2. REVIEW OF LITERATURE

2.1 Gowli buffaloes

Gowli buffaloes are maintained by the people of the Gowli tribe since long. However, these animals are not categorized under the recognized buffalo breeds of India. There are no recognized farms for these animals, either in public or private sector. There are no studies undertaken on morphology, biochemical, cytogenetic or molecular genetics in these animals, although much work has been carried out on the other recognized buffalo breeds of India in these aspects.

Littlewood (1936) classified the local buffaloes of South India into four categories as Toda, Paralakhemundi, South Kanara or Malabari, and the small non-descript local variety which may be seen everywhere. According to a report of the committee on reorganization of livestock farms of animal husbandry department in erstwhile Mysore state (Anon, 1962), no pure breeds of buffaloes existed in Mysore except for Pandharpuri found in large numbers in northern parts of Karnataka. The types of buffaloes found in Karnataka have been discussed by Jayashankar and Govindaiah (2001).

2.1.1 Buffalo Population in Karnataka

The details of the buffalo population of Karnataka as per the 17th Quinquennial livestock census conducted by the state Department of Animal Husbandry and Veterinary Services, Government of Karnataka (Anon, 2003) is given in Table 2.1.

Table 2.1: Buffalo population in Karnataka
<table>
<thead>
<tr>
<th>Category</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graded</td>
<td>25482</td>
<td>197623</td>
<td>223105</td>
</tr>
<tr>
<td>Indigenous</td>
<td>438729</td>
<td>3329097</td>
<td>3767826</td>
</tr>
<tr>
<td>Total</td>
<td>464211</td>
<td>3526720</td>
<td>3990931</td>
</tr>
<tr>
<td>Indigenous buffaloes in Belgaum, Uttar Kannada and Dharwar</td>
<td>88064</td>
<td>766812</td>
<td>854876</td>
</tr>
</tbody>
</table>

The buffalo census was conducted on the basis of breed/breed category for the first time in 2003. The total buffalo population was about 40 lakhs, with the indigenous buffaloes constituting 94.4 per cent of the total population, the rest of them being classified under graded buffaloes. The buffalo population in the breeding tract constitutes nearly 21.50 per cent of the total buffalo population in the state, of which the Gowli amme (buffaloes) constitute about 30 per cent of total buffalo population.

2.1.2 Habitat and Management

The major breeding tract of Gowli buffaloes is spread over in Belgaum, Uttar Kannada and Dharwar districts of Karnataka, which fall under the Northern Transition zone.

A tribal community called Gavali or Gowli exists in the northern districts of Karnataka, which derives its name from the occupation of rearing and milking of govu/cows including buffaloes. Gowli people live in small hamlets comprising of 20 to 30 families. The tribe is spread out in the thick woods of Western Ghats and in suburban areas of the taluks in Belgaum, Dharwar and Uttar Kannada districts of Karnataka. They are professional breeders and rearers of dairy animals especially buffaloes. Literacy level in this tribe is very poor and they have their own dialect. They
are friendly and hospitable. The buffaloes reared by the Gowli tribal people are popularly known as Gowli and are maintained in semiferal conditions.

### 2.1.3 Body measurements and functional characteristics

Gowli buffaloes are not maintained in sufficient numbers in any organized farms, and as such facts about them are rarely seen in the scientific literature.

Govindaiah and Rai (1984) conducted studies on Dharwar buffaloes. They observed that these buffaloes were medium in body built, dark skinned with lacing of dark sparse hairy coat. These buffaloes had a sweeping horn shape which projected laterally from the poll region, running down parallel to neck and shoulder and almost touching the ground. The ears were long and tubular. The buffaloes attained sexual maturity around 3 to 3½ years and calved at the age of 4 to 4½ years. The average values for morphological traits viz., height at withers, body length, heart girth and barrel girth were recorded as 115 ± 0.79, 122.91 ± 0.93, 170.86 ± 1.13 and 184.5 ± 1.17 cm, respectively. The milk yields in the females ranged from 500 to 1500 kg in lactation periods ranging from 265 to 325 days. They are regular calvers with a calving interval of 14 to 16 months. The mean lactation yield estimate was 765.2 ± 8.9 litres, ranging from 426.8 ± 27.4 to 1196.6 ± 57.1 litres. The mean lactation length observed was 266.7 ± 1.6 days and ranged from 163.0 ± 5.1 to 359.3 ± 5.3 days.

Sirothia et al. (2004) recorded a peak milk yield of 7.14 kg in Nagpuri buffaloes, while the daily yield and lactation yield were
5.15 kg and 1038.9 kg, respectively. In case of farm bred Surti buffaloes maintained at AICRP on buffaloes at Dharwar, Jahageerdar et al. (1997) observed values of 5.5 ± 0.1 kg and 1232 ± 38 kg for peak yield and total lactation yield, respectively.

2.2 Phenotypic characters in breed characterization

Breed characterization based on phenotypic characters has been a routine practice in the last several decades. FAO (1986) has developed detailed breed descriptors only for cattle. However, in case of cattle, due to introduction of exotic breeds for crossbreeding with indigenous cattle of India, there has been a dilution of the various characters among the noted breeds. Thus, this has necessitated the re-characterization of cattle available with the farmers to initiate conservation and improvement programmes of indigenous breeds of cattle in India.

India is the home of the best available buffalo germplasm in the world, exhibiting a large amount of genetic diversity amongst the existing breeds. Hence the question of crossbreeding does not arise in buffaloes. However, lack of scientific programmes with organized efforts for efficient improvement, absence of organized farms and uncontrolled large scale slaughter has led to deterioration in the genetic qualities of buffaloes. Apart from the recognized buffalo breeds, not much work has been done to characterize the other buffaloes spread in large numbers all over the country.

Studies on morphological traits have been carried out in certain recognized breeds of buffaloes viz., Murrah (Jawarkar and
Johar, 1975; Manik and Iqbalnath, 1981), **Paralakhemundi** (Patro and Mishra, 1986), Surti (Jogi and Patel, 1990), Ellichpuri (Ali et al., 1994), Mehsana of Gujarat (Singh et al., 1995), South Kanara (Shashidhara, 2002), Tarai buffaloes maintained by the farmers in Uttaranchal (Prasad, 2004) and Bhadawari of Uttar Pradesh (Sharma et al., 2004). Bhat et al. (1981) reviewed the physical and functional characteristics of the different livestock breeds including buffaloes, spread across the country.

Saini and Gill (1987) studied the relationship among different physical characteristics in Murrah type heifers and dry buffaloes, while Patil and Ulmek (2002) studied the influence of lactation number on some physical characteristics of Pandharpuri buffaloes.

Govindaiah and Rai (1984) indicated that the buffaloes seen in the Northern Districts of Karnataka state are variably called as Dharwari, and those strains of Dharwari buffaloes are referred as “Holesalu”, Mudalgi and Gowli buffaloes depending on locality or ownership.

In India, the National Bureau of Animal Genetic Resources (ICAR), Karnal, Haryana, has initiated Network projects under which survey, characterization and evaluation of different livestock breeds including buffaloes have been carried out. This is restricted to the recognized buffalo breeds available in India. One such completed work was the survey, evaluation and characterization of Nagpuri buffaloes in its breeding tract in Vidharbha region (Sirothia et al., 2004). Another network project completed was the survey and characterization of Jaffarabadi buffalo. Other buffalo breeds being studied are the Toda and Pandharapuri. Characterization of
local buffaloes was also undertaken under the NATP project “Animal Genetic Resources Biodiversity” (Prasad, 2004).

Sadana et al. (2004) have pointed out that, apart from established buffalo breeds, there are some defined populations which have potentials of a 'breed', and hence warrant greater attention. Perpetuated under geographic isolation and local milieu, these populations have developed adaptive characters which are sometimes unique, such as Chilika buffaloes. Some defined populations in buffaloes include Kujang, Chilika, Jerangi, Parlakhemundi, Manda, Kalahandi, Sambhalpuri (Orissa), swamp buffaloes (Assam), Kuttanad (Kerala), Godavari (Andhra Pradesh), South Kanara (Karnataka), Sikamese (Sikkim) and Tarai (Uttar Pradesh). The authors have stressed upon the need to carry out detailed studies on type, contribution and potential of these populations so as to consider them for inclusion in the listed breeds of the country.

2.3 Chromosomal Profile in Breed Characterization

Mammalian cytogenetics gained impetus with the discovery of colchicine, a mitotic spindle formation inhibitor (Levan, 1938), the discovery of sexual dimorphism in neurons of cat (Bar and Bertran, 1949) and the accidental discovery of hypotonic treatment (Hsu, 1952).

The basic short term tissue culture technique for the chromosome preparations from blood samples of human was reported by Hungerford et al. (1959) and Moorhead et al. (1960)
and it was successfully adapted to domestic animals (Nichols et al., 1962; Sasaki and Makino, 1962; Basrur and Gilman, 1964).

The discovery of 1:29 Robertsonian translocation in cattle by Gustavsson and Rockborn (1964) and the first heterozygous translocation in pigs by Henrickson and Backstorm (1964) renewed the interest and beginning of livestock genetics.

2.3.1 Technical developments in cytogenetics

2.3.1.1 Mitogen

Phytohaemagglutinin (PHA) was serendipitously discovered as mitogen by Nowell (1960) while using it to separate leucocytes from whole blood due to its powerful erythroagglutination property. PHA, extracted from red kidney bean (Phaseolus vulgaris) was found to stimulate the T-lymphocytes. Later on, pokeweed mitogen (PWM), a lectin from the roots of Phytolacca americana was discovered by Franes et al. (1964). PWM stimulated both T-cells and B cells.

PHA and PWM mitogens have been used widely to stimulate multiplication of buffalo lymphocytes in in vitro cultures (Rathnasabhapathy and Ganesh, 1980; Thiagarajan, 1987; Ramesha, 1988). However, PHA was found to agglutinate buffalo blood cells and consequently resulted in poor cultures compared to PWM (Bongso and Hilmi, 1982; Thiagarajan, 1987; Ramesha and Hegde, 1992). Joshi et al. (1999b) used both PHA and PWM, both of which yielded good results, but recorded PWM to be superior over PHA.
2.3.1.2 Hypotonic treatment

Hypotonic treatment was accidentally discovered by Hsu (1952), who observed that chromosomes could be clearly visualized in cells treated with hypotonic solution instead of isotonic solution. Hypotonic treatment causes the swelling of lymphocytes that enables better separation of chromosomes and better chromosomal spreads. A hypotonic potassium chloride solution was first recommended by Hungerford (1965). Most of the workers used 0.075 M potassium chloride as standard hypotonic solution. However, the duration of hypotonic treatment varied from eight to 30 minutes in different laboratories.

2.3.1.3 Tissue culture media and supplements

Fisher (1948) introduced a synthetic medium for tissue culture. He used dialysed plasma as a basal medium and systematically replaced it with a mixture of amino acids, which were biologically active nutrients. Morgan et al. (1950) prepared a completely defined medium that was capable of maintaining chick embryo cells alive for a few weeks, subsequently called as the TC-199 medium which was used widely in animal cell culture laboratories.

Buffalo lymphocytes were successfully cultured in TC-199 medium enriched with glutamine and L-cystine (Sethumadhavan, 1978; Rathnasabhapathy and Ganesh, 1980). Parker-199 medium (Gupta and Raychaudhuri, 1978) and RPMI 1640 medium (Bongso and Hilmi, 1982). Joshi and Govindaiah (1999) used 6 ml of culture
medium as against 10 ml commonly used, without affecting the results and also reduced the cost of the medium.

2.3.1.4 Role of serum

Serum was recognized as an essential component of the TC medium for induction and maintenance of growth of lymphocytes. Human inactivated serum (Gupta and Raychaudhuri, 1978), autologous plasma (Rathnasabhapathy and Ganesh, 1980), foetal calf serum (Bongso and Hilmi, 1982) and adult cattle serum (Goswami and Balakrishnan, 1983) had been incorporated in the tissue culture media at various levels to culture buffalo lymphocytes. However, Eldridge (1982) avoided the addition of serum or plasma from exogenous source. Likewise, Joshi and Govindaiah (1999) also avoided supplementing culture medium with exogenous serum/plasma and did not observe any adverse affect on the growth of the lymphocytes.

2.3.2 Standardization of chromosome nomenclature

The chromosome nomenclature of domestic animals was standardized during First International Conference on Domestic Animals held at University of Reading, England, in 1976 (Ford et al., 1980). It was recommended to follow strictly the systems developed for the description of human banded chromosomes. The chromosomes were distinguished by the following structural features viz., a) the length of each chromosome relative to the total length of the normal autosomes and X containing haploid set, b)
the ratio of length of longer arm relative to length of its short arm and c) the centromeric index expressed as the ratio of the length of shorter arm to the total length of the chromosome. The species discussed during the conference were cattle, sheep, goats, pigs, horses, cats and rabbits. However, the buffalo being dominant and an important domestic animal in Indian subcontinent and South East Asia was left untouched.

2.3.3 Evolution of buffalo chromosome:

The taxonomic classification of buffalo as cited by Cockrill (1974) is as follows:

Class : Mammalia
Order : Artiodactyla
Suborder : Ruminantia
Family : Bovidae
Sub family : Bovinae
Tribe : Bovini
Genus : a) Syncerus (African buffalo)
        b) Bubalus (Asian buffalo).

There are three distinct species of Asian buffaloes viz., Bubalus arni (water buffalo), Bubalus depressicornis (The Anoa of Indonesia) and Bubalus mindorensis (Tamarao of Phillipines). There are two subspecies of B. arni viz., river buffaloes of Indian subcontinent and swamp buffalo of South-East Asian origin.

There are two distinct species of African buffalo viz., Syncerus caffer caffer (Cape buffalo) and Syncerus caffer nanus (Congo buffalo).
Family bovidae is characterized by a remarkably constant *nombre fundamental (NF)*, varying only between 58 and 62. This feature was regarded as the strong evidence for hypothesis that the chief mechanism of karyotype evolution in the bovidae was Robertsonian fission or fusion (Wurster and Benirschke, 1968).

The banding homology among the chromosomes of goats, sheep and oxen was described by Evans *et al.* (1973). Based on banding pattern studies, homology was reported between the chromosomes of Australian swamp buffalo, cattle, sheep and goat (Toll and Halnan, 1976b), between Murrah buffalo and Sahiwal cattle (Gupta and Raychaudhuri, 1978) and between swamp and river buffalo (Bongso and Hilmi, 1982; Berardino and Iannuzzi, 1981).

Comparison of banding pattern of *Bubalus bubalis* (2n = 50) and *Bos taurus* (2n = 60) revealed similar autosomes in both species. In the former group, the five pairs of submetacentrics corresponded to centric fusion of chromosome between 1-29, 2-22, 8-19, 5-28 and 16-25 in the latter group (Berardino *et al.*, 1981).

Bongso and Hilmi (1982) reported that the numerical polymorphisms between river and swamp buffalo were due to a balanced tandem fusion between both members of chromosome 4 and 9 of the Murrah karyotype. This tandem fusion resulted in reduction of diploid number of swamp buffaloes by two and it was responsible for appearance of chromosome pair 4 as the largest autosomal metacentric pair.
The number of metacentric and acrocentric chromosomes in different species of buffaloes reviewed by Basu (1985) is presented in Table 2.2.
<table>
<thead>
<tr>
<th>Species</th>
<th>Diploid Number</th>
<th>Metacentric chromosomes</th>
<th>Acrocentric chromosomes</th>
<th>Sex chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swamp buffalo</td>
<td>48</td>
<td>10</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>River buffalo</td>
<td>50</td>
<td>10</td>
<td>38</td>
<td>2</td>
</tr>
<tr>
<td>African buffalo</td>
<td>52</td>
<td>8</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>Congo buffalo</td>
<td>54</td>
<td>6</td>
<td>46</td>
<td>2</td>
</tr>
</tbody>
</table>

(Basu, 1985).

### 2.3.4 Chromosome number and morphology in buffaloes

Pachakadze (1939) was first to study the chromosomes of water buffaloes in Russia and reported a diploid number of $2n = 56$. Later on, using testicular tissues, the chromosome number of water buffaloes was reported as $2n = 48$ (Malkino, 1944; Dutt and Bhattacharya, 1952; DeGirolamo, 1956). Subsequently, with the development of short term lymphocyte culture technique, many investigators established the chromosome number of Indian water buffaloes as $2n = 50$ (Fisher and Ulbrich, 1968; Chandra, 1968; Chakrabarti and Benjamin, 1980). However, there were few reports indicating the chromosome complement as $2n = 48$ in Murrah crossbred buffaloes (Sethumadhavan, 1978) and in Parlakhemundi buffaloes of Orissa (Bidhar et al., 1986). Yadav et al. (1988) also reported the presence of chromosome configuration typical to that
of swamp buffaloes in few buffaloes from Orissa and Assam. Joshi and Govindaiah (1997) also recorded a diploid chromosome number of 50 in South Kanara buffaloes of Karnataka, and the morphology of the chromosomes was found to by typical of river buffaloes.

In swamp buffaloes of South East Asia, a diploid number of $2n = 48$ was established by Ulbrich and Fisher (1967) and Dehonat and Ghanam (1971).

The Chinese buffaloes (Huang et al., 1937) and Australian swamp buffaloes (Toll and Halnan, 1976a) were found to possess a chromosome complement of $2n = 48$. Letts (1962) had indicated that the Australian swamp buffaloes were believed to be descendants of Asian buffaloes. However, Sri Lankan swamp buffaloes were found to possess a chromosome complement of $2n = 50$ (Scheurmann et al., 1974) and were believed to be descendants of Indian river buffaloes which might have acquired swamp buffalo habits (Bongso et al., 1977).

The African buffaloes from Kenya (S. caffer caffer) possessed a diploid number of $2n = 52$ (Ulbrich and Fisher, 1967), whereas Congo buffalo (S. caffer nanus) had a chromosome complement of $2n = 54$ (Wurster and Benirschke, 1967). Rasali et al. (1998) also observed a diploid chromosome complement of 50 in indigenous buffaloes of Western hills of Nepal.

The chromosome complement of Indian water buffalo was composed of 24 pairs of autosomes and one pair of sex chromosomes. The first four pairs of autosomes were submetacentric and the fifth pair was nearly metacentric in
morphology, with the rest of the autosomes being acrocentric. The X chromosome was the largest acrocentric while the Y chromosome was one of the smaller acrocentrics and was not always identifiable (Gupta and Raychaudhuri, 1978; Chakrabarti and Benjamin, 1980). Based on G-and C-band studies, Yadav and Balakrishnan (1982) attributed that Y chromosome was equal in length to that of chromosome pairs 19 and/or 20.

The diploid chromosome number of riverine, swamp and other buffaloes in various countries is presented in Table 2.3.

2.3.5 Chromosome Banding

Caspersson et al. (1969) were responsible for revolutionizing and heralding the era of chromosome banding with the introduction of fluorescent acridine derivative (quinacrine mustard) for staining the chromosome. They demonstrated that chromosomes were not homogenous organelles but were possessing very precise colouration qualities, which permitted identification of numerous structures and/or bands characteristic of each chromosome pair. Since then many banding techniques have been evolved to delineate the chromosomes in all species of livestock.

Table 2.3: Diploid chromosome number of riverine, swamp and other buffaloes in various countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Breed /type</th>
<th>No.(2n)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkey</td>
<td>Turkish Buffalo</td>
<td>50</td>
<td>Ulbrich and Fisher (1967)</td>
</tr>
<tr>
<td>Egypt</td>
<td>Water buffalo</td>
<td>50</td>
<td>Dehonat and Ghanam (1971)</td>
</tr>
<tr>
<td>Sri Lanka</td>
<td>Water buffalo</td>
<td>50</td>
<td>Scheurmann et al. (1974)</td>
</tr>
<tr>
<td>Country</td>
<td>Breed</td>
<td>Population</td>
<td>References</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>India</td>
<td>Water buffalo</td>
<td>50</td>
<td>Gupta and Raychaudhuri (1978)</td>
</tr>
<tr>
<td></td>
<td>Murrah</td>
<td>50</td>
<td>Yadav (1981)</td>
</tr>
<tr>
<td></td>
<td>Surti</td>
<td>50</td>
<td>Balakrishnan et al. (1988)</td>
</tr>
<tr>
<td></td>
<td>Jaffrabadi</td>
<td>50</td>
<td>Thiagarajan (1987)</td>
</tr>
<tr>
<td></td>
<td>Toda</td>
<td>50</td>
<td>Kumar and Yadav (1991)</td>
</tr>
<tr>
<td></td>
<td>Mehsana</td>
<td>50</td>
<td>Joshi and Govindaiah (1999)</td>
</tr>
<tr>
<td></td>
<td>South Kanara</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td>Iranian buffalo</td>
<td>50</td>
<td>Khavary (1978)</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Swamp buffalo</td>
<td>48</td>
<td>Balakrishnan et al. (1988)</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Water buffalo</td>
<td>48</td>
<td>Malkino (1944)</td>
</tr>
<tr>
<td>Australia</td>
<td>Swamp buffalo</td>
<td>48</td>
<td>Toll and Halnan (1976a)</td>
</tr>
<tr>
<td>Thailand</td>
<td>Swamp buffalo</td>
<td>48</td>
<td>Rommelt (1977)</td>
</tr>
<tr>
<td>Malaysia</td>
<td>Swamp buffalo</td>
<td>48</td>
<td>Bongso and Jainudeen (1979)</td>
</tr>
<tr>
<td>China</td>
<td>Chinese buffalo</td>
<td>48</td>
<td>Huang et al. (1987)</td>
</tr>
<tr>
<td>Africa</td>
<td>African buffalo</td>
<td>52</td>
<td>Ulbricht and Fisher (1967)</td>
</tr>
<tr>
<td></td>
<td>African buffalo</td>
<td>54</td>
<td>Cribui and Popescu (1980)</td>
</tr>
<tr>
<td>Congos</td>
<td>Congo buffalo</td>
<td>54</td>
<td>Wurster and Benirschke (1967)</td>
</tr>
</tbody>
</table>

Dutrillaux et al. (1971) were first to adopt an enzyme digestion of chromosomes using pronase as pre-treatment and obtained banding of human chromosomes. Summer et al. (1971) achieved G-band through incubation of slides in various salt solutions followed by staining with Giemsa (acetic/saline/Giemsa technique). Subsequently, Drets and Shaw (1971) modified this method by treating the chromosome preparations with sodium hydroxide (alkaline/Giemsa technique). A rapid G-band technique involving trypsin pretreatment of chromosome was proposed by Seabright (1971) which is the most common method being followed at present. The trypsin hydrolyses the protein component of nucleoproteins which was denatured during fixation and thus allowed giemsa to act on the exposed DNA (Wang and Fedroff, 1972). G-banding was also produced using protein denaturing substances like sodium dodecyl sulphate and sodium lauryl sulphate (Shiraishi and Yosida, 1972).
2.3.5.1 Standardized G-Banded karyotype

G-banded karyotypes in majority of domestic animals were standardized during the First International Conference in 1976 (Ford et al., 1980). The ultimate objective was the description of main G-band pattern of the chromosomes with sufficient details to permit the unequivocal identification of individual chromosomes. The description of G banded chromosome proceeded from the general to the particular order of whole chromosomes, parts or segments, down to the individual bands. The focal point of description of G banding patterns was centromere, since the nomenclature and definition of each chromosome pair emerged from this point. The chromatid arms of the chromosomes were given the normal designation of ‘p’ for short arm and ‘q’ for long arm. The Second International Conference held at Paris improved the G-banded standard karyotype established in the First International Conference at Reading convention with the aid of results obtained from prometaphase chromosome spreads (Anon., 1989).

Berardino et al. (1981) suggested that the existence of extensive homology in the banding pattern among the chromosomes of cattle and buffaloes would facilitate in establishing the standard karyotype of Murrah buffaloes of the Indian subcontinent.

2.3.5.2 G-banding in buffaloes

Toll and Halnan (1976b) reported G-band homologies among the chromosomes of Australian swamp buffaloes, cattle, sheep and
goats. Comparing the G-band homologies of Murrah with Indian cattle, Gupta and Raychaudhuri (1978) opined that the differences in configuration were due to Robertsonian translocation. Based on G banding pattern of Murrah and swamp buffaloes, Bongso and Hilmi (1982) confirmed the existence of numerical polymorphism arising due to a balanced tandem fusion between chromosomes 4 and 9 of Murrah karyotype.


Rasali et al. (1998) recognized Y-chromosome as a small acrocentric chromosome with a dark band at the terminal end of q arm in GTG banded metaphase field, which feature had also been recorded by Singh and Nivsarkar (1997) in Bhadawari buffaloes.

**2.3.6 Chromosomal morphometric parameters**

**2.3.6.1 Relative length**

The lymphocytes in culture respond to colchicine treatment depending on the stage of cell cycle. Consequently, the chromosomes tend to vary in length from cell to cell due to condensation process. Even among the members of a pair of
homologous chromosomes variations were recorded (Ford, 1973). Therefore, the concept of relative length at chromosomes was introduced as a parameter to compare the chromosome length reported in different karyotypes.

Gupta and Raychaudhuri (1978) studied the chromosome morphometric parameters in Murrah buffaloes and indicated that the largest autosome contributed 5.51 per cent while the smallest autosome was 1.96 per cent of the total female haploid set. The relative length of 'X' chromosome was 6.52 per cent and that of 'Y' chromosome was 1.89 per cent.

Yadav (1981) reported differences between animals or sex in relative lengths of chromosomes 1, 2, 18 and 24 in Murrah and Surti breeds. The relative length of X chromosome was more than six per cent of the total genome. More variability was also evinced in X and Y chromosomes.

In Paralakhemundi buffaloes of Orissa, the relative length of chromosomes ranged from 5.7 to 0.86 per cent (Bidhar et al., 1986). Sharar et al. (1989) observed mean relative lengths of 8.75, 1.86 and 4.72 per cent for the first and 24th pair of autosomes and X chromosome respectively, in Murrah buffaloes.

Kumar and Yadav (1991) compared the relative length in Mehsana, Murrah and Surti buffaloes and observed differences between the length of longer (1-5) and shorter (20-24) autosomes. The middle group of autosomes and X and Y chromosomes showed a close similarity in length. However, in Mehsana buffaloes, the submetacentric chromosomes were found to be
shorter and acrocentric chromosomes were longer than those observed in the other two Indian breeds of buffaloes.

Ramesha and Hegde (1992) reported relative length of 7.33, 2.13 and 6.70 per cent for the longest and shortest autosomes and X chromosome, respectively, in Surti buffaloes. The corresponding figures for local buffaloes were 7.01, 2.26 and 6.68 per cent, respectively.

Joshi and Govindaiah (1997) reported overall relative lengths of 6.80 ± 0.17, 1.67 ± 0.03, 6.46 ± 0.09 per cent for the first and 24th autosomes and X chromosomes in South Kanara buffaloes.

2.3.6.2 Centromeric index

The centromeric index is the ratio of short arm of the chromosome to its total length and is expressed in percentage. The centromeric index value indicates the position of centromere in the chromosome which enables to classify the chromosomes. Alteration in this index implies variation in the concerned chromosome pair.

Gupta and Raychaudhuri (1978) reported centromeric indices of 27.36, 30.70, 37.49, 32.56 and 40.45 per cent, respectively, for the first five biarmed pairs of chromosomes in Murrah buffaloes.

Yadav (1981) reported lowest centromeric index of 27.9 per cent for the first pair of chromosomes in Murrah and Surti
buffaloes. The 2nd and 4th had slightly higher index values of 32.3 and 33.6 per cent, respectively. The values for 3rd and 5th pairs were found to be the highest at 41.3 and 42.1 per cent, respectively. The centromeric index value for submetacentric chromosomes varied from 41.17 to 48.80 per cent in Paralakhemundi buffaloes of Orissa (Bidhar et al., 1986), while Sharar et al. (1989) observed centromeric index values of 38.17, 37.95, 35.97, 38.80 and 48.72 per cent, respectively, in biarmed chromosomes, one to five, in Murrah buffaloes.

Ramesha and Hegde (1992) reported centromeric index values of 27.35, 30.70, 35.00, 36.17 and 36.42 per cent, respectively, for the first five pairs of biarmed chromosomes in Surti buffaloes, while the corresponding figures for local non-descript buffaloes were 24.41, 28.45, 33.94, 33.02 and 38.37 per cent, respectively.

In South Kanara buffaloes, the centromeric values were found to range between 41.88 ± 0.08 for the 5th pair and 25.32 ± 0.43 for first pair of chromosomes by Joshi et al. (1999a), with the values being significantly affected by the genetic groups on 3rd and 5th pair of chromosomes.
2.4 Molecular genetic markers for genome analysis

A genetic 'marker' can be defined as any stable and inherited variation which is measurable or detectable by using a suitable method and can subsequently be used to detect the presence of a specific genotype or phenotype other than itself, which is otherwise not measurable or difficult to detect (Beckmann and Soller, 1987). The variations that crop up at various levels viz., morphological or anatomical, physiological, chromosomal, biochemical and DNA sequence level, can serve as genetic markers. Those markers detecting polymorphism directly at DNA sequence level are termed as molecular genetic markers (DNA markers).

Morphological or anatomical markers are called as "classical markers", while certain chromosomal abnormalities (numerical or structural), which serve as markers are called as "chromosomal markers". Apart from these, the variation in macromolecules present in body fluids and tissues that can be detected by immunological (e.g. blood groups, MHC, etc..) and electrophoretic (e.g., isoenzymes, milk proteins, blood proteins, etc..) methods, also serve as markers known as "biochemical" markers. Classical and chromosomal markers are not much useful for genome analysis, due to their low degree of polymorphism or heterozygosity. Besides, some of the lacunae like expression of concerned genes for identification, sex limited nature of genes during expression and finally dominance and pleiotropic effects of genes, which does not allow us to distinguish some of the genotypic classes at the phenotypic level, may hinder the use of biochemical markers as genetic markers.
2.4.1 Genomic markers

Rapid growth achieved in the field of molecular biology, in the recent past, has allowed the use of new class of markers called "molecular markers" or "genomic markers". One of the major areas of interest is the study of DNA markers for quantitative trait loci (QTLs).

The advent of polymerase chain reaction technique opened a new front in the field of molecular biology. The use of PCR by Saiki et al. (1988) to amplify a DNA sequence of interest and subsequent restriction enzyme analysis of the amplified product (PCR-RFLP) by Pinder et al. (1991) staged a lightning raid deep inside this field of molecular biology. Limitless number of genetic polymorphisms at the DNA sequence level has bestowed upon a number of genomic markers such as Randomly Amplified Polymorphic DNA (RAPD) markers (Williams et al., 1990; Welsh and McClelland, 1990), RFLPs (Botstein et al., 1980), minisatellites also known as Variable Number of Tandem Repeats (VNTR) (Jeffreys et al., 1985; Nakamura et al., 1987), microsatellites (Litt and Luty, 1989; Weber and May, 1989; Beckmann and Soller, 1990; Tautz, 1990; Fries et al., 1990), Amplified Fragment Length Polymorphism (AFLPs) (Vos et al., 1995) and Single Nucleotide Polymorphism (SNPs) (Marth et al., 1999).

A large amount of data has been piled up since the first demonstration of Restriction Fragment Length Polymorphism (Grodzicker et al., 1974), and the utility of such markers in human genetics (Botstein et al., 1980) and genetic improvement in plants (Beckmann and Soller, 1983; Burr et al., 1983) and in animals (Soller and Beckmann, 1983) has been studied extensively. Also,
more powerful and less laborious techniques like oligonucleotide polymorphisms are being landed onto this field to discover new types of genomic markers (Beckmann, 1988). Thus, these techniques helped in evolving sufficient polymorphic markers with which the entire genome of most species can be marked with DNA markers every 5 cM or less (Kappes et al., 1997).

2.4.1.1 Randomly Amplified Polymorphic DNA

A simple alternative technique that can be used for the amplification of variable DNA structures is the polymerase chain reaction (PCR) by randomly chosen primers applied at low annealing temperature.

PCR technique was first established by Williams et al. (1990), who used short decamer primers, and called the product Randomly Amplified Polymorphic DNA, and by Welsh and McClelland (1990, 1991), who employed primers of 20 to 30 nucleotides in length and named the procedure as arbitrarily primed PCR. This technique generates DNA fingerprints based on random amplification of DNA by PCR and these fingerprint products were called as Random Amplified Polymorphic DNA markers. RAPD technique involves use of short decamer oligonucleotide primers annealing at low temperature. The number and size of the RAPD products depend on the complimentarity of sequences of the particular primer and template DNA. Different primers produce different RAPD polymorphisms. These variations may be due to differences in spacing between primer binding sites as well as point mutations, insertions, deletions and inversions which allow or abolish primer binding sites (Gwakisa et al., 1994). The RAPD polymorphisms presumably are based on mismatches in primer binding sites or
insertion/deletion events, and therefore usually result in the presence or absence of amplified products from a single locus (Williams et al., 1990; Welsh and McClelland, 1991). RAPD polymorphisms may also arise, if secondary structures form around the primer annealing sites during the relatively low annealing temperature (Bowditch et al., 1993).

RAPD-PCR is a simple technique and easy to analyze. It doesn't require radioactively labelled nucleotides. RAPD markers follow Mendelian laws of segregation. It requires very small amount (in nanogrammes) of genomic DNA, and is subjected to PCR using random sequence oligonucleotide primers. In RAPD-PCR, only a single random oligonucleotide primer is employed, whereas in standard two primers mediated PCR amplification, two primers are required. RAPD doesn't require any prior knowledge of nucleotide sequence since primers are chosen arbitrarily and many organisms can be mapped with the same set of primers. It is based on the principle that, when the primer is short (e.g. 8 to 10 mer), there is a high probability that the genome contains several priming sites close to one another that are in an inverted orientation. The technique essentially scans a genome from these small inverted repeats and amplifies inverting DNA segments of variable length. The amplification products are then resolved on agarose gel electrophoresis resulting in DNA fingerprints (bands). Since, primers of arbitrary nucleotide sequences are used in this technique to access random segments of the genomic DNA to reveal polymorphism, these markers are named as Randomly Amplified Polymorphic DNA (RAPD) (Williams et al., 1990).

Williams et al. (1990) explained that nearly all RAPD markers were dominant, as DNA segments of same length were amplified
from one individual but not from another. The drawback of this technique was that it was not yet fully understood how the genetic variation observed was generated and consequently, it can be difficult to reconstruct evolutionary histories. The consistency of result was not guaranteed as minor differences in experimental conditions and different brands of Taq polymerases may give different amplification patterns and RAPD were dominant markers where heterozygotes were typically scored as homozygotes which decrease their information content (Meghen et al., 1994). Hence, RAPD may only be employed for analysis of genetic distances if reproducibility and Mendelian inheritances of the amplified DNA of interest are beyond doubt (Rothuizen and Wolferen, 1994). In spite of such considerations, it has been shown that the procedure of RAPD gives highly reproducible results under a standard protocol under optimum conditions (Welsh and McClelland, 1990; Welsh et al., 1991; Williams et al., 1990, Rothuizen and Wolferen, 1994).

2.4.1.1.1 Polymerase chain reaction

The polymerase chain reaction is a novel method for amplifying a selected DNA fragment. The technique has been widely acknowledged, within few years since its introduction (Saiki et al., 1988). In other words, PCR merely involves combining a genomic DNA with oligonucleotide primers, deoxyribonucleotide triphosphates, and the thermostable Taq DNA polymerase, in a suitable buffer. Then repeatedly heating and cooling the mixture for several hours until the desired amount of amplification is achieved (Saiki et al., 1989). This method was first invented by Kary Mullis and co-workers of the Cetus corporation, USA, in the year 1984 (Mullis and Fallona, 1987; Saiki et al., 1988). However, the principle of PCR had been discussed by Khorana and
colleagues a decade earlier (Kleppe et al., 1971; Panet and Khorana, 1974). In spite of its high sensitivity, the use of PCR was very limited until the thermostable DNA polymerase (Chien et al., 1976) became widely available.

**Principles and methodology of PCR**

The PCR is an *in vitro* technique, which allows the amplification of a specific deoxyribonucleic acid (DNA) region that lies between two regions of known DNA sequence. Knowledge of the nucleotide sequence of entire target DNA is also not required. However, it is essential to have the knowledge of the nucleotide sequence of short segments of 25 to 30 nucleotides, known as flanking sequences, at each end of target DNA. Two oligonucleotides, complementary to flanking sequences of each DNA strand are first synthesized. These oligonucleotides, acting as primers, which are also called as amplimers, are responsible for the specificity of target DNA.

The PCR programme commonly used for RAPD analysis with random decamer primer includes one minute template denaturing step at 94°C, one minute primer annealing step at 35°C and two minutes primer extension step at 72°C. Usually 45 cycles of these steps are run to obtain RAPD pattern. Several modifications were made later on to shorten the length of PCR programme. Gwakisa et al. (1994) and Kangfu and Pauls (1992) used a very shorter version of the programme. A five second denaturing, five second annealing and two minute extension duration, respectively, at 94°C, 35°C and 72°C for 40 cycles, were successfully used and obtained very reliable results. Nagaraja (1998) carried amplification with initial denaturation at 95°C for 2 minutes, followed by 30 second
denaturation at 94°C, 30 second annealing at 35°C and one minute extension at 72°C for 35 cycles. One final cycle of 2 minute denaturation, 2 minutes annealing and 10 minutes extension was included in the programme.

The technique of PCR, often regarded as cell-free molecular cloning, involves initial denaturation of DNA, in which the double stranded target DNA is heated to separate into single strands, which are annealed with primers, in which the single DNA strands are cooled and allowed to anneal with the amplimers (one for each strand) complementary to the flanking sequences, and followed by DNA amplification, in which on addition of enzyme DNA polymerase and the substrate deoxyribonucleotide triphosphates, the synthesis of new DNA strands occurs. These strands exist at this stage as double stranded DNA molecules.

Strand synthesis can be repeated, by repeating cycles again and again. The entire cycles are repeated many times. Since the products serve as template for the next, each successive cycle essentially doubles the amount of the desired DNA sequence.

In the early years of PCR development, DNA polymerase of E coli was used which had several disadvantages. This enzyme, being thermolabile, was destroyed in initial cycle of denaturation, for which high temperature is required. It had to be replenished after each denaturation step. Yield was also poor and the products were often heterogeneous in size (Scharf et al., 1986), probably because of "mis-priming". In addition some of the products were "shuffled" and consisted of the mosaics of different alleles of the target sequences (Saiki et al., 1988). Heat stable DNA polymerase such as Taq polymerase, derived from a bacterium Thermus
* aquaticus * that lives at 90°C, is now employed. The enzyme remains active after every heating step and need not have to be replenished. Also the enzyme is active at higher temperature at which annealing of the primers is more specific and DNA synthesis is more rapid. This resulted in a substantial improvement in the specificity and yield of the amplification, which ultimately led to the automation of PCR (Saiki *et al*., 1988).

### 2.4.1.1.2 DNA Isolation

The basic requirement for genome analysis is the isolation of DNA. Biological samples especially blood and semen have been shown to be the ideal sources of nucleated cells for the isolation of genomic DNA from farm animals.

The primary objective of the isolation process is to recover the maximum yield of high molecular weight DNA devoid of protein and other enzyme inhibitors (Sambrook *et al*., 1989). Common methods for the isolation of DNA from nucleated cells involve several extractions with phenol and chloroform (Blin and Stafford, 1976; Beckmann *et al*., 1986; Andersson *et al*., 1986). However, phenol was found to be corrosive and toxic and the extraction steps are time-consuming, limiting the number of samples that can be processed in a day (Montgomery and Sise, 1990).

Miller *et al*., (1988) developed high salt method which is rapid, safe and inexpensive for the purification of genomic DNA from human blood. This method involves salting out of the cellular protein by dehydration and precipitation with a saturated solution of sodium chloride, thereby avoiding the use of hazardous organic solvents like phenol and chloroform. The DNA obtained from this
simple technique yielded quantities comparable to those obtained from phenol-chloroform extractions. The OD260nm/OD280nm ratios were consistently 1.8 to 2.0 demonstrating good deproteinization.

High salt method was found to be suitable for purification of DNA from blood samples of cattle (Gwakisa et al., 1994; Gelhaus et al., 1995). High salt method with an additional chloroform extraction method was followed for the isolation of genomic DNA from the blood samples of cattle (Kantanen et al., 1995; Aravindakshan and Nainar, 1998; and Nagaraja et al., 2003) and buffaloes (Annapoorni, 1996; Aravindakshan and Nainar, 1998), with good results.

### 2.4.1.1.3 Yield and Purity of DNA

Montgomery and Sise (1990) observed that the average yields of DNA from 100 samples of 20ml of sheep blood were 640μg for high salt method. The OD ratio of OD260/OD280 ranged from 1.6 to 2.0, which was sufficiently pure for PCR reactions (Sambrook et al., 1989; Koh et al., 1998).

Senthil (1995) extracted DNA from blood samples of cattle by high salt method and obtained pure DNA in the range of 450 to 800 μg with an average of 625 μg per 15ml of blood. Using a similar protocol, Annapoorni (1996) obtained a yield of DNA from buffalo blood in the range of 210 to 602 μg with an average of 400 μg per 10 ml.

Aravindakshan (1997) obtained pure DNA in the range of 246 to 572 μg with an average of 360 μg per 10 ml of cattle blood and
300 to 707 μg with an average of 452 μg per 10ml of buffalo blood, while Nagaraja (1998) extracted about 400 μg of pure genomic DNA from 10-15 ml of cattle blood by using high salt method with an additional chloroform extraction step.

Shashidhara (2002) obtained mean yield of DNA ranging from 392 to 687.5 μg from 10ml of whole blood of Murrah and South Kanara by the high salt method.

2.4.1.1.4 Components of PCR

Template DNA

Optimal concentration of template DNA per reaction may vary substantially from typical conditions (25 ng per reaction) depending on the primer-template combination used (Carlson et al., 1991).

Ivinson and Taylor (1992) suggested the use of 100 to 500 ng of genomic DNA as target in the case of single copy genes, although they observed that anything between 100 to 1000 ng produced satisfactory amplification.

Nagarajan (1998) did not detect any changes in the reproducibility of RAPD fingerprints, with varied concentrations of template DNA ranging from 10 to 100 ng per 30 μl of reaction mixture with increments of 50 ng of DNA from different breeds of sheep.
**DNA Polymerase**

The most commonly used Taq DNA polymerase is isolated from a bacterium *Thermus aquaticus*. A recommended concentration of Taq DNA polymerase varied between 1 and 2.5 units (Lawyer *et al.*, 1989) per 100 μl reaction mixture when other parameters are optimum. While optimizing a PCR, Innis and Gelfand (1990) recommended the testing of enzyme concentration ranging from 0.5 to 5 units per 100 μl of reaction mixture and assaying the result by gel electrophoresis.

The outcome of a RAPD fingerprint pattern may depend on the type of polymerase used. Schierwater and Ender (1993) reported that no substantial variation could be attributed to various available commercial preparations of the same enzyme. However, Meunier and Grimont (1993) reported that variability in commercial preparations of Taq DNA polymerase was a major source of variation in RAPD results, while Cushwa *et al.* (1996) reported that Taq DNA polymerase and the stoffel fragment of Taq analysis preferentially amplify a different size range of fragments. Jayasankar and Dharmalingam (1997) observed that different fingerprints were generated when the same DNA template was amplified with two different commercial brands of Taq DNA polymerases.

**Deoxyribonucleotide triphosphates (dNTPs)**

High purity deoxyribonucleotide triphosphates (dNTPs) are supplied by several manufactures either as four individual stock solutions or as a mixture of all four dNTPs. Many stock solutions now supplied are already adjusted to pH 7.5 with sodium hydroxide
(NaOH). PCR is normally performed with dNTP concentrations around 100 μm. At lower dNTP concentrations (10 to 100 μm) Taq DNA polymerase has a higher fidelity (Innis et al., 1988). Innis and Gelfand (1990) recommended the usage of dNTPs at equivalent concentration to minimize misincorporation errors and also further recommended the use of the lowest possible dNTP concentration appropriate for the length and composition of the target sequence. Theoretically 20 μm of each dNTP in a 100 μl of reaction is sufficient to synthesize 2.5 μg of DNA or 10p.mol of a 400 bp sequence. The optimal concentration of dNTP depends on several factors including MgCl₂ concentration, reaction stringency, primer concentration and length of the amplified product.

**RAPD Primers**

A single oligonucleotide primer of arbitrary sequence (typically 10 bases) is employed for the PCR reaction. The minimum useful primer stretch is an oligonucleotide of nine bases, and GC content in an oligonucleotide of decamer should be at least 40 per cent to generate detectable levels of amplification products (Williams et al., 1990).

**Magnesium ion (Mg2+) concentration**

The concentration of MgCl₂ can have dramatic effect on the specificity and yield in a PCR reaction. Concentration of magnesium in the PCR reaction mixture affects both the polymerase activity as well as efficiency of annealing. Jayasankar and Dharmalingam (1997) reported that the concentration of primer and MgCl₂ were critical vis-à-vis resolution and reproducibility of RAPD markers.
Innis and Gelfand (1990) observed that in general, insufficient Mg2+ led to low yield while excess Mg2+ caused the accumulation of non-specific products. Thus an optimum concentration of MgCl$_2$ in the final mixture was essential, and usually varied within the range of 0.5 to 5 mM.

Concentrations of MgCl$_2$ used by various workers ranged from 0.4 mM (Riedy et al., 1992) to 1.5 or 2.0 mM (Williams et al., 1990; Scott et al., 1992), 4.0 mM (Welsh and McClelland, 1990, 1991; Welsh et al., 1991) and 5.0 mM (Kubota et al., 1992).
Other components of PCR buffer

PCR buffer that contains 10 to 50 mM Tris HCl with pH between 8.3 and 8.8 at 20°C was recommended by Innis and Gelfand (1990). Moderate concentrations of KCl stimulate the synthesis rate of Taq DNA polymerase by 50 to 60 per cent with an apparent optimum at 50 mM. It has been observed that inclusion of 10 per cent DMSO facilitates certain PCR assays, though it is not clear which parameters of PCR are affected (Gelfand and White, 1990). However, higher concentration of KCl inhibits the activity and no significant activity is observed in a DNA sequencing reaction at greater than 75 mM KCl (Innis et al., 1988), while, low concentrations have no effects on the activity of Taq polymerase (Gelfand, 1989).

Some researchers preferred to include gelatin (Williams et al., 1990; Usha et al., 1995) or bovine serum albumin (Morris et al., 1994) and non ionic detergents like Tween 20 and Nonidet P 40 (Kemp and Teale, 1994; Gwakisa et al., 1994) in stabilizing the enzyme, although these did not seem to be absolutely essential (Innis and Gelfand, 1990).

2.4.1.1.5 Conditions of PCR

Thermal cycling parameters

Thermal cycling parameters are critical to a successful PCR amplification. These parameters include the reaction volume, time and temperature of different steps, the number of cycles and the ramp time.
Reaction Volume

Small volumes are preferred for batch screening of large number of samples because it saves the cost of reagents. However, when a very few samples are to be processed, it is desirable to use larger volume of 25 to 50μl. For preparative work, like plasmid insert amplification, 100 μl has been found necessary (Taylor, 1992).

Template denaturation

The polymerase chain reaction (PCR) program commonly used for RAPD analysis with random decamers included a one minute template denaturing step at 94°C, a one minute primer annealing step at 36°C and a two minutes primer extension step at 72°C (Williams et al., 1990).

Yu and Pauls (1992) recommended that the denaturing time should be as short as possible, which can probably be attributed to the fact that Taq DNA polymerase has a limited life span at high temperatures. Several workers (Van Ejik et al., 1992; Hoj et al., 1993; Kantanen et al., 1995; Gelhaus et al., 1995) have used template denaturation conditions of 94°C for 30 seconds.

Annealing of primers

The temperature and time required for primer annealing depend upon the base composition, length and concentration of primer pairs. For the primers containing 50 to 80 per cent GC, 30 seconds of annealing time appeared to be sufficient to obtain a complete RAPD pattern (Yu and Pauls, 1992).
Primer extension

For amplification of PCR products shorter than 1.5kb, 30 seconds of extension time was sufficient but longer PCR products required a longer extension time. Fragments as large as 3 kb were amplified with one minute extension time (Yu and Pauls, 1992).

Number of cycles

The number of cycles is usually between 25 and 35. However, in RAPD studies many researchers used 40 to 45 cycles (Nagaraja et al., 2003; Appannavar, 2001). No differences in banding pattern were found among PCR products obtained from programs run for 40 or 45 cycles (Nagarajan, 1998).

Ramp time

The ramp time is that taken to change from one temperature to the next in the thermal cycler, and is dependent on the equipment used. Generally, the fastest ramp, which can be attained, is used for PCR. The actual ramp times can be determined by measuring the sample temperature with a thermocouple probe.

2.4.1.1.6 Inhibitors of PCR

Heparin is a potent inhibitor of PCR and therefore should not be used as an anti-coagulant while collecting blood, if PCR is to be performed on the extracted DNA. Other substances in the blood, probably porphyrin compounds, are also strong inhibitors of PCR and these can be eliminated from the DNA preparations by lysis of
the blood cells and centrifugation to pellet the white cells (Sambrook et al., 1989).

Ionic detergents such as SDS used for DNA extraction can inhibit Taq DNA polymerase at concentrations higher than 0.01 percent. Taq DNA polymerase is susceptible to proteinase K digestion and so it must be removed or inactivated by thermal denaturation at 95°C. Residual traces of phenol, which also inhibits PCR, can be removed by chloroform-isoamyl alcohol extraction and ethanol precipitation (Newton and Graham, 1994).

2.4.1.1.7 Reproducibility of RAPD fingerprinting

One important factor determining the applicability of RAPD technique for molecular genetic studies is the reproducibility of the reaction products. Since RAPD is based on low annealing temperature, many scientists have questioned the suitability of this technique (Riedy et al., 1992; Meunier and Griment, 1993). The absence of amplification at higher temperature indicated that binding of the primers to the genomic DNA in the first cycle is partly nonspecific (Rothiuizen and Wolferen, 1994). It was speculated that the reproducibility as well as heritability of discrete amplification patterns would be insufficient for practical application. However, experiments in plants and animals have shown that inspite of several considerations, the procedure of RAPD gave highly reproducible results (Welsh and McClelland, 1990; Carlson et al., 1991; Halward et al., 1992; Rothiuizen and Wolferen, 1994).
2.4.1.1.8 Applications of RAPD

RAPD technique is useful in genetic mapping, strain identification, population characterization, paternity and kinship testing, sexing, taxonomic and phylogenetic studies and other genetic diversity studies (Welsh and McClelland, 1990; Gwakisa et al., 1994; Bardin et al., 1995; Cushwa et al., 1996; Chung et al., 1999; and Horng and Huang, 2000). This technique provides a rapid and less expensive tool to compare at large number of loci (Bailey and Lear, 1994). RAPD was used to detect polymorphism in the absence of specific nucleotide sequence information in DNA from bacteria, fungi, human and animal origin (Welsh and McClelland, 1990; Williams et al., 1990).

Cattle

RAPD fingerprinting has been widely used for breed characterization in cattle. Gwakisa et al. (1994) used this technique to characterize three zebu breeds of cattle. Out of 141 random primers used, two primers could discriminate between the breed specific DNA pools.

Kemp and Teale (1994) reported polymorphism in a ruminant repetitive DNA sequence which differentiated B. indicus and B. taurus using ILO 526 and ILO 876. The two primers yielded amplification products characteristic of each of the components of the Bos indicus pool (Boran, Brahman and Massai zebu), but absent in amplifications of the components of the Bos taurus pool (N'Dama, Gambian N'Dama, Gabon N'Dama Friesian, Caribbean Creole). They showed that by pooling DNA of individuals
representing diverse populations, even where those populations were themselves genetically heterogenous, it was possible to identify population specific DNA polymorphism by RAPD technique, very rapidly. The two major subspecies of domestic cattle *Bos taurus* and *Bos indicus* were distinguished by RAPD markers. Primer ILO 526 amplified a specific 0.5 kb only in *Bos indicus* cattle of African origin and not in *Bos taurus*. They opined that these markers would assist in the detection of crossbreeding between *Bos indicus* and *Bos taurus* animals. However, contrary to this Nagaraja *et al.* (2003) failed to detect 0.50 kb in *Bos indicus* cattle of South Indian origin.

Genetic variations in bovine population and their association with production traits were demonstrated by Bardin *et al.* (1995) using RAPD technique. They obtained reproducible electrophoretic patterns of 24 to 32 main bands for each sample in Italian Friesian and Grigia Alpina bulls.

Teale *et al.* (1995) determined a distinct product of approximately 1.1 kb, generated by a primer ILO 1065 which could differentiate males of *Bos indicus* from those of *Bos taurus* breeds of cattle. The product was produced with template DNA derived from males of the Boran, West African zebu and Sahiwal breeds, all of which are of *Bos indicus* type. This product was not evident in the amplifications of templates derived from males of the N'Dama, Friesian and Jersey breeds, all of *Bos taurus* type, or in amplification products of any of the female template DNAs, whether of *Bos indicus* or *Bos taurus* type. Antoniou and Skidmore (1995) identified a bovine Y Specific 3.1 Kb marker by RAPD technique, which was further used as a probe in a southern blot experiment which hybridized strongly to three Y linked sequences.
The RAPD method was used to detect genetic variation and characteristics among the Korean Native, Holstein, Charolais, Aberdeen-Angus and Hereford cattle (Jeon et al., 1998). The degree of band sharing between Korean native and Holstein cattle, Charolais, Angus and Hereford cattle was 0.82, 0.82, 0.86 and 0.84, respectively. The lowest genetic distance (0.52) was found between Korean native and Holstein cattle.

Nagaraja et al. (2003) used RAPD technique for genetic characterization of seven Bos indicus and two Bos taurus cattle breeds. According to them, ten out of the forty four random decamer oligonucleotide primers amplified cattle DNA, whereas only six of these primers produced polymorphic fingerprints. They also noticed that Primer ILO 1065 did not amplify male specific 1.13 kb fragment contrary to earlier reports. Similarly primer ILO 1127 failed to amplify a Bos indicus specific 0.97 kb marker. Primer ILO 1065 amplified least polymorphic fingerprints and was considered unsuitable for RAPD analysis, while ILO 1127 amplified two RAPD fragments of size 1.8 kb and 1.4 kb in Bos indicus cattle pool. However, amplifications were not there in all the individual breeds constituting this pool. Primer ILO 876 produced a 0.95 kb product similar to the one reported in African zebu breeds, but found in both Bos indicus and Bos taurus cattle breeds.

Band sharing and mean average percentage differences (MAPD) calculated within and between breeds showed a higher degree of homogeneity within than across the breeds and indicated measurable divergence between different breeds. The MAPD values ranged from 19.14 between Hallikar and Kangayam to 32.14 between Amrithmahal and Kangayam (Nagaraja et al., 2003).
Horng and Huang (2000) showed that primer OPE - 01 (5' CCAAGGTCC-3') in Holstein cattle produced a specific band peculiar to male cattle only.

A comparative analysis by Rincon et al. (2000) between Holstein Friesian, Creole and Hereford using RAPD markers revealed that band sharing frequency between Creole and Hereford cattle was 0.77 and between Creole and Holstein Friesian was 0.81.

Thiyagarajan (2000) used nine arbitrary primers to characterize four Indian zebu cattle breeds by RAPD technique. Out of nine primers used, only five primers gave reproducible DNA fingerprints. In his study, apparent breed specific bands in Umblacherry (0.8 kb), Kangayam (1 kb), Red Sindhi (0.6 kb) and Ongole (1.4 kb) for primer ILO 1127; Umblacherry (1.3 kb), Kangayam (1.1 kb), Red Sindhi (0.8 kb) and Ongole (0.77 kb) for primer ILO 526; Kangayam (1.6 kb), Red Sindhi 1 (1.5 kb) and Ongole (0.73 kb to 0.76 kb) for primer ILO 868; Kangayam (1.4 kb), Kangayam (1.3 kb), Red Sindhi (1.7 kb) and Ongole (1.3 kb) for primer BG 85 clearly differentiated zebu breeds from each other.

Chung et al. (1998) studied parentage testing and conducted pedigree analysis in Holstein dairy cattle by using RAPD- PCR. The investigation revealed that the RAPD products in offspring were always present in one or both parents, indicating that the RAPD markers were inherited in Mendelian fashion.

Appannavar et al. (2002) used the RAPD technique for genetic characterization of Deoni breed of cattle. Among the eight primers
that amplified genomic DNA, six primers (ILO 526, ILO 868, ILO 876, ILO 1065, OPAV 15 and OPAX 19) were reported to produce polymorphic fragments between genomic DNA of different Deoni types. Further they reported that the primer ILO 1065 can be used to differentiate the three types of Deoni cattle.

**Buffalo**

Annapoorani (1996) identified apparently breed specific markers in buffaloes using the RAPD technique, wherein two primers produced RAPD bands specific to Murrah and Surti breeds and thereby discriminated between the breed specific DNA pools.

Rao et al. (1996) evaluated the potential use of random amplified polymorphic DNA (RAPD) as a source of development of alternative genetic markers for studying variation in buffalo (*Bubalus bubalis*) and other related species of the Artiodactyla family Bovidae, in order to ascertain genetic relationships and diversities. Fourteen arbitrary primers were used to amplify DNA fragments in four species such as Indian Zebu cattle (*Bos indicus*), buffaloes (*Bubalus bubalis*), sheep (*Ovis aries*) and goat (*Capra hircus*). Clear and distinct RAPD patterns with a higher level of polymorphism were detected between species, while fewer polymorphisms were found within the species. Species were subsequently scored for presence or absence of RAPD fragments and Jaccard's similarity coefficients were calculated to quantify the genetic divergence among the species. Wagner parsimony analysis of the RAPD data for 542 markers resulted in one most parsimonious tree which revealed very low similarity among the four species analysed.
Aravindakshan and Nainar (1998) used RAPD-PCR analysis for characterization of different breeds of cattle and buffaloes. They demonstrated that using short random primers of arbitrary nucleotide sequence, it is possible to identify reproducible DNA markers, characteristic of individual animals or breeds of cattle and buffaloes. Further, they reported that the genetic variation within Murrah and Surti animals was more or less similar as revealed by the similar proportion of polymorphic RAPDs (60 % vs 59.6 %). They observed lower interbreed band sharing than the intrabreed band sharing indicating that the genetic divergence was higher between breeds than within breeds. Further, they reported narrow genetic divergence between Murrah and Surti breeds (MAPD = 24.16 ± 3.55) than between Jersey crosses and Ongole breeds (MAPD = 28.10 ± 10.53).

In studies carried out by Shashidhara (2002) in South Kanara and Murrah buffaloes, four out of 19 random primers were found to produce polymorphic RAPD fingerprints. Primer ILO 1127 produced two fragments of 428 and 461 bp specific to female South Kanara buffaloes. Primers ILO 868, BG 86 and OPAC 4 produced Murrah breed specific RAPD fragments of 110 bp, 638 bp and 325 bp, respectively. The MAPD for the four primers between Murrah and South Kanara buffaloes was 25.85 ± 4.28.

Saifi et al. (2003) suggested that primers OPB 07, OPG 05, OPG 13 and BG 28 could be useful in exploring genetic polymorphisms in buffalo breeds as they amplified buffalo genome satisfactorily. They reported a Bhadawari specific amplicon of 2445 bp using primer OPG 11. Primer BG 27 was found to resolve two distinct breed specific bands of 905 and 2385 bp in Murrah breed pool and three bands (650, 1050 and 1465 bp) unique to
Bhadawari breed pool. Further studies by these workers revealed a genetic distance of 0.088 between Murrah and Bhadawari breeds.

Saifi et al. (2004) carried out RAPD PCR analysis with a battery of 11 random decamer primers to study band frequency (BF), genetic identity index (I) and mean average percentage difference (MAPD) between Bhadawari and Murrah breeds of buffaloes. The primers OPA04 and BG15 resolved a band of 460 bp, which was present only in animals of Bhadawari breed. Whereas, the primers OPA14, BG27 and BG28 produced Murrah specific fragments of sizes 730 bp and 1,230 bp, respectively. The estimate of genetic identity index was highest (0.845) with the primer OPA01 and the lowest (0.479) with the primer BG27. The genetic identity index pooled over the primers was 0.596 ± 0.037 between these two breeds. The highest MAPD estimate (53.9) between the two breeds was obtained with the primer BG27 and the lowest (14.3) with the primer OPA01. They concluded that the genetic identity index between these two breeds calculated on the basis of BF showed moderate level of genetic identity with the primers employed. MAPD calculated on the basis of uncommon bands also demonstrated lower to medium level of genetic difference between Bhadawari and Murrah breeds of buffaloes.

The potential use of RAPD technique was evaluated as a source of development of alternative genetic marker system for studying variation in buffaloes (Annapoorani, 1996; Aravindakshan and Nair, 1998), cattle (Gwakisa et al., 1994; Nagaraja et al., 2003; Appannavar et al., 2002), sheep (Clouscard et al., 1995; Kantanen et al., 1995; Cushwa et al., 1996; Dodgson et al., 1996; Nagarajan, 1998) horses (Bailey and Lear, 1994; Zelenaja et al., 1996), dogs (Rothuizen and Wolfren, 1994), pigs (Ovilo et al., 1998), goats
(Chung et al., 1999) chicken (Levin et al., 1993; Smith et al., 1996; Wei et al., 1997) and fish (Williams et al., 1996).

Rao et al. (1996) observed clear and distinct RAPD patterns with a higher level of polymorphism between species, and fewer polymorphisms within the species. RAPD analysis in different animal species by Joshi et al. (1998) showed that the RAPD profile was unique for each species in terms of the number and position of the bands, although some bands were common to cattle and buffaloes and to sheep and goats. Higher annealing temperature during the initial cycles of PCR amplification increases the specificity and permits species identification of suspected tissue samples.

Perusal of the literature on the application of RAPD technique clearly showed that this technique can be used for genetic characterization of different livestock breeds in the absence of specific nucleotide sequence information.