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檢定站種公豬精子成熟度檢測及應用

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### 檢定站種公豬精子成熟度檢測及應用

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本計畫目的為加倍公豬高飼料效率與產精遺傳同步選拔效率，藉由精子體能分析儀測定年青種公豬之精液濃度及精子粒線體完整度，以評估年青公豬產精能力與成熟度，期提早應用優質的高飼料效率種公豬於種豬繁殖及肉豬生產上，加速優質基因之擴散利用。測定之年青種公豬為財團法人中央畜產會種豬性能檢定站201607期、201609期、201610期及201611期完檢之杜洛克、藍瑞斯及約克夏等3個品種計257頭種公豬。種公豬於拍賣前20天採集精液，採集之新鮮精液儲存於 17 保溫攜回實驗室測定精液濃度及同步快速測定每頭公豬精液至少5,000隻精子之粒線體完整度，作為判別年青公豬產&#64029;能&#63882;指標。檢測結果顯示，杜洛克 (n=171)、藍瑞斯 (n=58) 及約克夏 (n=28) 公豬其各項分析項目之結果以平均值 ± 標準偏差表示，精液濃度分別為 $3.51 \pm 1.01$ 、 $4.01 \pm 0.99$ 及 $3.09 \pm 1.06$ 億/毫升；精子粒線體完整度分別為 $57.2 \pm 19.5$ 、 $52.2 \pm 27.8$ 及 $54.0 \pm 28.5\%$ 。

關鍵語：種豬、精子、粒線體完整性 級

Application of flow cytometer to evaluation of sperm maturity in breeding pig of the pig performance testing station

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The objective of this study is conducted to measure the sperm concentration and mitochondrial integrity by using flow cytometer to evaluate the semen productive ability and maturity of high feed efficiency young boar, and try to apply the elite young boar for the reproduction of breeding stock and the production of hog early. Finished test boars from 3 breeds (Duroc, Landrace and Yorkshire) in class 201607, 201609, 201610, and 201611 of the Pig Performance Testing Station of National Animal Industry Foundation were used at this project. We collected the semen 20 days before the auction and stored at 17 . The collected semen were immediately analyzed the sperm concentration and mitochondrial integrity at least 5,000 sperm each semen to assess the semen productive ability of young boar. The results showed that the sperm concentration and mitochondrial integrity of the young boars from Duroc (n=171), Landrace (n=58) and Yorkshire (n=28) were  $351 \pm 101$  (106/ml),  $401 \pm 99$  (106/ml),  $309 \pm 106$  (106/ml) and  $57.2 \pm 19.5$  (%),  $52.2 \pm 27.8$  (%),  $54.0 \pm 28.5$  (%), respectively.

Key Words: Breeding pig, Sperm, Mitochondrial Integrity

## 生長檢定豬與體型比賽豬之選種性狀差異分析

### 生長檢定豬與體型比賽豬之選種性狀差異分析

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我國杜洛克(D)、藍瑞斯(L)及約克夏(Y)等國際三大豬種之拍賣會前檢測性狀，因仔豬生長所在環境而有不同。生長檢定豬為70日齡仔豬於新化檢定站進行生長性能及飼料效率檢定至體重110Kg或180日齡、並於225日齡拍賣前有採精測試及體型評鑑。而體型比賽豬則於種豬場飼養至240~290日齡，經台灣區種豬產業協會派員到場進行採精照相及精子濃度檢測，體型比賽會前一天有體重及三點背脂厚度測量，以及拍賣會前有體型評鑑。本研究利用2000年至2017年9月等拍賣成交豬隻，總計15,969頭，包括D8,545頭、L5,892頭、與Y1,532頭，分析檢定公豬拍賣之最高成交價及拍賣年，在D為2005年661,500元、L為2005年521,000元、Y為2017年220,000元；而體型比賽公豬拍賣之最高成交價及拍賣年，在D為2006年700,000元、L為2006年260,000元、Y為2017年130,000元，顯示D及L豬種更新需求在2005~2006年間最迫切，而Y豬種更新需求在2017年間最迫切。不分品種及性別，每頭豬皆有出生系譜、乳頭數、同胎公母仔豬頭數、親代產仔時日齡及雌親懷孕天數、及其自身基因型資料與供選種用檢測性狀。生長檢定豬重於飼料效率，而體型比賽豬重於體長寬高及步態。分析比較兩種拍賣豬，體型比賽豬有體重背脂兩性狀說明生長速率及肩胛部背脂厚度優勢，但多為一胎一頭參加，不如生長檢定豬至少同胎兩頭以上送檢，以及檢定豬有六個月期間生物安全監測計畫。因標購人群也有所不同，體型比賽豬標購人多為肉豬飼養場，體型比賽豬之拍賣成交價平均低於生長檢定豬。例如於2016年，在體型比賽會之期均人均購買種公豬費是57,124元(179人/10期)與購買種女豬費是36,113元(84人/10期)，而在檢定站之期均人均購買種公豬費是82,896元(309人/8期)與購買種女豬費是32,332元(35人/4期)。期均人均購買費顯示標購人重視生長期飼料效率大於配種期精液性狀。

關鍵語：種豬、檢定性狀、拍賣會

PREFERENCES FOR BREEDING TRAITS IN PIG AUCTION BETWEEN GROWTH PERFORMANCE TEST AND BODY CONFORMATION CONTEST

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Duroc(D), Landrace(L) and Yorkshire(Y) as the major three international breeds in Taiwan, piglets had various traits for auction. Piglets at 70 days of age were tested for growth performance at Hsinhua Station and was off at 110kg (100kg for gilt) of body weight or by

180 days of age. Prior to the auction age at 225 days old, tested pigs were tested on semen collection and body conformation. Ready-to-use boars were raised at breeding farm for the semen collection test on 240~290 days of age by the Association with a photo taking. One day ahead the auction, body weight and three point of backfat thickness were taken with the body conformation contest prior to the auction. A total of 15,969 head, including of 8,545 D, 5,892 L and 1,532 Y sold during the period from 2000 to September of 2017. The highest price paid and sold year at Hsinhua Station were 661,500NT\$ in D on 2005, 521,000NT\$ in L on 2005, and 220,000NT\$ in Y on 2017. As at Body Contest, 700,000NT\$ in D on 2006, 260,000NT\$ in L on 2006, and 130,000NT\$ in Y on 2017. Pricing results indicated that needs of D and L breeding pigs for the replacement were in 2005~2006 but more needs for Y breeding pigs in 2017. Each pig had birth pedigree, teat number, male and female littermates, age of parents and maternal gestation length, gene markers and traits measured. Feed efficiency is the key trait in growth performance test and more littermates on test for biosecurity program at six month period. Body conformation contest had body weight with emphasis on the backfat thickness on shoulder point and locomotion but few had littermates. Due to the buyer population on the utilization of breeding pigs at the auction between Station and Contest, the price paid to each breeding pig at the Contest was lower as comparison with that at the Station. It may due to the breeding pig from the Contest was to produce hogs in majority. The mean of money spend by each buyer per auction in 2016, 57,124NT\$ for boars (179 buyer in 10 auction) and 36,113NT\$ for gilts (84 buyer in 10 auction) at the Contest, but 82,896NT\$ for boars (309 buyer in 8 auction) and 32,332NT\$ for gilts (35 buyer in 4 auction) at the Station. In summary, the mean of money spend by each buyer per auction indicated that buyer more focus on the feed efficiency of growth period than the semen traits of mating period.

Key Words: Breeding pig, Traits, Auction

## 臺灣豐和黑毛烏骨雞之微衛星遺傳標記多態性分析

### 臺灣豐和黑毛烏骨雞之微衛星遺傳標記多態性分析

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為評估臺灣豐和黑毛烏骨雞選育族群的遺傳變異，本試驗利用 FAO (2011) 建議使用的 24 組雞微衛星標記組分析 48 隻臺灣豐和黑毛烏骨雞第 G5 世代種雞個體 DNA。24 組微衛星標記皆有多態型的基因型。共檢測到 89 個對偶基因，平均每個基因座具有 3.7 個對偶基因 (2~9 個)，其期望異質度介於 0.010 到 0.828，平均為 0.517，觀測異質度介於 0 到 0.688，平均為 0.408，而多態性訊息含量平均為 0.452。在選用的 24 組微衛星標記組中有 10 組呈現高度多態性資訊 (PIC > 0.5)，有 11 組呈現中度多態性資訊 (0.5 > PIC > 0.25)，3 組呈現低度多態性資訊 (PIC < 0.25)。  
關鍵語：黑毛烏骨雞、遺傳多樣性、微衛星遺傳標記

Genetic diversity analysis of TW Fengho black silkie chicken by microsatellite markers

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In order to evaluate genetic variation of TW Fengho black silkie chicken flock. we use a set of 24 microsatellite markers recommended by FAO to analyze 48 candidate bred chickens from this flock. All the microsatellites were polymorphic. The average allelic number was 3.7, ranged from 2 to 9 per locus. The expected heterozygosity ranged from 0.010 to 0.828, and the average expected heterozygosity was  $0.517 \pm 0.187$  (mean  $\pm$  SD). The observed heterozygosity of the population ranged from 0 to 0.688, and the average observed heterozygosity was  $0.408 \pm 0.213$ . The estimated average polymorphic information content (PIC) was  $0.452 \pm 0.171$ . In 24 markers, ten markers were highly informative with polymorphism information content (PIC  $\geq 0.50$ ), eleven markers were reasonably informative ( $0.5 > \text{PIC} \geq 0.25$ ) and the other three markers were slightly informative (PIC

Key Words: Black silkie chicken, Genetic diversity, Microsatellite marker

#### 豐輝下營紅牌土雞之微衛星遺傳標記多態性分析

#### 豐輝下營紅牌土雞之微衛星遺傳標記多態性分析

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為評估豐輝下營紅牌土雞選育族群的遺傳變異，本試驗利用 FAO (2011) 建議使用的 24 組雞微衛星標記組分析 48 隻豐輝下營紅牌土雞第 G5 世代種雞個體 DNA。24 組微衛星標記皆有多態型的基因型。共檢測到 90 個對偶基因，平均每個基因座具有 3.8 個對偶基因 (2 至 8 個)，其期望異質性度介於 0.118 到 0.822，平均為  $0.492 \pm 0.202$ ，觀測異質性度介於 0 到 0.688，平均為  $0.349 \pm 0.195$ ，而多態性訊息含量平均為  $0.434 \pm 0.194$ 。在選用的 24 組微衛星標記組中有 6 組呈現高度多態性資訊 (PIC  $\geq 0.5$ )，有 11 組呈現中度多態性資訊 ( $0.5 > \text{PIC} \geq 0.25$ )，7 組呈現低度多態性資訊 (PIC

關鍵語：雞、遺傳多樣性、微衛星遺傳標記

#### Genetic diversity analysis of Fenghui Xiaying Red-Brand country chicken by microsatellite markers

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In order to evaluate genetic variation of Fenghui Xiaying Red-Brand country chicken flock. we use a set of 24 microsatellite markers recommended by FAO to analyze 48 candidate bred chickens from this flock. All the microsatellites were polymorphic. The average allelic number was 3.8, ranged from 2 to 8 per locus. The expected heterozygosity

ranged from 0.118 to 0.822, and the average expected heterozygosity was  $0.49 \pm 0.20$ . The observed heterozygosity of the population ranged from 0 to 0.688, and the average observed heterozygosity was  $0.349 \pm 0.195$ . The estimated average polymorphic information content (PIC) was  $0.434 \pm 0.194$ . In 24 markers, six markers were highly informative with polymorphism information content (PIC  $> 0.50$ ), eleven markers were reasonably informative ( $0.5 > \text{PIC} > 0.25$ ) and the other seven markers were slightly informative (PIC  $< 0.25$ ).

Key Words: Chicken, Genetic diversity, Microsatellite marker

## 民間種雞場家禽白血病J病毒的監測

### 民間種雞場家禽白血病J病毒的監測

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家禽白血病 (avian leucosis, AL) 是由家禽白血病毒 (avian leucosis virus, ALV) 引起, 其中的 J 亞群 (subgroup J ALV; ALV-J) 於 1988 年出現, 造成養雞業者的嚴重損失。為了解民間種雞場種雞群是否感染家禽白血病 J 病毒, 從 2011 年至 2017 年逐批監測進駐行政院農業委員會畜產試驗所創新育成中心的種雞場選育族群候選種雞是否感染家禽白血病 J 病毒。採集以含抗凝劑 EDTA-K3 隻採血器採集雞隻翼靜脈 2 毫升全血, 低溫寄送國立臺灣大學獸醫專業學院禽病學研究室進行雞白血病檢測, 每批採集 23 隻候選種雞, 全血抽取 DNA 進行 PCR (primer H5/H7) 檢測家禽白血病 J 病毒。監測 4 家民間種雞場種, 4 個雞種。包括 16 批紅羽土雞共 365 隻、1 批 23 隻黑羽土雞、8 批商用烏骨雞共 181 隻及 1 批 23 隻藍殼蛋黑羽烏骨雞, 共計 592 隻候選種雞。檢測結果在 2011 年有 1 批商用烏骨雞 23 隻送檢樣品中有 5 隻檢出家禽白血病 J 病毒, 並已將該陽性雞隻立即淘汰, 而在其他所有送檢樣品皆呈陰性反應。因此, 繼續監測種雞群是否潛藏家禽白血病 J 病毒仍有其必要性。

關鍵語: 家禽白血病、家禽白血病J病毒、雞、監測

Avian leucosis J-virus monitoring in private chicken breeder farms

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Avian leucosis is caused by Avian leucosis viruses (ALVs). are prevalent in the poultry industry worldwide and cause severe economic losses. The subgroup J of ALV (ALV-J) has emerged as an important pathogen of meat-type chickens since 1989 and causes serious economic losses in commercial poultry industry. In order to monitor ALV-J disease in the private selection country chicken flocks of those stationed in the Innovation Incubation Center of LRI-COA, we collected candidate bred chicken blood samples by batch, 23 samples per batch, used the blood collection with anticoagulant EDTA-K3 from 2011to 2017. Each bird was collected 2 ml of whole blood from wing vein. Blood samples were sent to Lab of Poultry Diseases in School of Veterinary Medicine National Taiwan University for ALV-J

detection. Four breeding farms including four country chicken breeds were monitored in this program. Totally 592 candidate bred chicken blood samples were detected including 365 Red Feathered country chicken from 16 batches, 23 Black Feathered country chickens of one batch, 181 Commercial Silky chickens from 8 batches and 23 Black Silky chickens for one batch. Only five samples of one batch were detected ALV-J positive in one farm. The positive birds were eliminate immediately. Others of all were ALV-J negative. Thus, it is necessary to continuously monitor the chicken breeding flocks harbored ALV-J seriously.

Key Words: Avian leucosis, ALV-J, Chicken, Monitoring

## 畜試紅公豬各世代生長性能

### 畜試紅公豬各世代生長性能

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本試驗旨在調查畜試紅公豬 R1 到 R6 代檢定豬生長性能，開檢日齡為  $70 \pm 3$  天，完檢日齡為  $150 \pm 3$  天，平均檢定天#63849; 78.1~79.6 天，試驗結果顯示，R1~R6 代公豬檢定性能在 70 日齡平均開檢體重 29.05~30.82 公斤、150 日齡平均完檢體重 93.88~96.65 公斤及平均日增重 0.80~0.86 公斤，且在 6 個世代皆無顯著差#63842; ( $P > 0.05$ )。公豬#64043;#63934;效#63841;以 R4 代  $2.44 \pm 0.03$  與 R1~R3 代 (2.63~2.65) 比較有顯著差#63842; ( $P < 0.05$ )，R3 代 2.10 公分則比其餘各世代背脂厚 ( $P < 0.05$ )  
關鍵語：生長性能、畜試紅豬、檢定

The growth performance in LRI red boars of six generations

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The purpose of this study was to investigate the growth performance in LRI red boars of the generation R1 to R6. The data collection of growth performance started at  $70 \pm 3$  days of age and ended at  $150 \pm 3$  days of age, which gives 78.1~79.6 days for average testing period. The results showed that the body weight at 70 days of age (BW70), the body weight at 150 days of age (BW150), average daily gain (ADG; from 70 to 150 days of age) were 29.05~30.82 kg, 93.88~96.65 kg and 0.80~0.86 kg, respectively. There was no significant difference on BW70, BW150 and ADG in the LRI red boars from generation R1 to R6. The feed efficiency (FE) in red boars of the R4 generation ( $2.44 \pm 0.03$ ) was better than those of the R1, R2 and R3 generations (2.63~2.65) ( $P < 0.05$ )

Key Words: Growth performance, LRI red boar, Performance testing

## POU1f1基因型對努比亞山羊肉質性能表現之分析

## POU1f1基因型對努比亞山羊肉質性能表現之分析

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本試驗之目的為利用腦下垂體特異性轉錄因子(pituitary transcription factor, POU1f1)的基因型，對10頭母努比亞山羊背最長肌的肉質表現進行評估，肉質表現包括物理性質的色澤L a, b值、保水性、蒸煮失重，韌度及硬度與化學性質的胺基酸與脂肪酸分析。初步結果：3頭POU1f1基因型CT之母努比亞山羊，其平均屠宰體重與日齡分別為 $48.3 \pm 3.0$  kg與 $462.3 \pm 55.4$ 日，而7頭POU1f1基因型CC之母努比亞山羊，其平均屠宰體重與日齡分別為 $44.6 \pm 3.5$  kg與 $421.1 \pm 25.1$ 日，在平均屠宰體重與日齡性狀上，兩者無顯著差異，化學性質亦無顯著差異。物理性質在色澤 a, b值、保水性、蒸煮失重等性狀，POU1f1基因型CT的個體都顯著低於CC個體，綜合上述結果顯示POU1f1 基因型對母努比亞山羊肉質性狀有影響，可供未來努比亞山羊基因選種之參考。

關鍵語：努比亞羊、肉質性能、基因型

## ANALYSIS OF GENOTYPE IN POU1f1 GENE WITH MEAT QUALITY PERFORMANCE OF NUBIAN GOAT

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The aim of this study was to apply the genotypes of pituitary transcription factor (POU1f1) genes to evaluate its relationship with meat quality performance of Longissimus muscle from 10 female Nubian goats. Meat performance includes the physical properties of color L a, b value, water retention rate, cooking weight loss, toughness and hardness, and the chemical properties of analysis in amino acid and fatty acid. The preliminary results, the average slaughter weight and age of 3 head female Nubian goats with POU1f1 CT were  $48.3 \pm 3.0$  kg and  $462.3 \pm 55.4$  days, respectively. While the average slaughter weight and age of 7 head female Nubian goats with POU1f1 CC were  $44.6 \pm 3.5$  kg and  $421.1 \pm 25.1$  days, respectively. There was no significant difference between the two groups on the average slaughter weight and age. There was also no significant difference between the two groups in the chemical properties of analysis in amino acid and fatty acid. Individuals with genotype POU1f1 CT were significant lower than with POU1f1 CC in the following physical property traits: color a, b value, water retention rate, cooking weight loss. The results are potentially useful in meat quality selection program for Nubian goat farmer in the future.

Key Words: Nubian goat, Meat quality performance, Genotype

## 異地保種小型豬族群基因多樣性分析

## 異地保種小型豬族群基因多樣性分析

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本研究目的在建立異地保種小型豬族群之遺傳背景資料。將19頭豬粒線體DNA部分D環區片段之PCR產物進行定序，可得長度為616 bp之DNA片段序列，序列進行比對後，發現所有序列皆相同，再將此序列以比對工具BLASTN與NCBI GenBank進行比對，結果最為相近的序列為先前上傳登錄至GenBank的蘭嶼豬粒線體DNA序列。以12種微衛星遺傳標記分析19頭蘭嶼豬DNA，基因多樣性參數平均交替基因數、觀測異質度、期望異質度及多態性訊息量數值分別為4.5、0.52、0.57及0.51，由多態性訊息量數值可得知族群的基因多樣性程度屬高度 ( $> 0.5$ )，但仍需採用計畫性配種制度方能維持族群之基因多樣性。此外，在12種標記的分析中，可發現標記SW2008僅有一種交替基因，未來可當作此族群後裔的驗證標記。

關鍵語：異地保種、小型豬、基因多樣性

Genetic diversity of miniature pig population under ex situ conservation

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The study was to establish the genetic background data of the ex-situ miniature pig population. The PCR products of the partial mtDNA D-loop from 19 pigs were sequenced to obtain a 616-bp fragment. After the DNA sequences were aligned, all the sequences were found to be identical. The closely related sequences found in the NCBI GenBank were those Lanyu pigs previously registered before by BLASTN analysis. The genetic parameters of average number of alleles, observed heterozygosity, expected heterozygosity and polymorphism information content of 19 pigs were analyzed by 12 microsatellite markers. The values of the genetic parameters were 4.5, 0.52, 0.57, and 0.51, respectively. The value of polymorphism information content showed that the genetic diversity of the population is high ( $> 0.5$ ). However, it still needs to adopt the planned breeding system in order to maintain the genetic diversity of ex-situ groups. In addition, it was found that marker SW2008 had only one allele and could be used as a marker for the descent certification in the future.

Key Words: Ex-situ conservation, Miniature pig, Genetic diversity

## 乳牛第11凝血因子缺失症基因型頻率分析

## 乳牛第11凝血因子缺失症基因型頻率分析

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乳牛第11凝血因子缺失症 (FXID) 為一種隱性遺傳疾病，此病症源於位在第27號染色體上的第11凝血因子基因exon 12插入了一個76-bp的DNA片段，因而造成原有蛋白質功能喪失。本研究自6場乳牛場分別收集16、92、63、112、145及113頭乳牛血液樣品，並萃取其DNA後冷凍保存備用。檢測方法與條件經確認後，再進行541頭乳牛DNA樣品之基因檢測，結果發現3個樣品基因型為雜合型，其餘皆為正常型，雜合型頻率為0.55%，低於波蘭 (2.9%)、土耳其 (1.8%) 及美國 (1.2%) 的研究報告。此3頭雜合型牛隻來自同一乳場，且該場非種牛場。儘管FXID雜合型頻率甚低，但仍有嚴密監控之必要，以防止此一不良基因經由進口冷凍精液或活體牛隻持續進入我國乳牛族群。

關鍵語：第11凝血因子、遺傳缺陷、基因型

Frequency of factor XI deficiency genotype of dairy cows

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The bovine factor XI deficiency (FXID) is a recessive genetic disorder caused by a 76-bp DNA fragment inserted to FXI exon 12 on bovine chromosome 27, resulting in a loss of protein function. In this study, 16, 92, 63, 112, 145 and 113 dairy cow blood samples were collected from six dairy farms, and the DNA was extracted and stored. After the identification method and conditions were confirmed, and then 541 DNA samples were examined. The results showed that the genotypes of the three samples were carriers and the others were normal. The carrier frequency was 0.55% lower than those of Poland (2.9%), Turkey (1.8%) and the United States (1.2%). The three carrier cattle were from the same farm, and fortunately, it is not a breeding farm. Although the FXID carrier frequency is very low, it still needs to closely monitor the imported frozen semen or live cattle and prevent this defective gene transmitting to cattle population continuously.

Key Words: Factor XI, Genetic defect, Genotype

活性污泥多源酯 $\beta$ -glucuronidase基因次選殖與表現分析

活性污泥多源酯 $\beta$ -glucuronidase基因次選殖與表現分析

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由活化污泥多源基因體中所得10個推定的酯 $\beta$ -glucuronidase基因 (est1, est2, est3, est4b, est5, est6, est10, est11, est12A, est13) 已成功藉由PCR選殖至pET-52b(+) 3C/LIC表現載體上。重組質體分別命名為pET52b-Est1, -Est2, -Est3, -Est4B, -Est5, -Est6, -Est10, -Est11, -Est12A, -Est13。10個推定的酯 $\beta$ -glucuronidase基因可在Escherichia coli BL21 Star (DE3)中大量表現，但除rEst6外，其

他表現的酯#37238;以不溶性形式存在。當使用Escherichia coli ArcticExpress (DE3)作為表現宿主並在低溫下培養時，大多數酯#37238;除了Est4B之外被大量表現為可溶形式。然而，只有在E. coli BL21 Star (DE3)中表達的rEst6可以用Strep#8226;Tactin純化試劑組純化而得。雖然推定的酯#37238;可以在E. coli ArcticExpress (DE3)中表達為可溶形式，但問題為大多數酯#37238;無法使用Strep#8226;Tactin純化試劑組和His#8226;Bind純化試劑組純化。當使用Strep#8226;Tactin純化試劑組時，Cpn60伴隨著純化的標的酯#37238;出現。因此，有必要克服這些新型酯#37238;基因的表現和純化的問題，如此方能進行酯#37238;的生物化學性質分析。

關鍵語：活性污泥、酯#37238;、次選殖

#### Subcloning and expression of esterase genes from an activated sludge metagenome

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The ten putative esterase genes (est1, est2, est3, est4b, est5, est6, est10, est11, est12A, est13) derived from an activated sludge metagenome were successfully cloned into pET-52b(+) 3C/LIC expression vector by PCR. The recombinant plasmids were designated pET52b-Est1, -Est2, -Est3, -Est4B, -Est5, -Est6, -Est10, -Est11, -Est12A, and -Est13, respectively. The ten putative lipolytic genes could be overexpressed in Escherichia coli BL21 Star (DE3), but the expressed enzymes except rEst6 (recombinant Est6) existed in insoluble form. Most of the lipolytic enzymes except Est4B were overexpressed as soluble form when using Escherichia coli ArcticExpress (DE3) as the expression host and cultured at low temperature. However, only purified rEst6 expressed in E. coli BL21 Star (DE3) could be obtained with Strep#8226;Tactin purification kit. Although the putative lipolytic enzymes could be expressed as soluble form in E. coli ArcticExpress (DE3), the problem was that most of the enzymes could not be purified with Strep#8226;Tactin purification kit and His#8226;Bind purification kit. Otherwise, the purified target enzymes accompanied by Cpn60 when using Strep#8226;Tactin purification kit. Therefore, it is necessary to overcome the problems of the expression and purification of these novel lipolytic genes and only then can the biochemical properties of these esterases be characterized in the future.

Key Words: Activated sludge, Esterase, Subcloning