1187 Development of a light-weight, microwavable equine artificial vagina. K. Bennett-Wimbush*, B. Raimonde, and P. Stull, *Ohio State University Agricultural Technical Institute, Wooster, Ohio USA*.

A light-weight equine artificial vagina (AV) incorporating reusable gel packs (Consolidated Products and Services, Inc. Braintree, MA) as the heat source was constructed and tested for use in teaching breeding laboratories. A Missouri model AV was fitted with a specially constructed leather outer case which housed reusable gel packs. Gel packs were microwaved in order to achieve an internal AV temperature of at least 44° C. The internal size diameter was adjusted using air. Five trials were conducted to compare the length of time that the internal AV temperature remained in breeding range (44 to 52° C) for the gel-pack AV vs. a Missouri model AV. Both AV models were set up with approximately the same internal diameter and placed outside together in ambient temperatures which ranged from -2 to 8° C. The experiment started when the internal AV temperature reached between 44 and 46° C. A thermometer (Animal Reproduction Systems, Chino, CA) was placed 30 cm inside each AV and the time that each AV remained in the breeding range was recorded. Data was analyzed using student t-test GLM, SAS. The gel-pack AV held its internal temperature longer ($p < .01$) than the Missouri model AV with means of 39.8 ± 7.2 and 18.4 ± 2.7 minutes respectively. Additionally the gel-pack AV weighed 2.8 kg when adjusted for breeding while the Missouri model weighed 4.4 kg., an increase of 57%. Although breeding trials were not conducted, the gel-pack AV was used several times during semen collection with no observable problems. This modified AV offers an alternative to the traditional AV models. Its lighter weight and increased heat retention may be beneficial for students learning collection techniques or on farms where personnel is limited and the AV must be set up in advance.

Key Words: Horse, Semen collection, Artificial vagina

Milk Synthesis

1188 Conjugated linoleic acids (CLA), *trans* fatty acids, and lipid content in milk from Holstein cows fed a high- or low-fiber diet with two levels of linseed oil. J. Loor, A. Ferlay, A. Ollier, M. Doreau, and Y. Chilliard*, *Unite de Recherche sur les Herbivores, INRA-Theix, 63122 St.-Genes Champanelle, France*.

To determine effects on lipid content and fatty acid profiles of milk in response to altered rumen fermentation and 18:3*n*-3 availability, four Holstein cows were fed a high (65:35 forage to grain; HF) or low (35:65; LF) fiber [derived from grass hay] diet without (HFN, LFN) added oil or with linseed oil (HFO, LFO) at 3% of DM. A 4 × 4 Latin square design was implemented for 4 wk. Milk yield (26.7 kg/d) and DMI (20.2 kg/d) were not affected by treatments. Milk fat percentage and yield, however, were lower in response to feeding LFN or LFO (2.31%, 625 g/d) compared with HFN or HFO (3.38%, 870 g/d). Yield of total CLA in milk averaged 6 g/d due to feeding HFN or LFN, and increased to 13 g/d in response to HFO or LFO. *Cis*9,*trans*11-18:2 accounted for 85-90% of total CLA. Its yield was not affected by fiber level but increased by 116% in response to linseed oil. Feeding low-fiber diets resulted in greater yield of *cis*11,*trans*13- and *cis*9,*cis*11-CLA. Linseed oil supplementation further increased yield of *cis*9,*cis*11-CLA, but also *trans*11,*trans*13-CLA. The *trans*10,*cis*12- isomer of CLA was not detectable under any feeding conditions. Yield of *trans*11,*cis*15-18:2, an intermediate during hydrogenation of 18:3*n*-3, was 1 g/d in cows fed HFN or LFN compared with 10 g/d due to feeding HFO or LFO. Total *trans*-18:1 yield in milk averaged 19 g/d when cows were fed HFN, increased to 30 g/d in response to LFN, and peaked at 89 g/d due to feeding HFO or LFO. Greater yield of *trans*10-18:1 (10 vs. 2 g/d) accounted for the increase in total *trans*-18:1 when LFN was fed compared with HFN. In contrast, increases in *trans*11-18:1 (24 vs. 8 g/d) and *trans*13+14-18:1 (40 vs. 4 g/d) yields were primarily responsible for the greater *trans*-18:1 yield when linseed oil was fed. Milk fat depression was only observed when diets induced a marked increase in milk *trans*10-18:1 but was not related to any increase in *trans*10,*cis*12-CLA.

Key Words: low-fiber, linseed oil, *trans*-fatty acids

1189 Intestinal supply of *trans*10,*cis*12-18:2 lowers milk fat output in Holstein cows fed a high- or low-fiber diet with two levels of linseed oil. J. Loor, A. Ferlay, M. Doreau, and Y. Chilliard*, *Unite de Recherche sur les Herbivores, INRA-Theix, 63122 St.-Genes Champanelle, France*.

To assess effects of enhanced *trans*10,*cis*12-18:2 (10/12CLA) availability on milk fat content and fatty acid profiles in milk, four Holstein cows fed a high (65:35 forage to grain; HF) or low (35:65; LF) fiber [derived from grass hay] diet without (HFN, LFN) added oil or with linseed oil (HFO, LFO) at 3% of DM were infused (0.208 g/h) with 10/12CLA for 5 d via the duodenum. A 4 × 4 Latin square with repeated measures was utilized. Diets were fed for 4 wk prior to each 5-d infusion period. Infusion of 10/12CLA did not affect DMI or milk yield. Prior to infusion, milk fat concentrations ranked by treatment were HFO (3.82%) and HFN (3.28%) > LFN (2.73%) and LFO (2.43%). Milk fat yield averaged 936 or 766 g/d in cows fed high- or low-fiber diets. Yield of *trans*10-18:1 in milk through d 5 of infusion was 9 or 3 g/d when low- or high-fiber diets were fed. *Trans*11-18:1 and *cis*9,*trans*11-18:2 yields averaged 24 and 10 g/d for HFO or LFO compared with 9 and 4 g/d for HFN or LFN through d 5. Although 10/12CLA was not detectable in milk fat prior to infusion, it averaged 0.2% of total fatty acids and 0.9 g/d by d 5. Secretion of this CLA isomer was associated with a 36% decrease in milk fat percentage and yield across diets. A 42% decrease in yield of saturated 4:0 to 16:0 contributed to the reduction in milk fat output. Infusion of 10/12CLA regardless of diet decreased the ratios [indicators of Δ9 desaturase action] of *cis*9-18:1 to 18:0 (0.65 to 0.59) and *cis*9,*trans*11-18:2 to *trans*11-18:1 (0.34 to 0.27) in milk fat. Results suggest 18:1 and CLA isomers with a *trans*10- double bond may be involved in milk fat depression. If production of *trans*10,*cis*12-18:2 in the rumen is shown to be high enough to bypass further hydrogenation, it could affect mammary lipid metabolism by simultaneously reducing *de novo* synthesis and desaturation of long-chain fatty acids.

Key Words: low-fiber, linseed oil, *trans*10,*cis*12-18:2

1190 A dynamic model of concentrate supplementation effects on milk production in high producing ewes. Reza Imamidoost*[1] and John Cant[1], [1]*University of Guelph*.

A computer model was developed to predict lactational performance responses of ewes to concentrate supplementation, whether on pasture or stall-fed, given concentrate once per day or in multiple feedings, and suckling one lamb or up to six. The model considers effects of concentrate supplementation on forage intake, rumen pH and metabolizable energy and protein supply. The user defines ewe bodyweight, feed composition and concentrate feeding times and amounts. The reference ewe has free access to pasture and water. On consumption, forages and concentrates enter into lag pools for 2.0h and 0.24h, respectively. Carbohydrates then enter rumen pools of digestible fiber, indigestible fiber, or non-structural carbohydrate, from which they are degraded or pass to the lower gut. Rumen pools of organic acid from carbohydrate fermentation and buffer from rumination are simulated to determine rumen pH. The pH, in turn, affects fiber degradation rates. Forage intake continues during daylight hours of 5:00 AM to 9:00 PM until rumen dry matter exceeds 1.3% Body Weight, or organic acid concentration exceeds 130 mM. Daily milk production is calculated from the postruminal flow of digestible cabohydrate, absorption of rumen organic acids and intake of protein and fat. The model predicted the substitution effect on forage intake of increasing rates of concentrate supplementation, the temporal pattern of rumen pH fluctuation with multiple concentrate feedings per day, the increase in dry matter intake when concentrate meals increases.

Key Words: Milk Production, Lactating Sheep, Modelling

1191 Effects of two levels of protein and conjugated linoleic acid (CLA) prills on performance, milk composition and fatty acid profile of dairy cows[1]. M.A.S. Gama*[2], S.R. Medeiros[2], L.J.M. Aroeira[3], and D.D.P. Lanna[2], [1]*Supported by FAPESP and Agribrands Int.*, [2]*LNCA-ESALQ/USP, SP, Brazil*, [3]*CNPGL-EMBRAPA, MG, Brazil*.

Forty-eight 7/8 Holstein X Zebu cows in early lactation (30±5d) were assigned to four treatments in a factorial arrangement for six weeks: 1) control diet (CD) plus Lac100; 2) CD plus CLA; 3) high protein

diet (HPD) plus Lac100; 4) HPD plus CLA. Calcium salts of soybean oil (Lac100, Yakult) and CLA prills (25% of CLAs, Agribands Int.) were fed at 400g/cow.d. Cows were housed in a free-stall with calan gates. Corn silage and concentrates (corn, soybean meal, soybean hulls and corn gluten meal) were used to formulate diets to supply 100% (CD) or 115% (HPD) of the CNCPS protein requirements. Milk fat content was reduced (P<.05) by CLA prills but fat production was unchanged due to a slight increase in milk production. Milk urea nitrogen increased (P<.01) in HPD diets while milk production was 2 kg/d higher (P>.1). The c9,t11 CLA content in milk fat increased (P<.001) with Lac100 supplementation. This indicates that Lac100 (rich in linoleic acid) was dissociated in the rumen. The t10,c12 CLA content in milk fat was increased in all treatments after the start of lipid supplementation (P<.001). This suggests that t10,c12 CLA was produced in the rumen of Lac100 supplemented cows under the conditions of this experiment, consitent with the low milk fat content. Amounts of absorbable t10,c12 from all diets were enough to decrease milk fat content. Prills were inefficient in delivering absorbable CLA isomers.

Parameter	Treatment					Effect[a]	
	CD + Lac100	CD + CLA	HPD + Lac100	HPD + CLA	CV, %	CLA	Diet
Milk Yield, kg/d	22.9	23.7	25.3	25.4	22.9	ns	ns
Milk Fat, %	3.02	2.76	3.14	2.90	19.9	<.05	ns
Milk Protein, %	2.91	2.89	2.89	2.85	8.4	ns	ns
Milk Fat, kg/d	0.70	0.66	0.78	0.75	29.6	ns	<.1
Milk Protein, kg/d	0.67	0.68	0.72	0.73	20.8	ns	ns
Milk Urea N, mg/dl	16.7	17.1	18.8	19.2	19.5	ns	<.01
DMI, %PV	3.54	3.38	3.56	3.35	11.2	ns	ns
BSC (1-5)	3.13	3.19	3.20	3.14	15.4	ns	ns
CLA isomers, %							
c9,t11	0.59	0.38	0.57	0.35	37.1	<.001	ns
t10c12	0.05	0.06	0.04	0.05	102.3	ns	ns

[a]Main effects (interactions were not significant) ns = Not significant (P>.1)

Key Words: CLA, Fatty acids, Dairy cow

1192 Effect of histidine and histamine on mammary blood flow in lactating dairy cows. T. G. Madsen[1], D. R. Trout[2], S. Cieslar*[2], M. O. Nielsen[1], and J. P. Cant[2], [1]*The Royal Veterinary and Agricultural University, Copenhagen, Denmark,* [2]*University of Guelph, Guelph, Ontario, Canada.*

Studies have indicated that concentration of individual nutrients affect mammary blood flow (MBF). In particular low histidine concentration has been shown to increase MBF and it has been suggested that this effect is mediated by histamine. Histamine has vasoactive effects in a number of tissues and in vitro studies have indicated a vasoconstrictive effect in mammary tissue. The objective of the present study was to examine the effect of arterial histidine concentration and histamine on MBF. The experiment was set up as a 4 x 4 Latin square design with four multiparous Holstein cows in mid lactation. The four treatments were arranged in a 2 x 2 factorial fashion, where the two factors were; 1) infusion of an amino acid solution resembling the milk protein profile with or without histidine (40 g/h) and 2) infusion of chlorpheniramine a histamine H1-receptor blocker (150 mg/h). The solutions were infused continuously into the external iliac artery supplying one udder half between morning and evening milking. The cows were milked twice daily at 8:00 and 18:00 h and fed a total mixed ration (14.4 % CP). Arterial and milk vein blood samples were taken simultaneously three times in the afternoon at 15:30, 16:30 and 17:30 h. MBF was measured according to the dilution technique with para-amino hippuric acid as marker. Neither the exclusion of histidine from the solution nor the inclusion of chlorpheniramine affected MBF to the infused udder half (Mean = 327 L/h). Exclusion of histidine from the infusion solution tended to drop the protein contents in milk from the infused udder half (P=0.0650) from 4.10 to 3.88 %, indicating that histidine was the limiting amino acid for synthesis. The inclusion of chlorpheniramine into the infusion solution tended to decrease feed intake (P=0.0984), indicating a systemic effect, and decreased milk production (P=0.0634) in the infused udder half from 4.6 to 3.5 kg/10 h. In conclusion, the arterial histidine concentrations in the present experiment did not affect MBF and histamine is not involved in regulation of MBF.

Key Words: Histidine, Histamine, Mammary blood flow

1193 Enzyme regulation of mammary fatty acid synthesis in vitro. T Wright*, J Cant, and B McBride, *University of Guelph.*

Acetyl coenzyme A (CoA) carboxylase has been described as the rate-limiting enzyme for the synthesis of mammary produced fatty acids. It is, however, only one of several enzymes involved in this process. More recently, a technique known as metabolic control analysis has determined that the control over pathway flux can be shared by enzymes in a biological pathway. The purpose of this experiment was to inhibit enzyme activity and produce data for control analysis. Mammary tissue was isolated from lactating Holstein cows averaging 21 kg milk/d. Tissue homogenate was prepared by grinding the tissue under liquid nitrogen to a powder that was stored at #70°C. Tissue was homogenized in two volumes of isotonic sucrose containing a protease inhibitor and centrifuged at 15 000 x g. The incubation solution (3.0 ml) contained 80 mM Tris-HCl, 0.80 mM $MnCl_2$, 20 mM $NaHCO_3$, 0.05 mM CoA, 1.7 mM ATP, 10 mM sodium citrate, 0.05 mM glucose 6-phosphate, 1.7 mM sodium acetate, 4.2 mM glutathione, 0.05 mM NADP, 20 mg/ml fatty acid free bovine serum albumin, approximately 1 μCi of 1-^{14}C acetate, and approximately 3 mg of mammary protein. Incubations were conducted for 1h at pH 7.0 and synthesized fatty acids were extracted three times using petroleum ether. One ml of petroleum ether was then analyzed by liquid scintillation spectroscopy and the incorporation of acetate into fatty acids was calculated. Incubations with this system were done with added concentrations of avidin, an inhibitor of the biotin dependent enzyme acetyl CoA carboxylase. Inhibition of the system in this manner progressively reduced acetate incorporation into fatty acids with increasing avidin concentration. Fatty acid synthesis rates were reduced by 9.3% to 72.6% using avidin concentrations from 1 to 10 μg/ml. Data from this system indicated that this model is useful for metabolic control analysis to determine the proportion of control shared by the enzymes present in this in vitro system.

Key Words: acetyl CoA carboxylase, fatty-acid, mammary

1194 Effect of breed, parity, and stage of lactation on milk fat content of CLA in the dairy cow. J.A. Kelsey*[1], B.A. Corl[1], R.C. Collier[2], and D.E. Bauman[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Arizona, Tucson, AZ.*

Conjugated linoleic acid (CLA) has been shown to possess a variety of health benefits in biomedical studies with animal models. Foods of ruminant origin are the major dietary source of CLA. Some milk fat CLA originates from CLA that escapes complete rumen biohydrogenation, but the major source is endogenous synthesis via Δ^9-desaturase from *trans*-11 $C_{18:1}$. The four primary substrates for Δ^9-desaturase are $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, and *trans*-11 $C_{18:1}$. The ratio of these and their products (desaturase index) serves as a proxy for Δ^9-desaturase activity. Diet has a major influence on milk fat CLA, however the effect of animal-related aspects is largely unknown. Our objectives were: 1) to determine the influence of breed, parity and stage of lactation on milk fat content of CLA and 2) to examine variation among individuals in milk fat content of CLA and desaturase index. Holstein (n = 116) and Brown Swiss (n = 106) cows (University of Arizona herd) were fed the same traditional TMR diet and milk was sampled on the same day to eliminate diet and seasonal effects. Cows ranged from 7 to 522 DIM and varied in parity (primiparous = 97 and multiparous = 125). Fatty acid analysis demonstrated that stage of lactation and parity had minimal effect on CLA. Breed differences were significant (p<0.05), but of small magnitude; CLA averaged 4.4 ± 0.1 vs 4.1 ± 0.1 mg/g fatty acid for Holsteins and Brown Swiss, respectively. Similarly, *trans*-11 $C_{18:1}$ concentration was higher in Holsteins than Brown Swiss (11.4 ± 0.2 vs 9.5 ± 0.2 mg/g fatty acid). Overall, the proportion of fatty acids that were <C16, C16, and >C16 were 20.7 ± 0.2, 30.7 ± 0.1, and 48.7 ± 0.3 for Holstein, and 22.5 ± 0.2, 30.7 ± 0.1, and 46.8 ± 0.3 for Brown Swiss. There was a three-fold variation among individuals in milk fat content of CLA and in the desaturase index for all desaturase pairs. Overall,

results indicate that breed, parity and stage of lactation had only minor effects on CLA concentration, but substantial individual variation existed in CLA content and desaturase index of milk fat.

Key Words: CLA

1195 Effect of feeding or abomasal infusion of canola oil on feed intake, digestion and milk fatty acid composition in late-lactation Holstein cows. P.K. Chelikani*, J.A. Bell, and J.J. Kennelly, *University of Alberta, Edmonton, Canada*.

Our objectives were to determine the effects of feeding or abomasal infusion of canola oil on feed intake, rumen fermentation, nutrient digestibility and milk fatty acid composition in late-lactation Holstein cows. Five ruminally and duodenally cannulated late lactation Holstein cows averaging 249 DIM (SE 14 d) at the beginning of the experiment were used in a 3 x 5 incomplete latin square design. The treatments were 1) Control (C): basal diet, 2) Control + abomasal infusion (I) of 1 kg/d of canola oil, and 3) Control + supplementation of canola oil at 1kg/d in the feed (F). Abomasal infusion of canola oil (I) resulted in a 2.7 kg reduction ($P<0.05$) in DMI compared to the control treatment (C), and a 3.2 kg reduction ($P<0.01$) in DMI compared to feeding canola oil (F). Rumen fluid concentrations of total VFA ($P<0.05$), propionate ($P<0.05$), and acetate ($P=0.07$) were reduced with I compared to C or F. Mean concentrations of ammonia and rumen pH did not differ ($P>0.10$) among treatments. There were no differences ($P>0.10$) among treatments for either ruminal or total tract digestibilities of DM, OM, NDF, ADF and cellulose. Milk yield and 4% FCM tended ($P=0.09$) to increase with F compared to C or I, but the yields of fat and protein did not differ ($P>0.10$) among treatments. Saturated fatty acids (FA) in milk were reduced ($P<0.01$) by 16% with F, and 30% with I. The proportions of medium chain FA were reduced by 25% with F and 36% with I. This decrease was primarily due to a reduction ($P<0.01$) of C14:0 by 17% with F, 32% with I; and reduction of C16:0 by 29% with F and 39% with I. Compared to C, total C18:1 increased 41% with F and 44% with I, while cis-9 C18:1 increased 26% with F and 44% with I. Compared to C, trans-11 C18:1 increased 196% with F ($P<0.01$) but was unaffected by I ($P>0.10$). The proportion of cis-9 trans-11 C18:2 (CLA) increased (<0.01) by 172% with F compared to C, but was unaffected by I. The proportions of C18:2 and C18:3 were increased ($P<0.01$) 2-fold with I relative to C. These results suggest that feeding canola oil did not have a major impact on intake or rumen fermentation characteristics but there was a significant effect on milk fatty acid profiles especially CLA.

Key Words: Canola oil, Feed intake & Digestibility, CLA

1196 Effect of different levels of mixed corn plant and tomato pomace on milk production and composition in Holstein dairy cows. R. Tahmasbi, H. Nasiri moghadam, A. Naserian, and B. Saremi*, *Ferdowsi University Of Mashhad, Mashhad, Khorasan, Iran*.

Production of tomato pomace is seasonal and corresponds to the time when corn is harvested for silage. We conducted experiments to determine whether a blend of whole corn plant and wet tomato pomace will ferment properly and to determined the feeding value of whole corn plant and wet tomato pomace mixture silage. The target of this study was to determine the chemical characteristics and nutritive value of this mixed silage and its effect on production performance of Holstein dairy cows. This study was conducted in a change over design by 3 treatments and 3 periods (each period length 21 days). Nine Holstein multiparous dairy cows averaging 31 3.5 kg daily milk production and 65 19 days in milk were used. Whole corn plants were chopped from a single field, mixed with 0, 7.5 and 15% DM tomato pomace and ensiled in 3 surface-walled clamp silos. Increasing the level of tomato pomace decreased cottonseed meal content, but all diets had the same energy and protein content. Dry matter intake and intake of other nutrients were not affected by treatments. Increasing level of tomato pomace had a significant effect on crude protein digestibility ($P\leq0.05$) but dry matter, organic matter, ADF and NDF digestibility did not show significant differences. Rumen pH, BUN and blood glucose were not affected by treatments, but total plasma protein showed significant differences between treatments ($P\leq0.05$). Milk production and its performance were increased but an increase in level of tomato pomace decreased feed intake. However, these differences were not significant. Milk protein, fat, NPN, and MUN percentages were not affected by treatment. We suggest that wet tomato pomace can be blended with corn plant before ensiling without undesirable effects on Holstein dairy cow performance. This has economical benefits because addition of tomato pomace will decrease cost of diet.

Key Words: Dairy cows, Mixed corn silage and tomato pomace, Milk production and composition

1197 Bovine leukemia virus in mammary epithelial cells: Effects on mitosis and lactogenesis. D. Motton* and G. Buehring, *University of California, Berkeley, CA*.

Bovine Leukemia Virus (BLV) is an oncogenic retrovirus that causes B cell leukemias in infected cows. About 92% of U. S. dairy herds are infected but only about 1% of infected cattle develop bovine leukosis and are culled from the herd. A major concern is whether BLV infection of dairy cows alters milk yield. Although several studies have examined the effect of BLV on milk production in vivo, the results were inconclusive. No in vitro studies have been done. The discovery of BLV in mammary epithelial cells (MEC) of infected cows raises the possibility that the virus could affect these cells directly. The purpose of this study was to use an in vitro system to determine if BLV could alter milk yield by altering cell number and/or milk production per cell. A short-term cell line was established from the MEC of a BLV-negative cow. This cell line and a proven casein producer mouse cell line, Comma-D, were successfully transfected with a plasmid containing the entire BLV genome. The transfected cells test positive for the presence of BLV by PCR, whereas the parental (control) lines test negative. The BLV-positive lines do not replicate viral proteins as indicated by immunocytochemistry. The BLV-containing bovine MEC line has altered growth properties: reduced population doubling time, higher saturation density, and increased longevity. The Comma-D line is an already transformed cell line and growth properties did not change after transfection with BLV. Both the bovine and mouse MEC#s undergo differentiation on collagen gels and both lines, when transfected with BLV, displayed a decreased production of casein as analyzed by ELISA and Western blot, when compared to control cell lines without BLV. In addition, both the BLV-containing Comma-D cell line and the BLV-containing bovine MEC#s exhibited little or no casein mRNA when tested by RT-PCR. These data indicate that BLV may enhance cell proliferation and longevity and perhaps in this way increase milk production. However, the direct inhibition of casein synthesis by BLV could contribute to decreased milk production. Our results suggest that the effects of BLV infection on milk production may not be related solely to overall animal health, but may be directly mediated at a cellular level.

Key Words: BLV, Mammary, Casein

1198 Quality control of PCR products for DNA array production by real-time PCR. W. Luo, J.L. Smith, K.M.S. Smuga-Otto*, and L.G. Sheffield, *University of Wisconsin, Madison*.

DNA arrays are a useful technique for analysis of hundreds or thousands of mRNAs simultaneously. DNA fragments used for array production are frequently produced by PCR amplification of plasmid inserts. As a quality control step, gel electrophoresis analysis is usually performed to ensure adequate DNA concentration and appropriate amplification. Recently, we developed a method of assessing the quality of amplified DNA fragments using real-time PCR and melting curve analysis. DNA amplifications were performed in the presence of SYBR Green, which fluoresces in the presence of double stranded DNA. Increase in fluorescence with each cycle of amplifiction was indicative of DNA amplification. Fluorescence intensity of less than 1.5 relative units was not detectable by PAGE analysis followed by ethidium bromide staining. Those samples also had less than 20 ng DNA (by Hoechst 33258 dye binding) and were unacceptable for array production. When amplification was completed, a melting curve analysis was performed and derivative of fluorescence plotted against temperature to estimate melting point. Appropriately amplified fragments (those that gave a single, well-defined band on PAGE analysis and had >20 ng DNA) also had a single sharp melting point with a large derivative value (dFluorescence/dTemperature > 1.6). Presence of multiple peaks, no peaks or low dfluorescence/dtemperature were indicative of multiple bands or no amplification. This method provides a rapid and easily automated way to assess quality of hundreds or thousands of DNA fragments prior to array production. The only major caveat is that removal of residual SYBR Green dye should be verified prior to using arrays.

Key Words: DNA Array, Quality Control, Polymerase Chain Reaction