ISOGEN LIFE SCIENCE
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stratec molecular

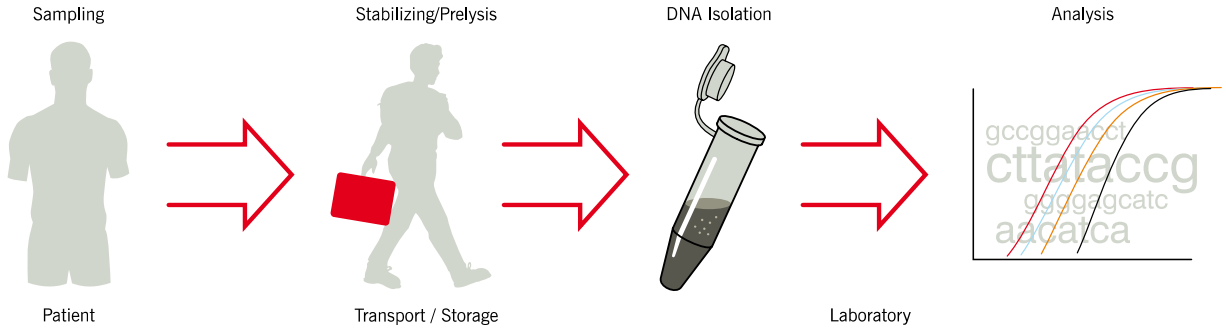
Sample Collection, DNA Stabilization and Purification of Saliva, Swab and Stool Samples

PSP® - Technology
a unique sample stabilization method for molecular diagnostics

The PSP® (**P**re-analytical **S**ample **P**rocessing) product portfolio is based on STRATEC Molecular's unique and proprietary non-chaotropic chemistry. It comprises an integrated sample management system from sample collection, DNA stabilization, transportation, storage through to purification – all conveniently combined in different kits.

Based on the unique PSP® buffer compositions two product lines are established

SalivaGene Product Line | **PSP® Stool Product Line**



Both product lines are characterized by following features:

- Reliable stabilization and storage of host and pathogen DNA in stool, saliva or swab samples at room temperature without DNA degradation
- Reliable sample collection in easy-to-handle collection devices
- Preservation of bacterial titer at the time of sampling – through optimized pre-lysis of bacterial cell wall
- Inactivation of DNases and RNases
- Non-invasive sample collection eliminates the risk of pathogen infections
- No cooled transportation is needed
- Collected samples are liquid and can easily be integrated in existing lab workflows allowing:
  - combination with liquid handling robots
  - use of standard lab ware
  - easy processing
- Suitable sets of extraction kits for a variety of purposes are available

All purification protocols are careful optimized for a fast and efficient isolation of highly purified DNA from host and pathogens.

SalivaGene Product Series

Saliva and Swab collection for stabilization of DNA

The SalivaGene Collector, the *Premium* Collector and the Collection Module II simplify saliva collection and are designed to provide highest integrity and yields of DNA. For the collection and DNA stabilization from swab samples the SalivaGene Comfort Set device is available. The patented lyophilized or liquid stabilization buffer – SalivaGene DNA Stabilizer (PSP® technology) eliminates cooling of samples and stabilizes genomic DNA for 12 months at room temperature. All Collection modules are CE-IVD compliant devices.*) **)

BENEFITS

Effortless collection

- Intuitive use and handling of sample collection tubes for assisted collection of saliva and swab samples
- Fast, painless & non-invasive sample collection
- Reduces puncture-associated infection risks
- Ideal for needle-phobic people and children

Long-term room temperature stability of samples

- Less costs and easy sample management
- No cold chain during transport reduces transportation and storage costs
- Easy storage without refrigeration for up to 12 months
- High quality DNA from saliva and swab samples without degradation

Saliva and buccal swab applications

- Predictive
- Pharmacogenomics
- HLA typing
- DNA profiling
- Diagnostic
- Metagenomics
- Genealogical DNA tests

Collection method comparison

	Blood collection	Oral collection	
Attributes	Venous blood	SalivaGene buccal swabs sample	SalivaGene saliva sample
Non-invasive collection	x	✓	✓
Standardized format for low and high-throughput processing	✓	✓	✓
Liquid sample	✓	✓	✓
Specimen stability at room temperature	Days	1 Year	1 Year
No cold chain required	x	✓	✓
Average DNA yield	4-6 µg	Up to 2 µg	Up to 150 µg
Sample size	200 µl	swab ***	2 mL

*) *The SalivaGene Collector Premium* is for research use only*
**) *Not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized*
***) *Stabilized in 650 µl stabilization buffer*

1. SalivaGene Collector *Premium**

The SalivaGene Collector *Premium* is a high-end sample collection device featuring the collection components as well as a convenient packaging for shipment by mail. Reliable and painless assisted collection at point-of-care can be realized. The collection tube fits directly with all common robotic systems and is barcode labelled for automated processing. (RUO only!)

2. SalivaGene Collector

The SalivaGene Collector includes the collection devices for assisted sample collection in plastic bags for lower cost per sample. This product is offered in packages of 50 collectors or as bulk ware (as requested) for high sample numbers in high-throughput processing, e.g. for clinical studies. The barcode labelled primary tube is also compatible with all common robotic platforms.

3. SalivaGene Collection Module II

The SalivaGene Collection Module II is a simple, alternative version for saliva sample collection and stabilization using a liquid DNA stabilization buffer (DNA is also stable for 12 months at room temperature).

SalivaGene Comfort Set

The SalivaGene Comfort Set includes the collection components in a blistered package with barcode labelled primary tubes, prefilled with the stabilization buffer for easy-to-use buccal cell swab collection and DNA protection, optimal in high-throughput processing, e.g. for clinical studies. The barcode labelled primary tube is compatible with all common robotic platforms.

**) only in bulk quantities or as OEM product available*

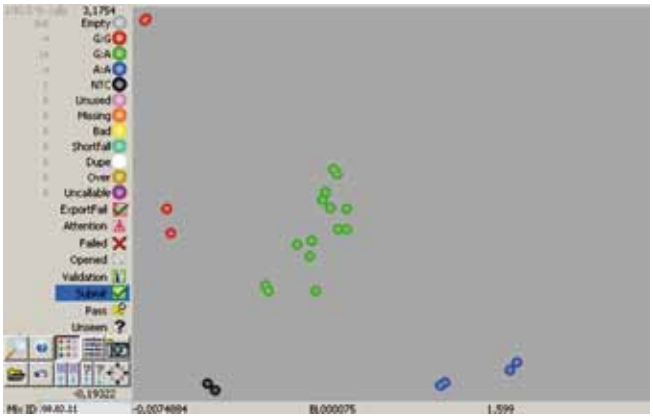
Not all products are available in the USA.

Application Examples

The SalivaGene DNA stabilizer inactivates DNases and prevents degradation of DNA. It also preserves the microorganism titer and pre-lyses bacteria. Genomic and bacterial DNA can be isolated from the stabilized samples using the different STRATEC Molecular purification kits.

1. SNP profiling for pharmacogenomics

Fig. 1: Genotyping results for SNP rs9934438 in *VKORC1* Gen



Genotyping of selected SNPs (Single Nucleotide Polymorphisms) for 11 randomly selected individuals was performed using a KASP assay system (KBioscience, Herts, UK). Genomic DNA, isolated from saliva collected in the SalivaGene Collector Premium using the InviGenius, was tested in duplicates. A genotyping accuracy rate of 100% was obtained. The genotyping success rate was between 91 and 100%.
Genotyped samples marked in red are homozygous for the G-, those marked in blue are homozygous for the A-allele. Heterozygous samples are marked green. Two negative controls (marked in black) were included on each genotyping plate.

The gene product of VCORC1 is involved in metabolism of coumarin derviative drugs (Warfarin®, Marcumar®).

Data kindly provided by D. Steinberger, bio.logis - Center for Human Genetics, Frankfurt am Main, Germany.

2. Bacteria detection

Tab.1: Customized application from stabilized sputum samples

Bacterial DNA from different species was isolated from 500 µl sputum sample, pretreated and stabilized using the SalivaGene Collection Module II. DNA was extracted on the KingFisher Flex96 using the InviMag® SalivaGene DNA Kit/ KF96 and a competitor kit. The sputum sample was mixed 1:1 with NAC buffer (cat.no. 1033221100) and heated for 10 min at 95 °C before adding the stabilization buffer. DNA eluates were amplified using the commercial PCR detection assays from Diagenode Diagnostics (Belgium) on the Illumina Eco Real-Time PCR instrument. The SalivaGene® extraction method showed better CT values for the Legionella samples and comparable values for the Mycoplasma and Chlamydophila samples.

Sample	Competitor Kit	InviMag SalivaGene DNA Kit/ KF96
Mycoplasma pneumoniae	34.82	34.43
Sample R 541 1215 413	34.54	33.80
Internal control		
Chlamydophila pneumonia	30.41	32.03
Sample RV 540 1215 403	34.22	34.07
Internal control		
Legionella species	36.28	32.16
Sample RV 536 1215 361	31.94	31.67
Internal control		
Legionella pneumophila	37.91	33.78
Sample RV 536 1215 361	31.94	31.67
Internal control		

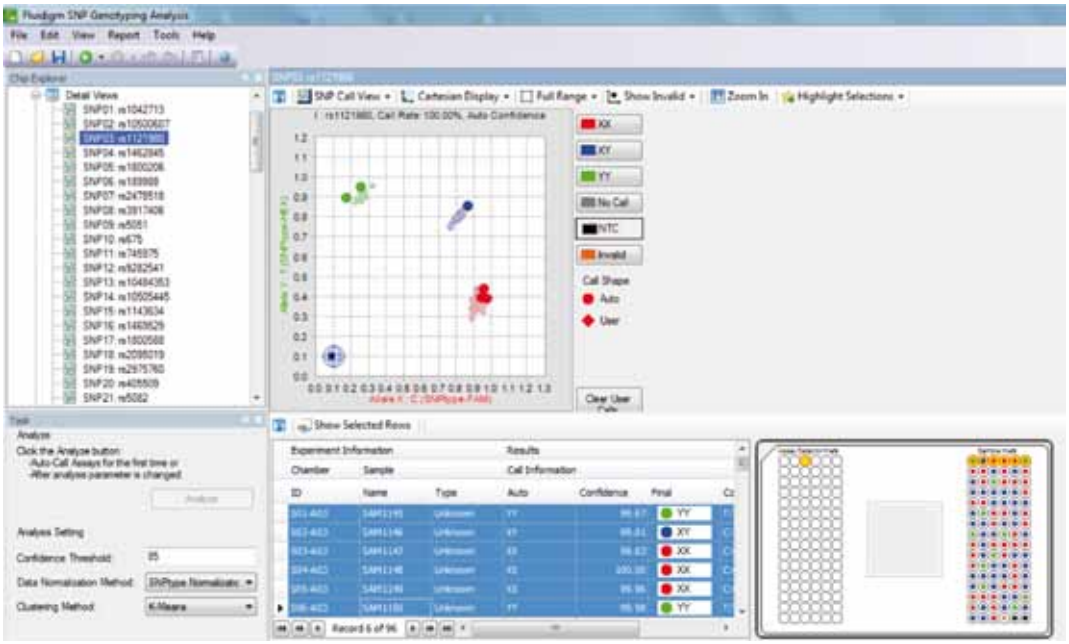
Data kindly provided by A. Reinecke, Medizinisches Labor Rostock, Germany.

3. Genotyping of SNPs related to metabolic disorders and sport genetics

The response to nutrition, training and exercise as well as the risk for certain lesions are to a large extent determined by the status of SNPs in certain genes. The analysis of the SNP status allows generating profiles of the respective individual and advising in regards to training programs. The sampling for this application needs to be robust and simple. The use of SalivaGene Collection devices and the Saliva Stabilizer enables automated and robust SNP genotyping e.g. using Fluidigm Dynamic Arrays IFC. The well-defined clusters are indicative of pure and high-quality DNA, as also verified by the 260/280 ratios.

Fig.2: Application example for sport genetics

2 ml saliva samples were collected using the SalivaGene Collection Module II and the DNA's were isolated from 800 µl stabilized saliva samples using the PSP® SalivaGene DNA HTS 96 Kit / C on a centrifuge. The DNA's were analyzed using Dynamics assays from Fluidigm Corporation.



DNA quantification

	ID	Concentration (ng/uL)	260 / 280	260 / 230
1	SAM145	9.88	3.22	6.45
2	SAM146	68.90	1.94	1.53
3	SAM147	86.37	1.21	0.86
4	SAM148	31.73	0.85	0.80
5	SAM149	19.66	2.21	1.02
6	SAM150	92.51	1.88	1.32

Genotyping results

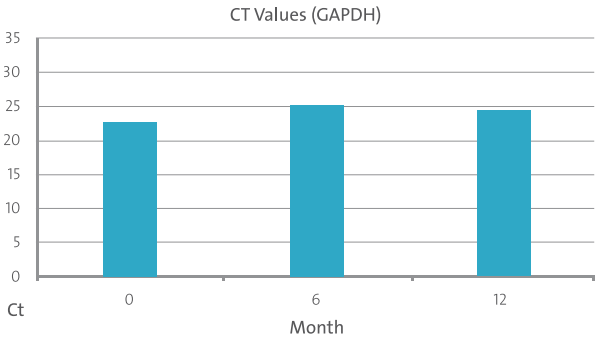
Assay	Gene	Related to	RS	SNP
A03	FTO	obesity	rs1121980	CT
A18	90	ancestry	rs2095019	AC
A52	TRHR	sport performance	rs16892496	AC

Flexible DNA purification using the SalivaGene product series

1. Spin columns - PSP® SalivaGene DNA Kit

Manual spin filter kit based on STRATEC Molecular´s expertise in nucleic acid purification.

Fig. 3: Stability study - Real-time PCR of stabilized saliva samples

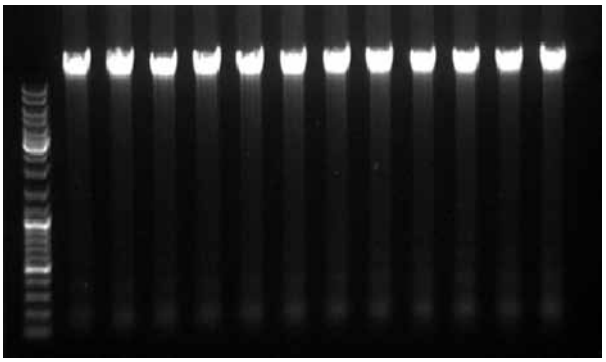


Mean CT values of real-time PCR reactions using primers for GAPDH of DNA aliquots from 200 µl of saliva after 0, 6 and 12 months of storage in the SalivaGene Collector at room temperature (average of 10 different samples, single determinations). DNA was manually isolated using the PSP® SalivaGene DNA Kit.

2. Fully automated on the InviGenius® - InviMag® SalivaGene DNA Kit/ IG

SalivaGene Collector tubes (barcode labelled) place directly into the sample loading racks of the InviGenius® and InviGenius® *Plus* for automated sample tracking.

Fig. 4: Seamless integration into automated workflows



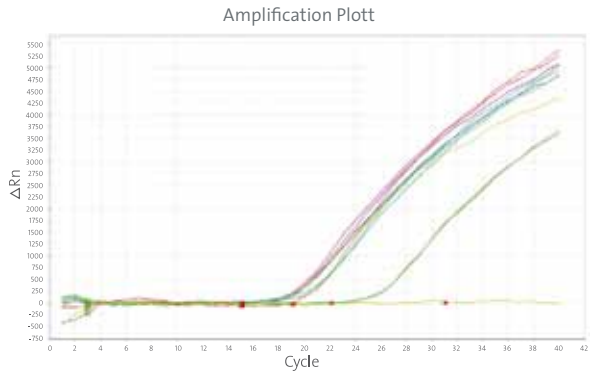
Genomic DNA was automatically purified on the InviGenius® from 1.6 ml of stabilized saliva samples (pooled samples of ten different donors) using the SalivaGene® Collector. 10 µl were analyzed on a 0.8 % agarose gel stained with ethidium bromide.

(Marker - GeneRuler™ DNA Ladder, Fermentas)

3. Semiautomated in 96 well format

For use on the KingFisherFlex 96 from Thermo Fisher Scientific.

Fig. 5: Automated DNA isolation in 96 well format



Genomic DNA was isolated using the InviMag® SalivaGene DNA Kit/ KF96 from SalivaGene® stabilized saliva samples (Collection Module II) from 8 different donors stored at room temperature for six weeks. The GAPDH sequence was amplified in a real-time PCR using the StepOnePlus™ Real-Time PCR System from Applied Biosystems. 2.5 µl of the eluted DNA were used as template.

(Yellow lane – negative control; green lane: positive control)

4. Fully automated in 96 well format

SalivaGene Collector tubes or SalivaGene Comfort Set swab collection tubes (barcode labelled) place directly into the sample loading racks of any Hamilton platform for automated sample tracking.

Fig 6: Influence of contact time of the swab with the buccal on the yield

2 Swabs of the SalivaGene Comfort Sets were used per person for 10 sec and 30 sec (each for one buccal side) and add to the stabilization reagent. The DNA extraction from 200 µl stabiliized sample were performed on the Starlet using the Invisorb® DNA Swab HTS 96 Kit /STARlet. 10 µl of the eluate was loaded onto the agarose gel. Result: 30 sec treatment shows a clearly higher yield (15-30 %) and sensitivity (0.5- 1 CT)

sample	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8
seconds	10	30	10	30	10	30	10	30	10	30	10	30	10	30	10	30

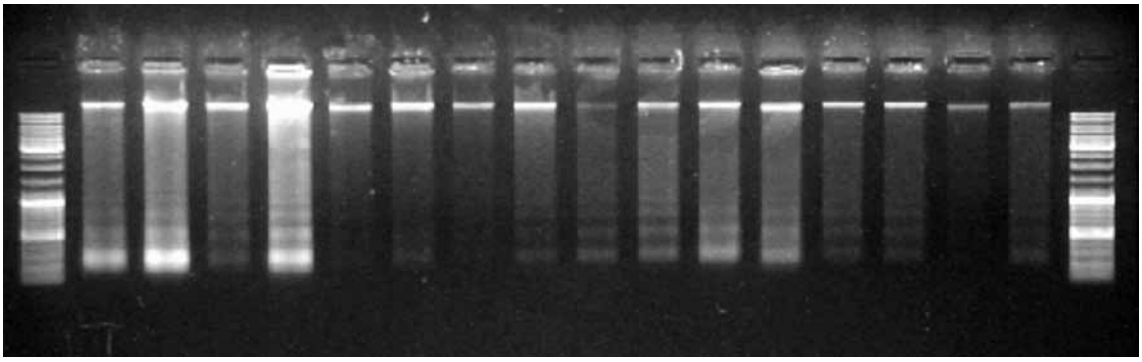
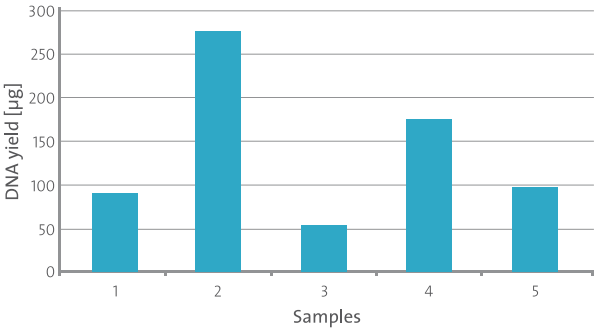


Fig. 7: High DNA yields from saliva samples



Five different saliva samples were stabilized using the SalivaGene® Collector. The genomic DNA was isolated using the standard precipitation protocol and measured on a Nanodrop™.

Comparison of DNA yields from stabilized saliva samples using different extraction methods

Purification method	Sample volume	Average DNA yields*
Spin columns	500 µl	25 – 50 µg
Semi-automated on KingFisher® mL and 96	500 µl	20 – 40 µg**
Fully automated on the InviGenius®	1.6 ml	150 µg
Fully automated on STARlet®	50 µl	2 - 4 µg
Precipitation	2 ml	150 µg
DNA from blood (spin columns)	200 µl	4 – 12 µg

**) with SalivaGene Collector*

Extraction kit overview

Collection Sets*	SalivaGene Swab Comfort**	SalivaGene Collection Module II**	SalivaGene Collector* / Premium*
Total sample volume	670 µl	4 ml (2 ml : 2 ml)	2 ml undiluted
PSP® SalivaGene DNA Kit	500 µl	500 µl	500 µl
Invisorb® SalivaGene DNA HTS 96 Kit / C	500 µl	300 µl	300 µl
InviMag® SalivaGene DNA Kit / KF96	500 µl	500 µl	500 µl
InviMag® SalivaGene DNA Kit / IG	comming soon	1,6 ml	1,6 ml
Invisorb® DNA Swab HTS 96 Kit / Starlet	200 µl	100 µl	50 µl

**) with lyophilzed stabilization buffer*
***) with liquid stabilization buffer*

Ordering information

Product	Package size	Catalogue number
Collection modules		
SalivaGene Collector	50 pieces	1035211200
SalivaGene Collection Module II	50 pieces	1035210700
SalivaGene Buccal Swab	50 pieces	1035230200
SalivaGene Swab Comfort	50 pieces	1035231200
Isolation kits - Manual use		
PSP® SalivaGene DNA Kit	50 purifications 250 purifications	1035200200 1035200300
PSP® SalivaGene Precipitation Kit	50 purifications	1035290200
PSP® SalivaGene DNA HTS 96 Kit/ C	4 x 96 purifications 24 x 96 purifications	7035360300 7035360400
Isolation kits - Semiautomated use		
InviMag® SalivaGene DNA Kit/ KF96	1 x 96 purifications 5 x 96 purifications	7435060100 7435060200
Isolation kits - Fully automated use		
InviMag® SalivaGene DNA Kit/ IG	4 x 96 purifications	2435260100
Invisorb® DNA Swab HTS 96 Kit /STARlet*	4 x 96 purifications 24 x 96 purifications	7135330300 7135330400

PSP® Stool Product Series

Stool DNA Collection and Preservation

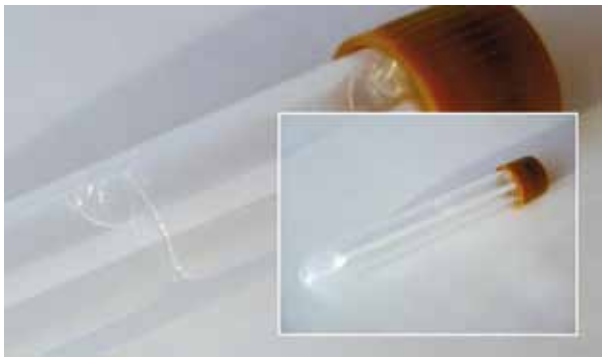
The ideal stool DNA collection and preservation tube (Stool Collection Module) - prefilled with the DNA Stool Stabilizer simplifies stool collection and is designed to provide highest DNA yields and quality. The patented stabilization buffer - DNA Stool Stabilizer (PSP® technology) eliminates cooling of samples and stabilizes genomic DNA for 3 months at room temperature.

The Stool Collection Tubes prefilled with Stool DNA Stabilizer is a user friendly stool collection and DNA protection device. It allows a convenient and hygienic collection of up to 1 g of stool, using spoon attachment.

BENEFITS

Effortless collection in user-friendly Stool Collection Tubes

- Sample collection tubes for easy stool collection and efficient DNA preservation using DNA stabilization buffer
- Fast, painless & non-invasive sample collection
- Reduces foul odors
- No need to immediately process samples
- Available in different formats



Long-term stability of samples – highest integrity of DNA at RT

- Stool DNA preserved for 3 month at 15 - 30°C
- Exellent yield and integrity
- PCR - amplificable (PCR inhibitor free)
- Less costs and easy sample management
- No cold chain during transport reducing transportation and storage costs
- Low variability between replicates compared to frozen
- Preservation shows no bias

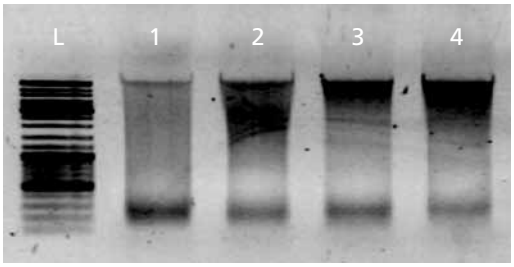


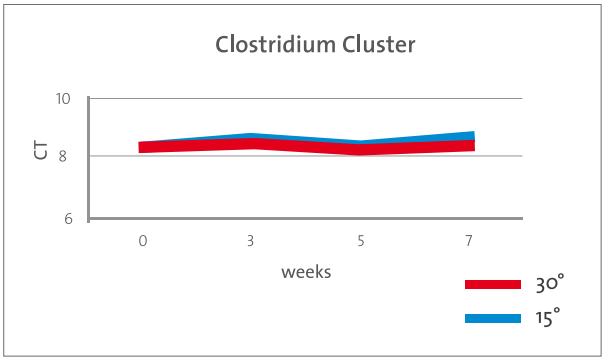
Fig 8: High integrity of DNA

DNA was isolated using the PSP® Spin Stool Plus Kit and 1.4 ml of preserved stool sample at the indicated time point. 10 µl of the eluted DNA was loaded onto the gel. High integrity stool DNA was isolated from the preserved sample that was stored at 25 °C for 14 days at RT.

Stable over a wide temperature range

Excellent preservation of biological information even with temperature fluctuations (15-30°C)

Fig 9: Influence of temperature on DNA stability



Stool from the same healthy donor was collected using the Stool Collection Tubes. Samples were then incubated at 15°C and at 30 C for 7 weeks. DNA was isolated from 1,4 ml aliquotes at the day of collection after 3, 5 and 7 weeks using the PSP® Spin Stool Plus Kit. 2 µl of the eluate was used in the real time PCR - Reaction (RIDA®GENE Gut Balance) from R-Biopharm.

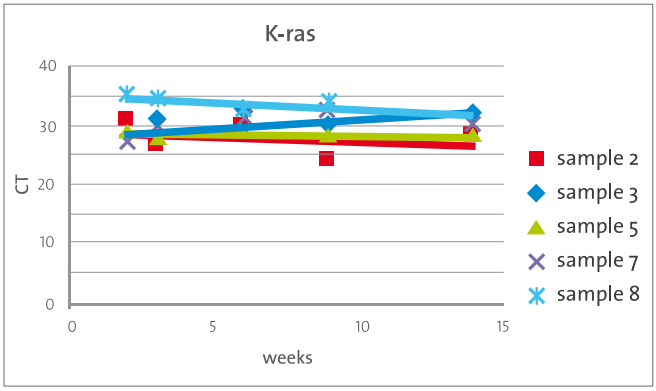
Suitable for microbiome and microbiota diversity studies

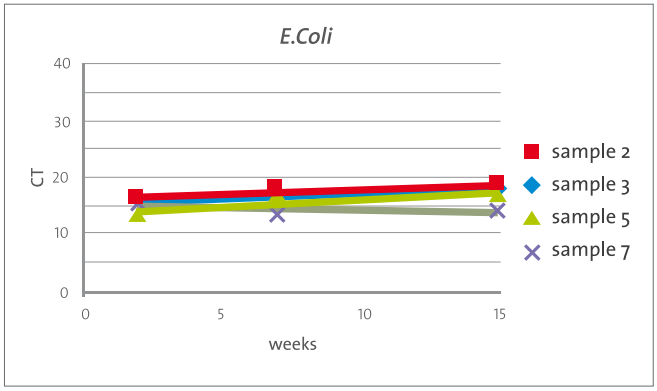
Human (Kras), two gastrointestinal gram negative bacteria (*E.coli* and *Bacteroides ssp.*) and a gastrointestinal gram positive bacterium (*Chlostridium cluster*) were monitored from stool samples stored at ambient temperature with Stratec's Stool DNA Stabilizer. Real-time PCR results demonstrate that DNA was preserved and stable over a 15 week period.

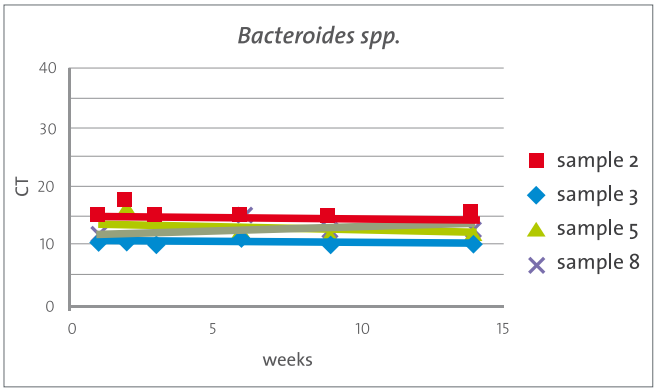
Fig. 10: Stability test

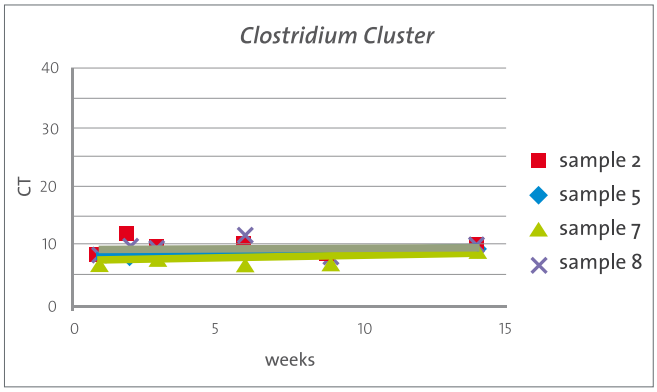
1.4 ml of preserved stool samples were tested on day 1, day 2, day 7, day 18, day 49 and day 105 for DNA isolation using Stratec's PSP Spin Stool DNA Plus Kit. Next 2 µl of eluate was used in the real time PCR reaction (internal Kras and E.coli real time PCR - Assay and RIDA®GENE Gut Balance real time PCR assay from R-Biopharm)*

**) System for detection and differentiation of Bacteroides ssp. and Chlostridium Cluster XIVa-DNA from human stool samples)*









Stool applications

- Pathogen detection
- Microbiome and microbiota diversity studies
- Tumor marker detection
- Clinical studies
- Metagenomics
- DNA profiling
- Paternity tests
- Genealogical DNA tests

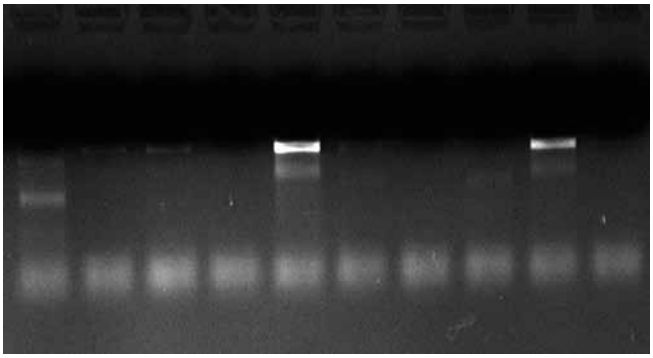
Application Examples

1. Veterinary pathogen detection

Fig. 11: Detection of *Chlamydiaceae* from bird excremental samples

DNA was isolated from fecal samples of birds with the PSP® Spin Stool DNA Kit. The isolated DNA was used in PCR and nested PCR (Kaltenböck et. al., 1997, J. Clin. Microbiol. 35, 1835-1841) for the detection of infections with Chlamydiaceae, (Dr. Nieper, LUA Sachsen)

PCR



nested PCR

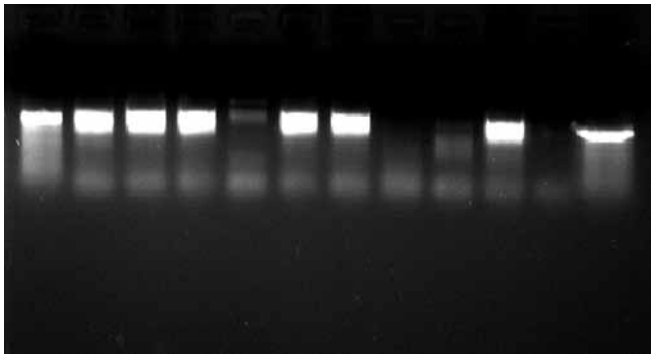
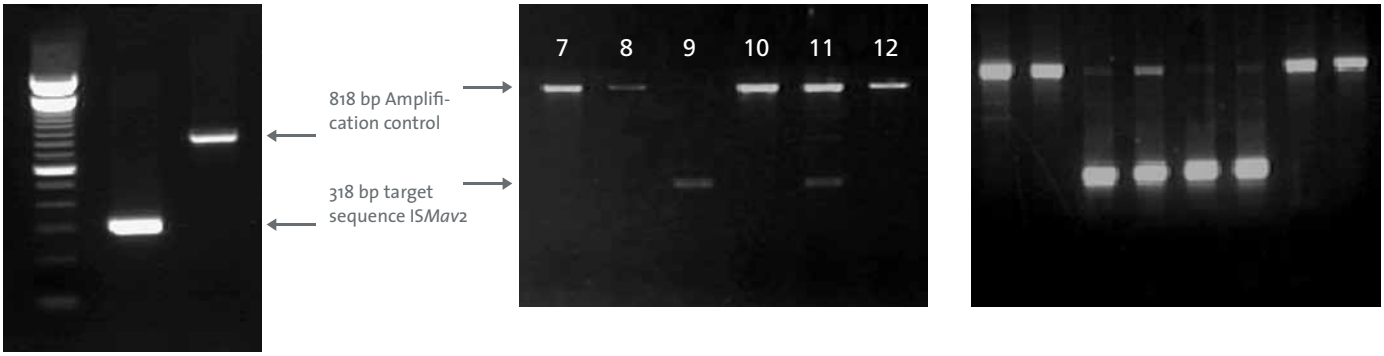


Fig. 12: Detection of *Mycobacterium avium ssp. paratuberculosis* from cattle excremental samples

DNA was isolated from stabilized clinical and from spiked stool samples from cattle with the PSP® Spin Stool DNA Plus Kit and used for PCR amplification of the M. avium ssp. paratuberculosis target sequence ISMav2 (with the Bactotype® Amplification Kit Mycobacterium paratuberculosis). The sensitivity lies at 102 cells/g stool (6-fold repeat) (Dr. F. Kloep, Biotype AG).

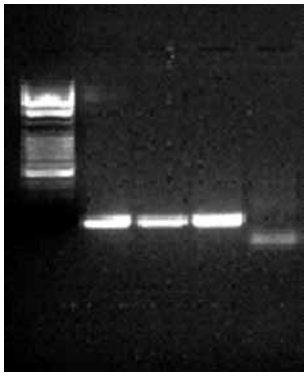
Clinical samples

Spiked samples



2. Human pathogen detection

Fig.13: Detection of *Helicobacter pylori* in human stool samples



urease-sequence

cagA-sequence

Amplification of different target sequences of extracted bacterial DNA (H.pylori) from human stool samples stored at room temperature (without cooling) for 3 days with Stool Stabilizer in a Stool Collection Tube

Extraction kit overview

Collection Set	Stool Collection Module
Total sample volume	8,00 ml = about 1 g stool sample
PSP® Spin Stool DNA *Plus* Kit	1,4 ml
InviMag Stool DNA Mini Kit/ KFml	600 µl
InviMag Stool DNA Kit /KF Duo	600 µl
InviMag Stool DNA Kit/ KF96	600 µl

Ordering information

Product	Package size	Catalogue number
Collection modules		
Stool Collection Tubes with Stool Stabilizer (Tube regular, thick diameter)	50 pieces 250 pieces	1038111200 1038111300
Stool Collection Tubes with Stool Stabilizer (Tube with conical bottom, thin diameter)	50 pieces	1038111700
Isolation kits		
PSP® Stool DNA *Plus* Kit	50 purifications 250 purifications	1038110200 1038110300
InviMag Stool DNA Mini Kit/ KFml	75 purifications 300 purifications	2438110200 2438110400
InviMag Stool DNA Kit /KF Duo w/o plastic	8 x 12 purifications 40 x 12 purifications	2438130150 2438130250
InviMag® Stool DNA Kit/ KF96	1 x 96 purifications 5 x 96 purifications	7438300100 7438300200
For 200 mg fresh or frozen stool samples		
PSP® Stool DNA Kit	50 pieces 250 pieces	1038100200 1038100300

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