# STUDY ON RELATIONSHIP OF REX RABBIT RAPD MARKER AND REPRODUCTIVE PERFORMANCES

**Ren Keliang[1]*, Li Yanping[1], He Dongchang[1], Wu Xinsheng[2], Zai Ping[3], Liang Quanzhong[1], Zhangg Lijun[1], Zhou Shenghua[1], Cao Liang[1]**

[1]Institute of Animal and Veterinary Sciences, Shanxi Academy of Agricultural Sciences, Taiyuan 030032, China
[2]Yangzhou University, Yangzhou 22500, China
[3]Jiangsu Institute of Animal and Veterinary Sciences, Nanjing 210000, China
*Corresponding author: gj@caaa.cn, leesinglee@gmail.com

## ABSTRACT

RAPD (Random Amplified Polymorphic DNA) marker was applied to study the relationship between some reproductive performances in Rex Rabbit. In the study 15 random primers were selected to PCR for genomes DNA and to detect the amplification product using agarose gel electrophoresis. The study showed some relationship between four primers (OPA1, OPA7, OPA14 and OPA15) with productive performance of Rex Rabbit. From them nine, six, eight and eight bands were obtained respectively. Two groups with or without and №.2 band from OPA1 showed significant ($P<0.05$) and highly significant ($P<0.01$) differences in the birth weight and the birth litter size. As for the two groups with or without №.4 band from OPA7 showed distinguished ($P<0.05$) or significant distinguished ($P<0.01$) differences in the litter size, living litter size, birth weight and birth litter size. The two groups with or without №.6 band from OPA14 showed significant differences ($P<0.05$) in the birth weight and birth litter size. The two groups with or without №.2 band from OPA15 showed distinguished significant differences ($P<0.01$) in litter size, living litter size and birth litter size.

**Key words**: Rex Rabbit, RAPD, Reproductive performance.

## INTRODUCTION

RAPD (random amplified polymorphic DNA) is a technology of genetics marker. It was established through PCR technology to test polymorphic DNA of genome by Williams *et al.* (1990) and Welsh *et al.* (1990). It uses a series of random base sequence oligonucleotide single-strands (with 10 bases) as random primers to amplify target genomes DNA. The amplification product is electrophoretically separated through polyacrylamide or gel agarose and detected polymorphic DNA through methods of stained with thidium bromide (EB), silver, or radiograph. As the technology is simple, quick, sensitive, little DNA consumption and low cost, it has been widely utilized in genetics analysis in animal, plant and microorganism and shown good results in population genetics diversity, genetic map construction, gene mapping and forecast of genetic distance and heterosis. At present, there are many studies using RAPD technology to analyses population genetics diversity, and study economic traits. For example, Li Hui (1999) and Gong Daoqing *et al.* (2002) studied RAPD marker for body fat trait in broiler type chicken, Jiang Li (2004) found RAPD marker significantly correlated with slaughter characteristics in goose, Li Xianglong *et al.* (1999) found RAPD marker which correlated with body weight and body size in local Qinglong goat; Shi Qishun *et al.* (2001) forecasted heterosis of growth and meat traits and markers related with daily gain in commercial pig. Gong Daoqing *et al.* (2005) found RAPD marker correlated with sebum weight (rate) and abdominal fat weight (rate) in meat duck. RAPD technology is principally used to study blood relationship between varieties/species in the rabbit. For instance, Yang Liping *et al.* (2000) analyzed three domestic rabbit varieties/species; Pang Rongqing *et al.* (2000) analyzed far or close evolutionary blood relationship analyzed five rabbit populations; Chen Mingli *et al.* (2005) analyzed genetic relationship among three varieties/species. Up to now there is still no report that studied rabbit in correlating productive performance of the rabbit.

In the experiment, Rex Rabbit was studied to look for RAPD marker correlated with the reproductive performance and provide theoretical basis for selection of reproductive performance in Rex Rabbit.

## MATERIALS AND METHOD

Thirty five mating rabbits were chosen for the trial. Measures were taken of the litter size, living little size, birth weight and birth litter size.

### Blood sample collection and DNA extraction

Blood samples were collected from the vein near the ear edge. Heparin sodium was used as blood anticoagulant and method of modified phenol chloroform extraction was used to genome DNA extraction.

### PCR reaction and separation of RAPD product

*Random primers*
Fifteen random primers used in the study were synthesis by Shenggong bio-engineering Company, Ltd, in Shanghai.

*PCR reaction system*
The RAPD 20 μl reaction systemize as follows**:** 2.0 μL 10×Buffer ($Mg^{2+}$ included), 2μl dNTPs (2.5 mmol/l respectively), 1μl primer (100 pmol/μl), 0.2 μl Taq enzyme (5.0 U/μl), 1μl DNA (50 ng/μl), 13.8 μl $ddH_2O$.

*PCR amplification conditions*
Predenaturation was done for 7 minutes at 97° and for 1 minute at 94°; annealing for 1 minute at 36°, for 2 minutes at 72° and after 45 circulations was extended for 10 minute at 72°, and then stopped. Preservation was at 4°.

*Test for the amplification products*
RAPD products were taken from PCR instrument and tested with 1.4% gel agarose electrophoresis. The samples were electropgoresed at the voltage of 2-3V/cm for 3-3.5 h. The results were observed and photographed in gel imaging system.

### Statistical analysis

The experimental results were statistically analyzed by software of SPSS11.0.

The mathematical model is: $t = \dfrac{\overline{X_i} - \overline{X_j}}{S_{\overline{x_i} - \overline{X_j}}}$

Xi: mean of animals with bands, Xj: mean of animas without bands, $Sx_i$-$x_j$: standard deviation.

## RESULTS AND DISCUSSION

### Polymorphism of RAPD marker

Four part reproductive performance correlated primers were selected from the total 15 primers (Table 1): OPA1, OPA7,OPA14 and OPA15 (Figure 1). From them 9, 6, 8 and 8 bands have been detected respectively. The amplified bands of other primers are seldom.
Total primers sequences:
P1 5’-ACAGGTGCTG-3’ P2 5’-ACGCCAGAGG-3’ P3 5’-ACGGCGTATG-3’

| | | |
|---|---|---|
| P4 5’-CAGACAAGCC-3’ | P5 5’-CAGCTCACGA-3’ | P6 5’-CCCAGCTAGA-3’ |
| P7 5’-TGGCGCAGTG-3’ | P8 5’-CTGGGCACGA-3’ | P9 5’-GACTAGGTGG-3’ |
| P10 5’-GGTCTACACC-3’ | P11 5’-GTCGCCGTCA-3’ | P12 5’-TGCGCCCTTC-3’ |
| P13 5’-CTTCCCCAAG-3’ | P14 5’-TTCCGCCACC-3’ | P15 5’-TTCGAGCCAG-3’ |

**Table 1**: Four primers sequences and amplified bands

| Primer | Sequence | Bands |
|---|---|---|
| OPA1 | 5’-ACAGGTGCTG-3’ | 9 |
| OPA7 | 5’-TGGCGCAGTG-3’ | 6 |
| OPA14 | 5’-TTCCGCCACC-3’ | 8 |
| OPA15 | 5’-TTCGAGCCAG-3’ | 8 |

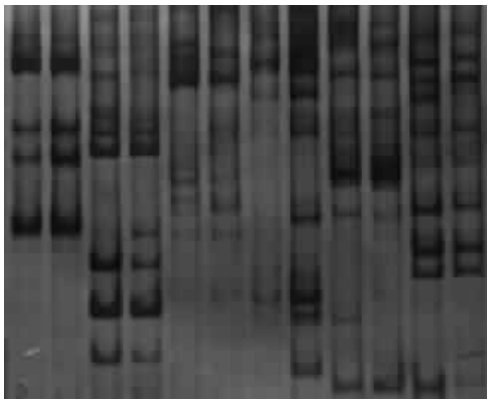

**Figure 1**: The part amplification results of OPA1

**Correlation between RAPD marker and reproductive performance**

Table 2 showed that there were significant (P<0.05) and highly significant (P<0.01) differences respectively in the birth weight and birth litter size between two groups with or without №.2 band from OPA1. There were distinguished (P<0.05) and significant distinguished (P<0.01) differences respectively in the litter size, living litter size, birth weight and birth litter size between two groups with or without №.4 band from OPA7. There were distinguished (P<0.05) and significant distinguished (P<0.01) differences respectively in birth weight and birth litter size between two groups with or without №.6 band form OPA14. There were distinguished (P<0.05) and significant distinguished (P<0.01) differences respectively in litter size, living litter size and birth litter size between two groups with or without №.2 band from OPA15.

**Table 2**: Comparison of reproductive performances between RAPD bands

| Primer | Bands | Performance | M0 | M1 |
|---|---|---|---|---|
| OPA1 | 2 | birth weight | 55.56±13.61(21)$^{a}$ | 67.26±17.98(14)$^{b}$ |
| | | birth litter size | 280.57±103.08(21)$^{a}$ | 412.93±159.91(14)$^{c}$ |
| OPA7 | 4 | litter size | 5.22±1.67(23)$^{a}$ | 6.33±1.67(12)$^{b}$ |
| | | living litter size | 5.13±1.18(23)$^{a}$ | 6.25±1.71(12)$^{b}$ |
| | | birth weight | 55.69±14.10(23)$^{a}$ | 68.98±17.30(12)$^{b}$ |
| | | birth litter size | 281.30±92.64(23)$^{a}$ | 433.58±170.67(12)$^{c}$ |
| OPA14 | 6 | birth weight | 55.53±12.21(23)$^{a}$ | 69.29±19.74(12)$^{b}$ |
| | | birth litter size | 298.91±106.04(23)$^{a}$ | 399.83±182.01(12)$^{b}$ |
| OPA15 | 2 | Litter size | 5.00±0.97(20)$^{a}$ | 6.40±1.60(15)$^{c}$ |
| | | living litter size | 4.90±0.97(20)$^{a}$ | 6.33±1.63(15)$^{c}$ |
| | | birth litter size | 279.40±90.11(20)$^{a}$ | 405.67±169.27(15)$^{c}$ |

Notes: M0 are groups without bands, M1 are groups with bands. The numbers in brackets are number of individual rabbits. In the same line, a and b means that there is significant difference (P<0.05) between them, while a and c means highly significant difference (P<0.01)

The theoretical base of statistical quantitative genetics is the quantitative performance. Controlled genes are slightly-effective multiple genes. Thus the multiple genes which control certain activities have to be studied in its entirety and it is hard to make a detail break-down for various genetics types or the effects. From the theory of quantitative genetics it is known that the correlation with quantitative performance is a result of inter-linkage of marker gene locus and QTL playing a role of the control for the performance, or by one-gene-multi-effects of the marker gene.

## CONCLUSIONS

The results showed that: OPA1, OPA7 and OPA14 are RAPD markers correlated with the performance of birth weight of the Rex rabbit; OPA1, OPA7,OPA14 and OPA15 are correlated with birth litter size, and OPA14 and OPA15 are correlated with the performances of litter size and living litter size. It showed that those markers might be linked with the dominant effect gene which controls those reproductive performances, or those markers might have the effect of one-gene-multi-effect. It can provide reliable theoretical basis for the molecule breeding of rabbit in our country. If those markers could be further utilized in construction of molecule linkage mapping, gene location and molecule marker can help selection as well.

## REFERENCES

Chen Liming, Zhao Weibo, Ying Huachun 2005. RAPD Analysis of Genetics Traits in WHBE Rabbit. *Journal of Zhejiang University (Edition of Life Science), 2005, 31 (4), 493-498.*

Gong Daoqing, Zhang Jun, Li Hui 2002. Detection of RAPD marker related with Fatness in Table Poultry. *Journal of Yangzhou University (Edition of Life Science), 23(3), 21-24.*

Gong Daoqing, Zhang Jun, Zhang Hong 2005. Study on the Relationship between RAPD Marker Polymorphism and Body Fatness Performance. *Chinese Journal of Animal Sciences, 41(12), 39-41.*

Jiang Li, Wang Jiwen 2004. Study on the Relationship between Polymorphism DNA Molecule marker and Slaughter Performances in Goose. *Chinese Journal of Animal Science, 40(9), 16-18.*

Li Hui, Zhu Xiaoping, Gong Daoqing 1999. Study on RAPD marker of Fatness in Table Poultry. *Chinese Agricultural Sciences, 32(3), 78-84.*

Li Xianglong, Tian Qingyi, Sun Naiquan 1999. Study on Relationship between Random Amplified Polymorphic DNA and Body Size. *Animal and Vet. Sciences in Heilongjiang, (9), 3-4.*

Pang Rongqing, Chen Chenggong, Liu Wanchen 2000. DNA Analysis on Random Amplified Polymorphism in Rabbit. *Chinese Journal of Veterinary Science, 20(2), 195-197.*

Ren Keliang 2002. Comprehensive Work of Modern Rex Rabbit Farming. *Taiyuan Shanxi Publish House of Science and Technology, April 2002.*

Shi Qishun, Liu Xiaochun, Wu Xiaolin 2001. Primitive Approach to the Relationship between the Heterotic Effect of Growth and Meat-purposed Performance and the Similarity Coefficient of RAPD Band Region in Commercial Hybrid Swine. *Journal of Genetics, 2(8), 722-729.*

Welsh J., McClelland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research, 18(24), 7213-7218.*

Williams G.K., Kubeiik A.R., Livak K.J. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research, 18(22), 6531-6535.*

Yang Liping, Zhang Yusheng, Lu Lianshan 2000. RAPD Genetics Analysis on Various Domestic Rabbit Varieties/Species. *Chinese Journal of Rabbit Farming, (3), 15-18.*