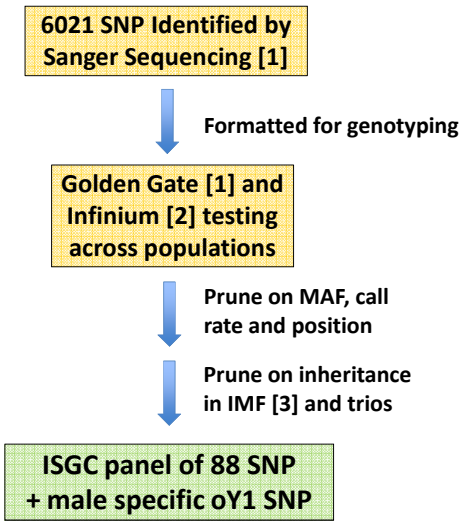
Development of a SNP Panel for Parentage Assignment in Sheep

James Kijas[1], John McEwan[2], Shannon Clarke[2], Hannah Henry[2], Jill Maddox[3], Russell McCulloch[1], Felice Driver[4], Katica Ilic[5], Mike Heaton[6] on behalf of the International Sheep Genomics Consortium[7]

[1] CSIRO Livestock Industries, Australia. [2] AgResearch, New Zealand. [3] University of Melbourne, Australia. [4] Meat and Livestock Australia. [5] Fluidigm Corporation, USA. [6] USDA, USA. [7] www.sheephapmap.org

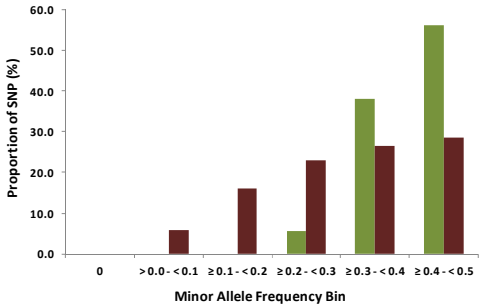
The use of accurate pedigrees is important for livestock production systems and research projects. We present the development and attributes of a SNP panel for the assignment of parentage in sheep.

Figure 1: Work Flow for SNP Panel Design



The genomic position and average minor allele frequency (MAF) of each SNP is given in Table 1. SNP were selected using population allele frequencies obtained from over 70 breeds sampled from 5 continents. The panel is biased towards high MAF markers (Figure 2) to ensure it will be of use across a wide range of breeds.

Figure 2: MAF



MAF was calculated by genotyping 2384 sheep from 74 breeds using the *ovine* SNP50 BeadChip [2]. MAF distributions are shown for all 49034 SNP on the BeadChip (red bars) and 89 SNP in the parentage panel (green bars, Table 1).

Four additional testing procedures (filters) have been applied to some, but not all SNP. Table 1 records the passage of SNP through each of the four additional filters.

Table 1: SNP ID, Genomic Location and Filter Testing

ISGC Parentage SNP	Chr	Mb Pos	Allele	MAF	Filter 1 Re-seq	Filter 2 Fluidigm	Filter 3 SQ_AgR	Filter 4 SQ_CLI	5k Chip	Filter problem
DU290101_408.1	1	7.8	A	0.337						3
DU518561_359.1	1	14.2	G	0.381						2
DU351298_316.1	1	69.6	A	0.445						
DU232924_365.1	1	95.8	G	0.250						
DU271929_382.1	1	97.5	A	0.483						4
DU502334_443.1	2	19.1	A	0.437						
DU469454_586.1	2	26.2	G	0.394						1
DU425907_184.1	2	50.1	G	0.358						
DU501115_497.1	2	62.8	A	0.239						
DU492516_411.1	2	63.4	T	0.478						
DU470875_383.1	2	91.5	G	0.357						
250506CS_*1	2	100.9	G	0.345						
DU191879_495.1	2	157.6	A	0.335						
DU480434_533.1	2	192.2	A	0.480						
DU260201_585.1	2	226.7	A	0.422						
DU503161_123.1	2	237.2	A	0.352						
DU425259_620.1	3	21.4	A	0.461						4
DU231007_156.1	3	59.0	G	0.463						2
DU225323_218.1	3	91.0	A	0.467						
DU260081_579.1	3	108.8	A	0.383						
DU394537_289.1	3	181.6	G	0.371						
CL635241_413.1	3	181.9	A	0.455						
DU408817_431.1	3	205.0	A	0.343						
DU202116_405.1	4	58.2	A	0.444						
DU460511_423.1	4	61.1	G	0.443						
DU305004_417.1	4	70.1	A	0.270						
DU369175_467.1	4	73.0	G	0.375						
DU446213_412.1	5	12.5	A	0.394						
DU444709_372.1	5	56.0	A	0.489						
DU453259_440.1	5	64.8	G	0.346						
DU194639_560.1	6	56.7	G	0.442						
CZ925803_293.1	6	100.8	A	0.443						
DU337465_337.1	6	106.0	A	0.338						
CL635944_160.1	6	115.0	A	0.490						4
DU467751_524.1	7	10.6	A	0.429						
DU499587_509.1	7	74.0	A	0.325						
CZ920950_468.1	7	74.8	A	0.456						
DU530067_219.1	7	100.0	G	0.327						
DU213735_493.1	8	6.6	A	0.437						
DU411204_551.1	8	13.8	A	0.361						
DU189970_325.1	9	86.6	C	0.374						
DU471913_499.1	9	91.1	G	0.490						
DU364754_308.1	9	93.9	A	0.397						
DU372582_268.1	9	94.4	G	0.247						2
DU468275_284.1	10	33.1	A	0.352						
DU310747_445.1	10	38.2	G	0.470						
DU269694_582.1	11	1.9	A	0.473						
DU433863_261.1	11	15.5	A	0.419						
DU417675_79.1	11	19.6	A	0.344						
DU508448_227.1	11	25.3	A	0.485						4
DU326572_241.1	11	59.5	A	0.446						
DU314655_578.1	12	26.7	A	0.365						
DU310703_497.1	12	75.3	A	0.492						1
DU275428_276.1	13	10.9	A	0.460						
DU435573_466.1	13	30.1	A	0.449						
DU411403_398.1	13	41.3	G	0.427						
DU462008_263.1	14	44.6	A	0.330						
DU223894_556.1	14	57.5	G	0.449						1
DU381045_479.1	14	60.7	A	0.403						
DU464373_638.1	15	2.3	A	0.467						
DU426312_454.1	15	44.4	G	0.375						
DU301502_402.1	15	73.7	G	0.441						
DU241306_191.1	15	78.6	G	0.279						
DU324670_456.1	17	10.2	A	0.400						
DU206327_107.1	17	14.4	A	0.499						
DU378819_632.1	17	22.3	A	0.475						3
DU511222_139.1	17	27.4	A	0.351						
DU300156_445.1	17	38.0	G	0.456						
DU463532_137.1	17	56.0	A	0.443						
DU492379_209.1	18	3.9	A	0.385						
DU488903_267.1	18	21.4	G	0.334						
DU325612_517.1	18	25.4	A	0.433						1
DU440765_491.1	18	60.5	A	0.474						3
DU345394_399.1	18	61.1	A	0.450						4
DU264531_279.1	19	0.6	A	0.388						
DU258053_237.1	19	57.1	A	0.400						
DU411432_523.1	19	57.2	C	0.406						
DU183112_480.1	20	31.1	A	0.453						
DU442373_141.1	20	48.4	A	0.342						
DU380983_440.1	21	28.3	G	0.451						
DU383863_376.1	21	38.2	G	0.443						1
DU196132_525.1	21	42.7	G	0.388						
DU413316_575.1	22	13.1	A	0.419						
DU302760_528.1	23	11.6	G	0.494						
DU313102_671.1	23	17.3	G	0.484						
CZ920359_258.1	24	3.2	G	0.382						
DU455254_479.1	25	0.1	G	0.453						
DU512685_259.1	25	1.2	G	0.495						
oY1	Y	0.0	G	0.320						

Table 1
The genomic location of each SNP (**Chr** / **Mb Pos**) is taken from the genome assembly version OAR2.0 available at [4]. SNP identifiers can be used to obtain additional information about each SNP [4]. The minor allele is given along with its frequency (**MAF**) in 2384 animals [2]. Four filters are described below, and irregularities arising from these additional tests are shown at right.

Filter1: Re-Sequencing
SNPs were re-sequenced from two nested PCR fragments produced from 96 diverse sires from 10 breeds. Passing SNPs could be reliably amplified and sequenced from genomic DNA without interference from nearby SNPs or other sequence features.

Filter2: Fluidigm Testing
SNP assays were designed using Early Access SNPType Assay Design Service and were tested on GT.96.96 microfluidic chip with SNPtype Assay Reagents. A panel of 95 animals was genotyped using genomic DNA. SNP assays exhibiting robust performance and high concordance rates against available SNP50 genotypes are shown.

Filter3: Sequenom Testing at AgResearch
Sequenom multiplexes were designed containing both parentage SNP (Table 1) and trait performance SNP unrelated to ISGC activities (not shown). Multiplexes were used to genotype pedigree material to prune SNP based on incorrect inheritance and call rate. SNP are shown that passed the filter.

Filter4: Sequenom Testing at MLA / CSIRO
Independent Sequenom multiplexes were designed by a second team. Multiplexes were used to genotype both high and low quality DNA samples. SNP are shown that passed both a call rate and concordance against SNP50 QC filter.

Key Points

- **We have identified a technically robust set of SNP suitable for parentage analysis in a wide variety of sheep.**
- **Disclosure of the SNP and their attributes is intended to promote uptake by commercial partners.**
- **The panel will be promoted to the International Society of Animal Genetics (ISAG) as the standard for testing in sheep.**
- **The SNP presented form the backbone of panels in beta-testing by GeneSeek [5] and Pfizer Animal Genetics [6].**
- **Wide applicability of the SNP panel opens the way for international level trace-back and product of origin testing.**

[1] Kijas et al. (2009) *PLoS ONE* 4:e4668
[2] Kijas et al. (2012) *PLoS Biology* (accepted pending minor revision)
[3] Crawford et al. (1995) *Genetics* 140:703-724.
[4] http://www.livestockgenomics.csiro.au/sheep/oar2.0.php
[5] http://www.neogen.com/GeneSeek/index.html
[6] http://www.pfizeranimalgenetics.co.nz/sites/PAG/aus/Pages/sheep.aspx

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