

***GATA1*-mutant clones are frequent and often unsuspected in babies with Down Syndrome: identification of a population at risk of leukemia, Roberts et al**

Supplementary Material and Methods

***GATA1* mutation analysis by pyrosequencing**

Presence of *GATA1* exon 2 mutations detected by NGS was tested by pyrosequencing using an independent aliquot of DNA. PCR primers and conditions are set out in Supplemental Table 7. 10 µl PCR product was run in triplicate or quadruplicate. Single-stranded PCR product was obtained using a biotinylated PCR primer (Supplemental Table 7), immobilised on streptavidin beads, denatured with NaOH and then annealed to a sequencing primer (Supplemental Table 8) by heating to 80°C for 2 minutes. Pyrosequencing was run on a PyroMark Q96 MD (Qiagen, Hilden, Germany) (dispensation orders - Supplemental Table 8). The proportion of mutant allele was measured for two to four bases in each sample. These were then averaged.

Sensitivity and specificity of deep sequencing were tested using serial DNA dilutions of a male ML-DS cell line with a hemizygous *GATA1* mutation and normal cord blood (Supplemental Table 1). Mutation quantitation from deep sequencing ($3\text{-}5 \times 10^5$ mapping reads analysed/sample) was compared with pyrosequencing. The limit of detection of mutant *GATA1* sequence was ~1% by pyrosequencing and ~0.3% by deep sequencing.

Blast cell immunophenotyping

Mononuclear cells (MNC) were isolated by Ficoll density gradient separation from peripheral blood samples from 7 DS neonates without TAM where next-generation-sequencing of DNA from the same samples was performed. Two patients were

subsequently found to have a *GATA1* mutation (silent TAM) and 5 had no *GATA1* mutations detected on next-generation sequencing. Results were compared with results in 8 anonymised samples from neonates without DS and 7 samples with a confirmed diagnosis of TAM as defined in the OIDS study. CD34+ enriched populations were purified using magnetic beads (Miltenyi, Biscy UK) from MNC and stained with PerCP-CD34 (BioLegend, San Diego, USA), PE-Cy5-CD7, PE-Cy7-CD33 and FITC-CD36 (eBioscience, Hatfield, UK). Dead cells were excluded using Hoechst 33258 (Invitrogen). Appropriate unstained, single-stained and fluorescence minus one controls were used to determine compensation, background staining and gating for each population. Analysis was performed using the FACS ARIA II (Becton Dickinson) and the FACS DIVA software (Becton Dickinson). The number of viable CD34+CD33+CD7+CD36+ cells as a percentage of CD34+ cells was calculated as a percentage of CD34+ cells in the original sample.

Legend to Supplementary Figure

Supplemental Figure 1: OIDSCS Blood Smear Review form

Blood smear review form used to report peripheral blood smears on anonymised samples from neonates recruited into the OIDSC study.

OIDSCS: Oxford Imperial Down Syndrome Cohort Study

Supplemental Figure 1: OIDSCS Blood Smear Review form**MORPHOLOGY REVIEW**PSID No:

Oxford Imperial Down Syndrome Cohort Study

Sex: Ethnic origin:

DOB: Gestational age: weeks Age at testing: days

AUTOMATED FULL BLOOD COUNT RESULT					
HB		WCC		PLT	
HCT		Neutrophils		MPV	
MCV		Lymphocytes			
MCH		Eosinophils			
RCC		Basophils			
NRBC		Monocytes			
		Blasts			

MANUAL DIFFERENTIAL COUNT					
	%		%		%
Neutrophils		Myeloblasts		Eosinophils	
Metamyelocytes		Lymphocytes		Basophils	
Myelocytes		Lymphoblasts		Monocytes	
Promyelocytes		Blasts – type unclear		NRBCs	

DEGREE OF DYSPLASIA (score)		None (0)	Some (1)	Moderate (2)	Plentiful (3)
NEUTROPHILS	Hypogranular				
	Agranular				
	Hypersegmentation				
	Pelger forms				
EOSINOPHILS	Abnormal (specify below)				
BASOPHILS	Abnormal (specify below)				
Comment:					

BLASTS	Cytoplasmic blebbing				
Comment:					

MONOCYTES	Elongated lobes				
Comment:					

RED CELLS	Macrocytes				
	Polychromasia				
	Target cells				
	Basophilic stippling				
	Dyserythropoietic erythroblasts				
Comment:					

PLATELETS	Giant forms				
	Megakaryocyte fragments				
Comment:					

OTHER COMMENTS

Slide reviewed by _____

Date _____

Supplemental Table 1: Quantitation of mutant clone size in dilutions of CMK cell line DNA by next-generation-resequencing (NGS) and pyrosequencing

Dilution	Total number of reads	Total number of reads after filtering	Total mapping reads after filtering	Reads likely to overlay mutation	Mutant reads	Wild type reads	Quantitation by NGS	Quantitation by pyro-sequencing
CMK 100%	2679094	2558535	1177664	395566	385354	5092	98·69%.	87·09%
CMK 15%	24513304	2156583	905243	307223	36996	265965	12·21%.	16·25%
CMK 5%	1775168	1600269	684422	245285	11543	230197	4·77%.	7·01%
CMK 1·5%	3042302	2827727	1205228	445318	4280	433617	0·97%.	1·94%
CMK 0·5%	3750306	3437119	1465754	507629	2608	497604	0·52%.	1·64%
CMK 0·16%	3058466	2895324	1285788	479693	933	470992	0·19%.	1·11%
CMK 0·1%	4119358	4215000	2002858	756599	1029	742103	0·13%.	0·69%
CMK 0·01%	2009850	1910057	819053	300732	240	295440	0·08%.	1·01%
CMK 0·001%	7275870	7138210	3238379	1224935	898	1205110	0·07%.	0·81%
CMK 0·0001%	5076054	4520778	1682721	537654	350	529928	0·06%.	1·13%
CB control 1	3026334	2765876	1181687	396585	310	390673	0·07%.	0·86%
CB control 2	1747636	1623810	687813	252487	204	248605	0·08%.	0·73%

CB: cord blood

Supplemental Table 2: Frequency of hematologic complications in DS neonates with and without TAM

Hematologic abnormality	Number of neonates		p value
	TAM (n=17)	DS without TAM (n=183)	
Thrombocytopenia ($<150 \times 10^9/L$)			
All babies	10/17	92/180	0.6162
Babies with IUGR	3/3	11/15	0.2279
Babies without IUGR	7/14	81/165	0.1034
Anemia (Hct < 0.4)	1	3	0.3010
Polycythemia (Hct >0.65)	1	44	0.1271
Increased blasts ($>10\%$)	17	6	$p<0.0001$

IUGR- intrauterine growth restriction

Hct- hematocrit

Supplemental Table 3: *GATA1* mutations in DS neonates

Patient ID.	Mutation detection by Sanger sequencing and/or DHPLC	Mutation detection by Deep Sequencing	Position* and sequence of mutation detected by Deep sequencing	Effect of mutation
DS1	WT	WT	N/A	N/A
DS2	WT	Not analysed	N/A	N/A
DS3	WT	Not analysed	N/A	N/A
DS4	WT	Not analysed	N/A	N/A
DS5	WT	Not analysed	N/A	N/A
DS6^	WT	WT	N/A	N/A
DS7	WT	Not analysed	N/A	N/A
DS8	WT	Not analysed	N/A	N/A
DS9	WT	Not analysed	N/A	N/A
DS10	WT	WT	N/A	N/A
DS11	WT	WT	N/A	N/A
DS12	WT	Not analysed	N/A	N/A
DS13	WT	Not analysed	N/A	N/A
DS14	WT	Not analysed	N/A	N/A
DS15	Not analysed	Not analysed	N/A	N/A
DS16	Not analysed	Not analysed	N/A	N/A
DS17	WT	WT	N/A	N/A
DS18	WT	WT	N/A	N/A
DS19	WT	WT	N/A	N/A
DS20	WT	Mutation	29 bp deletion at position 48649637 TTGGATGCAGCAGCTTC CTCCACTGCCCC	Frame shift
DS21	WT	Not analysed	N/A	N/A
DS22	WT	WT	N/A	N/A
DS23	WT	WT	N/A	N/A
DS24	WT	WT	N/A	N/A
DS25	WT	Mutation	13 bp duplication at position 48649679 GCTGCAGCTGCGG	Frame shift
DS26	WT	WT	N/A	N/A
DS27	WT	Not analysed	N/A	N/A
DS28	WT	Not analysed	N/A	N/A
DS29	WT	Not analysed	N/A	N/A
DS30	WT	Not analysed	N/A	N/A
DS31	WT	Not analysed	N/A	N/A
DS32	WT	Not analysed	N/A	N/A
DS33	WT	Not analysed	N/A	N/A

DS34	WT	Not analysed	N/A	N/A
DS35	WT	Not analysed	N/A	N/A
DS36	WT	Not analysed	N/A	N/A
DS37	WT	Not analysed	N/A	N/A
DS38	WT	Not analysed	N/A	N/A
DS39	WT	Not analysed	N/A	N/A
DS40	WT	Not analysed	N/A	N/A
DS41	WT	Not analysed	N/A	N/A
DS42	WT	Not analysed	N/A	N/A
DS43	WT	Not analysed	N/A	N/A
DS44	Not analysed	Not analysed	N/A	N/A
DS45	Not analysed	Not analysed	N/A	N/A
DS46^	WT	WT	N/A	N/A
DS47^	WT	WT	N/A	N/A
DS48	WT	WT	N/A	N/A
DS49	WT	Not analysed	N/A	N/A
DS50	WT	WT	N/A	N/A
DS51	WT	WT	N/A	N/A
DS52	WT	Not analysed	N/A	N/A
DS53	WT	WT	N/A	N/A
DS54	WT	Mutation	2bp deletion at position 48649606 (AG)	Frame shift
DS55	WT	WT	N/A	N/A
DS56	WT	WT	N/A	N/A
DS57	WT	Not analysed	N/A	N/A
DS58	WT	Not analysed	N/A	N/A
DS59	WT	Not analysed	N/A	N/A
DS60	WT	Not analysed	N/A	N/A
DS61	WT	Not analysed	N/A	N/A
DS62	WT	WT	N/A	N/A
DS63	WT	WT	N/A	N/A
DS64	WT	Not analysed	N/A	N/A
DS65	WT	Not analysed	N/A	N/A
DS66	WT	Not analysed	N/A	N/A
DS67	WT	Not analysed	N/A	N/A
DS68	WT	Not analysed	N/A	N/A
DS69	WT	Not analysed	N/A	N/A
DS70	WT	Not analysed	N/A	N/A
DS71	WT	Not analysed	N/A	N/A
DS72	WT	Not analysed	N/A	N/A
DS73	WT	Not analysed	N/A	N/A
DS74	WT	WT	N/A	N/A
DS75^	WT	WT	N/A	N/A
DS76	WT	Not analysed	N/A	N/A
DS77	WT	WT	N/A	N/A

DS78	WT	Not analysed	N/A	N/A
DS79	WT	Mutation	C>T at position 48649565	Introduction of stop codon
DS80	Not analysed	Not analysed	N/A	N/A
DS81	Not analysed	Not analysed	N/A	N/A
DS82	WT	WT	N/A	N/A
DS83	WT	Mutation	G>A at position 48649519	Loss of start codon
DS84	WT	Not analysed	N/A	N/A
DS85	WT	Mutation	Clone 1, G>A position 48649519 Clone 2, C>T position 48649565 Clone 3, del(AG) 48649606	Clone 1, loss of start codon Clone 2, introduction of stop codon Clone 3, frame shift
DS86	WT	Not analysed	N/A	N/A
DS87	WT	Not analysed	N/A	N/A
DS88	WT	Mutation	Clone 1, G>A position 48649519 Clone 2, C>G position 48649702	Clone 1, loss of start codon Clone 2, Introduction of stop codon
DS89	WT	WT	N/A	N/A
DS90	WT	WT	N/A	N/A
DS91	WT	WT	N/A	N/A
DS92	WT	WT	N/A	N/A
DS93	WT	WT	N/A	N/A
DS94	WT	Not analysed	N/A	N/A
DS95	WT	WT	N/A	N/A
DS96	WT	WT	N/A	N/A
DS97	WT	Mutation	AGT>C substitution at position 48649521	Frame shift
DS98	WT	WT	N/A	N/A
DS99	WT	WT	N/A	N/A
DS100	WT	Mutation	clone 1, 8 bp duplication CACCGCTG at position 48649675 clone2, G>A position 48649706	clone 1, Frame shift clone 2, loss of splice donor site
DS101	WT	Mutation	duplication CACCGCTG at position 48649675	Frame shift
DS102	WT	WT	N/A	N/A
DS103	WT		N/A	N/A

		Not analysed		
DS104^	WT	WT	N/A	N/A
DS105	WT	WT	N/A	N/A
DS106^	WT	WT	N/A	N/A
DS107	WT	WT	N/A	N/A
DS108 ⁺	WT	Mutation	Insertion C at position 48649618	Frame shift
DS109	WT	WT	N/A	N/A
DS110	WT	WT	N/A	N/A
DS111	WT	Not analysed	N/A	N/A
DS112	WT	WT	N/A	N/A
DS113	WT	Not analysed	N/A	N/A
DS114	WT	WT	N/A	N/A
DS115	WT	WT	N/A	N/A
DS116	WT	Not analysed	N/A	N/A
DS117	Not analysed	Not analysed	N/A	N/A
DS118	WT	Not analysed	N/A	N/A
DS119	WT	WT	N/A	N/A
DS120	WT	WT	N/A	N/A
DS121	WT	Not analysed	N/A	N/A
DS122	WT	Not analysed	N/A	N/A
DS123	WT	WT	N/A	N/A
DS124	WT	Not analysed	N/A	N/A
DS125	WT	Not analysed	N/A	N/A
DS126	WT	WT	N/A	N/A
DS127	WT	Not analysed	N/A	N/A
DS128	WT	WT	N/A	N/A
DS129	WT	WT	N/A	N/A
DS130	WT	WT	N/A	N/A
DS131	WT	Mutation	8bp duplication (CACCGCTG) at position 48649675	Frame shift
DS132	WT	Not analysed	N/A	N/A
DS133	WT	Not analysed	N/A	N/A
DS134	WT	Not analysed	N/A	N/A
DS135	Not analysed	Not analysed	N/A	N/A
DS136	WT	Not analysed	N/A	N/A
DS137	WT	Mutation	8bp duplication (CACCGCTG) at position 48649675	Frame shift
DS138	WT	WT	N/A	N/A
DS139	WT	WT	N/A	N/A
DS140	WT	WT	N/A	N/A
DS141	WT	WT	N/A	N/A
DS142	WT	WT	N/A	N/A

DS143	WT	WT	N/A	N/A
DS144	WT	Not analysed	N/A	N/A
DS145	WT	Not analysed	N/A	N/A
DS146	WT	Not analysed	N/A	N/A
DS147	WT	Not analysed	N/A	N/A
DS148	WT	WT	N/A	N/A
DS149	WT	WT	N/A	N/A
DS150	WT	WT	N/A	N/A
DS151	WT	WT	N/A	N/A
DS152	WT	Mutation	A>G mutation at position 48649496	Loss of splice acceptor site
DS153	WT	WT	N/A	N/A
DS154	WT	Not analysed	N/A	N/A
DS155	WT	Not analysed	N/A	N/A
DS156	WT	Not analysed	N/A	N/A
DS157	WT	Not analysed	N/A	N/A
DS159	Not analysed	Not analysed	N/A	N/A
DS160	Not analysed	Not analysed	N/A	N/A
DS161	WT	WT	N/A	N/A
DS162	WT	WT	N/A	N/A
DS163	WT	Mutation	G>A at position 48649519	Loss of start codon
DS164	WT	Not analysed	N/A	N/A
DS165	WT	Not analysed	N/A	N/A
DS166	WT	Not analysed	N/A	N/A
DS167	WT	WT	N/A	N/A
DS168	WT	Not analysed	N/A	N/A
DS169	WT	Not analysed	N/A	N/A
DS170	Not analysed	Not analysed	N/A	N/A
DS171	Not analysed	Not analysed	N/A	N/A
DS172	Not analysed	Not analysed	N/A	N/A
DS173	Not analysed	Not analysed	N/A	N/A
DS174	WT	WT	N/A	N/A
DS175	WT	WT	N/A	N/A
DS176	WT	WT	N/A	N/A
DS177	WT	Mutation	8bp duplication (CACCGCTG) at position 48649675	Frame shift
DS178	WT	WT	N/A	
DS179	WT	Mutation	2bp deletion (AG) at position 48649606	Frame shift
DS180	WT	WT	N/A	N/A
DS181	WT	Mutation	G>T at position 48649715	Introduction of stop codon
DS182	WT	Not analysed	N/A	N/A
DS183	WT	Not analysed	N/A	N/A

DS184	WT	Not analysed	N/A	N/A
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* Nucleotide 0 is the first nucleotide of *GATA1* exon 1 including exons and introns. NCBI reference NT_079573.4 (*Homo sapiens* chromosome X genomic contig, starting position 11496706) was used.

N/A = Not applicable

^ Neonates with blasts >10%

+ developed ML-DS, age 18 months

Supplemental Table 4: Frequency of hematopoietic cell morphologic abnormalities on blood smears from DS neonates with and without TAM

Morphologic abnormalities	Number of neonates (%)		p value
	TAM n=17	No TAM n=183	
Macrocytes	17 (100)	177* (97.3)	1.0000
Target cells	10 (58.8)	76* (41.7)	0.2054
Dyserythropoietic erythroblasts	10 (58.8)	70* (38.5)	0.1233
Basophilic stippling	2 (11.8)	11* (6.0)	0.3067
Giant platelets**	17 (100)	176 (96.3)	0.6038
Megakaryocyte fragments**	16 (94.1)	84 (45.9)	0.0002
Dysplastic neutrophils***	17 (100)	155* (85.2)	0.1358
Dysplastic monocytes***	16 (94.1)	176* (96.7)	0.4702

* RBC and WBC morphology was assessable in 182/183 cases.

** Giant platelets and megakaryocyte fragments were defined by their diameter of $>4 \mu\text{m}$ and $<8 \mu\text{m}$ respectively

*** Dysplastic neutrophils and monocytes were identified based on the EWOG criteria¹.

Supplemental Table 5: Impact of neonatal and pregnancy-related complications on hematologic findings in DS neonates (n=200)

	CHD (n=89)			Sepsis (n=10)			Preterm (n=44)			IUGR/PET (n=18)			Maternal DM (n=5)		
	Yes	No	p	Yes	No	p	Yes	No	p	Yes	No	p	Yes	No	p
Median Hct	.599	.589	0.3624	.627	.591	.1490	.571	.600	.0371	.583	.596	0.8852	.594	.595	0.5279
Median nRBC/100 WBC	6.5	4.0	0.2716	28	20	0.4664	2.5	6	0.2704	9	5	0.1911	20	5	0.3117
Median Platelets x 10 ⁹ /L	147	149	0.2302	117	158	0.1274	164	141	0.0306	113	149	0.0442	139	148	0.3297
Median WBC x 10 ⁹ /L	14.8	15.9	0.7000	17.9	17.4	0.8873	13.2	15.9	0.0250	16.1	14.9	0.4375	10.3	10.6	0.1317
Median Neuts x 10 ⁹ /L	9.5	9.9	0.1656	10.6	10.9	0.9052	7.7	10.8	0.0023	9.1	9.9	0.2565	11.3	10.7	0.9743
Median Blasts (%)	4	4	0.3086	7	4	0.0198*	5	4	0.3871	2	4	0.8982	5	4	0.7586

* Neonates without TAM: p=0.2431 (median blasts 6 v 4%, sepsis v no sepsis)

Neonates with TAM: p=0.7459 (median blasts 39 v 33%, sepsis v no sepsis)

CHD: Congenital Heart Disease. Preterm: gestational age at birth < 37 weeks; IUGR: intrauterine growth restriction; PET: Pre-eclamptic toxemia; DM Diabetes mellitus; nRBC: nucleated red blood cells; WBC: white blood cells; Neuts: neutrophils

Supplemental Table 6: Immunophenotype of peripheral blood blasts in neonates with DS

	% blasts on peripheral blood smear	CD34+ cells	
		% of total MNC	% CD33+CD7+CD36+
Silent TAM DS25 DS181	9 10	5.8 0.3	13.5 0.0
No GATA1 mutation DS26 DS55 DS112 DS114 DS115	4 2 2 6 4	2.0 1.0 0.4 0.46 0.70	32.6 0.71 0.15 0.0 0.17
TAM (n=7) mean \pm SEM (range)	43 \pm 3	38.6 \pm 14.7 (0.13-81.8)	28.6 \pm 6 (17.7-59.1)
Neonates without DS (n=13) mean \pm SEM (range)	(0-4)	0.74 \pm 0.2 (0.1-2.3)	0 \pm 0

MNC-mononuclear cells

Supplemental Table 7: *GATA1* mutation analysis by pyrosequencing- PCR

primers and conditions

Patient	Forward Primer	Reverse Primer	Annealing temperature
DST3	TCCTCCACACCAGAATCAGGGGTTTTCTT	AATGGAGTTACCTGGGGAGTGTCTGTAGGC	64 °C
DST4, DS25	TCCTCCACACCAGAATCAGGGGTTTTCTT	GAGTGTCTGTAGGCCTCAGCGTCCCTGTAG T	62.6 °C
DST7, DS83, DS88 Clone 1, DS152, DS163	GTGAAAGGATGTGGGGTGAAGGATTTCT T	GAGGACACCAGAGCAGGATCCACAACT	60 °C
DST8, DST9, DST10 DST12 clone 2 DST13 DS100, DS131, DS139, DS177, DS181	TCCTCCACACCAGAATCAGGGGTTTTCTT	GAGTGTCTGTAGGCCTCAGCGTCCCTGTAG T	62.6 °C
DST11 DS12	TGTCCTCCACACCAGAATCAGGGGTTTT	TGTCTGTAGGCCTCAGCGTCCCTGTAGTA	59°C
DST12 clone 1, DS54 DS179	CAGTTTGTGGATCCTGCTCTGGTGTCT	AGTGTCTGTAGGCCTCAGCGTCCCTGTAGT	64.8 °C
DST14 DST17 DS79	B-GCAGGTTAATCCCCAGAGGCT	GAAAACCCCTGATTCTGGTGTG	54.5°C
DST15	TACTACAGGGACGCTGAGGCCTACAGAC	CACAGTGGTATTCTGACCTAGCCAAGGATC C	64.8 °C
DS88 clone 2	B- CCTCCACACCAGAATCAGGGGTTTTCTT	CTCAATGGAGTTACCTGGGGAGTGTCTGTA G	64.8°C

Supplemental Table 8: *GATA1* mutation analysis by pyrosequencing- primers and dispensation orders

Patient	Sequencing Primer	Dispensation Order
DST2	CTACAGACACTCCCCAG	ATACTCGTACTCGATGAGTG
DST3	ACAGCCACCGCTGCA	TGCTCGCGCAGCTGCTACTGACAGACGCT
DST4	CAGTGCCGCAGCTGC	TAGCGTGCTGTGCAGCTGCGCAGTCGTGCTGTGCTCGCAGTGAGAGC
DST7	CCCCAGAGGCTCCAT	TGATGATCGTCGTGCT
DST8, DS181	GCCTACTACAGGGACG	CTAGCATACGCTAGACAGACTCACTCAGT
DST9	TCCTCCACTGCCCCG	AGCGTGAGCAGCACGCACGCTG
DST10	CTGCGGCACTGGCCT	TACTCAGTCGCAGTACTACGAGCACGCTG
DST11	GGGTTTTCTTCCCCTC	TTCTCGTCTGCTGAG
DST12 Clone 1, DS54 DS179	TCCTCCACACCAGAAT	TCGTCTACTGTCTCGTCTGCTGA
DST12 Clone 2, DS131 DS137 DS177	CCGAGCACAGCCACC	GCTGCAGCGTCGTGCGCAGCTGCGCACTGCTAC
DST13	ACTGCCCCGAGCACA	GCGCAGCTGCAGCACAGAGCTGCAGCGCTGCAGCTGCGC
DST14 DS79	GAGCAGGATCCACAA	ACTGAGCTCTAGCTCTAGTCAGTCAGAC
DST15	CTACAGACACTCCCCAG	ATGCTGTCTACTCATGAGTG
DS20	CTCTGGGCCTGAGGG	TCGATGATGCAGCAGCACTCTCACTGCGAGCACAGCACGCT
DS25	CAGTGCCGCAGCTGC	TAGCGTCGCAGCTGCAGCGTGCTGTGCTCG
DS83, DS88 Clone 1	CCCCAGAGGCTCCAT	TGAGTCTGAGTCTGCTCTGTCTGAC
DS88 Clone 2	CCTCAGCGTCCCTGT	TAGTCAGTACGACGTAGTGC
DS100	ACAGCCACCGCTGCA	GCGCTGCGCAGCTGCGCACTAC
DS152	CCCCTTCTGTCTCG	TCGTATGTACAGATCGAGAGC
DST17	CACAACTGGGGGAG	GGCTAGAGCTCGAGTCAGAC
DS163	CCCCAGAGGCTCCAT	ATTGGAGGTTT