

Step 1: Reverse transcription--cDNA synthesis from reverse primer mix.

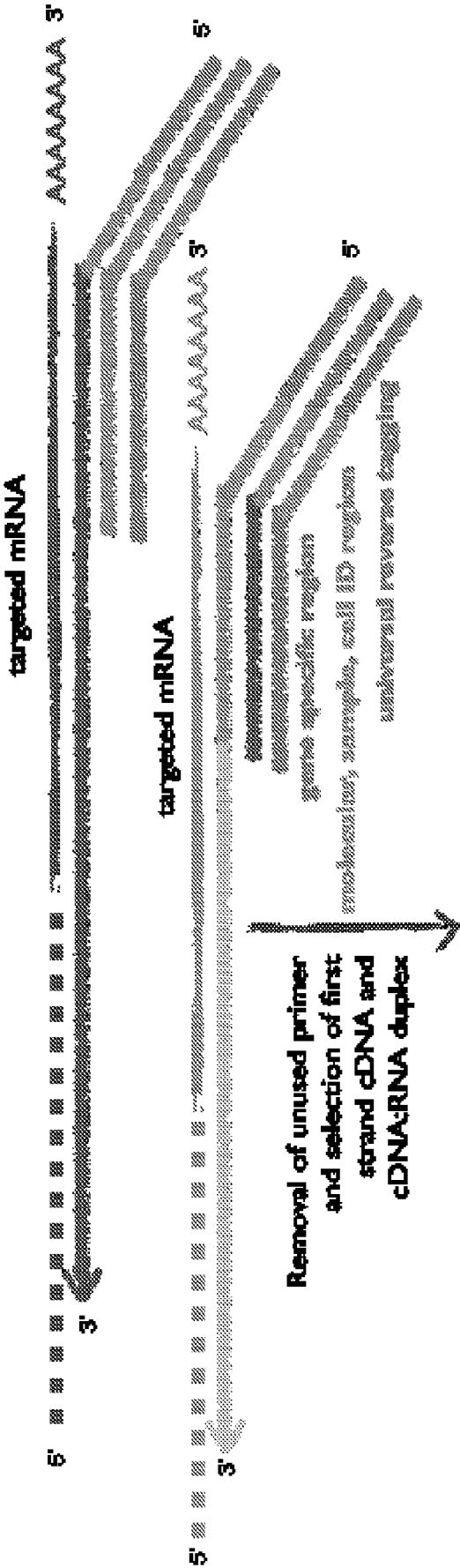


FIG. 1A

Step 2: One cycle second strand DNA synthesis from the forward primer mix.

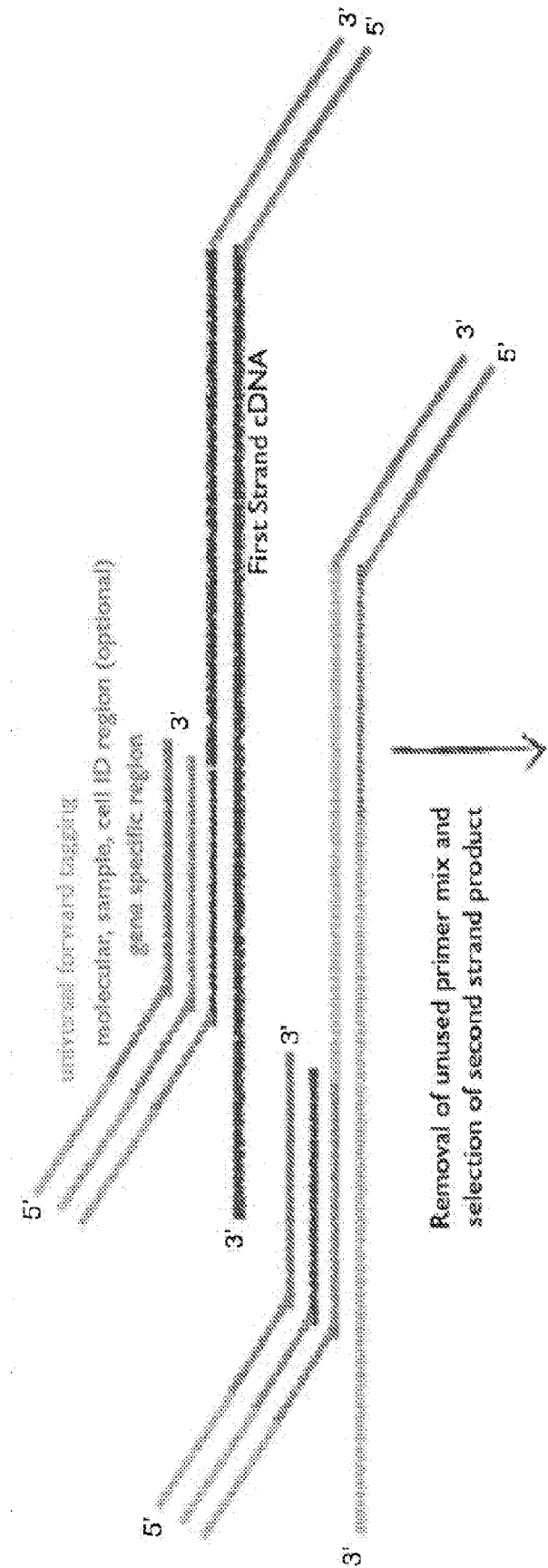
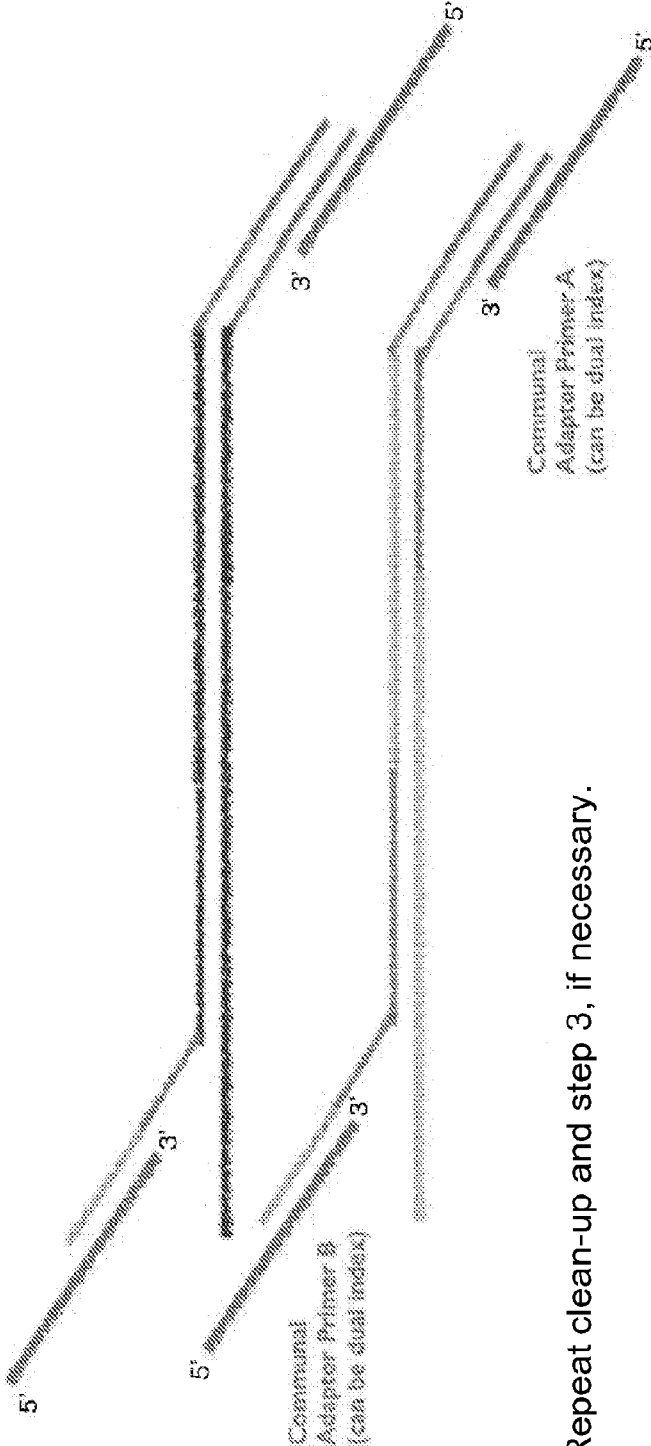


FIG. 1B

Step 3: Two-step PCR to complete the sequencing adaptor and amplify the tagged products from the first and second cycles.



Step 4: Repeat clean-up and step 3, if necessary.

FIG. 1C

Step 1: A first primer mix is used in one cycle tagging with gDNA. The first primer mix can be forward primers only, reverse primers only, or a mixture of forward and reverse primers used for strategic tagging.

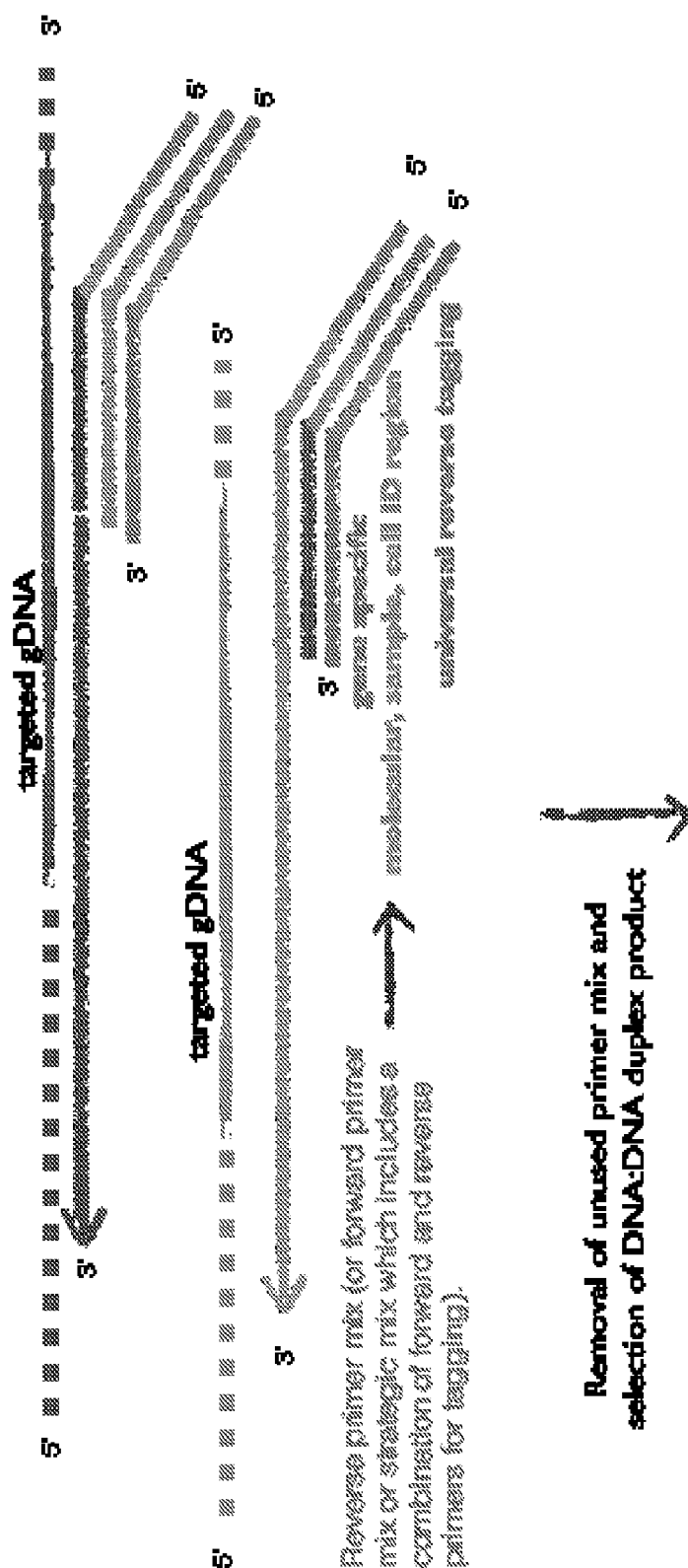


FIG. 2A

Step 2: One cycle second strand DNA synthesis with a second primer mix. The second primer mix can be forward primers only, reverse primers only, or a mixture of forward and reverse primers used for strategic tagging. The selection of the primers to include in the mix depends on what was used in the first cycle.

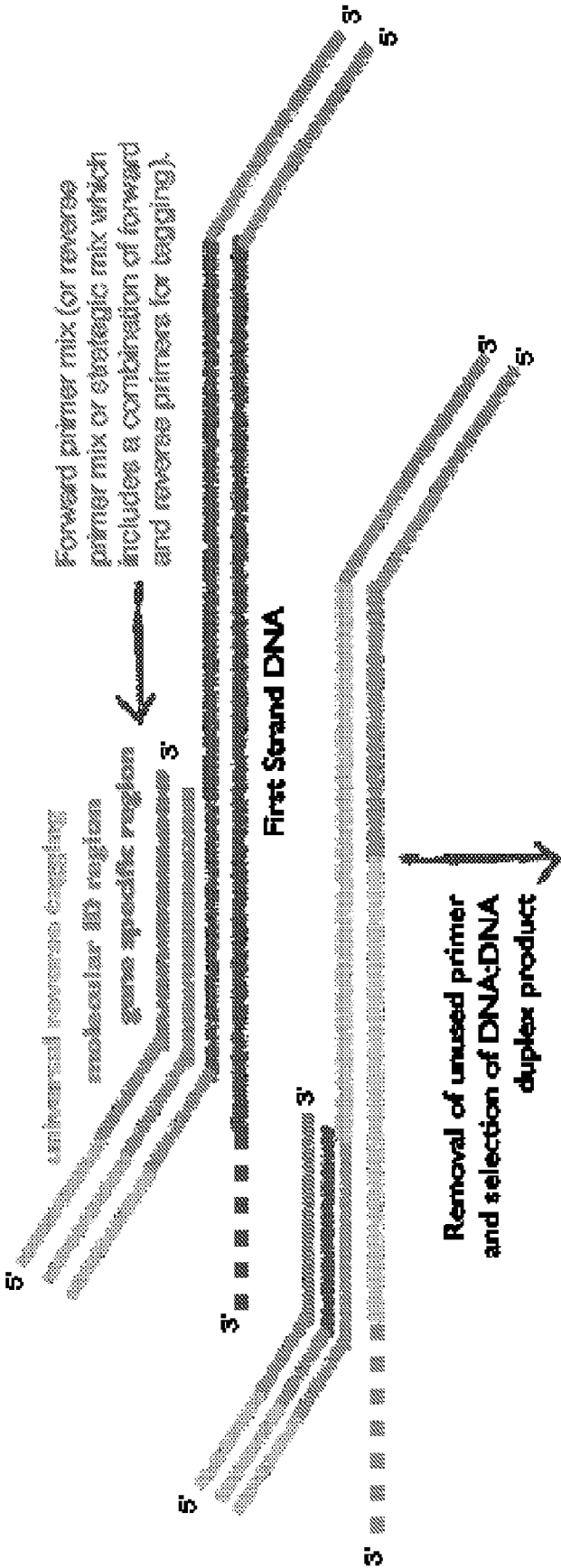
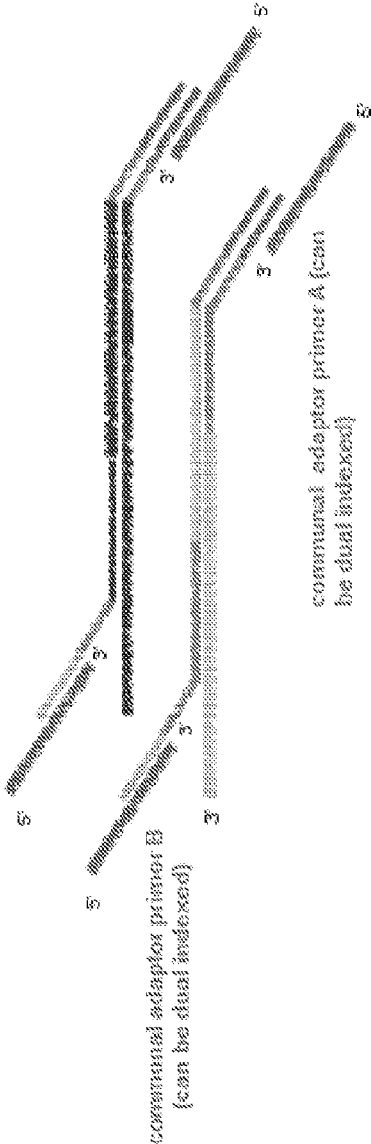


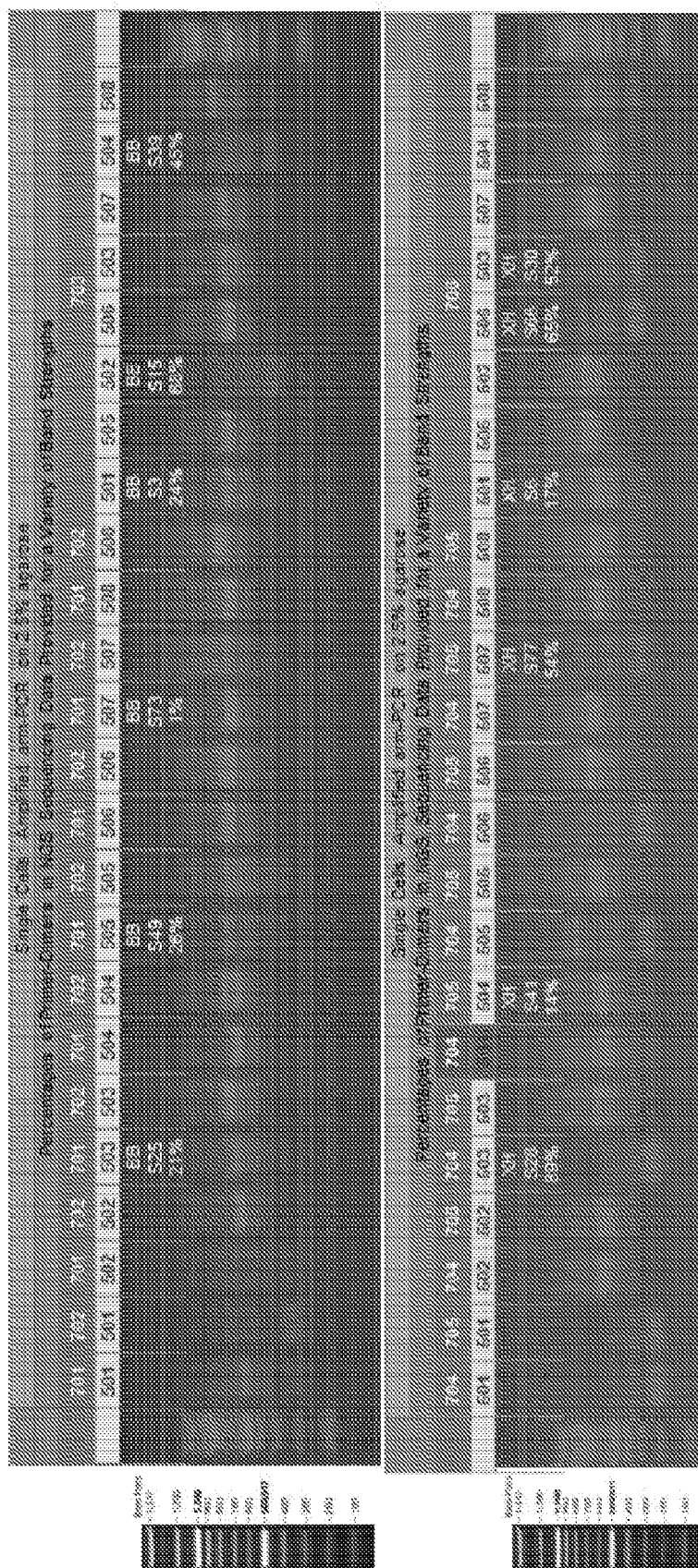
FIG.2B

Step 3: Two-step PCR to complete the sequencing adaptor and amplify the tagged products from the first and second cycles.



Step 4: Repeat clean-up and step 3 if necessary.

FIG. 2C



8/21

Primer Combination	Reverse Primer (R1)	Forward primer (R2)	Frequency	% in Trash	% for Total Reads	Sum % in Trash	Total reads in 12 samples reads as trash % trash	875062 270998 31%
FOXP3-Ri-09 + ITRAV_2	FOXP3-Ri-09	ITRAV_2	12	10.5%	3.2%	10.5%	10.5%	
BCL6Ri MD_11 + ITRAV_40	BCL6Ri MD_11	ITRAV_40	12	10.0%	3.1%	20.5%	20.5%	270998
ITRAC + ITRAV_2	ITRAC	ITRAV_2	12	6.1%	1.9%	26.6%	26.6%	31%
IL-17F-Ri-09 + ITRAV_41	IL-17F-Ri-09	ITRAV_41	12	5.3%	1.7%	32.1%	32.1%	
BCL6Ri MD_11 + ITRAV_23	BCL6Ri MD_11	ITRAV_23	12	4.6%	1.4%	36.7%	36.7%	
IL-17F-Ri-09 + ITRAV_2	IL-17F-Ri-09	ITRAV_2	12	2.6%	0.8%	39.2%	39.2%	
CTLA-4-Ri-09 + CD8-FI	CTLA-4-Ri-09	CD8-FI	10	3.1%	1.0%	42.3%	42.3%	
IL-17F-Ri-09 + ITRAV_34	IL-17F-Ri-09	ITRAV_34	10	2.2%	0.7%	44.5%	44.5%	
Eomes-Ri-09 + ITRAV_2	Eomes-Ri-09	ITRAV_2	7	2.1%	0.6%	46.6%	46.6%	
Eomes-Ri-09 + ITRAV_42	Eomes-Ri-09	ITRAV_42	5	2.1%	0.7%	48.7%	48.7%	
ITRBC + RUNX3FI MD	ITRBC	RUNX3FI MD	3	2.1%	0.7%	50.8%	50.8%	
IL-17F-Ri-09 + ITRAV_23	IL-17F-Ri-09	ITRAV_23	3	1.7%	0.5%	52.5%	52.5%	
FOXP3-Ri-09 + IL-22-FI	FOXP3-Ri-09	IL-22-FI	3	1.6%	0.5%	54.1%	54.1%	
FOXP3-Ri-09 + CD8-FI	FOXP3-Ri-09	CD8-FI	2	4.3%	1.3%	58.5%	58.5%	
Eomes-Ri-09 + ITRBV_15	Eomes-Ri-09	ITRBV_15	2	1.8%	0.6%	60.3%	60.3%	
TNFA-Ri-09 + ITRAV_42	TNFA-Ri-09	ITRAV_42	1	1.8%	0.6%	62.1%	62.1%	
ITRAC + ITRAV_34	ITRAC	ITRAV_34	1	1.0%	0.4%	63.5%	63.5%	

FIG. 3B

Time-domain production using variations of cycling conditions with same PCR protocols											
Forward:	H2AV_2				H2AV_40				Transfer control		
Reverse:	E-177-26-00				E-CLAR MD_11				Process control		
LMV marker	SOIT-PCR	R1010	SOIT-PCR	R1010	SOIT-PCR	R1010	SOIT-PCR	R1010	SOIT-PCR	R1010	
	1	2	3	4	5	6	7	8	9	10	11
											12

23

[illegible]

2542

AGTCTCTCAGCTGGTACACG
CGGTATAGTCTCTCAGCTGGTACACG

Abstract

22

[illegible]

82-175-24-08

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GCAGGTACAGGGTGCGCGTTTC

XXXXXXXXXXXXXXXXXXXX

2004

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DOC ID: 706_13

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HRA

2008-09-09 14:00

202

2000

ATGATAGGATGACAGGGGTGACCGAG;
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;GCAGTACGTGTGAGTCT
;TTRAV 41

80-2662X03

AIBZATTSBATTTCAGGTGCGT
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AICZATTTGACTTGAGGTGGCC

PAGE 2

2025

100

TADGTAGAGACCGGACGCTGCACCT
TADGTAGAGAGGTCGAGBACGTGCACCTCAGCTCCACAGGGG
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|||TRAV_23

2003

08-07-93

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FIG. 4B



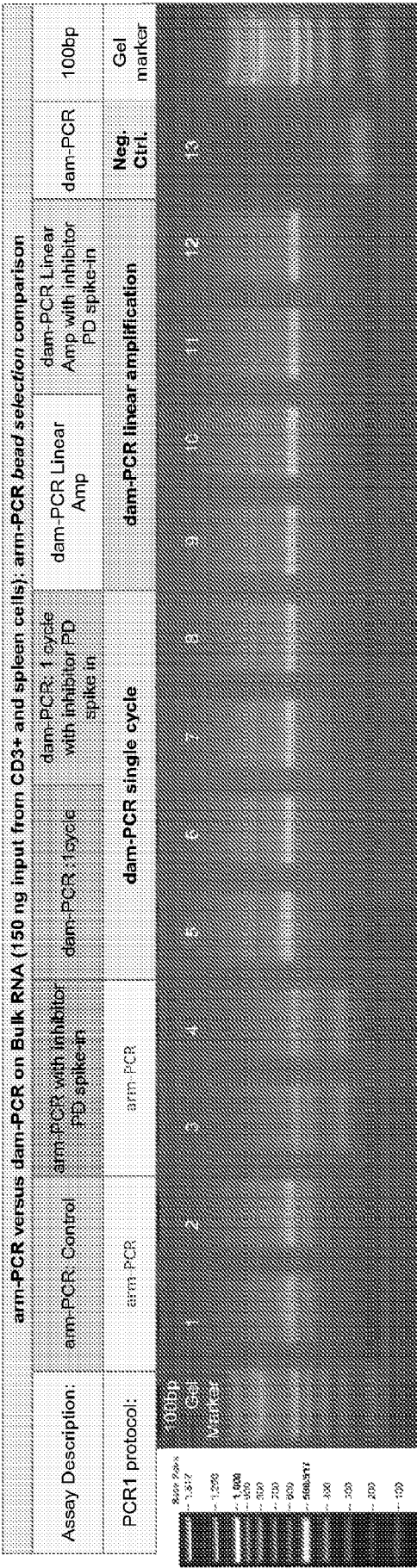


FIG. 7A

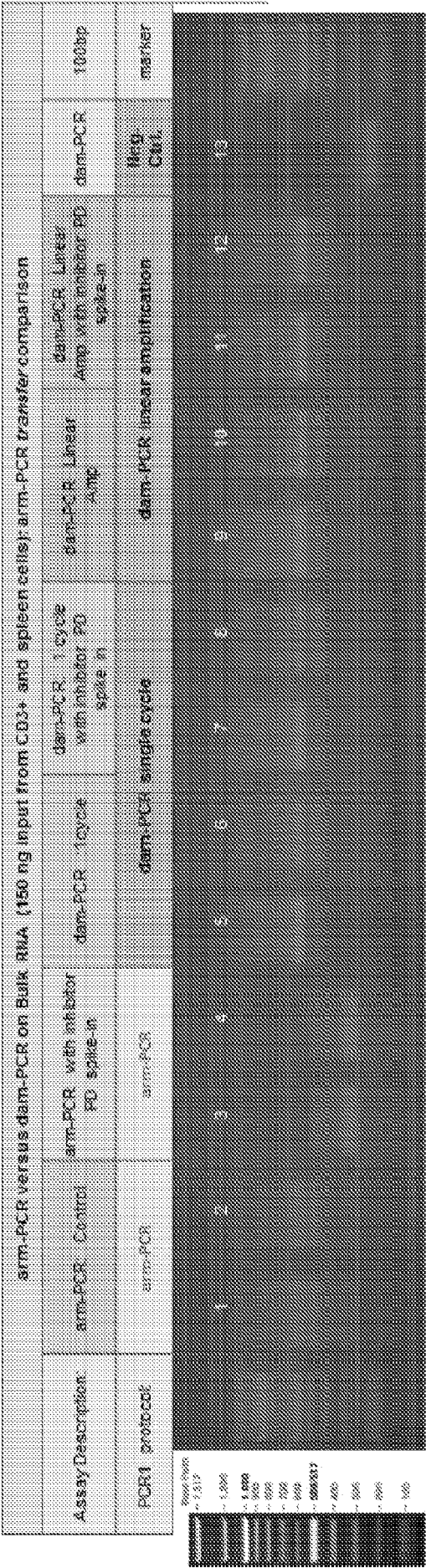


FIG. 7B

Primer Carry Over Clean-up Assessment										
Standard Curve of Primer Dimers						CES Clean-up				0.7% selection buffer to select
Test										
% of 2pmd input	0.50%	0.25%	0.10%	0.05%	0.03%	0.01%	0.01%	0	test	repeat
									negative control	negative control
									test	repeat
									3	4

FIG. 9

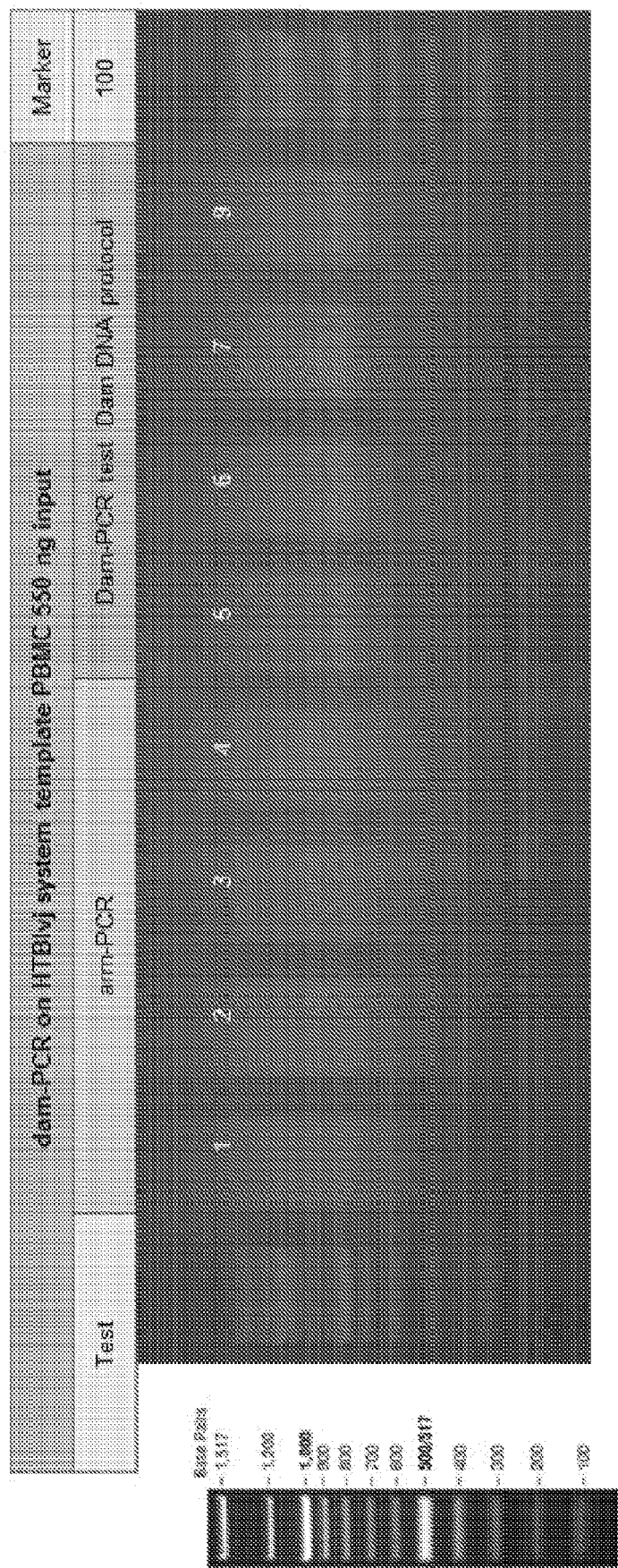


FIG. 10A

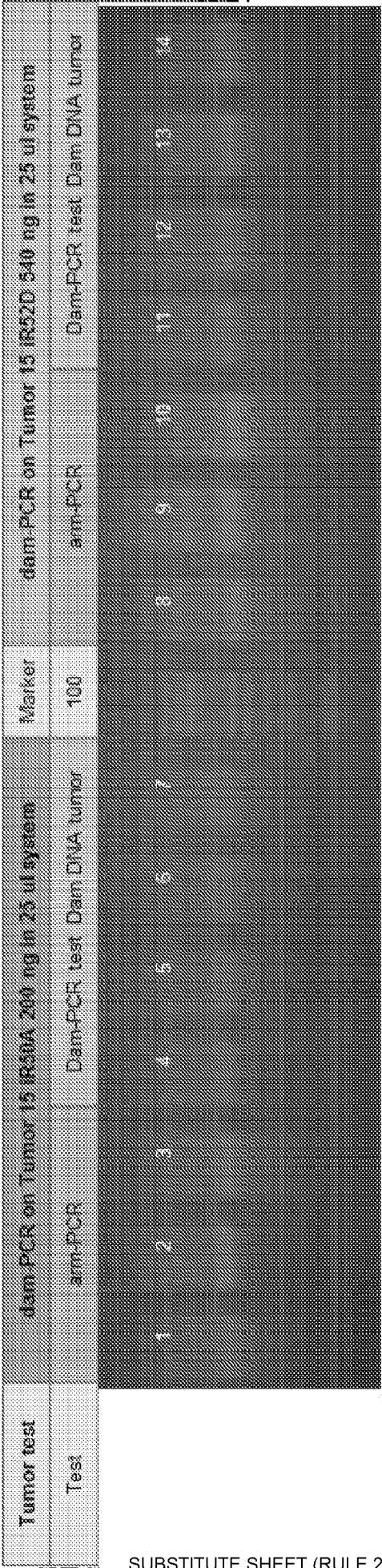


FIG. 10B

Larger Gene (gDNA) Targeted for Coverage with a Traditional Multiplex PCR Strategy

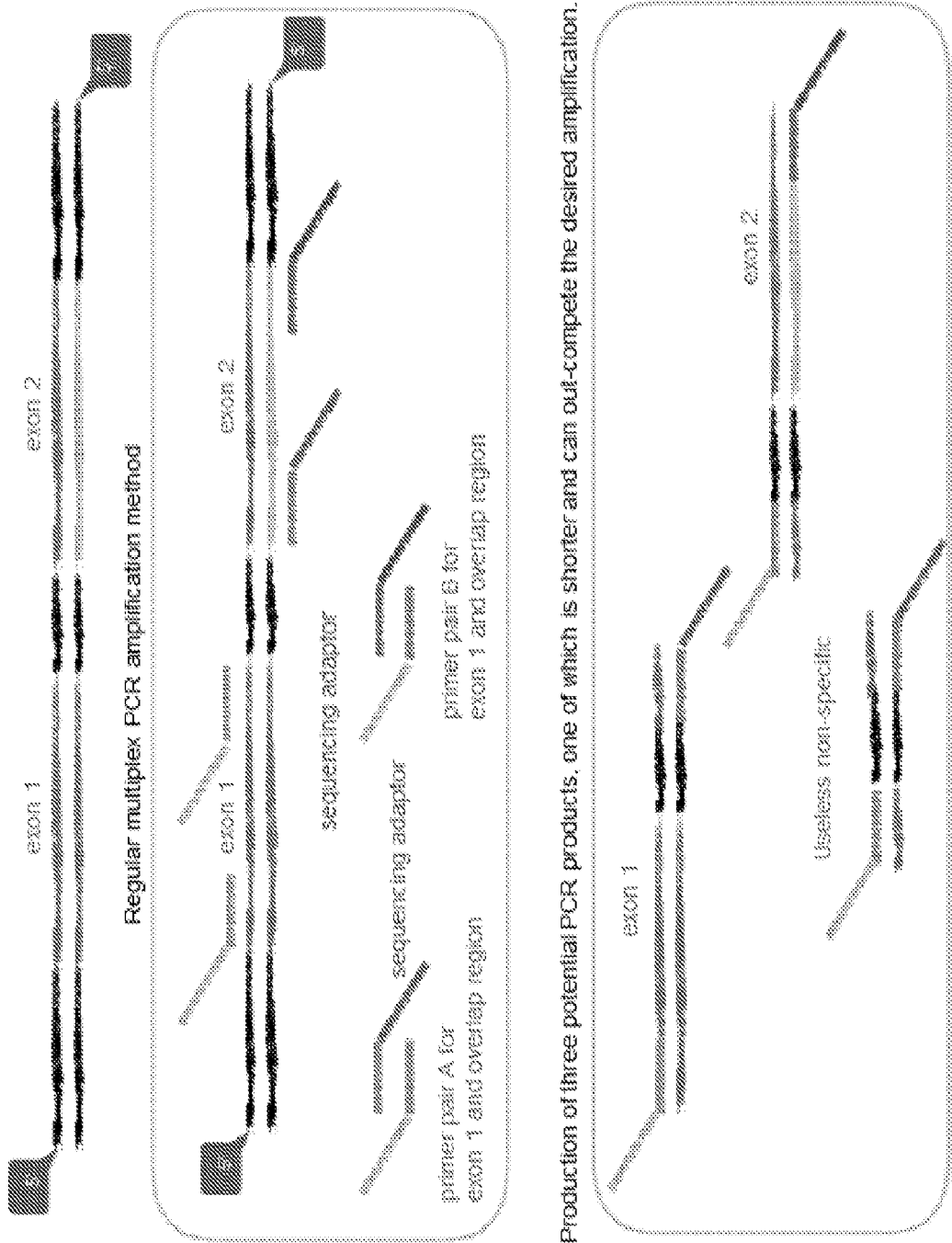


FIG. 11

20/21

Larger Gene (gDNA) Targeted for Coverage with dam-PCR

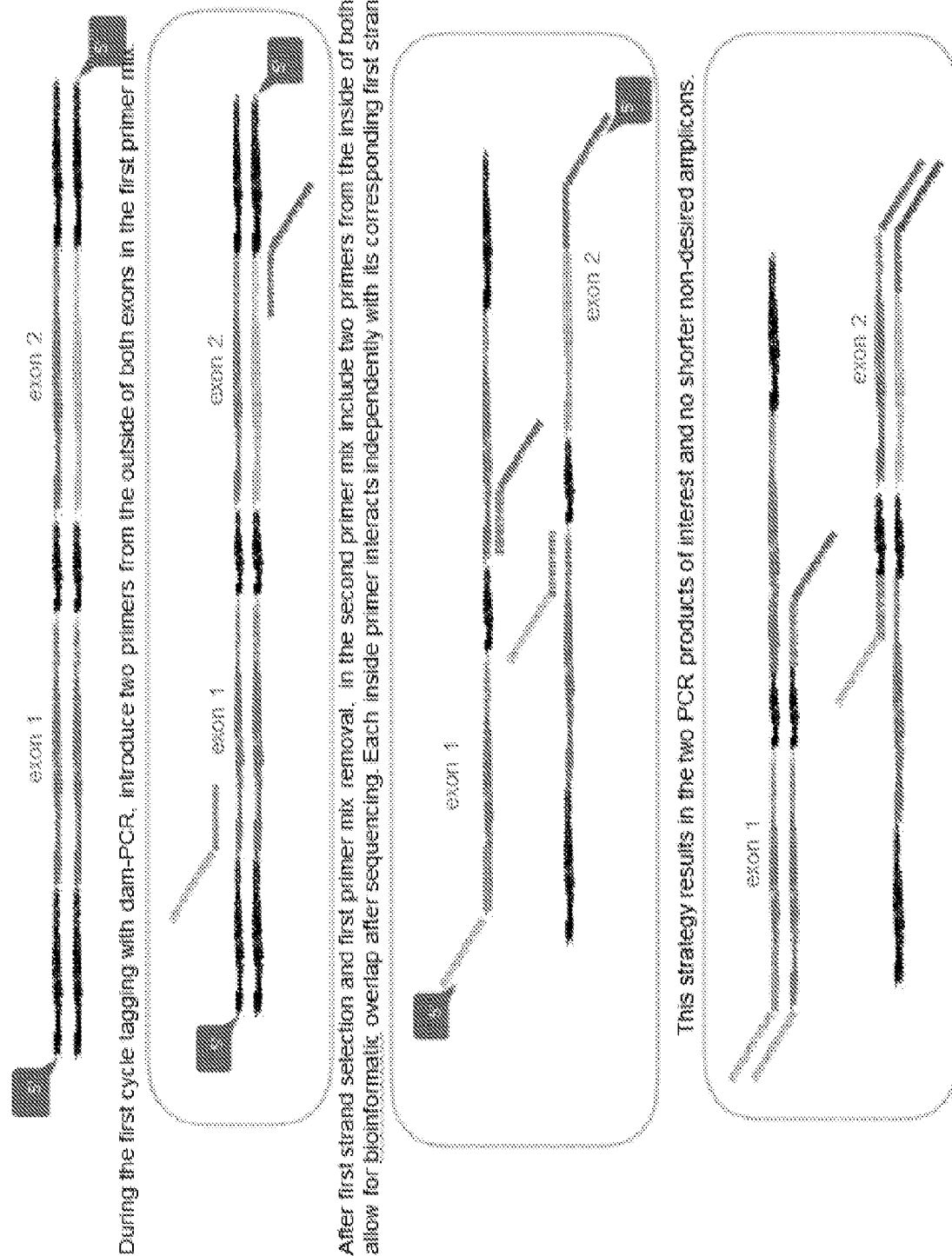
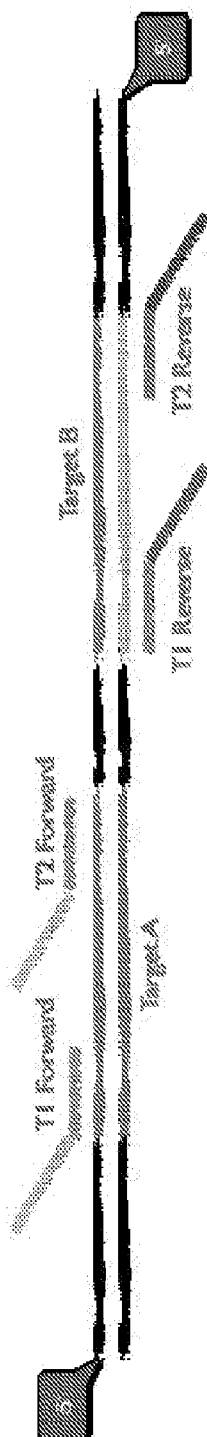


FIG. 12

21/21

dam-PCR gDNA amplification test on HLA overlap exon



DNA sample size 100 ng input	HSA 999-PCR amplification				dam-PCR amplification	Marker
Amplification target	Target A	Target B	Short insert	Long product	Target A	Target B
Forward primer in arm PCR	100%	100%	100%	100%	100%	100bp
Reverse primer in arm PCR	81%	79%	79%	71%	100%	dam PCR 1st cycle
	79%	79%	79%	71%	100%	dam PCR 2nd cycle

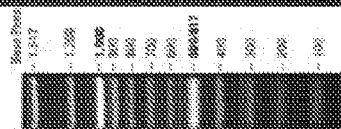


FIG. 13