

**Validation Sheet
for
GENESpin
Cat. No. 5224400605**

Setup: Extraction of DNA from different matrices. Of each matrix three samples are analyzed as follows: one sample sixfold and two samples onefold or – in case of raw materials – twofold.

The following parameters have been validated/verified and the respective performance criteria have been fulfilled:

Method Parameter:	Acceptance Criteria:	Results:
<u>DNA Concentration and Yield:</u> Extraction of DNA from different matrices. From each matrix three samples are analyzed as follows: one sample sixfold and two samples onefold or – in case of raw materials – twofold. <u>Processed Products/Feed/Meat:</u> Determination of the concentration of each DNA solution extracted by agarose gel electrophoresis and Pico Green ds DNA Quantification kit. Calculation of the yield. <u>Raw Materials:</u> RT-PCR on species single-copy gene, dermination of the species copy number.	Min. 10 ng/μL DNA Minimum 2000 DNA copies/μL	-fulfilled- -fulfilled-
<u>Repeatability Standard Deviation (RSD(r)):</u> Determination of the RSD(r) from the samples extracted sixfold. <u>Processed Products/Feed/Meat:</u> Pico Green Measurement. <u>Raw Materials:</u> RT-PCR on species single-copy gene with 5fold dilution of sample DNA.	RSD(r) of the DNA concentration and yield < 25%	-fulfilled- Exemption: Maize flour; however, in repetition RSD_r under 25% was achieved

Method Parameter:	Acceptance Criteria:	Results:
<u>Fragmentation state of DNA:</u> Determination of the fragmentation state by Agarose gel electrophoresis of aliquots of each DNA solution extracted (only for processed products, feed, meat)	Fragment length of the DNA should at least exceed the typical amplicon size of the test systems to be applied (approx. 200 bp).	-fulfilled-
<u>Purity/Absence of Inhibition:</u> <u>Processed Products/Feed/Meat:</u> Determination of absence of inhibition of the DNA aliquots extracted by real-time PCR assay with internal positive control (IPC) High-DNA samples were diluted to a concentrations of 20 ng/μl (100 ng/PCR reaction). Samples with a DNA concentration below 20 ng/μl were used undiluted. <u>Raw Materials:</u> RT-PCR on species single-copy gene undiluted and with 5fold dilution of sample DNA.	$CT_{IPC} \text{ sample} \leq CT_{IPC}$ Cut-off and dRn_{IPC} sample $\geq dRn_{IPC} \text{ Limit}$ Factor Species copy number of DNA undil. vs. diluted 1:5 is between 3,5 and 6,5. (Data not shown)	-fulfilled- Exemption: chocolate cake sample, which needed additional DNA cleaning (equivalent to GENE<i>Spin Clean</i> kit) -fulfilled-

Sample types

Processed products

Chocolate cake
Cereal mix
Toast bread

Feed

Poultry feed
Animal feed protein
Finished feed

Meat

Streaky bacon
Salami sausage
Beef meat

Raw Materials

Soy beans
Corn/Maize kernels
Corn/Maize flour

Determination of the DNA concentration and yield

sample no	Replicate	Concentration (ng/μl)	Yield (μg)
CHOCOLATE CAKE	I	8	0.8
CHOCOLATE CAKE	II	13	1.3
CHOCOLATE CAKE	III	10	1.0
CHOCOLATE CAKE	IV	12	1.2
CHOCOLATE CAKE	V	12	1.2
CHOCOLATE CAKE	VI	11	1.1
CEREAL MIX	I	23	2.3
TOAST BREAD	I	24	2.4
POULTRY FEED	I	113	11.3
POULTRY FEED	II	113	11.3
POULTRY FEED	III	112	11.2
POULTRY FEED	IV	115	11.5
POULTRY FEED	V	104	10.4
POULTRY FEED	VI	137	13.7
ANIMAL FEED PROTEIN	I	60	6.0
FINISHED FEED	I	132	13.2
STREAKY BACON	I	60	6.0
STREAKY BACON	II	46	4.6
STREAKY BACON	III	62	6.2
STREAKY BACON	IV	44	4.4
STREAKY BACON	V	64	6.4
STREAKY BACON	VI	58	5.8
SALAMI SAUSAGE	I	69	6.9
BEEF MEAT	I	290	29.0

Maize kernels: Overall mean copy nos: 68426 c hmgA / 5 µL
Mean sixfold sample: 94705 c hmgA / 5 µL

Maize flour: Overall mean copy nos: 49822 c hmgA / 5 µL
Mean sixfold sample: 57579 c hmgA / 5 µL

Soybeans: Overall mean copy nos: 126374 c lektin / 5 µL
Mean sixfold sample: 173554 c lektin / 5 µL

Repeatability Standard Deviation (RSD(r):

RSD(r) of the DNA concentration and yield

sample	Mean Yield	Standard deviation	Mean Concentration	Standard deviation	Relative standard deviation
CHOCOLATE CAKE	1.1 µg	0.2 µg	11.0 ng/µl	1.8 ng/µl	16.3%
POULTRY FEED	11.6 µg	1.1 µg	115.7 ng/µl	11.1 ng/µl	9.6%
STREAKY BACON	5.6 µg	0.9 µg	55.7 ng/µl	8.5 ng/µl	15.3%

Raw Materials:

Relative standard deviation of 6 species copy number measurements in 1:5 dilution of DNA:

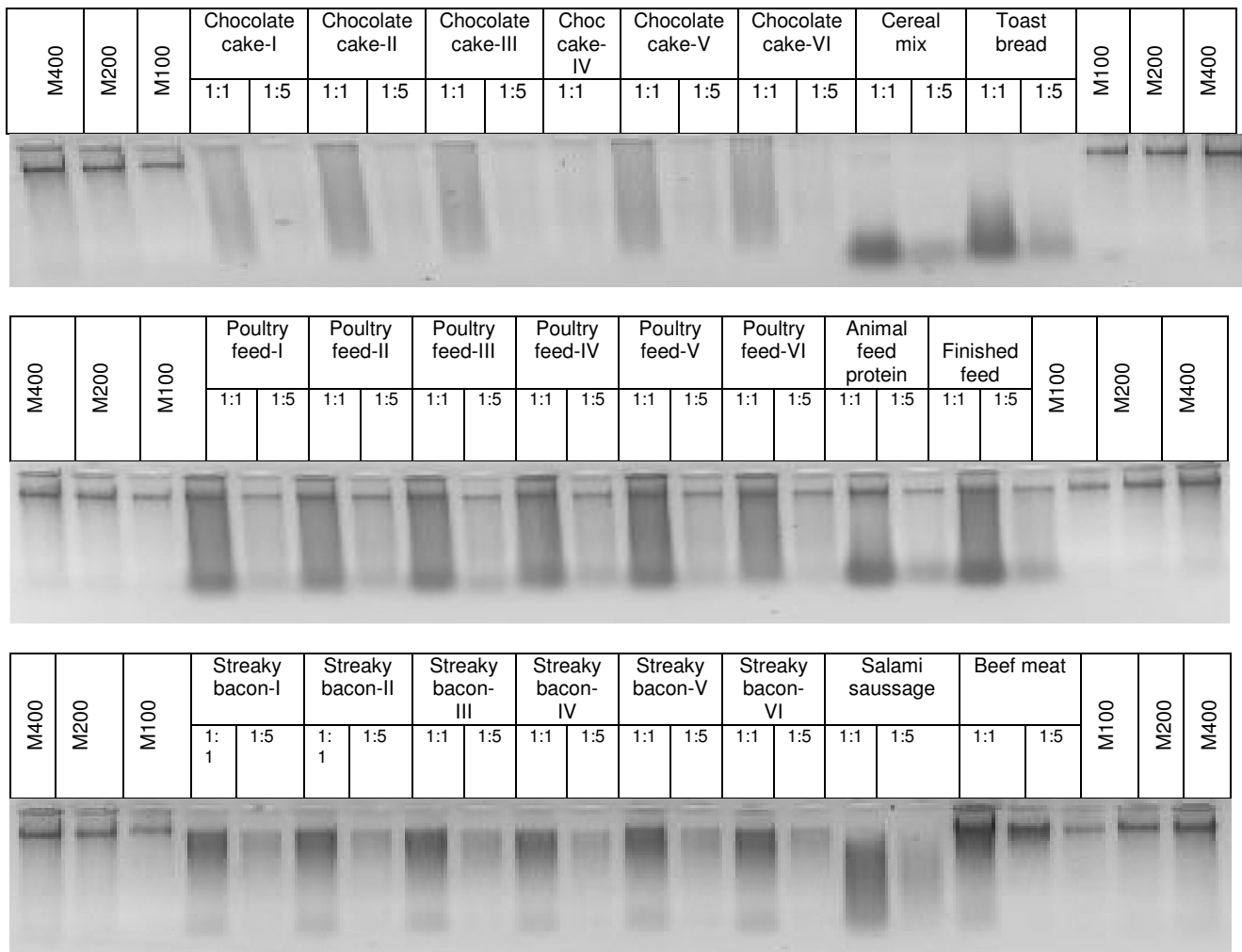
Maize kernels: 14%

Maize flour: 29% (repetition: 5%)

Soybeans: 5%

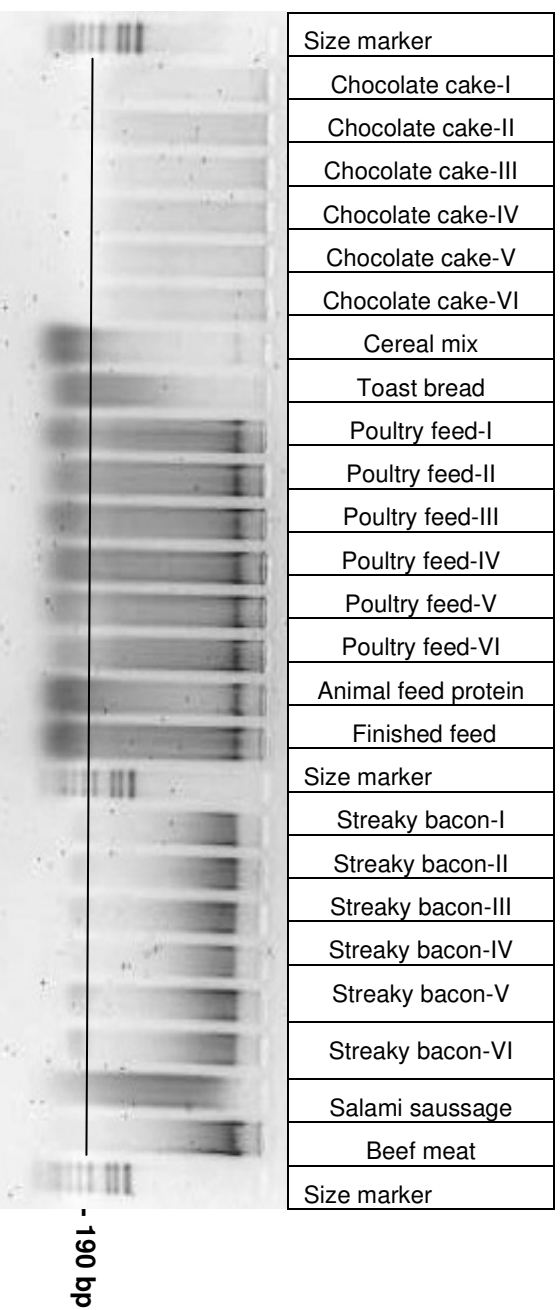
Amount of DNA

Weight marker (M) with 100, 200, 400 ng DNA



Fragmentation state of DNA

Size marker (bp): 501/489, 404, 353, 242, 190, 147, 110, 89, 67, 34/34/26



Purity/Absence of Inhibition

sample	replicate	CT _{IPC} sample	CT _{IPC} Cut-off	dRn _{IPC} sample	dRn _{IPC} Cut-off	Result
CHOCOLATE CAKE	I	38.0	37.5	0.37	0.18	inhib.
CHOCOLATE CAKE	II	40.5	37.5	0.21	0.18	inhib.
CHOCOLATE CAKE	III	38.6	37.5	0.35	0.18	inhib.
CHOCOLATE CAKE	IV	38.1	37.5	0.39	0.18	inhib.
CHOCOLATE CAKE	V	39.3	37.5	0.31	0.18	inhib.
CHOCOLATE CAKE	VI	37.8	37.5	0.37	0.18	inhib.
CEREAL MIX	I	33.3	37.5	0.52	0.18	not inhib.
TOAST BREAD	I	36.2	37.5	0.38	0.18	not inhib.
POULTRY FEED	I	34.0	37.5	0.48	0.18	not inhib.
POULTRY FEED	II	33.5	37.5	0.49	0.18	not inhib.
POULTRY FEED	III	33.5	37.5	0.47	0.18	not inhib.
POULTRY FEED	IV	34.0	37.5	0.47	0.18	not inhib.
POULTRY FEED	V	33.7	37.5	0.48	0.18	not inhib.
POULTRY FEED	VI	33.5	37.5	0.49	0.18	not inhib.
ANIMAL FEED PROTEIN	I	35.2	37.5	0.51	0.18	not inhib.
FINISHED FEED	I	33.5	37.5	0.51	0.18	not inhib.
STREAKY BACON	I	33.3	37.5	0.54	0.18	not inhib.
STREAKY BACON	II	33.8	37.5	0.51	0.18	not inhib.
STREAKY BACON	III	33.2	37.5	0.55	0.18	not inhib.
STREAKY BACON	IV	33.6	37.5	0.52	0.18	not inhib.
STREAKY BACON	V	32.9	37.5	0.52	0.18	not inhib.
STREAKY BACON	VI	34.2	37.5	0.48	0.18	not inhib.
SALAMI SAUSAGE	I	33.9	37.5	0.52	0.18	not inhib.
BEEF MEAT	I	33.3	37.5	0.49	0.18	not inhib.

Result after purification of sample CHOCOLATE CAKE with DNA Cleaning Columns:

sample no	replicate	CT _{IPC} sample	CT _{IPC} Cut-off	dRn _{IPC} sample	dRn _{IPC} Cut-off	Result
CHOCOLATE CAKE	I	34.3	38.2	0.28	0.09	not inhib.
CHOCOLATE CAKE	II	34.8	38.2	0.28	0.09	not inhib.
CHOCOLATE CAKE	III	34.3	38.2	0.30	0.09	not inhib.
CHOCOLATE CAKE	IV	34.3	38.2	0.29	0.09	not inhib.
CHOCOLATE CAKE	V	34.4	38.2	0.28	0.09	not inhib.
CHOCOLATE CAKE	VI	34.3	38.2	0.28	0.09	not inhib.