

Assessment of genetic diversity among supermarket tea biology



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Contents

- 16

Tea works is the most popular non-alcoholic drink harvest across the universe. Tea was discovered more than 2000 old ages ago in China but is of course distributed throughout the whole Asiatic Monsoon part (Banerjee, 1992) . The chief category of cultivated tea consist of *Camellia sinensis* (L.) O. Kuntze with little foliage, *Camellia assamica* with large foliage and *Camellia assamica* ssp. *lasiocalyx* (Planchon ex Watt) with the intermediate foliage size (Tapan, 2000) . The ' China type ' *C. sinensis* and ' Assam type ' *C. assamica* originate around Southwest China, Myanmar and Northeast India (Assam) severally, but the native scope is obscured by a history of cultivation and debut by adult male (Sealy, 1958) . Tea is extremely heterogenous and all the above taxa freely interbreed ensuing in a Cline widening from utmost China types to those of Assam beginning (Wight, 1959) .

The tea workss have been cultivated widely in many Asiatic and African states where they contribute significantly to the local economic system (Freeman et al. , 2004) . The tea production can be categorised into six types, such as Green tea, Black tea, Oolong tea, White tea, Yellow tea and Dark tea harmonizing to their different processing processs as shown in Figure 1 (Yao et al. , 2008) . Green tea, Black tea and Oolong tea are more popular in the universe (Yao et al. , 2008) . China is presently the foremost manufacturer, consumer and exporter of commercial tea, particularly for Green and Oolong tea (Yao et al. , 2008) . On the other manus, India, Kenya

and Sri Lanka are the largest manufacturers and exporters of Black tea (Yao et al. , 2008) .

Figure 1. Major Tea Processing stairss with matching types (Hilal and Engelhardt, 2007) .

1. 3 Tea Benefits

Tea is a popular drink due to its stimulatory belongings and physiological maps (Chen, 1999) . It is reported to move against a figure of abnormalcies including artherosclerosis, radiation harm, antioxidative, anticancer, antiulceric, antiviral and bactericidal (Chen, 1999) .

Darjeeling Tea

Darjeeling Tea is the finest tea, because it ' s alone spirit and has traditionally been prized above all other black teas, particularly in the United Kingdom and other states ([hypertext transfer protocol: //www. darjeelingtea. com/](http://www.darjeelingtea.com/)) . It is cultivated, grown, processed and manufactured within the defined district of hilly countries (viz. : Sadar sub-division, Kalimpong sub-division and Kurseong subdivision) of Darjeeling territory in the province of West Bengal in India ([hypertext transfer protocol: //www. darjeelingtea. com/](http://www.darjeelingtea.com/)) . The quality and repute of Darjeeling Tea are basically attributable to its geographical beginning and processing. About 86 registered Tea Estates (gardens) located within the demarcated district cultivate *Camellia sinensis* tea assortment ([hypertext transfer protocol: //www. darjeelingtea. com/](http://www.darjeelingtea.com/)) .

About 40 million kilogram is sold as “ Darjeeling Tea ” ([hypertext transfer protocol: //www. darjeelingnews. net/](http://www.darjeelingnews.net/)) when the existent production capacity of Orthodox Darjeeling Tea varies from 9-10 million kilogram

([hypertext transfer protocol: //www. darjeelingtea. com/](http://www.darjeelingtea.com/)) . The low measure with really high quality pushes up the demand to do monetary value escalation ([hypertext transfer protocol: //www. darjeelingnews. net/](http://www.darjeelingnews.net/)) . As a consequence, teas of non-Darjeeling beginnings blended with Darjeeling Tea are sold to the consumer through abuse of the word “ Darjeeling ” ([hypertext transfer protocol: //www. darjeelingnews. net/](http://www.darjeelingnews.net/)) . The stairss involved in tea production (processing, sweeping purchasing, blending, transportation and repackaging allows for alteration in beginning ([hypertext transfer protocol: //www. darjeelingnews. net/](http://www.darjeelingnews.net/)) . This restricts the growing of other tea merchandises such as Assam.

1. 5 DNA Molecular Markers

Deoxyribonucleic acid analysis can be used to supervise tea beginning from manufacturer to consumers. DNA molecular markers provide a good and enlightening attack to gauge the familial diverseness and familial relationship of tea cultivars (Yao et al. , 2008) . Tea diverseness has been studied with random amplified polymorphous DNA (RAPD) markers (Wachira 1995 ; Chen et al. , 1998 ; Liyanage et al. , 2001 ; Wachira et al. , 2001 ; Kaundun & A ; Park, 2002) , amplified fragment length polymorphism (AFLP) markers (Wachira et al. , 2001 ; Balasaravanan et al. , 2003) and limitation fragment length polymorphism (RFLP) markers (Matsumoto et al. , 2002, Kaundun and Matsumoto, 2003) .

However, RAPDs and AFLP techniques generate dominant markers and in the instance of RAPDs there are serious inquiries referring duplicability between research labs (Freeman et al. , 2004) . The usage of RFLP is limited by their awkwardness, the demand for ringer bank and demand for big sums of DNA (<https://assignbuster.com/assessment-of-genetic-diversity-among-supermarket-tea-biology/>

Tapan, 2002) . The demand of radioactive labelling and comparatively better quantitative and qualitative DNA demand besides limits the use of AFLP in every research lab (Tapan, 2002) .

Mahipal Singh et Al. (1999) , demonstrated DNA extraction from processed tea but the DNA is unsuitable for RAPDs and AFLP because during treating the tea degrades much of the cells and their Deoxyribonucleic acid, and merely the debauched Deoxyribonucleic acid can be isolated. As PCR engineering discoveries increased usage in familial analysis, extra novel fluctuations of this technique are emerging (Tapan, 2002) .

1. 5. 1 Microsatellite or Simple Sequence Repeat (SSR)

Microsatellites or simple sequence repetitions (SSR) have gained attending late as an alternate agencies of qualifying complex eucaryotic genomes (Tapan, 2002) . These are normally 2-5 bp long, short DNA sequence motive that occur at multiple sites (Wang et al. , 1994) and uncover a high grade of allelomorphic diverseness which can be typed via PCR elaboration of genomic sections flanked by reciprocally oriented (5 ' or 3 ' terminal) , closely separated microsatellite repetitions (Schlotterer et al. , 1991) . The PCR merchandises therefore generated reveal multiple polymorphous merchandises, which can be resolved on agarose gel cataphoresis (Tapan, 2002) . Simple sequence repetition (SSR) markers have besides been developed in tea works (Kaundun and Matsumoto 2002 ; Freeman et al. , 2004) and the SSR primers are available for diverseness surveies in tea works. The CEQ 8000 Genetic Analysis System was used to analyze the samples along with SSR markers. CEQ is good suited for SSR analysis

because of its ability to reproducibly size Deoxyribonucleic acid fragments.
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Accurate genotyping of SSRs by fragment sizing relies on the changeless comparative migration of indistinguishable allelomorphs, and prior cognition of the spectrum of most or all possible evident sizes that are derived from those comparative migrations (Basu 2005) .

1. 6 The Present Project

In the present survey, SSR markers were used (I) to analyze familial relationship among Darjeeling tea from supermarkets ain trade names and other trade names and (two) to analyze familial relationship among Darjeeling tea and assorted blends.

2. 0 Materials and Methods

2. 1 Tea Samples

The descriptions of 16 tea samples bought for the present survey are given in Table 1. *Camelia assamica* was used as positive control.

Table 1. Tea Samples

Detail/Brand/Source

Type

Description

Whittard

Darjeeling

High Quality

Twinnings

Darjeeling

Charles digby harrods

Darjeeling

Limiters

Green Tea Single Estate

Waitrose

Darjeeling

Tesco

Darjeeling

Supermarket ain trade names

Sainsbury

Darjeeling

Wild Needle Tea

Chinese Green Tea

Assorted High quality Tea

Whittard

Assam

Whittard

Oolong

Whittard

White Tea

Tea Institute

Camelia assamica (Assam)

Whittard

Earl Grey

Assorted Blends

PG tips

Blend

Typhoo

Blend

Tetley

Blend

2. 2 DNA Extraction and Purification

Genomic DNA was extracted from dried tea foliage samples utilizing a modified protocol by Dellaporta et Al. 1983. Dried tea foliage (about 15mg) put into a 1. 5ml eppendorf tubing and 400Aµl of extraction buffer added (8Aµl mercaptoethanol and 10ml Extraction buffer Appendix) were grind utilizing a bluish plastic stamp and votex briefly. The samples in the eppendorf tubing were incubated at 65oC for 30 proceedings with intermittent shaking and inverting after which were extractors at 13000 revolutions per minute for 10 proceedings. Supernatant were decanted into new 1. 5ml eppendorf tubings and centrifuged once more at 13000 revolutions per minute for 5 proceedings to take leaf dust. The supernatant

was decanted into new 1.5 ml eppendorf tubings and an equal volume of cold isopropyl alcohol (200 μ l) was added to precipitate the Deoxyribonucleic acid and so centrifugate at 13000 for 10 proceedings. The supernatant was poured away and 200 μ l of 70 % ethyl alcohol added to clean the DNA pellet and extractor at 13000 revolutions per minute for 5 minutes. The Deoxyribonucleic acid pellets were air-dried for 10 proceedings and re-suspended in 50 μ l H₂O.

The Deoxyribonucleic acid extracted was purified utilizing the Promega Wizard Clean-Up Kit (Appendix 1) harmonizing to maker's instructions and the unity and intactness of DNA were checked on 1 % agarose gels.

2.3 PCR Amplification

Eight SSR primer brace labelled with the Beckman dye for sensing and analysis on the CEQ 8000 Genetic Analysis System were used for PCR elaboration of all the 16 tea samples and H₂O (negative control). 0.2 ml PCR reaction mastermix for each microsatellite primer brace was prepared. The chemical constituents and volume of the mastermix is shown in Table 2. An aliquot 17 μ l of the mastermix was pipette into 17 ten 0.2 ml PCR tubings (96 Wellss home base) and 3 μ l for each tea genomic DNA added (diluted to ~10ng/ μ l).

Table 2: Contentss for 20 μ l reaction volume for 16 Tea samples. (Example Casmin10)

Chemical

Volume for one sample (μ l)

Mastermix Total volume (μl)

(($\times 17$) + 5 %)

MQ H₂O

11.35

193

10 ten NEB PCR buffer inc. MgCl to 1.5 millimeters

2

34.05

20 μM Camsin M10FGreen

0.1

1.75

20 μM Camsin M10R

3

51.05

10mM dNTPs

0.4

6.85

NEB Taq polymerase

0.15

2.6

Tea genomic DNA (~10ng/A μ l)

3

Entire volume

20

The contents were assorted gently and centrifuged briefly to roll up the reaction. The undermentioned PCR rhythms were performed:

One rhythm consists of initial denaturation at 94oC for 3 proceedings.

35 rhythms of 45 seconds denaturing at 94oC, 1 minute tempering at 60 oC (temperature harmonizing to Table 3) and 1 minute extension at 72oC and so a concluding extension at 72oC for 7 proceedings with a soak temperature at 4oC.

Table 3. Microsatellite Word picture

Microsatellite

Size Range (bp)

Annealing Temperature (oC)

Dye tag Colour

CasminM9

CasminM10

CasminM12

P3

P7

P16

P18

P19

195-235

179-219

135-190

190-225

293-325

173-200

233-259

162-198

55

55

55

55

55

55

60

60

Black

Green

Black

Green

Green

Blue

Blue

Black

2. 4 Agarose Gel Electrophoresis

2. 4. 1 Gel Preparation

The 2. 5 % agarose gel make up of 500 milliliter (250ml TBE in each flask) was prepared by runing 10g agarose (5g in each flask) in 0. 5x TBE buffer (89 mM Tris-Hcl (pH 8. 3) , 89 millimeter boracic acid, 5 millimeter EDTA) in a microwave. The content was cool for 10 to 15 proceedings and 2. 5A μ l Ethidium Bromide added to each flask and assorted gently by twirling the conelike flask. The gel mixture was poured into a maxi-gel tray with 8 combs and allowed to put.

2. 4. 2 Gel Loading and Running

5A μ l DNA size marker (2-Log ladder) used for set size was loaded in the first and last well of each row followed by 4A μ l PCR merchandise mixed with 2A μ l 6x lading buffer of 17 samples for each microsatellite marker. The PCR merchandises were so separated on 2. 5 % agarose gel tally at a changeless 120 V for 45 proceedings and detected by Ag staining (Panaud et al. , 1996) and gel images were recorded for analysis.

Analysis of SSR fragments

The Beckman Coulter CEQ 8000 Genetic Analysis System was used for the analysis of SSR fragments. The CEQ 8000 is a to the full automated familial analysis system. The system automatically fills the capillary array with a patented additive polyacrylamide (LPA) gel, denatures and loads the sample, applies the electromotive force and analyze the informations base on the dye signal. Dye D1 (ruddy) is reserved for usage in the size standard ladder. The staying three dyes used for primer labelling: D2 (Black) , D3

(green) , and D4 (Blue) with fluorescence emanation of the dyes in the order: D4 & gt ; D3 & gt ; D2.

Sample Preparation and lading into CEQ

The amplified PCR merchandises were separated by capillary cataphoresis utilizing a CEQ 8000 familial analysis system (Beckman Coulter Inc.) . For each tea sample 2 microsatellites PCR merchandises were assorted given 2 pools. An aliquot of entire volume of 217A μ l was prepared by blending 215A μ l of sample lading solution (SLS) and 2A μ l size criterion 400 (SS4000) for each row of 8 Wellss of 96 Wellss home base. 25A μ l of aliquot was pipette into each of the 8 Wellss of one row. To this was added 3A μ l of pooled PCR merchandises for each sample and layered with a bead of mineral oil to forestall vaporization of the reagents while running on the CEQ system. This was repeated for the all the pooled PCR merchandises for the remainder of the samples.

Datas Analysis

Data analysis was performed utilizing the CEQ 8000 Fragment Analysis package version 7. 0. 55 harmonizing to maker ' s recommendations (Beckman Coulter Inc) . The SSR sizes extremums generate were scored on presence (1) or absence (0) for each sample base on the four SSR primer braces in a binary manner. The information obtain was entered into excel and a dendrogram was constructed by the unweighted brace group method (UPMG) and Nei & A ; Li ' s similarity coefficient was estimated utilizing MVSP package. Multivariate Statistical Package (MSVP) ordination method makes no premises about the distribution of the random variables or about their population genetic sciences (Kovach, 2006) . Euclidian distance was chosen <https://assignbuster.com/assessment-of-genetic-diversity-among-supermarket-tea-biology/>

in penchant to other distance steps, as it does non category common absence of an allelomorph as a shared feature (Kovach, 2006) .

3. 0 Consequences and Discussion

3. 1 PCR-SSR Analysis

The Deoxyribonucleic acid from all the 16 Tea samples used for PCR reaction was quantified on a 1 % agarose gels and consequences are shown in Figure 2. Although clear sets were non observed, DNA was present for all the Tea samples.

A B C D E F G H I J K L M N O P Q R

Figure 2. Quantification of genomic Deoxyribonucleic acid from 16 tea samples. (A) , Whittard-Darjeeling (B) , Sainsbury-Darjeeling (C) , Tesco-Darjeeling (D) , Twinnings-Darjeeling (E) , Harrods-Darjeeling (F) , Chinese Green Tea (G) Whittard-Earl Grey (H) , Camelia assamica (Assam) (I) Whittard-Assam (J) , Whittard-Oolong (K) , Whittard-White Tea (L) , Clipper-Green Tea (M) , PG Tips (N) , Typhoo (O) , Tetley (P) , Waitrose-Darjeeling (Q) , Water (R) .

Microsatellite markers have been used for word picture in this survey, they are known for their carbon monoxide laterality, multi-allelic nature, duplicability, extended genome coverage and easiness of sensing by polymerase concatenation reaction with alone primer braces that flank the repetition motive (Sharma 2009) . Among the 8 primers used for DNA elaboration, merely 4 primers (M9, M10, M12 and P18) produce stable SRR polymorphous fingerprints runing in size from 1000bp to 3000bp base on 2-Log DNA ladder indicant for 1 % agarose gel as show in Figure 3 and 4.

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Figure 3. Amplification of genomic Deoxyribonucleic acid from 16 tea samples with primer M12 and P18. Tea samples are label in the order as 2-log DNA ladder (Appendix 2)

Figure 4. Amplification of genomic Deoxyribonucleic acid from 16 tea samples with primer M9 and M10. Tea samples are label in the order as 2-log DNA ladder (Appendix 2)

Among the other 4 primers, P3 and P16 produce unstable or really weak sets whilst the last two primers (P7 and P19) failed to magnify DNA for all the 16 tea samples as show in Figure 5 for primer P19. Hence these primers (P3, P16, P7 and P19) were excluded from capillary cataphoresis. The failure of these primers to magnify the Deoxyribonucleic acid could be attributed to inefficiencies during lading or wrong annealing temperature. The annealing temperature is a cardinal factor on the quality of SSR fingerprints and it had to be determined for each primer (Yao et al. 2008) . In this survey, the optimum annealing temperature of the SSR primers ranged from 55oC to 60oC as show in Table 3.

Figure 5. Faint Band/ absence of sets observed with primer P19.

3. 2 SSR-CEQ Fragment Analysis

Due to little fragment size of the Deoxyribonucleic acid and big pore size of the agarose gel, clear sets may non been observed therefore capillary cataphoresis was done for the four primers (M9, M10, M12 and P18) and CEQ 8000 Fragment Analysis package was used to analyze the SSR fragment sizes. SSR fragment sizes were automatically calculated to two denary topographic points by the CEQ 8000 Genetic Analysis System. However

whole figure sizes for extremums naming were used and these are reported in Table 4.

Primers

Dye Tag Colour

Size (bp)

Size (bp)

Size (bp)

Size (bp)

Size (bp)

Size (bp)

Size (bp)

Size (bp)

P18

Blue

238

240

241

242

M9

Black

195

196

197

198

204

206

207

208

M10

Green

167

168

169

170

172

M12

Black

167

168

169

170

171

172

173

174

Table 4. SSR Primers Sizes Scored and Dye tag coloring material

Clear extremums for allelomorphs were non observed for primer P18 in 5 tea samples although clear stria form for this primer was observed when cataphoresis was carried out on its PCR merchandises. This show as losing informations (changeless migration speed) in Figure 6 and may be due to the presence of a bubble in the Wellss of capillary home base during pipetting therefore forestalling the Deoxyribonucleic acid from being picked up when current is passed through. Thus the CEQ 8000 fragment size for primer P18 have been excluded from the informations used for bunch analysis. Extremums were nevertheless observed for the other primers as show in Figure 7 (primer P18) and Figure 8 (primer M9) .

Figure 6. Electropherogram for a sample considered as losing informations with Primer P18

Figure 7. Electropherograms demoing extremum sizes for Primer M10 to for Tea Samples

Figure 8. Electropherogram demoing peak sizes for Primer M9 to for Tea Samples

3. 3 Cluster Analysis

3. 3. 1 Familial Variation among all Tea Trade names

A dendrogram generated utilizing unweighted brace group method and step of similarity estimated utilizing Nei & A ; Li ' s similarity coefficient is shown in Figure 9. Similarity among the tea samples range from 0 % similarity coefficient between Whittard-Assam and the other 15 tea samples to a upper limit of 83 % similarity coefficient between Tetley and PG tips blend tea

samples. Three blend trade names made up of PG tips, Tetley, Typhoo and Clipper Green tea a high quality trade name separate into one group at 70 % similarity coefficient. Earl Grey blend (black tea blend with bergamot oil) is nevertheless different from the other three tea blends (49 % similarity coefficient) . Tesco and Twinnings Darjeeling tea samples has 80 % similarity coefficient.

Figure 9. UPGMA dendrogram screening Nei & A ; Li ' s similarity coefficient among all the Tea Brands utilizing SSR shapers. (Appendix 3)

3. 3. 2 Familial Variation among Darjeeling Tea Brands

Nei and Li ' s similarity coefficient was used to measure the diverseness among the Darjeeling tea sold under different trade name names. The familial relationship between the Darjeeling tea samples was portrayed diagrammatically in the signifier of a dendogram in Figure 10. At a minimal similarity coefficient of 14 % Darjeeling tea separate into one bunch whilst Chinese green tea and Camilia assamica (Assam) are in the 2nd bunch. Sainsbury Darjeeling tea is different from the other Darjeeling types (37 % similarity coefficient) . A maximal similarity coefficient of 80 % was observed between Tesco Darjeeling and Twinnings Darjeeling.

Figure 10. UPGMA dendrogram screening Nei & A ; Li ' s similarity coefficient among all the Darjeeling types utilizing SSR markers. (Appendix 3)

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3. 4 Principle Coordinate Analysis

Figure 11: Scatter plot of Principal Coordinates instance tons (Euclidean) (Appendix 4) .

Chief co-ordinate analysis for the first two axis give 48 % entire fluctuation as shown in Figure 11 with Axis 1 stand foring 28 % fluctuation and axis 2 stand foring 20 % fluctuation with instance mark (Appendix 4) . Axis 1 is strongly positively correlated to all the four blends used in the analysis. It ' s decrepit positively correlated to Clipper Green Tea, Twining Darjeeling and Harrods Darjeeling. Sainsbury Darjeeling and Whittard Darjeeling are decrepit negatively correlated to axis 1.

3. 5 Discussion

The bunch obtained of the assorted blends is consistent with predicted associations. Typically those trade names being sold under Darjeeling assortment should constellate together, nevertheless in this survey they did non constellate together. The tea samples used in the survey proved to be boring to work with and integral DNA fragments could non be extracted due industrial processing of the tea leaves, this besides meant that a high sum of dross were present. This survey demonstrated that SSRs offer a suited sensing of familial variableness and molecular survey of tea genotypes. Several characteristics of the protocol contributed to this including the PCR rhythms, runing and tempering temperatures. The SSR technique proved to

be fast and sufficiently dependable (Tapan 2002) . Though the figure of primers used in the concluding analysis was little, hence it is non to the full representative of the entire diverseness nowadays in the tea types used

4. 0 Decisions and Recommendations

Consequences of present probe reveal variableness in familial nature of the tea samples used in the current survey. However, this decision is base on the consequences generated from three SSR primers ; hence by sing more primers in future may uncover the existent familial diverseness among the tea samples. To gauge familial relationship among tea genotype it is of import to screen primers and select those that produced quotable and stable stria form (Tapan, 2002)

Further surveies (Biochemical) can be carried out complement molecular biological science techniques (Magoma 2000) . Tea contains a broad scope of phenolic compounds including flavanols, flavandiols, flavonoids, and phenolic acids ; these compounds may account for up to 30 % of the dry weight of the tea leaves harmonizing to the literature (Hilal and Engelhardt, 2007) . Surveies can beconducted on the chief quality constituents of tea such as tea polyphenols, catechins and amino acid content (<http://www.nbtea.co.uk/tea-manufacture>) . The type of antioxidants (polyphenol or flavenoids) differs - green tea has simple flavenoids called catechins, while black tea has more complex flavenoids called the aflavins and the arubigins ([hypertext transfer protocol: //www.nbtea.co.uk/tea-manufacture](http://www.nbtea.co.uk/tea-manufacture)) .

The chief advantage of this method of diverseness sensing is that it is robust, inexpensive and has high throughput. Besides it can be developed as

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one of the markers of tea quality and can be used to supervise the type of tea coming from the supermarkets and other processing companies (Magoma 2000) . In add-on, enfranchisement by the tea taste testers to hold the typical characteristics of Darjeeling tea such as olfactory property, coloring material, spirits, spirit and gustatory sensation may be considered for farther surveies.

5. 0 Mentions

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6. 0 APPENDIX

6. 1 APPENDIX 1: Wizard DNA Clean Up System

Purification without a Vacuum Manifold (Using 3 milliliter Luer – Lok Syringes)

Before You Begin

Thoroughly mix the ace DNA Clean-Up Resin before taking an aliquot. If crystals or sums are present, fade out by warming the rosin to 37EsC for 10 proceedings. The rosin itself is indissoluble. Cool to 25-30EsC before usage.

The sample volume must be between 50-500Aµl. If the sample volume is less than 50Aµl, conveying the volume at least 50Aµl with unfertile H2O. Pre-warm H2O for elution at 65-70EsC.

Binding of DNA

Use one WizardA® Minicolumn for each sample. Remove and set aside the speculator from the 3ml disposable syringe. Attach the syringe barrel to the Leur-Lok A® extension of each Minicolumn.

Add 1ml of Wizard DNA clean up resin to a 1.5ml micro extractor tubing. Add the sample to the Clean -up Resin and mix by inversion.

Pipette the WizardA® DNA Clean -Up Resin incorporating the edge DNA into the Syringe Barrel. Insert the syringe speculator easy and gently force the slurry into the Minicolumn with the syringe speculator.

Washing

Detach the syringe from the Minicolumn and take the speculator from the syringe. Reattach the syringe Barrel to the Minicolumn. Pipette 2ml of 80 % isopropyl alcohol into the syringe. Reinsert the speculator and force the solution through the Minicolumn.

Remove the Syringe Barrel and reassign the Minicolumn to a 1.5 milliliter micro-centrifuge tubing. Centrifuge the Minicolumn at maximal velocity in a micro-centrifuge for 2 proceedings to dry the resin.

Elution

Transfer the Minicolumn to a new micro extractor tubing. Apply 50Aµl of pre-warmed (65-70EsC) H₂O or TE buffer to the Minicolumn and delay for 1 minute. Centrifuge the Minicolumn fro 20 seconds at maximal velocity to elute the edge DNA.

Remove and fling the Minicolumn. The purified Deoxyribonucleic acid may be stored in the micro extractor tubing at 4EsC or -20EsC.

6. 2 APPENDIX 2:

2-Log DNA ladder

6. 3 APPENDIX 3:

CLUSTER ANALYSIS Darjeeling Types

Analyzing 19 variables x 9 instances

UPGMA

Nei & A ; Li ‘ s Coefficient

Objects

Node

Group 1

Group 2

Simil.

in group

1

Twinnings-D.

Tesco-D.

0. 8

2

2

Clipper-Green-tea

Node 1

0. 714

3

3

Node 2

Harrods D organic

0. 686

4

4

Whittard-D.

Node 3

0. 578

5

5

Node 4

Waitrose-D

0. 489

6

6

Node 5

Sainsbury-D.

0. 373

7

7

Camelia-assamica-assam

Chinese-green-tea

0. 333

2

8

Node 6

Node 7

0. 141

9

CLUSTER ANALYSIS ALL TEA BRANDS

Analyzing 19 variables x 16 instances

UPGMA

Nei & A ; Li ' s Coefficient

Objects

Node

Group 1

Group 2

Simil.

in

group

1

Tetley-Blend

PGtips-Blend

0. 833

2

2

Twinnings-Darjeeling

Tesco-Darjeeling

0. 8

2

3

Clipper-Green-tea

Typhoo-Blend

0. 783

2

4

Node 2

Whitard-white-tea

0. 714

3

5

Node 3

Node 1

0. 701

4

6

Node 5

Charles digby harrods Darjeeling

0. 674

5

7

Whittard-Darjeeling

Camelia-assamica-assam

0. 667

2

8

Node 6

Node 4

0. 622

8

9

Waitrose-Darjeeling

Whittard-Oolong

0. 533

2

10

Node 8

Earl Grey Blend

0. 487

9

11

Node 7

Node 10

0. 421

11

12

Node 11

Sainsbury-Darjeeling

0. 406

12

13

Node 12

Node 9

0. 395

14

14

Node 13

Chinese-green-tea

0. 185

15

15

Node 14

Whittard-Assam

0

16