

Supplementary material

Table S1. Values obtained for each individual RNA purification, including amount, purity and efficiency.

Collector	Kit	Total Volum (µl)	Total RNA (ng)		A _{260/280}	Efficiency (ng total RNA/µl saliva)	collector	Kit	Total RNA (ng)		A _{260/280}	Efficiency (ng total RNA/µl saliva)	collector	Kit	Total RNA (ng)		A _{260/280}	Efficiency (ng total RNA/µl saliva)	
50ml tube	MV	20	87.12	4207.1		8.41	Salimetrics	MV	29.04	457.04	1	0.91	Oragene	MV	514.8	1628.8	1.84	3.26	
50ml tube	MV	50	4120		1.9		Salimetrics	MV	428		2.54		Oragene	MV	1114		2.22		
50ml tube	MV	20	10.56	798.56	0.32	1.60	Salimetrics	MV	510.84	1528.84	1.74	3.06	Oragene	MV	545.82	1355.82	1.55	2.71	
50ml tube	MV	50	788		1.81		Salimetrics	MV	1018		1.36		Oragene	MV	810		2.15		
50ml tube	MV	20	275.88	4725.9	1.81	9.45	Salimetrics	MV	907.5	2017.5	1.8	4.04	Oragene	MV	2158.86	5108.86	1.86	10.22	
50ml tube	MV	50	4450		1.8		Salimetrics	MV	1110		1.42		Oragene	MV	2950		2.04		
50ml tube	MV	20	506.22	1966.2	2.37	3.93	Salimetrics	MV	113.52	473.52	1.81	0.95	Oragene	MV	1716.66	2338.66	2.17	4.68	
50ml tube	MV	50	1460		2.1		Salimetrics	MV	360		0.84		Oragene	MV	622		2.14		
50ml tube	MV	20	458.7	2870.7	2.03	5.74	Salimetrics	MV	1178.1	2078.1	1.85	4.16	Oragene	MV	939.84	1393.84	1.67	2.79	
50ml tube	MV	50	2412		1.73		Salimetrics	MV	900		1.49		Oragene	MV	454		1.54		
		Average	2913.7		5.83				Average	1311		2.62				Average	2365.2		4.73
50ml tube	NS	20	1707.2	1.73	1.90		Salimetrics	NS		114.4	1.14	0.13		Oragene	NS		2786.4	1.98	3.10
50ml tube	NS	20	338.4	1.67	0.38		Salimetrics	NS		396.8	1.78	0.44		Oragene	NS		912	1.71	1.01
50ml tube	NS	20	1107.2	1.8	1.23		Salimetrics	NS		659.2	1.63	0.73		Oragene	NS		12029.6	1.62	13.37
50ml tube	NS	20	688	1.71	0.76		Salimetrics	NS		81.6	0.78	0.09		Oragene	NS		4294	1.85	4.77
50ml tube	NS	20	282.4	2.04	0.31		Salimetrics	NS		449.6	1.9	0.50		Oragene	NS		4991.2	1.58	5.55
		Average	824.64		0.92				Average	340.32		0.38				Average	5002.64		5.56
50ml tube	MR	20	512.8	1.83	0.85		Salimetrics	MR		0	2.25	0.00		Oragene	MR		542	2.08	0.90
50ml tube	MR	20	51.2	2.78	0.09		Salimetrics	MR		444.8	2.03	0.74		Oragene	MR		1624	1.78	2.71
50ml tube	MR	20	929.6	1.84	1.55		Salimetrics	MR		760.8	1.99	1.27		Oragene	MR		5144	1.9	8.57
50ml tube	MR	20	436.8	1.98	0.73		Salimetrics	MR		101.6	1.53	0.17		Oragene	MR		4469.6	1.72	7.45
50ml tube	MR	20	944.8	1.98	1.57		Salimetrics	MR		701.6	0.36	1.17		Oragene	MR		1398	0.83	2.33
		Average	575.04		0.96				Average	401.76		0.67				Average	2635.52		4.39

Table S2. P-values corresponding to the comparison of the total RNA amount obtained by the different combinations of saliva collectors and miRNA extraction kits applying the Wilcoxon Signed Rang test. In red, p< 0.05 (statistically not significant); in green, statistically significant p-values.

		50ml tube			Salimetrics			Oragene		
		MV	NS	MR	MV	NS	MR	MV	NS	MR
50ml tube	MV	-	0,0317	0,0317	0,151	0,0079	0,0079	0,6915	0,421	0,548
	NS	-	-	0,691	0,309	0,222	0,421	0,0556	0,0317	0,151
	MR	-	-	-	0,222	0,421	0,548	0,0079	0,0317	0,0317
Salimetrics	MV	-	-	-	-	0,0317	0,0952	0,421	0,556	0,421
	NS	-	-	-	-	-	0,841	0,0079	0,0079	0,0159
	MR	-	-	-	-	-	-	0,0079	0,0079	0,0317
Oragene	MV	-	-	-	-	-	-	-	0,421	1
	NS	-	-	-	-	-	-	-	-	0,309
	MR	-	-	-	-	-	-	-	-	-

The Wilcoxon Signed Rang test was used. In red, p< 0.05 (statistically not significant); in green, statistically significant p-values.

Table S3. P-values corresponding to the comparison of the miRNA extraction efficiency obtained by the different combinations of saliva collectors and miRNA extraction kits applying the Wilcoxon Signed Rang test. In red, p< 0.05 (statistically not significant); in green, statistically significant p-values.

		50ml tube			Salimetrics			Oragene		
		MV	NS	MR	MV	NS	MR	MV	NS	MR
50ml tube	MV	-	0,0159	0,0079	0,151	0,00794	0,00794	0,6905	0,691	0,548
	NS	-	-	1	0,0952	0,2222	0,5476	0,00794	0,0317	0,0317
	MR	-	-	-	0,0952	0,1412	0,5476	0,00794	0,0317	0,0317
Salimetrics	MV	-	-	-	-	0,00794	0,09524	0,4206	0,151	0,841
	NS	-	-	-	-	-	0,4206	0,00794	0,00794	0,00794
	MR	-	-	-	-	-	-	0,00794	0,0317	0,03175
Oragene	MV	-	-	-	-	-	-	-	0,691	0,463
	NS	-	-	-	-	-	-	-	-	0,691
	MR	-	-	-	-	-	-	-	-	-

The Wilcoxon Signed Rang test was used. In red, p< 0.05 (statistically not significant); in green, statistically significant p-values.

Table S4. Spike-in 3 and spike-in 6 Cp values obtained by the different saliva collectors and miRNA extraction kit combinations. Values are shown in Average and standard deviation (SD).

Spike-in	miRNA extraction kit	Saliva collector					
		50ml tube		Salimetrics		Oragene	
		Cq Average	Cq SD	Cq Average	Cq SD	Cq Average	Cq SD
UniSp3	MV	18,74	0,20	18,61	0,40	18,55	0,30
	NS	18,74	0,17	18,71	0,14	18,49	0,27
	MR	18,74	0,30	18,72	0,05	18,45	0,20
UniSp6	MV	18,56	0,56	18,39	0,55	18,29	0,19
	NS	18,37	0,29	18,26	0,36	18,38	0,39
	MR	18,24	0,22	17,97	0,14	18,57	0,56

Table S5. P-values corresponding to the comparison of the Cp values obtained from miRNA expression for the nine different combinations of collectors and extraction kits applying the Wilcoxon Signed Rang test. In red, p< 0.05 (statistically not significant); in green, statistically significant p-values.

hsa-iR-223-3p										
		MV			NS			MR		
		50ml ¹	Salim. ²	Orag. ³	50ml	Salim.	Orag.	50ml	Salim.	Orag.
MV	50ml		0,955	0,0635	0,336	0,0939	0,0007	0,0059	0,0059	0,0046
	Salim.			0,0343	0,0499	0,0148	0,0006	0,0019	0,0011	0,00054
	Orag.				0,3154	0,762	0,166	0,286	0,237	0,123
NS	50ml					0,574	0,0086	0,0281	0,0148	0,00312
	Salim.						0,0343	0,0499	0,0281	0,0266
	Orag.							0,829	0,356	0,529
MR	50ml								0,141	0,274
	Salim.									0,460
	Oragene									
hsa-miR-24-3p										
		MV			NS			MR		
		50ml	Salim.	Orag.	50ml	Salim.	Orag.	50ml	Salim.	Orag.
MV	50ml		0,189	0,161	0,152	0,336	0,0001	0,00031	0,0031	0,0001
	Salim.			0,0266	0,0104	0,01041	0,00005	0,00031	0,001933	0,00005
	Orag.				0,633	0,897	0,0115	0,00055	0,008729	0,00072
NS	50ml					0,721	0,0021	0,00109	0,007362	0,00005
	Salim.						0,0014	0,00109	0,01003	0,00005
	Orag.							0,02052	0,1418	0,041
MR	50ml								1	0,515
	Salim.									0,894
	Oragene									
hsa-miR-191-5p										
		MV			NS			MR		
		50ml	Salim.	Orag.	50ml	Salim.	Orag.	50ml	Salim.	Orag.
MV	50ml		0,1206	0,0097	0,867	0,955	0,0097	0,0401	0,0205	0,0019
	Salim.			0,0025	0,083	0,130	0,0007	0,00062	0,0029	0,00005
	Orag.				0,0062	0,0062	0,970	0,477	0,762	0,280
NS	50ml					0,798	0,0009	0,0104	0,0207	0,00018
	Salim.						0,0016	0,0148	0,0148	0,00018
	Orag.							0,307	0,450	0,140
MR	50ml								0,752	0,083
	Salim.									0,173
	Oragene									
hsa-miR-30c-5p										
		MV			NS			MR		
		50ml	Salim.	Orag.	50ml	Salim.	Orag.	50ml	Salim.	Orag.
MV	50ml		0,613	0,0068	0,613	0,354	0,0001	0,0140	0,0093	0,0021
	Salim.			0,0006	0,083	0,021	0,00005	0,0011	0,0011	0,0006
	Orag.				0,01554	0,02052	0,6842	0,5938	0,7221	0,273
NS	50ml					0,4418	0,00009	0,01041	0,01476	0,0008

	Salim.						0,00055	0,065	0,0281	0,0029
	Orag.							0,360	0,829	0,307
MR	50ml								0,328	0,068
	Salim.									0,351
	Orag.									

hsa-iR-375-3p

		MV			NS			MR		
		50ml	Salim.	Orag.	50ml	Salim.	Orag.	50ml	Salim.	Orag.
MV	50ml		0,628	0,0075	0,181	0,573	0,118	0,0126	0,0293	0,0196
	Salim.			0,0645	0,536	0,955	0,417	0,0721	0,0939	0,0874
	Orag.				0,0014	0,0044	0,0147	0,965	0,965	0,241
NS	50ml					0,959	0,101	0,0011	0,0355	0,0018
	Salim.						0,696	0,010	0,105	0,083
	Orag.							0,0014	0,237	0,0091
MR	50ml								0,674	0,286
	Salim.									0,625
	Oragene									

hsa-miR-27a-3p

		MV			NS			MR		
		50ml	Salim.	Orag.	50ml	Salim.	Orag.	50ml	Salim.	Orag.
MV	50ml		1	0,161	0,0093	0,014	0,0008	0,00031	0,014	0,0001
	Salim.			0,034	0,0011	0,0029	0,0004	0,00015	0,0104	0,00005
	Orag.				0,101	0,146	0,0002	0,00005	0,0085	0,00001
NS	50ml					0,959	0,011	0,0011	0,083	0,0021
	Salim.						0,154	0,0019	0,105	0,016
	Orag.							0,0019	0,398	0,212
MR	50ml								0,674	0,674
	Salim.									0,790
	Oragene									

hsa-iR-26b-5p

		MV			NS			MR		
		50ml	Salim.	Orag.	50ml	Salim.	Orag.	50ml	Salim.	Orag.
MV	50ml		0,429	0,333	0,667	0,889	0,267	0,178	0,044	0,909
	Salim.			0,945	0,534	0,628	0,950	0,020	0,00067	0,328
	Orag.				0,259	0,383	1	0,014	0,00031	0,252
NS	50ml					1	0,397	0,054	0,0037	0,681
	Salim.						0,281	0,040	0,0037	0,681
	Orag.							0,0104	0,00015	0,199
MR	50ml								0,574	0,236
	Salim.									0,059
	Orag.									

The Wilcoxon Signed Rang test was used. In red, p< 0.05 (statistically not significant); in green, statistically significant p-values. ¹ 50 ml tube, ² Salimetrics, ³ Oragene.