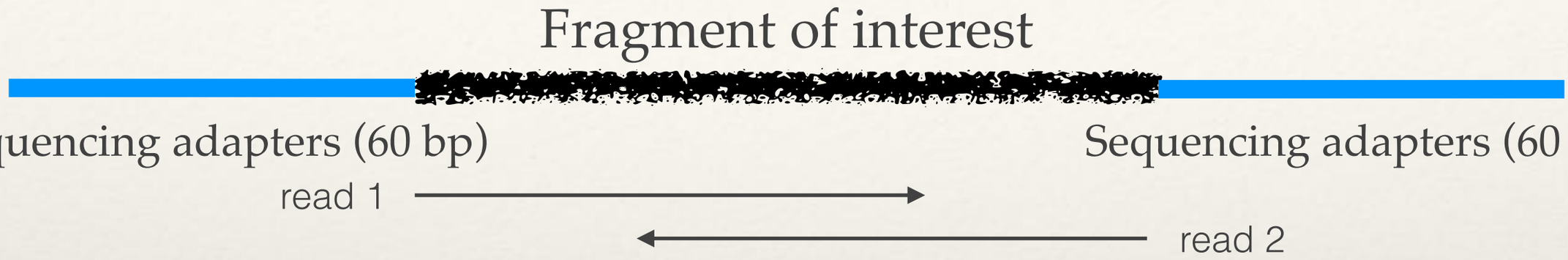

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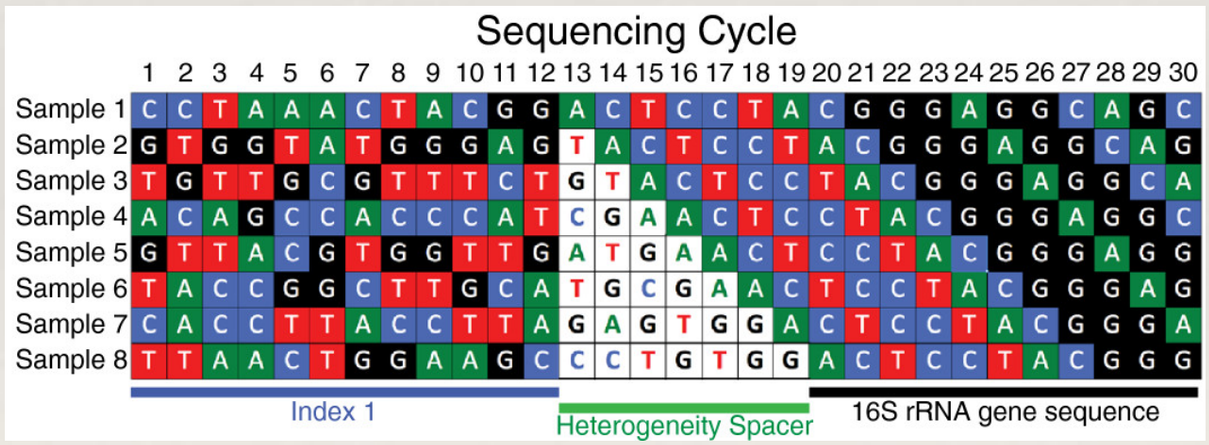
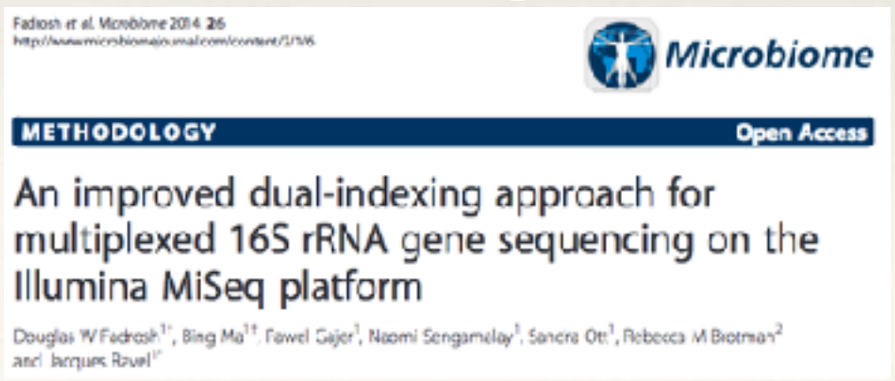


500 + samples

3500 + samples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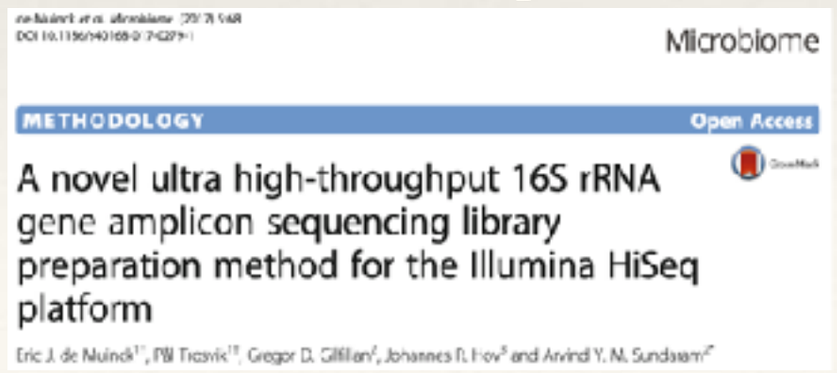
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