

# The use of chloroplast markers for the traceability of certified sustainably produced cocoa (*Theobroma cacao*) in the chocolate industry

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# *Theobroma cacao*: Food of the Gods

The cocoa tree was first domesticated at least 3500 years ago in the rain forests of Central and South America.



[http://exhibits.martindale.com/edu/chocolate/images/content\\_img/CacaoGod.jpg](http://exhibits.martindale.com/edu/chocolate/images/content_img/CacaoGod.jpg)



It became the drink of the wealthy 16th and 17th century Europeans ruling class

*The cup of Chocolate by Jean-Baptiste Charpentier 1768*



PARIS EXHIBITION, 1889, GOLD MEDAL awarded to J. S. FRY & SONS.



"HAVE A CUP WITH ME."

# FRY'S PURE Concentrated COCOA

This preparation of Cocoa is exceedingly Soluble and easily digested. It makes an agreeable thin drink, and is a most delightful beverage for Breakfast or Supper.

*LANCET*.—"Pure, and very soluble."

*MEDICAL TIMES*.—"Eminently suitable for Invalids."

*Sir C. A. CAMERON*.—"I have never tasted Cocoa that I like so well."  
*President Royal College of Surgeons, Ireland.*

J. S. FRY & SONS, BRISTOL, LONDON, & SYDNEY.

MAKERS TO  
T. M. THE  
KING & QUEEN

# FRY'S

MAKERS TO  
H. M.  
QUEEN ALEXANDRA

DESPERATION. PACIFICATION. EXPECTATION. ACCLAMATION. REALIZATION.  
"IT'S FRY'S"

# CHOCOLATE

**FRY'S**

GOLD MEDAL PARIS 1889

GOLD MEDAL PARIS 1889

# CHOCOLATE

Creams = Cakes = Fancy Boxes.

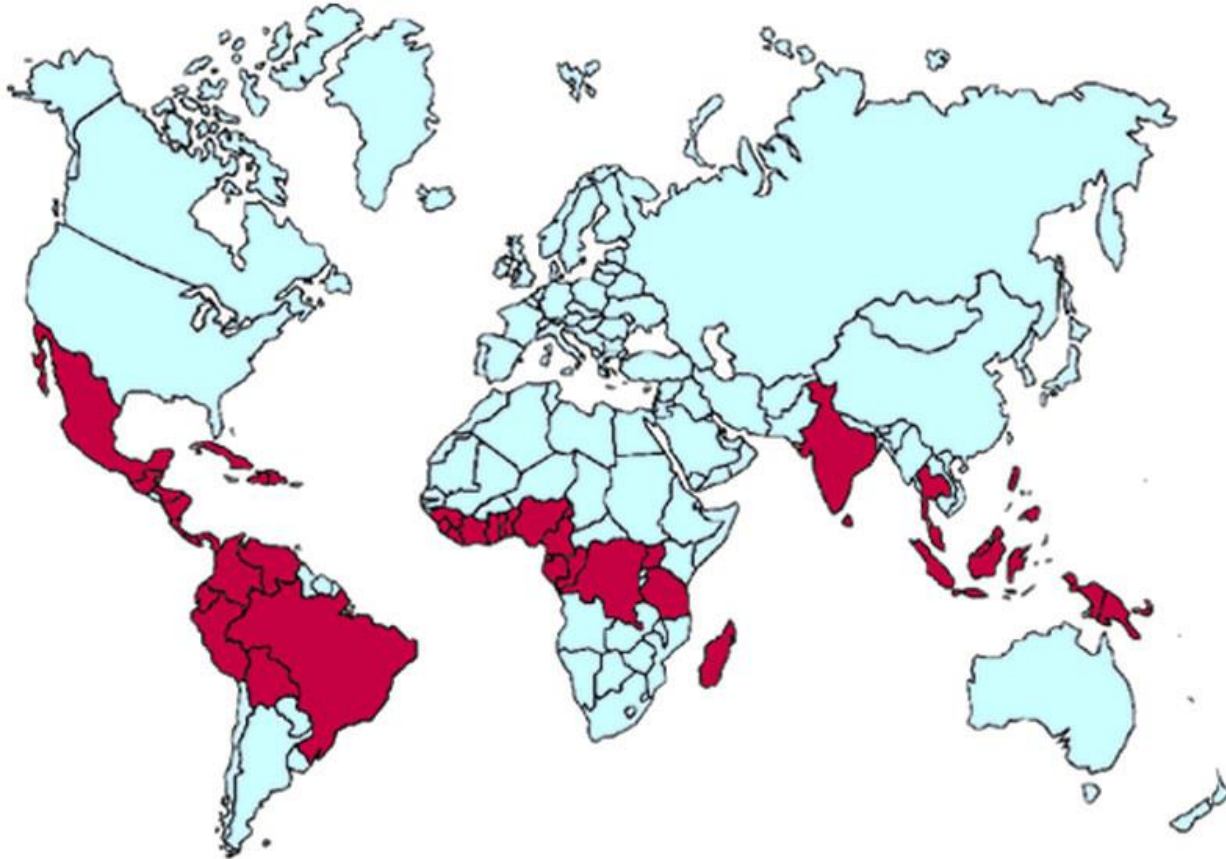
# Increase demand for chocolate products



Cacao cultivation is restricted to the hot, humid belt 10 to 20 degrees north and south of the equator.

As the popularity of chocolate spread, European countries quickly established plantations in these regions.

It has been now introduced as a crop to the equatorial regions of West Africa and South East Asia. Cocoa is still typically produced in many small individual farms, where it is the main source of revenue.



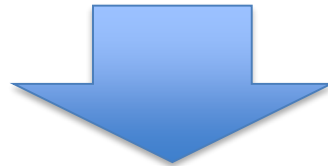


# Tracking



Recently the chocolate industry has changed due to a higher demand for certified cacao (Fair Trade). Chocolate companies have stated that by 2020, all their cacao will be certified sustainable. There is therefore a need for methodologies enabling the characterisation and tracking of products along the cacao supply chain.

# Case Study

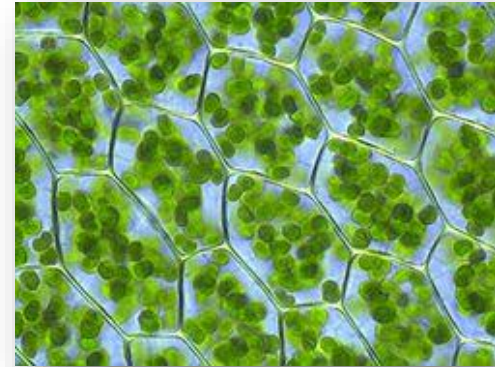


Assessing diagnostic DNA biomarkers specific to cacao farming region that can be tested on all derived products along the certified cacao supply chain

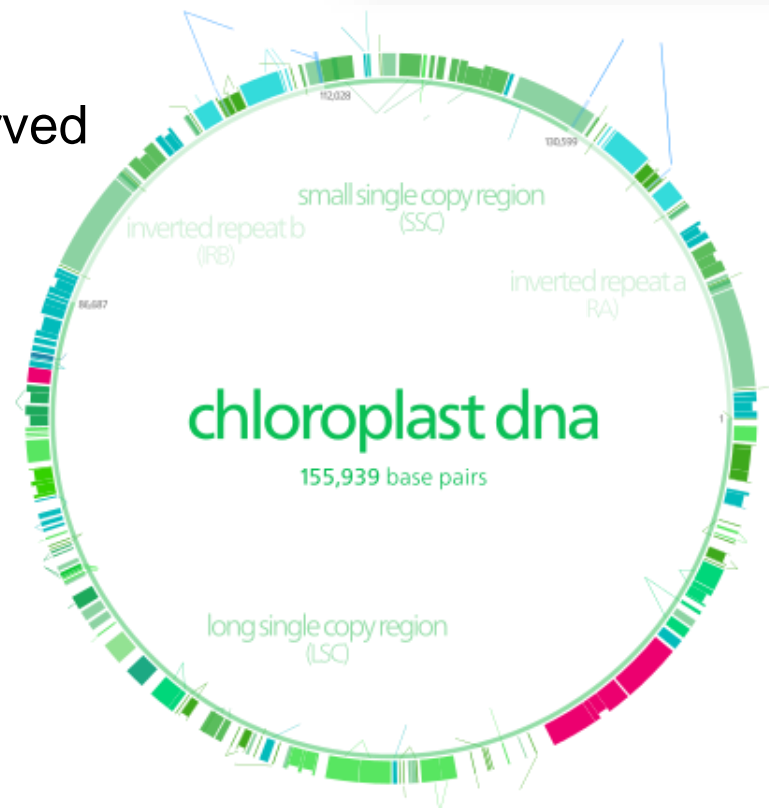


# Chloroplast cpSSR

- 4 chloroplast SSR assays designed from published cocoa sequences.
  - High copy number.
  - Intact DNA more likely to be preserved following chocolate production.
  - Maternally inherited
  - Haploid genome



Conse... Identity	CCCCCTCTACTATACTA-TTTTTTTTTTTATTTC1
1. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
2. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
3. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
4. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
5. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
6. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
7. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
8. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
9. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
10. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
11. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1
12. ...	CCCTCTACTATACTA-TTTTTTTTTTTATTTC1



# DNA extracted from:

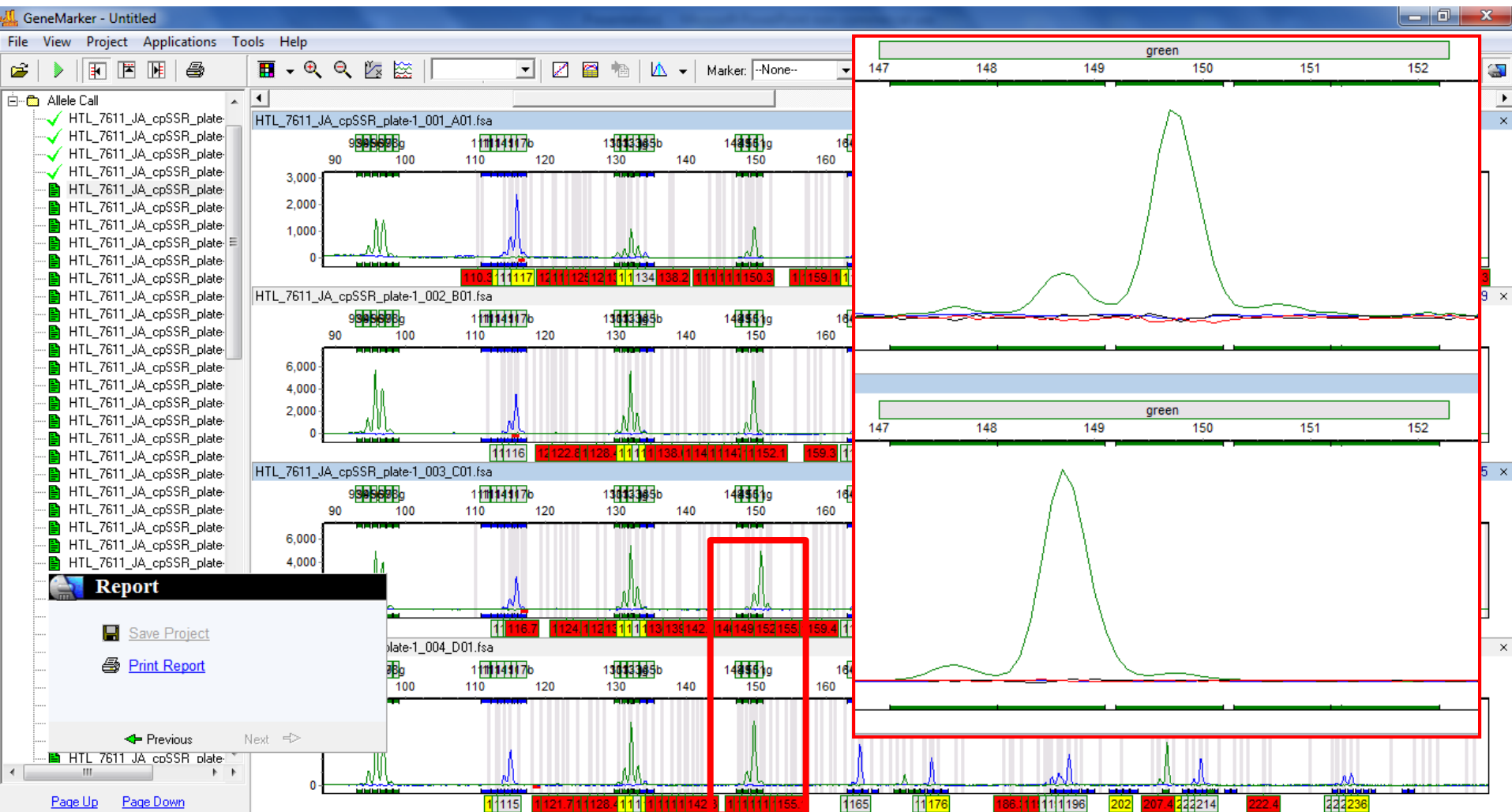
- *Theobroma cacao* reference panel
  - 159 accessions from The International Cocoa Quarantine Centre at the University of Reading (ICQCR)
- Chocolate
  - Bulk cocoa (Mars, Nestle, Cadbury) from West Africa.
  - Fine cocoa (Hotel Chocolat) from Peru, Ecuador, Venezuela, Trinidad and Madagascar)
- Cacao beans
  - 6 farms from Côte d'Ivoire





# cpSSR screen reference panel

- Capillary analysis *GeneMarkers*
  - Multiplex of loci (Hex and Fam label)



# Reference panel

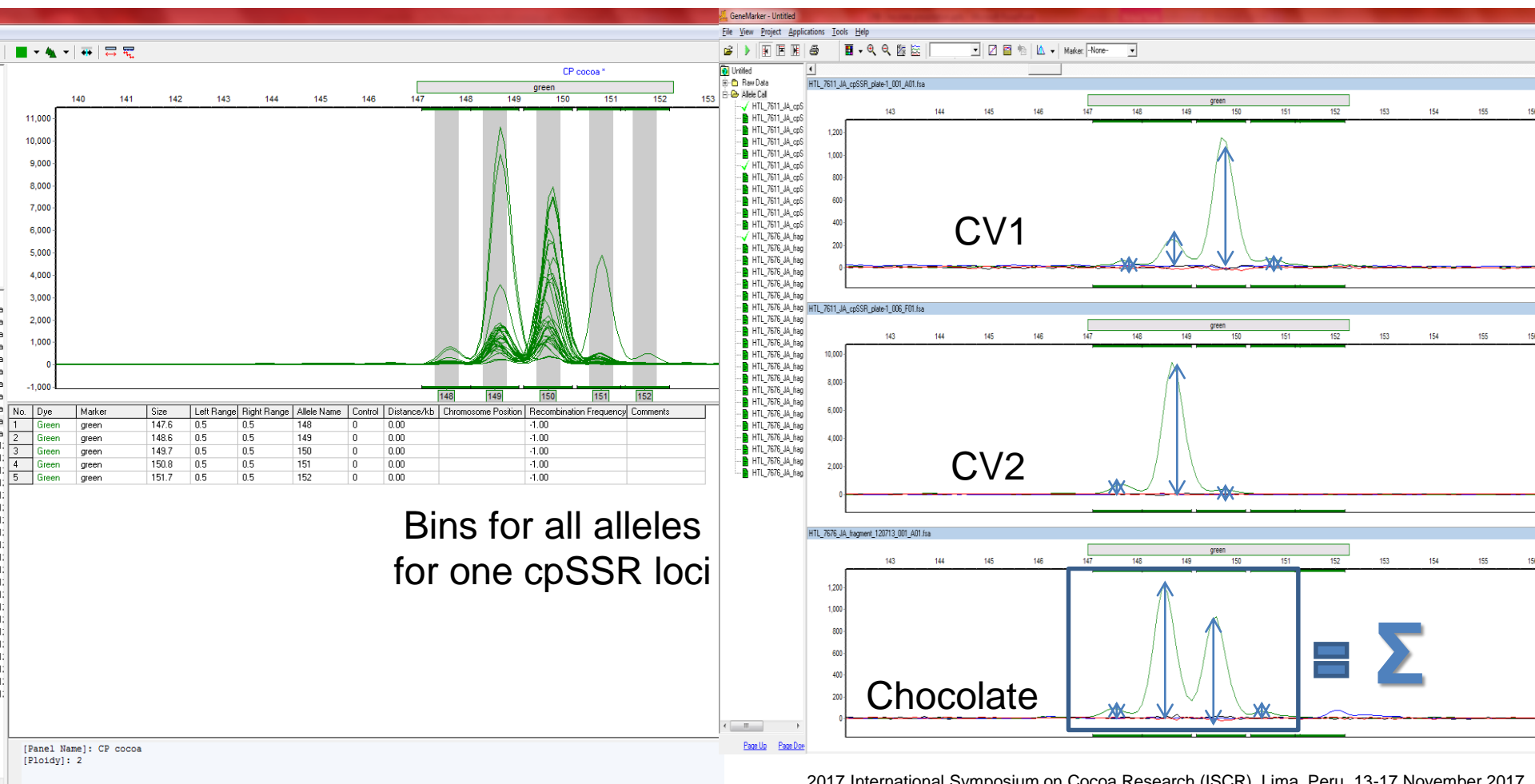
<b>cpSSR locus</b>	<b>cpSSR1</b>	<b>cpSSR2</b>	<b>cpSSR3</b>	<b>cpSSR4</b>
<b>Na</b>	2	2	2	4
<b>h</b>	0.172	0.202	0.397	0.551

<b>Haplotypes</b>	<b>Ha %</b>	<b>Amplicon size (bp)</b>			
H1	1.9	209	149	171	276
H2	4.4	215	149	172	275
H3	5.1	215	149	172	276
H4	11.9	209	150	171	275
H5	13.2	209	150	171	277
H6	63.5	209	150	172	278



# cpSSR screen chocolate and beans

- Data normalised to quantify putative cultivar origin
  - Peak height proportion ( $\updownarrow$  for one loci /  $\Sigma$  for this loci)
  - PCA analysis of transformed data.



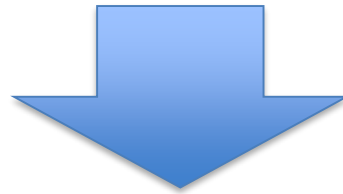
# Chocolate and cacao beans analysis using cpSSR markers





# Conclusion

- Potential to support cocoa certification programmes for the Fine cocoa/premium cocoa market
- Not likely to be appropriate for the characterisation of bulk cocoa production.



- Different markers/approaches needed

# *Merci*

*Pedro Lafargue Molina*

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*Andrew Daymond*

*Kirsten Skøt*

*Martin Gilmour*