Chromatin Immunoprecipitation Assay

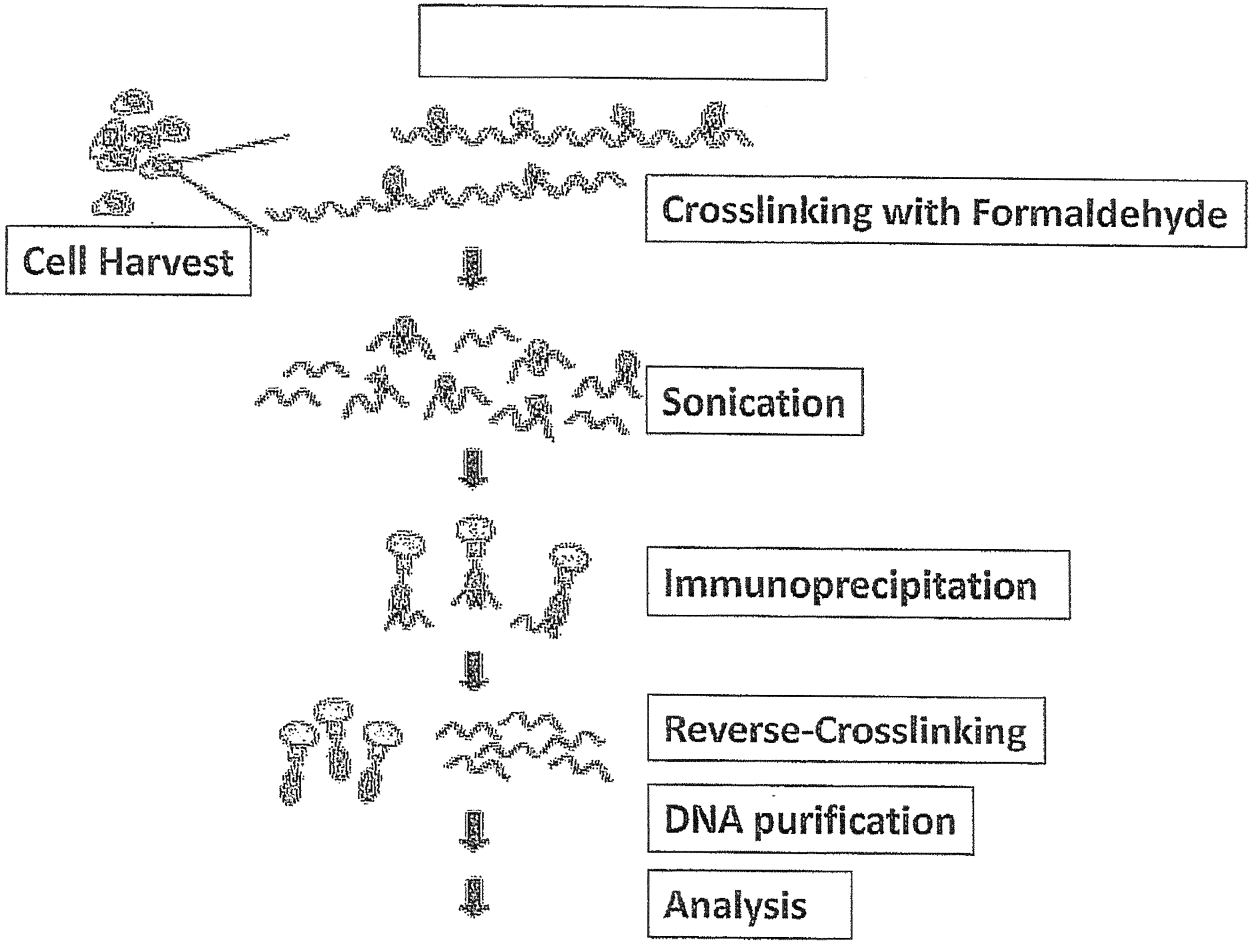
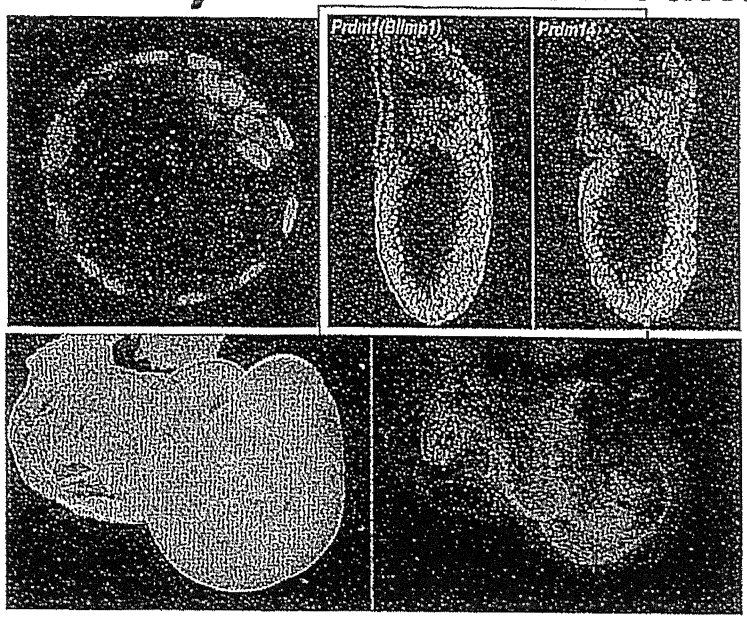


Figure 1

ChIP assay with limited sample



- Early embryo
- Rare cell population
- Human sample

Figure 2

Modifications on ChIP Protocol for Small-Scale Assay

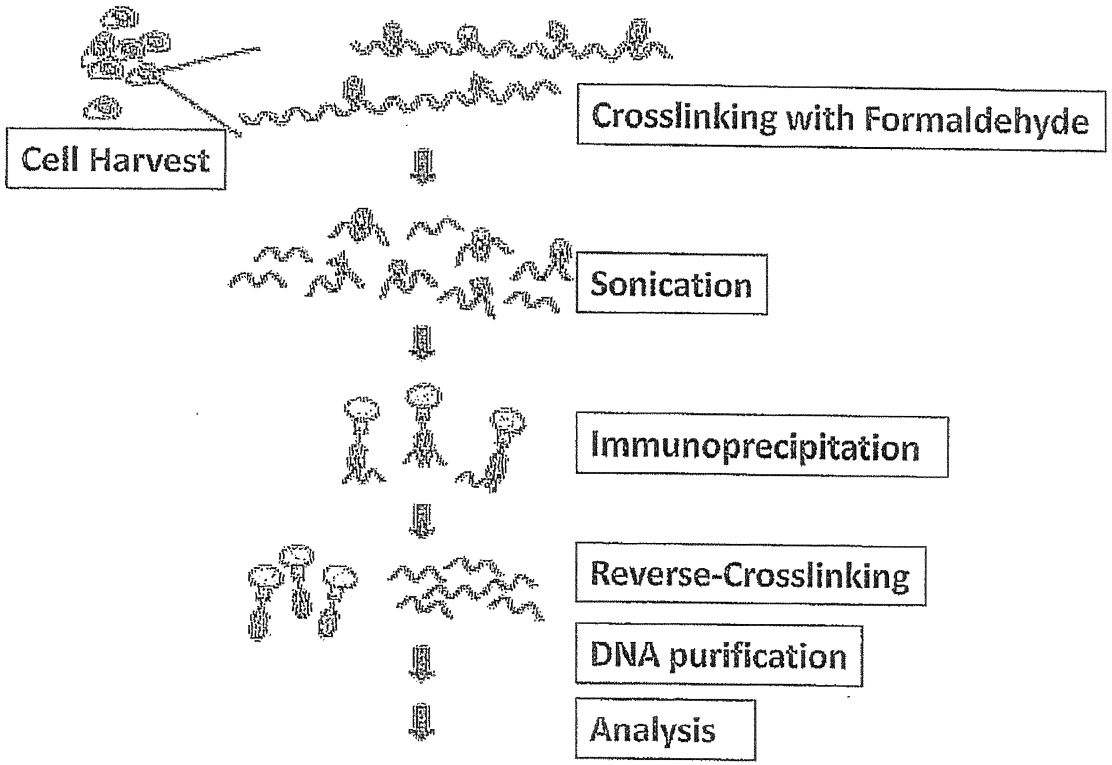


Figure 3

Modifications on ChIP Protocol for Small-Scale Assay

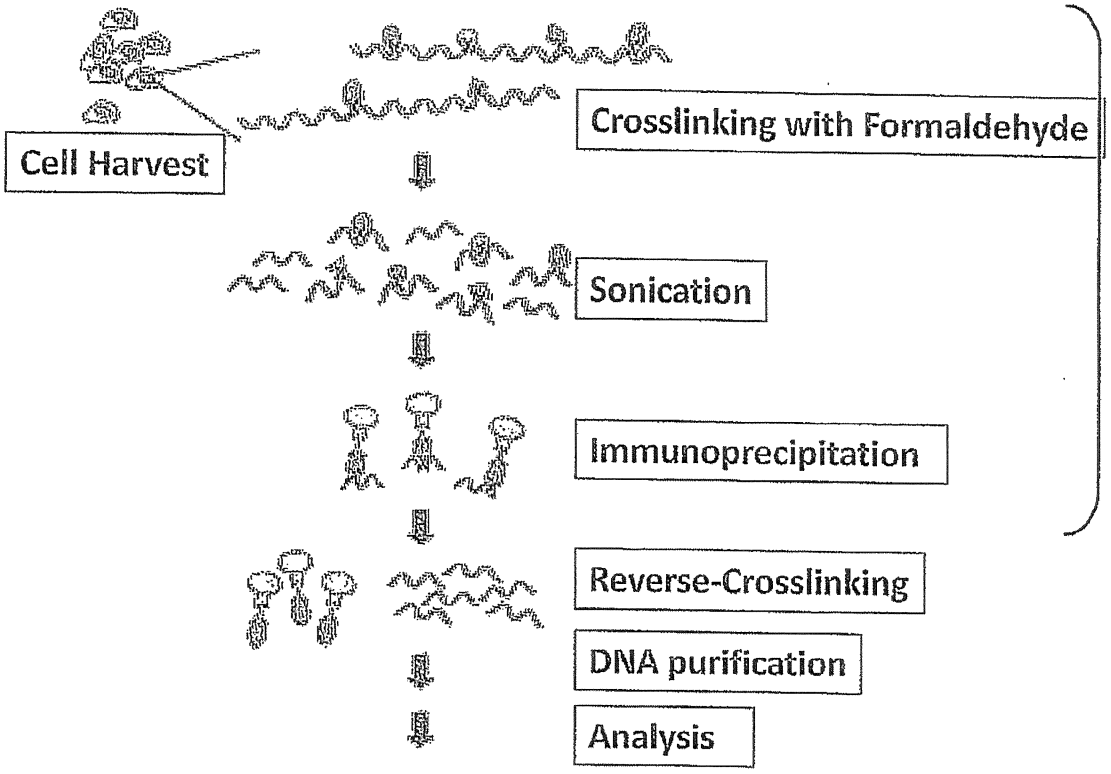


Figure 4

Modifications on ChIP Protocol for Small-Scale Assay

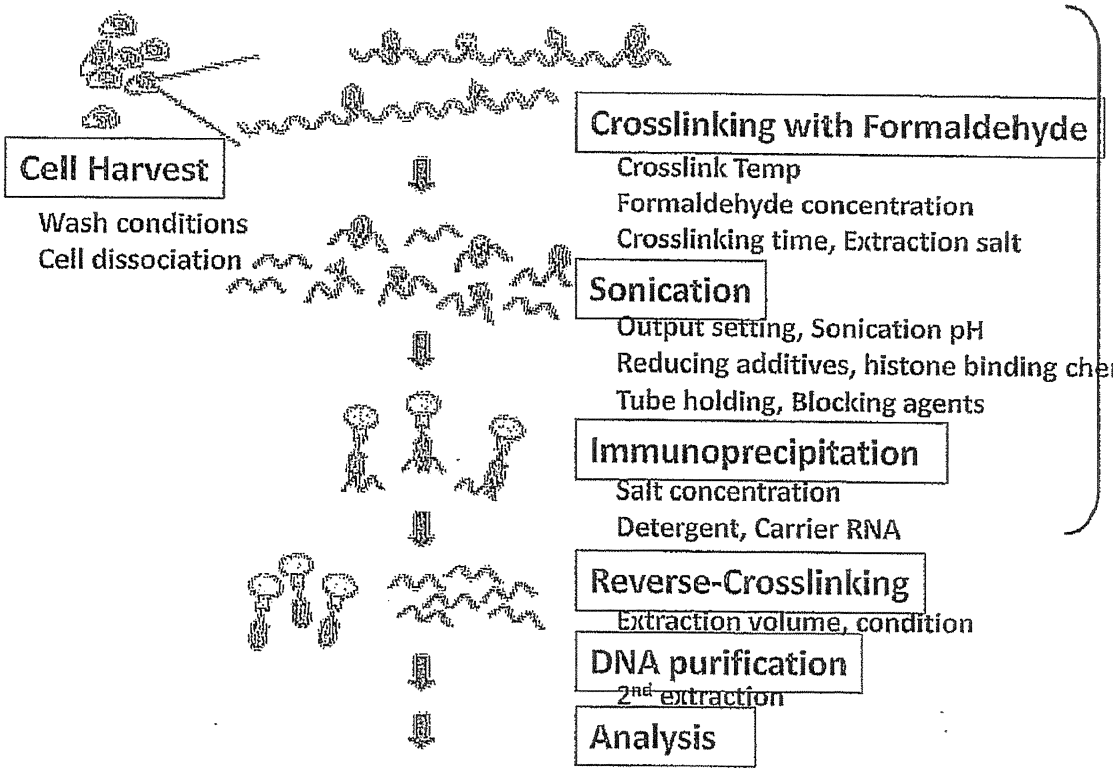


Figure 5

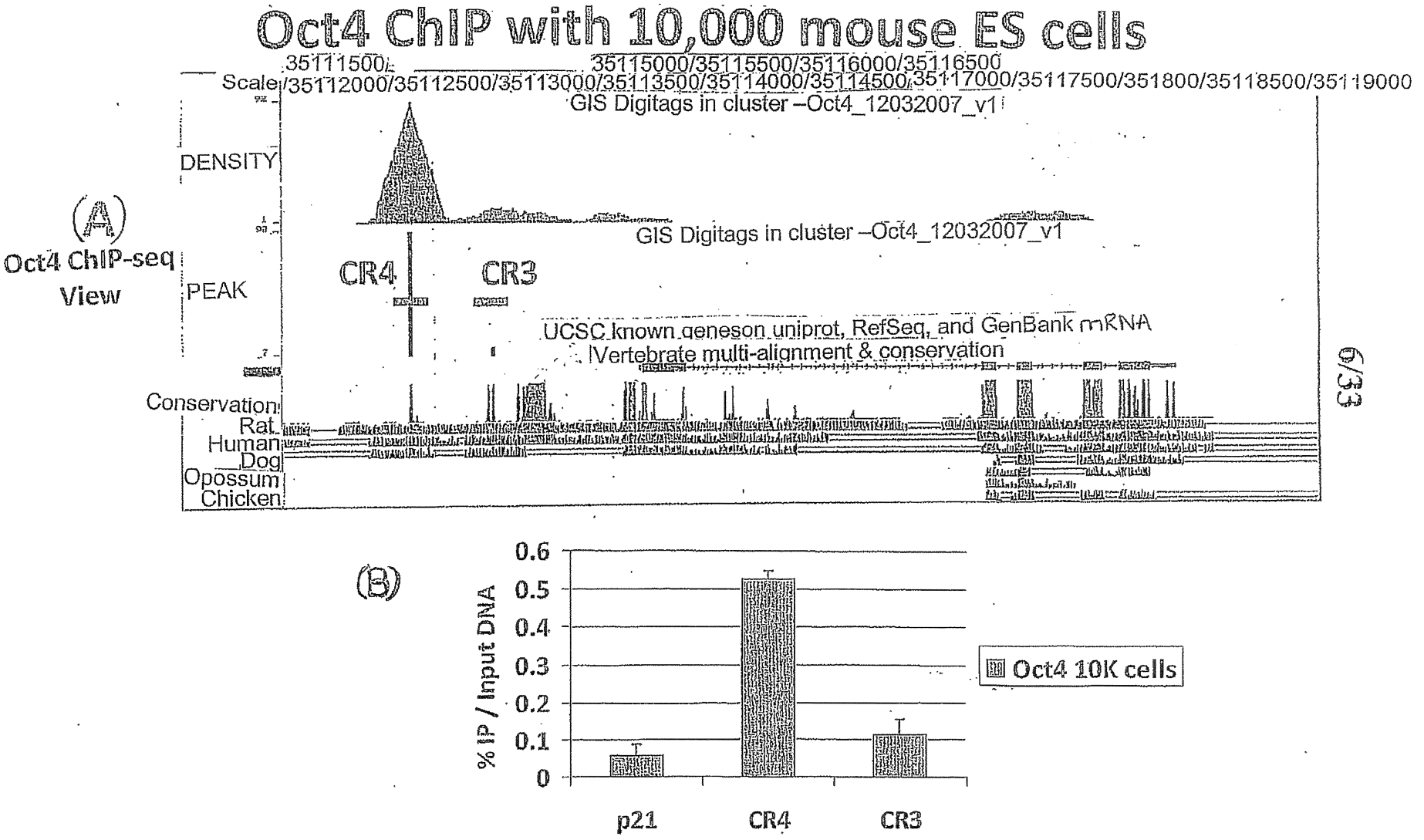

Oct4 ChIP with 10,000 mouse ES cells
(A)
Oct4 ChIP-seq View
35111500
35115000/35115500/35116000/35116500
Scale
/35112000/35112500/35113000/35113500/35114000/35114500/35117000/35117500/351800/35118500/35119000
GIS Digitags in cluster –Oct4_12032007_v1
DENSITY
GIS Digitags in cluster –Oct4_12032007_v1
PEAK
CR4
CR3
UCSC known genes on uniprot, RefSeq, and GenBank mRNA
Vertebrate multi-alignment & conservation
Conservation
Rat
Human
Dog
Opossum
Chicken
(B)
% IP / Input DNA
0.6
0.5
0.4
0.3
0.2
0.1
0
p21
CR4
CR3
Oct4 10K cells

Figure 6

Whole Genome Amplification (*WGA*)

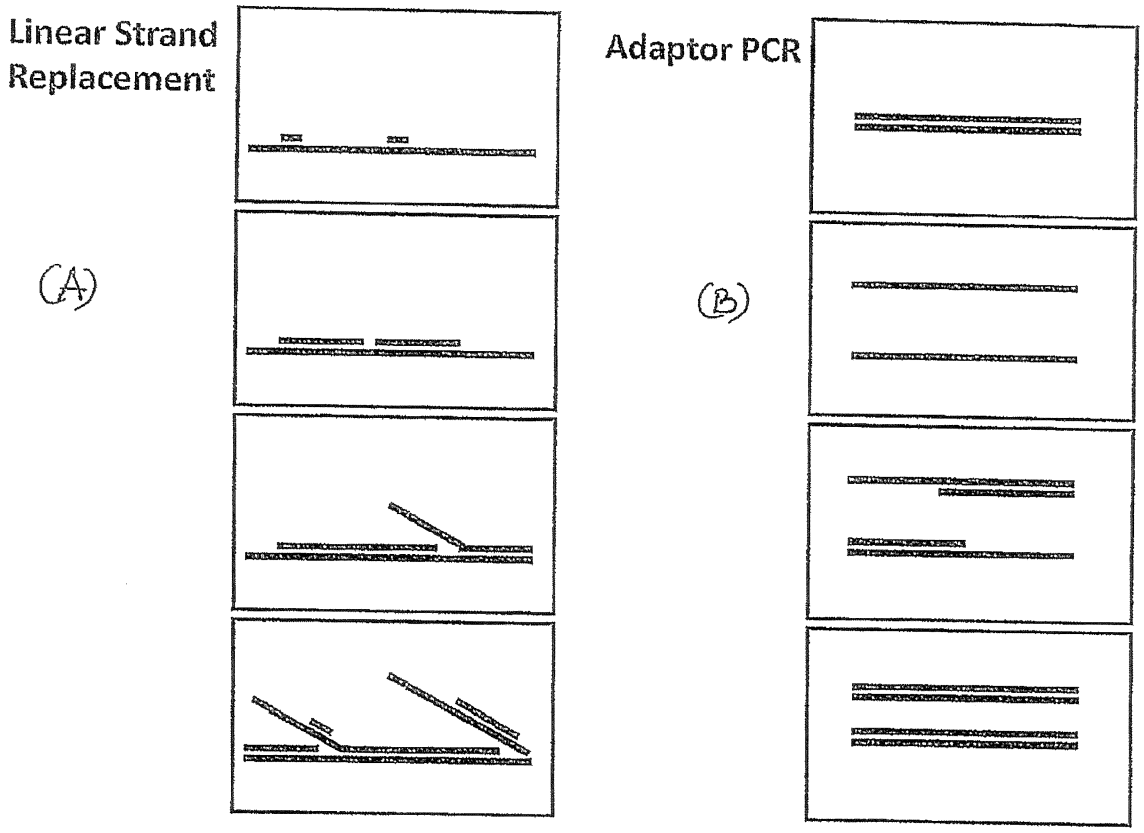
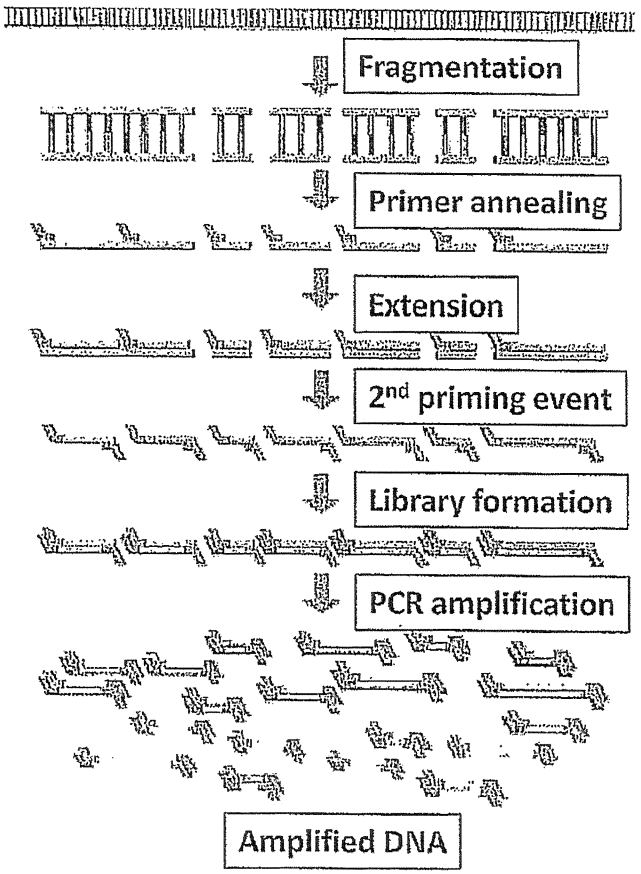


Figure 7

Degenerate Oligonucleotide Primed-PCR (*DOP-PCR*)

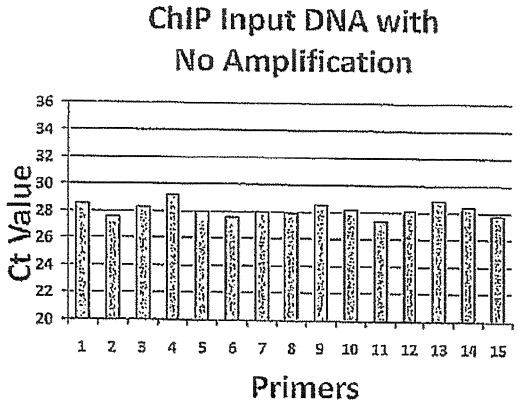


Library synthesis
Linear amplification
with random oligo primer

Amplification
Exponential amplification
with common primer

Figure 8

WGA Test with ChIP Input DNA



30 cycles

Figure 9

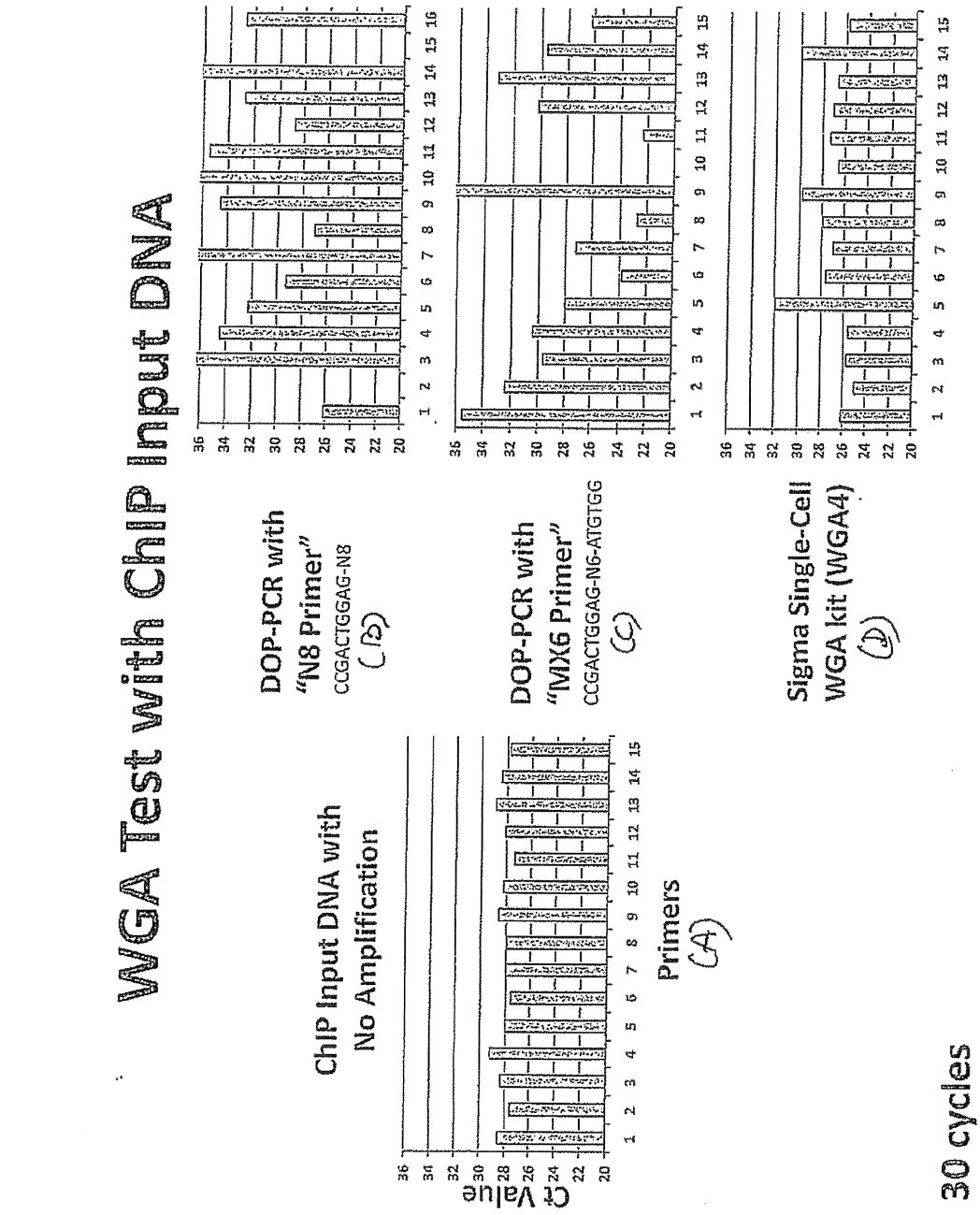
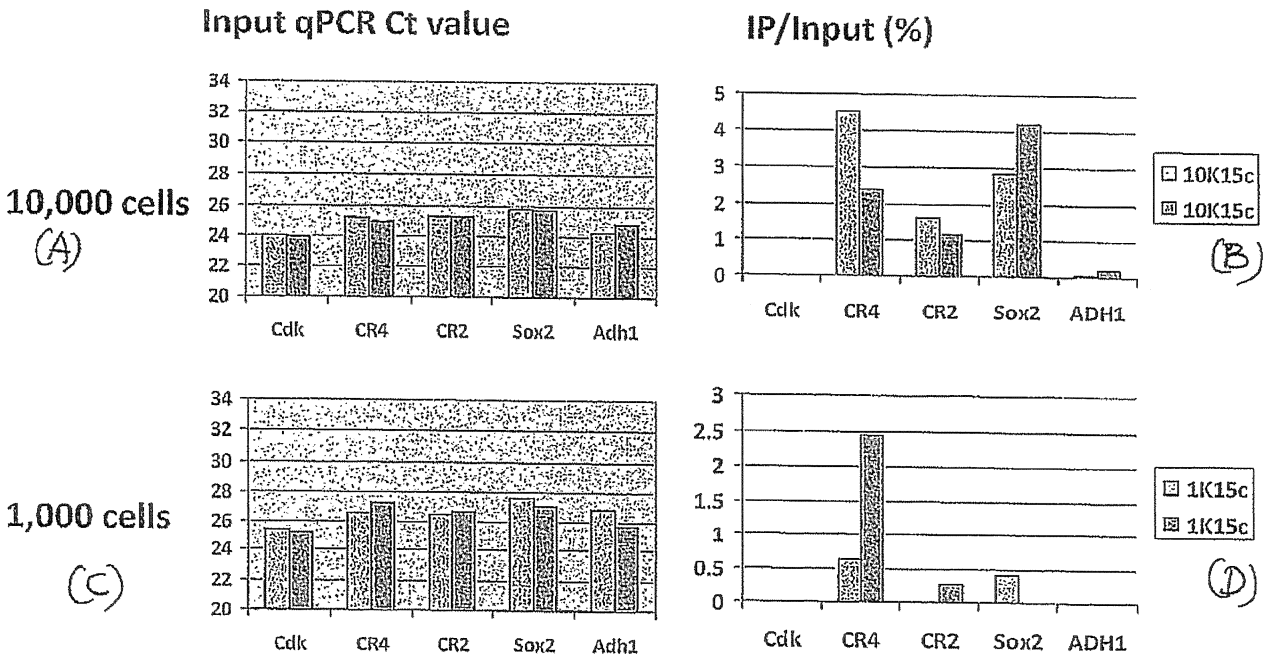

WGA Test with ChIP Input DNA
ChIP Input DNA with
No Amplification
(A)
Ct Value
Primers
DOP-PCR with
"N8 Primer"
CCGACTGGAG-N8
(B)
DOP-PCR with
"MX6 Primer"
CCGACTGGAG-N6-ATGTGG
(C)
Sigma Single-Cell
WGA kit (WGA4)
(D)
30 cycles

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

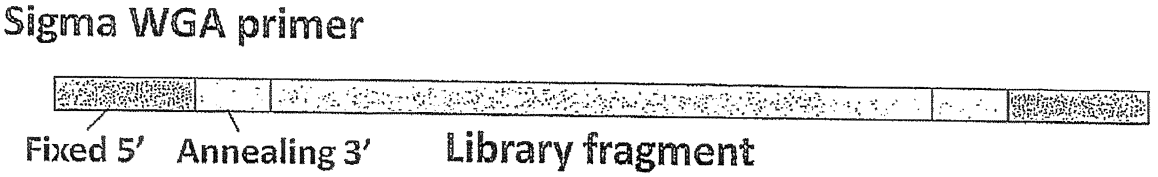


Figure 12

Bpm I Restriction Enzyme

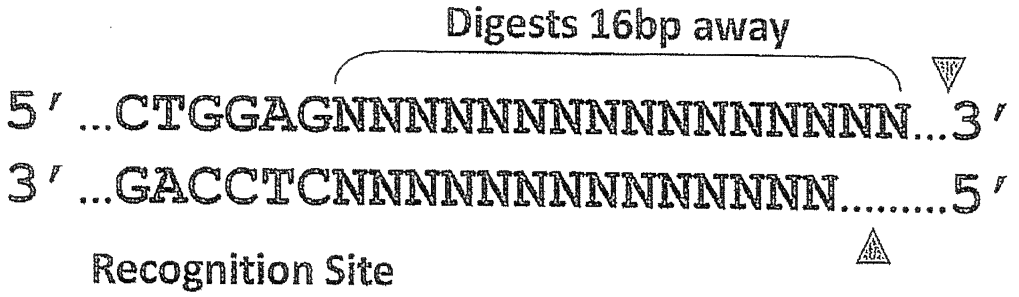


Figure 13

Processing ChIP Material for Solexa Sequencing

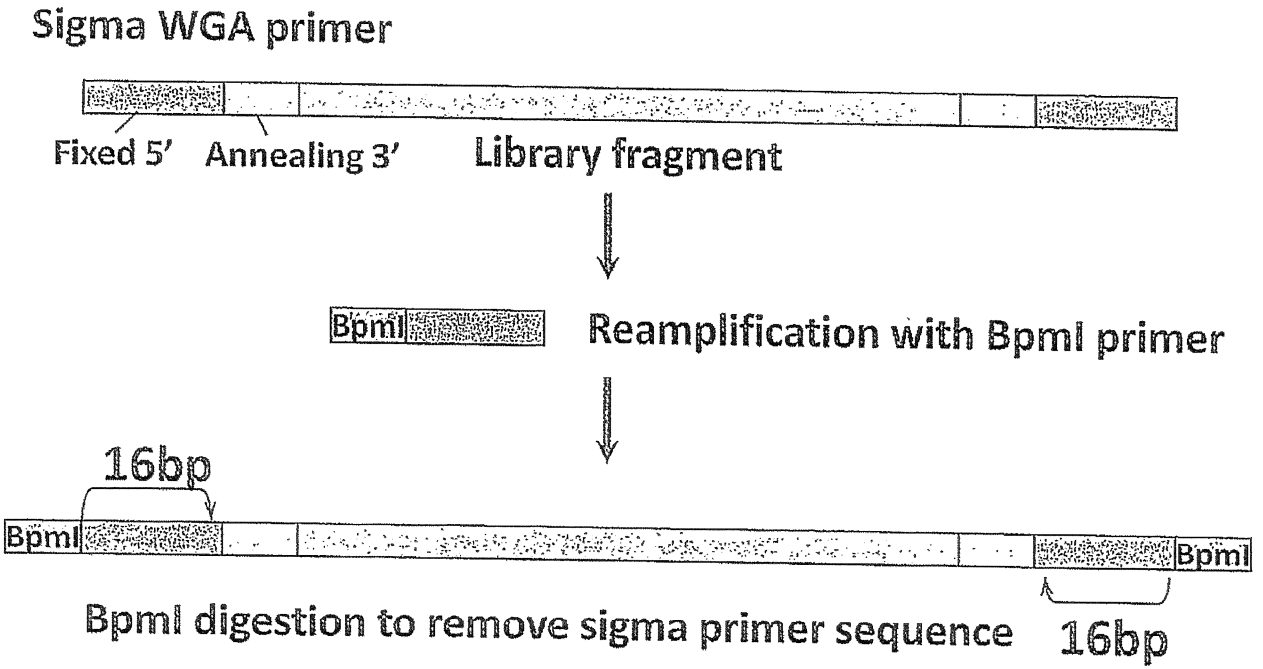


Figure 14

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

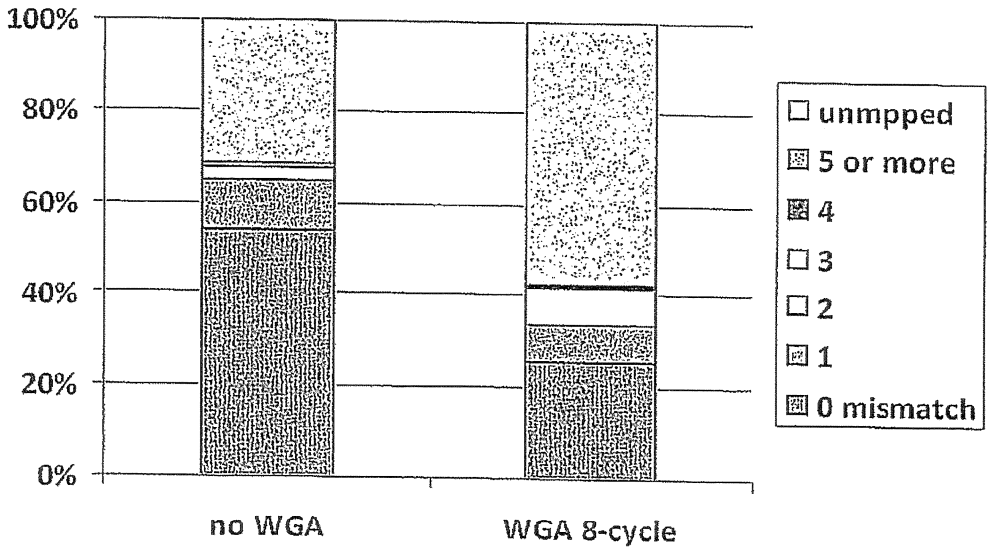


Figure 15

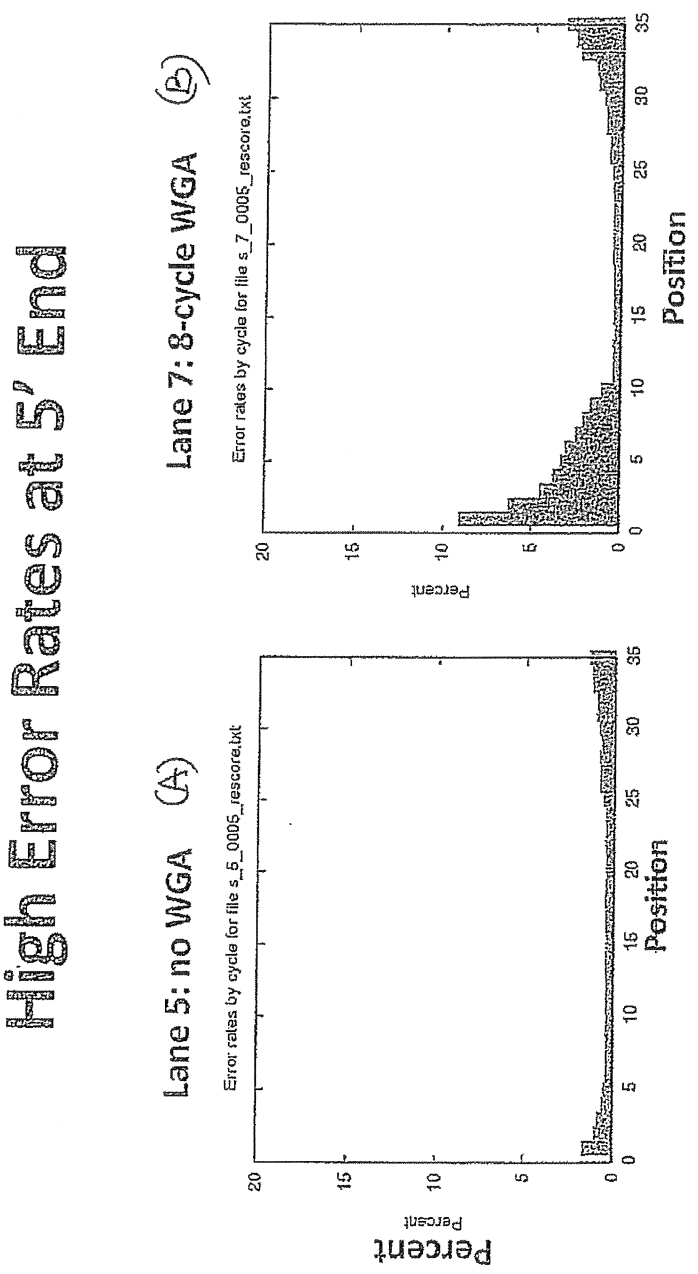

High Error Rates at 5' End
Lane 5: no WGA
(A)
Error rates by cycle for file s_5_0005_rescore.txt
Percent
Position
Lane 7: 8-cycle WGA
(B)
Error rates by cycle for file s_7_0005_rescore.txt
Percent
Position

Figure 16

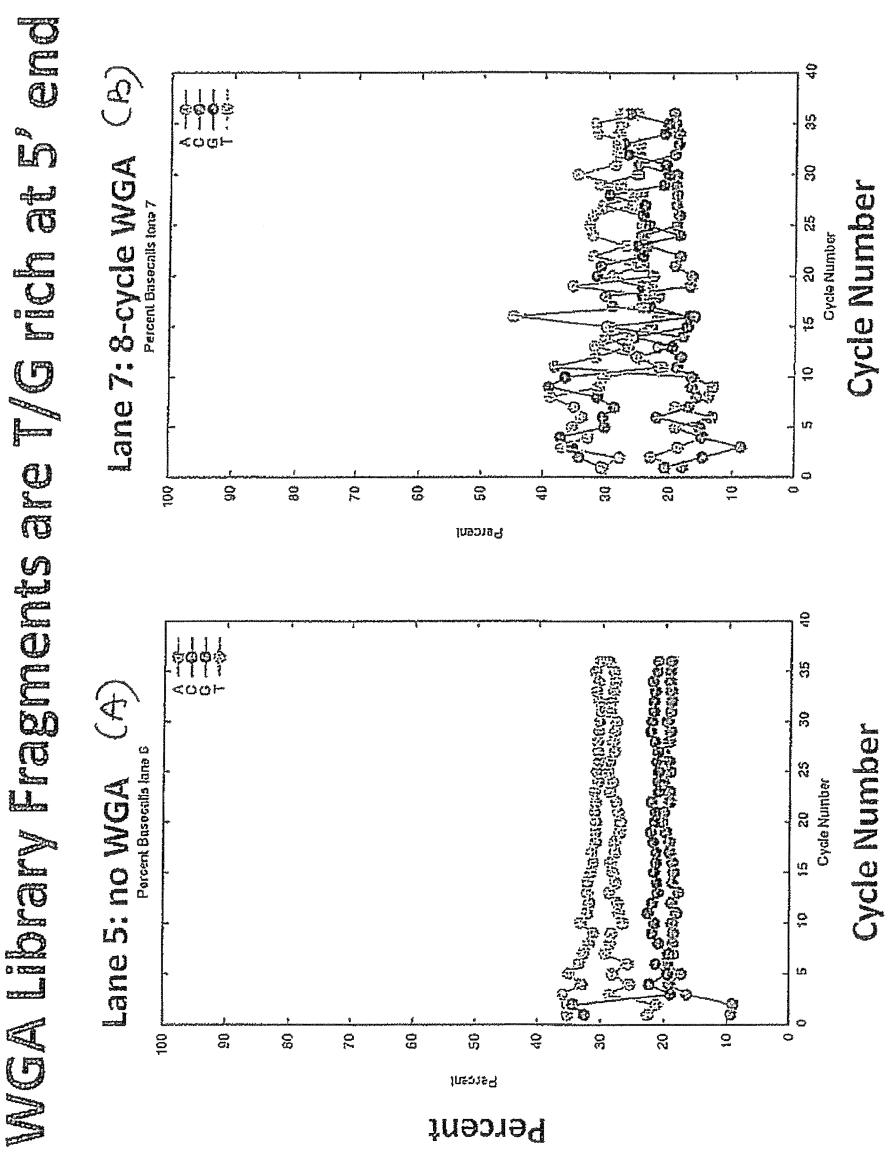

WGA Library Fragments are T/G rich at 5' end
Lane 5: no WGA (A)
Lane 7: 8-cycle WGA (B)
Percent Basecalls lane 5
Percent Basecalls lane 7
A
C
G
T
Percent
Cycle Number

Figure 17

Topo-Cloning and Sequencing of Sigma-WGA Fragment

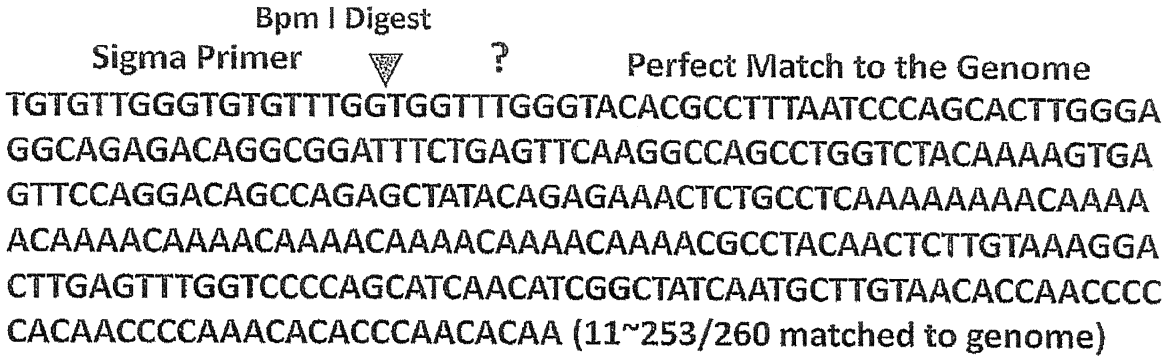


Figure 18 30-cycle WGA fragments

Topo-Cloning and Sequencing of Sigma-WGA Fragment

Bpm I Digest

Sigma Primer ▽ ? Perfect Match to the Genome

TGTGTTGGGTGTGTTTGGTGGTTTGGGTACACGCCTTTAATCCCAGCACTTGGGA
GGCAGAGACAGGCGGATTTCTGAGTTCAAGGCCAGCCTGGTCTACAAAAGTGA
GTTCCAGGACAGCCAGAGCTATACAGAGAAACTCTGCCTCAAAAAAAACAAAA
ACAAAACAAAACAAAACAAAACAAAACAAAACGCCTACAACTCTTGTAAAGGA
CTTGAGTTTGGTCCCCAGCATCAACATCGGCTATCAATGCTTGTAACACCAACCCC
CACAACCCCAAACACACCCAACACAA (11~253/260 matched to genome)

▽

TGTGTTGGGTGTGTTTGGTGTGGTGGTGGAAAATTTAGAAATGTCCACTGTAGG
ACGTGGAATATGGCAAGAAAACTGAAAATCATGGAAAATGAGAAACATCCACTT
GACGACTTGAAAAATGACGAAATCACTAAAAAACGTGAAAAATGAGAAATGCC
CACTAAAGGACCTGGGATATGGCGAGTAAACTGAAAATCACGGAAAATGAGAA
ATACACACTTTAGGACGTGAAATATGGCGAGGAAAACTGAAAAAGGTGGAAAAT
ATAGAAATGTCCACGGTAGGACGTGGAATATGGCAAGAAAACCAAAAACCAAAC
ACACCCAACACAA (9~298/298 matched to genome)

30-cycle WGA fragments

Figure 19

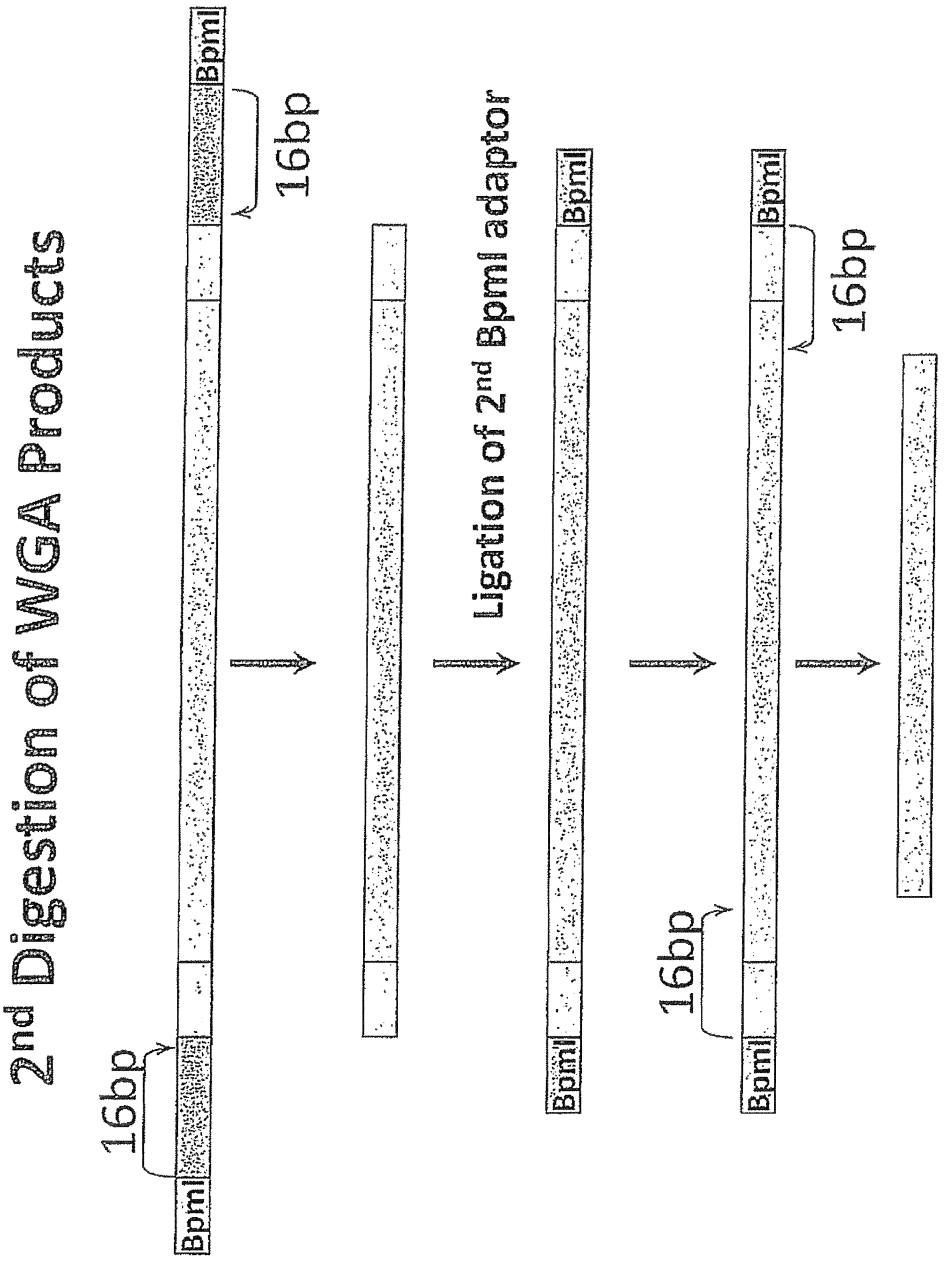

2nd Digestion of WGA Products
16bp
BpmI
16bp
BpmI
Ligation of 2nd BpmI adaptor
BpmI
BpmI
16bp
BpmI
BpmI
16bp

Figure 20

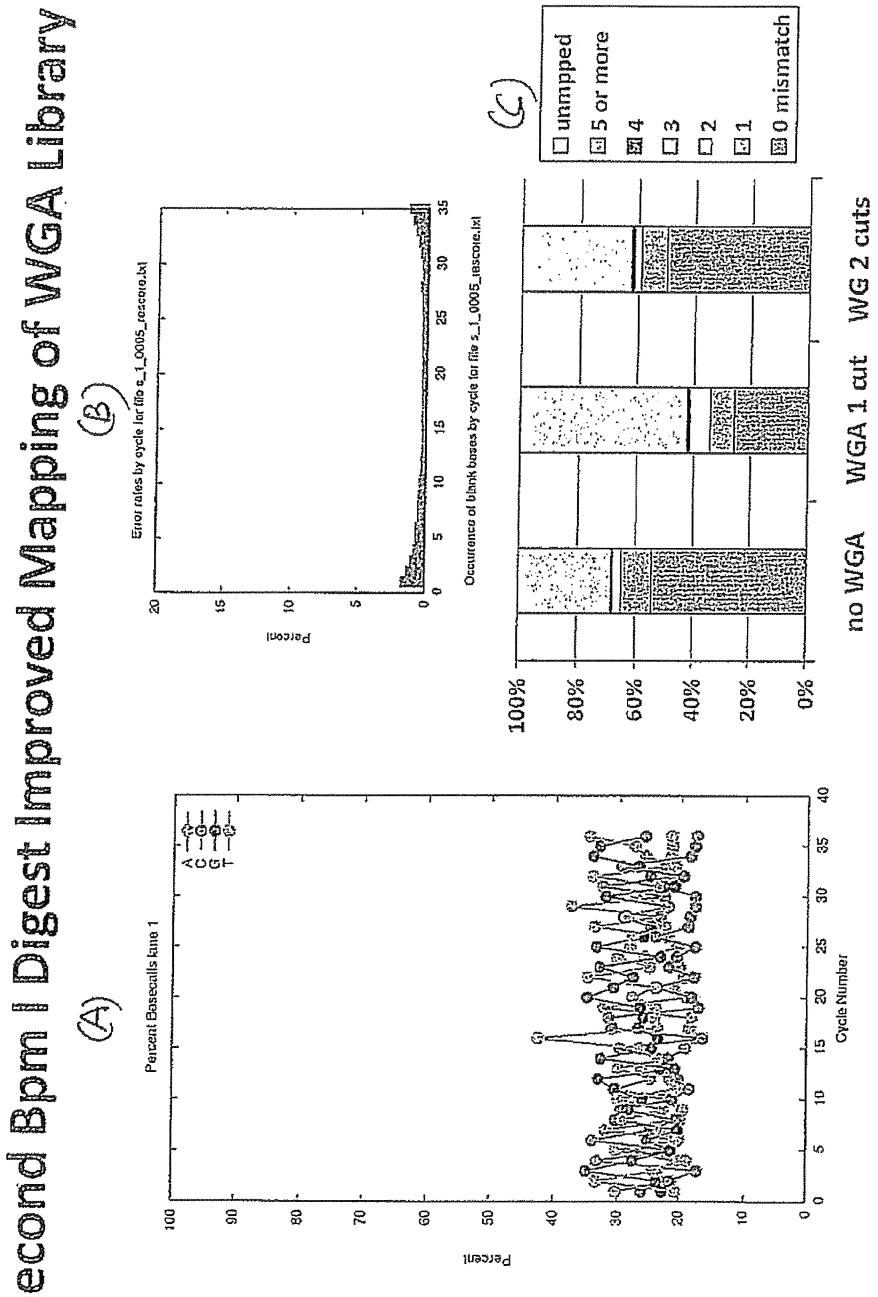
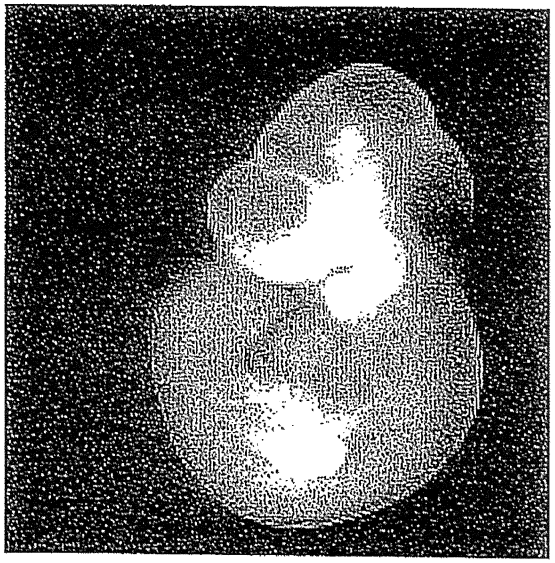

Second Bpm I Digest Improved Mapping of WGA Library
(A)
Percent Basecalls lane 1
A
C
G
T
Percent
Cycle Number
(B)
Error rates by cycle for file s_1_0005_rescore.txt
Percent
Occurrence of blank bases by cycle for file s_1_0005_rescore.txt
(C)
unmpped
5 or more
4
3
2
1
0 mismatch
100%
80%
60%
40%
20%
0%
no WGA
WGA 1 cut
WG 2 cuts

Figure 21

ChIP with embryo tissue



11.5 dpc mouse embryo

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

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

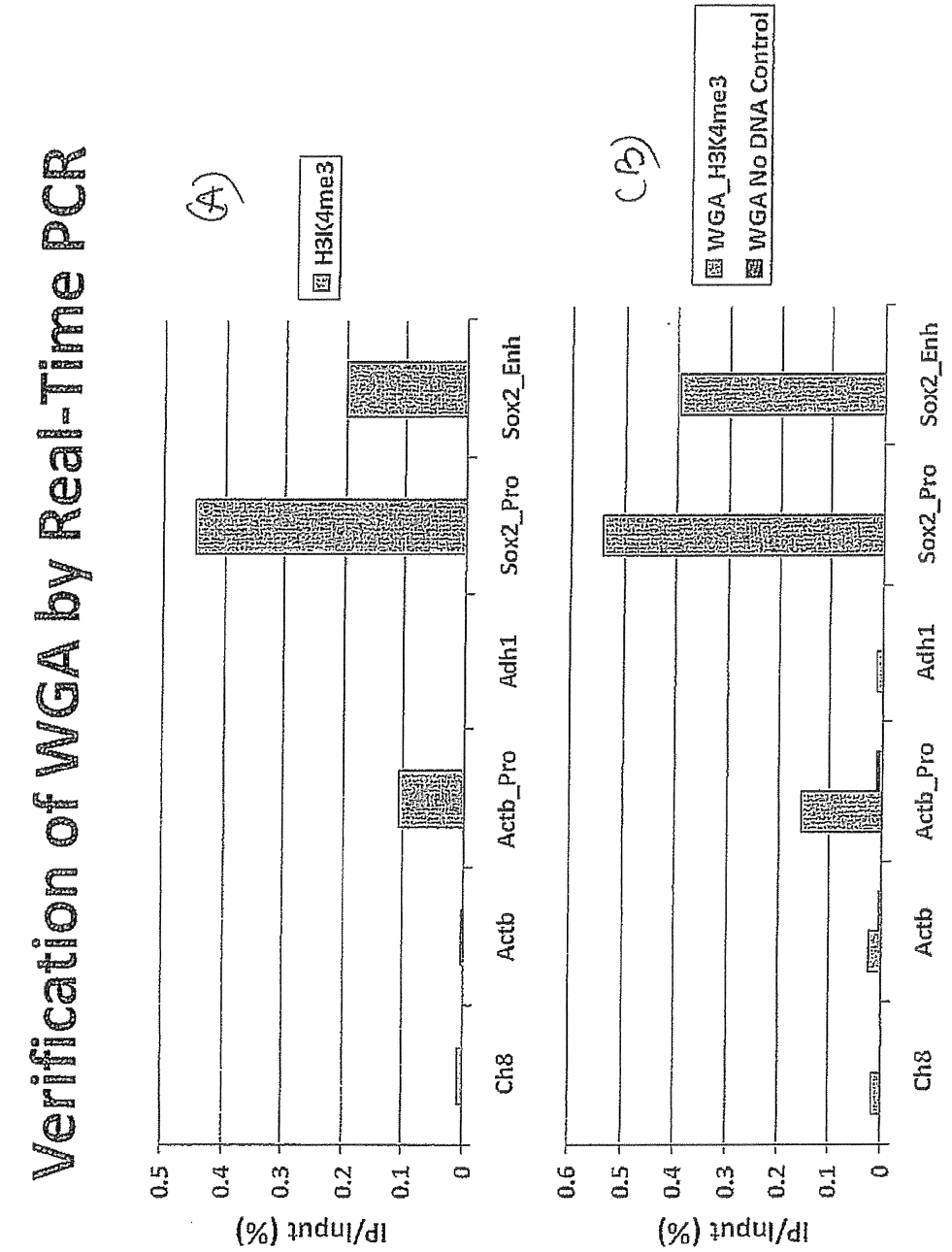

Verification of WGA by Real-Time PCR
(A)
H3K4me3
IP/Input (%)
0.5
0.4
0.3
0.2
0.1
0
Ch8
Actb
Actb_Pro
Adh1
Sox2_Pro
Sox2_Enh
(B)
WGA_H3K4me3
WGA No DNA Control
IP/Input (%)
0.6
0.5
0.4
0.3
0.2
0.1
0
Ch8
Actb
Actb_Pro
Adh1
Sox2_Pro
Sox2_Enh

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

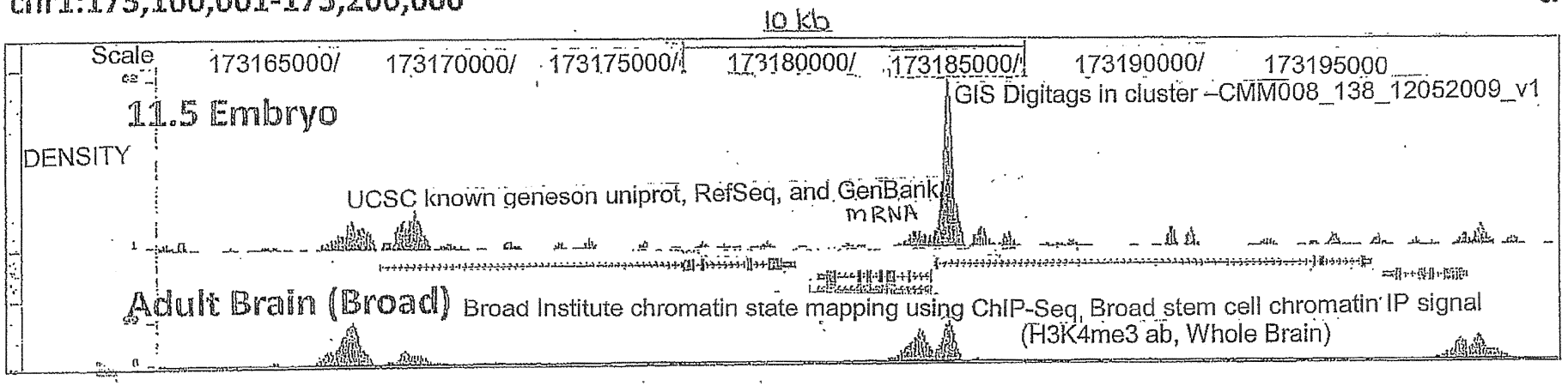
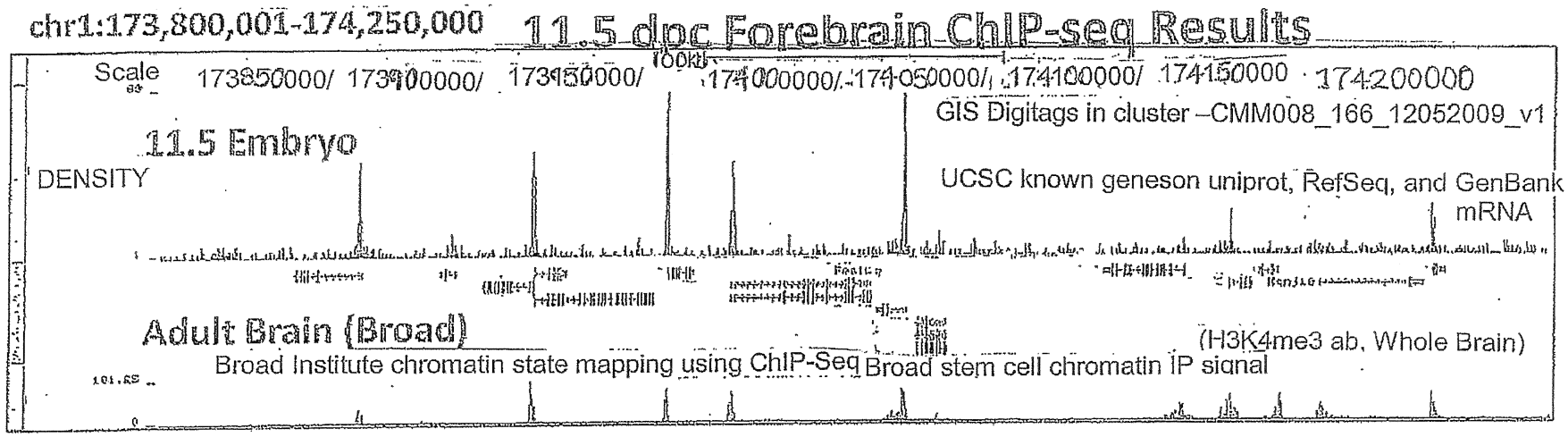
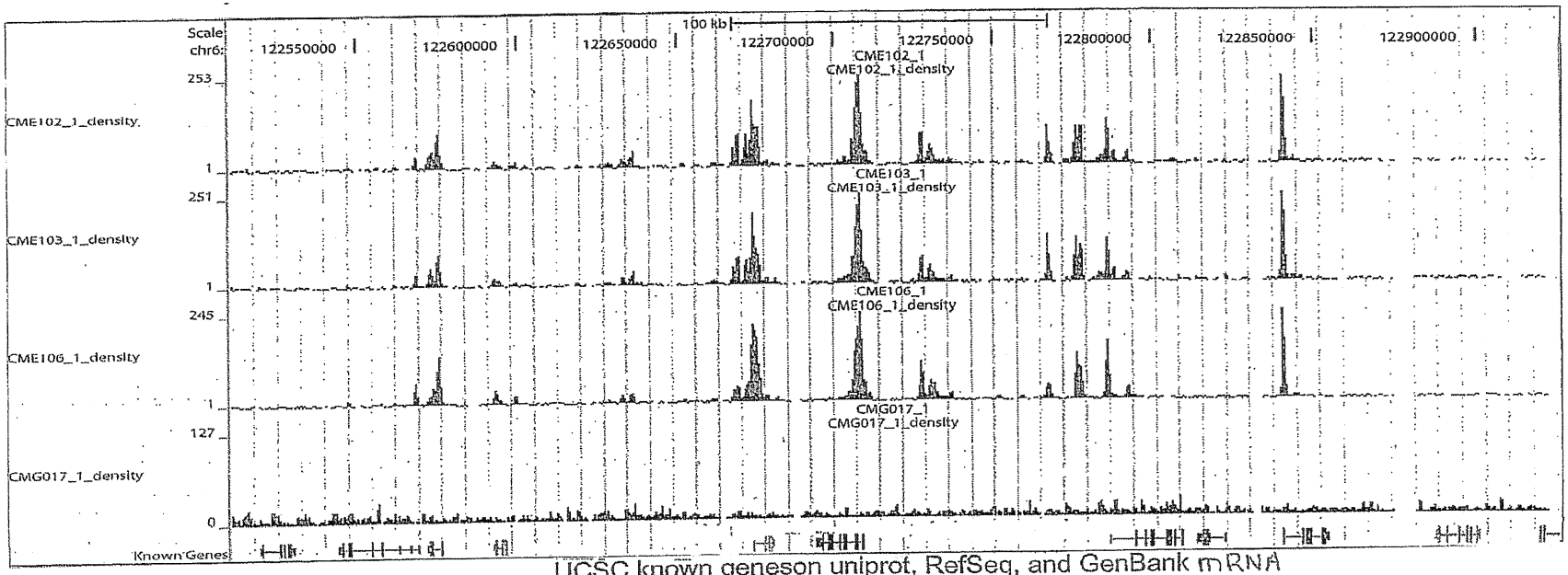


Figure 24



A



UCSC known geneson uniprot, RefSeq, and GenBank mRNA

B

Figure 25

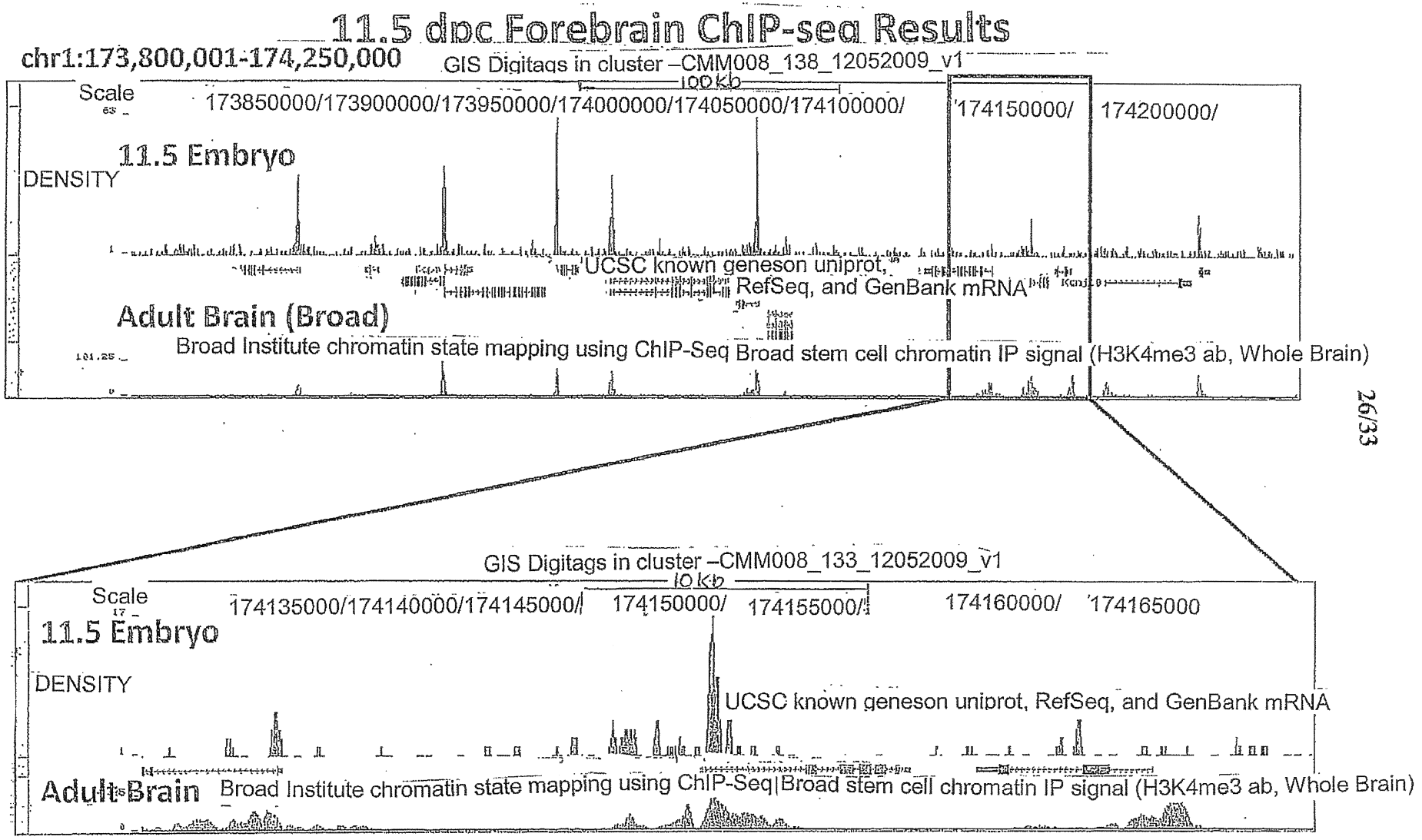

11.5 dpc Forebrain ChIP-seq Results
chr1:173,800,001-174,250,000
GIS Digitags in cluster –CMM008_138_12052009_v1
Scale
100 kb
173850000/173900000/173950000/174000000/174050000/174100000/
174150000/
174200000/
11.5 Embryo
DENSITY
UCSC known geneson uniprot, RefSeq, and GenBank mRNA
Adult Brain (Broad)
Broad Institute chromatin state mapping using ChIP-Seq Broad stem cell chromatin IP signal (H3K4me3 ab, Whole Brain)
GIS Digitags in cluster –CMM008_133_12052009_v1
Scale
10 kb
174135000/174140000/174145000/
174150000/
174155000/
174160000/
174165000
11.5 Embryo
DENSITY
UCSC known geneson uniprot, RefSeq, and GenBank mRNA
Adult Brain
Broad Institute chromatin state mapping using ChIP-Seq Broad stem cell chromatin IP signal (H3K4me3 ab, Whole Brain)

Figure 26

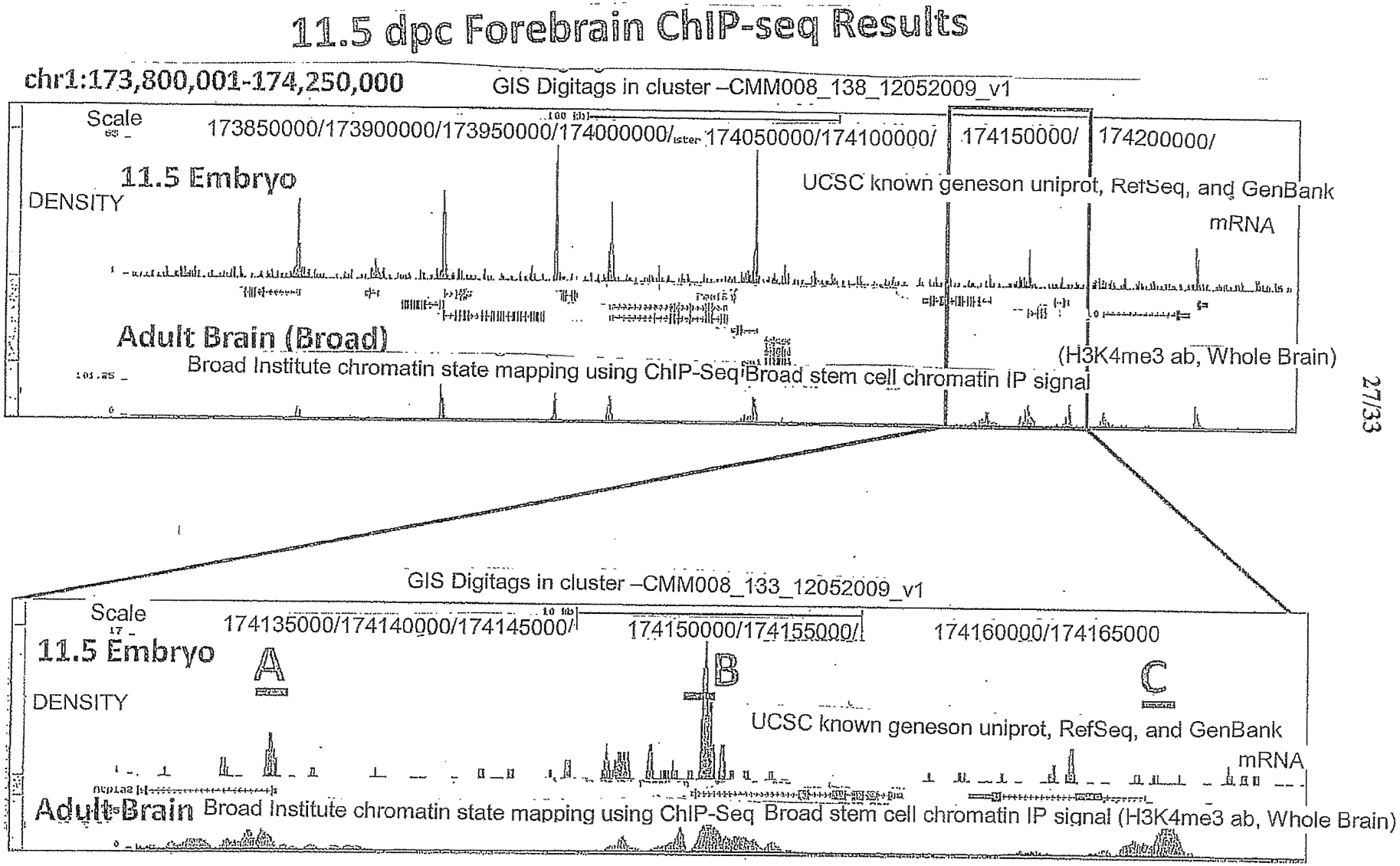
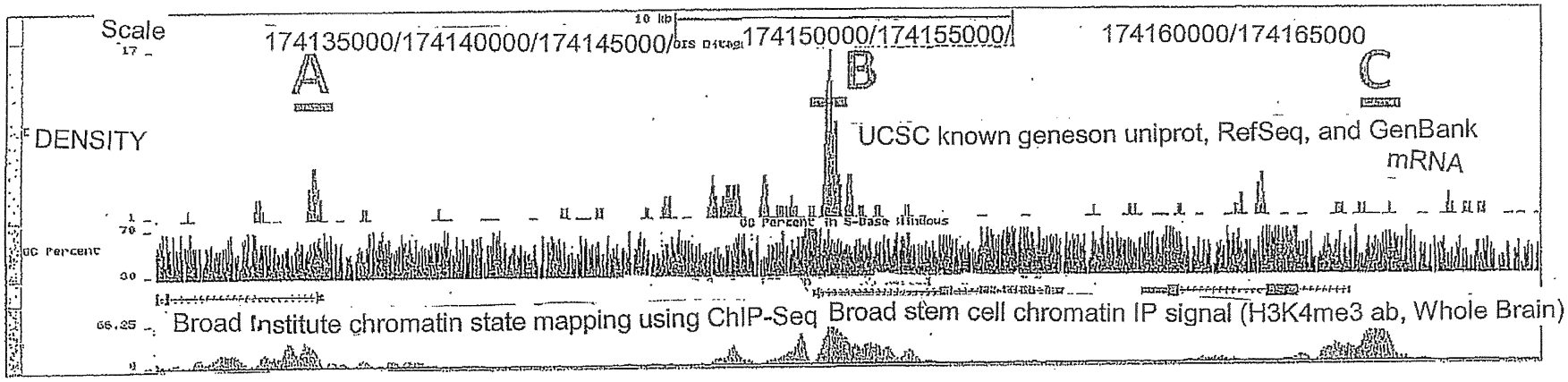

11.5 dpc Forebrain ChIP-seq Results
chr1:173,800,001-174,250,000
GIS Digitags in cluster –CMM008_138_12052009_v1
Scale
173850000/173900000/173950000/174000000/
174050000/174100000/
174150000/
174200000/
11.5 Embryo
DENSITY
UCSC known geneson uniprot, RefSeq, and GenBank
mRNA
Adult Brain (Broad)
Broad Institute chromatin state mapping using ChIP-Seq
Broad stem cell chromatin IP signal
(H3K4me3 ab, Whole Brain)
GIS Digitags in cluster –CMM008_133_12052009_v1
Scale
174135000/174140000/174145000/
174150000/174155000/
174160000/174165000
11.5 Embryo
A
B
C
DENSITY
UCSC known geneson uniprot, RefSeq, and GenBank
mRNA
Adult Brain
Broad Institute chromatin state mapping using ChIP-Seq
Broad stem cell chromatin IP signal (H3K4me3 ab, Whole Brain)

Figure 27

Verification of ChIP-seq Results by Real-Time PCR



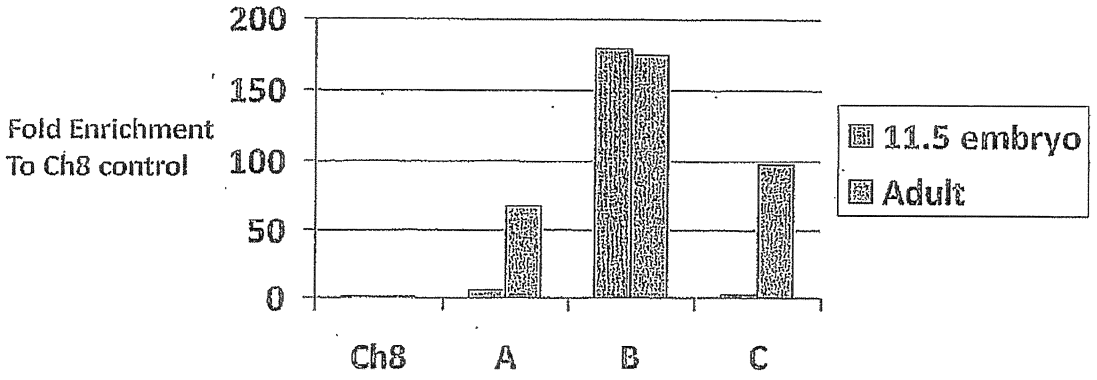
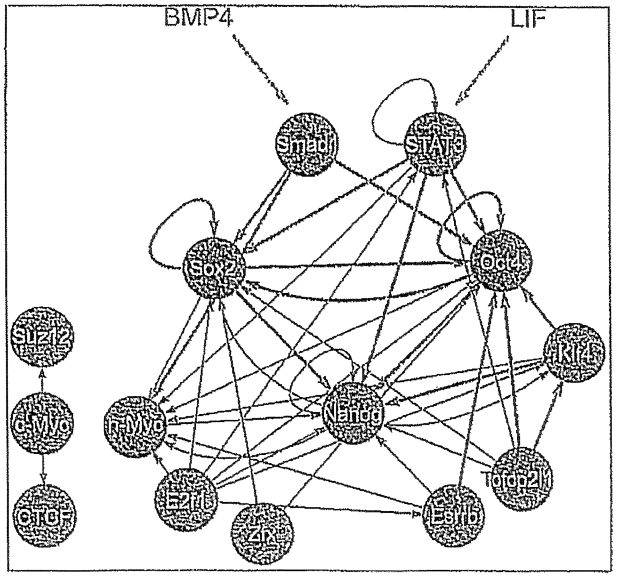


Figure 28

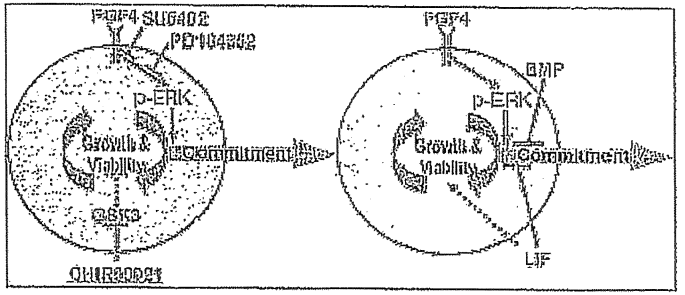
Maintenance of Embryonic Stem Cells

BMP/Serum + LIF



Chen et al., 2008

3 inhibitor cocktail



Ying et al., 2008

Figure 29

Mouse Blastocyst

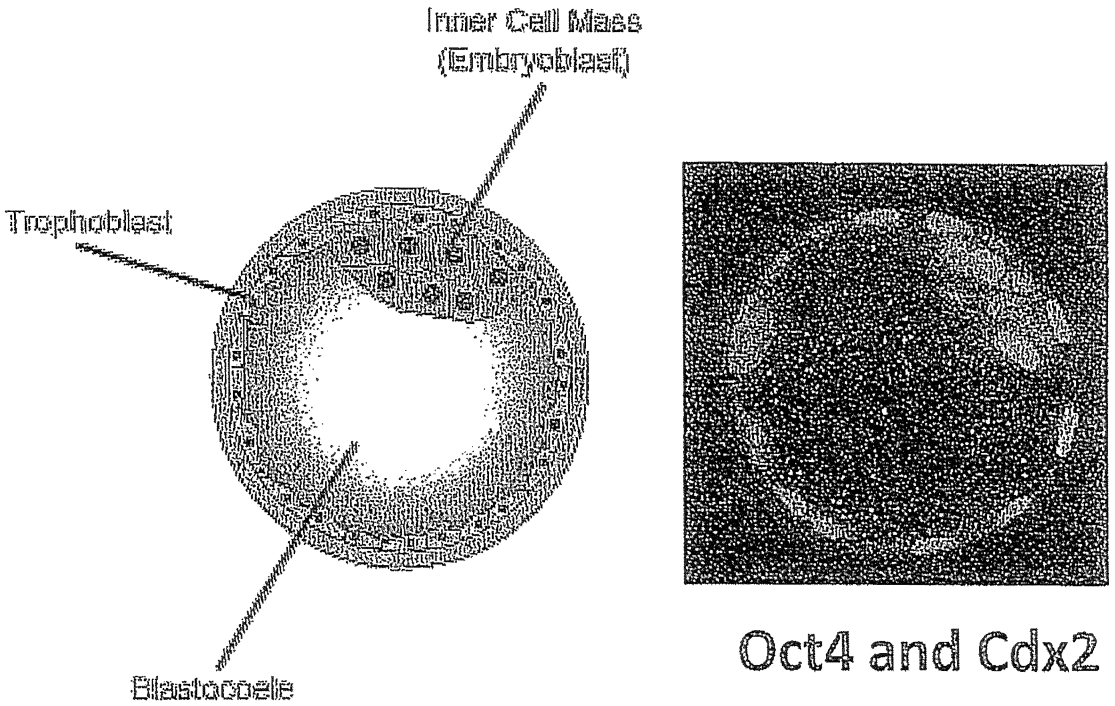


Figure 30

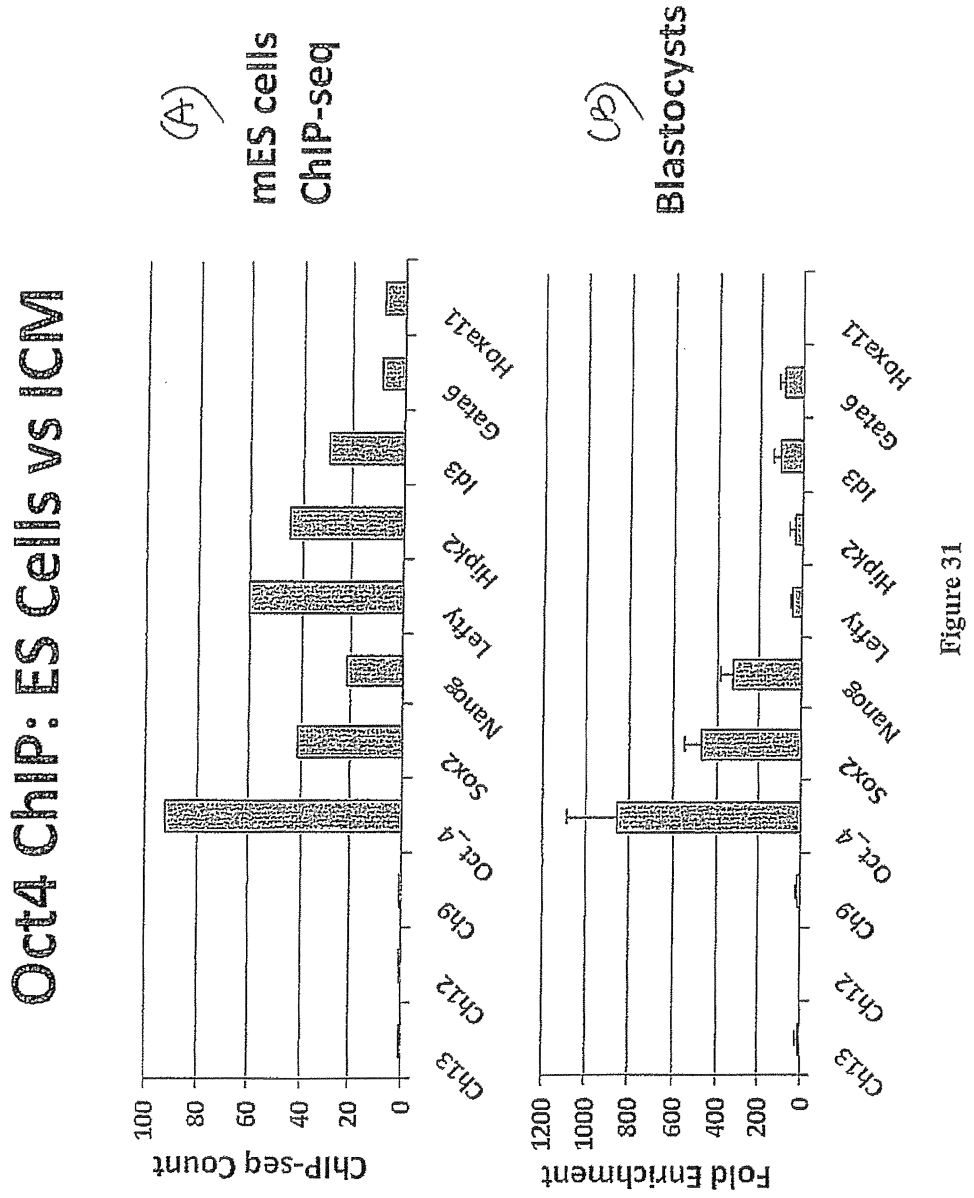
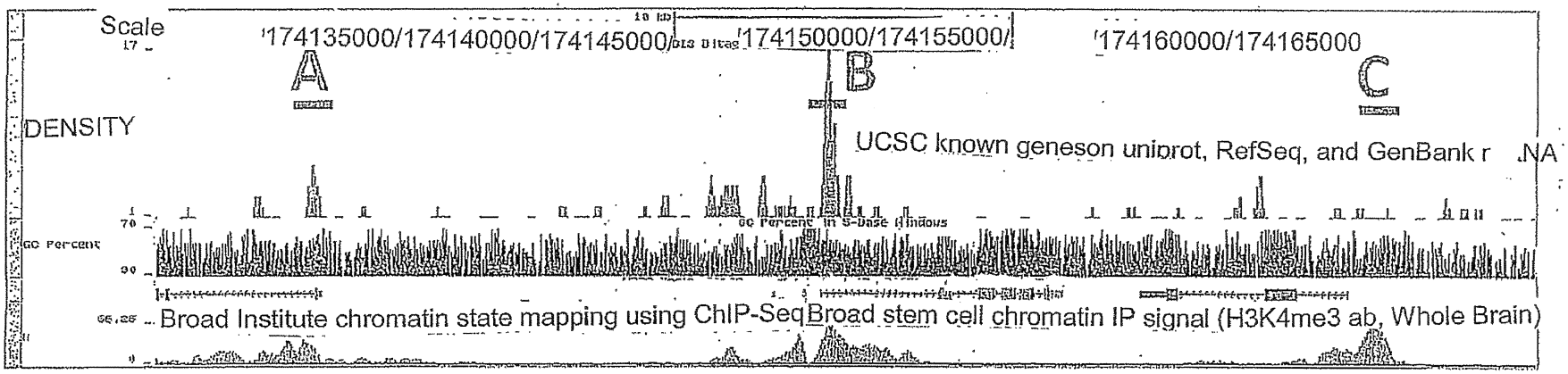

Oct4 ChIP: ES Cells vs ICM
(A)
mES cells
ChIP-seq
ChIP-seq Count
100
80
60
40
20
0
Ch13
Ch12
Ch9
Oct_4
Sox2
Nanog
Lefty
Hipk2
Ida
Gata6
Hoxa11
(B)
Blastocysts
Fold Enrichment
1200
1000
800
600
400
200
0
Ch13
Ch12
Ch9
Oct_4
Sox2
Nanog
Lefty
Hipk2
Ida
Gata6
Hoxa11

Figure 31

Verification of ChIP-seq Results by Real-Time PCR



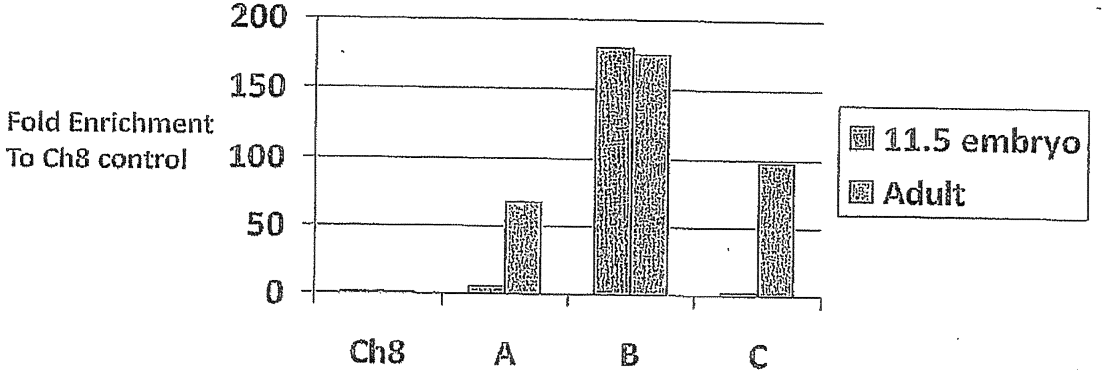


Figure 32

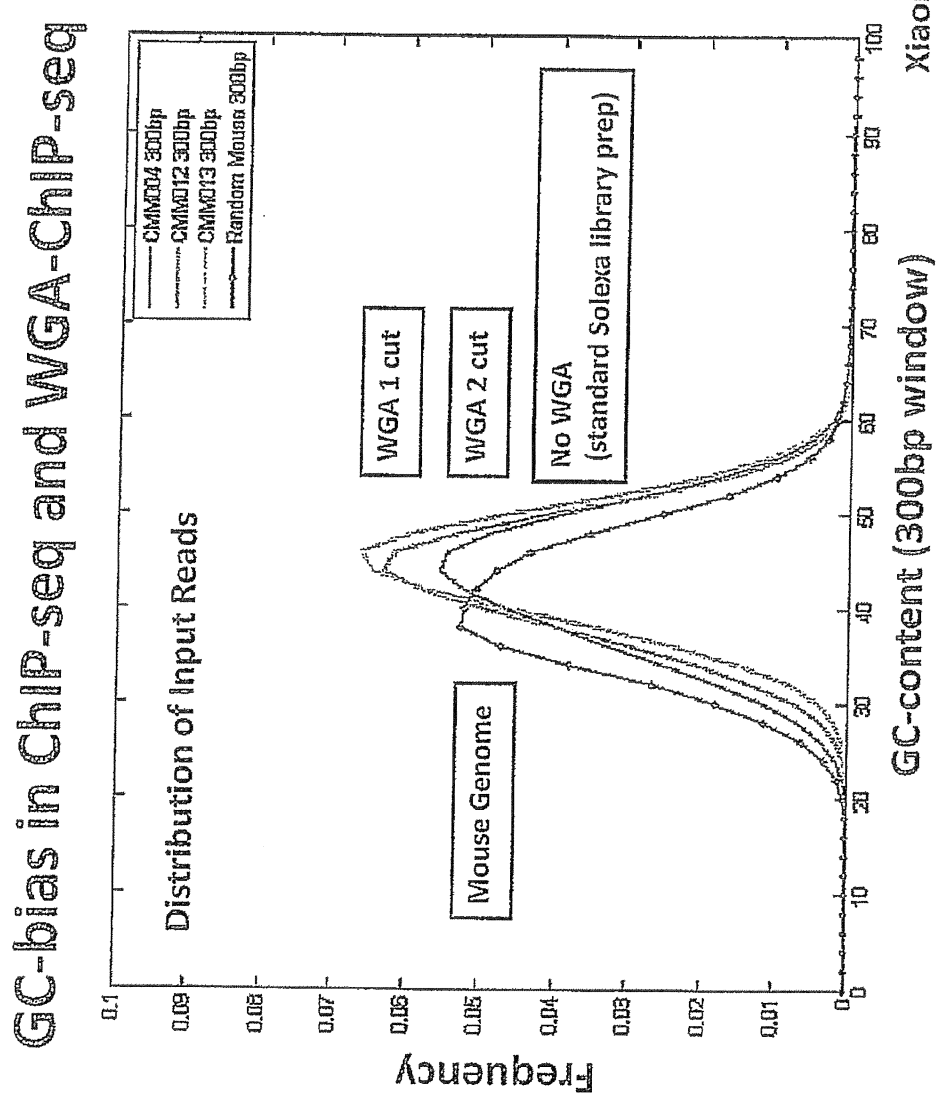

GC-bias in ChIP-seq and WGA-ChIP-seq
Distribution of Input Reads
CMM004 300bp
CMM012 300bp
CMM013 300bp
Random Mouse 300bp
WGA 1 cut
WGA 2 cut
No WGA
(standard Solexa library prep)
Mouse Genome
Frequency
GC-content (300bp window)
Xiaoming Hu

Figure 33