

Chromatin Immunoprecipitation Assay

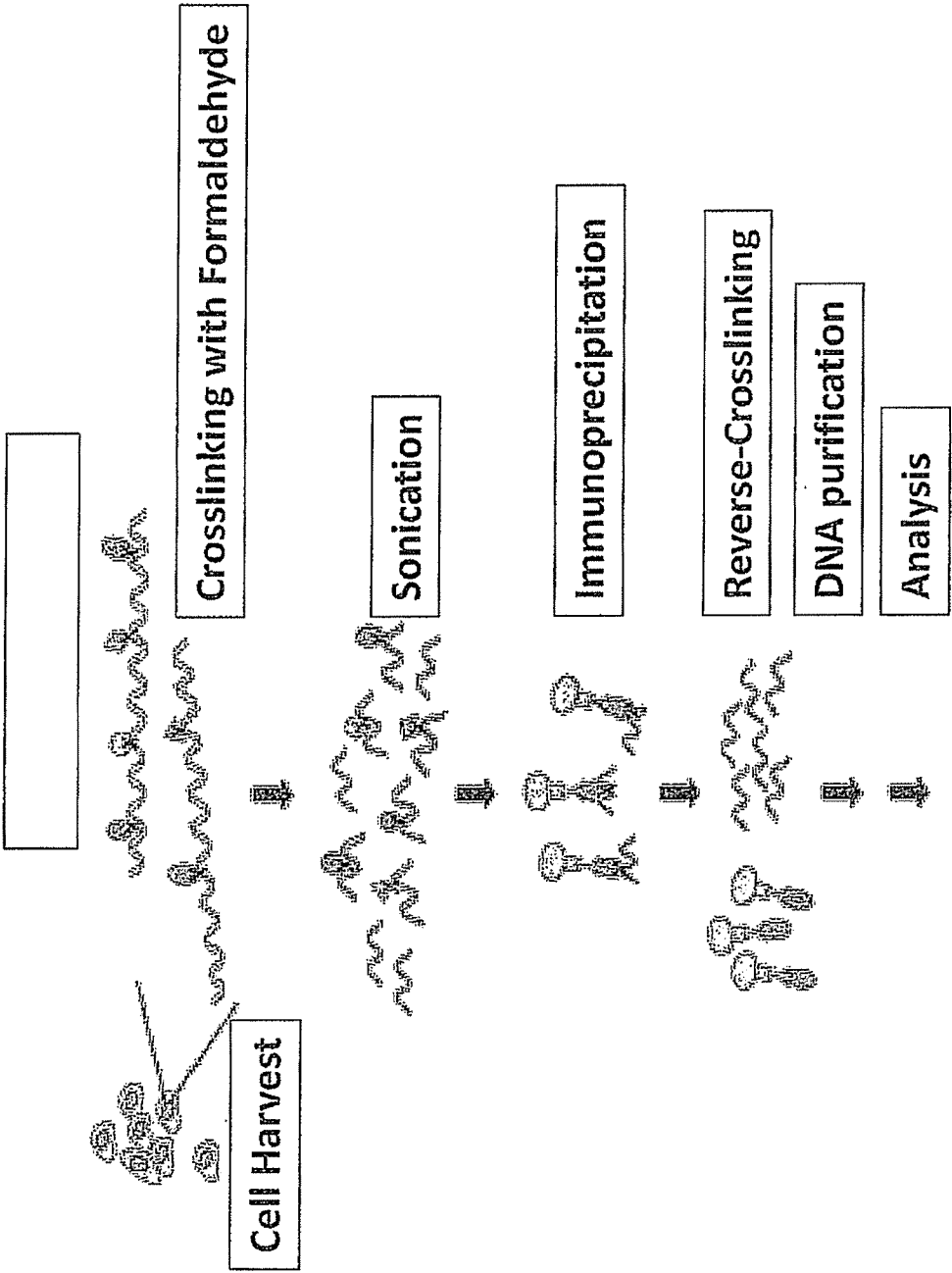
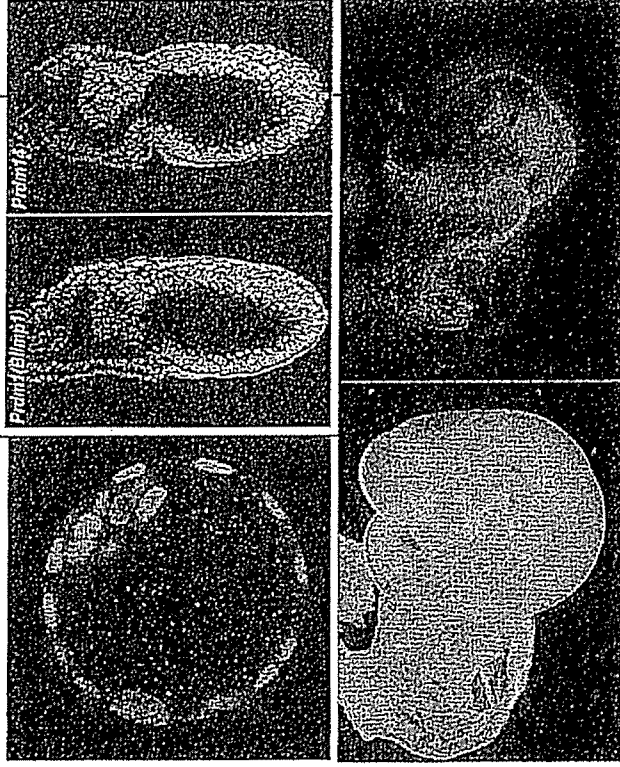


Figure 1

ChIP assay with limited sample



- Early embryo
- Rare cell population
- Human sample

Figure 2

Modifications on ChIP Protocol for Small-Scale Assay

3/33

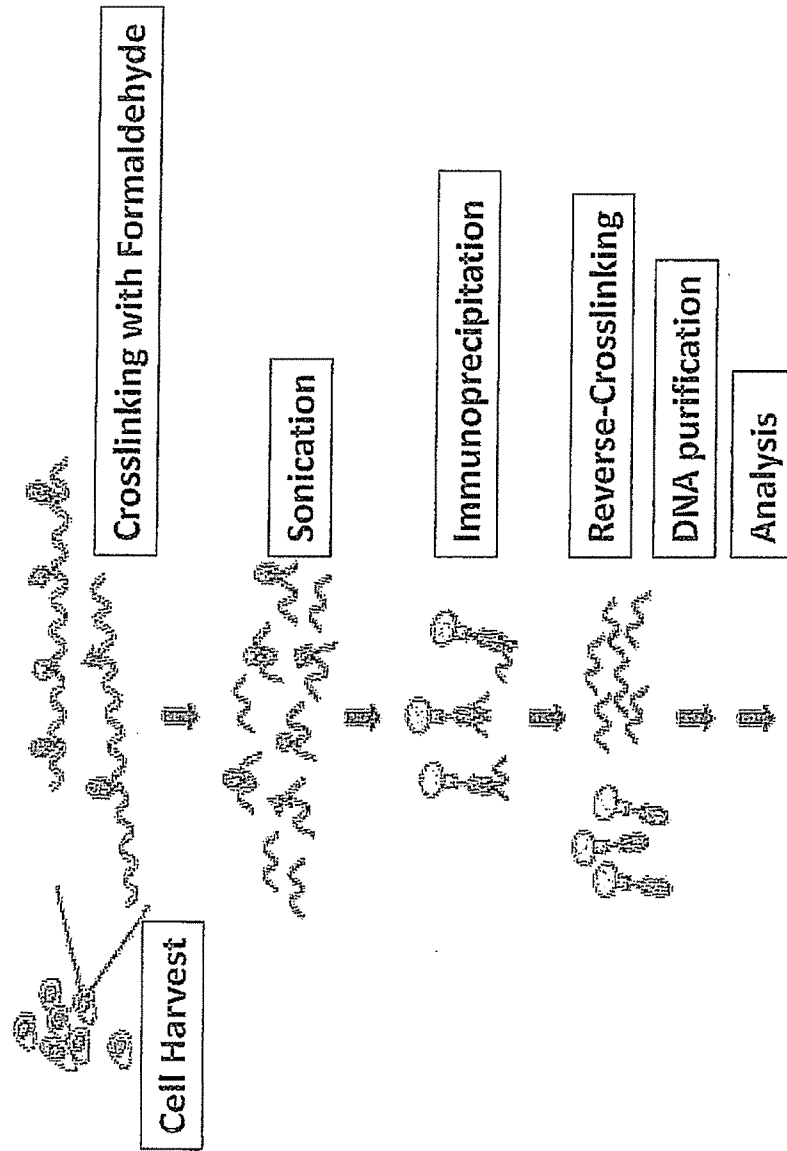


Figure 3

Modifications on ChIP Protocol for Small-Scale Assay

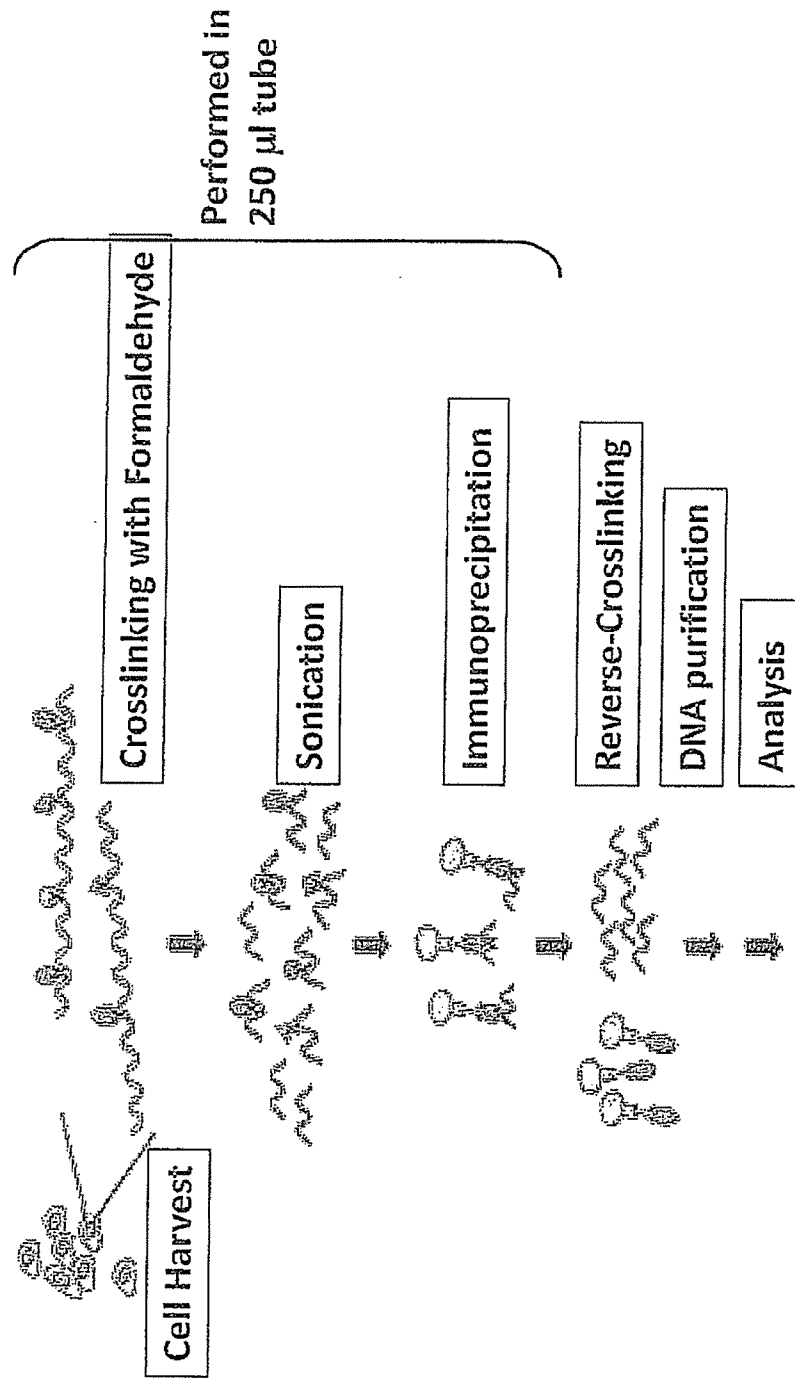


Figure 4

Modifications on ChIP Protocol for Small-Scale Assay

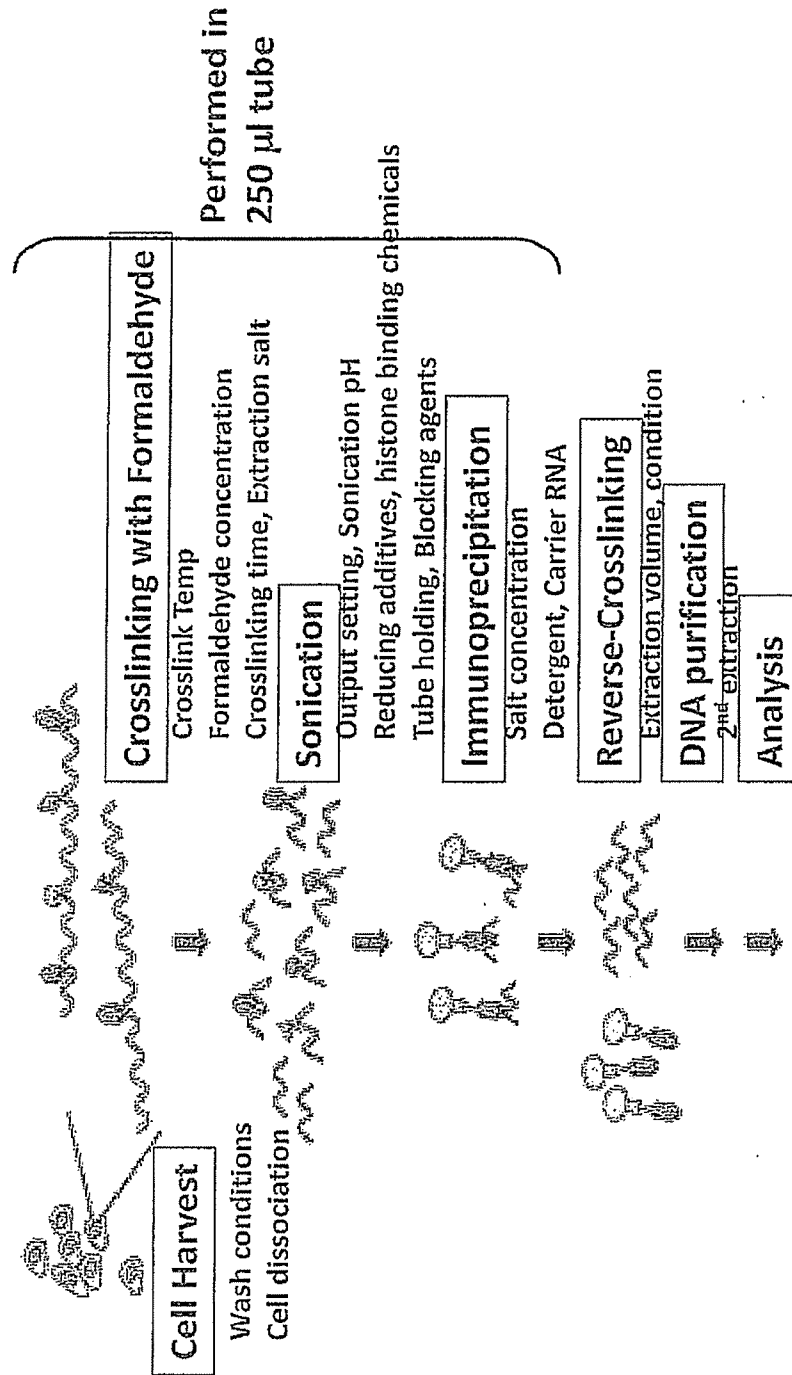
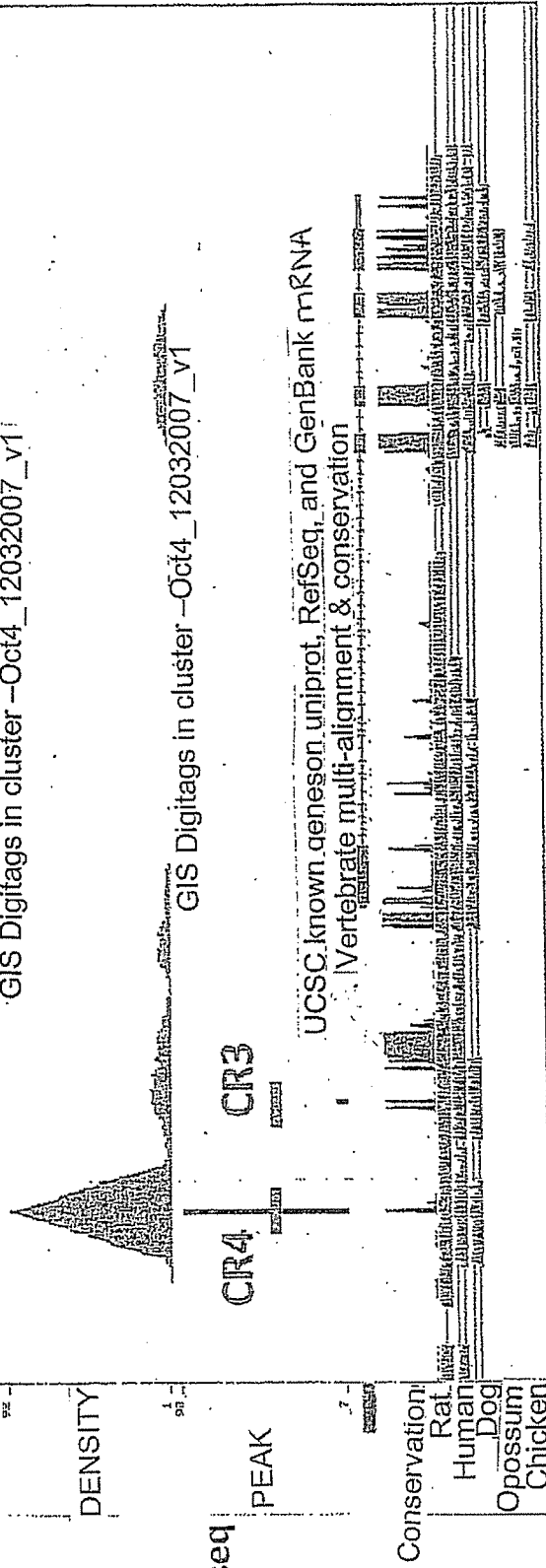


Figure 5

Oct4 ChIP with 10,000 mouse ES cells

Scale: 351115000
 35112000/35112500/35113000/35113500/35114000/35114500/35117000/35117500/35118000/35118500/35119000
 GIS Digtags in cluster -Oct4_12032007_v1



6/33

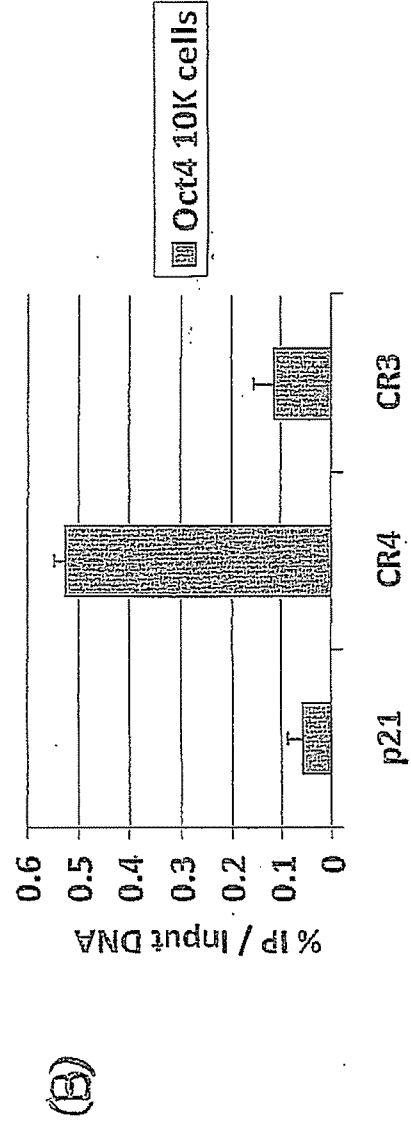


Figure 6

Whole Genome Amplification (WGA)

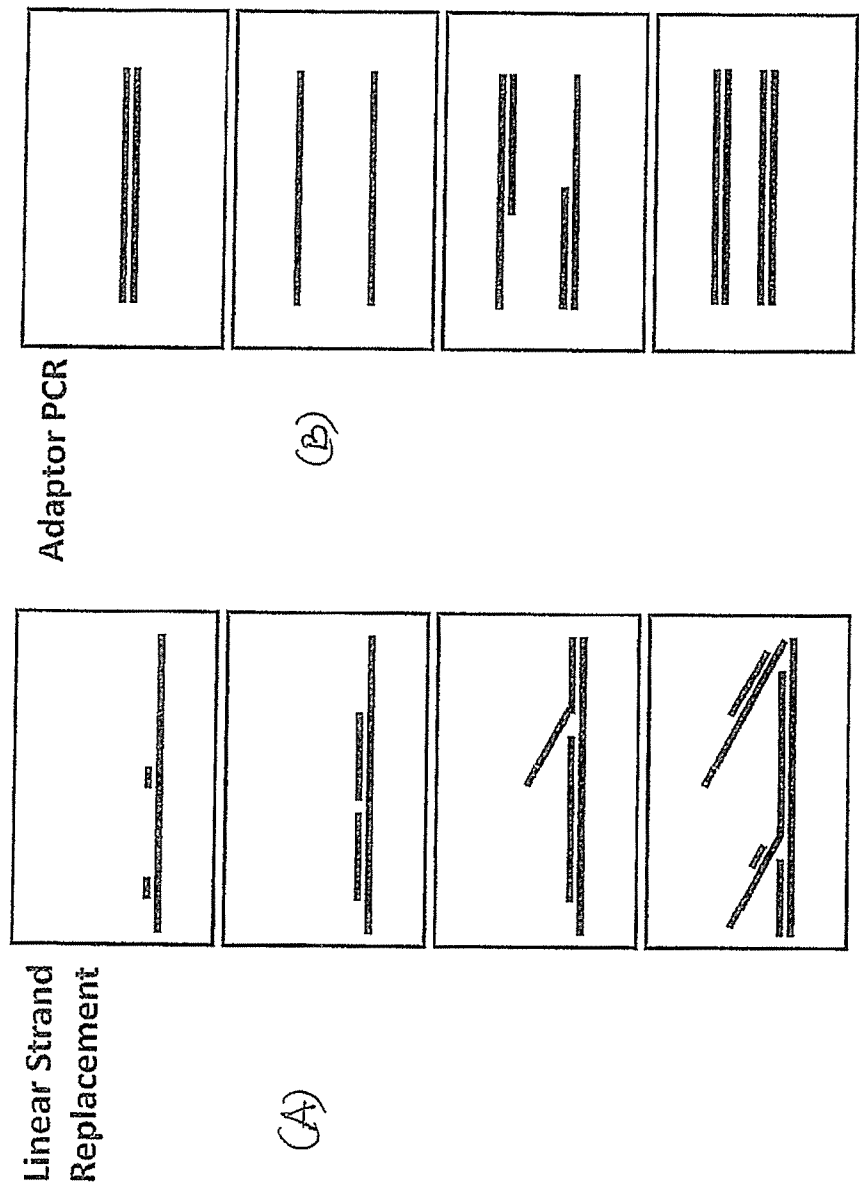


Figure 7

Degenerate Oligonucleotide Primed-PCR (DOP-PCR)

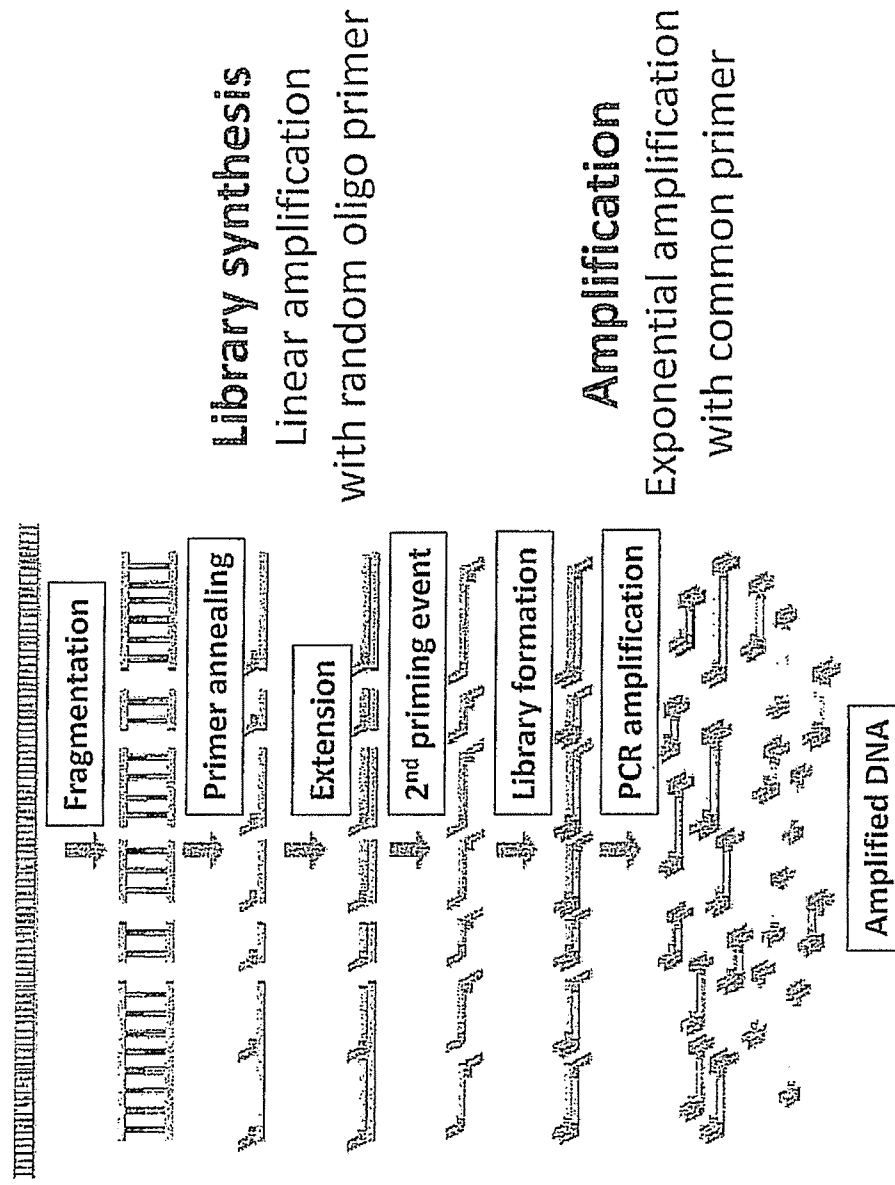
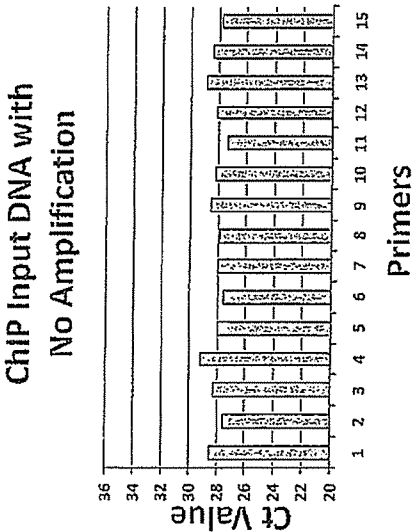


Figure 8

WGA Test with CHIP Input DNA

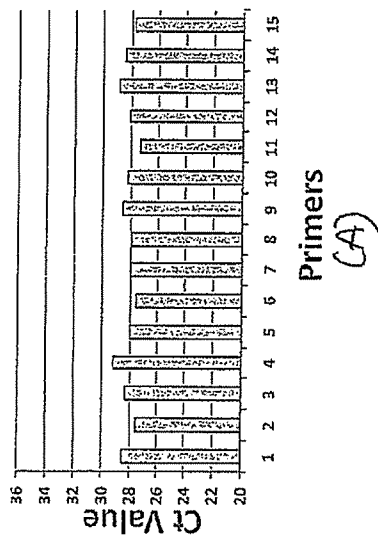


30 cycles

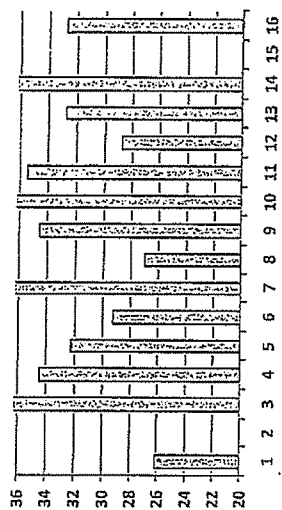
Figure 9

WGA Test with CHIP Input DNA

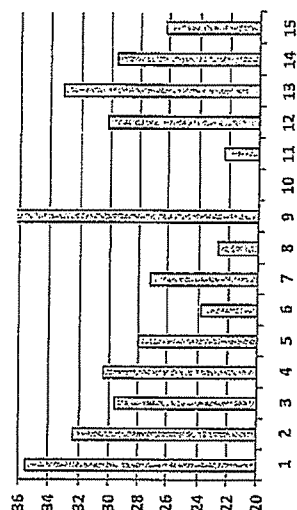
CHIP Input DNA with
No Amplification



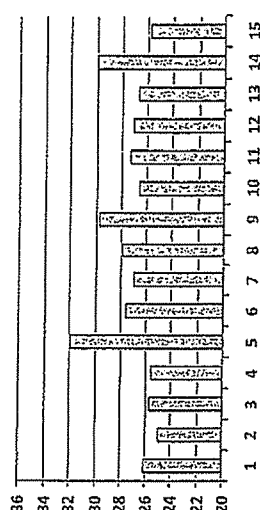
DOP-PCR with
"N8 Primer"
CCGACTGGAG-N8
(B)



DOP-PCR with
"MX6 Primer"
CCGACTGGAG-N6-ATGTGG
(C)



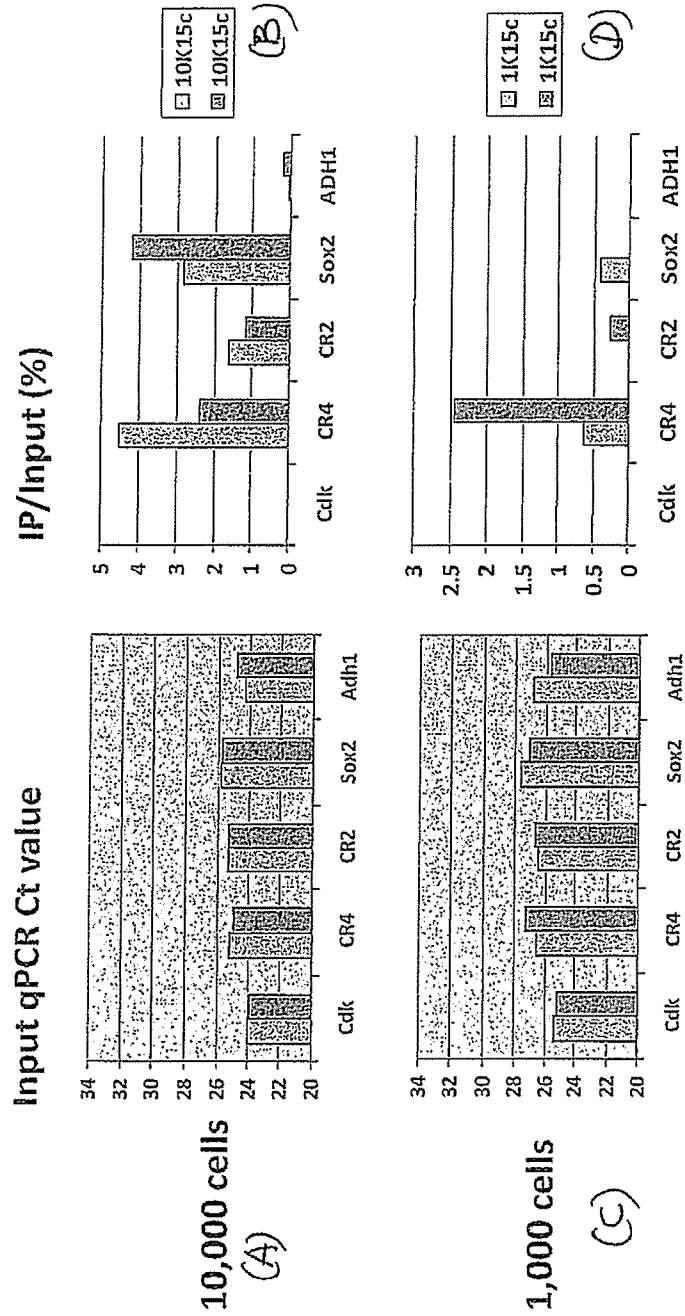
Sigma Single-Cell
WGA kit (WGA4)
(D)



30 cycles

Figure 10

Oct4 CHIP-qPCR assay with 10K and 1K mES cells

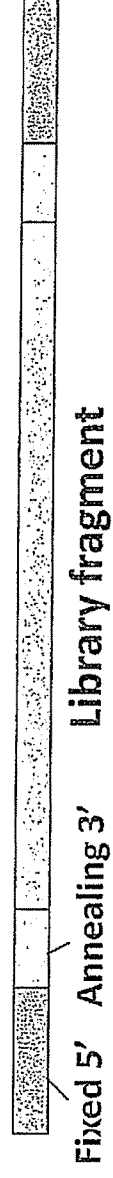


WGA 15 cycle amplification, two harvests for 10K, 1K

Figure 11

Processing ChIP Material for Solexa Sequencing

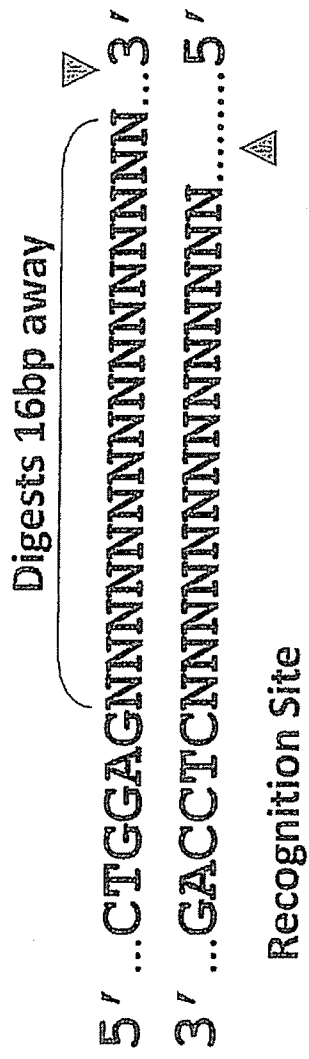
Sigma WGA primer



12/33

Figure 12

Bpm I Restriction Enzyme



13/33

Figure 13

Processing ChIP Material for Solexa Sequencing

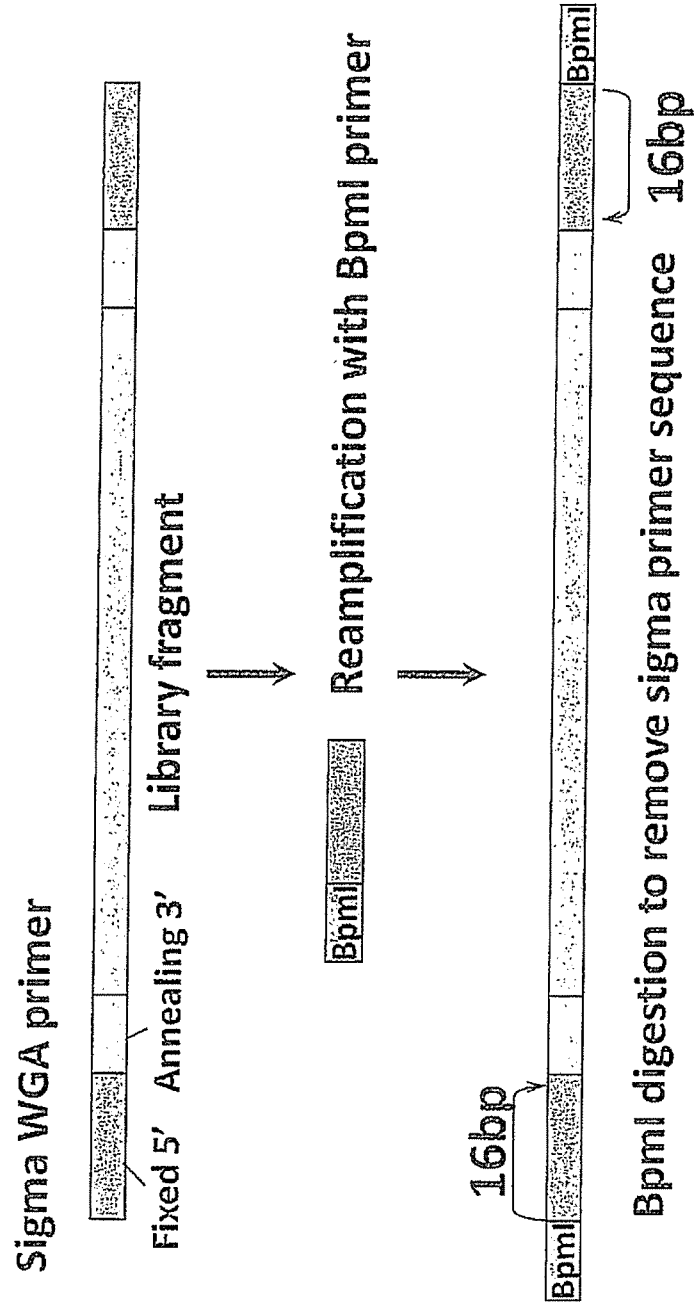


Figure 14

Low Mapping Efficiency of WGA Library

	Passed Filter	Mapped
Lane 5: no WGA	11,590,772	7,904,285
Lane 7: WGA 8 cycles	12,399,781	5,247,571

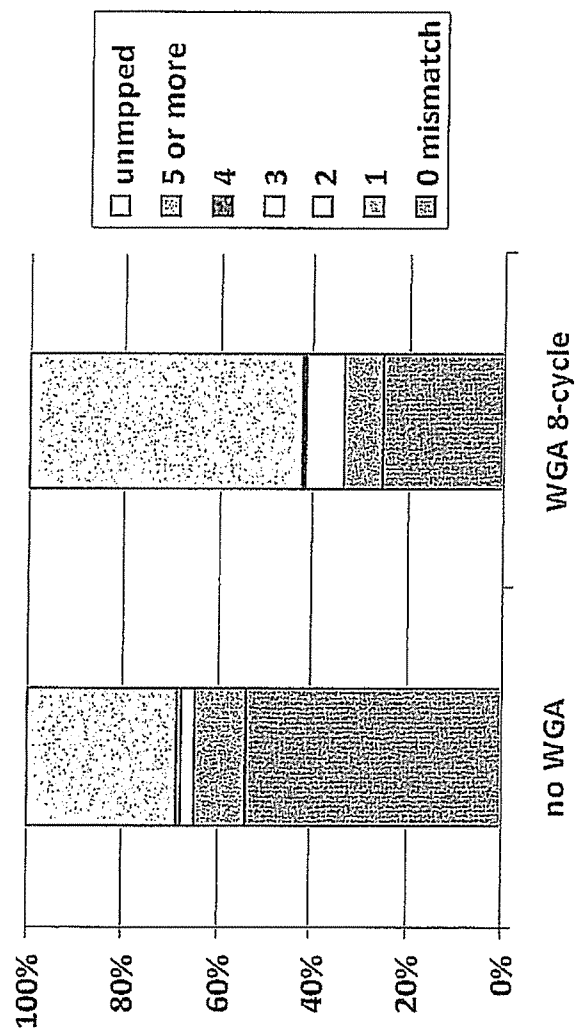


Figure 15

High Error Rates at 5' End

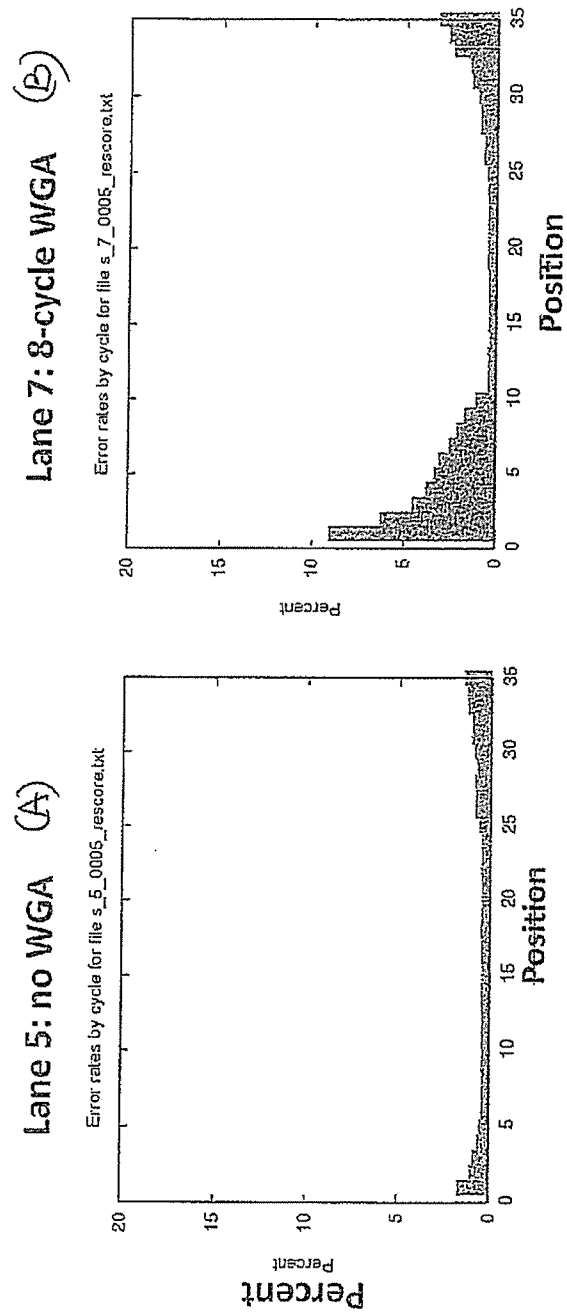


Figure 16

WGA Library Fragments are T/G rich at 5' end

17/33

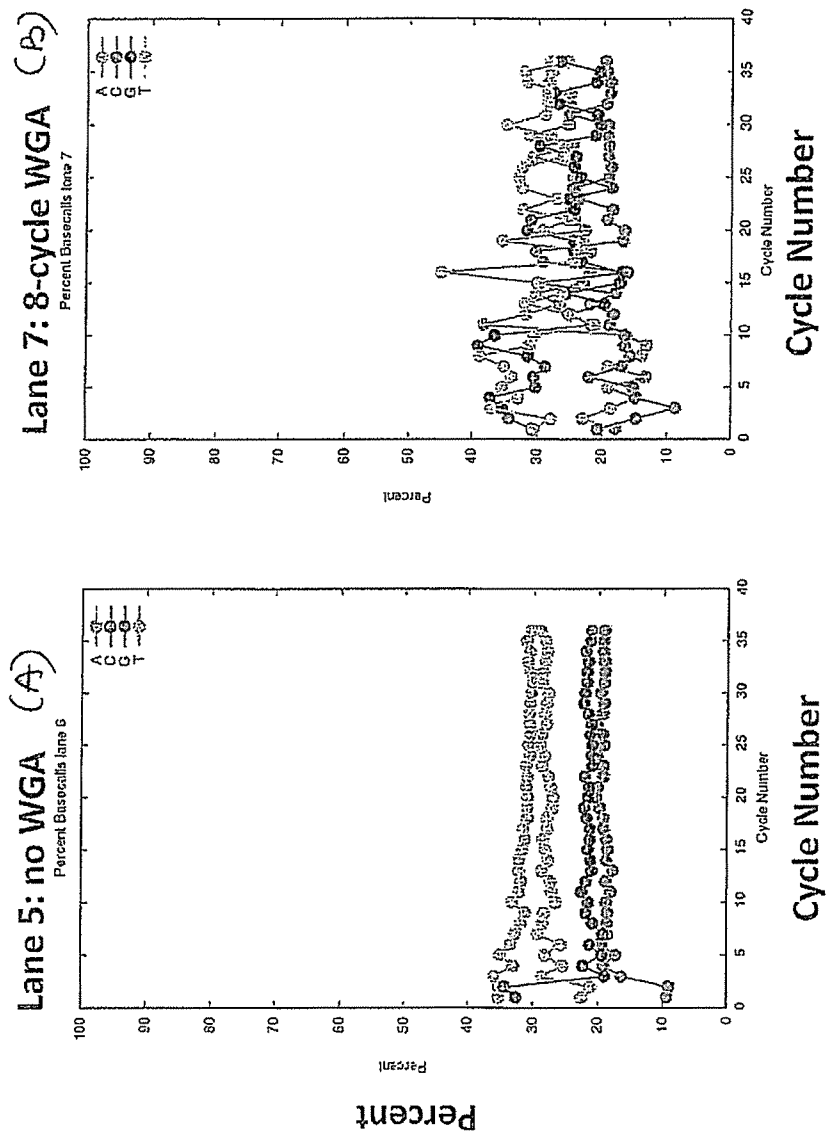


Figure 17

Topo-Cloning and Sequencing of Sigma-WGA Fragment

Bpm I Digest

Sigma Primer	▼	?	Perfect Match to the Genome
TGTGTTGGGTGTTTGGTGGTTTGGGTACACGCCCTTAAATCCCAGCACCTTGCGA			
GGCAGAGACAGGCGGATTCTGAGTTCAAGGCCAGCCTGGTCTACAAAGTGA			
GTTCCAGGACAGCCAGAGCTATACAGAGAACTCTGCCCTCAAAAACAAAA			
ACAAACAAACAAACAAACAAACAAACAAACGCTACAACTCTGTAAAGGA			
CTTGAGTTTGGTCCCCAGCATCAACATCGGCTATCAATGCTTGTAACACCAACCCC			
CACAACCCCAACACACCCCAACACAA			(11~253/260 matched to genome)

18/33

Figure 18

30-cycle WGA fragments

Topo-Cloning and Sequencing of Sigma-WGA Fragment

Bpm I Digest

Sigma Primer

▼

?

Perfect Match to the Genome

TGTGTTGGGTGTGTTTGGTGGTTGGGTACACGCCCTTTAATCCAGCACCTTGGGA
GGCAGAGACAGGCGGATTCTGAGTTCAAGGCCAGCCTGTGCTACAAAGTGA
GTTCCAGGACAGCCAGACTATACAGAGAACTCTGCCTCAAAACAAACAAA
ACAAACAAACAAACAAACAAACAAACAAACGCTACAACTCTTGTAAGGA
CTTGAGTTTGGTCCCGCATCAACATCGGCTATCAATGCTTGTAACACCAACCCC
CACAAACCCCAACACACCCCAACACAA (11~253/260 matched to genome)

▼

TGTGTTGGGTGTGTTTGGTGGTGGTGGAAATTTAGAAATGTCCACTGTAGG
ACGTGGAATATGGCAAGAAACCTGAAATCATGGAAATGAGAAACATCCACTT
GACGACTTGAAATGACGAAATCACTAAACGTAACGTAACGTAACGTAACGTA
CACTAAAGGACCTGGGATATGGCGAGTAACCTGAAATCACGGAATGAGAA
ATACACACTTTAGGACGTGAAATATGGCGAGGAAACCTGAAAGGTGGAAAT
ATAGAAATGTCCACGGTAGGACGTGGGAAATATGCAAGAAACCAACCAAC
ACACCAACACAA (9~298/298 matched to genome)

30-cycle WGA fragments

Figure 19

2nd Digestion of WGA Products

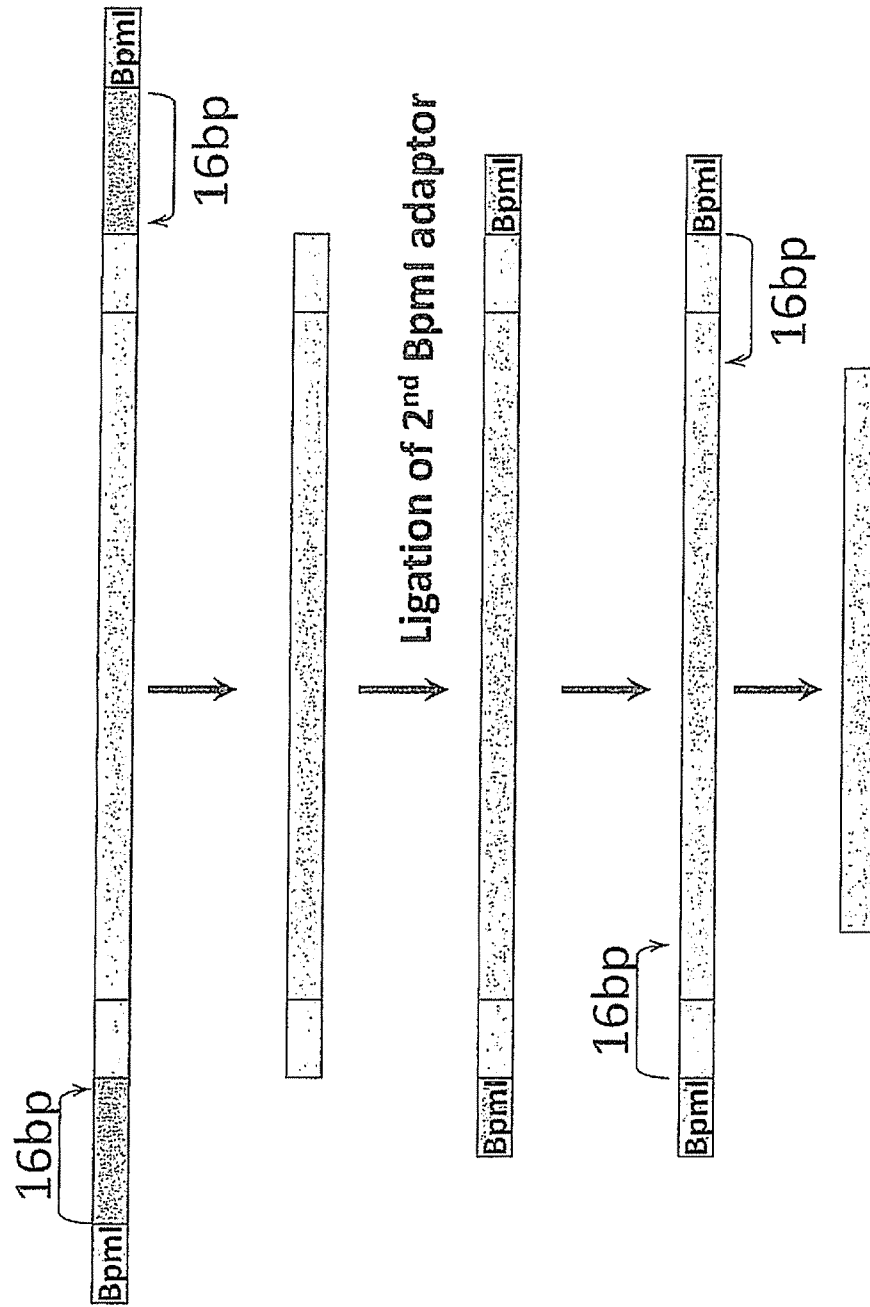


Figure 20

Second Bpm I Digest Improved Mapping of WGA Library

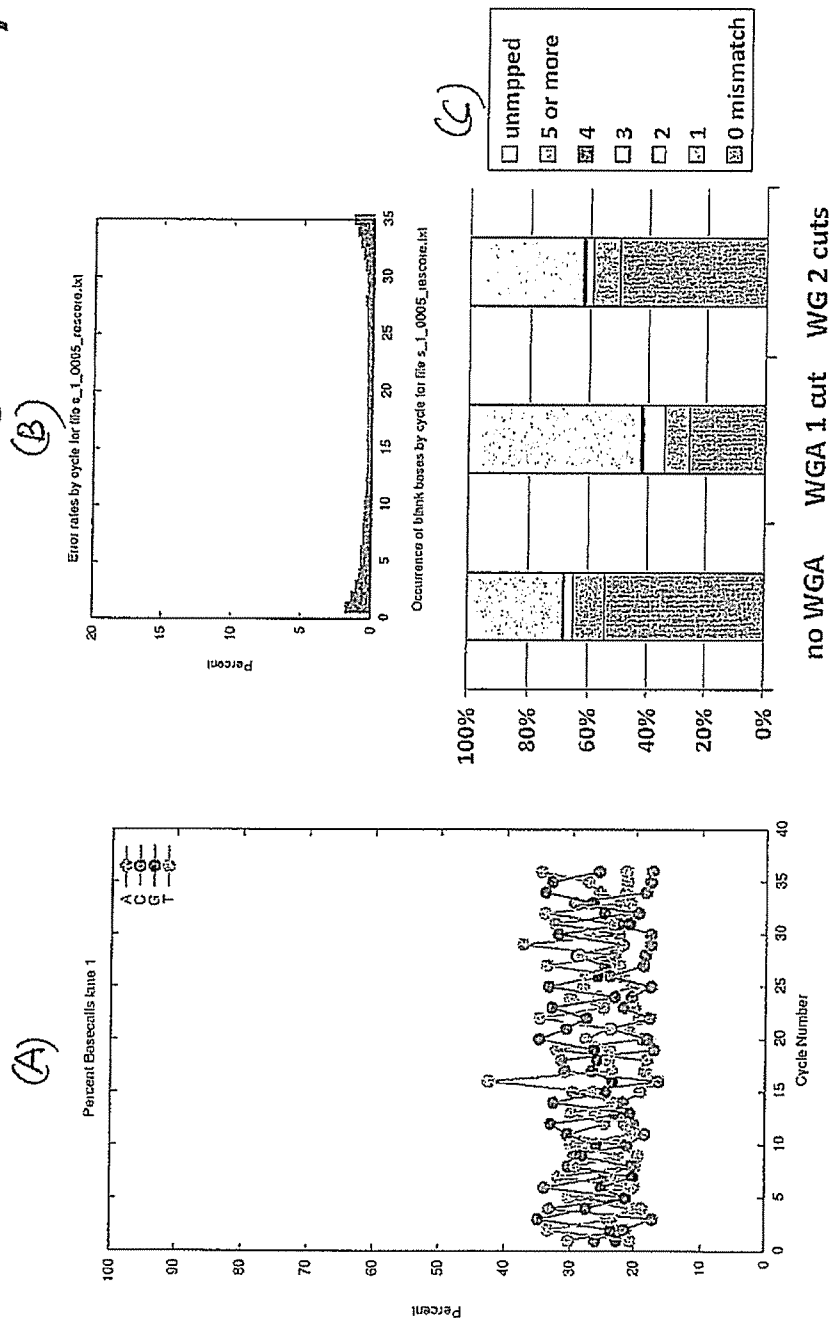
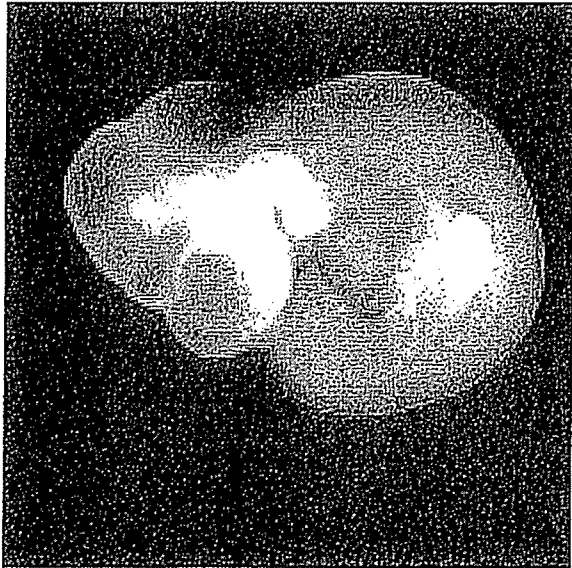


Figure 21

ChIP with embryo tissue



11.5 dpc mouse embryo

One forebrain from 11.5-day embryo
= ~300,000 cells

22/33

Targets		ChIP DNA/ forebrain	Embryos for 15 ng DNA
Histone H3	K4 me1	1.8 ng	10
	K27 Ac	1.4 ng	12
	K27 me3	~ 250 pg	60
	K4 me3	?	???

10 - 100X less DNA for Transcription Factors

Figure 22

Verification of WGA by Real-Time PCR

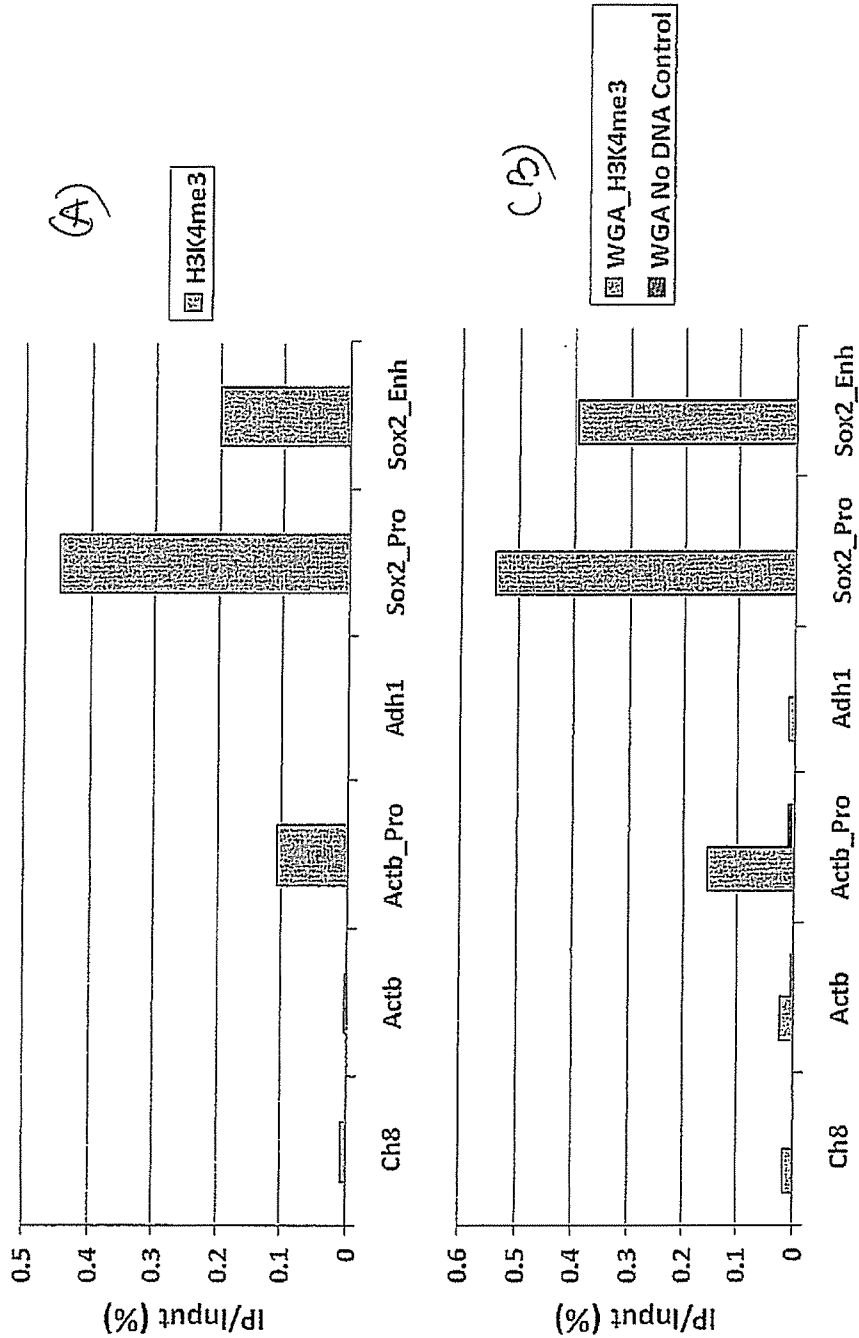


Figure 23

11.5 dpc Forebrain H3K4me3 ChIP-seq Results

	FDR	Total Peaks	Threshold
10.6M mapped reads from	0.05	160,020	7
2 lanes of Solexa Sequencing	0.01	112,225	8
	0.001	81,797	9

24/33

chr1:173,160,001-173,200,000

10 kb

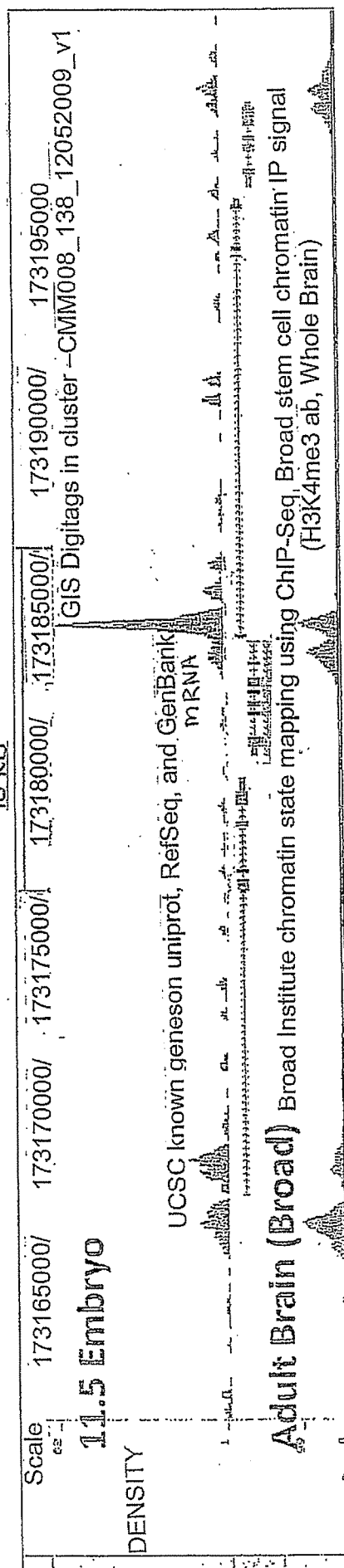


Figure 24

chr1:173,800,001-174,250,000 11.5 dpc Forebrain ChIP-seq Results

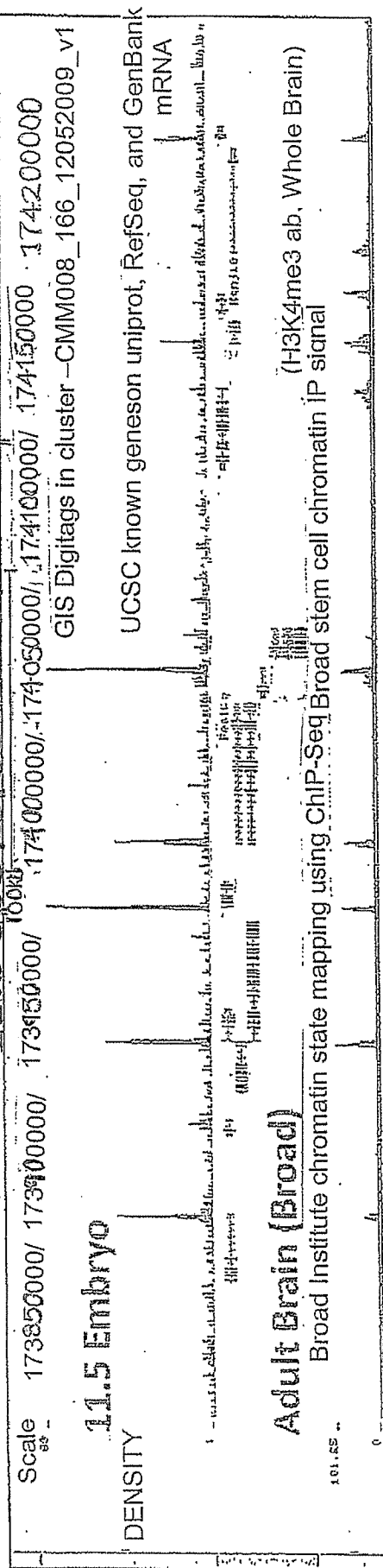
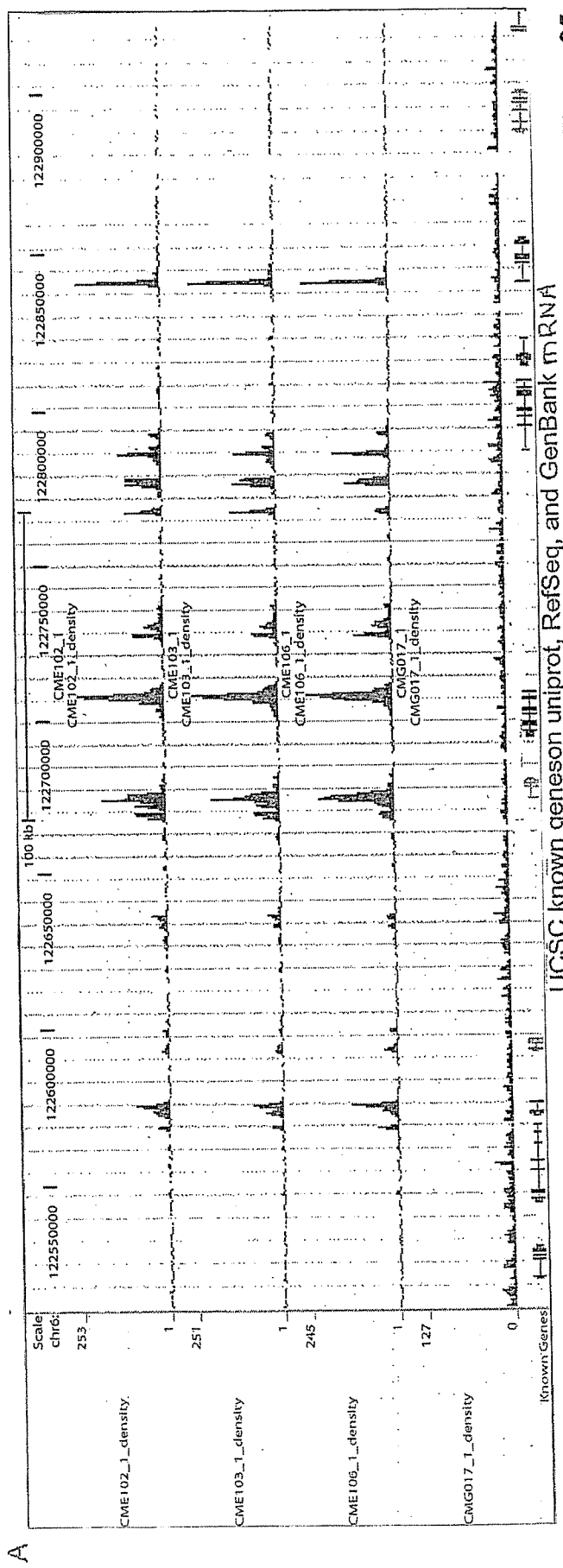
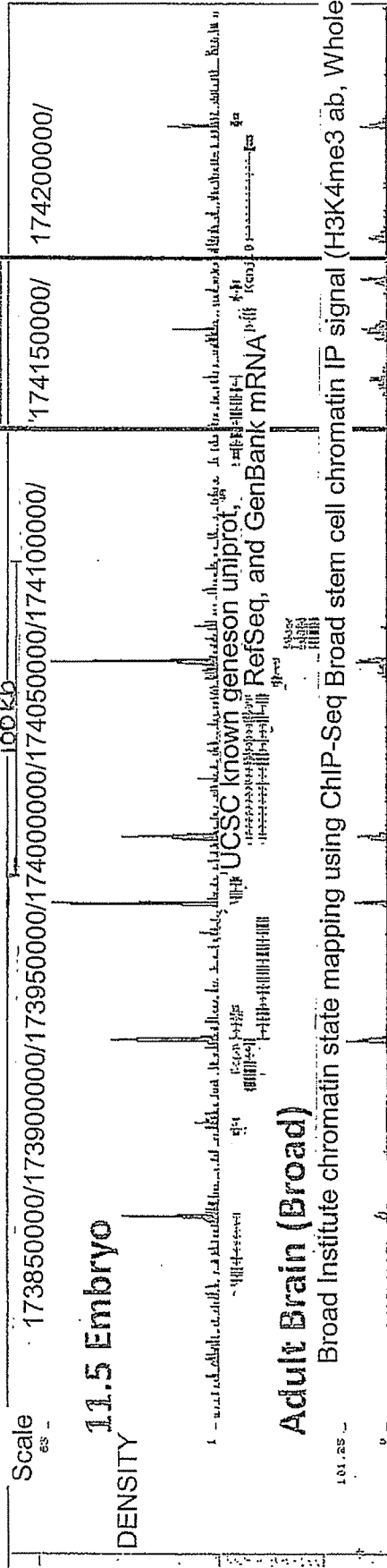


Figure 25

11.5 dpc Forebrain ChIP-seq Results

chr1:173,800,001-174,250,000 GIS Digits in cluster -CMM008_138_12052009_v1



26/33

GIS Digits in cluster -CMM008_133_12052009_v1

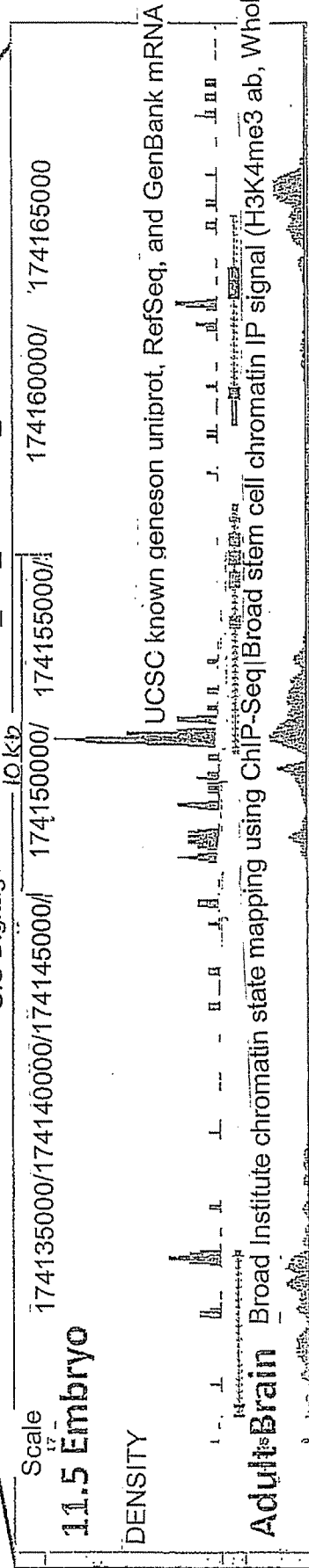
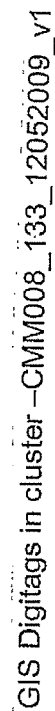
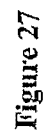


Figure 26

27/33

GIS Digitags in cluster -CMM008_138_12052009 v1



Verification of ChIP-seq Results by Real-Time PCR

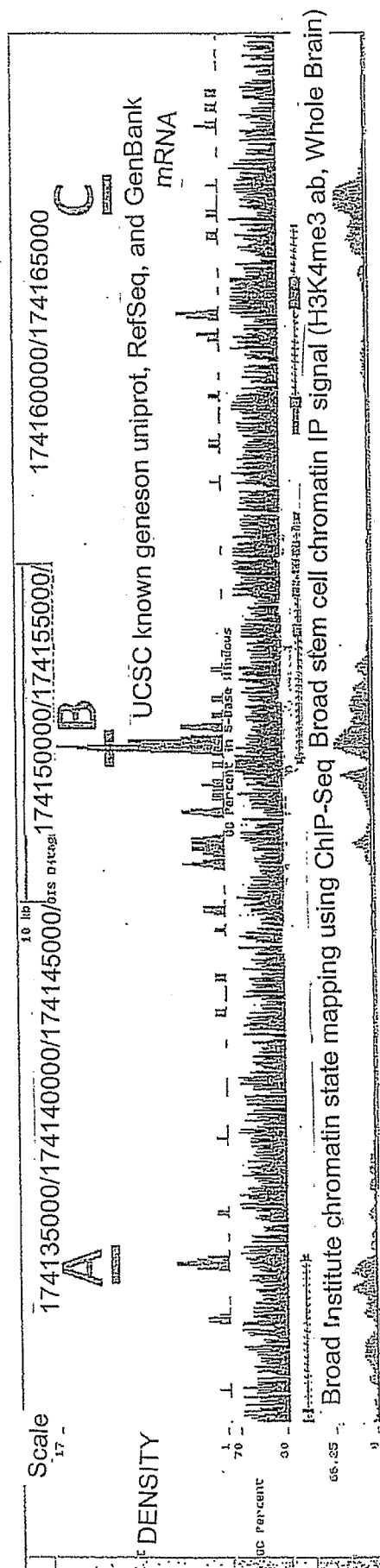
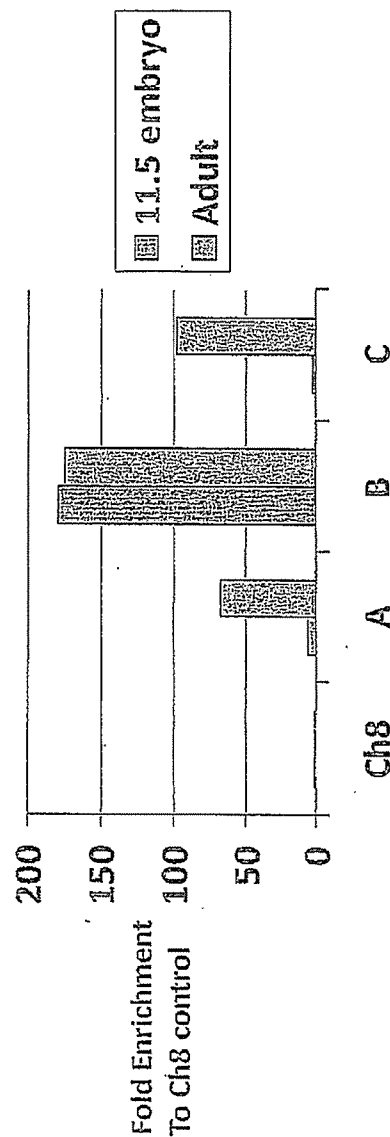
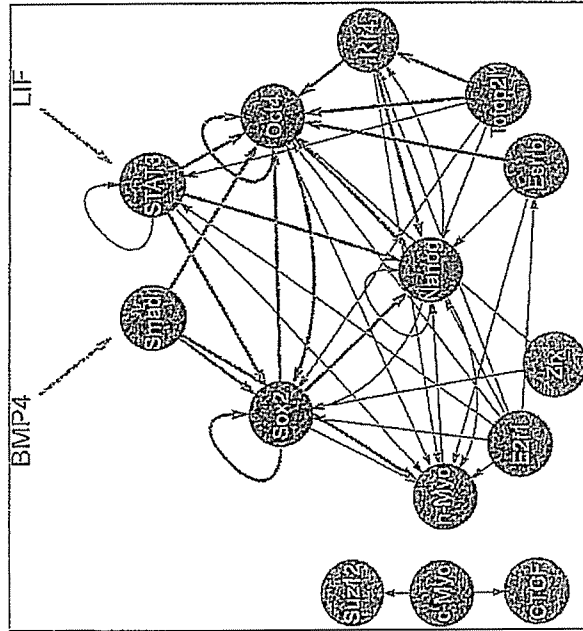


Figure 28

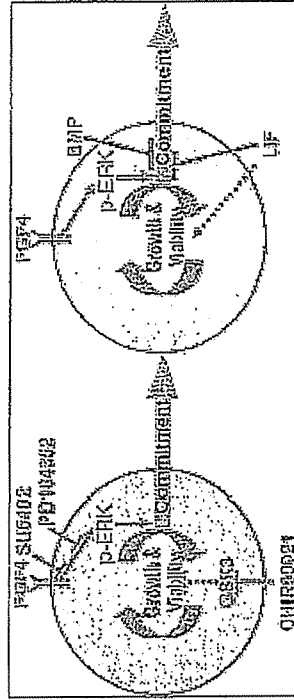
Maintenance of Embryonic Stem Cells

BMP/Serum + LIF



Chen et al., 2008

3 inhibitor cocktail



Ying et al., 2008

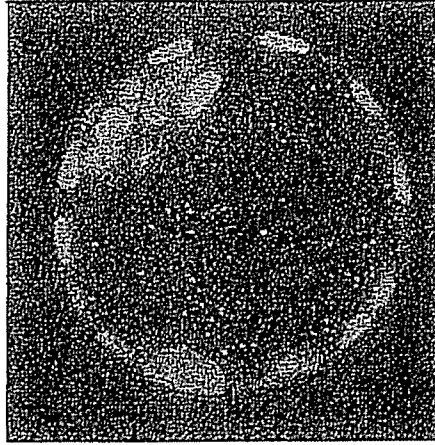
Figure 29

Mouse Blastocyst

Inner Cell Mass
(Embryoblast)

Trophoblast

Blastocoel



Oct4 and Cdx2

Figure 30

Oct4 ChIP: ES Cells vs ICM

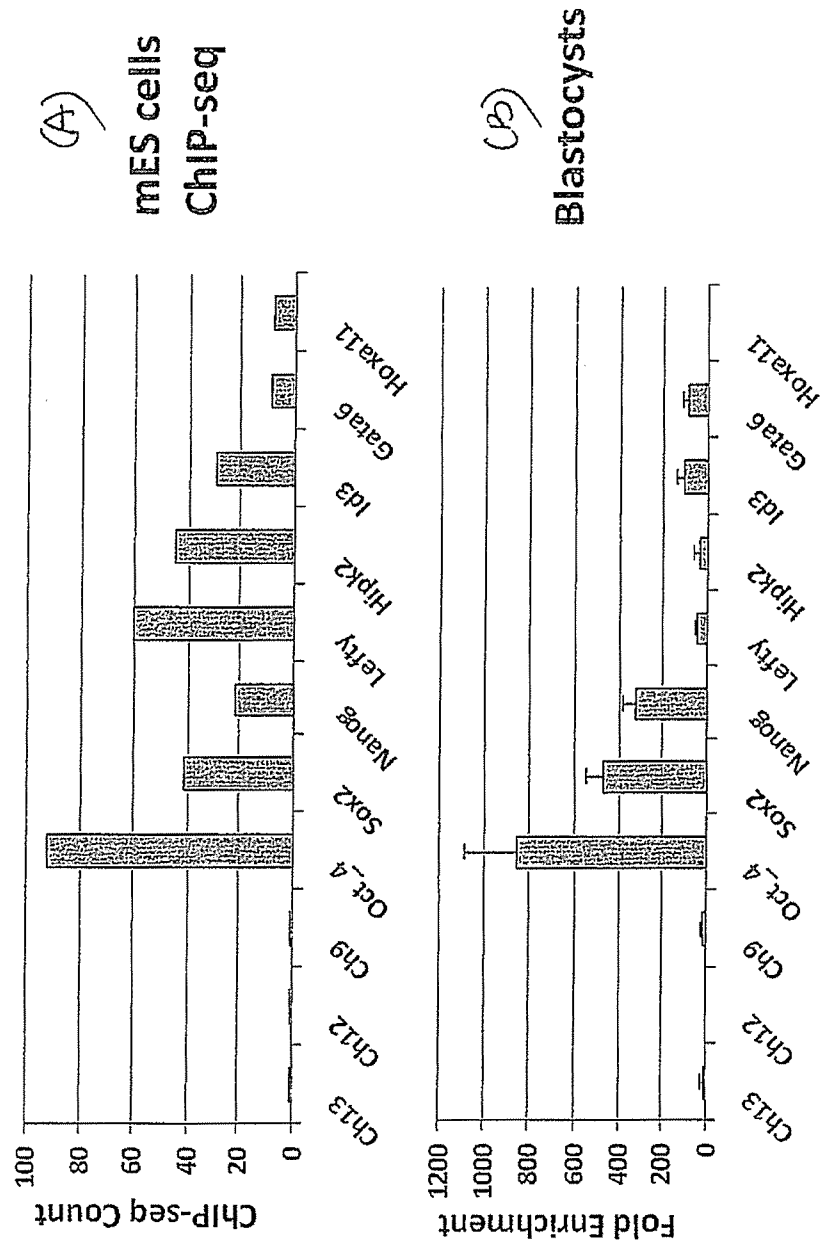


Figure 31

Verification of ChIP-seq Results by Real-Time PCR

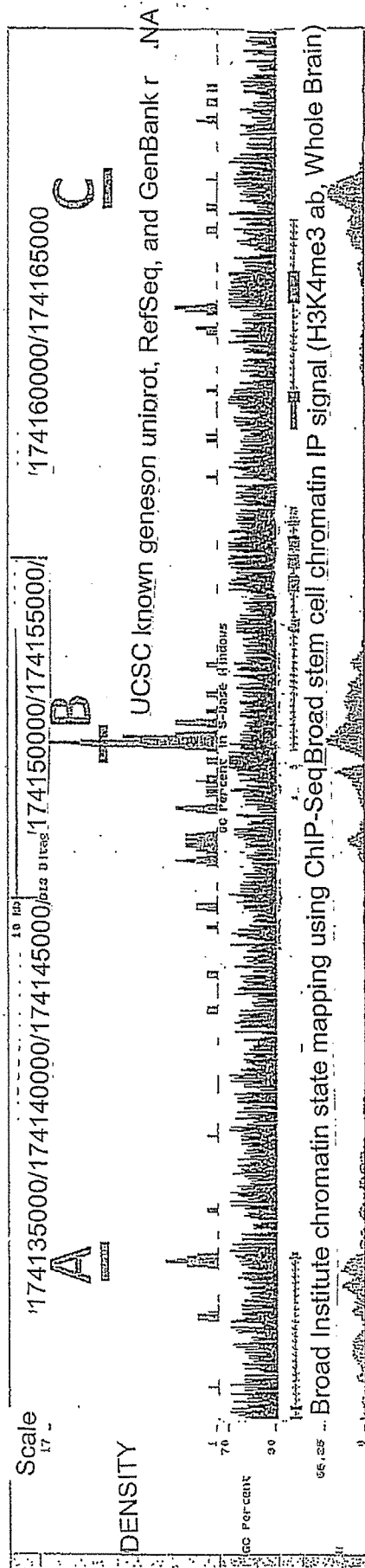
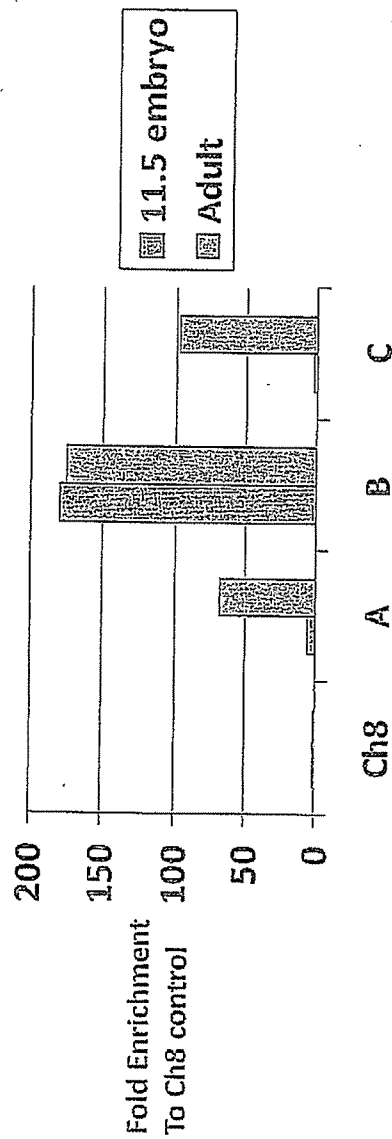


Figure 32

GC-bias in ChIP-seq and WGA-ChIP-seq

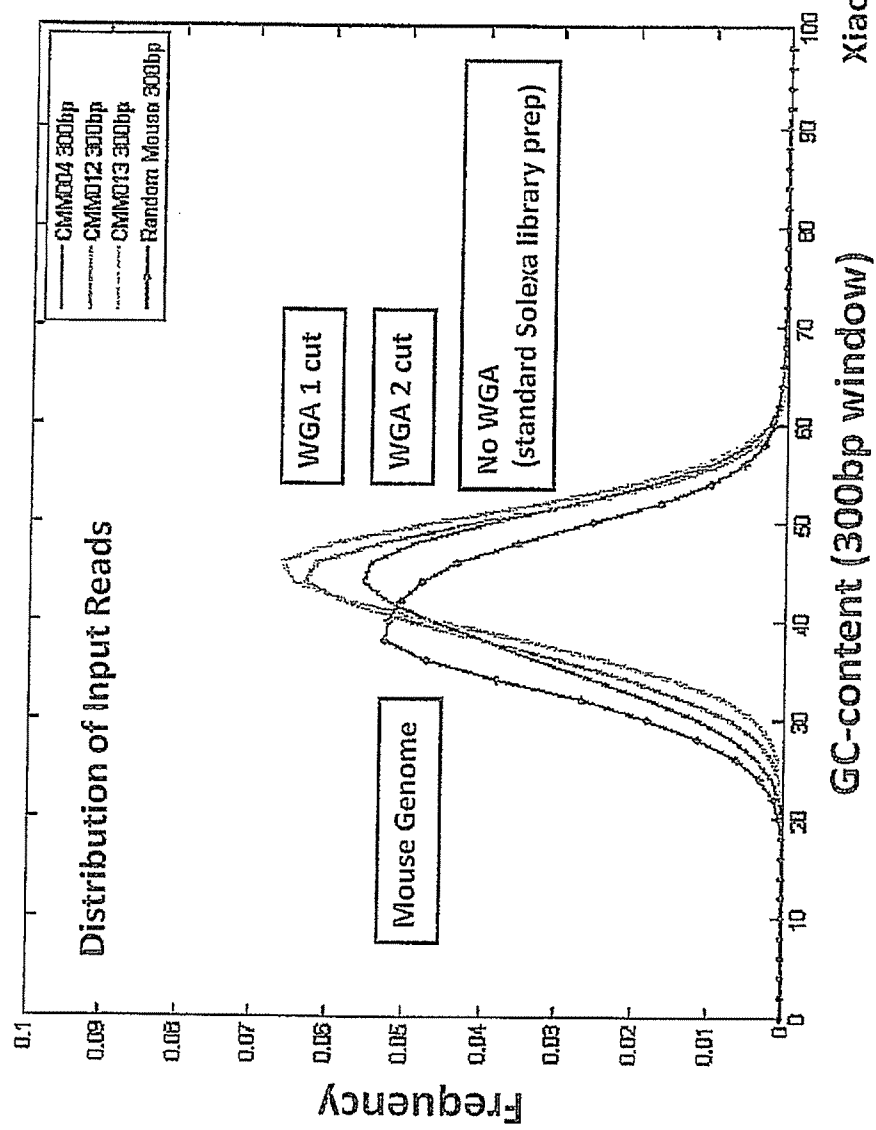


Figure 33