1 Pipeline of experiments

1)DNA samples,barcode,and common adapter pairs are plated and dried;

2)Samples are then digested with restrict enzyme;

3)Adapters are ligated to the ends of genomic DNA fragments;

4)Pooling and purification;

5)PCR: appropriate primers with binding sites on the ligated adapters are added and PCR is performed to increase the fragment pool;

6-7)Cleaned up PCR products, checked fragment sizes of the resulting library on a DNA analyzer.And sequence DNA.

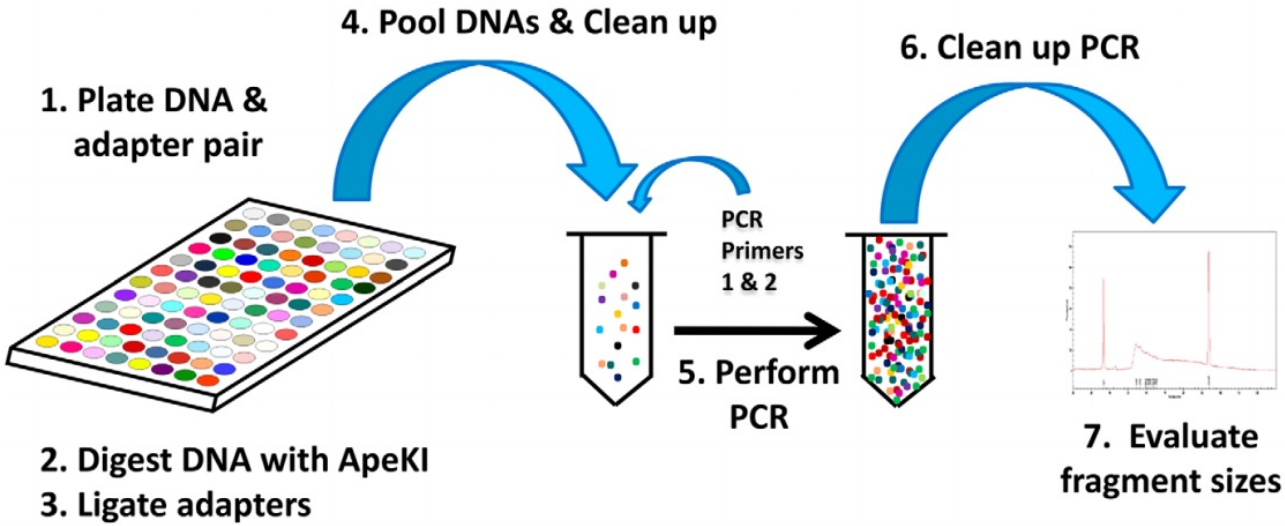


Figure.1 Pipeline of experiments

2 Bioinformatics analysis

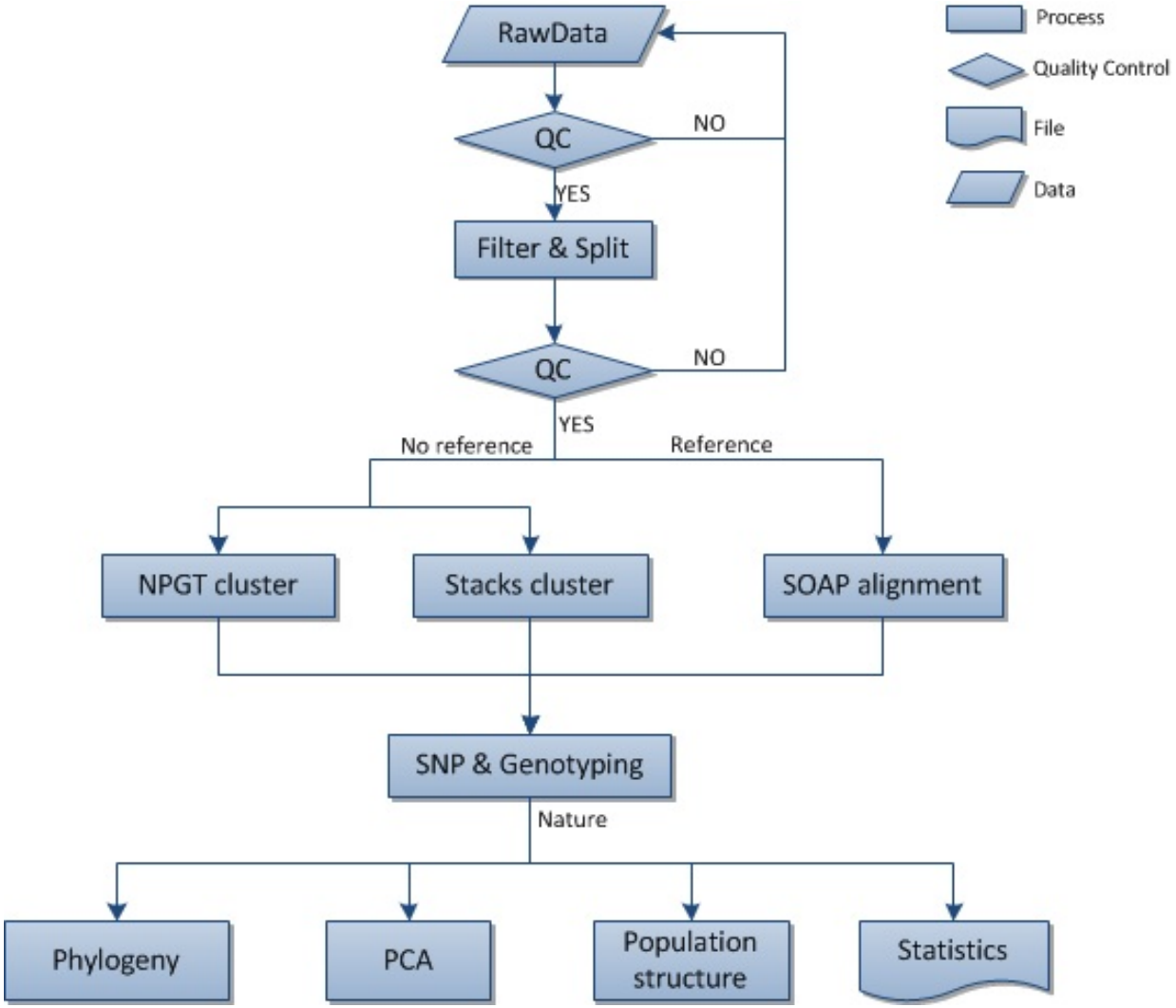


Figure.2 The flow chart of Bioinformatic analysis

2.1 Data statistics

52.61 Gb clean data was generated in this project. Data statistics of some samples was shown as following (Please read Data.readme_en.txt for definition of each column.):

Table.1 Data statistics(Clean_Data)

Sample name	Read number (M)	Base number (Mb)	GC (%)	Q20 (%)	Q30 (%)								
1HL_10A	6.37	615.01	42.61	95.68	90.80								
1HL_11A	7.14	688.72	42.69	95.72	90.91								
1HL_12A	5.05	487.29	42.57	95.61	90.70								
1HL_13A	5.35	516.55	42.84	95.63	90.72								
1HL_14A	4.03	387.00	42.69	95.59	90.64								
1HL_15A	4.39	421.69	42.50	95.59	90.62								
1HL_16A	4.67	447.97	42.62	95.63	90.74								
1HL_17A	7.31	701.52	42.51	95.61	90.70								
1HL_19A	7.24	694.65	42.76	95.64	90.73								
1HL_1A	4.52	436.26	42.36	95.61	90.66								
...	...	...	...	...	...	...	...	...	...	...	...	...	...

*Data statistics of all samples:Data.stat.xls

2.2 SNP Detection

The information about SNPs in one sample was shown as following(Please read SNP.readme_en.txt for definition of each column.):

Table.2 SNP statistics

Sample name	Total	Homo	Hete	Homo rate (%)	Hete rate (%)
1HL_10A	8058	6755	1303	83.83	16.17
1HL_11A	8392	7033	1359	83.81	16.19
1HL_12A	7422	6405	1017	86.30	13.70
1HL_13A	7580	6499	1081	85.74	14.26

1HL_14A	6374	5552	822	87.10	12.90						
1HL_15A	6683	5706	977	85.38	14.62						
1HL_16A	7041	6043	998	85.83	14.17						
1HL_17A	8419	7050	1369	83.74	16.26						
1HL_19A	8543	7141	1402	83.59	16.41						
1HL_1A	6919	5949	970	85.98	14.02						
...	...	...	...	...	...	...	...	...	...	...	...

* SNPs in all samples:SNP.stat.xls

2.3 Genotyping

The genotyping result was shown as following (Please read genotype.noref.readme_en.txt for definition of each column.):

Table.3 Genetying result-noref

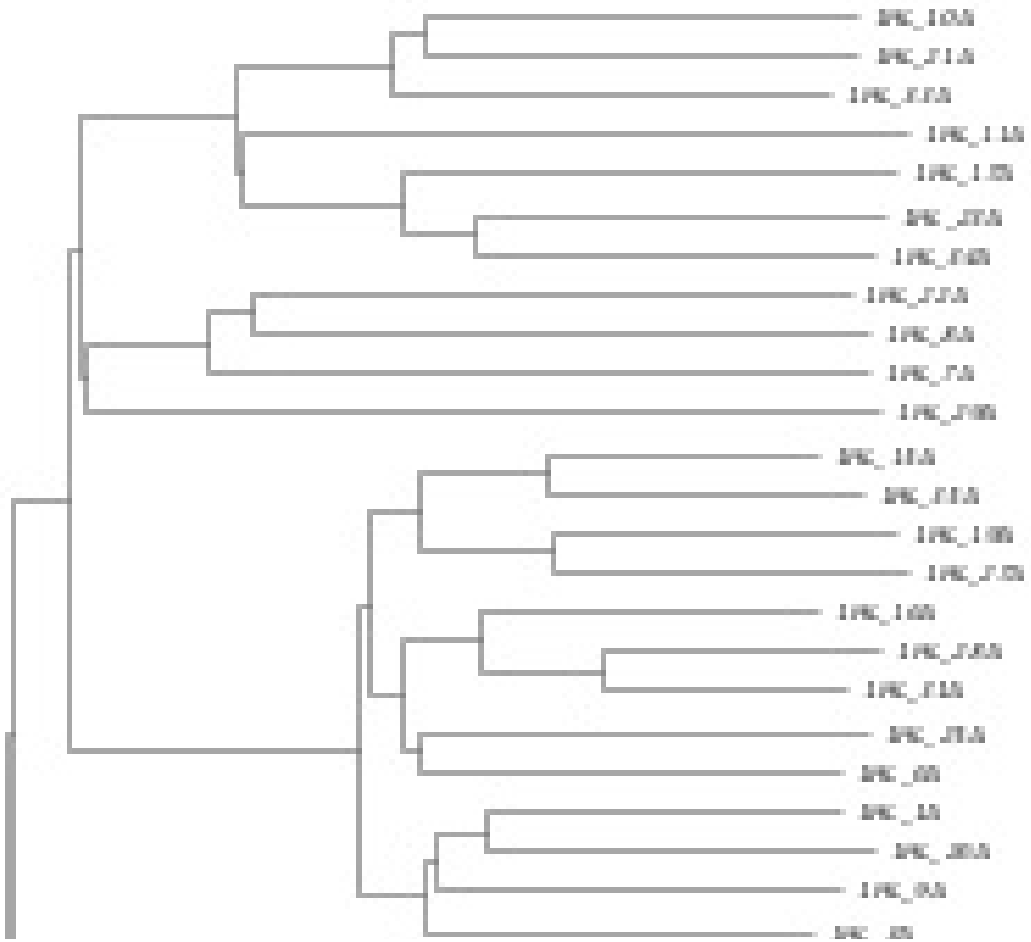
ID	Consensus_Seq	pos	1SN_9A
record_385	CAGCCCTTATTAGGCCACCTGAGTRAACTGATGTGACCTATATTGTAATTAGTTTTCATCCATCATTGTTAAGTCATATGTA	25	A
record_795	CAGCTTCCGATGCGTCGGAGAAGATCAGCACTTYTGGATCTGATACTAAGCTCAGTGATTGTGGAACAAACATCCGTGGAAT	34	Y
record_1907	CAGCTGAAGCATCCAGCAAAGCACGAACAAAGCAGAGAGACACCTACAACACCAAAGTGAGARGAGGAACGGTTAAGGCTGG	63	G
record_2412	CAGCCCTTCACCGGTAATGGTGACGTCTCAATATGAATGAAATATTCTCGACGGGWCGAAAAACAACAAATAATCAATCAAT	56	-
record_3293	CAGCACCTCAGGGCTATCCGTTTCTAACACGGGTACGGGCCMGCGGGGGTTCACGGGCAGTGACCTCCCTTGCACGTTACAA	42	-
record_4526	CTGCACTTGACCACAATAAATGTGATCTTCCTTTAGATTTAATATATATCTGTCTGCCTTCATRAACGTTGATTTTTAAAAT	64	R
record_4591	CAGCTGTATCATCTGACCCCAGAAGAAGCCGGYTTGATAGGAAGCTCTCTTGATGTGTGCTTCTCGAGTTGGAGGAATGGCC	33	C
record_5127	CTGCGTTGTCATGCAAGCATTCTTGACTCCCATGCTCACGAGCCATAGTTACAATGCCGGAATCGCRCATTGGTTTATTCGC	67	R
record_5132	CAGCTTAAGATTTTGTGCATTTTTCAGTTGTGYTCTCTCTACTTTCGATTCCTTGATCCGCCCTTGAAGGAGACCTTCATGT	33	C

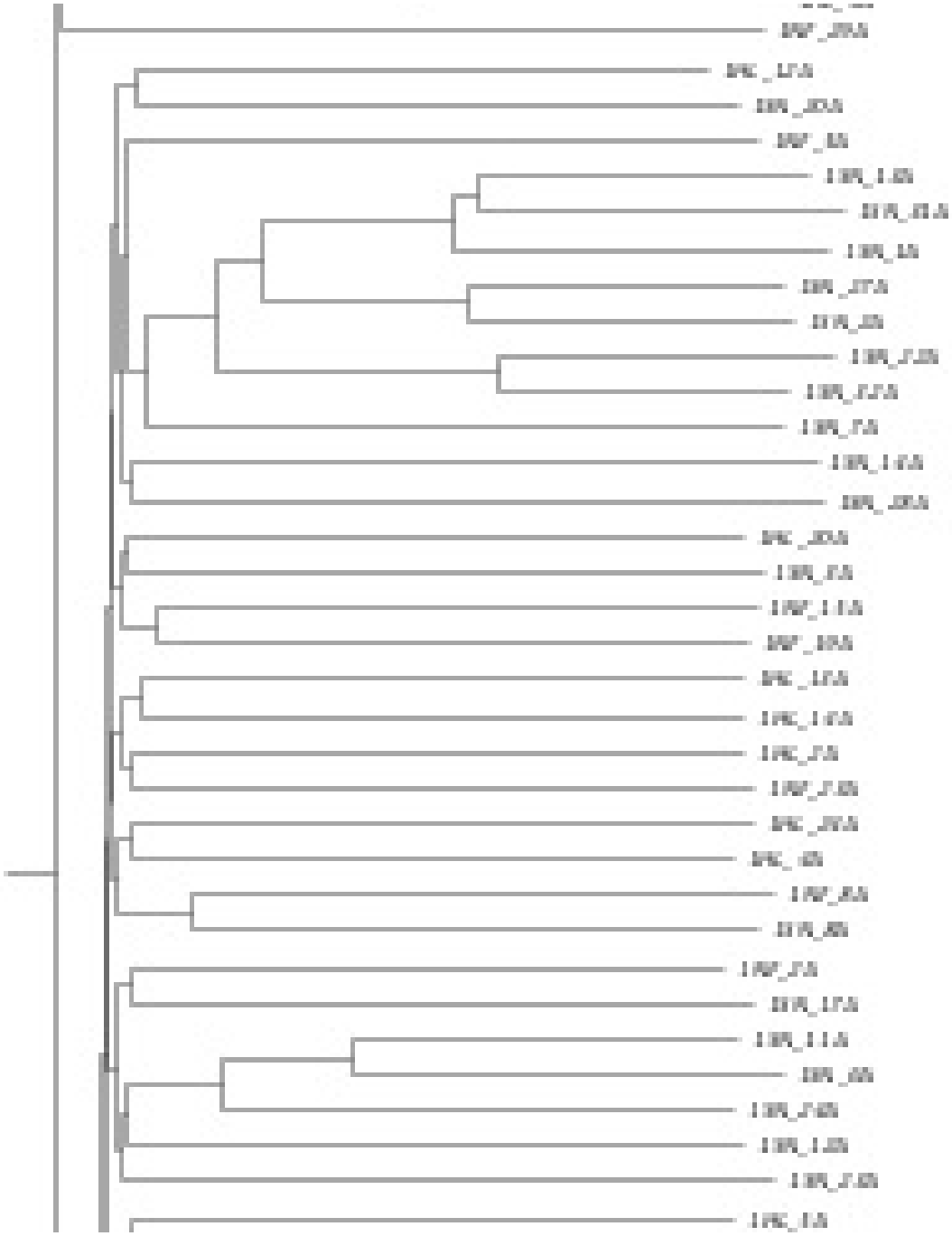
record_5456	CAGCTTGTTTACGAGGCCCTGCYCGCACTTTCTACATAAGTCTCCCCGCAGAATTCAGACAGCCATATAACTTGTTAATTAC	23	Y
...	...	...	...

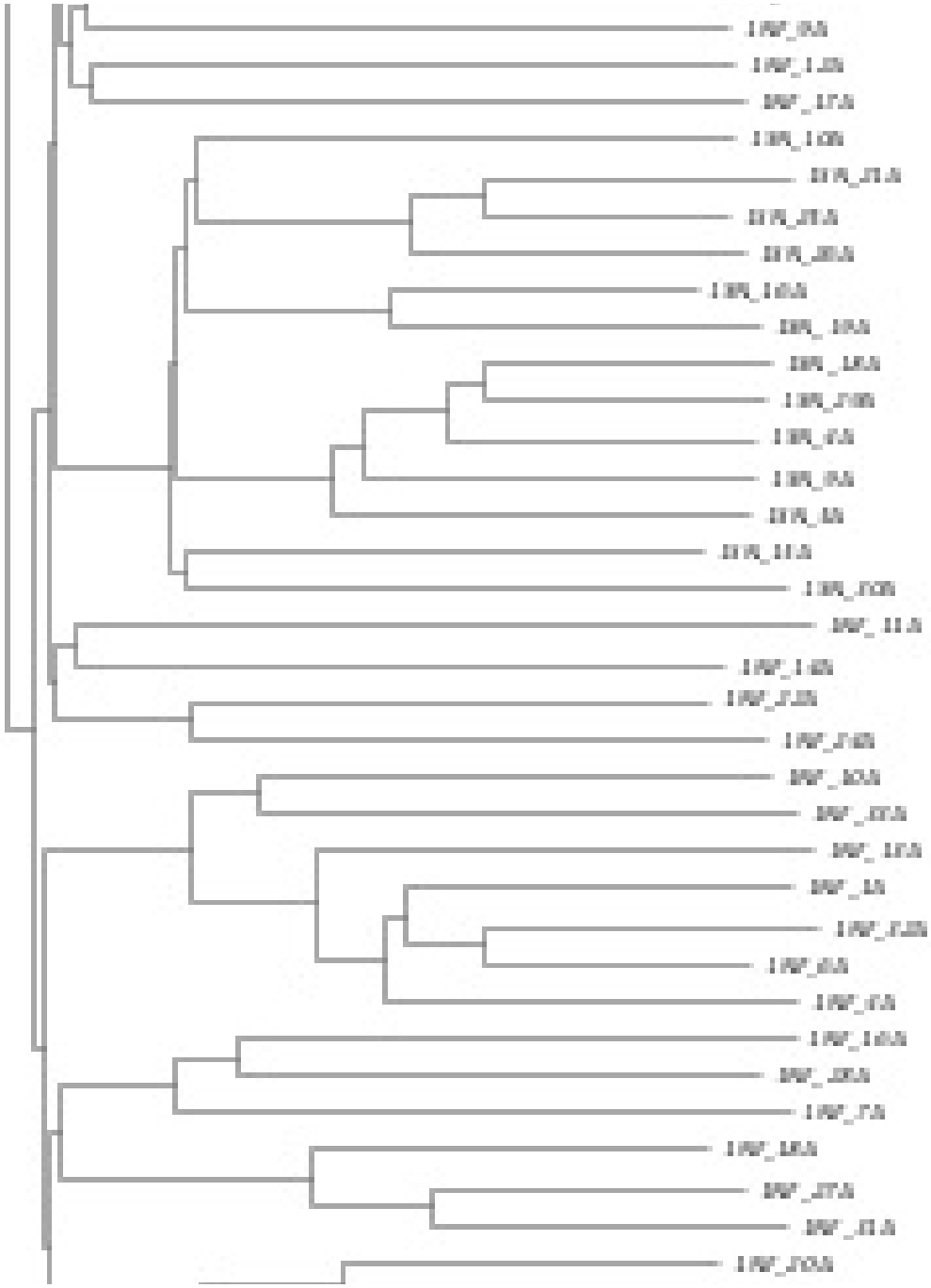
* Genotyping result of all samples:Genotype.xls

2.4 Phylogeny analysis

According to the SNPs we got, the evolution relationship between groups can be infered and shown in phylogenetic tree as following:







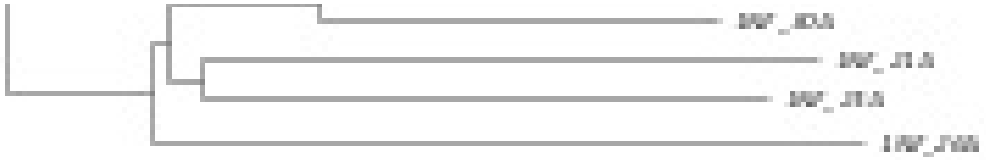


Figure.3 Phylogenetic tree.

2.5 PCA

PCA (Principal Component Analysis) is a mathematical procedure that uses orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables call principal components, which have the largest possible variances. Based on different SNPs between samples, we could separate samples into different subgroups by PCA. The analysis result was shown below:

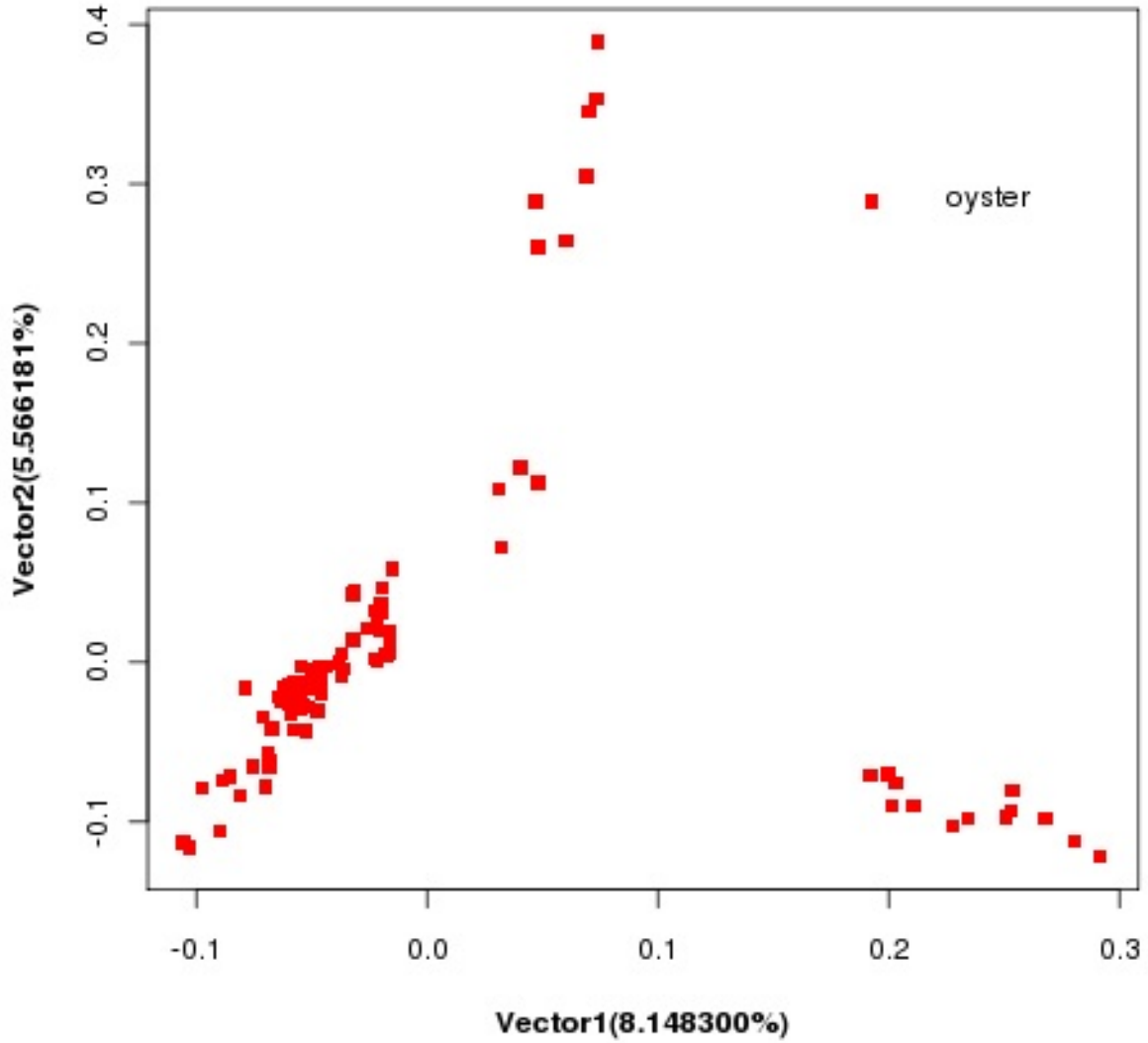


Figure.4 PCA.

Note:Dots in different color represent in different subgroups.