

# GENETIC REFLECTIONS



This clone of leading cutting horse producer Royal Blue Boon carries the same genetic code as the original, and maybe it has a few similar cutting moves as well.

ANNIE LAMBERT

## Advances in reproduction technology allow for genetic duplicates of great horses.

by Annie Lambert

**T**he precocious filly bears no distinction from her peers as she bucks, plays and runs circles around her surrogate dam in the pasture. But the blue roan is anything but typical. She is the first commercially cloned horse and the spittin' DNA-image of her original—the great Royal Blue Boon.

On February 19 at Royal Vista Farm Southwest, Purcell, Okla., James Bailey, DVM, supervised the birth of the first commercially cloned horse in the United States. The clone is a genetic duplicate of the legendary cutting horse producer Royal Blue Boon.

Elaine Hall, Weatherford, Texas, owner of Royal Blue Boon and the clone, was not prepared for the emotions associated with the new filly she has tentatively named Royal Blue Boon Too. Hall met “Too” for the first time when the filly was just 3 days old and found herself nearly at a loss for words to

describe the moment.

“First I looked at her and I thought, well this is just a daughter of Royal Blue Boon somehow,” Hall said. “It was so unbelievable...I kind of looked at her and I just said, ‘Oh my gosh, oh my gosh!’ It is just overwhelming what science can do.”

This first commercial clone can thank two Texas-based companies for her birth...or should that be rebirth? ViaGen Inc., Austin, and Encore Genetics Ltd., Weatherford, joined forces last year with intentions to produce the first commercially cloned horses in the United States. Partners in Encore, the marketing end of the alliance, include Jim Ware, Milt Bradford and reproduction specialist Brad Stroud, DVM, all from Weatherford.

And while many may ponder the moral and ethical slant on cloning, Drs. Bailey and Stroud view it from purely a scientific standpoint. Issues of stem cell

research and human cloning exist in a different world, far removed from that which produced “Too.”

“I think the technology is a reality and that there will be more cloning,” Dr. Bailey, a managing partner at Royal Vista, said. “I believe it will become a more valuable tool for the management of genetics and animal husbandry. It is inevitable that it will happen, and I certainly have no fear of that process.”

“Cloning is a tool like artificial insemination and embryo transfer,” Dr. Stroud added. “Actually it is a simpler tool than either of those when you sit back and give it full perspective. We’re not creating anything new. We are copying something that already exists. It is up to the breeders and the animal owners within these respective industries to determine how they can use that tool.”

### Heart & Soul

Hall’s first reaction to the concept of cloning 26-year-old Royal Blue Boon was a mixed bag of questions, thoughts and emotions. And being admittedly rather old-fashioned, Hall was at first taken aback by the entire subject.

“I was very confused and didn’t know exactly how I felt about it myself,” Hall admitted. “I went to my



BRAD STROUD, DVM

**Breeder Elaine Hall made history when she cloned her 26-year-old friend Royal Blue Boon.**

daily living source book, which is the Bible, and I found where God has given humans dominion over all of the earth—the fish, the cattle and the birds in the sky—all of the animals. I realized that God was giving us the high technology that we have now to go forth with the process of cloning.”

Hall knew Royal Blue Boon (now well beyond her reproductive years) was a superior individual; her show and production records stand as indelible evidence. Royal Blue Boon earned \$381,764 in the cutting pen between 1983 and 1987. Her progeny list reads like a Who’s Who of cutting, with 16 offspring earning nearly \$2.6 million, for an average of \$162,224.

After contemplating the idea, Hall stepped outside her comfort zone and into a new era of bloodstock reproduction. She knew at least a few other breeders would embrace the concept of cloning. She was also well aware that perching near the top of any equine endeavor meant keeping in step with the times.

“If you’re going to stay in this business and be a competitor, then you have to go with what is offered, or you’re going to be left in the dust,” Hall said. “I really felt it would be an injustice to Royal Blue Boon to leave her in the dust.”

### **Applied science**

The word clone congers up nightmarish tales (largely from old science fiction movies) of duplicates grown in pods and emerging in an adult form as a carbon copy of the original. But that just ain’t so!

A clone can be nothing more than a genetic duplicate of its original. Little “Too” and her original, Royal Blue Boon, share identical DNA—the same as twin sisters would. The two are separated only by time. The fact that Royal Blue Boon and her new clone share an “absolute and indisputable” genetic code has been verified by the University of California at Davis.

And just as identical twins are often very different,

“Too” may develop a significantly different persona than that of Royal Blue Boon due to the varied circumstances of her different life.

“Most people in the animal industries are going to clone with the sense of preserving genetics for future reproduction,” Bailey said. “Some people may clone pets out of fondness and for that pet to continue (being with them), but it is only a genetic match.”

Stroud pointed out it is important that people are not misled into thinking any clone will be just like the original animal in all ways. Personality traits are not necessarily duplicated along with the genetic code.

The science of this reproduction technique is not a difficult concept to grasp. Cloning begins with a small tissue biopsy from the original animal that is to be reproduced. The English pea-sized biopsies are usually taken from an area on the neck under the mane.

“Directly underneath the epithelial cells is subcutaneous tissue, which is partly composed of fibroblast cells,” Stroud explained. “Fibroblasts are the cells we use for the cloning process.”

Every somatic (non-sex) cell in a body has the same genetic code. Stroud stressed that although skin, bone and cardiac cells have different functions and visibly appear different, they still have the same nucleus and chromosomes. Their genetic makeup is identical.

“We theoretically could take a cell from any part of 220 cell types in the body and do the same thing,” Stroud said. “But we know that epithelial skin biopsies work wonderfully, so that’s what we’re dealing with. They are easy and relatively noninvasive to harvest.”

The tissue sample is shipped to ViaGen’s lab where it goes through a culture process. In proper culture conditions, the cells are stimulated to mitotically divide and begin to replicate themselves.

During the next step, called enucleation, the nucleus that contains the DNA from a donor egg is removed, leaving only the cytoplasm. A newly grown fibroblast cell containing the nucleus from the animal being cloned is transferred into the enucleated oocyte (egg). The cytoplasm, the remaining exterior of the donor egg, responds to the new nucleus by reprogramming it into embryonic tissue as though the egg had been fertilized by sperm.

All cells have a cytoplasm and a nucleus, Stroud reminded. When the remaining cytoplasm from the donor egg acknowledges the nucleus from the animal being cloned, it is fooled into thinking it is a freshly fertilized zygote.

“A zygote is the union of a sperm and egg with two sets of chromosomes,” Stroud said. “An egg has one set of chromosomes and a sperm has one set, and once they both unite, they fuse and there are two sets. The cytoplasm says, ‘My two sets of chromosomes are there. Let’s replicate those two sets; let’s start mitosis.’”

“One cell divides into two daughter cells that are genetically identical. Each of those two daughter cells replicates their own DNA; all of those cells retain the same genetic code of the original sperm and egg. In the case of a clone, it is the DNA from what we stuck into it.”

The cytoplasm of the egg cell has the power to reprogram the adult skin cell nucleus from the animal being cloned, and then revert it back into the embryonic state.

After maturing for a few days in an incubator, the donor egg with new DNA from the animal being cloned is transferred into a recipient mare, the same as a traditional embryo transfer. The cloned foal is born

after a normal gestation period.

**Simplistic in nature**

“It isn’t something everyone will be doing at home,” Bailey said with a laugh, referring to the cloning process. “But, it is very simplistic by nature. On the opposite side of that, the molecular mechanisms that control all these processes are rather complex. Fortunately, the physical act of doing this can be done very quickly. And with trained technicians, it is very repeatable.”

Once the original tissue biopsy has been received by the labs at ViaGen, it is between one and two weeks before the embryo, with its planned genetic code, is ready to be implanted into a recipient mare through embryo transfer.

Stroud, a pioneer and a foremost authority in embryo transfer, commercial in vitro fertilization and ultrasound, has an extensive background in the cattle industry. According to Stroud, about one in five bovine clone embryo transfers produces a live calf. Those numbers improve in equines partly because the physiology of the horse lends itself to multiple implants.

Four of Royal Blue Boon’s clone embryos resulted in initial pregnancies. Two were lost to normal reproductive problems. A second clone of Royal Blue Boon is due to arrive in mid-June.

“Sixty percent of the recipient mares that have cloned embryos became pregnant,” Bailey said. “Those rates are pretty good when compared to normal embryo transfer procedures where we expect around 80 percent.”

It is possible to hypothesize, yet difficult to prove, that the higher percentage of loss in the clone embryos could be caused by a reprogramming error on the part of the cytoplasm. And Mother Nature probably plays a role, according to Stroud.

“It’s really a good thing, because it is probably kicking out those embryos that are biologically inept,” Stroud said. “The problem could be from the egg cell that came from the donor ovary, and it could be that that egg was just not the correct compliment to the DNA material transferred into it.

“That egg is a powerful little instrument because it is doing the reprogramming. So there can be problems there, and there can be problems in the recipient

**“Original” Records**

Cloned Original	Earnings	# Performers	Total Progeny Earnings	Average
Royal Blue Boon	\$381,764	16	\$2,595,577	\$162,224
Playboys Ruby	\$268,441	10	\$1,509,428	137,965
Laney Doc	\$245,310	12	\$1,261,881	105,157
Bet Yer Blue Boons	\$350,615	6	\$362,740	60,457
Tap O Lena	\$502,145	5	\$309,573	61,915

mare. We have a certain amount of attrition with normal embryo transfer. It is certainly higher with clones that we’ve seen so far. But we really don’t know why each one failed.”

**Shake of a lamb’s tale**

Dolly, the sheep, debuted in 1996 as the first mammal cloned from an adult cell, and the media ran with the news—sometimes turning fact into fiction or folly. Understandably, misconceptions, fears and false impressions of cloning grew out of untruthful or exaggerated reports. Many of the wild speculations have been countered, according to Stroud, who thinks education is the key to acceptance.

“Since Dolly, there have been roughly a dozen other animal species cloned, so the release of a newly cloned species isn’t that big of news anymore,” Stroud said. “The general fear of animal cloning—I won’t say it is over, but I think it has largely been addressed.”

An example of unfounded speculation was the rumor that clones had a compromised longevity factor. That stemmed from Dolly’s untimely death at the age of 6-and-a-half years, when she was euthanized because of complications from arthritis and respiratory problems.

“Dolly did die earlier than the average Dorset sheep that has a lifespan of 10 to 12 years,” Stroud acknowledged. “What is not (widely) known is that they cloned more copies—possibly a half dozen—of Dolly, and those animals are still alive, healthy and doing fine. ViaGen actually bought those animals, to keep them alive, just to let them live a full life.”

ViaGen specializes in advanced livestock technologies and has cloned more animals from a broader range of species than anyone else in the field. Their scientists include Dr. Irina Polejaeva, who was involved with the production of Dolly and is the company’s chief scientific officer.

“The process of cloning is state-of-the-art repro-

**Royal Blue Boon Progeny List**

Royal Blue Boon earned her title as the No. 1 producer of cutting horses, with progeny earnings of \$2,595,577—10 performers of 16 offspring averaged \$162,224.

Red White And Boon	(88 G x Smart Little Lena)	\$904,437
Bet Yer Blue Boons	(90 M x Freckles Playboy)	350,615
Autumn Boon	(94 M x Dual Pep)	259,685
Duals Blue Boon	(92 S x Dual Pep)	197,449
Peptoboonsmal	(92 S x Peppy San Badger)	180,487
Peppys From Heaven	(87 M x Peppy San Badger)	142,956
Royal Red Boon	(DNA 87 M x Smart Little Lena)	126,454
TH Royal Red Peppy	(92 M x Peppy San Badger)	122,313
Royal Blue Dually	(91 M x Dual Pep)	86,394
Peek A Boon	(95 M x Smart Little Lena)	81,143

ductive technology, one that allows the breeder to preserve genetics and expand the reproductive potentials of their superior animals,” Polejaeva said.

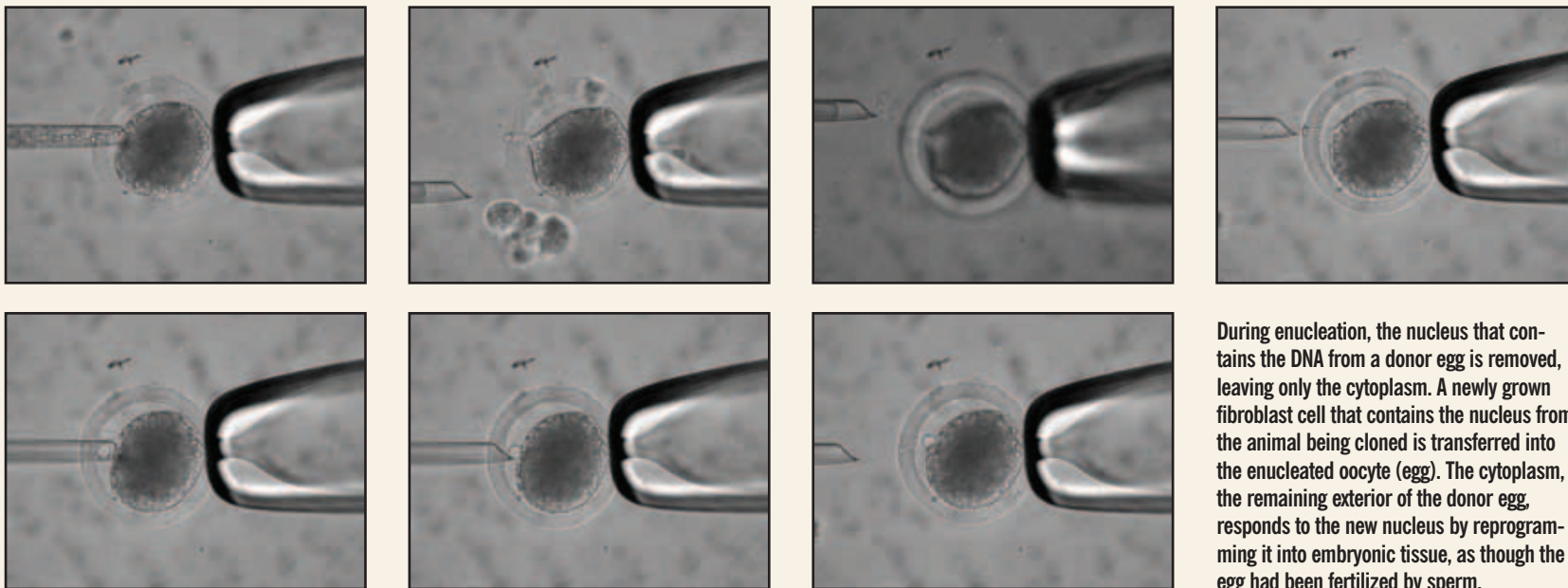
ViaGen is currently the only entity outside of Australia and New Zealand with the rights to the cloning technology licensed from the Roslin Institute in Edinburgh, Scotland. As a subsidiary of Exeter Life Sciences, Phoenix, Ariz., ViaGen currently provides commercial cloning services for horses, cattle and pigs.

ViaGen also does gene banking. The process of taking a biopsy from a donor horse and preserving those cells in a frozen state for future use is a pre-cloning option for breeders to preserve genetics beyond the normal life of an animal.

**Pay as you grow**

The cost of one clone, “the first ordered foal,” is \$150,000. Any additional ordered clones are \$90,000 each. Not an inexpensive proposition, to be sure, but what price can be put on having a young Royal Blue Boon in your broodmare band? This is especially the

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Royal Blue Boon Too may be a clone, but she eats, runs, plays, bucks, is curious and looks as normal as any other foal.

case considering she is the No. 1 all-time leading dam and possesses phenomenal production abilities.

According to Encore's Jim Ware, the payments are structured, and provisions regarding the health of the foal are detailed within the contract. The customer makes a \$15,000 (10 percent) payment when they decide to clone a horse.

"That guarantees you a live foal," Ware explained. "In other words, they are going to continue to try until you get a pregnancy. That initial \$15,000 carries over to the next year if you don't get a live foal the first year. The next 10 percent is not due until the recipient mare is 120 days in foal. And that second payment is refundable if you do not have a live foal. The balance is due 60 days after the foaling date."

Often, multiple embryos from one DNA source are successfully transferred to recipient mares. Within the industry, those extras are termed "overs." The first over has a price of \$30,000, while the second and any remaining are \$20,000 each.

Encore stepped into ViaGen's world in January 2005. Both Ware and Bradford have extensive backgrounds in the sale and promotion of performance horses, so assuming the marketing end of this new reproduction method was a lateral move.

"Obviously, we are pretty excited about it," Ware said of Encore's projects. "Relying on Dr. Stroud's expertise, we were confident that this was going to work. But we were a little apprehensive and conservative with the details until we had a live, healthy foal on the ground."

### More on the way

While Royal Blue Boon Too may hold the distinction of being the first commercially cloned horse, she is far from being an only child, so to speak.

On March 9, another recipient mare gave birth to a healthy clone of Tap O Lena, owned by Phil and Mary Ann Rapp of Weatherford. Tap O Lena was a superior show horse that earned over \$500,000 during

an illustrious career. The daughter of Doc O'Lena and Tapeppyoka Peppy (by Doc's Oak) has produced five offspring with average earnings of \$61,915.

Trainer and breeder Lindy Burch, Weatherford, is all but jumping up and down in anticipation of two clones of her champion mare Bet Yer Blue Boons, the highest money-earning daughter of Royal Blue Boon. By Freckles Playboy, "Bet" earned \$350,615 during her show career. The red roan mare has produced six performing foals with total progeny earnings of \$362,741.

Playboys Ruby is the only horse Phil Rapp ever owned that came running when he called, but he'll have to wait awhile to see if that trait is genetic or acquired! "Ruby," by Freckles Playboy out of Lenachick (by Doc O'Lena), will have just one clone this year. If the duplicate produces like the original, the results will be golden. Ruby earned \$268,441 herself and has produced 10 winners with earnings of \$1,509,428. Ruby is the second all-time leading producer of cutting horses.

There are multiple Laney Doc clones in recipient mares that will hopefully do as well as their original. Laney Doc, by Doc Quixote out of Christmas Four (by Christmas Star), earned \$245,310. Her 12 performing foals averaged \$105,157 for total progeny earnings of \$1,261,881. Laney Doc, No. 3 on the all-time leading dam's list, was owned by EE Ranches of Texas Inc., Whitesboro, Texas.

### Future stars

Hall is strictly a breeder, not a trainer or rider, so her purpose in cloning Royal Blue Boon varies slightly from Burch's reasons for perpetuating the genetic code of Bet Yer Blue Boons.

"Too' will be used for reproduction purposes only," Hall said. "There is no need to train her; that has all been proven and done. There's no need to take the risk of getting her hurt."

Hall did admit she might spare a short moment to wonder how "Too' might have performed in the cutting pen, but she stopped herself short, vowing she would "not even think about it."

"I've interacted with her, and she has a great personality," Hall said about "Too." "She's very inquisitive, smart and just a happy, normal, wonderful little foal bouncing around with a surrogate mother who loves her dearly."

Hall plans to take her foal home when she is about 60 days old. Once weaned, her surrogate mama will become a permanent pasture mate for the original Royal Blue Boon.

Hall has been too overwhelmed with the entire clone episode to even consider whom she might cross her new edition of Royal Blue Boon with in the future.

"I'll breed her to some lucky, young stallion!" Hall said with a laugh.

Burch, with National Cutting Horse Association earnings of \$2,775,252, can't wait to get a saddle on both of the Bets that she anticipates hitting the ground soon.

"I didn't have any real moral issue or problem with cloning a horse," said Burch, who holds a master's degree in mammalian physiology. "Cloning doesn't strike fear in me like it does a lot of people. It may be because of my biological background. I think animals are completely different than cloning people—those are two very different issues."

Bet Yer Blue Boons is now 16 and reproductively challenged because she has only one ovary. She is an



Lindy Burch holds two surrogate mares that are due with clones of Bet Yer Blue Boons, the highest-earning daughter of Royal Blue Boon.



Dr. James Bailey and Elaine Hall introduced Royal Blue Boon to her clone, a genetic twin separated only by time.

“oocyte mare,” an individual that must have immature eggs collected directly from her ovary (using technology developed by Colorado State University).

“It didn’t take me very long to figure out that, if I could prolong Bet’s productivity and not lose her genotype—that genetic map—it would certainly be an advantage,” Burch said. “I hope Bet outlives me, but she may not.”

Burch completely understands why Hall would not care about showing “Too.” With her background in the cutting pen, however, the trainer cannot wait to compare the duplicates to the original Bet Yer Blue Boons.

“I want to start mine, and I have no qualms about that,” Burch said with a grin. “I didn’t have to think very long about it...of course I’m going to ride them! I just want to see if they are the same or better.

“I trained Bet 14 years ago. I’m a lot different than I was, hopefully smarter and a better trainer. But the environment won’t be the same. There will be a lot of different issues—a lot of variables that you can’t control. So it won’t be exactly the same upbringing, of course.”

With the help of her friend and journalist Sally Harrison, Burch plans to keep a video and audio diary detailing the progression of the Bet clones through their lives.

“I just think it will be a great experiment to see how much they are alike, or not,” Burch said. “We’ll be able to extrapolate how much the different environment, or a difference in habitat, has to do with it. I know we can draw some conclusions...a real study would be 100 of them, not one or two. But it will be interesting.”

Burch has gene-banked DNA from her syndicated stallion, Bet On Me 498, in consideration of his reproductive longevity and value.

“I feel he is a candidate,” Burch said. “I’m not doing it now, but Heaven forbid if something ever happened to him.”

Bearly Doc, a 1976 Doc Bar mare, is one Burch holds near and dear. Bearly Doc earned over \$100,000 and was the 1983 NCHA Open Reserve World Champion. Before being pensioned at Melinda and Jerry Black’s Valley Oak Ranch in California, the 30-year-old mare produced 10 performers with earnings of \$329,232 from 13 foals.

“Horses like Bearly Doc could be worthy of cloning,” Burch said. “I don’t think it will end up where I’ll want to clone everything I have. I think they have to be an extraordinary horse.”

### Best bet

Burch opines that cloning horses—the best-proven performers and producers—will expand the gene pool; not narrow it, as some critics might surmise. The Royal Blue Boon line of horses has produced extraordinarily sound horses, a point Burch punctuated.

“The first time I injected Bet, she was 14 years old,” Burch said. “And she went down the road and won numerous competitions on hard ground, bad ground—in all sorts of environments.”

And no matter how different nurturing might affect the outcome of Royal Blue Boon’s clones and grand-clones, Hall knows she still has the undiluted genetic impact of the blue roan in her breeding program.

“There’s 26 years’ difference in time between Royal Blue Boon and ‘Too,’” Hall emphasized. “‘Too’ will be crossed on different stallions with new and different bloodlines. Royal Blue Boon’s extended reproduction is going to prove to be an outcross.”

Stroud doesn’t agree with the argument that cloning will narrow the gene pool, citing that it is giving proven genetic lines an extension, an opportunity to cross with future pedigrees.

“There is no way other than through cloning to share the genotype of Royal Blue Boon,” Stroud said. “It opens up a lot of opportunities for genetic diversification and genetic preservation. Older mares and older sires basically now infuse some new blood, if you will, back into the newer bloodlines, giving you more genetic diversification.”

Burch is also heartily behind the search for outcrosses to widen the cutting horse gene pool, and agrees that cloning may come to the rescue with the older, proven bloodlines. Bet Yer Blue Boons’ clones, she thinks, can provide a solid genetic source for young stallions not yet proven.

Burch knows you can work forever trying to find that one superstar horse, but also knows that those kind don’t come along very often. As a trainer, she predicted she might live another 50 or, hypothetically, even 150 years without finding another Bet Yer Blue Boons in her barn.

“My point is that you can wait a lifetime,” Burch said. “We breed horses every day—some of them are good, some aren’t, some are great. But if you have a proven dyed-in-the-wool mold of outstanding genetics that can be duplicated, I’m not going to sit back and miss that opportunity.”

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