



ANALYSIS OF THE ANTI-HAIR LOSS EFFECTS OF 4 SAMPLES IN HUMAN FOLLICLE DERMAL PAPILLA CELLS



FINAL REPORT

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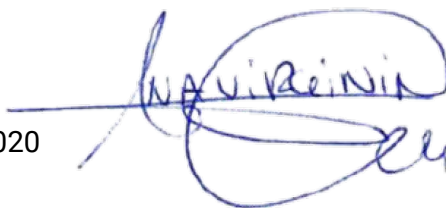
**ANALYSIS OF THE ANTI-HAIR LOSS EFFECTS
OF 4 SAMPLES IN HUMAN FOLLICLE DERMAL
PAPILLA CELLS**

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Executive Summary

GOAL: To determine the *in vitro* effects of 4 different samples (Mezcla 2, Mezcla 3, Mezcla 4, and Mezcla 5) on *SRD5A1* (5 α -Reductase type 1), *SRD5A2* (5 α -Reductase type 2) and *SRD5A3* (5 α -Reductase type 3) gene expression, after treatment in Human Follicle Dermal Papilla Cells (HFDPC).

METHODOLOGY: For gene expression analysis, HFDPC cells were treated with Mezcla 2, Mezcla 3, Mezcla 4, or Mezcla 5 for 24 hours at concentrations 0.001 % and 0.005 %, determined from previous studies. After that, total RNA was purified, quantified and it was used to synthesize complementary DNA (cDNA). This cDNA from treated or untreated cells (control) was used to determine the relative gene expression of *SRD5A1*, *SRD5A2* and *SRD5A3* through RT-qPCR. *Actin* (*ACT*) was used as reference gene. Data was statistically analysed.

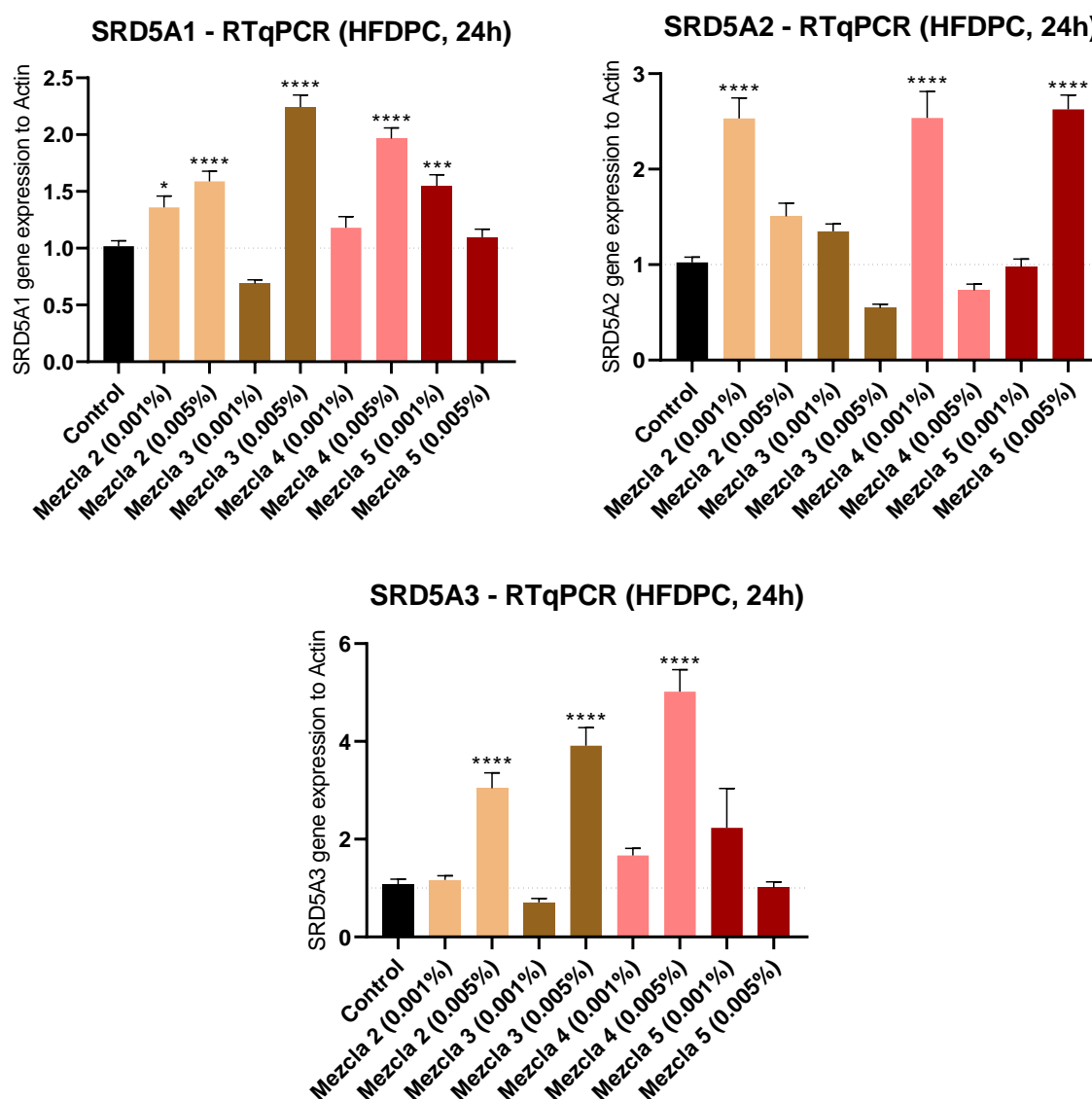
RESULTS: Results indicated that treatment with Mezcla 2 at 0.001 % concentration significantly increased the gene expression of *SRD5A1* and *SRD5A2*, whereas the treatment at 0.005 % significantly increased *SRD5A1* and *SRD5A3*, compared to the untreated control.

In the same way, the treatment with Mezcla 3 at 0.005 % concentration significantly increased the gene expression of *SRD5A1* and *SRD5A3*, compared to the untreated control. On the other hand, the treatment with Mezcla 3 at 0.001 % concentration decreased *SRD5A1* and *SRD5A3* gene expression by 32.8 ± 7.4 % and 36.9 ± 13.4 %, respectively, even though results were not statistically significant ($p > 0.05$), compared to the untreated control. The treatment with Mezcla 3 at 0.005 % concentrations also decreased the expression of *SRD5A2* by 47.8 ± 7.4 % ($p > 0.05$).

With regard to Mezcla 4, results indicated the treatment with Mezcla 4 at 0.001 % concentration significantly increased the expression of *SRD5A2*, whereas the treatment at 0.005 % concentration significantly increased the expression of *SRD5A1* and *SRD5A3*. On the other hand, the treatment with Mezcla 4 at 0.005 % concentration inhibited the expression of *SRD5A2* by 29.2 ± 8.5 %, even though results were not statistically significant ($p > 0.05$), compared to the untreated control.

Last, for Mezcla 5, results indicated the treatment at 0.001 % concentration significantly increased the expression of *SRD5A1*, whereas the treatment at 0.005 % significantly increased the expression of *SRD5A2*, compared to the untreated control.

CONCLUSION: The *in vitro* treatment with Mezcla 2, Mezcla 3, Mezcla 4, or Mezcla 5 at concentrations 0.001 % or 0.005 %, do not display any significant inhibition of *SRD5A1* (5 α -Reductase type 1), *SRD5A2* (5 α -Reductase type 2), or *SRD5A3* (5 α -Reductase type 3) gene expression, after 24 hours of treatment in Human Follicle Dermal Papilla Cells (HFDPC), compared to the untreated control. Specifically, most of the tested conditions resulted in a significant increase of gene expression, meaning the samples are not displaying an anti-hair loss effect through this biological pathway, but the opposite.



1 Title

Analysis of the anti-hair loss effects of 4 samples in Human Follicle Dermal Papilla Cells.

2 Introduction

Androgenetic alopecia (AGA) or also known as Male-pattern hair loss (MPHL) is the most common type of hair loss, affecting women (50 % of menopausal women and a large number of women of childbearing age, around 25 %), as well as males (over 70 % of adult men) [McElwee and Saphiro, 2012]. It occurs due to an underlying susceptibility of hair follicles to shrinkage due to the combined effect of two factors: Genetic predisposition (several loci are involved including AR, EDA2R/Chr. X-WNT10A/2q35, etc.) and hormonal stimulation [Liang et al., 2013; Rinaldi et al., 2016]. It is known that both genetic and environmental factors play a role, but many causes of AGA remain unknown.

The hair growth cycle consists of an anagen phase (a growth period of 2-6 years on average), a catagen phase (a period of involution, about 1-3 weeks) and a telogen phase (a rest period of about 1-3 months) [Geyfman et al., 2014] as shown in Figure 1. With androgenetic alopecia, under androgenic stimulation, there is a progressive reduction of the average duration of the anagen phase, at each hair growth cycle. The hair follicles become progressively smaller and the hair, shorter and thinner, is absent for longer periods (increased interval between the loss of the hair in telogen and its replacement with new hair), which contributes to worsen the thinned appearance. Hereditary predisposition determines the sensitivity of the follicle to male hormones and thus influences the age of onset and severity of the clinical picture [Ramos-E-Silva and Pirmez, 2013].

Androgens control the proliferation of human hair, which responds to hormones differently depending on the body location [Thornton et al., 1991]. Dermal papilla cells (DPCs) of the beard, armpit, and scalp hair of people who are genetically predisposed to baldness were shown to be androgen target cells [Randall, 2007]. The binding of androgens to their androgen receptors (ARs) decreases the anagen phase of the hair cycle. DPCs have particularly saturable ARs and are proliferated from androgen-responsive follicles. Compared to testosterone (T), 5 α -dihydrotestosterone (DHT) has an approximately five-fold higher

affinity for the AR [Rastegar et al., 2015].

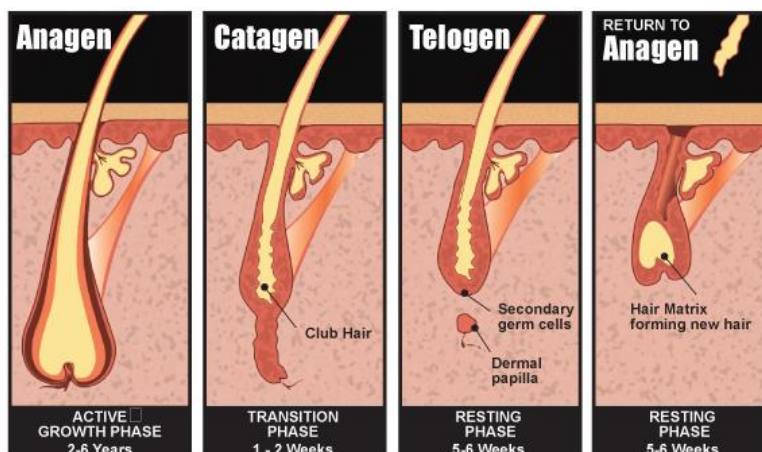
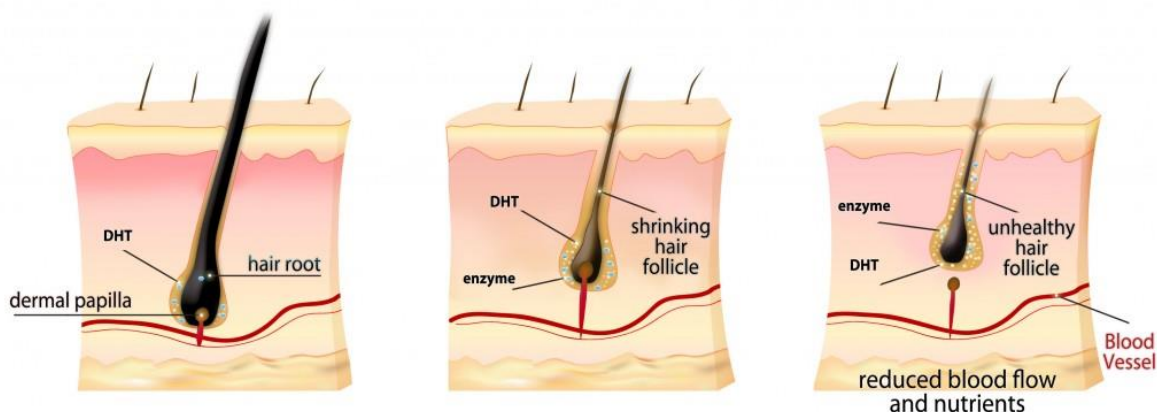


Figure 1. Hair growth phases. Graphical representation about hair growth phases (Anagen, Catagen and Telogen).

Enzymes 5 α -reductases convert testosterone to 5 α -dihydrotestosterone [Russell and Wilson, 1994] and this conversion enhance the androgenic signal via two mechanisms: First, DHT cannot be aromatized to estrogen and, therefore, its effect are solely androgenic and in second place, in vitro DHT binds to the AR with a higher affinity than testosterone does, preventing its usual action. A schematic representation of this process is shown in Figure 2. 5 α -reductases participate in 3 metabolic pathways: bile acid biosynthesis, androgen and estrogen metabolism, and prostate cancer. It is produced in many tissues in males and females, in the reproductive tract, testes and ovaries, skin, seminal vesicles and pilosebaceous units of hair follicles, among others [Agís-Balboa et al., 2006]. There are three isoenzymes of 5 α -reductase: steroid 5 α -reductase type 1, 2 and 3 (SRD5A1, SRD5A2 and SRD5A3) [Yamana et al., 2010]. Specifically, isoenzymes type 1 and 2 are highly present at pilosebaceous units in papillae of individual hair follicles [Bernard, 1994]. For these reasons, 5 α -reductase inhibitors (5-ARIs) have been widely used in the treatment of androgenic alopecia. These agents inhibit the enzyme activity, decreasing conversion of testosterone to DHT, leading to increased testosterone and estradiol; thus preventing hair follicles to shrink and reduce the flow of blood and nutrients.



DHT causes hair follicles to shrink, which reduces the flow of blood and nutrients to the hair follicle

Figure 2. Representation of androgenetic alopecia process. Schematic representation about the process occurred in androgenetic alopecia, where DHT causes hair follicles to shrink, which reduced the flow of blood and nutrients to the hair follicle, causing alopecia.

It is widely documented that 5 α -reductase enzymes are involved in androgenetic alopecia and that 5 α -reductase inhibitors are beneficial treatments for anti-hair loss due to androgenetic alopecia. For these reasons, in this assay, the capacity of Mezcla 2, Mezcla 3, Mezcla 4, and Mezcla 5 to inhibit the expression of *SRD5A1* (5 α -Reductase type 1), *SRD5A2* (5 α -Reductase type 2) and *SRD5A3* (5 α -Reductase type 3) through RT-qPCR, was assessed *in vitro* after 24 hours of treatment in Human Follicle Dermal Papilla Cells (HFDPC).

3 Products tested

The following products were received in Bionos on 30/11/2020 at room temperature, and labelled as indicated:

Internal Code	Sample	Abbreviation
P.1805	Octanoic acid 99 % Code 129390010 Lot A0417435	Caprílico
P.1806	Decanoic acid 99 % Lot 10222156	Cáprico
P.1807	Lauric acid 99% Code 167280010 Lot A0417432	Láurico
P.1808	Myristic acid 99 % Code 156960010 Lot A0417108	Mirístico
P.1863	Palmitic acid 98 % Code 129700010 Lot A0416993	Palmítico
P.1864	Palmitoleic acid 98 % Lot A18XC6153	Palmitoleico
P.1865	Stearic Acid 97 % Code 174490010 Lot A0423400	Esteárico
P.1866	Oleic Acid 99 % Lot: M22G032	Oleico

Mezcla 2, Mezcla 3, Mezcla 4, and Mezcla 5 were prepared in Bionos Biotech's laboratory, according to the following table:

	MEZCLA 2	MEZCLA 3	MEZCLA 4	MEZCLA 5
Caprílico (C8:0)	1,00%	1,00%	1,00%	1,00%
Cáprico (C10:0)	1,00%	1,00%	1,00%	1,00%
Láurico (C12:0)	5,70%	3,20%	3,16%	19,70%
Mirístico (C14:0)	4,40%	5,50%	12,70%	9,70%
Palmítico (C16:0)	75,10%	27,10%	13,00%	14,80%
Palmitoleico (C16:1)	1,90%	18,50%	1,70%	0,20%
Esteárico (C18:0)	1,90%	0,30%	2,30%	3,80%
Oleico (C18:1)	9,10%	43,40%	36,60%	49,80%

Table 1. Table of composition for the 4 different samples. Analytical composition of the 4 different samples used for the experiments. Mezcla 4 was completed with water until 100 % formulation.

Samples were stored at room temperature in our facilities to avoid alteration until the start of the experiment and dilutions were freshly prepared each time.

4 Registration date

Study begins: 13/10/2020

Study ends: 15/12/2020

Experimental phase begins: 01/12/2020

Experimental phase ends: 11/12/2020

5 Platform

Human Follicle Dermal Papilla Cells, HFDPC.

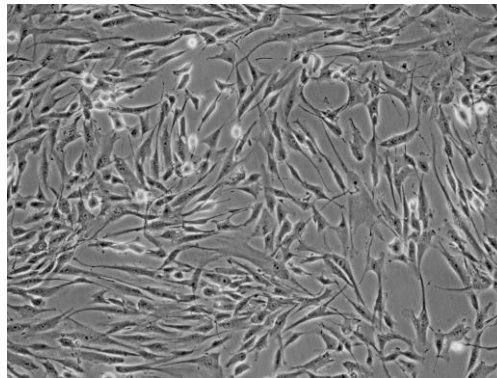


Figure 3. Human Follicle Dermal Papilla Cells. Microscope image of Human Follicle Dermal Papilla Cells (HFDPC), used during the studies.

6 Material and methods

6.1 Analytical equipment

Stereoscopic microscope, incubator, refrigerated centrifuge, statistical analysis software, laminar flow hood, Bürker chamber, micropipettes, tips, pipettes, propipette, rack, quantifier Nano-Drop spectrophotometer, Quant studio 5 (Applied Biosystem) Quantitative real-time PCR, vortex, precision balance, heating block and consumables.

6.2 Reagents

HFDPC culture medium (Promocell), nutrient solution mix (Promocell), Phosphate buffered saline (Sigma), Trypan Blue Solution (Bio-Rad), Ethanol (Sigma-Aldrich), MTT reagent (Invitrogen), DMSO (SIGMA), RNase free-DNase (Qiagen), PrimeScript RT Reagent (Perfect Real Time- Takara Clontech), Oligonucleotides for RT-PCR amplification of *SRD5A1*, *SRD5A2*, *SRD5A3* and β -*ACT*, SYBR® qRT-PCR, liquid nitrogen.

6.3 Procedure

For seeding cells, cell numbers and viability were determined using Trypan-Blue staining and counting in a Bürker chamber under the microscope.

For the main gene expression assay, HFDPC cells were cultured at a 300.000 cells/well density in a 6-well plate, in growth medium. 24 hours later, the medium was removed and Mezcla 2, Mezcla 3, Mezcla 4, or Mezcla 5 at 0.001 % and 0.005 % concentration were added to cells. After 24 hours of incubation period, cells were washed with PBS buffer and collected in lysis buffer to proceed with RNA extraction. Total RNA was extracted using RNeasy kit (Qiagen) and treated with DNase-I to remove any contamination from genomic DNA. RNA quality and quantity were checked in a Nano-Drop spectrophotometer, and 500 µg of total RNA was used to synthesize cDNA, using First-strand Synthesis kit (Takara-Clontech). The suitability of each primer pair used in this study for RT-qPCR, *SRD5A1*, *SRD5A2*, *SRD5A3* and *ACT* was previously evaluated to determine melting curves, efficiency of amplification and specificity of the primers. Finally, quantitative PCR (qPCR) was performed in a real time PCR machine (QuantStudio 5, Applied BioSystem).

To perform raw data analysis, we used the Pfaffl method [Pfaffl, 2001] to calculate the gene relative expression ratio to *ACT* (internal control- housekeeping gene). Mathematical

model of relative expression ratio in real-time PCR is shown in Figure 4. Statistical analysis to determine significant changes was performed using Student's t-test. For all data a level of 5% or less ($p < 0.05$) was taken as statistically significant.

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta\text{CP}_{\text{target}}(\text{control} - \text{sample})}}{(E_{\text{ref}})^{\Delta\text{CP}_{\text{ref}}(\text{control} - \text{sample})}}$$

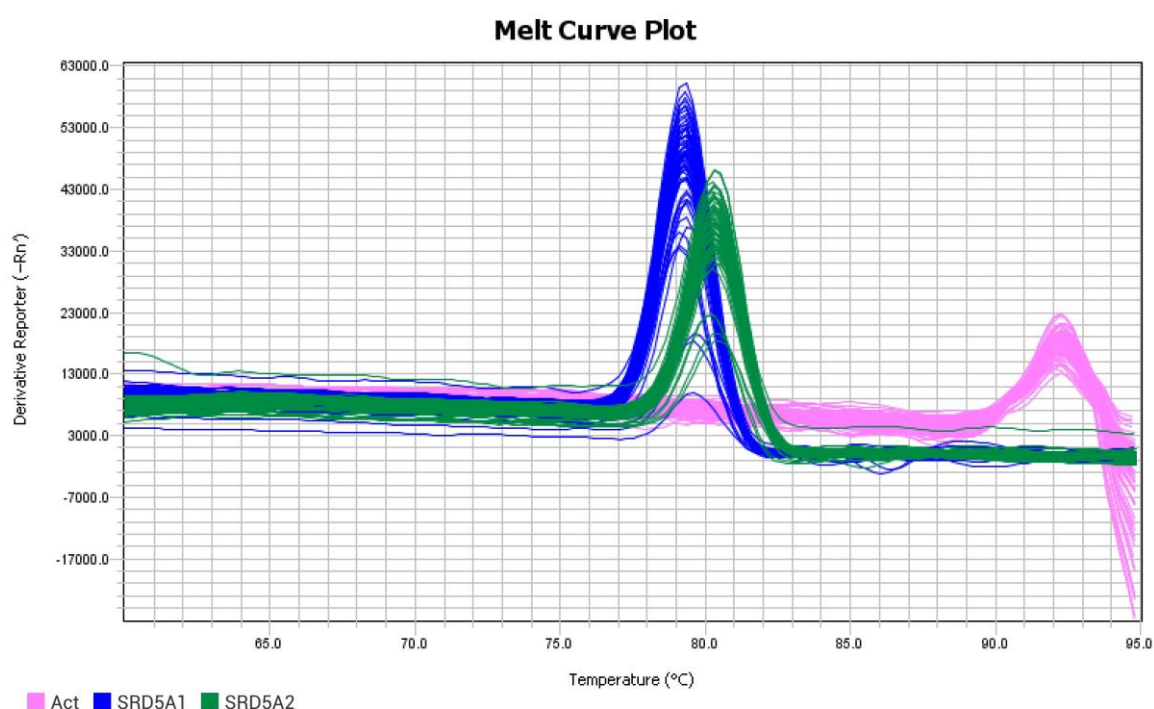
Figure 4. Mathematical model of relative expression ratio used in real-time PCR data analysis. The ratio of a target gene is expressed in a sample versus a control in comparison to a reference gene. E_{target} is the real-time PCR efficiency of target gene transcript; E_{ref} is the real-time PCR efficiency of a reference gene transcript; $\Delta\text{CP}_{\text{target}}$ is the CP deviation control – sample of the target gene transcript; $\Delta\text{CP}_{\text{ref}}$ = CP deviation of control – sample of reference gene transcript.

7 Results

The anti-hair loss capacity of 4 different samples was assessed on HFDPC in culture by quantifying the expression of *SRD5A1*, *SRD5A2* and *SRD5A3* through RT-qPCR. 0.001 % and 0.005 % concentrations were selected from previous experiments.

7.1 Primer pair validation

To evaluate the correct amplification and specificity of primer pairs for the genes of interest, melting curves for each primer pair were performed. Results showed an efficient amplification of the genes, with a single peak in the melting point, indicating the high specificity of the primers and their suitability for real-time application, as they do not form primer-dimer structures. The melting curves for each primer pair used in the assay are shown below. As shown, all the oligos amplify one amplicon and the melting temperatures are in the same range.



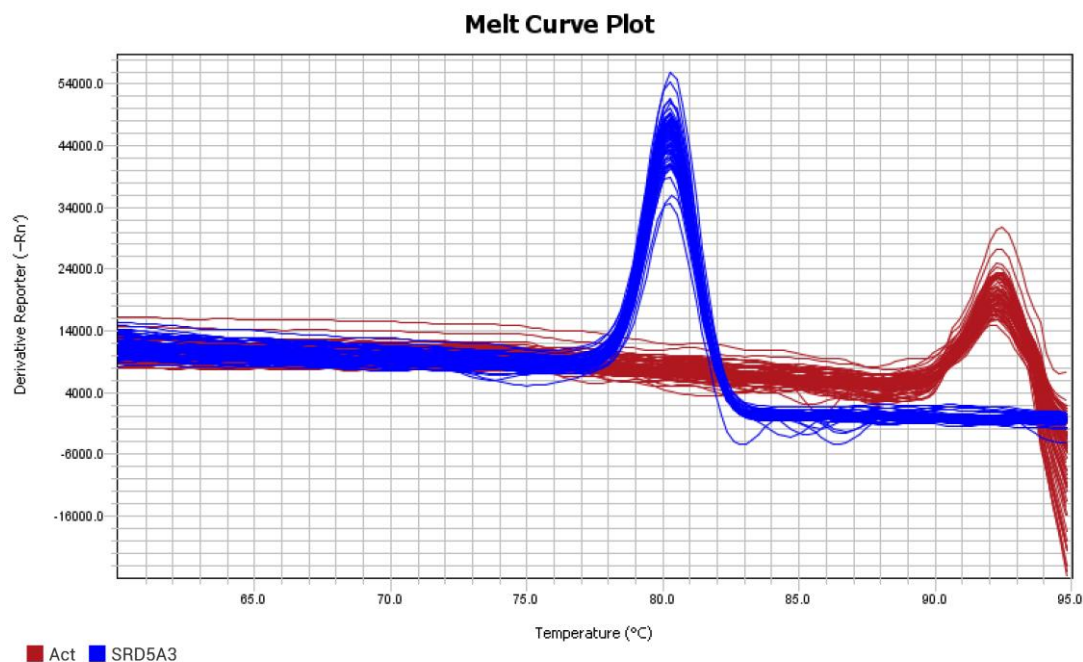
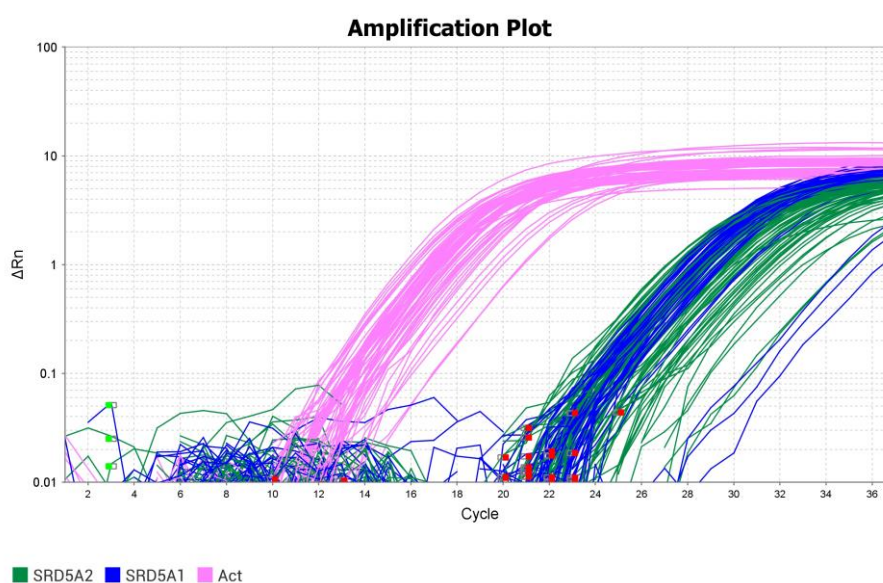
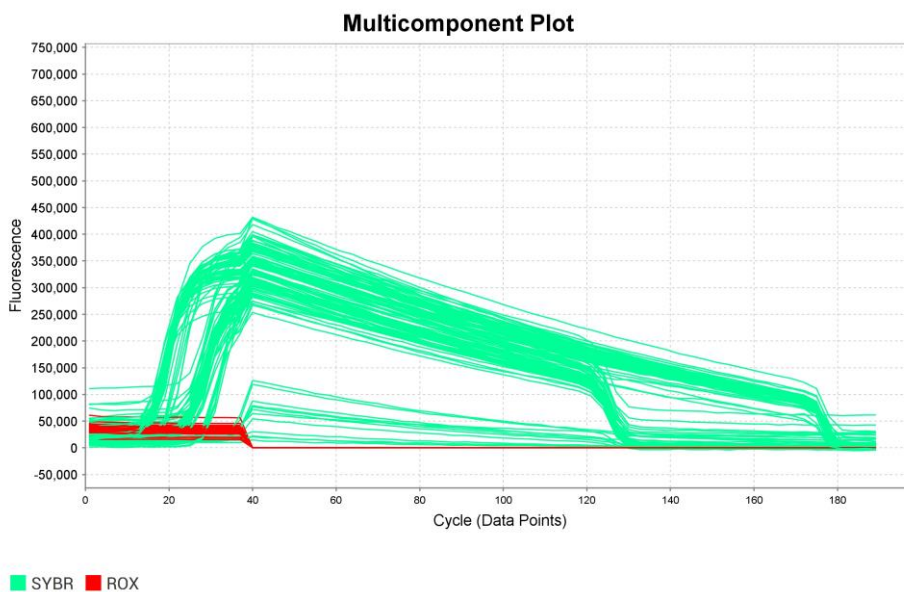
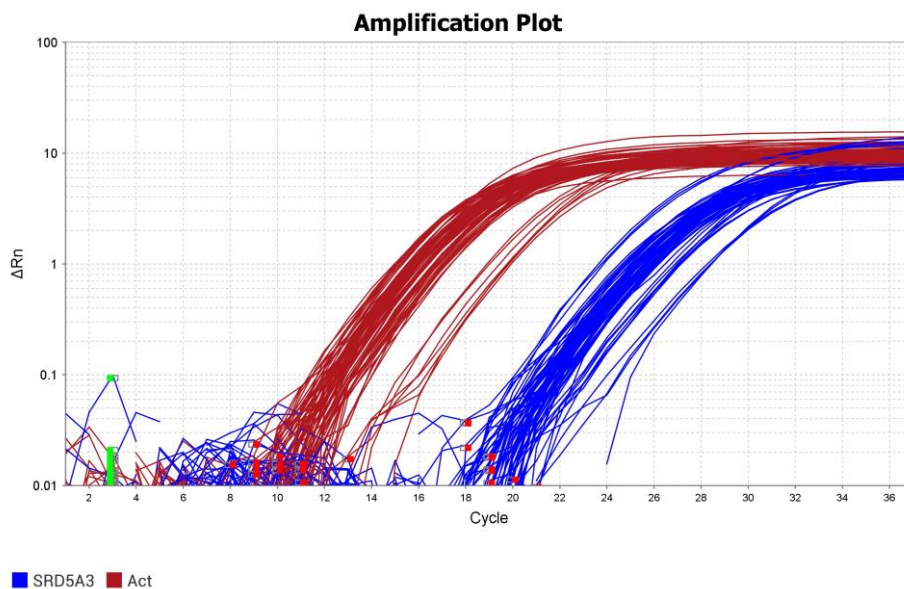


Figure 5. Melt Curve Plot. Melting curves showing a single peak for all the oligos used in this qPCR assay.

In Figure 6, it is shown a plot of the technical parameters considered for the analysis of the data. All together the parameters indicate that the efficiency of the PCR reaction was optimal.





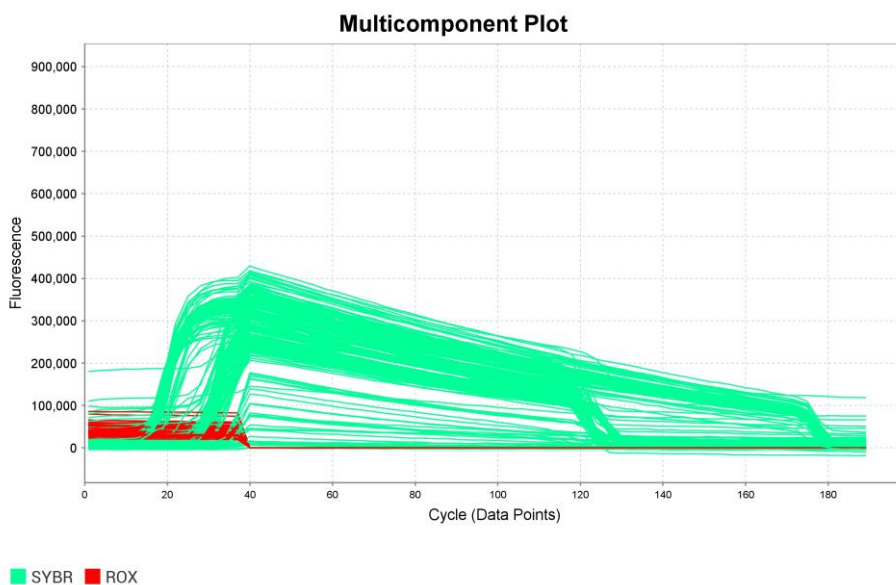


Figure 6. Parameters evaluated to check technical quality of the RT-qPCR reaction. Amplification plot vs cycle represent the magnitude of normalized fluorescence signal generated by the reporter at each cycle during PCR amplification; in plot amplification Ct vs Well, Ct indicates the PCR cycle number at which the fluorescence meets the threshold in the amplification plot; good Ct values should stay in the range >8-35<; the plot Fluorescence vs Cycle indicates the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference. All these parameters allow identifying and examining irregularity in the amplification.

7.2 Gene expression quantification by qPCR

mRNA expression levels were determined after treatment with the product tested in Human Follicle Dermal Papilla Cells (HFDPC) for 24 hours. *SRD5A1*, *SRD5A2*, *SRD5A3* and *ACT* (internal control) were amplified using four technical replicates of cDNAs. All the results are shown in Figure 7 and Table 2.

Results indicated that treatment with Mezcla 2 at 0.001 % concentration significantly increased the gene expression of *SRD5A1* and *SRD5A2*, whereas the treatment at 0.005 % significantly increased *SRD5A1* and *SRD5A3*, compared to the untreated control.

In the same way, the treatment with Mezcla 3 at 0.005 % concentration significantly increased the gene expression of *SRD5A1* and *SRD5A3*, compared to the untreated control. On the other hand, the treatment with Mezcla 3 at 0.001 % concentration decreased *SRD5A1* and *SRD5A3* gene expression by 32.8 ± 7.4 % and 36.9 ± 13.4 %, respectively, even though results were not statistically significant ($p > 0.05$), compared to the untreated control. The treatment with Mezcla 3 at 0.005 % concentrations also decreased the expression of *SRD5A2* by 47.8 ± 7.4 % ($p > 0.05$).

With regard to Mezcla 4, results indicated the treatment with Mezcla 4 at 0.001 % concentration significantly increased the expression of *SRD5A2*, whereas the treatment at 0.005 % concentration significantly increased the expression of *SRD5A1* and *SRD5A3*. On the other hand, the treatment with Mezcla 4 at 0.005 % concentration inhibited the expression of *SRD5A2* by 29.2 ± 8.5 %, even though results were not statistically significant ($p > 0.05$), compared to the untreated control.

Last, for Mezcla 5, results indicated the treatment at 0.001 % concentration significantly increased the expression of *SRD5A1*, whereas the treatment at 0.005 % significantly increased the expression of *SRD5A2*, compared to the untreated control.

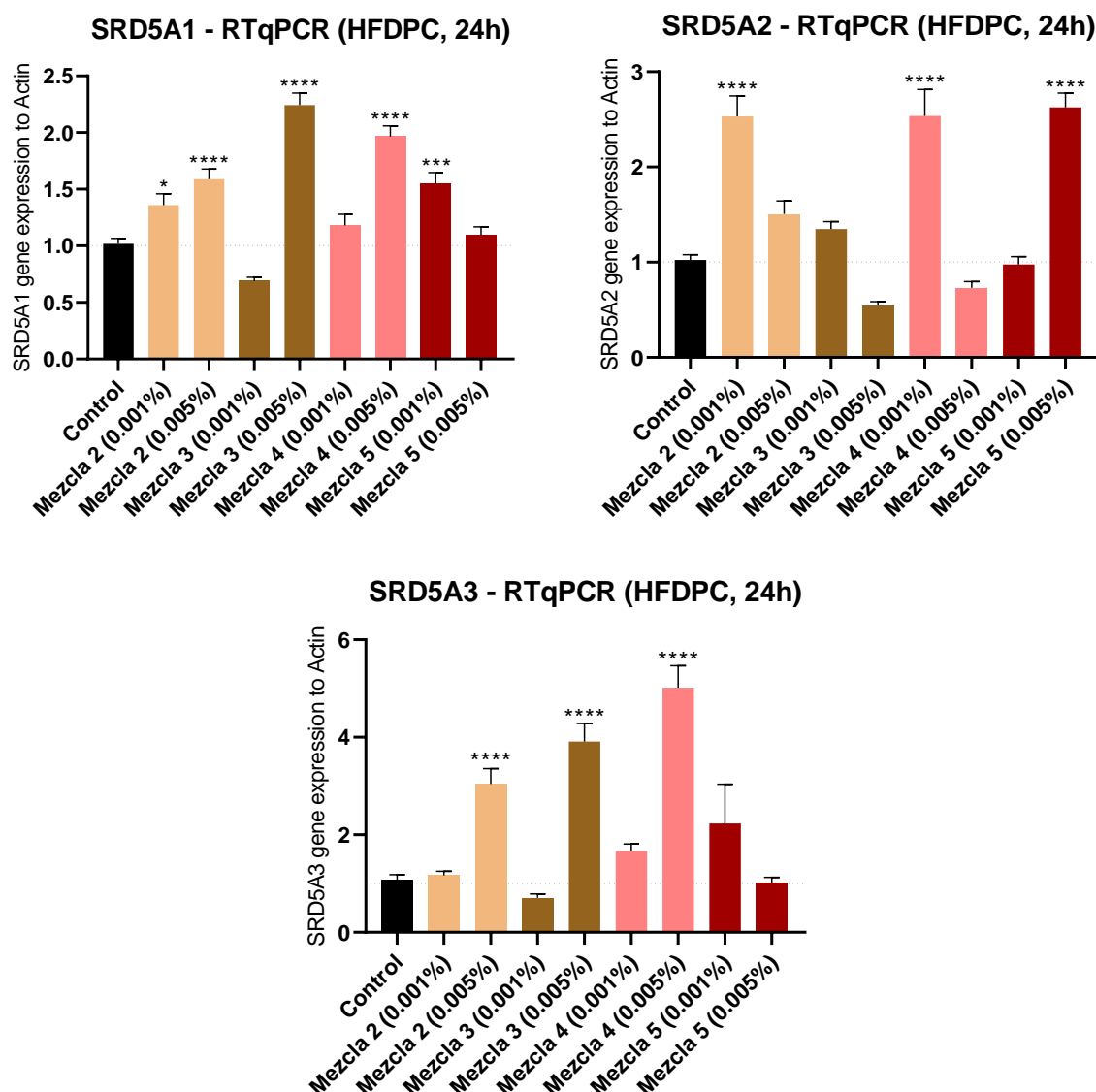


Figure 7. Gene expression results. Bar graphs showing SRD5A1, SRD5A2 and SRD5A3 gene expression results after treating HFDPC cells with Mezcla 2, Mezcla 3, Mezcla 4, or Mezcla 5, at 0.001 % and 0.005 % concentrations, during 24 hours, compared to untreated Control. * Represents statistical significance with p value < 0.05 . *** Represents statistical significance with p -value < 0.001 . **** Represents statistical significance with p value < 0.0001 .

SRD5A1 – RT-qPCR								
Dunnett's multiple comparisons test	Mean Diff,	95% CI of diff,	Significant?	Summary	Adjusted P Value	A-?		
Control vs. Mezcla 2 (0.001%)	-0,3414	-0,6513 to -0,03161	Yes	*	0,0234	B		
Control vs. Mezcla 2 (0.005%)	-0,5700	-0,8798 to -0,2602	Yes	****	<0,0001	C		
Control vs. Mezcla 3 (0.001%)	0,3284	-0,05108 to 0,7078	No	ns	0,1223	D		
Control vs. Mezcla 3 (0.005%)	-1,224	-1,604 to -0,8449	Yes	****	<0,0001	E		
Control vs. Mezcla 4 (0.001%)	-0,1618	-0,4965 to 0,1728	No	ns	0,7103	F		
Control vs. Mezcla 4 (0.005%)	-0,9478	-1,258 to -0,6379	Yes	****	<0,0001	G		
Control vs. Mezcla 5 (0.001%)	-0,5318	-0,8664 to -0,1971	Yes	***	0,0003	H		
Control vs. Mezcla 5 (0.005%)	-0,07944	-0,3893 to 0,2304	No	ns	0,9867	I		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	q	DF
Control vs. Mezcla 2 (0.001%)	1,018	1,359	-0,3414	0,1141	16	16	2,992	111
Control vs. Mezcla 2 (0.005%)	1,018	1,588	-0,5700	0,1141	16	16	4,996	111
Control vs. Mezcla 3 (0.001%)	1,018	0,6891	0,3284	0,1397	16	8	2,350	111
Control vs. Mezcla 3 (0.005%)	1,018	2,242	-1,224	0,1397	16	8	8,761	111
Control vs. Mezcla 4 (0.001%)	1,018	1,179	-0,1618	0,1232	16	12	1,313	111
Control vs. Mezcla 4 (0.005%)	1,018	1,965	-0,9478	0,1141	16	16	8,306	111
Control vs. Mezcla 5 (0.001%)	1,018	1,549	-0,5318	0,1232	16	12	4,315	111
Control vs. Mezcla 5 (0.005%)	1,018	1,097	-0,07944	0,1141	16	16	0,6962	111

SRD5A2 – RT-qPCR								
Dunnett's multiple comparisons test	Mean Diff,	95% CI of diff,	Significant?	Summary	Adjusted P Value	A-?		
Control vs. Mezcla 2 (0.001%)	-1,508	-2,032 to -0,9845	Yes	****	<0,0001	B		
Control vs. Mezcla 2 (0.005%)	-0,4823	-1,006 to 0,04132	No	ns	0,0857	C		
Control vs. Mezcla 3 (0.001%)	-0,3244	-0,9658 to 0,3169	No	ns	0,6623	D		
Control vs. Mezcla 3 (0.005%)	0,4769	-0,08870 to 1,042	No	ns	0,1395	E		
Control vs. Mezcla 4 (0.001%)	-1,514	-2,080 to -0,9486	Yes	****	<0,0001	F		
Control vs. Mezcla 4 (0.005%)	0,2916	-0,2320 to 0,8153	No	ns	0,5591	G		
Control vs. Mezcla 5 (0.001%)	0,04606	-0,5195 to 0,6117	No	ns	0,9997	H		
Control vs. Mezcla 5 (0.005%)	-1,604	-2,128 to -1,080	Yes	****	<0,0001	I		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	q	DF
Control vs. Mezcla 2 (0.001%)	1,023	2,531	-1,508	0,1932	16	16	7,805	115
Control vs. Mezcla 2 (0.005%)	1,023	1,505	-0,4823	0,1932	16	16	2,496	115
Control vs. Mezcla 3 (0.001%)	1,023	1,348	-0,3244	0,2366	16	8	1,371	115
Control vs. Mezcla 3 (0.005%)	1,023	0,5462	0,4769	0,2087	16	12	2,285	115
Control vs. Mezcla 4 (0.001%)	1,023	2,537	-1,514	0,2087	16	12	7,255	115
Control vs. Mezcla 4 (0.005%)	1,023	0,7314	0,2916	0,1932	16	16	1,509	115
Control vs. Mezcla 5 (0.001%)	1,023	0,9770	0,04606	0,2087	16	12	0,2207	115
Control vs. Mezcla 5 (0.005%)	1,023	2,627	-1,604	0,1932	16	16	8,302	115

SRD5A3 – RT-qPCR								
Dunnett's multiple comparisons test	Mean Diff,	95% CI of diff,	Significant?	Summary	Adjusted P Value	A- ?		
Control vs. Mezcla 2 (0.001%)	-0,09050	-1,147 to 0,9665	No	ns	0,9997	B		
Control vs. Mezcla 2 (0.005%)	-1,966	-3,023 to -0,9087	Yes	****	<0,0001	C		
Control vs. Mezcla 3 (0.001%)	0,3687	-0,6883 to 1,426	No	ns	0,9183	D		
Control vs. Mezcla 3 (0.005%)	-2,838	-3,895 to -1,781	Yes	****	<0,0001	E		
Control vs. Mezcla 4 (0.001%)	-0,5901	-1,647 to 0,4668	No	ns	0,5486	F		
Control vs. Mezcla 4 (0.005%)	-3,942	-4,999 to -2,885	Yes	****	<0,0001	G		
Control vs. Mezcla 5 (0.001%)	-1,156	-2,450 to 0,1387	No	ns	0,1021	H		
Control vs. Mezcla 5 (0.005%)	0,06569	-0,9913 to 1,123	No	ns	0,9998	I		
Test details	Mean 1	Mean 2	Mean Diff,	SE of diff,	n1	n2	q	DF
Control vs. Mezcla 2 (0.001%)	1,076	1,166	-0,09050	0,3919	16	16	0,2309	127
Control vs. Mezcla 2 (0.005%)	1,076	3,042	-1,966	0,3919	16	16	5,016	127
Control vs. Mezcla 3 (0.001%)	1,076	0,7073	0,3687	0,3919	16	16	0,9408	127
Control vs. Mezcla 3 (0.005%)	1,076	3,914	-2,838	0,3919	16	16	7,241	127
Control vs. Mezcla 4 (0.001%)	1,076	1,666	-0,5901	0,3919	16	16	1,506	127
Control vs. Mezcla 4 (0.005%)	1,076	5,018	-3,942	0,3919	16	16	10,06	127
Control vs. Mezcla 5 (0.001%)	1,076	2,232	-1,156	0,4800	16	8	2,408	127
Control vs. Mezcla 5 (0.005%)	1,076	1,010	0,06569	0,3919	16	16	0,1676	127

Table Analyzed	SRD5A1	SRD5A1	SRD5A1	SRD5A1	SRD5A1	SRD5A1	SRD5A1	SRD5A1
Column B	Mezcla 2 (0,001%)	Mezcla 2 (0,005%)	Mezcla 3 (0,001%)	Mezcla 3 (0,005%)	Mezcla 4 (0,001%)	Mezcla 4 (0,005%)	Mezcla 5 (0,001%)	Mezcla 5 (0,005%)
vs.	vs,	vs,	vs,	vs,	vs,	vs,	vs,	vs,
Column A	Control	Control	Control	Control	Control	Control	Control	Control
Unpaired t test								
P value	0,0049	<0,0001	0,0002	<0,0001	0,1244	<0,0001	<0,0001	0,3671
P value summary	**	****	***	****	ns	****	****	ns
Significantly different? (P < 0.05)	Yes	Yes	Yes	Yes	No	Yes	Yes	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=3,041, df=30	t=5,553, df=30	t=4,452, df=22	t=11,96, df=22	t=1,588, df=26	t=8,954, df=30	t=5,267, df=26	t=0,9157, df=30
How big is the difference?								
Mean ± SEM of column A	1,018	1,018	1,018	1,018	1,018	1,018	1,018	1,018
Mean ± SEM of column B	1,359	1,588	0,6891	2,242	1,179	1,965	1,549	1,097
Difference between means	0,3414 ± 0,1123	0,5700 ± 0,1026	-0,3284 ± 0,07375	1,224 ± 0,1024	0,1618 ± 0,1019	0,9478 ± 0,1059	0,5318 ± 0,1010	0,07944 ± 0,08675
95% confidence interval	0,1121 to 0,5707	0,3604 to 0,7796	-0,4813 to - 0,1754	1,012 to 1,437	-0,04766 to 0,3713	0,7316 to 1,164	0,3242 to 0,7393	-0,09773 to 0,2566
R square	0,2356	0,5069	0,4740	0,8667	0,08840	0,7277	0,5162	0,02719

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Table Analyzed	SRD5A2	SRD5A2	SRD5A2	SRD5A2	SRD5A2	SRD5A2	SRD5A2	SRD5A2
Column B	Mezcla 2 (0,001%)	Mezcla 2 (0,005%)	Mezcla 3 (0,001%)	Mezcla 3 (0,005%)	Mezcla 4 (0,001%)	Mezcla 4 (0,005%)	Mezcla 5 (0,001%)	Mezcla 5 (0,005%)
vs.	vs,	vs,	vs,	vs,	vs,	vs,	vs,	vs,
Column A	Control	Control	Control	Control	Control	Control	Control	Control
Unpaired t test								
P value	<0,0001	0,0031	0,0031	<0,0001	<0,0001	0,0019	0,6360	<0,0001
P value summary	****	**	**	****	****	**	ns	****
Significantly different? (P < 0.05)	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=6,755, df=30	t=3,220, df=30	t=3,327, df=22	t=6,459, df=26	t=6,145, df=26	t=3,412, df=30	t=0,4790, df=26	t=10,09, df=30
How big is the difference?								
Mean ± SEM of column A	1,023	1,023	1,023	1,023	1,023	1,023	1,023	1,023
Mean ± SEM of column B	2,531	1,505	1,348	0,5462	2,537	0,7314	0,9770	2,627
Difference between means	1,508 ± 0,2233	0,4823 ± 0,1498	0,3244 ± 0,09751	-0,4769 ± 0,07383	1,514 ± 0,2464	-0,2916 ± 0,08547	-0,04606 ± 0,09617	1,604 ± 0,1589
95% confidence interval	1,052 to 1,964	0,1764 to 0,7882	0,1222 to 0,5267	-0,6287 to - 0,3251	1,008 to 2,021	-0,4662 to - 0,1171	-0,2437 to 0,1516	1,279 to 1,929
R square	0,6034	0,2568	0,3347	0,6161	0,5922	0,2796	0,008746	0,7725

Table Analyzed	SRD5A3	SRD5A3	SRD5A3	SRD5A3	SRD5A3	SRD5A3	SRD5A3	SRD5A3
Column B	Mezcla 2 (0,001%)	Mezcla 2 (0,005%)	Mezcla 3 (0,001%)	Mezcla 3 (0,005%)	Mezcla 4 (0,001%)	Mezcla 4 (0,005%)	Mezcla 5 (0,001%)	Mezcla 5 (0,005%)
vs.	vs,	vs,	vs,	vs,	vs,	vs,	vs,	vs,
Column A	Control	Control	Control	Control	Control	Control	Control	Control
Unpaired t test								
P value	0,5106	<0,0001	0,0100	<0,0001	0,0026	<0,0001	0,0574	0,6813
P value summary	ns	****	**	****	**	****	ns	ns
Significantly different? (P < 0.05)	No	Yes	Yes	Yes	Yes	Yes	No	No
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=0,6659, df=30	t=5,909, df=30	t=2,752, df=30	t=7,382, df=30	t=3,278, df=30	t=8,511, df=30	t=2,005, df=22	t=0,4147, df=30
How big is the difference?								
Mean ± SEM of column A	1,076	1,076	1,076	1,076	1,076	1,076	1,076	1,076
Mean ± SEM of column B	1,166	3,042	0,7073	3,914	1,666	5,018	2,232	1,010
Difference between means	0,09050 ± 0,1359	1,966 ± 0,3327	-0,3687 ± 0,1340	2,838 ± 0,3844	0,5901 ± 0,1800	3,942 ± 0,4632	1,156 ± 0,5764	-0,06569 ± 0,1584
95% confidence interval	-0,1871 to 0,3681	1,286 to 2,645	-0,6423 to - 0,09506	2,052 to 3,623	0,2224 to 0,9578	2,996 to 4,888	-0,03956 to 2,351	-0,3892 to 0,2578
R square	0,01457	0,5378	0,2015	0,6449	0,2637	0,7072	0,1545	0,005699

Table 2. Statistical analysis of the results shown in Figure 7.

8 Discussion and Conclusions

Androgenetic alopecia (AGA) or also known as Male-pattern hair loss (MPHL) is the most common type of hair loss, affecting women (50 % of menopausal women and a large number of women of childbearing age, around 25 %), as well as males (over 70 % of adult men). It occurs due to an underlying susceptibility of hair follicles to shrinkage due to the combined effect of two factors: Genetic predisposition (several loci are involved including AR, EDA2R/Chr. X-WNT10A/2q35, etc.) and hormonal stimulation. It is known that both genetic and environmental factors play a role, but many causes of AGA remain unknown.

Enzymes 5 α -reductases convert testosterone to 5 α -dihydrotestosterone and this conversion enhance the androgenic signal via two mechanisms: First, DHT cannot be aromatized to estrogen and, therefore, its effect are solely androgenic and in second place, in vitro DHT binds to the AR with a higher affinity than testosterone does, preventing its usual action. There are three isoenzymes of 5 α -reductase: steroid 5 α -reductase type 1, 2 and 3 (*SRD5A1*, *SRD5A2* and *SRD5A3*).

It is widely documented that 5 α -reductase enzymes are involved in androgenetic alopecia and that 5 α -reductase inhibitors are beneficial treatments for anti-hair loss due to androgenetic alopecia. For these reasons, in this assay, the capacity of **Mezcla 2, Mezcla 3, Mezcla 4, and Mezcla 5** to inhibit the expression of *SRD5A1* (5 α -Reductase type 1), *SRD5A2* (5 α -Reductase type 2) and *SRD5A3* (5 α -Reductase type 3) through RT-qPCR, was assessed *in vitro* after 24 hours of treatment in Human Follicle Dermal Papilla Cells (HFDPC).

Results indicated that **treatment with Mezcla 2 at 0.001 % concentration significantly increased the gene expression of SRD5A1 and SRD5A2**, whereas the treatment at **0.005 % significantly increased SRD5A1 and SRD5A3**, compared to the untreated control.

In the same way, the treatment with **Mezcla 3 at 0.005 % concentration significantly increased the gene expression of SRD5A1 and SRD5A3**, compared to the untreated control. On the other hand, the treatment with **Mezcla 3 at 0.001 % concentration decreased SRD5A1 and SRD5A3 gene expression by 32.8 ± 7.4 % and 36.9 ± 13.4 %**, respectively, even though results were **not statistically significant ($p > 0.05$)**, compared to the untreated control. The treatment with **Mezcla 3 at 0.005 % concentrations also decreased the expression of SRD5A2 by 47.8 ± 7.4 % ($p > 0.05$)**.

With regard to Mezcla 4, results indicated the **treatment with Mezcla 4 at 0.001 % concentration significantly increased the expression of SRD5A2**, whereas the treatment at **0.005 % concentration significantly increased the expression of SRD5A1 and SRD5A3**. On the other hand, the treatment with **Mezcla 4 at 0.005 % concentration inhibited the expression of SRD5A2 by 29.2 ± 8.5 %**, even though results were **not statistically significant ($p > 0.05$)**, compared to the untreated control.

Last, for **Mezcla 5**, results indicated the **treatment at 0.001 % concentration significantly increased the expression of SRD5A1**, whereas the treatment at **0.005 % significantly increased the expression of SRD5A2**, compared to the untreated control.

In conclusion, the *in vitro* treatment with Mezcla 2, Mezcla 3, Mezcla 4, or Mezcla 5 at concentrations 0.001 % or 0.005 %, do not display any significant inhibition of **SRD5A1 (5 α -Reductase type 1)**, **SRD5A2 (5 α -Reductase type 2)**, or **SRD5A3 (5 α -Reductase type 3)** gene expression, after 24 hours of treatment in Human Follicle Dermal Papilla Cells (HFDPC), compared to the untreated control. Specifically, **most of the tested conditions resulted in a significant increase of gene expression**, meaning the **samples are not displaying an anti-hair loss effect through this biological pathway**, but the opposite.

9 References

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10 Registry and Regulation

The final report, the raw data and the assay protocol have been saved in computer format, and a copy on paper. All the information provided from the Client, volunteers and generated by Bionos Biotech will be considered as *confidential*. The information about materials, reagents and protocols adopted by Bionos Biotech SL during the assays is confidential and will not be shared with third parts.

The whole process involving this assay was performed under **Quality Management System UNE-EN-ISO 9001/2015**.

Article 20 of the EC Cosmetic Products Regulation 1223/2009 (CPR) frames the requirements for cosmetic claims. Furthermore, cosmetic claims have to comply with **EU Regulation 655/2013** that provides the Common Criteria to ensure that the information conveyed to the end-users through claims is useful, understandable, and reliable so that consumers can make informed decisions.

The **third Common Criterion, 'Evidential support'**, states that "claims for cosmetic products, whether explicit or implicit, shall be supported by adequate and verifiable evidence regardless of the types of evidential support used to substantiate them, including where appropriate expert assessments. Evidence for claim substantiation shall take into account the state-of-the-art practices. Where studies are being used as evidence, they shall be relevant to the product and the benefit claimed, shall follow well-designed, well-conducted methodologies (valid, reliable, and reproducible) and shall respect ethical considerations."

Experimental studies include (but are not limited to) studies *in silico*, *in vitro*, *ex-vivo*, with instrumental or biochemical methods, studies conducted on volunteers, investigator evaluations, sensory evaluations, etc. Different types of experimental studies can be used to provide data on the performance of cosmetic products. It is useful to take into consideration existing relevant guidelines, e.g. guidelines relating to instrumental clinical techniques, other European or international guidelines or standards (e.g. CEN, ISO, etc.).

Such studies should comprise methods which are **reliable and reproducible**. The studies should follow a well-designed and **scientifically valid methodology** according to best practices. The criteria used for evaluation of product performance should be defined with accuracy and chosen in accordance with the aim of the test. The experimental aspect of studies calls for reliance on knowledge and awareness of statistical principles in the design and analysis of the study, e.g. in terms of number of subjects, test samples, etc. This is necessary in order to ensure that the studies achieve scientifically and statistically valid conclusions.

A study protocol should be drawn up and validated in order to enable the study to be conducted and monitored appropriately, thereby ensuring its quality. Whatever the type of study, it is important that the person conducting the study has the appropriate qualifications, has training and experience in the field of the proposed study and has high ethical qualified standards and professional integrity.

Test facilities should maintain a quality assurance system, including standardised operating procedures. A monitoring system should be set up for each study in order to ensure that the protocol and the operating procedures are correctly followed.

Data processing and the **interpretation of results** should be fair and should not overstep the limits of the test's significance. Data recording, transformations and representation in tabular or graphical form should be transparent or clearly explained if complex. It should not be designed to overstate the effect(s) measured. Appropriate statistical analysis of the data should be performed.

Attachments

qPCR expression data after analysis

SRD5A1								
Control	Mezcla 2 (0.001%)	Mezcla 2 (0.005%)	Mezcla 3 (0.001%)	Mezcla 3 (0.005%)	Mezcla 4 (0.001%)	Mezcla 4 (0.005%)	Mezcla 5 (0.001%)	Mezcla 5 (0.005%)
1	0,79	1,684	0,572	1,834	0,654	1,462	1,548	0,606
1,342	1,06	2,26	0,768	2,462	0,878	1,962	2,078	0,813
1,399	1,104	2,355	0,8	2,566	0,915	2,044	2,165	0,847
1,167	0,922	1,966	0,668	2,141	0,764	1,706	1,807	0,707
0,745	1,052	1,236	0,551	1,82	1,007	1,704	1,166	0,977
1	1,412	1,659	0,74	2,443	1,352	2,287	1,565	1,312
1,042	1,471	1,729	0,771	2,545	1,409	2,383	1,631	1,367
0,87	1,228	1,443	0,643	2,124	1,176	1,989	1,361	1,14
0,715	1,022	1,154			1,222	1,895	1,074	0,857
0,96	1,372	1,549			1,64	2,544	1,441	1,15
1	1,429	1,614			1,709	2,65	1,502	1,199
0,835	1,193	1,347			1,426	2,212	1,253	1
0,857	1,566	1,101				1,346		1,136
1,15	2,103	1,478				1,807		1,525
1,198	2,191	1,54				1,882		1,589
1	1,828	1,285				1,571		1,326

SRD5A2								
Control	Mezcla 2 (0.001%)	Mezcla 2 (0.005%)	Mezcla 3 (0.001%)	Mezcla 3 (0.005%)	Mezcla 4 (0.001%)	Mezcla 4 (0.005%)	Mezcla 5 (0.001%)	Mezcla 5 (0.005%)
1	4,16	2,462	1,377	0,453	2,712	0,735	1,394	2,127
0,91	3,786	2,24	1,254	0,412	2,468	0,669	1,269	1,936
0,711	2,957	1,75	0,979	0,322	1,927	0,522	0,991	1,512
1,052	4,375	2,589	1,449	0,477	2,852	0,773	1,466	2,237
1,099	2,151	1,648	1,558	0,729	1,678	0,878	1,013	2,767
1	1,958	1,5	1,418	0,663	1,527	0,799	0,922	2,518
0,781	1,529	1,171	1,107	0,518	1,193	0,624	0,72	1,966
1,156	2,262	1,733	1,638	0,767	1,765	0,923	1,065	2,91
1,407	2,318	0,99		0,603	3,901	0,451	0,785	3,232
1,281	2,11	0,901		0,548	3,55	0,41	0,715	2,942
1	1,648	0,703		0,428	2,772	0,321	0,558	2,297
1,48	2,438	1,041		0,634	4,102	0,474	0,826	3,399
0,951	2,398	1,459				1,123		3,319
0,865	2,183	1,328				1,022		3,021
0,676	1,704	1,037				0,798		2,359
1	2,522	1,534				1,181		3,491

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SRD5A3								
Control	Mezcla 2 (0.001%)	Mezcla 2 (0.005%)	Mezcla 3 (0.001%)	Mezcla 3 (0.005%)	Mezcla 4 (0.001%)	Mezcla 4 (0.005%)	Mezcla 5 (0.001%)	Mezcla 5 (0.005%)
1	1,374	5,569	0,593	6,797	1,954	5,203	0,32	1,893
0,998	1,371	5,556	0,592	6,781	1,95	5,19	0,32	1,888
0,553	0,76	3,078	0,328	3,757	1,08	2,876	0,177	1,046
0,614	0,844	3,419	0,364	4,173	1,2	3,194	0,197	1,162
1,002	1,705	4,122	0,629	3,581	1,493	6,02	5,322	0,916
1	1,701	4,112	0,628	3,573	1,49	6,005	5,309	0,914
0,554	0,943	2,278	0,348	1,98	0,825	3,327	2,942	0,506
0,615	1,047	2,53	0,386	2,199	0,917	3,695	3,267	0,562
1,809	1,367	2,826	1,359	4,232	2,34	5,457		1,516
1,805	1,364	2,82	1,355	4,222	2,334	5,444		1,512
1	0,756	1,562	0,751	2,339	1,293	3,016		0,838
1,111	0,839	1,735	0,834	2,598	1,436	3,35		0,931
1,629	1,451	2,863	0,995	5,178	2,637	8,696		0,784
1,625	1,448	2,856	0,993	5,165	2,631	8,674		0,782
0,9	0,802	1,582	0,55	2,862	1,458	4,806		0,433
1	0,891	1,758	0,611	3,179	1,619	5,338		0,481

Raw data from qPCR before analysis

Sample Name	Target Name	CT	Ct Mean
C1	Act	15,930	15,910
C1	Act	15,890	15,910
P2 0.005% 1	SRD5A1	27,634	27,234
P2 0.005% 1	SRD5A1	26,833	27,234
P4 0.005% 1	SRD5A1	27,901	28,025
P4 0.005% 1	SRD5A1	28,150	28,025
P1 0.001% 1	SRD5A2	25,337	25,438
P1 0.001% 1	SRD5A2	25,538	25,438
P3 0.001% 1	SRD5A2	24,896	24,935
P3 0.001% 1	SRD5A2	24,974	24,935
C2	Act	16,402	16,372
C2	Act	16,341	16,372
P4 0.005% 2	SRD5A1	27,308	27,277
P4 0.005% 2	SRD5A1	27,246	27,277
P1 0.001% 2	SRD5A2	26,633	26,595
P1 0.001% 2	SRD5A2	26,557	26,595
P3 0.001% 2	SRD5A2	26,939	26,745
P3 0.001% 2	SRD5A2	26,551	26,745
C3	Act	15,554	15,579
C3	Act	15,603	15,579
P2 0.001% 3	Act	15,022	15,099
P2 0.001% 3	Act	15,177	15,099
P4 0.001% 3	Act	16,327	16,331
P4 0.001% 3	Act	16,364	16,331
P4 0.001% 3	Act	16,302	16,331
P2 0.005% 3	SRD5A1	27,457	27,348
P2 0.005% 3	SRD5A1	27,240	27,348
P4 0.005% 3	SRD5A1	26,560	26,666
P4 0.005% 3	SRD5A1	26,772	26,666
P1 0.001% 3	SRD5A2	26,457	26,255
P1 0.001% 3	SRD5A2	26,052	26,255
P3 0.001% 3	SRD5A2	25,455	25,706
P3 0.001% 3	SRD5A2	25,957	25,706
C4	Act	15,707	15,757
C4	Act	15,806	15,757
P2 0.001% 4	Act	15,141	15,150
P2 0.001% 4	Act	15,159	15,150
P2 0.005% 4	SRD5A1	27,667	27,579
P2 0.005% 4	SRD5A1	27,491	27,579
P4 0.005% 4	SRD5A1	26,563	26,670
P4 0.005% 4	SRD5A1	26,776	26,670
P1 0.001% 4	SRD5A2	26,436	26,444
P1 0.001% 4	SRD5A2	26,452	26,444
P1 0.005% 1	Act	17,894	17,955
P1 0.005% 1	Act	18,016	17,955

P3 0.005% 1	Act	16,840	16,805
P3 0.005% 1	Act	16,770	16,805
C1	SRD5A1	27,262	27,156
C1	SRD5A1	27,050	27,156
P4 0.001% 1	SRD5A1	33,717	34,389
P4 0.001% 1	SRD5A1	35,240	34,389
P4 0.001% 1	SRD5A1	34,210	34,389
P2 0.005% 1	SRD5A2	28,080	29,384
P2 0.005% 1	SRD5A2	29,520	29,384
P2 0.005% 1	SRD5A2	30,552	29,384
P4 0.005% 1	SRD5A2	26,763	26,503
P4 0.005% 1	SRD5A2	26,532	26,503
P4 0.005% 1	SRD5A2	26,215	26,503
P1 0.005% 2	Act	16,820	16,716
P1 0.005% 2	Act	16,640	16,716
P1 0.005% 2	Act	16,689	16,716
P3 0.005% 2	Act	17,769	17,505
P3 0.005% 2	Act	17,589	17,505
P3 0.005% 2	Act	17,156	17,505
C2	SRD5A1	28,213	28,043
C2	SRD5A1	27,873	28,043
P2 0.001% 2	SRD5A1	27,372	27,426
P2 0.001% 2	SRD5A1	27,479	27,426
P2 0.005% 2	SRD5A2	32,571	32,526
P2 0.005% 2	SRD5A2	32,482	32,526
P4 0.005% 2	SRD5A2	26,090	26,066
P4 0.005% 2	SRD5A2	26,042	26,066
P1 0.005% 3	Act	16,601	16,622
P1 0.005% 3	Act	16,661	16,622
P1 0.005% 3	Act	16,604	16,622
P3 0.005% 3	Act	17,341	17,320
P3 0.005% 3	Act	17,427	17,320
P3 0.005% 3	Act	17,192	17,320
C3	SRD5A1	27,437	27,309
C3	SRD5A1	27,182	27,309
P2 0.001% 3	SRD5A1	27,228	27,152
P2 0.001% 3	SRD5A1	27,076	27,152
P4 0.001% 3	SRD5A1	27,389	27,417
P4 0.001% 3	SRD5A1	27,446	27,417
P2 0.005% 3	SRD5A2	28,997	28,970
P2 0.005% 3	SRD5A2	28,943	28,970
P4 0.005% 3	SRD5A2	24,850	25,041
P4 0.005% 3	SRD5A2	25,233	25,041
P1 0.005% 4	Act	16,437	16,496
P1 0.005% 4	Act	16,555	16,496
P3 0.005% 4	Act	18,385	18,695

P3 0.005% 4	Act	18,977	18,695
P3 0.005% 4	Act	18,722	18,695
C4	SRD5A1	27,254	27,226
C4	SRD5A1	27,198	27,226
P2 0.001% 4	SRD5A1	27,245	27,256
P2 0.001% 4	SRD5A1	27,268	27,256
P2 0.005% 4	SRD5A2	29,097	29,464
P2 0.005% 4	SRD5A2	29,460	29,464
P2 0.005% 4	SRD5A2	29,835	29,464
P4 0.005% 4	SRD5A2	25,208	25,413
P4 0.005% 4	SRD5A2	25,663	25,413
P4 0.005% 4	SRD5A2	25,368	25,413
P1 0.001% 1	Act	15,919	15,957
P1 0.001% 1	Act	15,995	15,957
P3 0.001% 1	Act	14,779	14,837
P3 0.001% 1	Act	14,895	14,837
P1 0.005% 1	SRD5A1	28,383	28,450
P1 0.005% 1	SRD5A1	28,457	28,450
P1 0.005% 1	SRD5A1	28,510	28,450
P3 0.005% 1	SRD5A1	27,495	27,504
P3 0.005% 1	SRD5A1	27,514	27,504
C1	SRD5A2	27,318	27,447
C1	SRD5A2	27,576	27,447
P1 0.001% 2	Act	16,130	16,163
P1 0.001% 2	Act	16,163	16,163
P1 0.001% 2	Act	16,195	16,163
P3 0.001% 2	Act	16,043	15,955
P3 0.001% 2	Act	15,820	15,955
P3 0.001% 2	Act	16,003	15,955
P1 0.005% 2	SRD5A1	27,730	27,657
P1 0.005% 2	SRD5A1	27,584	27,657
P3 0.005% 2	SRD5A1	27,945	27,982
P3 0.005% 2	SRD5A1	28,020	27,982
C2	SRD5A2	27,740	27,773
C2	SRD5A2	27,684	27,773
C2	SRD5A2	27,896	27,773
P2 0.001% 2	SRD5A2	26,060	26,698
P2 0.001% 2	SRD5A2	27,332	26,698
P2 0.001% 2	SRD5A2	26,701	26,698
P1 0.001% 3	Act	15,838	15,931
P1 0.001% 3	Act	16,023	15,931
P3 0.001% 3	Act	16,052	16,133
P3 0.001% 3	Act	16,139	16,133
P3 0.001% 3	Act	16,208	16,133
P1 0.005% 3	SRD5A1	26,933	27,662
P1 0.005% 3	SRD5A1	28,299	27,662
P1 0.005% 3	SRD5A1	27,754	27,662
P3 0.005% 3	SRD5A1	27,841	27,644

P3 0.005% 3	SRD5A1	27,447	27,644
C3	SRD5A2	26,638	26,623
C3	SRD5A2	26,608	26,623
P2 0.001% 3	SRD5A2	26,141	26,175
P2 0.001% 3	SRD5A2	26,208	26,175
P4 0.001% 3	SRD5A2	27,385	27,818
P4 0.001% 3	SRD5A2	27,882	27,818
P4 0.001% 3	SRD5A2	28,188	27,818
P1 0.001% 4	Act	16,218	16,169
P1 0.001% 4	Act	16,120	16,169
P1 0.005% 4	SRD5A1	27,536	27,604
P1 0.005% 4	SRD5A1	27,563	27,604
P1 0.005% 4	SRD5A1	27,713	27,604
P3 0.005% 4	SRD5A1	29,548	29,513
P3 0.005% 4	SRD5A1	29,478	29,513
C4	SRD5A2	27,361	27,366
C4	SRD5A2	27,372	27,366
P2 0.001% 4	SRD5A2	25,950	26,048
P2 0.001% 4	SRD5A2	26,145	26,048
P4 0.005% 1	Act	16,055	16,055
P1 0.001% 1	SRD5A1	27,446	27,544
P1 0.001% 1	SRD5A1	27,643	27,544
P3 0.001% 1	SRD5A1	26,620	26,695
P3 0.001% 1	SRD5A1	26,818	26,695
P3 0.001% 1	SRD5A1	26,649	26,695
P1 0.005% 1	SRD5A2	28,161	28,193
P1 0.005% 1	SRD5A2	28,225	28,193
P3 0.005% 1	SRD5A2	28,839	28,787
P3 0.005% 1	SRD5A2	28,734	28,787
P2 0.005% 2	Act	19,828	19,847
P2 0.005% 2	Act	19,867	19,847
P4 0.005% 2	Act	15,925	15,997
P4 0.005% 2	Act	16,069	15,997
P1 0.001% 2	SRD5A1	27,225	27,336
P1 0.001% 2	SRD5A1	27,447	27,336
P3 0.001% 2	SRD5A1	27,098	27,191
P3 0.001% 2	SRD5A1	27,285	27,191
P1 0.005% 2	SRD5A2	28,019	27,533
P1 0.005% 2	SRD5A2	27,034	27,533
P1 0.005% 2	SRD5A2	27,544	27,533
P3 0.005% 2	SRD5A2	29,305	29,230
P3 0.005% 2	SRD5A2	29,155	29,230
P2 0.005% 3	Act	16,934	16,977
P2 0.005% 3	Act	17,020	16,977
P4 0.005% 3	Act	15,256	15,197
P4 0.005% 3	Act	15,138	15,197
P1 0.001% 3	SRD5A1	27,160	27,146
P1 0.001% 3	SRD5A1	27,133	27,146

P3 0.001% 3	SRD5A1	27,088	27,090
P3 0.001% 3	SRD5A1	27,092	27,090
P1 0.005% 3	SRD5A2	28,180	28,174
P1 0.005% 3	SRD5A2	28,168	28,174
P3 0.005% 3	SRD5A2	28,984	30,006
P3 0.005% 3	SRD5A2	31,027	30,006
P2 0.005% 4	Act	17,169	17,196
P2 0.005% 4	Act	17,224	17,196
P4 0.005% 4	Act	15,556	15,607

P4 0.005% 4	Act	15,615	15,607
P4 0.005% 4	Act	15,649	15,607
P1 0.001% 4	SRD5A1	26,786	26,768
P1 0.001% 4	SRD5A1	26,750	26,768
P1 0.005% 4	SRD5A2	27,496	27,488
P1 0.005% 4	SRD5A2	27,481	27,488
P3 0.005% 4	SRD5A2	30,176	30,065
P3 0.005% 4	SRD5A2	29,954	30,065

Sample Name	Target Name	CT	Ct Mean
C1	Act	15,074	15,074
C1	Act	15,075	15,074
P2 0.001% 1	Act	14,500	14,563
P2 0.001% 1	Act	14,626	14,563
P2 0.005% 1	SRD5A3	23,058	23,126
P2 0.005% 1	SRD5A3	23,193	23,126
P4 0.005% 1	SRD5A3	24,667	24,580
P4 0.005% 1	SRD5A3	24,493	24,580
C2	Act	16,136	16,127
C2	Act	16,117	16,127
P2 0.001% 2	Act	14,508	14,514
P2 0.001% 2	Act	14,520	14,514
P2 0.005% 2	SRD5A3	27,461	27,543
P2 0.005% 2	SRD5A3	27,625	27,543
P4 0.005% 2	SRD5A3	25,113	24,971
P4 0.005% 2	SRD5A3	24,830	24,971
C3	Act	15,231	15,573
C3	Act	15,567	15,573
C3	Act	15,922	15,573
P2 0.001% 3	Act	15,079	15,032
P2 0.001% 3	Act	14,985	15,032
P4 0.001% 1	Act	15,863	15,863
P4 0.001% 2	Act	16,166	16,166
P4 0.001% 3	Act	15,900	15,900
P2 0.005% 3	SRD5A3	23,749	23,861
P2 0.005% 3	SRD5A3	23,973	23,861
P4 0.005% 3	SRD5A3	24,889	24,849
P4 0.005% 3	SRD5A3	24,810	24,849
C4	Act	15,478	15,390
C4	Act	15,314	15,390
C4	Act	15,378	15,390
P2 0.001% 4	Act	15,096	15,104
P2 0.001% 4	Act	15,111	15,104
P2 0.005% 4	SRD5A3	24,222	24,073
P2 0.005% 4	SRD5A3	24,108	24,073
P2 0.005% 4	SRD5A3	23,889	24,073

P4 0.005% 4	SRD5A3	25,180	25,122
P4 0.005% 4	SRD5A3	25,064	25,122
P1 0.005% 1	Act	18,378	18,431
P1 0.005% 1	Act	18,483	18,431
P3 0.005% 1	Act	16,666	16,601
P3 0.005% 1	Act	16,536	16,601
C1	SRD5A3	25,251	25,273
C1	SRD5A3	25,296	25,273
P2 0.001% 1	SRD5A3	25,648	25,516
P2 0.001% 1	SRD5A3	25,384	25,516
P4 0.001% 1	SRD5A3	27,737	27,704
P4 0.001% 1	SRD5A3	27,670	27,704
P1 0.005% 2	Act	16,443	16,568
P1 0.005% 2	Act	16,693	16,568
P3 0.005% 2	Act	16,848	16,785
P3 0.005% 2	Act	16,609	16,785
P3 0.005% 2	Act	16,899	16,785
C2	SRD5A3	26,371	26,322
C2	SRD5A3	26,273	26,322
P2 0.001% 2	SRD5A3	25,382	25,381
P2 0.001% 2	SRD5A3	25,380	25,381
P1 0.005% 3	Act	16,262	16,311
P1 0.005% 3	Act	16,315	16,311
P1 0.005% 3	Act	16,355	16,311
P3 0.005% 3	Act	16,596	16,613
P3 0.005% 3	Act	16,674	16,613
P3 0.005% 3	Act	16,568	16,613
C3	SRD5A3	24,973	24,917
C3	SRD5A3	24,861	24,917
P2 0.001% 3	SRD5A3	24,882	24,789
P2 0.001% 3	SRD5A3	24,798	24,789
P2 0.001% 3	SRD5A3	24,687	24,789
P4 0.001% 3	SRD5A3	22,964	23,687
P4 0.001% 3	SRD5A3	23,986	23,687
P4 0.001% 3	SRD5A3	24,112	23,687
P1 0.005% 4	Act	15,829	15,820
P1 0.005% 4	Act	15,896	15,820

P1 0.005% 4	Act	15,736	15,820
P3 0.005% 4	Act	19,300	19,314
P3 0.005% 4	Act	19,327	19,314
C4	SRD5A3	24,821	24,885
C4	SRD5A3	24,949	24,885
P2 0.001% 4	SRD5A3	25,282	25,310
P2 0.001% 4	SRD5A3	25,337	25,310
P1 0.001% 1	Act	15,619	15,599
P1 0.001% 1	Act	15,434	15,599
P1 0.001% 1	Act	15,743	15,599
P3 0.001% 1	Act	15,448	15,308
P3 0.001% 1	Act	15,168	15,308
P1 0.005% 1	SRD5A3	25,994	26,152
P1 0.005% 1	SRD5A3	26,142	26,152
P1 0.005% 1	SRD5A3	26,320	26,152
P3 0.005% 1	SRD5A3	24,419	24,421
P3 0.005% 1	SRD5A3	24,423	24,421
P1 0.001% 2	Act	15,365	15,428
P1 0.001% 2	Act	15,383	15,428
P1 0.001% 2	Act	15,538	15,428
P3 0.001% 2	Act	15,023	14,930
P3 0.001% 2	Act	14,837	14,930
P1 0.005% 2	SRD5A3	24,561	24,724
P1 0.005% 2	SRD5A3	24,886	24,724
P3 0.005% 2	SRD5A3	24,367	24,394
P3 0.005% 2	SRD5A3	24,406	24,394
P3 0.005% 2	SRD5A3	24,410	24,394
P1 0.001% 3	Act	15,171	15,304
P1 0.001% 3	Act	15,417	15,304
P1 0.001% 3	Act	15,324	15,304
P3 0.001% 3	Act	15,327	15,243
P3 0.001% 3	Act	15,190	15,243
P3 0.001% 3	Act	15,213	15,243
P1 0.005% 3	SRD5A3	24,960	25,011
P1 0.005% 3	SRD5A3	25,062	25,011
P3 0.005% 3	SRD5A3	24,448	24,363
P3 0.005% 3	SRD5A3	24,389	24,363
P3 0.005% 3	SRD5A3	24,254	24,363
P1 0.001% 4	Act	15,432	15,323
P1 0.001% 4	Act	15,213	15,323
P3 0.001% 4	Act	16,068	15,823
P3 0.001% 4	Act	15,567	15,823

P3 0.001% 4	Act	15,833	15,823
P1 0.005% 4	SRD5A3	24,640	24,502
P1 0.005% 4	SRD5A3	24,364	24,502
P3 0.005% 4	SRD5A3	26,457	26,392
P3 0.005% 4	SRD5A3	26,327	26,392
P2 0.005% 1	Act	15,076	15,692
P2 0.005% 1	Act	16,307	15,692
P4 0.005% 1	Act	15,473	15,302
P4 0.005% 1	Act	15,130	15,302
P1 0.001% 1	SRD5A3	25,225	25,339
P1 0.001% 1	SRD5A3	25,342	25,339
P1 0.001% 1	SRD5A3	25,450	25,339
P3 0.001% 1	SRD5A3	24,678	24,540
P3 0.001% 1	SRD5A3	24,512	24,540
P3 0.001% 1	SRD5A3	24,431	24,540
P2 0.005% 2	Act	18,915	19,185
P2 0.005% 2	Act	19,231	19,185
P2 0.005% 2	Act	19,408	19,185
P4 0.005% 2	Act	14,570	14,646
P4 0.005% 2	Act	14,721	14,646
P1 0.001% 2	SRD5A3	25,037	24,857
P1 0.001% 2	SRD5A3	24,706	24,857
P1 0.001% 2	SRD5A3	24,829	24,857
P3 0.001% 2	SRD5A3	24,485	24,551
P3 0.001% 2	SRD5A3	24,617	24,551
P2 0.005% 3	Act	15,741	15,744
P2 0.005% 3	Act	15,747	15,744
P4 0.005% 3	Act	15,276	15,251
P4 0.005% 3	Act	15,226	15,251
P1 0.001% 3	SRD5A3	25,027	25,052
P1 0.001% 3	SRD5A3	25,077	25,052
P3 0.001% 3	SRD5A3	24,385	24,216
P3 0.001% 3	SRD5A3	24,046	24,216
P2 0.005% 4	Act	16,158	16,246
P2 0.005% 4	Act	16,335	16,246
P4 0.005% 4	Act	14,413	14,571
P4 0.005% 4	Act	14,730	14,571
P1 0.001% 4	SRD5A3	24,990	24,984
P1 0.001% 4	SRD5A3	25,129	24,984
P1 0.001% 4	SRD5A3	24,834	24,984
P3 0.001% 4	SRD5A3	24,827	24,623
P3 0.001% 4	SRD5A3	24,418	24,623