

**Annual Report to
Environmental Protection Authority
for**

Activities under ERMA 200223

AgResearch Ltd

For the 12 months ending
30th June 2017

**Prepared by Tim Hale
Operator / Manager
AgResearch Ruakura
Animal Containment Facility**

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Summary of Activities for the period 1st July 2016 to 30th June 2017

This summary provides the information required by control 11 (Annual reporting) of the HSNO Act approval ERMA200223.

Outdoor Development Activities

All outdoor development activities being carried out within the Animal Containment Facility at Ruakura comply with the requirements of the ERMA200223 approval.

Cattle, still alive, which were field tested or developed under the GMF98009 or GMD02028 approvals continue to be maintained under the ERMA200223 approval and all new cattle development activities are now only being undertaken under the ERMA200223 approval.

Goat development and maintenance activities now only involve animals developed under the ERMA200223 approval.

Cattle and Goat activities, other than the maintenance or growing of animals, have been the calving of recipient cows, flushing eggs from fertile animals, kidding of recipient goats, milking of transgenic animals (goats) and the transfer of embryos to recipient animals.

Some initial ET work in sheep has not as yet progressed to live offspring.

These transferred embryos fall within the approved organism description for the ERMA200223 approval and are for either the production of human therapeutic proteins, or for the study of gene function.

All activities have been undertaken with the approval of the Ruakura Animal Ethics Committee and all activity approvals have been renewed during the year.

Further details on development activities are provided within the following Science, Management and Ethics reports.

Unforeseen adverse effects resulting from the genetic modifications

There have been no unforeseen adverse effects identified during this period.

Iwi liaison group relationship development and management activities

The ERMA200223 Liaison Group has still not officially met since December 2011.

As advised in previous annual reports, at the request of a group of Ngati - Wairere elders the Liaison meetings were put on hold, while representation and membership of the Liaison group was discussed within the Hapu.

Following some correspondence and individual contact, this group of Ngati - Wairere elders was invited and did visit Ruakura in October 2012 and a process to progress representation was discussed. Unfortunately due to circumstances outside of AgResearch influence, despite numerous attempts, no progress has been made in resolving this directly to date.

Informal contact within the last year with two original monitoring group members and Tainui Group Holdings continues to provide some updating on activities at Ruakura.

AgResearch's Portfolio Leader – Māori Agribusiness who has local affiliations, has been working to build a relationship with Ngati Wairere for Liaison Group and other Ruakura initiatives of interest to Ngati Wairere and Tainui. He has acknowledged this is slow going.

Additional Supporting Information

The following reports are supporting information provided to expand on the previous summary and provide evidence of wider compliance with ERMA200223 Controls and MAF/ERMA New Zealand Standard 'Containment Standard for Field Testing of Farm Animals'.

This additional supporting information is also provided to enable equivalence to the previous annual reporting for the inactive GMF98009 approvals.

Science Report

Overexpression of a testes specific transgene to investigate the process of fertilization

- Semen from trained bucks was collected, processed and frozen
- Frozen semen samples were analysed for transgene effects on sperm motility by computer-aided sperm analysis (CASA).
- The motility of sperm from the transgenic goats was affected in a similar way as compared to control semen from transgenic mice fully validated for showing strong distortion of the 'transgenic' sperm and inferior to non-transgenic sperm for fertilising an oocyte.

Overexpression of recombinant therapeutics in the milk of transgenic goats

- Offspring from two transgenic lines were born following natural mating or multiple ovulation and embryo transfer.
- For one transgenic line we have produced a small nucleus herd of transgenic males and females. For the second line we currently have only three females and multiple ovulation and embryo transfer has been repeated to increase transgenic animal numbers and generation of some transgenic bucks.
- Transgenic females have been mated to provide access to natural lactation milk following kidding.
- Semen from one transgenic buck was collected, processed and frozen.
- The transgene insertion sites for both transgenic lines has been characterised. In one line that transgenes are integrated on chromosome 21 and in our second line on chromosome 23.

Cattle engineered for the knockdown of the milk protein beta-lactoglobulin

- Analysis of induced milk produced by F1 offspring with three, two or one transgene insertion sites demonstrated effective knockdown of BLG comparable to the founder animal with all three insertion sites. Effective knockdown was observed in all cases which was irrespective of the number of insertion sites present.
- More detailed analysis of the milk composition is in progress
- The heifers have been mated for production and analysis of natural lactation milk.

Cattle engineered for the disruption or deletion of the gene for the milk protein beta-lactoglobulin

- One male and one female calf were successfully edited for a 9 bp deletion of the beta-lactoglobulin gene to disrupt its function. Analysis of induced milk produced by the female showed absence of full length beta-lactoglobulin in the milk.

- Several calves were born that were generated from two independent cell lines edited for an insertion of the gene for human lysozyme in one of the two copies of the beta-lactoglobulin gene. The insertion will disrupt the beta-lactoglobulin gene and essentially replace its function with the human lysozyme gene. Calves from one line were verified for the correct site-specific integration of human lysozyme. The calves from the second line showed correct integration at the start of the human lysozyme gene but could not be confirmed for correct integration at the end of the human lysozyme gene.
- Calves with the correct integration of the human lysozyme gene will be induced into lactation at 6 months of age.

Overexpression of bovine casein and human myelin basic protein (hMBP) in transgenic (TG) cattle

- Animals were farmed to maintain these transgenic lines.

Overexpression of the histone demethylase KDM4B in transgenic (TG) cattle

- Fetal fibroblasts that overexpressed the histone demethylase KDM4B were used for cloning, alongside with non-induced control cells.
- Cloned blastocysts from both groups were transferred into surrogate heifers and have been developing until D90 to date.
- No difference was observed between the experimental groups, indicating that histone tri-methylation is not a major stumbling block for epigenetic reprogramming in cattle.

Overexpression of the histone demethylase KDM6B in transgenic (TG) cattle

- Fetal fibroblasts that overexpressed the histone demethylase KDM6B were used for cloning to rejuvenate the senescent donor cell line.
- Cloned blastocysts were transferred into surrogate heifers and two fetuses recovered on D42 of gestation.
- Rejuvenated cell clones were expanded and frozen away for future studies.

Generating germline-deficient sheep for embryo complementation

- *DAZL* and *NANOS2* genes were knocked out by CRISPR-mediated genome editing in male ovine fetal fibroblasts (OFFs) and used for cloning.
- OFF knockout-derived cloned blastocysts were obtained at significantly higher rates from *DAZL* than *NANOS2* null donor cells.
- For embryo complementation with a reporter cell line, wildtype OFFs were modified to constitutively overexpress a red fluorescent protein (RFP).
- One clonal RFP cell line was used for aggregation with cloned *DAZL*^{-/-} and *NANOS*^{-/-} embryos for germline replacement.
- A total of 50 blastocysts were produced from *DAZL*, *NANOS2* and *RFP* cell lines and transferred into recipient ewes.

On Farm Management Summary for year ending 30/06/2017

Animal Numbers 01/07/2016– 30/06/2017 (Births exclude still born or animals which die soon after birth reported in Animal Ethics Reports, Aged In and Out records changes in animal age¹)

Stock Class	Open (1/07/16)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/17)
Casein (ERMA200223)									
MA Cows	11				1				12
R1yr Heifers	1				1	1			1
Heifer Calves	0	1				1			0
R2yr (+) Bulls	3						3		0
Bull Calves	0								0
Total Casein	15	1	0	0	2	2	3	0	13
MBP (ERMA200223)									
MA Cows	1						1		0
Bull Calves	0	1					1		0
Total MPB	1	0	0	0	0	0	1	0	0
rhLF (ERMA200223)									
Total rhLF	0	0	0	0	0	0	0	0	0
BLg - (ERMA200223)									
MA Cows	0								0
R2yr Heifers	0				12				12
R1yr Heifers	12				8	12			8
Heifer Calves	2	8				8			2
R1yr + Bulls	2				1				3
Bull Calves	1	2				1			2
Total BLg -	17	10	0	0	21	21	0	0	27
Erbix (ERMA200223)									
MA Cows	1								1
Total Erbix	1	0	0	0	0	0	0	0	1
Conventional Cattle									
MA Cows	65		11	59	48		7		58
R2yr Heifers	48					48			0
Other classes	2	8		5			0		5
Total Conventional	115	8	11	64	48	48	7	0	63
Cattle Total	149	19	11	64	71	71	11	0	104
Cattle developed under ERMA approvals (Tg and non Tg progeny)									41
Goats									
Erbix & Enbrel (ERMA200223)									
Ma Doe	27				4		10		21
R2yr Doe	4				6	4	2		4
R1yr Doe	6				15	6			15
Doe Kid	1	15				15		1	0
Buck Kid	2	18				5	14	1	0
R1yr Male +	3				5		2		6
Total Erbix	43	33	0	0	30	30	28	2	46
non Med inherit (ERMA200223)									
R1yr + Male	14						14		0
Total TCR	14	0	0	0	0	0	14	0	0
Conventional Goats									
MA Doe	29				12		30	5	6
R2yr Doe	13				18	12	1		18
R1yr Doe	18				26	18	2	2	22
Male R1yr +	4				9		4		9
Kids	0	35				35			0
Total Conventional	64	35	0	0	65	65	37	7	55
Goat Total	121	68	0	0	95	95	79	9	101
Goats developed under ERMA approvals (Tg and non Tg progeny)									46

¹ Aligns with normal livestock reconciliation aging practice.

Stock Class	Open (1/07/16)	Births	Transfer In	Transfer Out	Aged In	Aged Out	Killed	Deaths	Closing (30/06/17)
Sheep									
Conventional Sheep									
MA Ewes	0		5						5
Ewe Hgts	0		57				0		57
Total Conventional	0	0	62	0	0	0	0	0	62
Sheep developed under ERMA approvals (Tg and non Tg progeny)									0

The preceding table provides animal numbers over the reporting period in the development lines and are linked to the EPA approval. This includes transgenic and non-transgenic animals (progeny) and the conventional animals which are used to support the programmes.

For cattle there has been one movement of conventional animals (11 in total) into the facility during the period. 64 conventional animals were moved out of the facility, the 11 moved on as the NonTg science flushing had finished and 48 females who were found to be unsuitable (temperament wise) or which had never been used to hold GM embryos and 5 steer calves who were the progeny of non Tg cows present on the facility for flushing practice purposes which were allowed to retain their pregnancies at the conclusion of earlier work.

11 cattle of varying ages have been euthanased (killed); these animals have been disposed of in offal holes on-site, having been identified as surplus or now unsuitable animals, or following veterinary advice during this period.

For goats there has been no movement of animals onto the facility (apart from approved exit and returns for surgery purposes) during the period.

79 goats of varying ages have been euthanased (killed) and 9 goats died during the period; these animals have also been disposed of in offal holes on-site, as now surplus or unsuitable animals, or following veterinary advice.

The start of a new phase of work meant 62 conventional female sheep were moved into the facility.

For management purposes, as previously identified, the facility is treated as a separate small farm within the main Ruakura Farm. It is fully self-contained apart for some machinery requirements and specialist staffing.

Animals on the facility continue to be managed in a way which is considered normal farming practice and is accepted by the majority of farmers in New Zealand.

This consists of daily shifts and restricted intakes depending on the age of the animal and its feed requirements. Examples are stage of pregnancy, lactating or rearing calf or kid, empty, young growing animals, etc.

65 cattle, 15 goat and 55 sheep recipients have been used for ET (embryo transfer), with others being mated with artificial insemination or bucks, on a rotational basis during the period. Suitable goats have also been mated using non transgenic bucks to generate additional offspring, female only are normally kept, to be available as future recipients. All animals are regularly monitored for live weight and health status.

All animals have been grazed mainly on pasture, with supplementary feeding of hay or balage when required and been outside in the natural environment.

Goats can at times receive a higher proportion of their daily intake as supplementary feed, as concentrates, to reduce their impact on pasture availability for cattle and have access to covered shelter in inclement weather.

Surplus pasture is conserved when possible for use in periods of low growth, as balage or hay and there was only minimal purchasing of extra supplement (meal) required this season following favourable weather conditions which enabled maintenance of an adequate annual feed supply.

Regular pasture renewal is carried out with at least 10% of the facility receiving some form of renovation annually. Mineral supplementation is carried out using a mineral dispensing system through the water troughs for assisting Facial Eczema control and other normal mineral deficiencies during identified periods of risk, as occurs on many farms.

Maintenance fertiliser applied this season contained no nitrogen and no selective additional Nitrogen (Urea) has been used on areas not used for milk/waste irrigation during the year.

Milk Production 16/17 season

No GM cows were calved for milk production, any that did calve just reared their own progeny this season, so no cows were milked in the facility this season.

The milk from the couple of GM goats which were milked was either used to feed kids, for science analysis or frozen on the facility.

This has meant there was no milk stored this year for disposal irrigation.

Ruakura Animal Ethics Committee Reports

RAEC # 13848 - Production and characterisation of transgenic cattle, generic application

Ruakura Animal Ethics Committee Report: Third Quarter 2016

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval July to September 2016 in relation to the conditions for approval of Application 13848 "Production and characterisation of transgenic cattle, generic application".

A) Casein Plus cattle

A.1 Status of transgenic casein cattle

Multiple generations (F0-F3) of the transgenic casein lines have been produced with the animal born on 31st July 2003 being the oldest (now 13 years).

A.2 Treatments and activities during reporting interval

14034, F3, 6.9.16 calved healthy female calf. See end of report for health treatments.

A.3 Culling and losses during reporting interval

14031, 14032, 14033, F4 bulls surplus to requirements and euthanased 12.8.16.

B) hMBP cattle

B.1. Status of transgenic hMBP cattle

One F1 animal born in 2013 now represents this transgenic line.

B.2 Treatments and activities during reporting interval

Calved 12.9.16, healthy male Hereford cross calf. See end of report for health treatments.

B.3 Culling and Losses during reporting interval

Nil

C) Erbitux cattle

11001 healthy and now 5 years old.

D) Beta-lactoglobulin (BLG) knockdown (kd) cattle

D.1. Status of transgenic BLGkd cattle

12 x 2015 born F1 animals dried off after hormonally induced lactation. 2 x 2015 male animals healthy. January born 2016 calves (two female, one male) continue to grow well.

Group 80 recipients calved, and 6 female calves were born in early September. These were from a cell line that was modified to disrupt the endogenous milk whey protein gene for beta-lactoglobulin through the insertion of the gene for the human milk whey protein lysozyme. The human lysozyme gene was inserted in such a way that it essentially replaced, and was then expressed, instead of the endogenous beta-lactoglobulin. Bovine beta-lactoglobulin is a non-essential milk protein and is a major cause for triggering strong allergic reactions to cows' milk. In contrast, lysozyme, is a known beneficial whey

protein with antimicrobial attributes. The main aim of the study was swapping an undesirable whey protein with a beneficial protein to improve the quality of the milk. Nuclear transfer embryos from this cell line were generated and transferred in December (9.12.16 and 16.12.16). The aim was to generate live calves to i) characterize and confirm the precise genetic modification in the calves and ii) investigate their milk characteristics.

One calf died shortly after birth and post mortem revealed enlarged adrenal and thyroid glands with poorly developed kidneys and liver (typical of a hydrops allantois). One calf was euthanased on 30.9.16 due to sudden onset of severe respiratory distress. Post mortem revealed a consolidation of the lungs which had possibly been present since birth.

Recipients: no synchronies or embryo transfers carried out in this reporting period. Group 81 (transferred in May 2016) had 7 live pregnancies confirmed by ultrasound scanning in late July.

Health Treatments: All cattle on the ACF received annual vaccinations for leptospirosis, bovine viral diarrhoea and clostridial disease during July and August. In addition they received copper, cobalt, magnesium and copper supplementation. All animals received a topical anthelmintic to treat internal and external parasites.

Ruakura Animal Ethics Committee Report: Fourth Quarter 2016

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval October to December 2016 in relation to the conditions for approval of Application 13848 "Production and characterisation of transgenic cattle, generic application".

A) Casein Plus cattle

A.1 Status of transgenic casein cattle

Multiple generations (F0-F3) of the transgenic casein lines have been produced with the animal born on 31st July 2003 being the oldest (now 13 years).

A.2 Treatments and activities during reporting interval

Calf born in September disbudded and primary vaccinations administered.

A.3 Culling and losses during reporting interval

Nil

B) hMBP cattle

B.1. Status of transgenic hMBP cattle

One F1 animal born in 2013 now represents this transgenic line.

B.2 Treatments and activities during reporting interval

Calf born in September disbudded and primary vaccinations administered.

B.3 Culling and Losses during reporting interval

Nil

C) Erbitux cattle

11001 healthy and now 5 years old.

D) Beta-lactoglobulin (BLG) knockdown (kd) cattle

D.1. Status of transgenic BLGkd cattle

2015 born F1 animals were healthy during the reporting period, and were synchronised and mated to AI with a follow up natural mating with an easy calve Hereford bull.

January born 2016 calves (two female, one male) continued to grow well. Females mated with 2015 born animals.

Group 80 calves were born in early September, and were disbudded and primary vaccinations administered during the reporting period. One calf was euthanased in early October due to continued problems with swollen joints that had not responded to treatment with antibiotics and anti-inflammatories. Post mortem revealed cellular fluid deposits in all the joint spaces and very small kidneys and a rounded liver. Diagnosis was compromised renal and hepatic function with accumulation of immune complexes in the joint cavities.

Recipients: Two groups (82&83) of synchronies and embryo transfers were carried out in this reporting period. Group 82 (Re-derivation BEF3_RFP, or bovine embryos overexpressing red fluorescent protein) had one pregnancy confirmed in November and the embryo was recovered for tissue rejuvenation in the laboratory. Group 83 (Re-derivation of EF5_KDM6B cell clone (non-induced)) had one pregnancy confirmed in December, and the foetus was recovered.

Group 81, a further run of BLG disruption lysozyme insertion embryos, (transferred in May 2016) was scanned again in mid December with 6 live pregnancies still present, two were suspected to have large placental cotyledons and were noted for ongoing monitoring for development of hydrops (expected calving early February 2017).

Culling and Losses: one recipient was found dead in the paddock (death due to misadventure).

Health Treatments: no routine health treatments in this reporting period.

Ruakura Animal Ethics Committee Report: First Quarter 2017

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval January to March 2017 in relation to the conditions for approval of Application 13848 "Production and characterisation of transgenic cattle, generic application".

A) Casein Plus cattle

A.1 Status of transgenic casein cattle

Multiple generations (F0-F3) of the transgenic casein lines have been produced with the animal born on 31st July 2003 now the oldest (rising 14 years).

A.2 Treatments and activities during reporting interval

A.3 Culling and losses during reporting interval

Nil

B) hMBP cattle

B.1. Status of transgenic hMBP cattle

One F1 animal born in 2013 now represents this transgenic line.

B.2 Treatments and activities during reporting interval

13005 injured hip and hock joint in back left leg in January (paddock trauma, unknown origin). Given separate paddock with calf and treatment with anti-inflammatories.

B.3 Culling and Losses during reporting interval

13005 euthanased 14.2.17 on humane grounds as chronically lame with no resolution of injury described in B2. Her calf was weaned at this point.

C) Erbitux cattle

11001 healthy and now rising 6 years old.

D) Beta-lactoglobulin (BLG) knockdown (kd) cattle

D.1. Status of transgenic BLGkd cattle

2015 born F1 animals were ultrasound pregnancy scanned. Two animals were empty, ten in calf.

16001 was confirmed as non pregnant (after mating October to December) and started on a programme for hormonal induction into lactation in March. 16003 was confirmed pregnant.

Group 80 calves had further routine calfhood vaccinations and anthelmintic treatment.

Group 81(BLG disruption lysozyme insertion embryos) were calved in early February.

Five dams had normal or assisted vaginal calvings, one dam was euthanased and a calf recovered. The calf was suffering from the physical effects (swollen abdomen with fluid retention, liver, kidney, adrenal malfunction) of hydrops allantois and was euthanased shortly after birth. The other five calves suckled their dams and were ear biopsied and disbudded in March. Dams and calves were treated with topical fly control preparations.

Recipients: no use of non pregnant recipients in this reporting interval.

Health Treatments: no routine health treatments in this reporting period.

Ruakura Animal Ethics Committee Report: Second Quarter 2017

Transgenic Cattle

Summarised below is the status of the various cattle groups and their offspring and any losses that have occurred during the reporting interval April to June 2017 in relation to the conditions for approval of Application 13848 "Production and characterisation of transgenic cattle, generic application". Approval passes to Application 14236 on 1st July 2017

A) Casein Plus cattle

A.1 Status of transgenic casein cattle

Multiple generations (F0-F3) of the transgenic casein lines have been produced with the animal born on 31st July 2003 now the oldest (rising 14 years).

As at 30.6.17 there are 13 animals made up of 12 Mixed Age cows and 1 R1yr heifer.

A.2 Treatments and activities during reporting interval

Routine anthelmintic treatment.

A.3 Culling and losses during reporting interval

Nil

B) hMBP cattle

B.1. Status of transgenic hMBP cattle

Nil

C) Erbitux cattle

One founder animal, 11001 healthy and now rising 6 years old.

C.2 Treatments and activities during reporting interval

Nil

D) Beta-lactoglobulin (BLG) knockdown (kd) and knockout (ko) cattle

D.1. Status of transgenic BLGkd cattle

10 x 2015 born F1 animals are in calf, due to calve late September 2017. 1603 also in calf.

As at 30.6.17 there are 27 animals made up of 12 R2yr heifers, 8 R1yr heifers, 2 heifer calves plus 3 R1yr bulls and 2 bull calves.

D.2 Treatments and activities during reporting interval

Group 81 calves weaned and given anthelmintic treatment

D.3 Culling and losses during reporting interval

Nil

Recipients: As at 30.6.17, 58 mixed age cows.

Group 84: 30 recipients synchronised and embryos transferred into on 17th May.

Health Treatments: no routine health treatments in this reporting period.

Ruakura Animal Ethics Committee Report: Third Quarter 2016

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval July to September 2016 in relation to the conditions for approval of Applications 13561 and 13787 "Development of transgenic goats for production of Biosimilars" and "Development of transgenic goats showing non-Mendelian inheritance" respectively.

1. Biosimilars

A) Erbitux Goats

A.1 Status of transgenic Erbitux Goats

Two generations (F0-F1) of the transgenic Erbitux lines have been produced with the founder Erbitux animals (F0) as the oldest, rising 5 years old (born 2011).

A.2 Treatments and activities during reporting interval

Founder doe, 1227, were mated with AI and Saanen buck for super ovulation programme in June, and on flushing and transfer day, embryos were transferred into 5 does.

Routine annual vaccinations for leptospirosis and clostridial disease.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

A.3 Culling and losses during reporting interval

Nil

B) Enbrel Goats

B.1. Status of transgenic Enbrel Goats

Two generations (F0-F1) of the transgenic Enbrel lines have been produced with the founder Enbrel animals (F0) as the oldest, rising 4 years old (born 2012).

B.2 Treatments and activities during reporting interval

Routine annual vaccinations for leptospirosis and clostridial disease.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

B.3 Culling and Losses during reporting interval

Nil

2. Non Mendelian Inheritance

C) TCR Goats

C.1. Status of transgenic non-Mendelian inheritance (TCR) Goats

One generation (F0) of the transgenic TCR lines have been produced with the oldest, being or rising 2 years old (born 2014).

C.2 Treatments and activities during reporting interval

Routine annual vaccinations for leptospirosis and clostridial disease.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

C.3 Culling and Losses during reporting interval

Nil

3. Recipients

Routine annual vaccinations for leptospirosis and clostridial disease.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

Does kidded from natural mating in August/September.

Culling and Losses during reporting interval:

Unwanted male kids were euthanased as born.

One doe euthanased with acute mastitis, a second with an udder that could not be suckled from, and a third due to malpresentation of kid at parturition.

Two female kids euthanased, one with a traumatic front leg injury and one with atresia coli.

Ruakura Animal Ethics Committee Report: Fourth Quarter 2016

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval October to December 2016 in relation to the conditions for approval of Applications 13561 and 13787 "Development of transgenic goats for production of Biosimilars" and "Development of transgenic goats showing non-Mendelian inheritance" respectively.

1. Biosimilars

A) Erbitux Goats

A.1 Status of transgenic Erbitux Goats

Two generations (F0-F1) of the transgenic Erbitux lines have been produced with the founder Erbitux animals (F0) as the oldest, rising 5 years old (born 2011).

A.2 Treatments and activities during reporting interval

Founder doe, 1227, was synchronised, superovulated, mated with AI and natural mating. She only produced one embryo to this programme, which was placed into a synchronised recipient.

Three does pregnant with embryos from 1227's previous superovulation produced three healthy female kids.

Kids disbudded and primary vaccinations given.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

A.3 Culling and losses during reporting interval

7 x Erbitux F0 and F1 does euthanased due to chronic foot lameness.

B) Enbrel Goats

B.1. Status of transgenic Enbrel Goats

Three F1 animals are 2 years old.

B.2 Treatments and activities during reporting interval

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

B.3 Culling and Losses during reporting interval

Three founder animals euthanased due to chronic foot lameness.

2. Non Mendelian Inheritance

C) TCR Goats

C.1. Status of transgenic non-Mendelian inheritance (TCR) Goats

One generation (F0) of the transgenic TCR lines have been produced with the oldest, being 2 years old (born 2014).

C.2 Treatments and activities during reporting interval

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

C.3 Culling and Losses during reporting interval

Nil

3. Recipients

Does finished kidding from natural mating.

Kids all disbudded and primary vaccinations started. August born kids weaned as reached 15kg liveweight.

All animals treated for flies.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

Culling and Losses during reporting interval:

12 does were euthanased due to chronic lameness problems. One doe had a fractured back leg and was euthanased.

Ruakura Animal Ethics Committee Report: First Quarter 2017

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval January to March 2017 in relation to the conditions for approval of Applications 13561 and 13787 "Development of transgenic goats for production of Biosimilars" and "Development of transgenic goats showing non-Mendelian inheritance" respectively.

1. Biosimilars

A) Erbitux Goats

A.1 Status of transgenic Erbitux Goats

Two generations (F0-F1) of the transgenic Erbitux lines have been produced with the founder Erbitux

animals (F0) as the oldest, rising 6 years old (born 2011).

A.2 Treatments and activities during reporting interval

Founder doe embryo placed in recipient in November did not gestate and produce a kid.
November 2016 born buck had abscess in testicle which resolved with antibiotic and anti-inflammatory treatment. Origin was suspected to be from a traumatic wound (negative for *Brucella ovis*).

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

A.3 Culling and losses during reporting interval

Nil

B) Enbrel Goats

B.1. Status of transgenic Enbrel Goats

Three F1 animals are rising 3 years old (born 2014).

B.2 Treatments and activities during reporting interval

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

B.3 Culling and Losses during reporting interval

Nil

2. Non Mendelian Inheritance

C) TCR Goats

C.1. Status of transgenic non-Mendelian inheritance (TCR) Goats

One generation (F0) of the transgenic TCR lines have been produced with the oldest, rising 3 years old (born 2014).

C.2 Treatments and activities during reporting interval

Aaron trained bucks for semen collection and 9 bucks were successfully collected from, by Animal Breeding Services.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

C.3 Culling and Losses during reporting interval

1435 euthanased due to chronic lameness.

1427 died in paddock (cast).

3. Recipients

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks.

Culling and Losses during reporting interval:

Ruakura Animal Ethics Committee Report: Second Quarter 2017

Transgenic Goats

Summarised below is the status of the various goat groups and their offspring and any losses that have occurred during the reporting interval April to June 2017, in relation to the conditions for approval of Applications 13561 and 13787 "Development of transgenic goats for production of Biosimilars" and "Development of transgenic goats showing non-Mendelian inheritance" respectively. Approval for both these applications passing to Application 14328 "Production and Characterization of Transgenic Goats, generic application" on 1st July 2017.

1. Biosimilars

A) Erbitux Goats

A.1 Status of transgenic Erbitux Goats

Two generations (F0-F1) of the transgenic Erbitux lines have been produced with the founder Erbitux animals (F0) as the oldest, rising 6 years old (born 2011).

As at 30th June 2017 there were 39 animals made up of 34 mixed age females and 5 males.

A.2 Treatments and activities during reporting interval

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks. Any individual lameness treated as occurred.

Superovulation programme carried out on four founder Erbitux animals, embryos transferred into synchronised recipients. 7 F1 animals were also synchronised and inseminated laparoscopically with Saanen semen.

A.3 Culling and losses during reporting interval

Founder animal euthanased due to ongoing failure for superovulation programme and chronic locomotion problems due to skeletal limb anatomy. F1 animal euthanased also for ongoing lameness problems. Male with fractured hind leg also euthanased.

B) Enbrel Goats

B.1 Status of transgenic Enbrel Goats

As at 30th June 2017 there were 7 animals made up of 6 females and one male.

B.2 Treatments and activities during reporting interval

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks. Any individual lameness treated as occurred.

B.3 Culling and Losses during reporting interval

Two bucks were euthanased as no longer required.

2. Non Mendelian Inheritance

C) TCR Goats

C.1. Status of transgenic non-Mendelian inheritance (TCR) Goats

One generation (F0) of the transgenic TCR lines have been produced with the oldest, rising 3 years old (born 2014). As at 30th June 2017, no animals from this line remain (see C.3).

C.2 Treatments and activities during reporting interval

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks. Any individual lameness treated as occurred.

C.3 Culling and Losses during reporting interval

One buck died with parasitic diarrhoea. Remaining 14 bucks were euthanased as no longer required.

3. Recipients

As at 30th June 2017, there were 55 non transgenic animals, made up of 46 mixed age does and 9 rising one year old bucks.

All drenched at required intervals dependant on worm counts, and feet checked and trimmed every 6-8 weeks. 25 younger animals were synchronised as recipients for Erbitux superovulation programme (see A.2).

Culling and Losses during reporting interval

One 16 born doe became stuck in a culvert and died. Access to the culvert has now been prevented. One doe was found dead in the paddock and post mortem showed an acute pneumonia.

19 animals were culled for age, udder and feet problems.



Ministry for Primary Industries External Audit Report:

Facility	AgResearch - Ruakura
Location	Ruakura Research Centre 10 Bisley Rd, Hamilton
Facility Codes	TF02501 (364)
Operator	Tom Richardson
Primary Verifier	Crystal Lange

Audit conducted By Crystal Lange

Audit Date August 30 2016

Audit report Date September 6 2016



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Executive Summary

The external audit of AgResearch Ruakura's transitional and containment facility in Hamilton for the period March 2016 – September 2016 was carried out by the Ministry for Primary Industries (MPI) August 30th 2016.

This was the first assessment of the Ruakura site as a single transitional and containment facility. The report is written in the same format as previous reports for continuity. This enables cut & paste options for AgResearch's own external reporting requirements and draws a line between research units using multiple HSNO approvals and those using single approvals. If this format does not meet your requirements reverting to the previous individual reporting format is still a valid option.

There were minor structural issues noted with the Dairy Science building reflecting both the age of this facility and the wet winter. Minor issues were noted with internal reporting and record keeping. Two non-compliances were issued and three recommendations made. There were no concerns raised with the animal containment facilities.

Overall the audit outcome was satisfactory, with the delegated Managers able to demonstrate good knowledge and control of containment principals. MPI is **satisfied** that AgResearch Ruakura is operating in compliance with the requirements of the facility and operator approvals.

Audit (Inspection) Process & Scope

The AgResearch site at the Ruakura is a transitional and containment facility, approved as such under the Biosecurity Act 1993 because it holds or processes goods that are risk goods as defined by the Biosecurity Act 1993 or may contain new organisms as defined by Hazardous Substances and New Organisms (HSNO) Act 1996.

The facility is approved to Environmental Protection Authority (EPA) Standards 154.03.02: *Containment Facilities for Microorganisms 2007a*, 154.03.06: *Containment Standard for the Field Testing of Farm Animals*, Standard 154.03.03: *Containment Facilities for Vertebrate Laboratory Animals*, 155.04.09 *Containment Facilities for Plants 2007* and MAFBNZ Standard 154.02.17 *Transitional Facilities for Biological Products*. These standards specify the structural and operational requirements for transitional and containment facilities receiving and holding risk goods or new organisms.

The purpose of this inspection was to determine if the facility and operator approvals held by AgResearch and the HSNO Act approvals are being complied. Consequently, the scope of the inspection includes the standards and HSNO approvals. Verification of compliance is largely achieved by verifying that the AgResearch manual, which specifies the measures by which AgResearch meets standard and approval requirements, is being complied with.

This inspection was a scheduled external audit was conducted by Crystal Lange (MPI), with [REDACTED], Tim Hale and [REDACTED] (delegated Facility Managers). The inspection process included a review of onsite records where applicable, including staff training and internal audit records, registers, and physical inspection of the containment areas.

This report is written by standard so that each Manager can extract the parts that are specifically relevant to their own area.

References:

MAFBNZ Standard: *Transitional Facilities for Biological Products*
EPA Standard: *Facilities for Microorganisms and Cell Cultures: 2007a*
EPA Standard: *Containment Standard for the Field Testing of Farm Animals*
EPA Standard: *Containment Facilities for Vertebrate Laboratory Animals*
EPA Standard: *Containment Facilities for Plants 2007*
Australian Standard & New Zealand Standard (AS/NZS) 2243.3: 2002 - *Safety in Laboratories: Microbiological aspects and containment facilities*
Facility Manual: version 1.0 January 2016
Approvals granted under the HSNO Act
MPI permits to import and associated Import Health Standards (IHS's)



Findings

Animal Containment Facility

ERMA200223

Compliance with the controls of ERMA200223 was assessed. No issues were noted. N.B. Controls 8, 12, 14 and 15 are not applicable to this review.

Control 1

AgResearch is meeting the requirements of all applicable controls.

Control 2

Two of the permissible species are in use for research purposes.

Control 3

Practices comply with EPA, Animal Ethics and MPI requirements.

Control 4

The Requirements of the Standard are being met:

The internal audit was completed to schedule, the report was comprehensive and noted no areas of concern. The stock register was being reconciled in preparation of the Annual report, random sampling was used for checking animal records, staff were knowledgeable of animal locations and tag numbers verified during the farm walk.

Control 5

Sheep are used to graze the perimeter; sheep are not used as an experimental species.

Control 6

There have been no reported breaches of containment or any suspicious activity. Internal fencing, gates and races were well maintained, the perimeter fencing secure and entry gates monitored during daily activities.

Control 7

Animal records are maintained electronically. All health and treatment records were available to view.

Control 9

Visible identification was seen on goats and cows. Staff are familiar with stock and are able to visually determine the status of each animal.

Control 10

Practice complies with this control.

Control 11

The draft report was almost complete at the time of this visit.

Control 13

Contact is being maintained with Iwi.

Summary

The purpose of this audit was to verify and confirm compliance with the facility and operator approvals and thereby EPA/MPI Standards, HSNO and CTO approvals.

Overall the audit outcome was satisfactory, with increased monitoring required in the research laboratory area (including glasshouses).



Name: Crystal Lange
Travelling Technical Supervisor
Verification Services



MPI contact details

AUCKLAND REGION	• CENTRAL REGION
<p>Chris Rodley Containment Verifier Ministry for Primary Industries Verification Services Airport Oaks PO Box 53030, Auckland Airport AUCKLAND 2150</p> <p>Ph: 09 909 3520 Fax: Mob: 021 739 214 Email: Christopher.rodley@mpi.govt.nz</p>	<p>• Crystal Lange • Containment Verifier • Ministry for Primary Industries • Verification Services • Ruakura Research Centre, • PO Box 966 • HAMILTON 3240 • • Ph: 07 957 8319 • Fax: 07 957 8317 • Mob: 029 957 8319 • Email: crystal.lange@mpi.govt.nz •</p>
GREATER WELLINGTON REGION & NELSON / MARLBOROUGH	SOUTHERN REGION
<p>Mike Aitkenhead Specialist Advisor Ministry for Primary Industries Verification Services PO Box 2526 WELLINGTON 6140</p> <p>Ph: 04 894 4209 Fax: 04 894 4201 Mob: 029 894 4209 Email: mike.aitkenhead@mpi.govt.nz</p>	<p>Christchurch Office Ministry for Primary Industries Verification Services Private Bag 4765 CHRISTCHURCH 8140 Ph: 03 943 1777 Andrew McKay 029 943 1793 Email: andrew.mckay@mpi.govt.nz</p> <p>Craig Mason 029 943 1783 Email: craig.mason@mpi.govt.nz</p>



Ministry for Primary Industries External Audit Report:

Facility	AgResearch - Ruakura
Location	Ruakura Research Centre 10 Bisley Rd, Hamilton
Facility Codes	TF02501 (364)
Operator	Tom Richardson
Primary Verifier	Crystal Lange
Audit conducted By	Crystal Lange
Audit Date	February 21 and 27 2017
Audit report Date	March 1 2017



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Executive Summary

The external audit of AgResearch Ruakura's transitional and containment facility in Hamilton for the period October 2016 – March 2017 was carried out by the Ministry for Primary Industries (MPI) February 21 & 29 2017.

This was the second assessment of the Ruakura site as a single (combined) transitional and containment facility. The report is written in the same format as previous reports for continuity.

No issues were noted with records or structural compliance for either of the animal containment units. There were minor structural issues noted in the Microbiological laboratories reflecting in some cases the age of the facilities, in others a lack of use or poor laboratory hygiene. Of more concern is the disregard of procedures for removing rooms from a transitional or containment facility and the Operating Managers failure to identify this.

The Operating Manager also voiced concerns with the additional delays in handing over his role in the management of the containment facility. This audit was booked to suit the availability of the Compliance Advisor as part of this handover, this person was not available on the day. The Operating Manager is conflicted with the pressures applied by his manager, other AgResearch management, and his own desire to return to core research. MPI is concerned that extending this situation will exacerbate the issues noted in this and previous audit reports and result in reduced diligence over time.

MPI is also aware AgResearch staff are dealing with increasing workloads and workplace uncertainty, this is as applicable for research staff as it is for the Delegated Facility Operators. Succession planning needs to be given serious attention particularly as the contract for one Delegated Facility Operator ceases later this year.

Succession planning is essential for this site. MPI would recommend reviewing the Delegated Operator roles; it is possible that a part time compliance role would be of assistance in supporting these staff. This model is exhibited to varying degrees at a number of CRIs and Universities in New Zealand.

Another identified area of risk to AgResearch is the number of small lease holders (tenants) who are using AgResearch resources (Permits to Import, inventory/transfers, laboratory inspections) to obtain goods and services without being able to control how these goods are managed operationally. Leased laboratories are assessed during the external audits at a cost to AgResearch as they remain within the AgResearch Containment facility.

MPI would recommend a risk assessment being undertaken by AgResearch in relation to tenants, as well as its own transitional and containment laboratories and identify any that are no longer required for this purpose and removing them from the containment foot print. This would reduce the internal inspection requirements and ongoing maintenance.

Overall the audit outcome was satisfactory, with some areas of concern. MPI will continue to monitor progress and in the meantime the facility and operator approvals will be continued.

Sincerely



Crystal Lange
Travelling Technical Supervisor
Verification Services



Audit (Inspection) Scope

The AgResearch site at the Ruakura is a transitional and containment facility, approved as such under the Biosecurity Act 1993 because it holds or processes goods that are risk goods as defined by the Biosecurity Act 1993 or may contain new organisms as defined by Hazardous Substances and New Organisms (HSNO) Act 1996.

The facility is approved to Environmental Protection Authority (EPA) Standards 154.03.02: *Containment Facilities for Microorganisms 2007a*, 154.03.06: *Containment Standard for the Field Testing of Farm Animals*, Standard 154.03.03: *Containment Facilities for Vertebrate Laboratory Animals*, 155.04.09 *Containment Facilities for Plants 2007*, 154.02.08 *Transitional & Containment Facilities for Invertebrates* and MAFBNZ Standard 154.02.17 *Transitional Facilities for Biological Products*. These standards specify the structural and operational requirements for transitional and containment facilities receiving and holding risk goods or new organisms.

The purpose of this inspection was to determine if the facility and operator approvals held by AgResearch and the HSNO Act approvals are being complied with. Consequently, the scope of the inspection includes the standards and HSNO approvals. The Plants 2007 standard was not included in this audit.

This report is written by standard so that each Manager can extract the parts that are specifically relevant to their own area.

References:

MAFBNZ Standard: *Transitional Facilities for Biological Products*
EPA Standard: *Facilities for Microorganisms and Cell Cultures: 2007a*
EPA Standard: *Containment Standard for the Field Testing of Farm Animals*
EPA Standard: *Containment Facilities for Vertebrate Laboratory Animals*
EPA Standard: *Transitional and Containment Facilities for Invertebrates*
Australian Standard & New Zealand Standard (AS/NZS) 2243.3: 2002 - *Safety in Laboratories: Microbiological aspects and containment facilities*
Facility Manual: version 1.0 January 2016
Approvals granted under the HSNO Act
MPI permits to import and associated Import Health Standards (IHS's)

Audit Process

This inspection was a scheduled external audit was conducted by Crystal Lange (MPI), with [REDACTED] Tim Hale (delegated Facility Operators) and [REDACTED] (delegated Facility Operator and Operating Manager). The inspection process included a review of onsite records where applicable, including staff training and internal audit records, registers, and physical inspection of the containment areas.

This report is written by standard so that each delegated Operator can extract the parts that are specifically relevant to their own area.

Audit Findings

Animal Containment Facility

ERMA200223

Compliance with the controls of ERMA200223 was assessed. No issues were noted.
N.B. Controls 8, 12, 14 and 15 are not applicable to this review.

Control 1

AgResearch is meeting the requirements of all applicable controls.

Control 2

Two of the permissible species are in use for research purposes.

Control 3

Practices comply with EPA, Animal Ethics and MPI requirements.

Control 4

The Requirements of the Standard are being met:

The internal audit was completed to schedule, the report noted one recommendation. The stock register had been reconciled, animals were viewed and four ear tag numbers selected for record review. Perimeter checks are undertaken regularly and noted in the day book along with non-routine visits. Training for new staff is underway; currently limited to access of the farm office and non-containment areas while the induction phase is under way.

Control 5

Sheep are used to graze the perimeter; sheep are not used as an experimental species.

Control 6

There have been no reported breaches of containment or any suspicious activity. Perimeter fencing was secure, internal fencing, gates and races were well maintained. Entry gates are monitored during daily activities.

Control 7

Animal records are maintained electronically. All health and treatment records were available to view.

Control 9

Visible identification was seen on goats and cows. Staff are familiar with stock and are able to visually determine the status of each animal.

Control 10

Practice complies with this control.

Control 11

The draft report was submitted on time and final report accepted by the EPA and advised in writing on December 1 2016.

Control 13

Contact is being maintained with Iwi.

MPI contact details

AUCKLAND REGION	• CENTRAL REGION
<p>Chris Rodley Travelling Technical Supervisor Ph: 09 909 3520 Mob: 021 739 214 Email: Christopher.rodley@mpi.govt.nz</p> <p>Marisa Sorce Travelling Technical Supervisor Ph: 09 909 9059 Mob: 021 724789 Email: marisa.sorce@mpi.govt.nz</p>	<p>• Crystal Lange Travelling Technical Supervisor Ministry for Primary Industries Verification Services Ruakura Research Centre, PO Box 966 HAMILTON 3240</p> <p>• Ph: 07 957 8319 Fax: 07 957 8317 Mob: 029 957 8319 Email: crystal.lange@mpi.govt.nz</p>
GREATER WELLINGTON REGION & NELSON / MARLBOROUGH	SOUTHERN REGION
<p>Mike Aitkenhead Specialist Advisor Ministry for Primary Industries Verification Services PO Box 2526 WELLINGTON 6140</p> <p>Ph: 04 894 4209 Fax: 04 894 4201 Mob: 029 894 4209 Email: mike.aitkenhead@mpi.govt.nz</p>	<p>Christchurch Office Ministry for Primary Industries Verification Services Private Bag 4765 CHRISTCHURCH 8140 Ph: 03 943 1777 Andrew McKay 029 943 1793 Email: andrew.mckay@mpi.govt.nz</p> <p>Craig Mason 029 943 1783 Email: craig.mason@mpi.govt.nz</p>