Cutting-edge solutions for disease diagnostics caused by pathogens and the human microbiome

Matthieu Pesant, Ph.D.
NGS Product Manager / Scientific Support Specialist Takara Bio Europe

Agenda

- Introduction to pathogens and microbiome in human diseases
- Main methodologies for pathogen/microbiome analysis
- Application examples
  - Taxonomic studies by 16S rRNA
  - Metagenomics by shotgun sequencing
  - High throughput antibiotic resistance genes by qPCR
  - Host/pathogen interaction analysis by single-cell RNA-seq
  - Viral genome sequencing for public health management

Introduction to pathogens and microbiome in human disease

Some definitions

- **Microbes** : microorganisms (either unicellular or multicellar) like bacteria, virus, fungi…
- **Microbiota** : all the microbes living a specific environment (soil, water, human body…)
- **Microbiome** : collection of all the genomes of the microorganisms of a specific environment
- **Metagenomics** : study of all microorganism genomes present in a sample

Introduction to pathogens and microbiome in human disease

- Pathogens (bacteria and virus) cause infectious diseases
- Human microbiome is linked to a wide array of diseases among which
  - Cancers
  - Diabetes
  - Obesity
  - Inflammatory bowel disease
- Growing interest in understanding microbiome dysregulation and impact on disease

nature medicine

FOCUS | REVIEW ARTICLE

https://doi.org/10.1038/s41591-019-0377-7

The microbiome, cancer, and cancer therapy

Beth A. Helmink[1], M. A. Wadud Khan[1], Amanda Hermann[1], Vancheswaran Gopalakrishnan[1,3] and Jennifer A. Wargo[1,2,3]*

Current Diabetes Reports (2018) 18: 55

https://doi.org/10.1007/s11892-018-1020-6

GENETICS (AP MORRIS, SECTION EDITOR)

CrossMark

The Gut Microbiome as a Target for the Treatment of Type 2 Diabetes

Ömrüm Aydin[1,2] · Max Nieuwdorp[2,3,4] · Victor Gerdes[1,2]

frontiers in Microbiology

REVIEW

published: 25 September 2018

doi: 10.3389/fmicb.2018.02247

The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease

*Tao Zuo[1,2] and Siew C. Ng[1,2]**

frontiers in Physiology

MINI REVIEW

published: 08 January 2018

doi: 10.3389/fphys.2017.01122

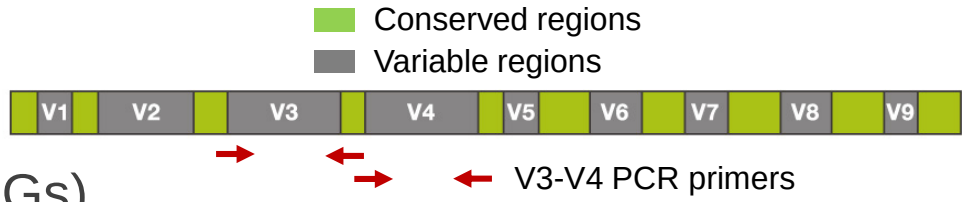
Commensal Homeostasis of Gut Microbiota-Host for the Impact of Obesity

Pengyi Zhang[1,2†], Xiangjing Meng[3†], Dongmei Li[2], Richard Calderone[2], Dewei Mao[1] and Bo Sui[1]*

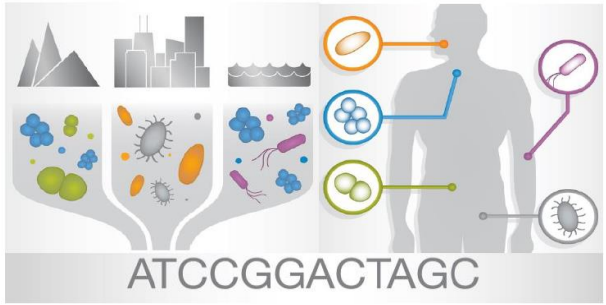
Main methodologies for pathogen/microbiome analysis

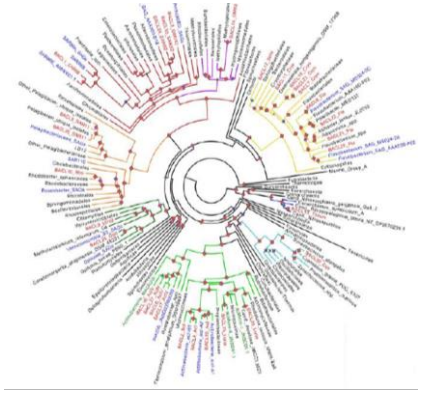
Main research methods for pathogens and microbiome analysis

- **PCR / qPCR**
  - 16S rRNA gene amplification
  - Antibiotic resistance genes (ARGs)



- **NGS**
  - 16S rRNA sequencing
  - Metagenomics by whole genome sequencing (shotgun sequencing)
  - metatranscriptomics





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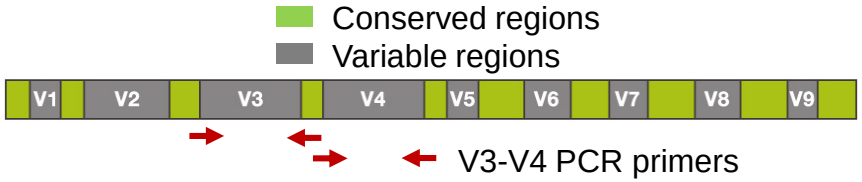
Application examples

- Taxonomic studies by 16S rRNA
- Metagenomics by shotgun sequencing
- High throughput antibiotic resistance genes by qPCR
- Host/pathogen interaction analysis by single-cell RNA-seq
- Viral genome sequencing for public health management

Taxonomic analysis:16S rRNA amplification and sequencing



SCIENTIFIC REPORTS

OPEN

The biodiversity Composition of Microbiome in Ovarian Carcinoma Patients

Received: 11 April 2017
Accepted: 3 September 2018
Published online: 08 February 2019

Bo Zhou, Chaoyang Sun, Jia Huang, Meng Xia, Ensong Guo, Na Li, Hao Lu, Wanying Shan, Yifan Wu, Yuan Li, Xiaoyan Xu, Danhui Weng, Li Meng, Junbo Hu, Qinglei Gao, Ding Ma & Gang Chen

Extraction, amplification of bacterial genomic DNA and 16S rRNA

PCR was performed with 10 ng template, 0.3 μl **ExTaq Polymerase (TaKaRa Bio**, Japan), 2 μl 10 × Extaq Buffer, 0.8 μl Forward Frimer (5 μM), 0.8 μl Reverse Primer (5 μM)

Taxonomic analysis:16S rRNA amplification and sequencing

- **Ex Taq DNA Polymerase** is a blend of enzymes: **TaKaRa Taq polymerase** and **proof reader**.
- Ex Taq DNA Polymerase combines the proven performance of Takara Taq polymerase with the proofreading activity, for high-sensitivity, high-efficiency PCR reactions.
- Features
  - **5'-3' exonuclease activity**: can be used for qPCR
  - **3'-5' exonuclease activity (proofreading)** : higher fidelity
  - Longer amplification than regular Taq (20kb gDNA, 30 kb plasmid)
  - Dedicated mainly to PCR with **difficult templates & high yield**
  - Generates T/A overhangs

- **To date > 6100 published papers using Ex Tag for 16S rRNA amplification** in various research fields including human disease

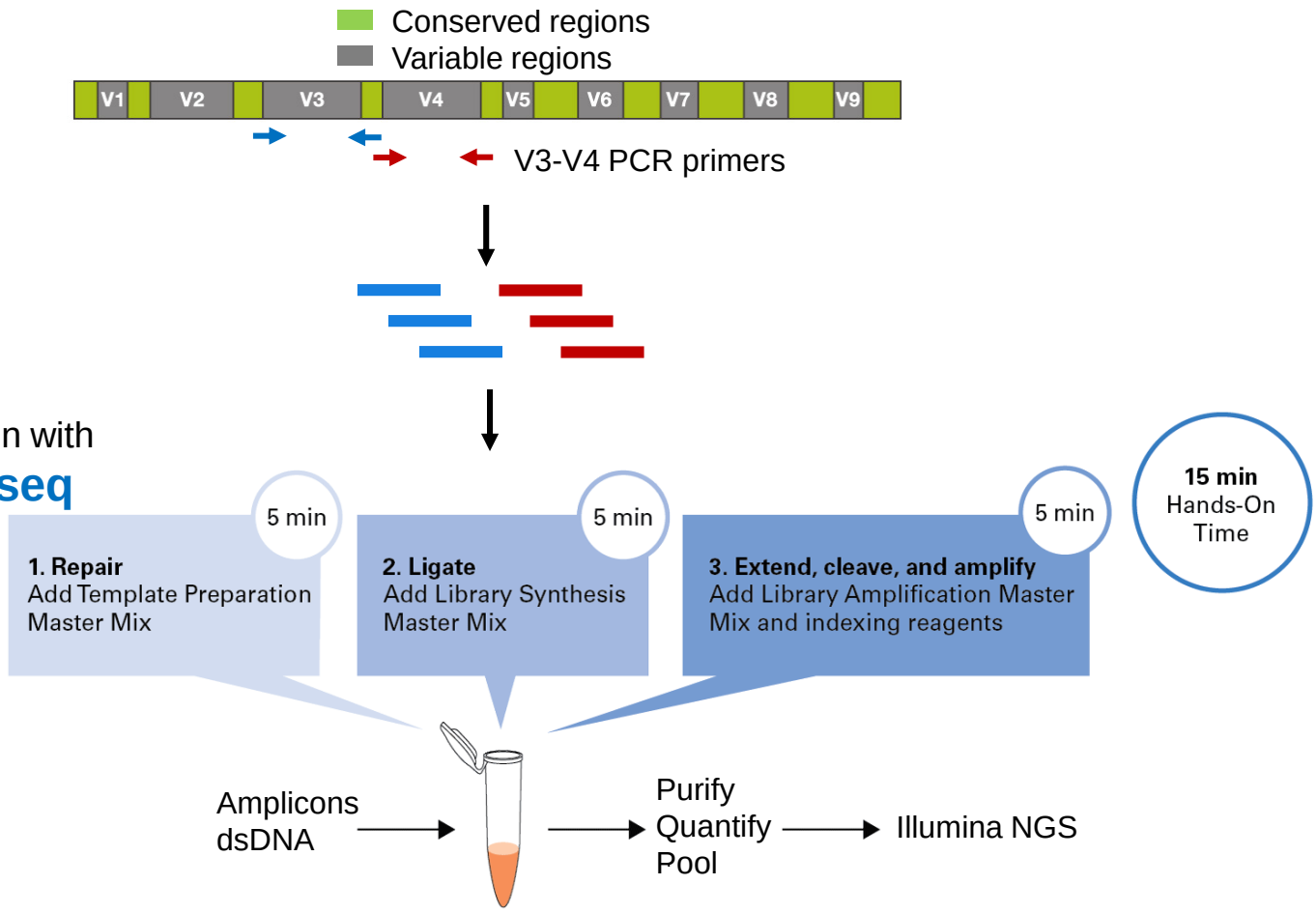
Taxonomic analysis:16S rRNA amplification and sequencing

16S rRNA sequencing workflow

Targeted 16S rRNA PCR **Ex Taq DNA Polymerase**

Targeted 16S rRNA amplicons

NGS library preparation with **ThruPLEX DNA-seq**



Application examples

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Metagenomics by shotgun sequencing

ThruPLEX DNA-seq workflow: 1 tube, 2 hours, 3 steps
For shotgun metagenomics

npj | Biofilms and Microbiomes

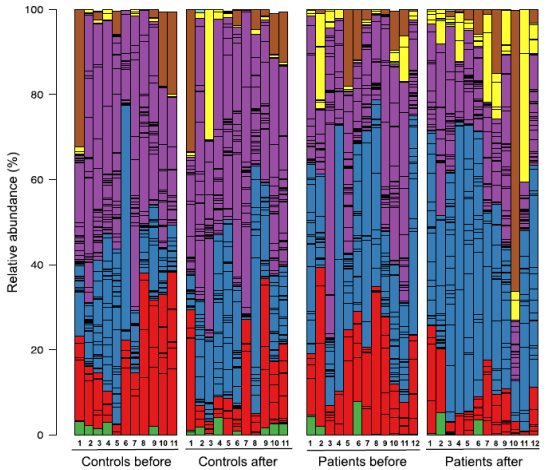
www.nature.com/npjbiofilms

ARTICLE OPEN

The ketogenic diet influences taxonomic and functional composition of the gut microbiota in children with severe epilepsy

Marie Lindefeldt[1], Alexander Eng[2], Hamid Darban[3], Annelie Bjerkner[4], Cecilia K Zetterström[5], Tobias Allander[4], Björn Andersson[3], Elhanan Borenstein[2,6,7,8,9], Maria Dahlin[1] and Stefanie Prast-Nielsen[10]

Microbial diversity analysis of gut microbiota by shotgun sequencing

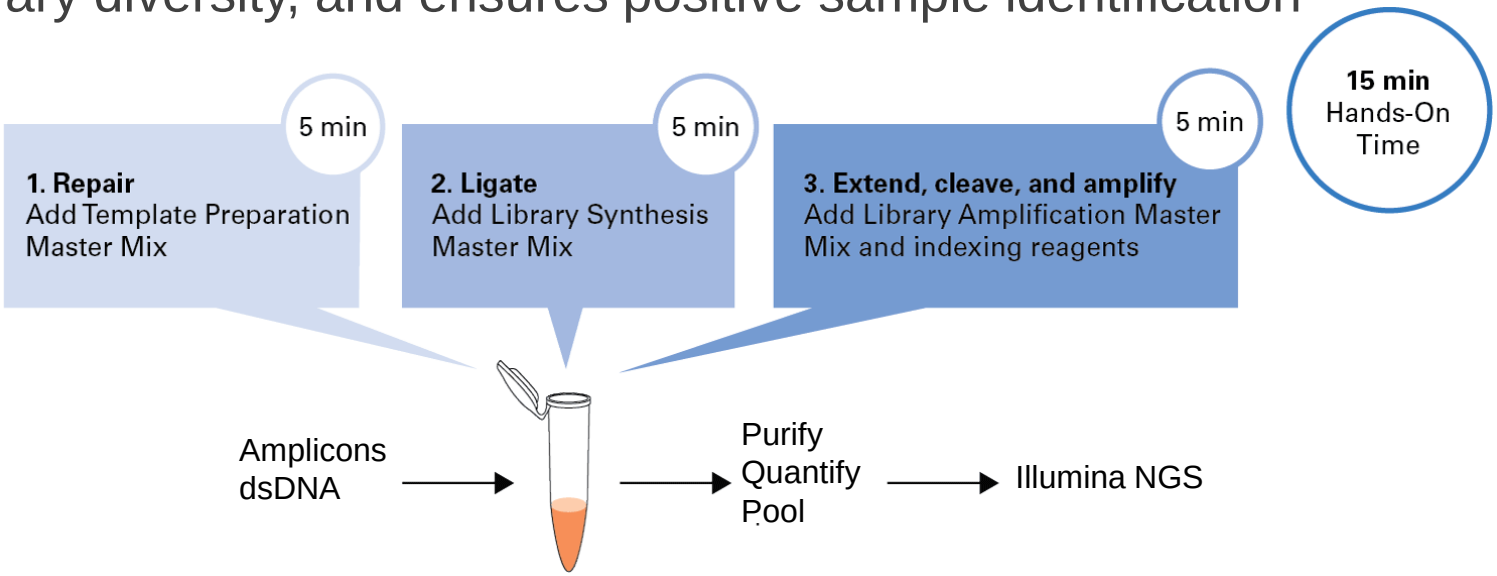


DNA library preparation and sequencing. DNA was quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). 250 ng DNA was sheared in the Covaris® S2 instrument (Covaris, Inc.) to an insert size of approximately 650 bp. Fifty nanograms of sheared DNA was used for preparation of sequencing libraries with the **ThruPLEX® DNA-seq Kit**

Taxonomic analysis:16S rRNA amplification and sequencing

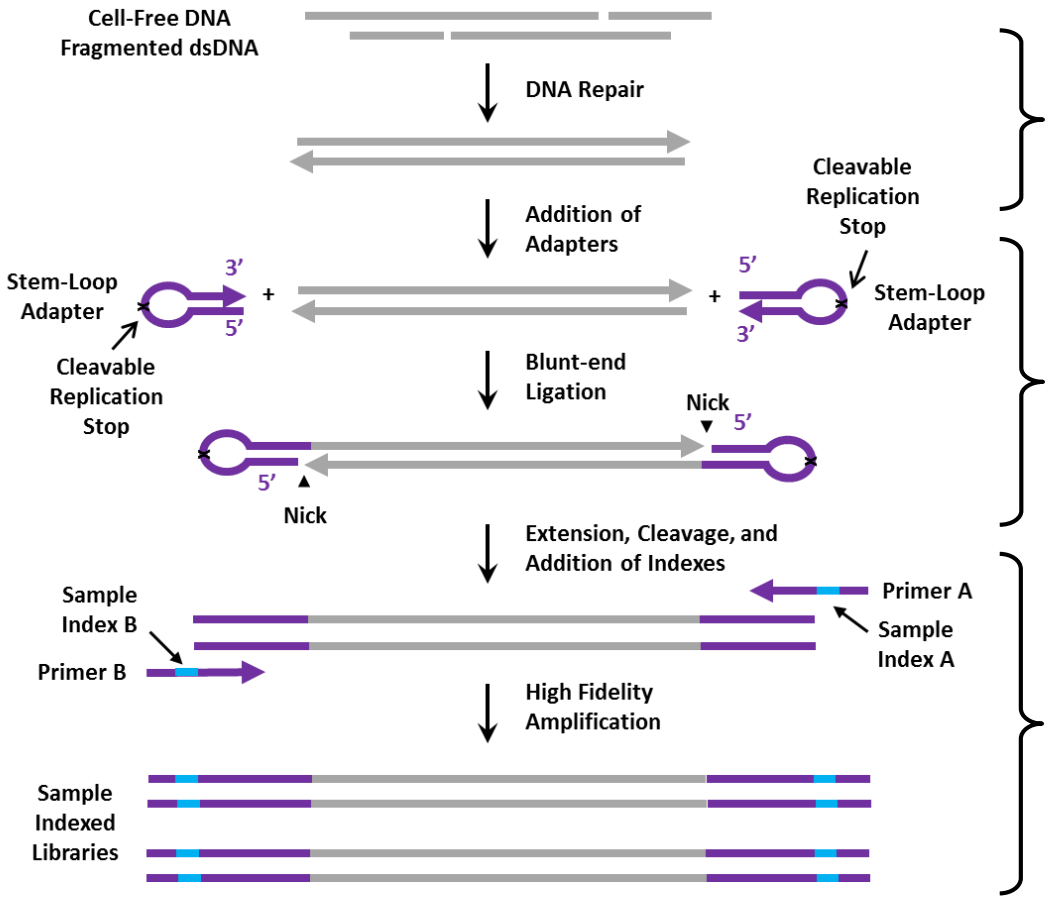
ThruPLEX DNA-seq workflow: 1 tube, 2 hours, 3 steps

- **Fast—**15 minutes of hands-on time
- **Simple—**3 easy steps in the same PCR tube or well; no intermediate sample purification or sample transfer steps
- **Benefits—**reduces sample loss and risk of contamination, preserves library diversity, and ensures positive sample identification



Taxonomic analysis:16S rRNA amplification and sequencing

ThruPLEX DNA-Seq: 1 tube, 2 hours, 3 steps



Step 1: Repair

Fragmented double-stranded DNA is repaired in a highly efficient process.

Step 2: Ligate

Background is reduced using double-stranded adaptors with no single-stranded tails. Blunt end ligation occurs with high-efficiency. Blocked 5'ends reduce adaptor-adaptor ligation.

Step 3: Extend, Cleave, and Amplify

Background is further reduced by destroying unused adaptors after ligation.

Application examples

- Taxonomic studies by 16S rRNA
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High throughput antibiotic resistance genes by qPCR

- Antibiotics widely used: reduction in human morbidity and mortality
- One emerging problem with antibiotics is the development of antibiotic-resistant bacteria
- Bacteria can acquire antibiotic resistance genes (ARGs) over time due to selective pressure
- antibiotic use in agriculture can also contribute to bacteria developing ARGs

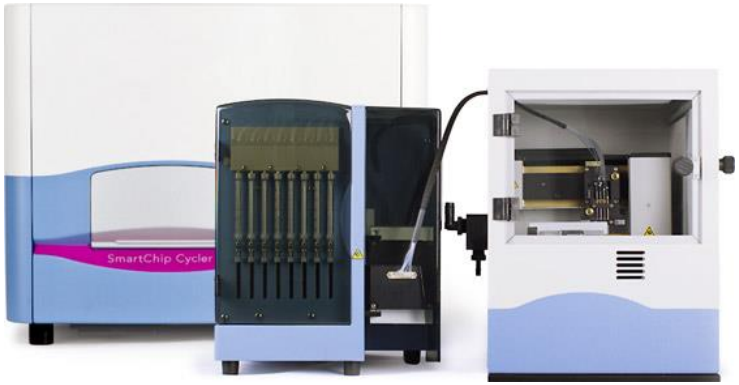
-> to transmit antibiotic-resistant strains

- currently nearly 400 types of ARGs that have been identified

High throughput antibiotic resistance genes by qPCR

- 400 ARGs identified
- Need to perform high-throughput real-time PCR screens: many targets, many samples
- Flexibility required to add new targets to the screens

SmartChip Real-Time PCR System

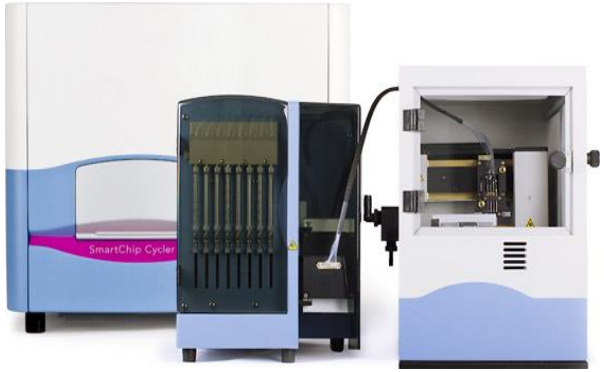


High throughput antibiotic resistance genes by qPCR

- >200 reactions benefits from a high-throughput method to simplify and lower costs for your workflow
- The SmartChip™ Real-Time qPCR System enables high-throughput qPCR that:
  - Saves time : 5,184 reactions in <4 hours
  - Saves money :100 nl reactions -> 200X reduction in reaction volumes and cost
  - Provides flexibility (14 different sample/gene combinations)

Supported chip configurations														
Assays	12	24	36	48	54	72	80	96	120	144	216	248	296	384
Samples	384	216	144	108	96	72	64	54	42	36	24	20	16	12

https://www.takarabio.com/learning-centers/automation-systems/smartchip-real-time-pcr-system-introduction



•**SmartChip MultiSample NanoDispenser**
- High-precision, nanoliter-volume liquid handler
- Dispenses 5,184 reactions in 40 minutes
- Enclosed humidified environment ensures reproducibility

•**SmartChip Real-Time PCR Cycler**
- Fast real-time analysis—2 hours from sample to data
- Supports probe- or dye-based assays
- Filters for FAM, VIC, ROX, and SYBR dyes
- Integrated analysis software

High throughput antibiotic resistance genes by qPCR

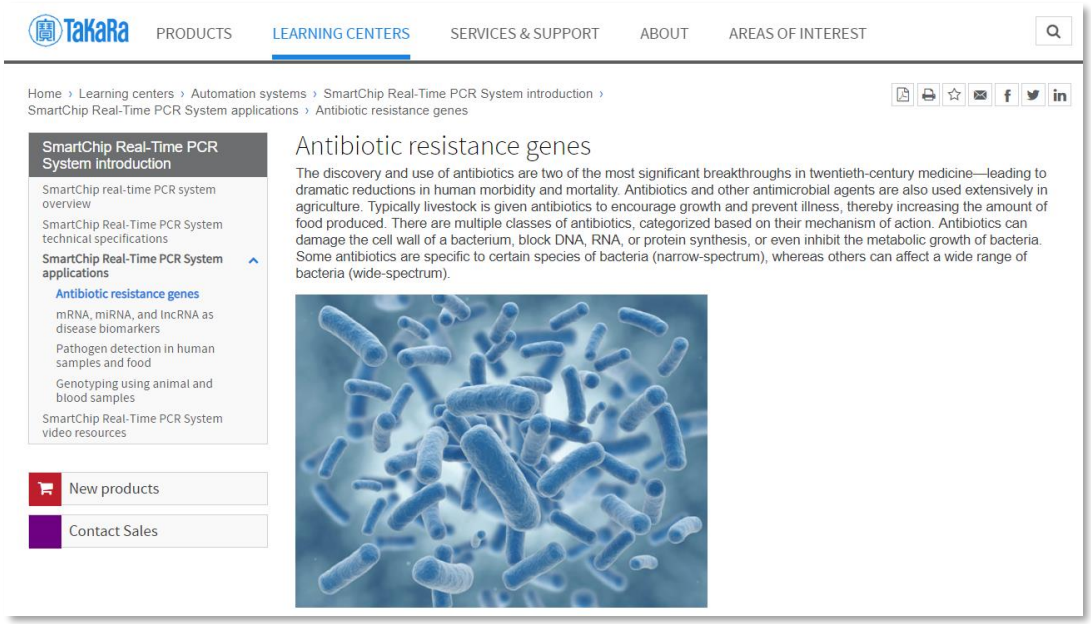
SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence

Katariina M. M. Pärnänen[1]*, Carlos Narciso-da-Rocha[2]*, David Kneis[3]*, Thomas U. Berendonk[3], Damiano Cacace[3], Thi Thuy Do[4], Christian Elpers[5], Despo Fatta-Kassinos[6], Isabel Henriques[7], Thomas Jaeger[8], Antti Karkman[1], Jose Luis Martinez[9], Stella G. Michael[6], Irene Michael-Kordatou[6], Kristin O'Sullivan[10], Sara Rodriguez-Mozaz[11], Thomas Schwartz[8], Hongjie Sheng[12,13], Henning Sørum[10], Robert D. Stedtfeld[13], James M. Tiedje[14], Saulo Varela Della Giustina[11], Fiona Walsh[4], Ivone Vaz-Moreira[2], Marko Virta[1†], Célia M. Manaia[2†]

qPCR array
The qPCR array contained 384 primer sets…
The qPCR array uses a microfluidic **SmartChip Multisample Nanodispenser (Takara)**



We maintain a list of key publications on antibiotic resistance research enabled by the SmartChip Real-Time PCR system

https://www.takarabio.com/smartchip-real-time-pcr-system-applications/antibiotic-resistance-genes

Application examples

- Taxonomic studies by 16S rRNA
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- Host/pathogen interaction analysis by single-cell RNA-seq
- Viral genome sequencing for public health management

Host/pathogen interaction analysis by single-cell RNA-seq

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

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Cellular Response to Infection

West Nile Virus-Inclusive Single-Cell RNA Sequencing Reveals Heterogeneity in the Type I Interferon Response within Single Cells

Justin T. O'Neal, Amit A. Upadhyay, Amber Wolabaugh, Nirav B. Patel, Steven E. Bosinger, Mehul S. Suthar

Rozanne M. Sandri-Goldin, Editor

DOI: 10.1128/JVI.01778-18 Check for updates

- New WNV-inclusive single-cell RNA sequencing (scRNA-seq) approach for studying WNV infection at the single-cell level
- Variation in antiviral gene expression and viral abundance across cells
- Demonstrated the feasibility and utility of WNV-inclusive scRNA-seq as a high-throughput technique for single-cell transcriptomics and WNV RNA detection

Host/pathogen interaction analysis by single-cell RNA-seq

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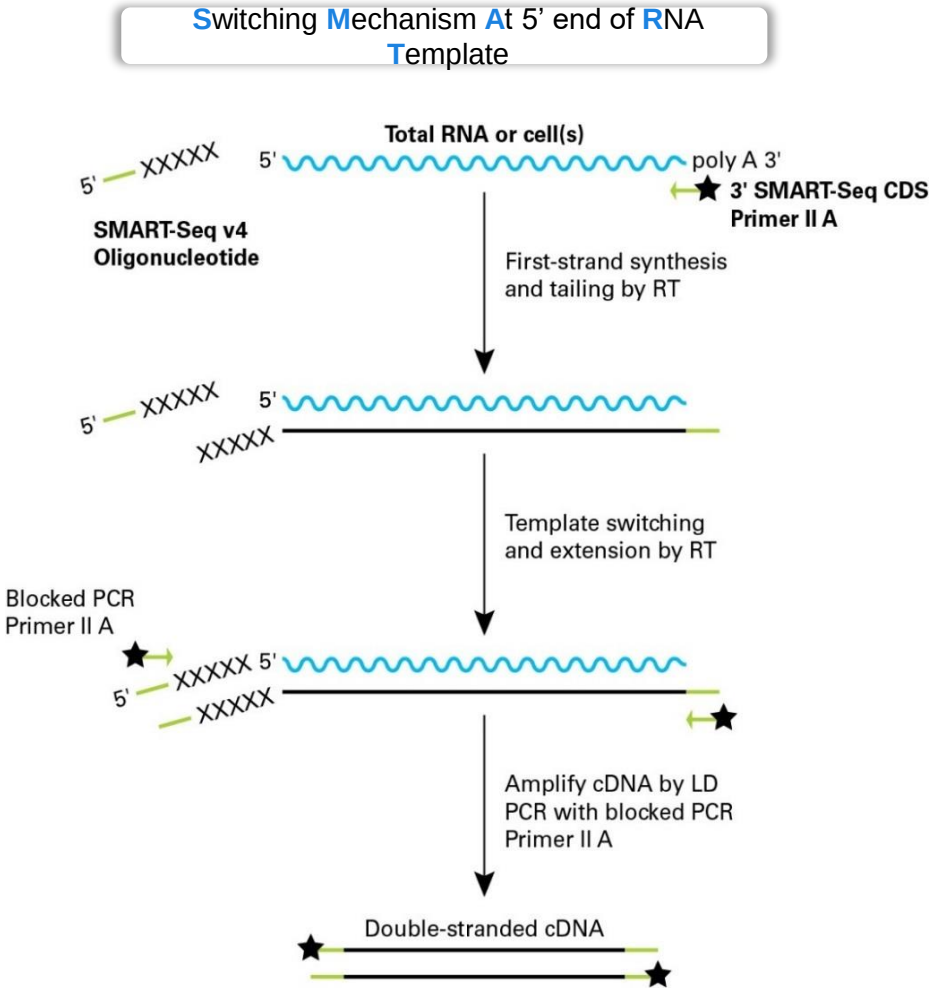
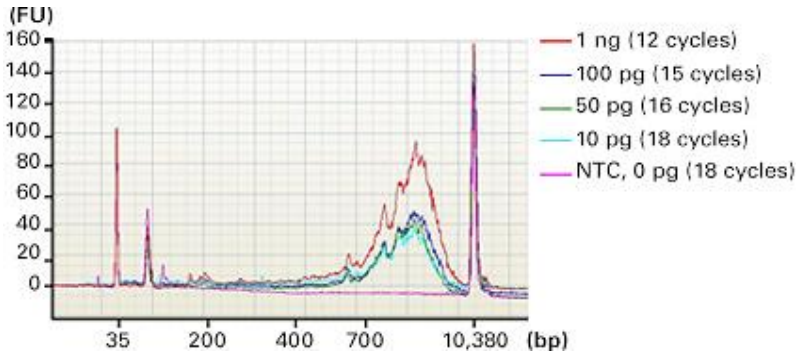
Single-cell RNA sequencing (scRNA-seq). SMART-Seq v4 Ultra Low Input RNA Kit (Takara) was used for cDNA preparation.

Host/pathogen interaction analysis by single-cell RNA-seq: SMART technology



SMART-Seq v4 Ultra™ Low Input RNA Kit for Sequencing

- Can work directly with cells (1-1000) or total RNA (10pg-10ng)
- Oligo dT-primed. No rRNA removal, no DNAse treatment required
- Single tube workflow.
- Generates full-length cDNA compatible with Ion Torrent or Illumina® library preparation



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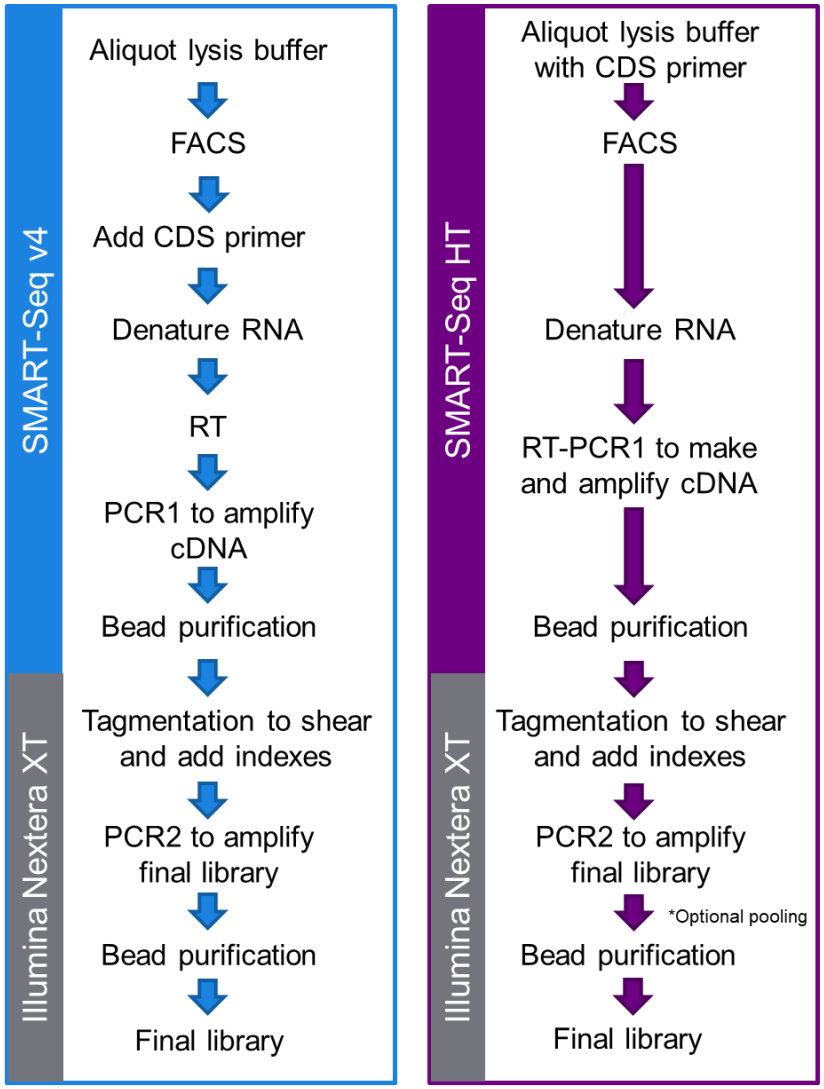
Host/pathogen interaction analysis by single-cell RNA-seq: SMART-Seq v4-SMART-Seq HT



- Generate ds full-length cDNA using oligo-dT priming from intact cells or high quality RNA

	v4	HT
Cells	1-1,000	1-100
Total RNA	10 pg – 10 ng	10 pg – 1 ng

- SMART-Seq HT as a streamlined version of SMART-Seq v4:
  - Optimized to work downstream of FACS
  - Has similar performance to SMART-Seq v4
  - More price competitive
  - Requires less hands on time
- **Requires only ¼ volume Nextera XT reagents**

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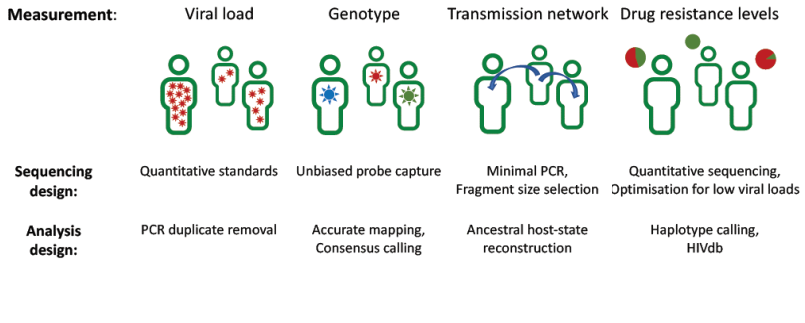
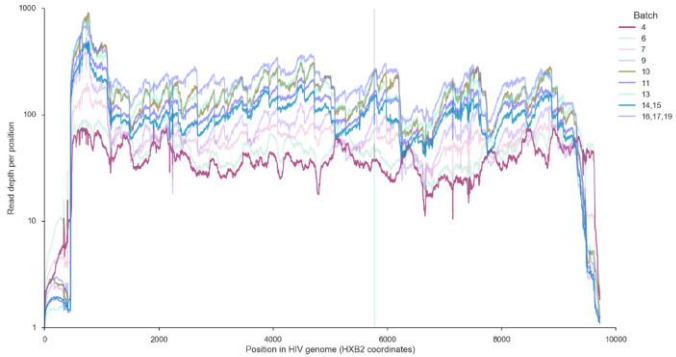
Viral genome sequencing for public health management - HIV

A comprehensive genomics solution for HIV surveillance and clinical monitoring in a global health setting

David Bonsall[a,b,*], Tanya Golubchik[a,b,*], Mariateresa de Cesare[b], Mohammed Limbada[c,d], Barry Kosloff[c,d], George MacIntyre-Cockett[b,a], Matthew Hall[a], Chris Wymant[a], M Azim Ansari[b,e], Lucie Abeler-Dörner[a], Ab Schaap[c,d], Anthony Brown[e], Eleanor Barnes[e], Estelle Piwowar-Manning[f], Ethan Wilson[g], Lynda Emel[g], Richard Hayes[d], Sarah Fidler[h], Helen Ayles[c,d], Rory Bowden[b], Christophe Fraser[a]





Read depth coverage across the HIV genome

(PopART) trial's goals are to estimate:

- Proportion of transmissions that occur during early and acute HIV infection
- Proportion of transmission events that occur from individuals living within or outside of the trial communities

Viral genome sequencing for public health management - HIV

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2.3. Laboratory methods

Libraries retaining directionality were prepared using the **SMARTer Stranded Total RNASeq Kit v2 — Pico Input Mammalian** (Clontech, Takara Bio) …

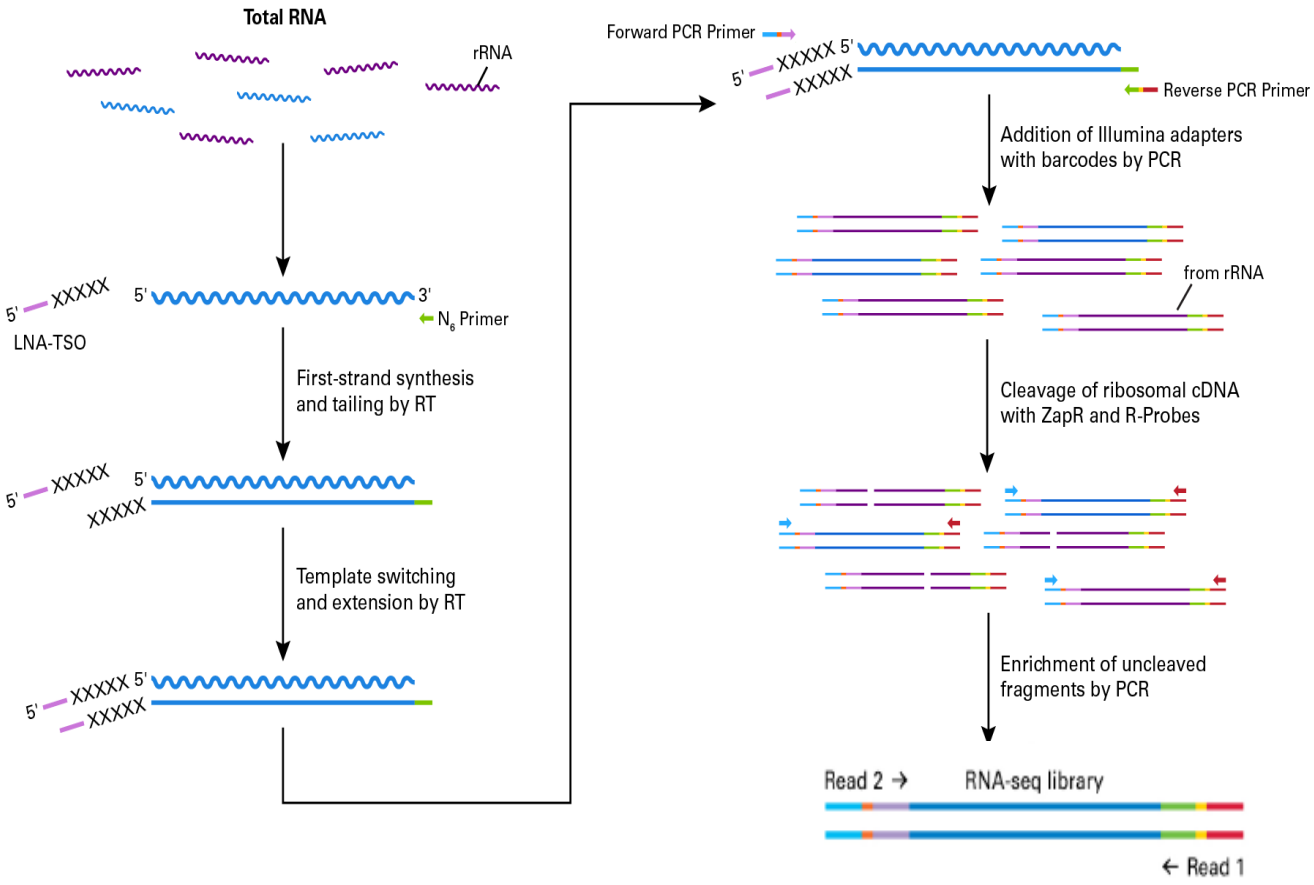
… A total of 500 ng of pooled libraries was hybridized to a mixture of custom HIV-specific biotinylated 120-mer oligonucleotides

The cost of the assay — approximately 45 USD per sample — compares favourably with existing VL and HIV genotyping tests, and provides the additional value of viral load quantification and inference of drug resistance with a single test.

Viral genome sequencing for public health management - HIV

SMARTer Stranded Total RNA-Seq Kit - Pico Input Mammalian v2

- Input 250 pg–10 ng of mammalian total RNA
- Removal of rRNA in the form of ribosomal cDNA using a novel proprietary technology
- RNA of any quality (RIN 2-10)
- Ligation-free addition of Illumina adapters
- From total RNA to Illumina libraries ready to sequence in 5 hours
- Strand information



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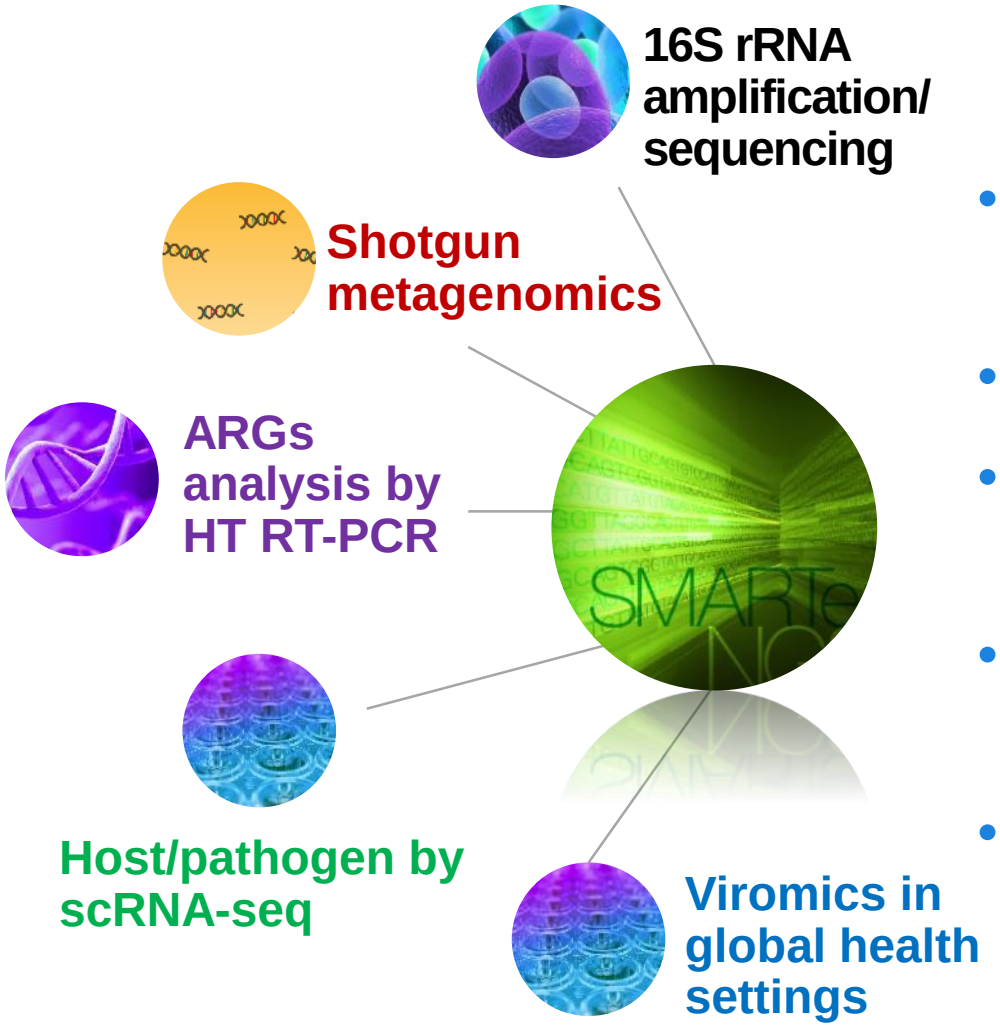
Solutions for disease diagnostics caused by pathogens and the human microbiome



- **Ex Taq / ThruPLEX DNA-seq**
- **ThruPLEX DNA-seq**
- **SmartChip Real-Time PCR System**
- **SMART-Seq v4 / SMART-Seq HT**
- **SMARTer Stranded Pico v2**

Ressources

https://www.takarabio.com/

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that's GOOD science!®

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