

Overcoming challenges in human saliva gene expression measurements

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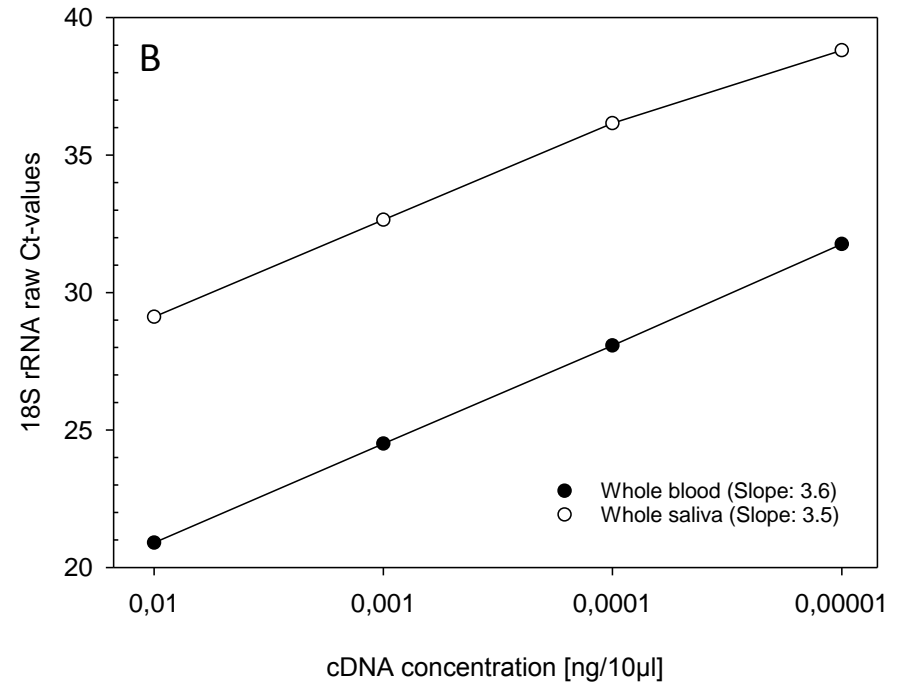
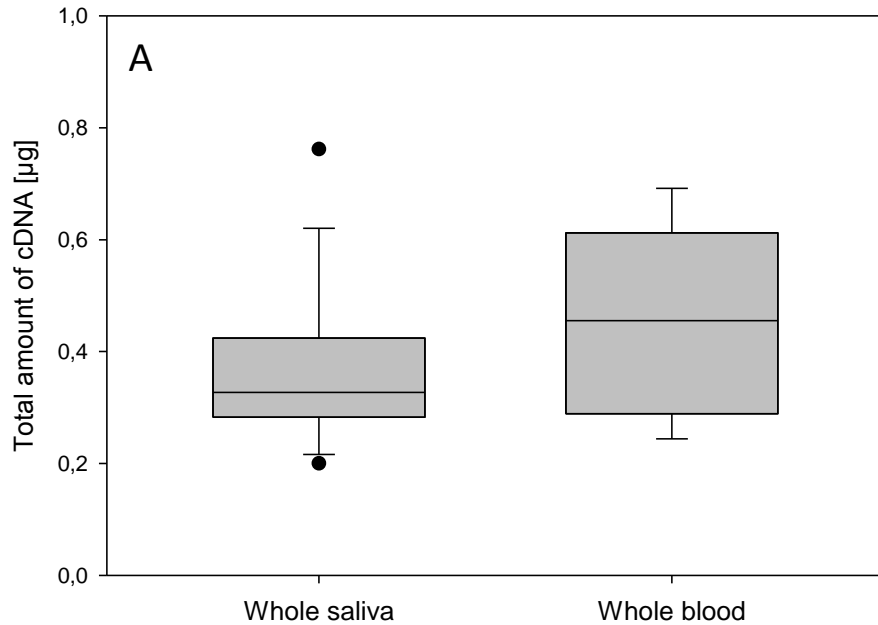
Number of tables: 3 (+1 supplement)

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Supplemental figure 1

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Supplemental figure 1

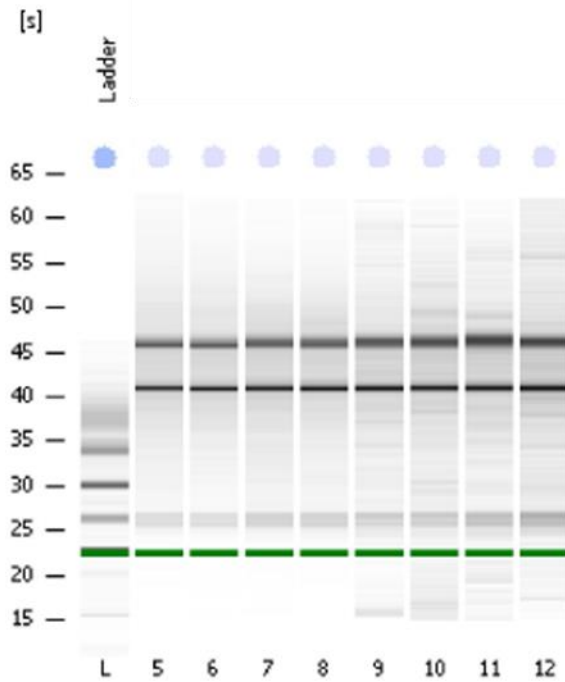
Box plots in (A) illustrate the total cDNA amounts (μg) measured fluorometrically (via Qubit 2.0 Fluorometer) from whole saliva ($n=12$) and whole blood ($n=6$). Input of 1 μg of total RNA was synthesized during cDNA synthesis previously.

Scatter plots in (B) display the results from cDNA dilution series and measurement of 18S rRNA raw Ct-values from whole saliva and whole blood samples using four different cDNA serial concentrations from 0.01 to 0.00001 $\text{ng}/10\mu\text{l}$. Slopes were calculated from the first-order linear regression analysis.

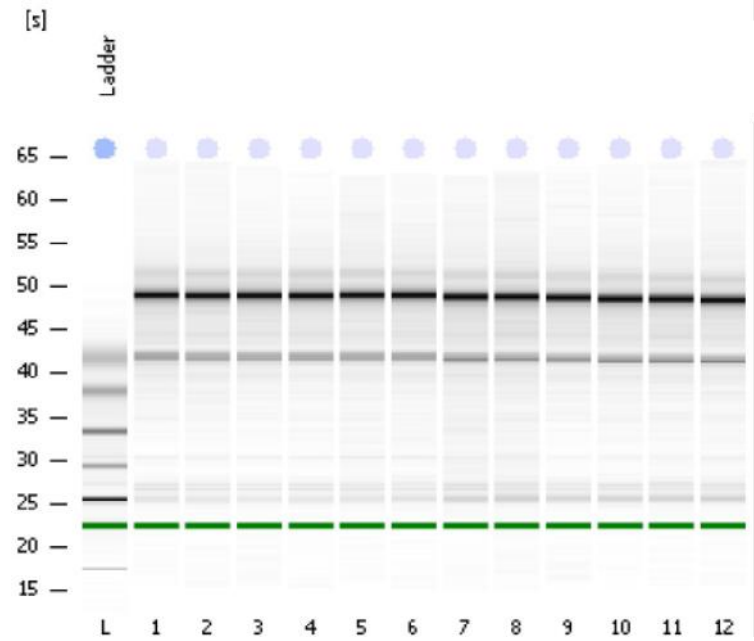
Supplemental figure 2

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A



B



Supplemental figure 2

These Gel-like images created by the Agilent bioanalyzer show the quality of isolated RNA and display bands of rRNA for eight randomly selected saliva samples as well as for six whole blood samples (duplicate measurements). They additionally depict the original versions of the cropped images of figure 1.

Supplemental table 1

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Gene	Sample ID	Pre-amplification	Measurements			Interpretation		
			Mean Ct values	<i>Difference observed vs expected</i>	Manufacturer predicts success	<u>Our findings:</u> works?	Agreement?	
<i>observed</i>	<i>expected</i>							
CDKN1A	1	<i>0x</i>	31.3					
		<i>10x</i>	21.3	21.3	-0.02	yes	yes	yes
		<i>14x</i>	17.5	17.3	0.20	yes	yes	yes
	2	<i>0x</i>	30.6					
		<i>10x</i>	20.6	20.6	-0.04	yes	yes	yes
		<i>14x</i>	16.9	16.6	0.26	yes	yes	yes
	3	<i>0x</i>	33.0					
		<i>10x</i>	22.9	23.0	-0.13	yes	yes	yes
		<i>14x</i>	19.0	19.0	0.01	yes	yes	yes
	4	<i>0x</i>	31.5					
		<i>10x</i>	21.5	21.5	-0.02	yes	yes	yes
		<i>14x</i>	17.8	17.5	0.31	yes	yes	yes
5	<i>0x</i>	33.9						
	<i>10x</i>	24.5	23.9	0.54	yes	yes	yes	
	<i>14x</i>	20.5	19.9	0.59	yes	yes	yes	
6	<i>0x</i>	32.6						
	<i>10x</i>	22.6	22.6	0.03	yes	yes	yes	
	<i>14x</i>	18.7	18.6	0.12	yes	yes	yes	
7	<i>0x</i>	33.1						
	<i>10x</i>	23.3	23.1	0.25	yes	yes	yes	
	<i>14x</i>	19.7	19.1	0.57	yes	yes	yes	
8	<i>0x</i>	34.9						
	<i>10x</i>	24.7	24.9	-0.25	yes	yes	yes	
	<i>14x</i>	20.8	20.9	-0.15	yes	yes	yes	
9	<i>0x</i>	34.9						
	<i>10x</i>	25.3	24.9	0.43	yes	yes	yes	
	<i>14x</i>	21.0	20.9	0.11	yes	yes	yes	
10	<i>0x</i>	35.0						
	<i>10x</i>	25.2	25.0	0.14	yes	yes	yes	
	<i>14x</i>	21.3	21.0	0.33	yes	yes	yes	
11	<i>0x</i>	n/a						
	<i>10x</i>	34.2	n/a	n/a	no	no	yes	
	<i>14x</i>	27.6	n/a	n/a	no	no	yes	
12	<i>0x</i>	n/a						
	<i>10x</i>	32.6	n/a	n/a	no	no	yes	
	<i>14x</i>	27.3	n/a	n/a	no	no	yes	

CDKN1A	mean	<i>0x</i>	33.1					
		<i>10x</i>	23.2	23.1	0.09	yes	yes	yes
		<i>14x</i>	19.3	19.1	0.24			

Gene	Sample ID	Pre-amplification	Measurements			Interpretation		
			Mean Ct values		Difference observed vs expected	Manufacturer predicts success	Our findings: works?	Agreement?
			observed	expected				
DDB2	1	<i>0x</i>	35.8					
		<i>10x</i>	26.7	25.8	0.97	no	no	yes
		<i>14x</i>	22.9	21.8	1.17	no	no	yes
	2	<i>0x</i>	36.1					
		<i>10x</i>	26.3	26.1	0.20	no	yes	no
		<i>14x</i>	22.6	22.1	0.52	no	yes	no
	3	<i>0x</i>	36.5					
		<i>10x</i>	27.7	26.5	1.20	no	no	yes
		<i>14x</i>	24.1	22.5	1.57	no	no	yes
	4	<i>0x</i>	36.3					
		<i>10x</i>	26.4	26.3	0.17	no	yes	no
		<i>14x</i>	22.5	22.3	0.29	no	yes	no
	5	<i>0x</i>	n/a					
		<i>10x</i>	30.5	n/a	n/a	no	no	yes
		<i>14x</i>	25.4	n/a	n/a	no	no	yes
	6	<i>0x</i>	n/a					
		<i>10x</i>	27.5	n/a	n/a	no	no	yes
		<i>14x</i>	23.6	n/a	n/a	no	no	yes
	7	<i>0x</i>	n/a					
		<i>10x</i>	27.9	n/a	n/a	no	no	yes
		<i>14x</i>	23.9	n/a	n/a	no	no	yes
	8	<i>0x</i>	36.1					
		<i>10x</i>	29.0	26.1	2.97	no	no	yes
		<i>14x</i>	24.1	22.1	1.98	no	no	yes
	9	<i>0x</i>	n/a					
		<i>10x</i>	n/a	n/a	n/a	no	no	yes
		<i>14x</i>	28.2	n/a	n/a	no	no	yes
	10	<i>0x</i>	n/a					
		<i>10x</i>	29.4	n/a	n/a	no	no	yes
		<i>14x</i>	25.8	n/a	n/a	no	no	yes
	11	<i>0x</i>	n/a					
		<i>10x</i>	n/a	n/a	n/a	no	no	yes
		<i>14x</i>	n/a	n/a	n/a	no	no	yes
	12	<i>0x</i>	n/a					
		<i>10x</i>	n/a	n/a	n/a	no	no	yes
		<i>14x</i>	n/a	n/a	n/a	no	no	yes

DDB2	<i>mean</i>	<i>0x</i>	36.1					
		<i>10x</i>	27.9	26.1	1.8	no	no	yes
		<i>14x</i>	24.5	22.1	2.33			

Genes	Sample ID	Pre-amplification	Measurements			Interpretation		
			Mean Ct values <i>observed</i>	<i>expected</i>	<i>Difference observed vs expected</i>	Manufacturer predicts success	Our findings: works?	Agreement?
FDXR	1	0x	n/a					
		10x	27.6	n/a	n/a			
		14x	23.0	23.6	-0.60	no	yes	no
	2	0x	36.7					
		10x	26.4	26.7	-0.26	no	yes	no
		14x	22.3	22.7	-0.35	no	yes	no
	3	0x	n/a					
		10x	28.7	n/a	n/a			
		14x	23.9	24.7	-0.72	no	yes	no
	4	0x	37.7					
		10x	27.2	27.7	-0.47	no	yes	no
		14x	22.7	23.7	-0.96	no	yes	no
5	0x	n/a						
	10x	30.5	n/a	n/a				
	14x	26.1	26.5	-0.40	no	yes	no	
6	0x	37.6						
	10x	30.0	27.6	2.32	no	no	yes	
	14x	24.4	23.6	0.73	no	yes	no	
7	0x	n/a						
	10x	28.6	n/a	n/a				
	14x	24.6	24.6	0.06	no	yes	no	
8	0x	n/a						
	10x	30.3	n/a	n/a				
	14x	25.9	26.3	-0.36	no	yes	no	
9	0x	n/a						
	10x	30.1	n/a	n/a				
	14x	25.5	26.1	-0.58	no	yes	no	
10	0x	n/a						
	10x	28.9	n/a	n/a				
	14x	25.2	24.9	0.31	no	yes	no	
11	0x	n/a						
	10x	n/a	n/a	n/a	no	no	yes	
	14x	n/a	n/a	n/a	no	no	yes	
12	0x	n/a						
	10x	31.0	n/a	n/a	no	no	yes	
	14x	n/a	27.0	n/a	no	no	yes	

FDXR	mean	0x	37.4					
		10x	28.4	27.4	1.02	no	yes	no
		14x	23.9	23.4	0.49			

Genes Sample ID Pre-amplification			Measurements			Interpretation		
			Mean Ct values <i>observed expected</i>	<i>Difference observed vs expected</i>	Manufacturer predicts success	<u>Our</u> findings: works?	Agreement?	
GAPDH	1	<i>0x</i>	31.4					
		<i>10x</i>	26.3	21.4	4.92	yes	no	no
		<i>14x</i>	23.4	17.4	6.05	yes	no	no
	2	<i>0x</i>	31.2					
		<i>10x</i>	25.7	21.2	4.50	yes	no	no
		<i>14x</i>	22.6	17.2	5.34	yes	no	no
	3	<i>0x</i>	31.9					
		<i>10x</i>	27.6	21.9	5.72	yes	no	no
		<i>14x</i>	24.7	17.9	6.78	yes	no	no
	4	<i>0x</i>	31.3					
		<i>10x</i>	27.0	21.3	5.70	yes	no	no
		<i>14x</i>	23.9	17.3	6.55	yes	no	no
5	<i>0x</i>	35.4						
	<i>10x</i>	30.8	25.4	5.41	no	no	yes	
	<i>14x</i>	27.5	21.4	6.03	no	no	yes	
6	<i>0x</i>	32.7						
	<i>10x</i>	28.4	22.7	5.73	yes	no	no	
	<i>14x</i>	25.4	18.7	6.69	yes	no	no	
7	<i>0x</i>	38.1						
	<i>10x</i>	37.3	28.1	9.20	no	no	yes	
	<i>14x</i>	34.5	24.1	10.43	no	no	yes	
8	<i>0x</i>	33.3						
	<i>10x</i>	28.6	23.3	5.35	yes	no	no	
	<i>14x</i>	25.3	19.3	5.98	yes	no	no	
9	<i>0x</i>	35.3						
	<i>10x</i>	30.9	25.3	5.53	no	no	yes	
	<i>14x</i>	27.7	21.3	6.42	no	no	yes	
10	<i>0x</i>	34.6						
	<i>10x</i>	30.3	24.6	5.71	yes	no	no	
	<i>14x</i>	27.2	20.6	6.62	yes	no	no	
11	<i>0x</i>	n/a						
	<i>10x</i>	n/a	n/a	n/a	no	no	yes	
	<i>14x</i>	n/a	n/a	n/a	no	no	yes	
12	<i>0x</i>	n/a						
	<i>10x</i>	n/a	n/a	n/a	no	no	yes	
	<i>14x</i>	35.8	n/a	n/a	no	no	yes	

GAPDH	mean	<i>0x</i>	33.5					
		<i>10x</i>	29.3	23.5	5.78	yes	no	no
		<i>14x</i>	26.2	19.5	6.69			

Supplemental table 1

The table depicts qRT-PCR results (single raw threshold cycle [Ct]-values, duplicate measurements not shown) of four gene targets (*CDKN1A*, *FDXR*, *DDB2* and *GAPDH*) for all saliva samples. Shown are the measured/observed mean Ct values for unamplified samples as well as for samples after 10X and 14X pre-amplification. The column for expected mean Ct values shows the calculated Ct values when estimating a 100% linear and unbiased 10X and 14X pre-amplification (steps by 10 and 14, taking the mean Ct value without pre-amplification). The ΔCt (observed vs expected) is shown for each gene and each pre-amplification. We categorized the data by whether unbiased pre-amplification should have worked according to the manufacturer (Ct value without pre-amplification <35, yes) or not (Ct value without pre-amplification >35, no). For *FDXR* the interpretation whether we could show linear pre-amplification, when no Ct value was available without pre-amplification, was done according to the steps between 10X and 14X pre-amplification. The data are further presented if pre-amplification worked linear (yes) or not (no) according to the amplification biases through deviating expression patterns between amplified and un-amplified material.