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Assessment of MDA efficiency for genotyping using cloned embryo biopsies

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ABSTRACT

The possibility to genotype embryos prior to implantation would have advantages for increasing the speed of selection of cattle. Reliable genotyping requires more DNA than can be obtained from biopsies of embryos, if they are to remain viable. Multiple displacement amplification (MDA) is a whole genome amplification technique used to increase the amount of DNA from biopsies for analysis. Reduced genome coverage resulting in Allele Drop Out (ADO) at heterozygous loci or missing genotypes are drawbacks of MDA.
The present article describes the correlation between the input DNA quantity or embryo biopsy size and MDA success. Missing genotypes and ADO drastically increased when fewer than 30–40 cells or the genomic equivalents were used. However, embryo viability was found to be reduced if biopsied with more than 10 cells. Therefore, *in vitro* cell culture was investigated as a means to increase the number of cells available and the genotyping reliability.

1. Introduction

Obtaining the genome-wide genotype of bovine embryos used in assisted reproduction techniques would benefit the cattle industry by increasing the rate of genetic gain in selection programmes. Genotyping at the time of embryo collection from super-ovulated donors, or at the time of blastocyst formation for embryos produced *in vitro* would allow the calculation of genomic breeding values for individual embryos and hence the selection of those with the highest breeding values. This approach has two advantages for the breeding industry: 1) a 9 month increase in the rate of genetic gain, and 2) only embryos with the desired genotypes would be implanted, therefore ensuring the best use of recipients. To carry out the genotyping, the embryos are usually biopsied at the blastocyst stage and the biopsied embryos then stored frozen until the genotyping and genomic breeding estimates are obtained.

The genotyping of a limited number of loci, *e.g.* for the sex determination is carried out directly on the embryo biopsy [1], however, genome wide genotyping requires more DNA than can be obtained from the few cells recovered by the embryo biopsies. Therefore an amplification step is necessary, which can be performed by various whole genome amplification (WGA) techniques. Amongst these techniques, multiple displacement amplification (MDA) has been shown to have the best performance [2,3]. MDA uses random primers that cover the genome to initiate DNA replication by the Phi29 polymerase. The Phi29 displacement activity unwinds the double helix and makes the DNA accessible to additional primers and polymerase, resulting in an exponential isothermal amplification of the template [4]. MDA efficiency is affected by DNA quality and quantity and stochastic effects which results in the uneven and incomplete amplification of the genome. The extent of genome amplification is not necessarily proportional to the starting amount of DNA. The unbalanced amplification of heterozygote loci can lead to Allele Drop Out (ADO). Moreover, amplification artefacts could cause the appearance of new alleles at non-polymorphic loci, a phenomenon known as Allele Drop In (ADI). The failure to amplify a genomic region will result in the loss of information.

The reported ADO rates for genome amplification of single cells vary from 4% up to 50% or more [2,5–9]. The completeness of WGA increases with the amount of starting material, although the minimum quantity of DNA that can be used in a MDA reaction has not been defined. Some authors claim reasonable quality (<10% errors) can be achieved with as few as 10 cells [5,10,11], whereas others suggest at least 100 genome equivalents are necessary [12]. MDA kits (Replig, QIAGEN and GenomiPhi V2, GE Healthcare) recommend the use of 1 to 10 ng of DNA, which corresponds to about 200 to 2000 genome equivalents.

In the present work SSR markers and the Illumina 54K SNP panel were used to define the correlation between the starting amount of template DNA and the quality of WGA, using DNA dilutions and bovine embryo biopsies. Cloned embryos derived from somatic cell nuclear transfer, and hence with identical genotype, were cultured *in vitro* up to the blastocyst stage then different sizes of biopsy taken. Cells from

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the biopsies were either used directly for WGA, or cultured further *in vitro* to obtain a greater number of cells for WGA. Results were confirmed in blind experiments with embryos of unknown genotype obtained from *in vitro* fertilization.

2. Results

2.1. WGA quality assessment from DNA dilutions

The quality of the WGA was assessed in relation to the quantity of input DNA, using serial dilutions of DNA corresponding to 600, 200, 100, 75, 50, 40, 30, 20, 15, 10 and 1 bovine genomic equivalent(s) (ge). The amplification products were genotyped using 12 SSR markers and the genotypes obtained were compared with that of the un-amplified control DNA. The quality of amplification was directly proportional to the amount of input DNA used as the WGA template (Fig. 1). Samples starting from more than 200 ge had the highest quality, with no differences in genotype with respect to the control. The quality decreased from 100 to 40 ge, although in all cases at least 94% accuracy of genotypes were obtained. Below 30 ge the quality of the amplification was reduced to values between 40% and 70%, with high variability among repetitions.

2.2. WGA quality assessment from embryo biopsies

Embryo biopsies containing 75, 40, 30, 20 and 10 cells obtained from cloned embryos, and therefore of identical genotypes, and biopsies cultured to 100 and 1000 cells (8 replicates per sample type) were tested as starting material for WGA. Samples starting from 30 or more cells gave genotypes matching that of the control, whereas the amplification quality was lower for samples starting from 20 and 10 cells, which had lower success and a more variable quality (91.25% and 82.50%, with a standard deviation of 19.44% and 35.00%, respectively) (Fig. 2).

The SSR genotyping of WGA products obtained from DNA dilutions and from embryo biopsies showed that 30 cells was the minimum biopsy size necessary to obtain high quality amplification (>94% of correct allele calls). A further increase in WGA quality may be expected using a larger number of input cells, however, biopsies of more than 30 cells are likely to reduce embryo viability. Biopsies of 20–30 cells were therefore cultured to increase cell numbers, prior to amplification.

2.3. Whole Genome Genotyping of WGA products

WGA products obtained from the least invasive biopsy (10 cells, 16 replicates), the best viability/WGA quality ratio (30 cells, 16 replicates) and the highest quality (cultured biopsies, 12 replicates) were compared by Whole Genome Genotyping (WGG) using the Illumina

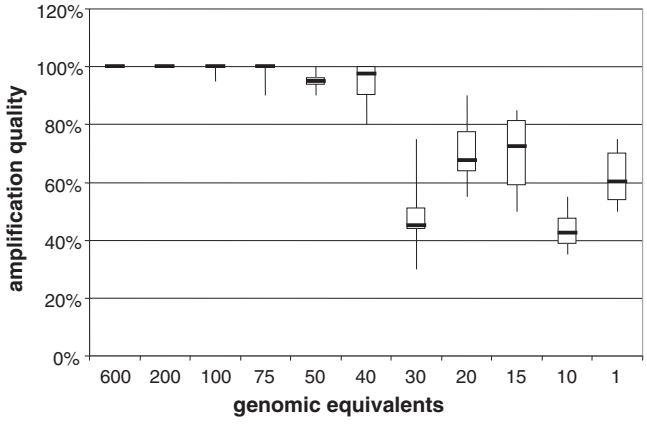


Fig. 1. The WGA product quality, assessed with SSR markers, is reported for serial dilutions of pure DNA, as box plots.

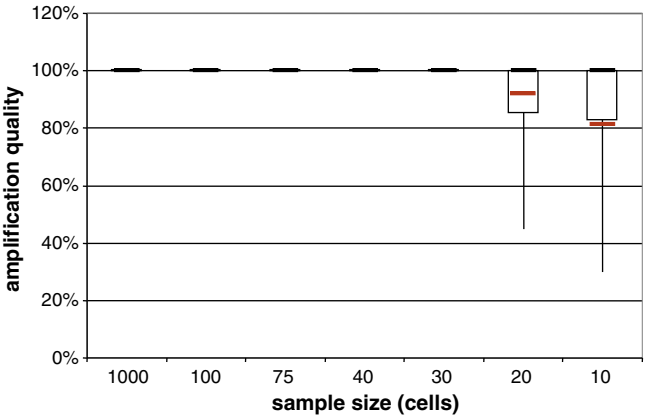


Fig. 2. The WGA product quality assessed with SSR markers is reported for cultured embryo (100 and 1000) and embryo biopsies of various sizes (10 to 75), as box plots. Red lines for the 10 and 20 cell series represent the average values.

Bovine 54K SNP panel. The Genome Coverage (GC), the Allele Drop Out (ADO) and the Allele Drop In (ADI), were used to evaluate the efficiency and the quality of amplification. More than 30% of the 10 cell samples (5/16) showed better than 85% genotyping success with an average of 72.45% across all the samples. Only 1 of the 30 cell samples had below 85% success and half of the samples were above 95%. The cultured biopsies had slightly higher GC with only 2 out of 12 samples having a genotyping success rate below 85% and with an average of 96.04% (Figs. 3 and 4).

The ADO decreased from 12.60% to 1.41% between 10 to 30 cells as the starting point. For the 10 cell series 5/16 of the samples showed ADO below 10%, while for the 30 cell series all samples had and ADO below 10% and 10/16 (62.50%) of the samples showed ADO below 1%. For the cultured biopsies 10/12 of the samples had an ADO below 1%. The ADI showed a comparable reduction from 10 to 30 cells and the cultured biopsies showed the lowest ADI (Figs. 3 and 4).

GC, ADO and ADI were correlated in all sets of samples (Fig. 3). This was particularly evident in the 10 cell samples, where the highest GC values corresponded to the lowest ADO and ADI. Considering all samples, the pair wise correlation between GC, ADO and ADI fitted with a linear curve (Fig. 5).

2.4. Blind genotyping

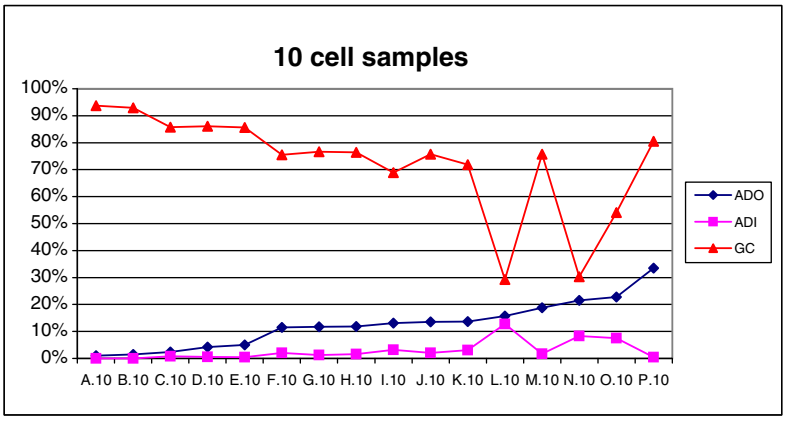
Ten cell biopsies of sixteen embryos with unknown genotype were analysed with the 54K SNP Illumina panel and ADO, ADI and GC were determined as described in Section 4.8. The average ADO was 13.75%, with 12.50% of the samples having an ADO lower than 1% and 37.50% of the samples between 1% and 10%. The average ADI was 3.34%, with 81.25% of the samples with a value below 1% and 6.25% between 1% and 10%. The average GC was 81.42%, with 75% of the samples above 85% and 6.25% of the samples above 95% (Fig. 4). As with the cloned embryos, the ADO and ADI were higher for samples with lower GC values (Fig. 3).

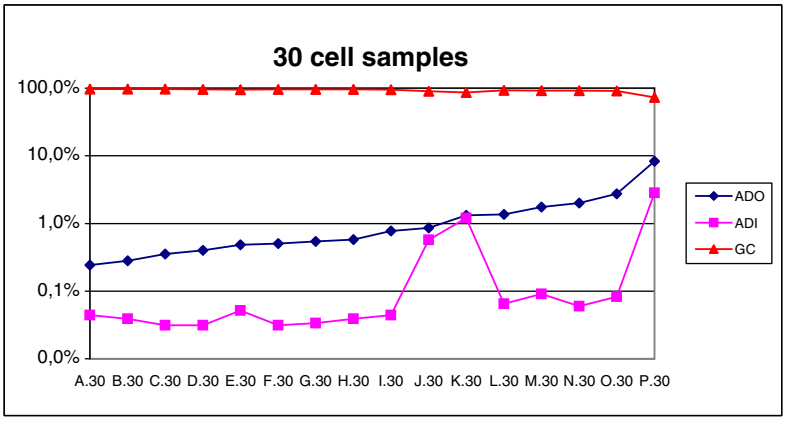
A Student *t* test was used to investigate if the differences between the two data sets obtained for 10 cells (blind and cloned, reported in Fig. 3) were statistically relevant. The results showed no significant difference between the two sample sets for ADO, ADI and GC.

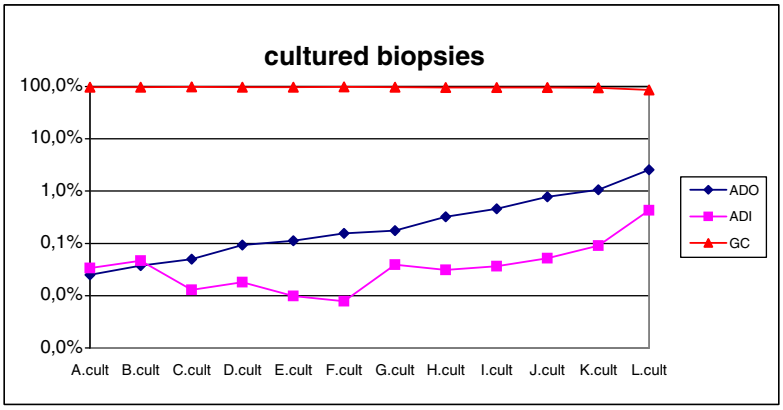
2.5. Biopsied embryo viability

The viability of a biopsied embryo is related to the size of biopsy, and is also affected by freezing, which is necessary to store embryos prior to transfer to the recipient. In order to assess the viability of embryos with different sizes of biopsy, embryos were frozen immediately after biopsy

	MISS (HET)	ADO	MISS (HOM)	ADI	MISS (TOT)	GC
10 cells						
A.10	18,59%	1,00%	1,14%	0,05%	6,27%	93,73%
B.10	21,49%	1,46%	1,06%	0,04%	7,07%	92,93%
C.10	28,53%	2,35%	8,28%	0,82%	14,23%	85,77%
D.10	32,92%	4,18%	5,94%	0,52%	13,87%	86,13%
E.10	33,96%	4,96%	6,23%	0,47%	14,38%	85,62%
F.10	42,96%	11,46%	16,86%	2,10%	24,53%	75,47%
G.10	43,97%	11,71%	14,76%	1,26%	23,35%	76,65%
H.10	42,35%	11,84%	15,82%	1,55%	23,62%	76,38%
I.10	47,36%	13,09%	24,33%	3,15%	31,10%	68,90%
J.10	43,56%	13,57%	16,18%	2,05%	24,23%	75,77%
K.10	44,75%	13,71%	21,22%	3,09%	28,14%	71,86%
L.10	70,60%	15,70%	70,74%	12,73%	70,70%	29,30%
M.10	43,39%	18,81%	16,30%	1,74%	24,27%	75,73%
N.10	69,60%	21,54%	69,75%	8,36%	69,70%	30,30%
O.10	53,84%	22,76%	42,65%	7,57%	45,94%	54,06%
P.10	40,47%	33,48%	10,66%	0,47%	19,42%	80,58%
AVERAGE	42,40%	12,60%	21,37%	2,87%	27,55%	72,45%
30 cells						
A.30	9,54%	0,24%	0,95%	0,04%	3,48%	96,52%
B.30	8,79%	0,28%	0,89%	0,04%	3,22%	96,78%
C.30	9,71%	0,36%	0,73%	0,03%	3,37%	96,63%
D.30	11,56%	0,40%	0,76%	0,03%	3,94%	96,06%
E.30	13,75%	0,49%	1,09%	0,05%	4,81%	95,19%
F.30	12,68%	0,50%	0,92%	0,03%	4,38%	95,62%
G.30	12,69%	0,54%	0,80%	0,03%	4,29%	95,71%
H.30	12,92%	0,58%	0,93%	0,04%	4,46%	95,54%
I.30	15,62%	0,77%	1,02%	0,04%	5,31%	94,69%
J.30	20,73%	0,86%	5,57%	0,57%	10,02%	89,98%
K.30	22,31%	1,32%	10,43%	1,20%	13,92%	86,08%
L.30	19,33%	1,36%	1,75%	0,06%	6,92%	93,08%
M.30	22,32%	1,74%	1,89%	0,09%	7,90%	92,10%
N.30	22,80%	2,01%	1,21%	0,06%	7,56%	92,44%
O.30	26,94%	2,74%	1,97%	0,08%	9,31%	90,69%
P.30	41,16%	8,30%	20,45%	2,82%	26,54%	73,46%
AVERAGE	17,68%	1,41%	3,21%	0,33%	7,46%	92,54%
cultured biopsies						
A.cult	5,00%	0,02%	1,46%	0,03%	2,50%	97,50%
B.cult	4,79%	0,04%	1,72%	0,05%	2,62%	97,38%
C.cult	3,83%	0,05%	0,96%	0,01%	1,80%	98,20%
D.cult	5,56%	0,09%	1,22%	0,02%	2,49%	97,51%
E.cult	4,60%	0,11%	1,01%	0,01%	2,07%	97,93%
F.cult	4,22%	0,16%	0,81%	0,01%	1,81%	98,19%
G.cult	5,50%	0,17%	1,70%	0,04%	2,82%	97,18%
H.cult	10,10%	0,32%	1,30%	0,03%	3,89%	96,11%
I.cult	8,97%	0,46%	1,68%	0,04%	3,82%	96,18%
J.cult	10,87%	0,77%	1,81%	0,05%	4,48%	95,52%
K.cult	12,15%	1,07%	2,05%	0,09%	5,02%	94,98%
L.cult	28,18%	2,54%	8,44%	0,43%	14,25%	85,75%
AVERAGE	8,65%	0,48%	2,01%	0,07%	3,96%	96,04%
blind						
5266M01	34,78%	36,28%	9,30%	0,47%	16,70%	83,30%
5266M02	31,45%	8,44%	0,66%	0,04%	12,34%	87,66%
8946F02	37,80%	24,57%	3,35%	0,13%	13,47%	86,53%
8946F06	20,57%	2,05%	0,31%	0,04%	6,68%	93,32%
8946F08	34,62%	22,25%	3,24%	0,20%	13,14%	86,86%
8946M01	15,04%	0,82%	0,49%	0,03%	5,32%	94,68%
8946M03	36,36%	51,40%	27,81%	1,43%	30,56%	69,44%
8946M04	35,68%	17,05%	0,56%	0,04%	14,58%	85,42%
8946M05	59,67%	9,23%	58,63%	36,91%	58,62%	41,38%
8946M07	15,93%	1,47%	0,21%	0,03%	5,43%	94,57%
8946M10	14,25%	0,65%	0,17%	0,02%	4,55%	95,45%
8946M11	19,72%	2,53%	0,28%	0,02%	6,52%	93,48%
8958F01	34,09%	11,28%	0,81%	0,03%	10,97%	89,03%
8958F02	24,15%	10,84%	0,84%	0,04%	8,33%	91,67%
8958F04	78,21%	12,06%	78,43%	13,94%	78,64%	21,36%
8958M03	33,89%	9,00%	1,27%	0,04%	11,46%	88,54%
AVERAGE	32,89%	13,75%	11,65%	3,34%	18,58%	81,42%







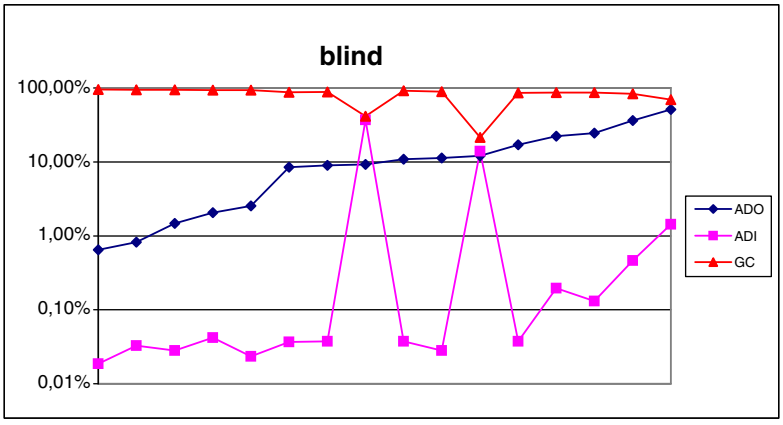
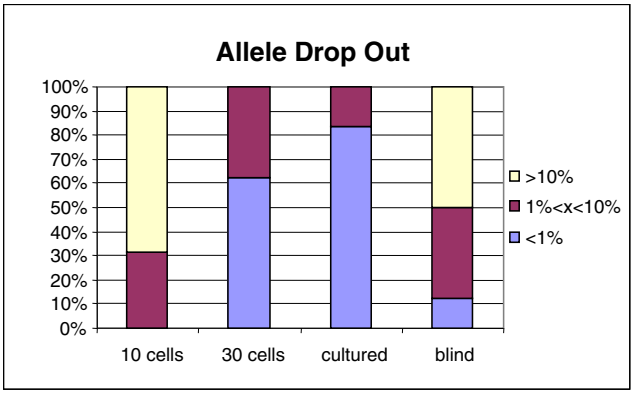
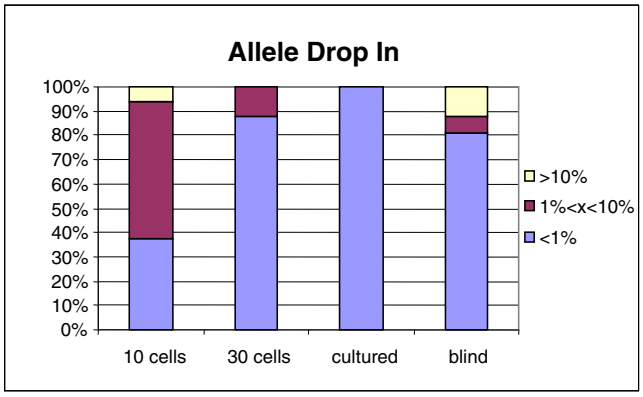
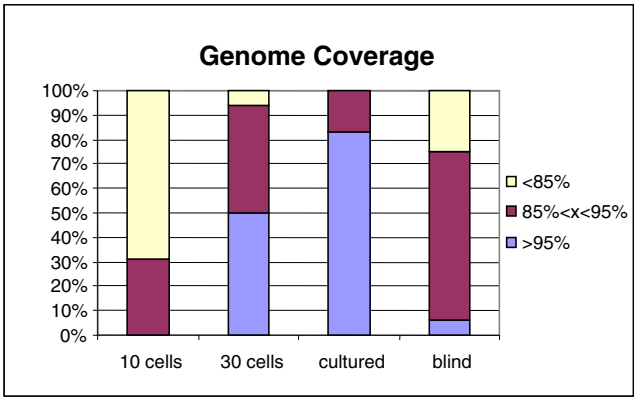


Fig. 3. The results from Whole Genome Genotyping with the Illumina Bovine 54K panel are reported. Data represent the Allele Drop Out (ADO), Allele Drop In (ADI) and the Genome Coverage (GC). Percentage of negative calls (MISS) among the homozygote, heterozygote or all loci is also shown (HET, HOM and TOT, respectively). Charts report the values ordered by growing ADO. In the three lower charts (30 cells, cultured and blind samples). Y axes values are reported on a logarithmic scale.







ADO	10 cells	30 cells	cultured	blind
<1%	0.00%	62.50%	83.33%	12.50%
1%<x<10%	31.25%	37.50%	16.67%	37.50%
>10%	68.75%	0.00%	0.00%	50.00%

ADI	10 cells	30 cells	cultured	blind
<1%	37.50%	87.50%	100.00%	81.25%
1%<x<10%	56.25%	12.50%	0.00%	6.25%
>10%	6.25%	0.00%	0.00%	12.50%

GC	10 cells	30 cells	cultured	blind
>95%	0.00%	50.00%	83.33%	6.25%
85%<x<95%	31.25%	43.75%	16.67%	68.75%
<85%	68.75%	6.25%	0.00%	25.00%

Fig. 4. The ADO, ADI and GC shown in Fig. 3 are reported grouped in series (<1%, included between 1% and 10%, >10%, for ADI and ADO; <85%, included between 85% and 95%, >95%, for GC).

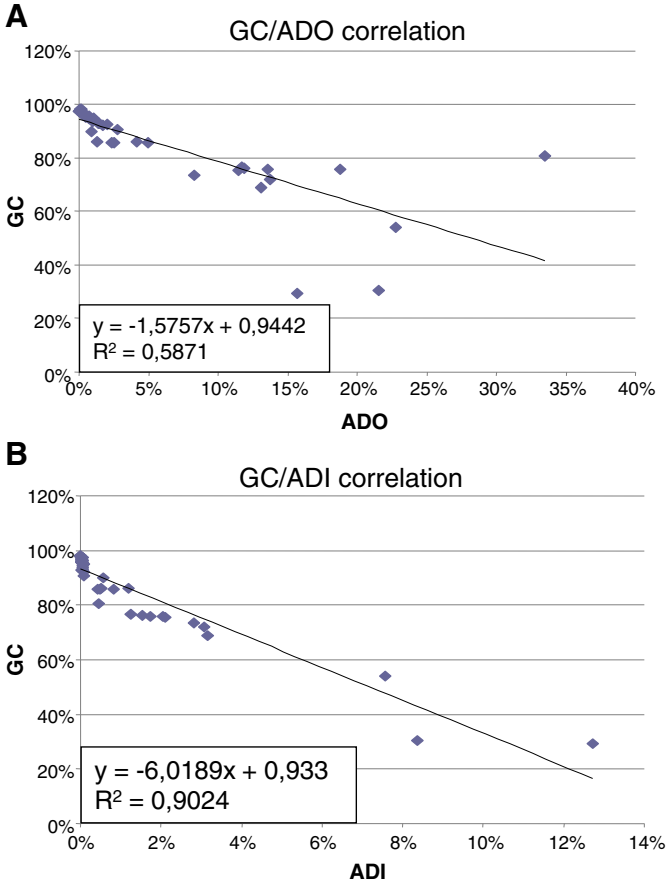


Fig. 5. The correlation between GC and ADO (Panel A) and GC and ADI (Panel B) are shown by plotting all the values reported in Fig. 3 and drawing regression lines.

of 10 cells (28 replicates) or 30 cells (32 replicates), then thawed and cultured *in vitro* for 48 h. The same procedure was used with un-biopsied embryos, for comparison (52 replicates). Embryos following the 10 cell biopsy showed a survival rate of 89% whereas viability of embryos from a 30 cell biopsy was reduced to 62%. Control un-biopsied embryos showed a survival rate of 90%.

3. Discussion

Genotyping an embryo from biopsied cells can facilitate the estimation of the genomic breeding values for that embryo and hence genetic selection could be achieved even before implantation ensuring the selection of the best embryos for breeding. For cattle this would significantly increase the rate of breeding improvement. However there is a trade off between genotyping success, the number of cells in the biopsy and embryo survival. Generally, the lower the number of cells, or starting DNA, the lower the success of the WGA, but the higher the survival rate of the embryos.

In the present work, the relationship between the amount of starting material and WGA amplification quality was investigated using dilutions of purified DNA and different numbers of cells from embryo biopsies. The results of genotyping with 12 SSR markers were comparable using cells or the same genome equivalents of purified DNA as starting material. The lower limit cut off before WGA problems were observed was approximately 30–40 cells or ge. The consistency of these results suggests that there is no inhibitory effect from components in the embryo lysate and hence a DNA purification step before the WGA is not necessary. The results presented here suggest that the pre-treatment of samples with proteinase K claimed to destroy amplification inhibitors before WGA and improve efficiency [13] also seems to be unnecessary.

The effect of splitting biopsies into smaller samples to be amplified independently then analysed as a MDA DNA pool was not investigated. Nevertheless, results reported here (Fig. 2) and in a previous

publication [5] would suggest a roughly linear relationship between biopsy size and average amplification quality, for samples with less than 30 cells. If so, the expected output of such of an approach would not be an improvement over the amplification of the whole sample.

The lower limit for DNA amplification is most likely because of stochastic effects which result in incomplete coverage of the genome and the uneven amplification of different regions which will result in missing genotypes. The unequal amplification of the DNA as one of the causes of ADO and negative calls was confirmed by the direct correlation between GC and ADO reported in this study. The appearance of new alleles (ADI) also seems to be higher at lower GC values and this presumably reflects an overall higher copying error rate, although the reason has not yet been completely understood [14].

To reduce the loss of vitality of the embryo after the biopsy, the biopsies should be restricted to as few cells as possible. The data presented here show that embryos with biopsies of 10 cells have a survival rate comparable to that of un-biopsied embryos and 27% higher viability than the embryos from which 30 cell biopsies are taken, while the former have an 11% higher chance of missing an allele in homozygote loci due to poor WGA. Pregnancy rates following the transfer of frozen thawed biopsied embryos suggest that there is only minor loss of viability following a biopsy of 10 cells, as compared with intact embryos [5]. To overcome the conflict between embryo viability and genotyping success the number of cells can be increased by *in vitro* cell culture. Using 20–30 cell biopsies cultured to about 1000 cells increases the reliability of WGA. However the additional time and costs required for the culture step makes this not a viable approach for commercial use.

When using unknown samples, ADO rate can be estimated from the GC represented by the call rate. If the genotypes of the two parents are known, the ADO, and consequently the genotyping reliability, can be estimated more accurately on the basis of the loci that are expected to be heterozygous. In current genomic selection programmes, high density SNP information is often recovered from low density genotyping using data from parents and relatives and statistical approaches to impute missing genotypes [15]. A similar approach can be used to verify and recover data for embryos genotyped using WGA.

4. Materials and methods

4.1. Production of cloned bovine embryos

Cloned embryos were produced as described by Galli et al. [16]. Briefly, bovine ovaries were collected from slaughtered cows, follicles over 3 mm in diameter were dissected, oocytes recovered and transferred to maturation medium (TCM-199 supplemented with 10% (v/v) foetal calf serum, gonadotropins (0.05 IU/ml FSH and 0.05 IU/ml LH) and growth factors (50 ng/ml long-EGF and 10 ng/ml bFGF) [17,18]. Oocytes were cultured at 38.5 °C and 5% CO_2 in humidified air for 16 h–20 h after which the cumulus cells were removed and the oocytes were enucleated by the aspiration of polar body and associated metaphase II plate in minimal volume of ooplasm. The somatic cells were obtained by trypsinisation of a fibroblast cell line derived from a skin biopsy of a Holsetin Fresian bull. A single somatic cell was fused to each enucleated oocyte and chemically activated to induce cell division. Developing embryos were cultured in a modified Synthetic Oviduct Fluid medium for 7–8 days after activation until they reached the blastocyst stage.

4.2. Embryo biopsy and culture of biopsied cells

Blastocyst stage embryos were biopsied using a microblade (Bioniche, Pulman Washington, USA) mounted on a Leitz (Germany) micromanipulator. The biopsied cells were either frozen prior to DNA extraction, genotyping, or cultured *in vivo* by transferring them into micro-drops of 40 μl culture medium consisting of DMEM-F12 supplemented with BSA (10 mg/ml) and N2 (Invitrogen) under oil. The biopsies were cultured for 4–5 days in order to obtain 100–2000 cells which were then frozen.

4.3. Embryo freezing

Following biopsy embryos were frozen in hepes-SOF containing 6 mg/ml of BSA and 1.5 M ethylene glycol, using an embryo freezer methanol bath and following a standard freezing curve (first holding at −6 °C, then cooling to −32 °C at 0.5 °C per minute, before plunging in liquid nitrogen). Embryo survival following biopsy and freezing was assayed by thawing a sample of embryos in water at 25 °C and culturing them for 48 h in SOF medium. Viability was measured as the rate of embryo survival (expanded or hatched blastocyst stage embryos/total embryos thawed) at 48 h.

4.4. In vitro fibroblast culture and DNA extraction

Primary fibroblasts were obtained from a skin biopsy which was cut into small pieces and the tissue fragments plated onto on gelatinised culture dishes in TCM199/DMEM (1:1) medium supplemented with 10% foetal calf serum. The same fibroblast cell line was used to generate all the cloned bovine embryos by somatic cell nuclear transfer, and for trail WGA DNA analyses. Genomic DNA was extracted using the Gentra Puregene kit (QIAGEN) following the manufacturer's instructions. DNA was quantified using a Nanodrop (Thermo Scientific). For dilution studies 1 genome equivalent was considered to be equivalent to 5 pg of DNA.

4.5. WGA amplification from embryo biopsies

Frozen embryo biopsies were thawed for 15′ at room temperature, then re-frozen at −20 °C for 30′. This slow freezing/thawing (repeated 3 times) facilitated the release of DNA. The WGA was carried out using the Repli-g Mini Kit (QIAGEN) following the manufacturer's instructions for 16 h at 30 °C. Following the reaction 5 μl were analysed on an EtBr stained agarose gel to verify the presence of an amplification product.

4.6. Blind samples

Sixteen embryos with unknown genotype, resulting from *in vitro* fertilization of egg cells from 3 donors with sperm cells from one bull, were sampled with 10 cell biopsies and analysed by WGG. The genotypes of the 4 parents were also assessed from genomic DNA extracted from blood samples or semen (see Section 4.4).

4.7. SSR markers

The survey of SSR markers was carried out by amplifying the 12 loci with the primers reported in Table 1, using about 50 ng of the WGA product per reaction. The PCR amplification conditions were: an initial denaturation (10′ at 94 °C) was followed by 31 cycles (45″ at 94 °C; 45″ at 59 °C; 60″ at 72 °C) and a final extension (30′ at 72 °C). The PCR products were analysed using an ABI PRISM 3100 Genetic Analyze and the genotypes assigned using ABI-PRISM Genescan and ABI-PRISM Genotyper software.

WGA products and 1 un-amplified control were genotyped for the 12 SSR markers. The control profile included 8 heterozygote loci and 4 homozygote loci, for a total of 20 alleles. The quality of the amplification was assessed as the average percentage, out of eight repetitions per dilution, of alleles that matched the control.

4.8. WGA quality parameters

WGA products from the cloned embryos were genotyped for the Illumina bovine 54K SNP panel. Genomic DNA extracted from the

Table 1
SSR markers and primers used in this study. The amplification range and the amplification failure rate per allele are reported per each SSR marker.

Marker — primer name	Sequence	Fluorochrome	Colour	Range	Amp failure per allele
TGLA227 F	CGA ATT CCA AAT CTG TTA ATT TGC T	VIC	Green	75–105 bp	10.4%
TGLA227 R	ACA GAC AGA AAC TCA ATG AAA GCA	–	–		
TGLA126 F	CTA ATT TAG AAT GAG AGA GGC TTC T	VIC	Green	109–125 bp	4.2%
TGLA126 R	TTG GTC TCT ATT CTC TGA ATA TTC C	–	–		
ETH3 F	GAA CCT GCC TCT CCT GCA TTG G	FAM	Blue	101–131 bp	16.7%
ETH3 R	ACT CTG CCT GTG GCC AAG TAG G	–	–		
BM2113 F	GCT GCC TTC TAC CAA ATA CCC	NED	Yellow	121–143 bp	17.4%
BM2113 R	CTT CCT GAG AGA AGC AAC ACC	–	–		
ETH225 F	GAT CAC CTT GCC ACT ATT TCC T	FAM	Blue	136–162 bp	21.9%
ETH225 R	ACA TGA CAG CCA GCT GCT ACT	–	–		
TGLA122 F	CCC TCC TCC AGG TAA ATC AGC	VIC	Green	135–187 bp	22.2%
TGLA122 R	AAT CAC ATG GCA AAT AAG TAC ATA C	–	–		
TGLA53 F	GCT TTC AGA AAT AGT TTG CAT TCA	NED	Yellow	152–188 bp	17.4%
TGLA53 R	ATC TTC ACA TGA TAT TAC AGC AGA	–	–		
BM1824 F	GAG CAA GGT GTT TTT CCA ATC	FAM	Blue	174–194 bp	19.4%
BM1824 R	CAT TCT CCA ACT GCT TCC TTG	–	–		
INRA23 F	GAG TAG AGC TAC AAG ATA AAC TTC	VIC	Green	196–222 bp	19.4%
INRA23 R	TAA CTA CAG GGT GTT AGA TGA ACT C	–	–		
ETH10 F	GTT CAG GAC TGG CCC TGC TAA CA	NED	Yellow	209–227 bp	12.2%
ETH10 R	CCT CCA GCC CAC TTT CTC TTC TC	–	–		
SPS115 F	AAA GTG ACA CAA CAG CTT CTC CAG	NED	Yellow	246–264 bp	6.3%
SPS115 R	AAC GAG TGT CCT AGT TTG GCT GTG	–	–		
BM1818 F	AGC TGG GAA TAT AAC CAA AGG	FAM	Blue	250–280 bp	1.4%
BM1818 R	AGT GCT TTC AAG GTC CAT GC	–	–		

donor fibroblasts was used as benchmark for the WGA product quality assessment.

Genome coverage (GC) was calculated from the call rate, *i.e.* the number of SNP with a positive call on the total number of SNP. The ADO rate was calculated with respect to the heterozygote loci (16055), the ADI rate was calculated with respect to the homozygote loci (38554).

For blind samples, only SNP located on the autosomes and that were correctly genotyped in both parents were considered. ADI was calculated based on the expected homozygote loci, *i.e.* those that resulted from loci homozygous for the same allele in the two parents. Similarly, ADO was calculated based on expected heterozygote loci, *i.e.* those that resulted from parental loci homozygous for different alleles. The GC was defined as the total number of positive calls on the whole set of loci.

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References

[1] M.C. Carneiro, P.L. Takeuchi, A. Araujo, R.B. Lobo, F.P. Elias, R.A. Vila, C.L. Miranda-Furtado, E.S. Ramos, Sexing single bovine blastomeres using TSPY gene amplification, Genet. Mol. Res. 10 (2011) 3937–3941.
[2] N.R. Treff, J. Su, X. Tao, L.E. Northrop, R.T. Scott Jr., Single-cell whole-genome amplification technique impacts the accuracy of SNP microarray-based genotyping and copy number analyses, Mol. Hum. Reprod. 17 (2011) 335–343.
[3] S. Hosono, A.F. Faruqi, F.B. Dean, Y. Du, Z. Sun, X. Wu, J. Du, S.F. Kingsmore, M. Egholm, R.S. Lasken, Unbiased whole-genome amplification directly from clinical samples, Genome Res. 13 (2003) 954–964.
[4] F.B. Dean, S. Hosono, L. Fang, X. Wu, A.F. Faruqi, P. Bray-Ward, Z. Sun, Q. Zong, Y. Du, J. Du, M. Driscoll, W. Song, S.F. Kingsmore, M. Egholm, R.S. Lasken, Comprehensive human genome amplification using multiple displacement amplification, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 5261–5266.
[5] P. Humblot, D. Le Bourhis, S. Fritz, J.J. Colleau, C. Gonzalez, C. Guyader Joly, A. Malafosse, Y. Heyman, Y. Amigues, M. Tissier, C. Ponsart, Reproductive technologies and genomic selection in cattle, Vet. Med. Int. 2010 (2010) 192787.
[6] A. Hellani, S. Coskun, A. Tbakhi, S. Al-Hassan, Clinical application of multiple displacement amplification in preimplantation genetic diagnosis, Reprod. Biomed. Online 10 (2005) 376–380.
[7] Z. Ren, C. Zhou, Y. Xu, J. Deng, H. Zeng, Y. Zeng, Mutation and haplotype analysis for Duchenne muscular dystrophy by single cell multiple displacement amplification, Mol. Hum. Reprod. 13 (2007) 431–436.
[8] P.J. Renwick, C.M. Lewis, S. Abbs, C.M. Ogilvie, Determination of the genetic status of cleavage-stage human embryos by microsatellite marker analysis following multiple displacement amplification, Prenat. Diagn. 27 (2007) 206–215.
[9] C. Spits, C. Le Caignec, M. De Rycke, L. Van Haute, A. Van Steirteghem, I. Liebaers, K. Sermon, Optimization and evaluation of single-cell whole-genome multiple displacement amplification, Hum. Mutat. 27 (2006) 496–503.
[10] A.H. Handyside, M.D. Robinson, R.J. Simpson, M.B. Omar, M.A. Shaw, J.G. Grudzinskas, A. Rutherford, Isothermal whole genome amplification from single and small numbers of cells: a new era for preimplantation genetic diagnosis of inherited disease, Mol. Hum. Reprod. 10 (2004) 767–772.
[11] J. Ling, G. Zhuang, B. Tazon-Vega, C. Zhang, B. Cao, Z. Rosenwaks, K. Xu, Evaluation of genome coverage and fidelity of multiple displacement amplification from single cells by SNP array, Mol. Hum. Reprod. 15 (2009) 739–747.
[12] L. Lovmar, M. Fredriksson, U. Liljedahl, S. Sigurdsson, A.C. Syvanen, Quantitative evaluation by minisequencing and microarrays reveals accurate multiplexed SNP genotyping of whole genome amplified DNA, Nucleic Acids Res. 31 (2003) e129.
[13] J. Polisseni, W.F. Sa, O. Guerra Mde, M.A. Machado, R.V. Serapiao, B.C. Carvalho, L.S. Camargo, V.M. Peters, Post-biopsy bovine embryo viability and whole genome amplification in preimplantation genetic diagnosis, Fertil. Steril. 93 (2010) 783–788.
[14] E. Giardina, I. Pietrangeli, C. Martone, S. Zampatti, P. Marsala, L. Gabriele, O. Ricci, G. Solla, P. Asili, G. Arcudi, A. Spinella, G. Novelli, Whole genome amplification and real-time PCR in forensic casework, BMC Genomics 10 (2009) 159.
[15] Y. Li, C. Willer, S. Sanna, G.a. Abecasis, Genotype imputation, Annu. Rev. Genomics Hum. Genet. 10 (2009) 387–406.
[16] C. Galli, I. Lagutina, I. Vassiliev, R. Duchi, G. Lazzari, Comparison of microinjection (piezo-electric) and cell fusion for nuclear transfer success with different cell types in cattle, Cloning Stem Cells 4 (2002) 189–196.
[17] C. Galli, G. Crotti, C. Notari, P. Turini, R. Duchi, G. Lazzari, Embryo production by ovum pick up from live donors, Theriogenology 55 (2001) 1341–1357.
[18] C. Galli, G. Lazzari, Practical aspects of IVM/IVF in cattle, Anim. Reprod. Sci. 42 (1996) 371–379.