

Il ruolo della genetica per l'autenticità di prodotto nelle filiere pane e pasta

Valeria Terzi, Caterina Morcia*, Giorgio Tumino*, Paolo Laino*,
Diego Breviario**,
Giuseppe Spano****

**CRA-GPG, Fiorenzuola d'Arda*

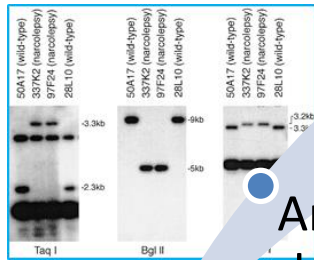
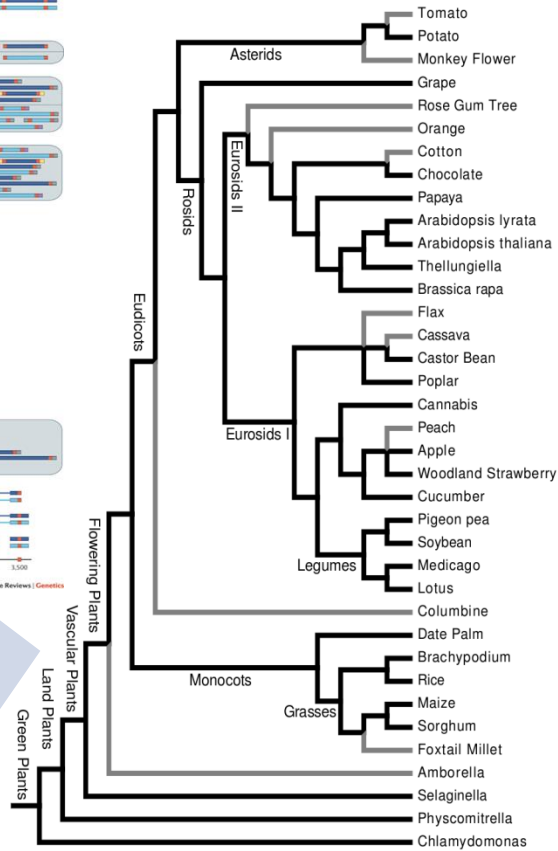
***IBBA-CNR, Milano*

****DIVA, Università degli Studi di Foggia*

Firenze, 28 novembre 2013

TRACCIABILITA' MOLECOLARE

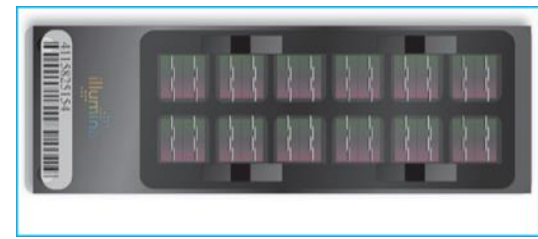
..in prodotti di filiera...



Anni '00:
centinaia di
marcatori

Anni '90:
decine di
marcatori

Oggi:
migliaia di
marcatori,
genomi



TRACCIABILITA' MOLECOLARE



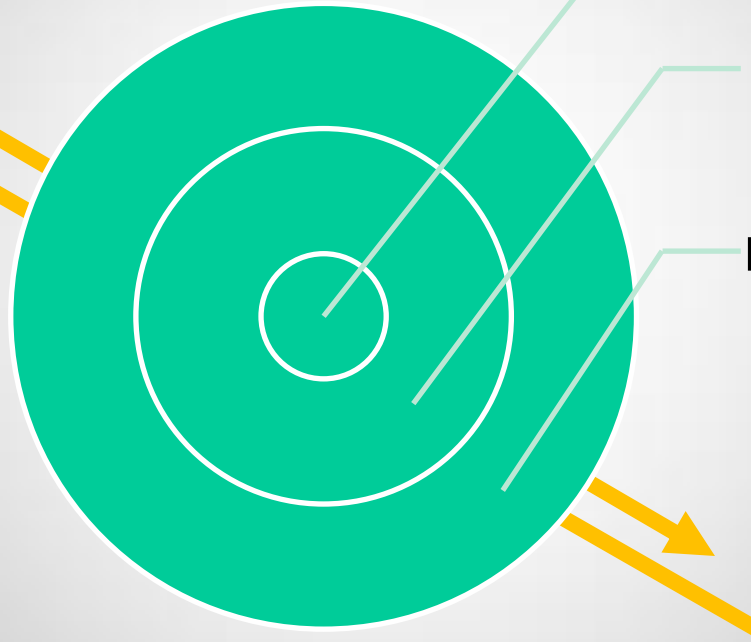
Specie

varietà

microbioma



TRACCIABILITA' MOLECOLARE



Specie

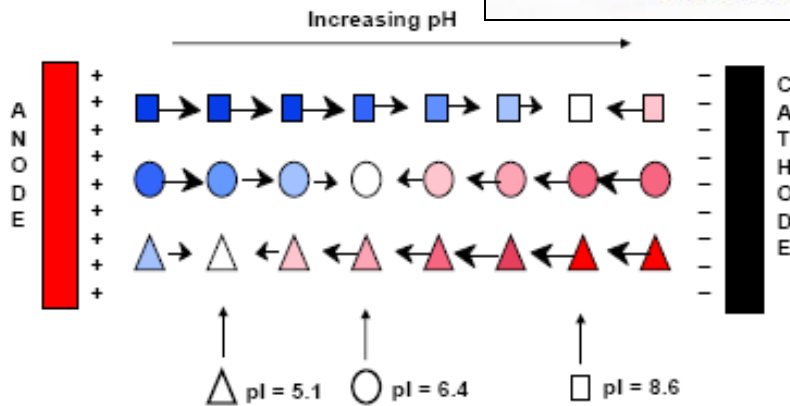
varietà

microbioma



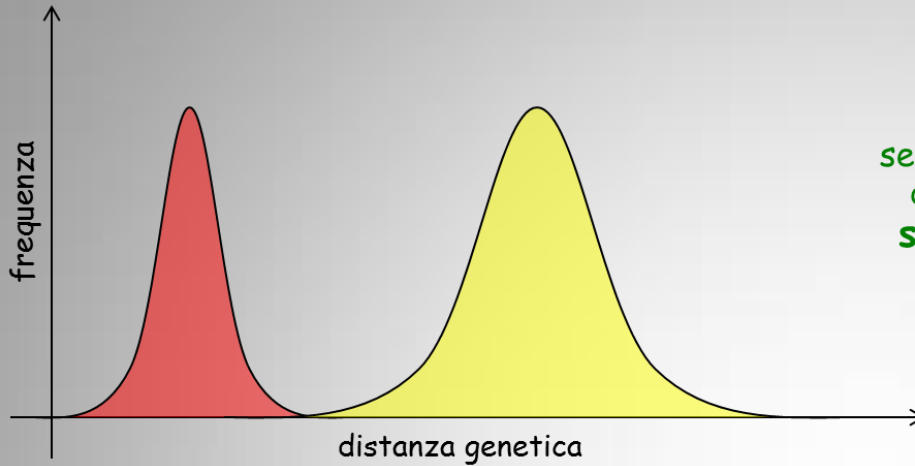
Tracciabilità di specie e qualità

frumento tenero
nelle semole????
3% valore soglia

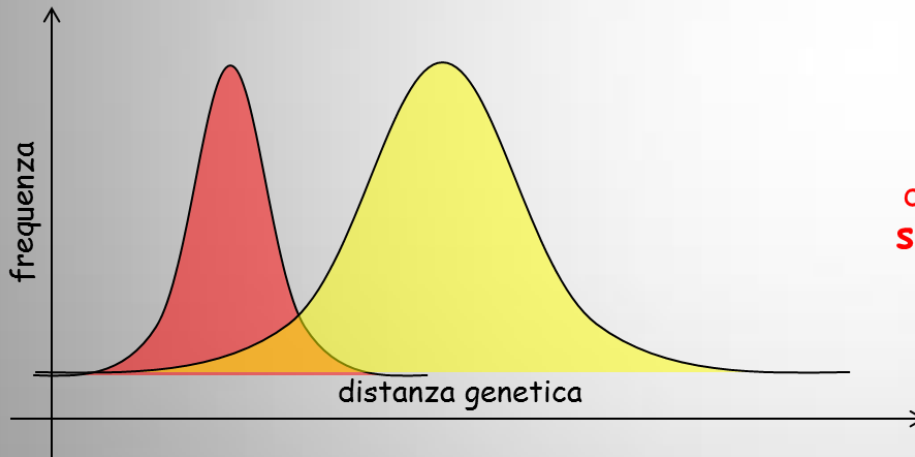


Metodo ufficiale = IEF proteine
di riserva della cariosside

DNA Barcoding



separazione tra i picchi delle
distanze interspecifiche:
sequenza adatta per il
barcoding!



sovrapposizione tra i picchi
delle distanze interspecifiche:
sequenza non adatta per il
barcoding!



Received 29 July 2002
Accepted 30 September 2002
Published online

Biological identifications through DNA barcodes

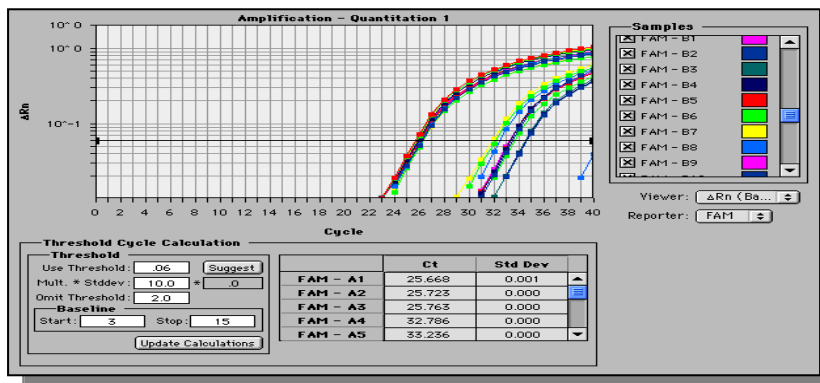
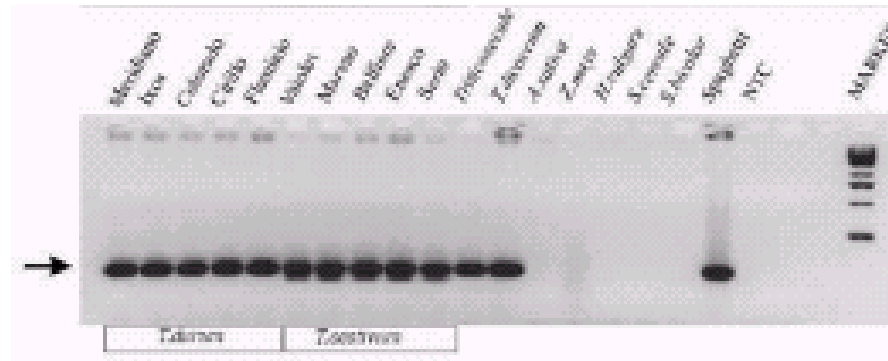
Paul D. N. Hebert*, Alina Cywinska, Shelley L. Ball
and Jeremy R. deWaard

Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Although much biological research depends upon species diagnoses, taxonomic expertise is collapsing. We are convinced that the sole prospect for a sustainable identification capability lies in the construction of systems that employ DNA sequences as taxon 'barcodes'. We establish that the mitochondrial gene cytochrome *c* oxidase I (COI) can serve as the core of a global biodiversity system for animals. First, we demonstrate that COI profiles, derived from the low-density sampling of higher taxonomic categories, ordinarily assign newly analysed taxa to the appropriate phylum or order. Second, we demonstrate that species-level assignments can be obtained by creating comprehensive COI profiles. A model COI profile, based upon the analysis of a single individual from each of 200 closely allied species of lepidopterans, was 100% successful in correctly identifying subsequent specimens. When fully developed, a COI identification system will provide a reliable, cost-effective and accessible solution to the current problem of species identification. Its assembly will also generate important new insights into the diversification of life and the rules of molecular evolution.

Keywords: molecular taxonomy; mitochondrial DNA; animals; insects; sequence diversity; evolution

Sequenze di proteine di riserva per la tracciabilità e quantificazione del DNA di frumento tenero nella filiera pasta



Journal of Cereal Science 38 (2003) 47–54

Journal of
CEREAL
SCIENCE

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Development of analytical systems based on real-time PCR
for *Triticum* species-specific detection and quantitation of bread
wheat contamination in semolina and pasta

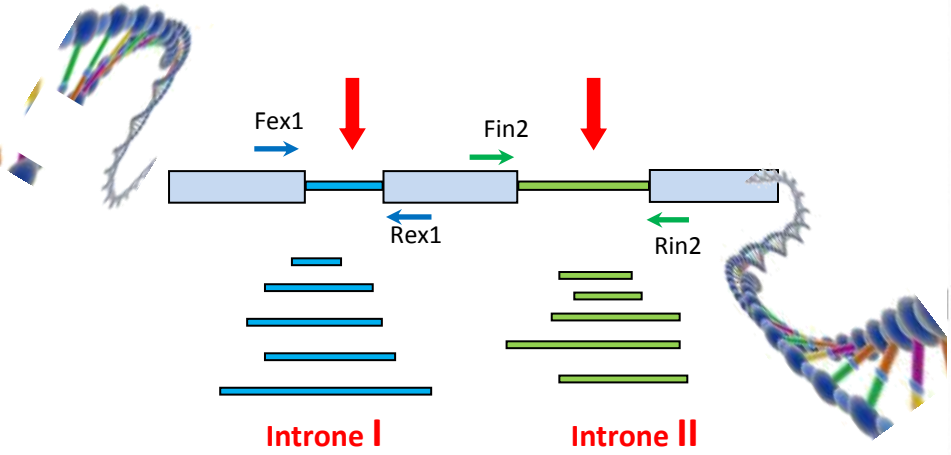
Valeria Terzi^{a,*}, Mauro Malnati^b, Martino Barbarera^c, A. Michele Stanca^a, Primetta Faccioli^d

^a Istituto Sperimentale per la Cerealicoltura, Via San Provasio 80, I-20037 Fiorano di Arda (PC), Italy
^b Unità di Zoologia (Entom. EMB), Istituto Nazionale San Raffaele, Via Olgettina 58, I-20132 Milano, Italy
^c Cerep Italia s.r.l., Via del Lavoro 6/R, I-48015 Castelnuovo di Reno (BO), Italy

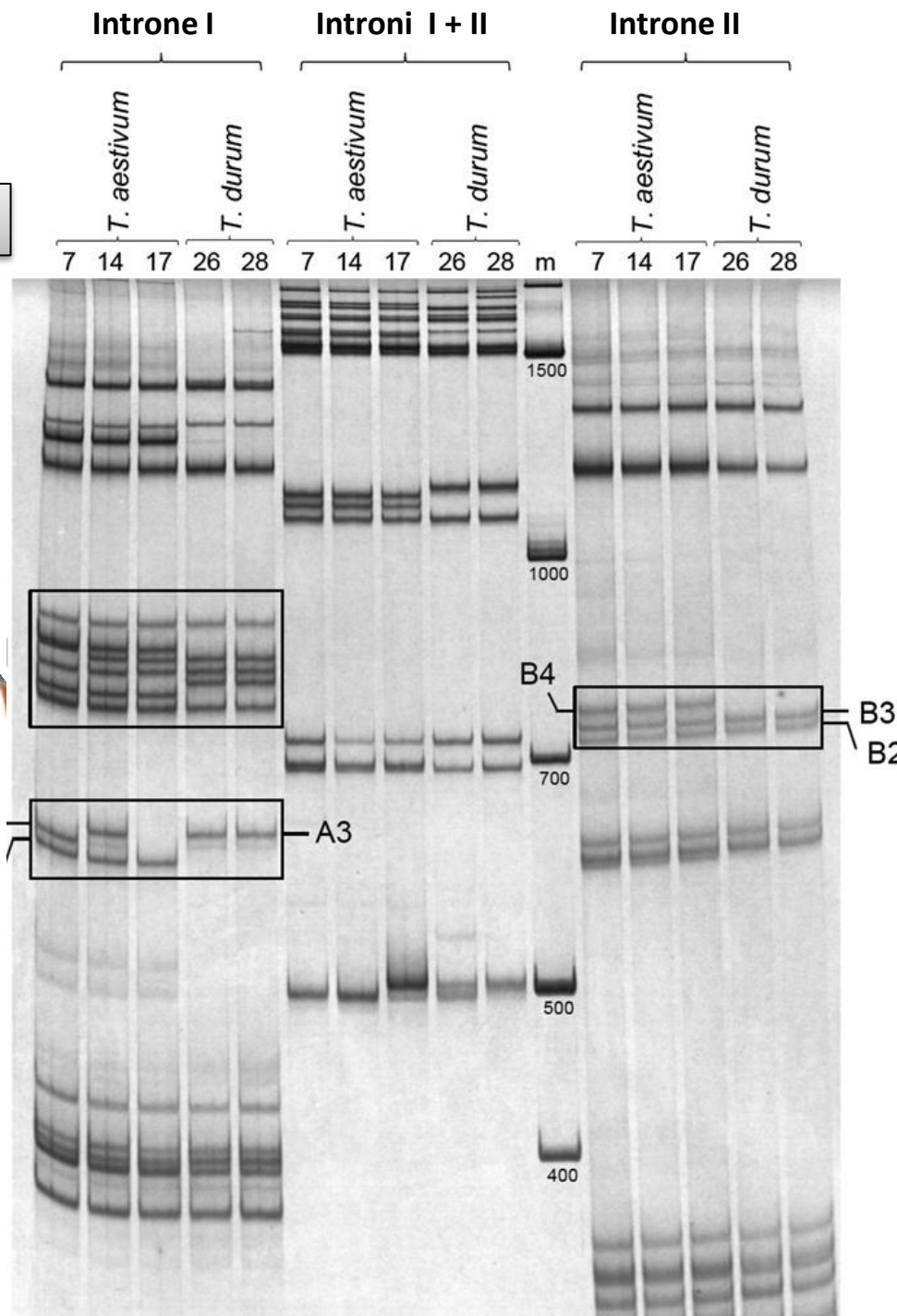
Received 5 August 2002; revised 7 November 2002; accepted 22 November 2002

Il Metodo TBP (Tubulin-Based-Polymorphism)

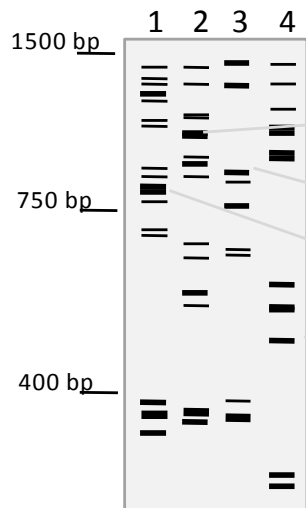
La Famiglia delle β -tubuline [5-20]



1. TBP amplification



2. TBP profile on PAGE



3. Diagnostic band sequencing and probe design

```

GTATACCCTCCCGCGTGGATCTGGACTTTTCCCTTCTCCCGAAATGGATCTCGAATG 60
ATTGGGGCGGTGTGATCTGCTCCCTTCTCTGTGTAAAGCGACTTGTAGATGCT 120
ATTAGATTAC TGGGTTTTCGCGCTCTAGACTCCGCGTTCCTCCCTTCCGAGGAG 180
TGGTCCGCGTGGTTCGAACTCTAGACTCTGACTCCAGCACTACCAGGATCAGGATACA 240
CTGTCCATTATGCTTGTCTACTTGACAGAGAGAGCGCTTTGGATGGTTATGTGGATCTA 300
ATC GCGGAGATCAGAGAAATAGCGGCATGGATTCGATATATGTCTGCTCCGTTGTGTTTC 360
CTC GGTATGATAGATCARTCTGATTCCTCCGCTTCTACGCGTATGATGATCTGTTG 420
CATTAAGTGTGTTTTACGTAGGCTTCAGCGATCATCTTCCGCTCATCTATGT 480
GTGAATCTATGTTTAAAGTAGTACTTTCCTACTCTCTACTCTCTGAGTAAAGTA 540
TGTAGTAAATCTAAAAGAGTCAAAAGCATCGGTAGAGTAGCATGCTCTGATGCGGATT 600
CTCAAGCACCAGTAGGATGCTTATGATGTGTGTGGATCAAGCATCAGTAGAGTAGCA 660
TGC TGA TGTGATTCCAGCAGCAGTAGGCTCTTAAAGTGTGTGTGATGAGCAAG 720
CATCGGTGATGAGATGCTGATGATGATGATGATGATGATGATGATGATGATGATGAT 780
TGATGATTCATATGTCAGTTTCCCTGATGATGATGATGATGATGATGATGATGATGAT 840
ATAACACTACTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 900
AAATTAACCTATGTTTTTCTATCCACAG 930
    
```

```

GTATTTGAATCGAA TTAATGTTGATAGT TTAGAATC CTATGGCAGGCTTACATGT 60
ATTAAATTCAGTTTTGGATATAGAAACAGCCACTTACTGTGTGATGAGATGAAATGTGT 120
TAGGCTACAGTATGATTTACCGCCATGATGATGATGATGATGATGATGATGATGATGAT 180
AGTATGCTATCTTTTTTATGCGCATGATGATGATGATGATGATGATGATGATGATGATGAT 240
AACCTGATCTATGCGAGCACTGGTGTGATGATGATGATGATGATGATGATGATGATGATGAT 300
ATTGTTTTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 360
GTAGAGATCTAGCAATAC TACTGATGATGATGATGATGATGATGATGATGATGATGATGAT 420
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GCATCTTTTATGTTTTTCAG 500
    
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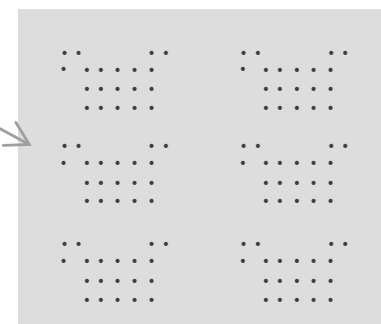
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GTATACCCTCCCGCGTGGATCTGGACTTTTCCCTTCTCCCGAAATGGATCTCGAATG 60
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ATTAGATTAC TGGGTTTTCGCGCTCTAGACTCCGCGTTCCTCCCTTCCGAGGAG 180
TGGTCCGCGTGGTTCGAACTCTAGACTCTGACTCCAGCACTACCAGGATCAGGATACA 240
CTGTCCATTATGCTTGTCTACTTGACAGAGAGAGCGCTTTGGATGGTTATGTGGATCTA 300
    
```

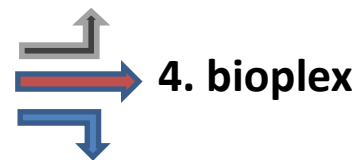
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TATTA CCGCAGTAATGCTATGCGAATGATGCGCGTGGGATCTCTGTTT 180
TTTATTGGCAGTATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 240
GACAACTTGGTATTA TCAAGAGTAGTACTAGGCTCTCGCTTGC 300
TTGTCATGGCACTGATTGCTTCCATATCTGTGTGAATTA TCTGATA 360
ATTAAC TACTGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 420
TATTTATATTCAGGATTAACCTCTGTCAGTTGAAATCATTATTTAA 480
TTTTCA G 500
    
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Casazza et al. Journal of Cereal science
56 (2012) 733-740

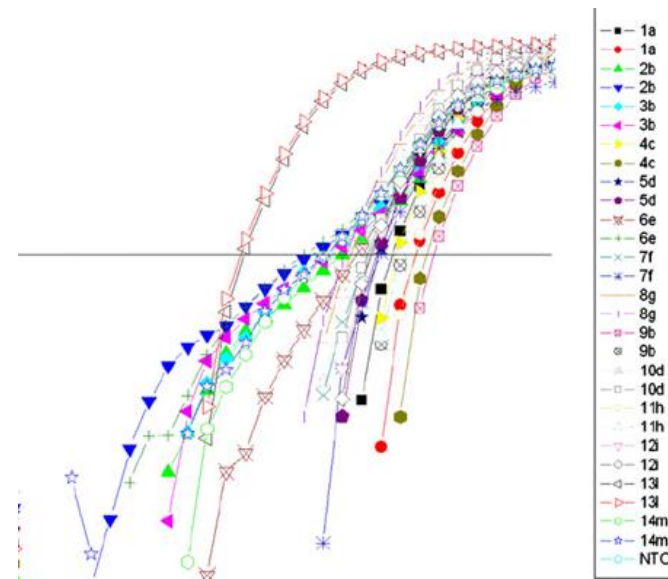


4. Array preparation

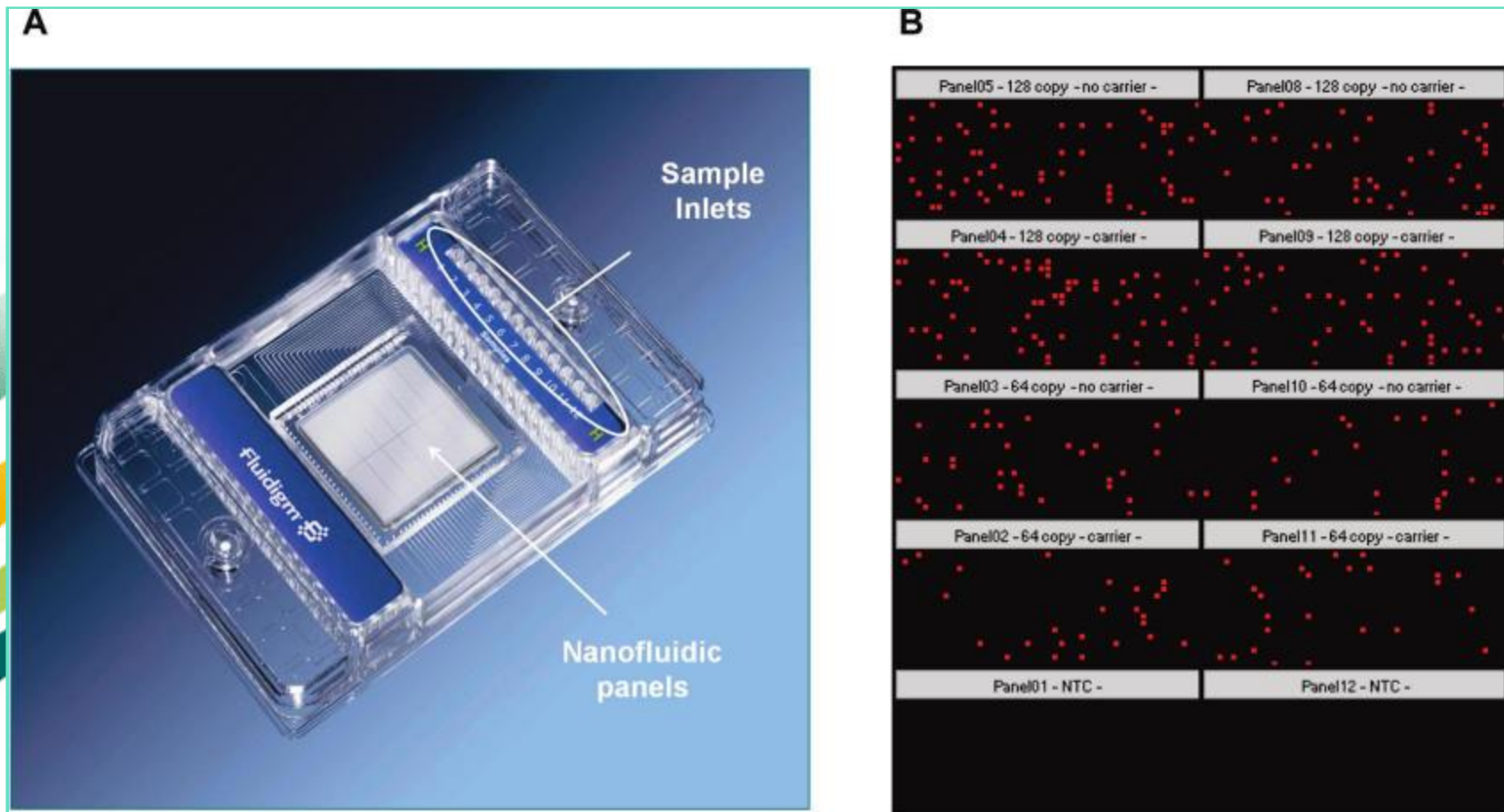


4. RT-PCR

SAMPLE	Mean Ct	Soft wheat amount %
1	32.07	0.4
2	28.05	5.9
3	28.24	5.2
4	32.49	0.3
5	30.66	1.0
6	28.01	6.1
7	30.60	1.0
8	29.21	2.7
9	32.87	0.2
10	29.89	1.7
11	32.30	0.3
12	30.41	1.2
13	23.73	100
14	28.07	5.9

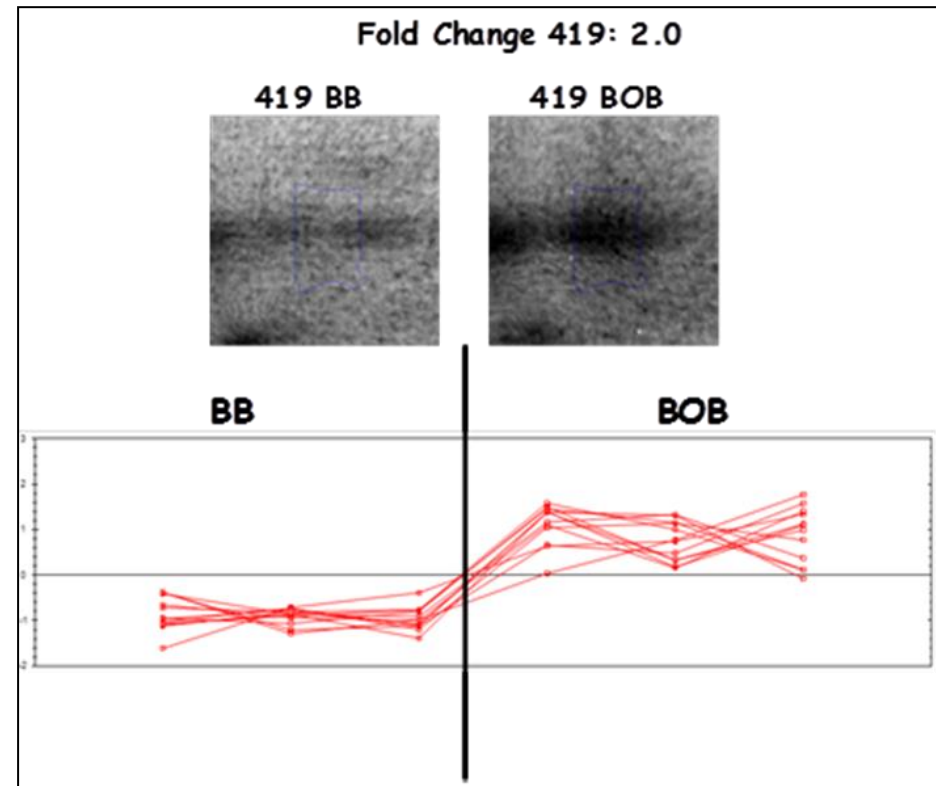


Un nuovo modo di fare qPCR: microfluidic digital PCR (dPCR)



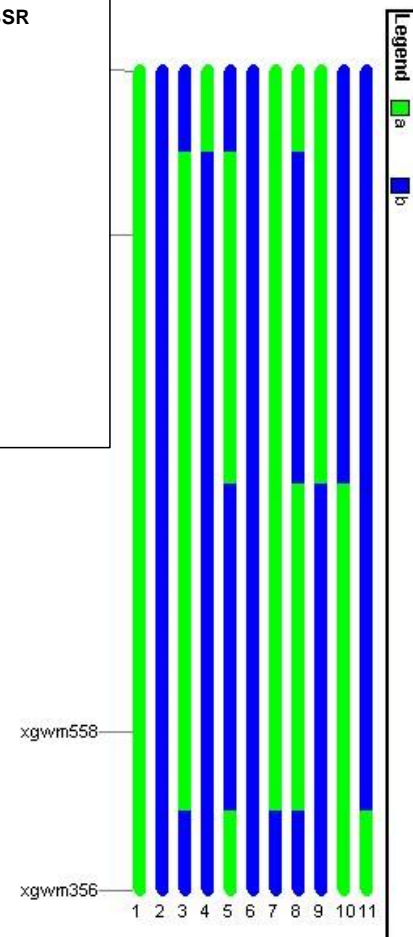
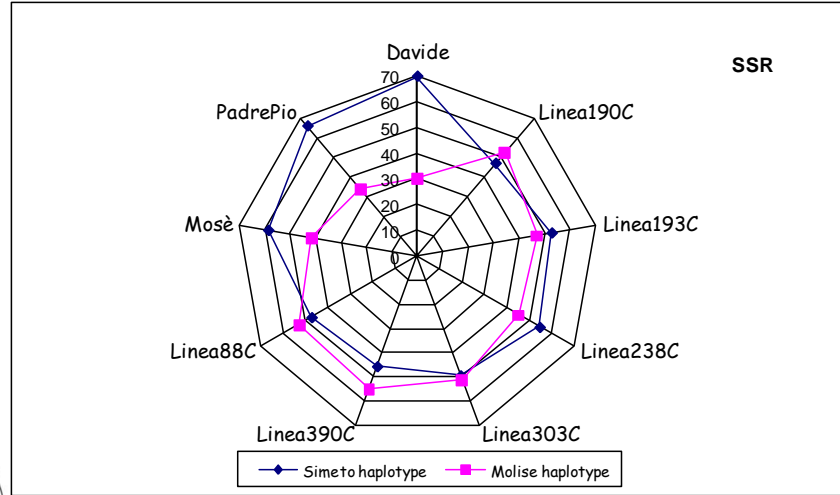
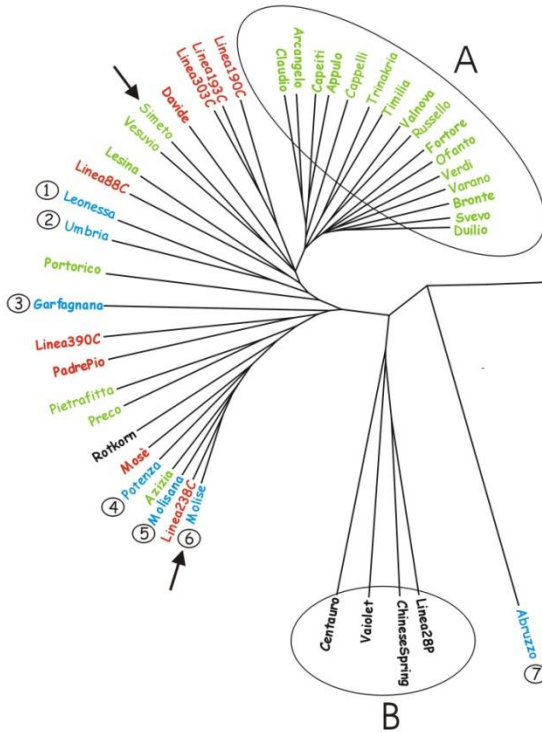
Sanders et al, Anal Chem, 2011

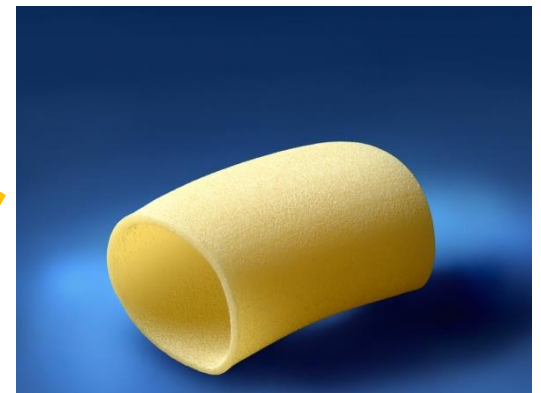
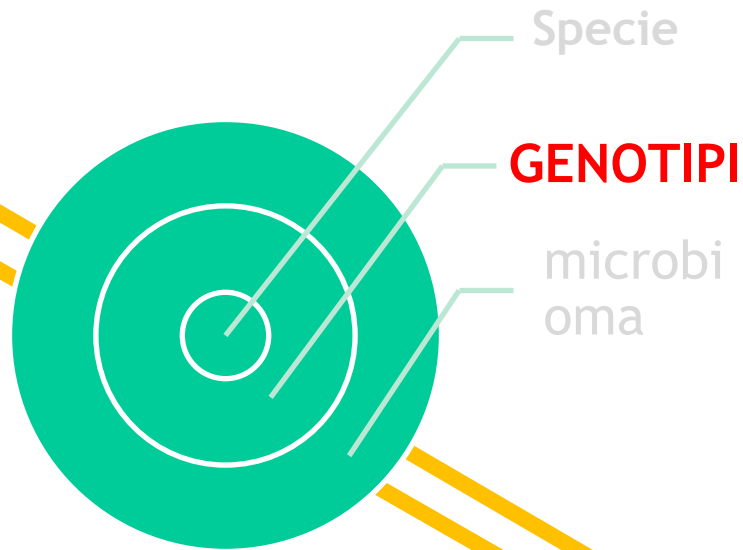
Analisi del proteoma del pane per la tracciabilità di specie



Assessment of genetic diversity in emmer (*Triticum dicoccon* Schrank) × durum wheat (*Triticum durum* Desf.) derived lines and their parents using mapped and unmapped molecular markers

Valeria Terzi · Caterina Morcia · A. Michele Stanca · Ludislaw Kusura · Clara Fenu · Pasquale Cattivelli · Nicola Di Fonzo · Primitiva Facioli





Tracciabilità di varietà

ARDA

Anno di iscrizione al Paese d'origine: 1988
 Anno di iscrizione al Registro nazionale italiano: 1985
 Genitori: Iari x ILE 51-43-3
 Coviatore: Istituto sperimentale per la Cerealicoltura - Roma, Italia

CARATTERI VEGETATIVI

Portamento a fave acrobatiche
 semi-risolto

Stelo:
 medio

Pigmentazione anticimica delle
 auricole:
 media

Glutine:
 +Bontà media
 glucosidica, media

CARATTERI DELLA SPIGA

Tipi:
 diffuso

Aristare:
 presente

Sagittatura delle ariste:
 medio

Portamento:
 acrobatico

Compattezza:
 alta

Glucosidica:
 medio o medio debole

Forma delle spighe:
 sterili,
 deboli

Lunghezza dell'articolazione
 del rachide:
 corto

Lunghezza delle glume in rapporto
 alla glume la inferiore:
 più corta

CARATTERI DEL SEME

Vestito:
 medio

Lunghezza dei peli della rachide:
 lunga

Sagittatura delle glume:
 acrobato

Vibosità del seme:
 medio

Aristazioni sulle nervature:
 presente

CARATTERI FENOLOGICI

Ritmo:
 intermedio

Classificazione della spigatura
 il fiorimento Agri:
 da 4 a 7 giorni più precoce

ARDA

ARDA

CORNEL



Protein chip

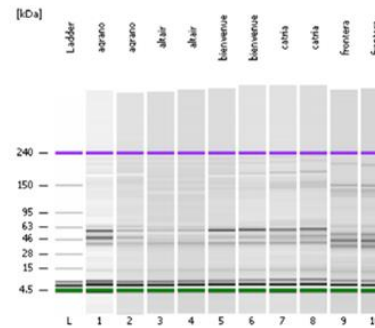
2100 expert_Protein 230_DE72901269_2008-11-06_11-06-50.xad

Page 1 of 12

Assay Class: Protein 230
 Data Path: C:\...2100 expert_Protein 230_DE72901269_2008-11-06_11-06-50.xad

Created: 11/6/2008 11:06:50 AM
 Modified: 11/6/2008 11:36:21 AM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DE72901269
 Serial #: DE72901269
 Firmware: C.01.069
 Type: G2939A

Assay Information:

Assay Origin Path: C:\Program Files\Agilent\2100 bioanalyzer\2100 expert\assays\protein\Protein 230 Series II.xad
 Title: Protein Analysis 14 - 230 kDa
 Version: 3.1
 Assay Comments: © Copyright 2003 - 2008 Agilent Technologies, Inc.

Chip Information:

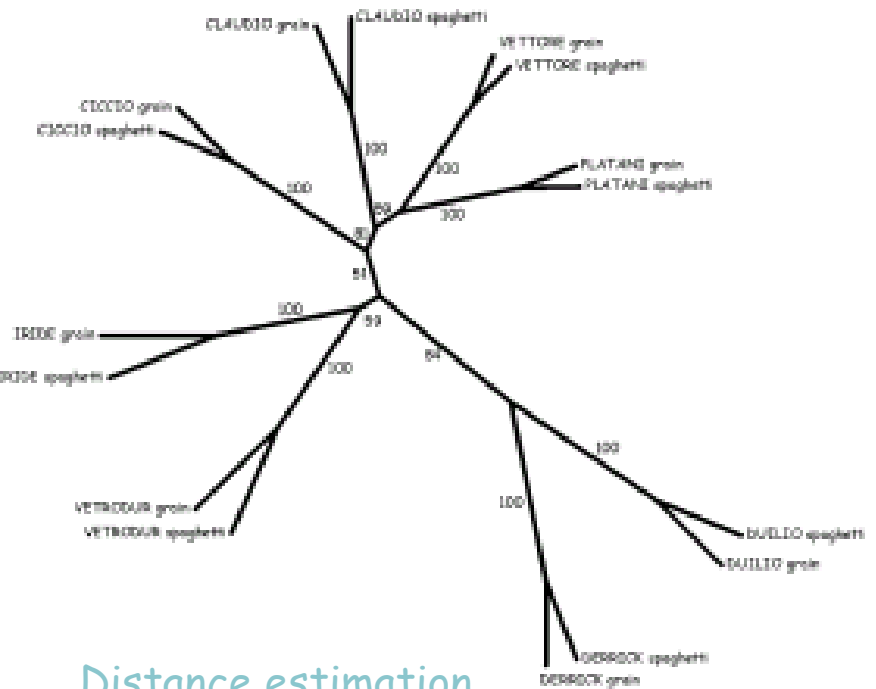
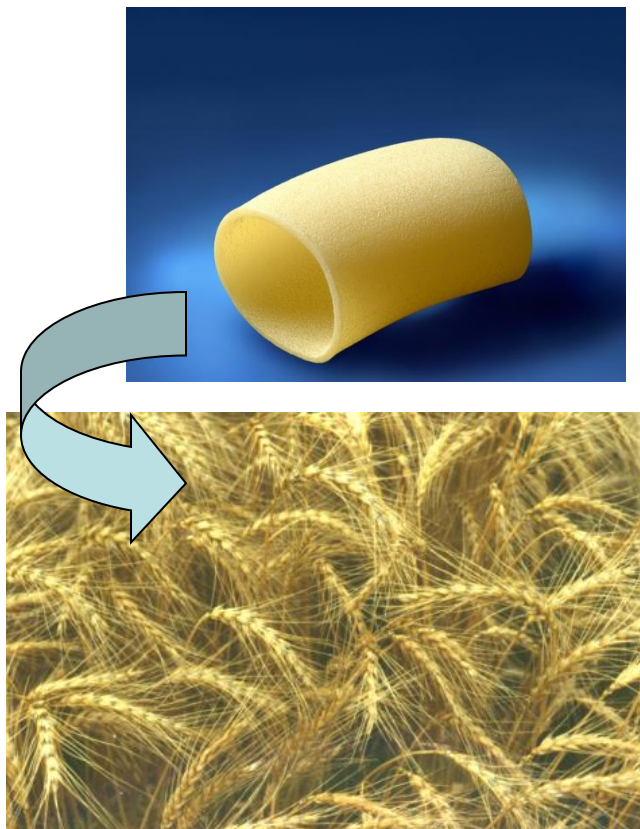
Chip Lot:
 Reagent Kit Lot:
 Chip Comments:



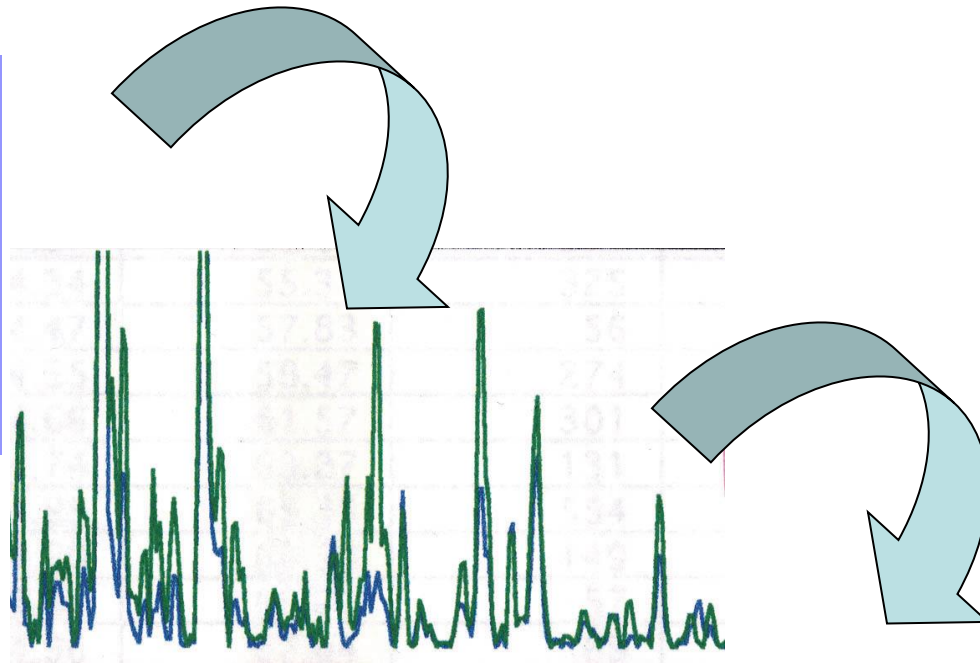
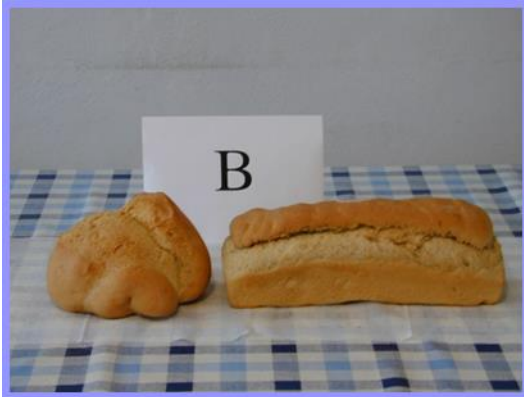
ORIGINAL PAPER

Valeria Terzi · Caterina Morcia · Davide Giovanardi ·
Maria Grazia D'Egidio · A. Michele Stanca ·
Primetta Faccioli

DNA-based analysis for authenticity assessment of monovarietal pasta

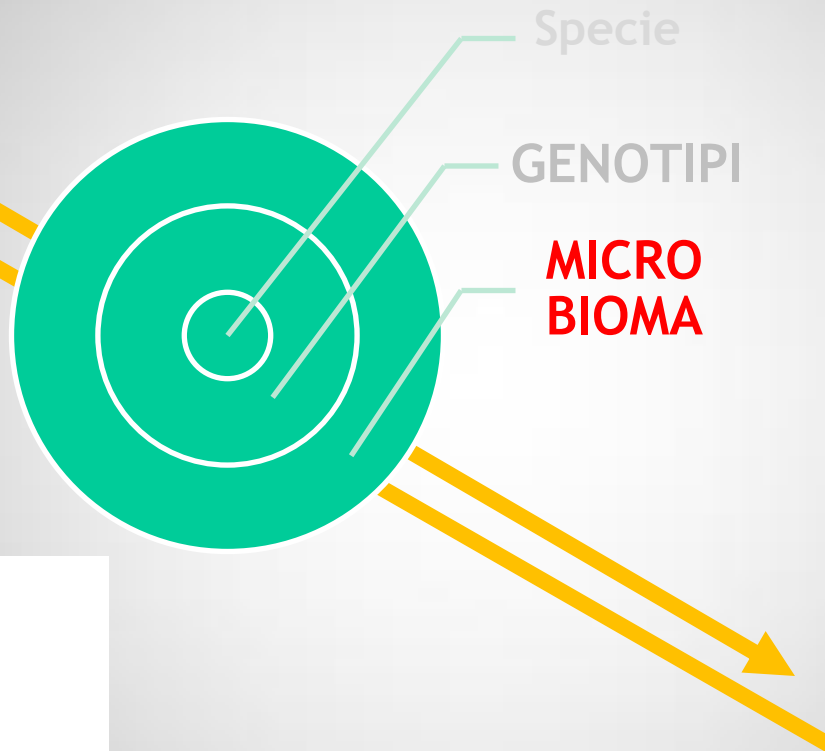


Tracciabilità di varietà



“Passaporto molecolare” per certificazione di autenticità varietale





Specie

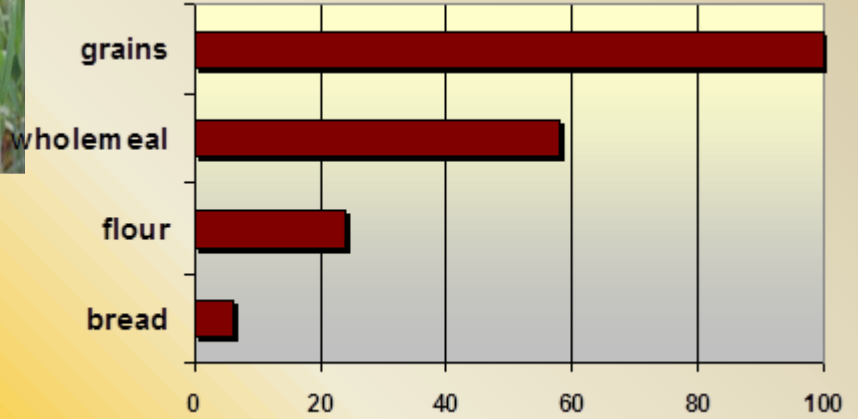
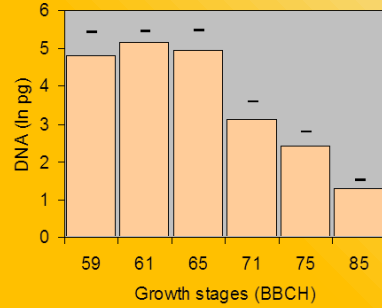
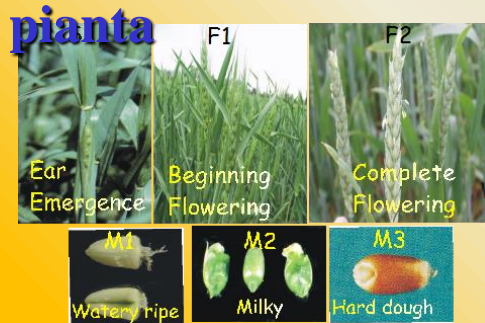
GENOTIPI

**MICRO
BIOMA**

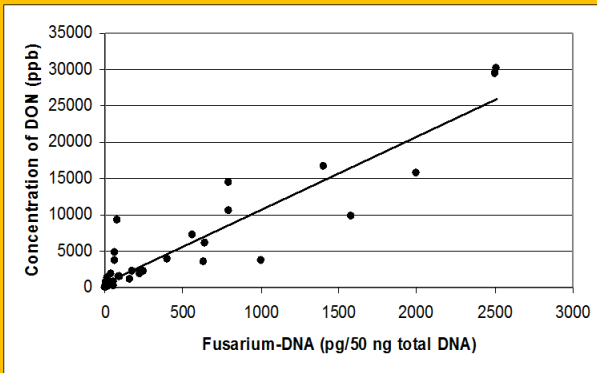


Tracciabilità DNA-based di *Fusarium* lungo la catena di produzione del pane

Fungus	Hours	Bilancia	Sagittario	Duilio	S. Carlo
<i>F. culmorum</i>	0				
	6				
	12				
	18				
	24				
	48				
	72				
	144				
	216				
288					
<i>F. graminearum</i>	0				
	6				
	12				
	18				
	24				
	48				
	72				
	144				
	216				
288					



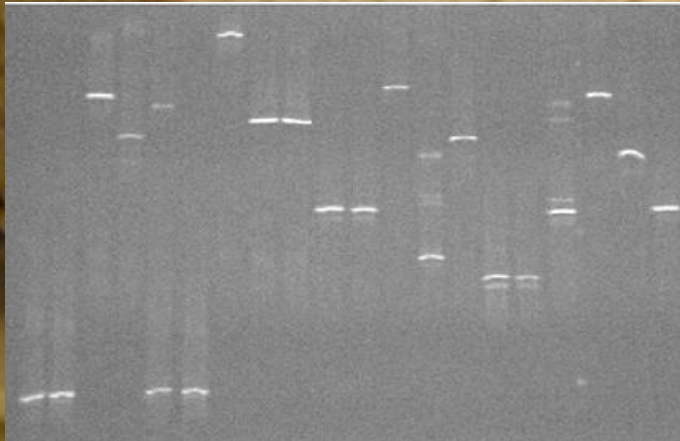
International Journal of Food Science and Technology
 Original article
Fusarium DNA traceability along the bread production chain
 Valeria Trenti,^{1*} Caterina Marzola,² Pinella Pascale,³ Nadia Piacchi,⁴ Vittorio Rossi,⁴ Mariacristina Cipolletti,⁵ Maria Corbelli,⁶ Diego Scuderi,⁷ R. Giovanni DeLuca⁸
 1 C.R.A. - Centro Ricerche Cereali, Via San Pietro 102, 20017 Fontanafredda (TV), Italy
 2 Istituto di Entomologia e Patologia vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 14, 29100 Parma, Italy
 3 C.R.A. - Istituto Sperimentale per la Cerealicoltura, Via R. Fieschi 1, 20078 San Agostino, Longiano (PR), Italy
 4 CRP, Centro Ricerche Produzioni Vegetali, Via Emilia Levante 10, 40138 Imola (BO), Italy
 (*Received 10 January 2006; Accepted in revised form 10 May 2006)



Coefficiente di correlazione tra il contenuto di DNA e la concentrazione di DON = 0.9291



Caratterizzazione della flora microbica degli impasti acidi.



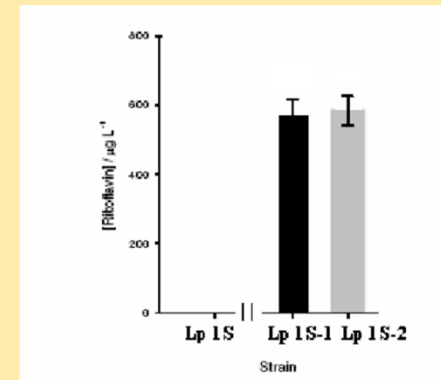
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1177F	5'GAGTACGacCGCAAGgt	16S V6
1258F	5'CATGTGGTTTAATTCTGAAGCaAC	16S V6
V6F	5'TCGAtGCAACGCGAAGAA	16S V6
1046R	5'CGACAACCATGCAGCACCT	16S V6
1391R	5'GACGGGCGGTGtGTaCA	16S V6
1731R	5'CGGGAACGTATTACCGCGGC	16S V6
1504R	5'TTTGTCAACCGCA	16S V6
1538R	5'TAAGGGGCATGATGATTTGACG	16S V6
V6R	5'ACATtTCACaACACGAGCTGACGA	16S V6
NL1F	5'GCATATCAATAAGCGGAGGAAAAG	26S
NL1shortF	5'GCATATCAATAAGCGGAGGAA	26S
NL4R	5'GGTCCGTGTTTCAAGACGG	26S

DGGE

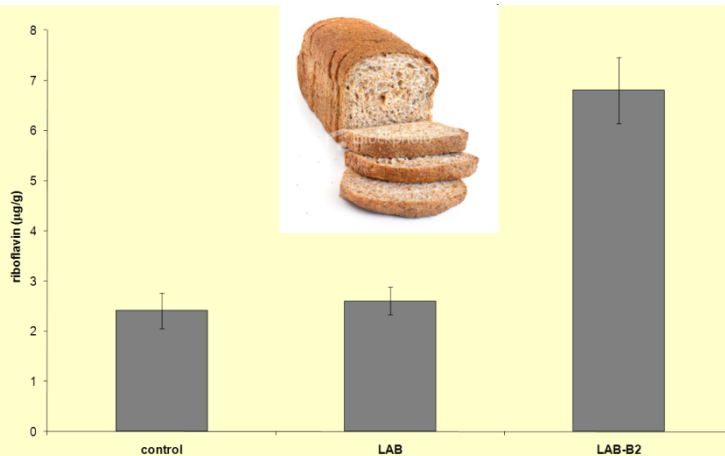
Sequenziamento

Riborich (Riboflavin enriched bread and pasta)

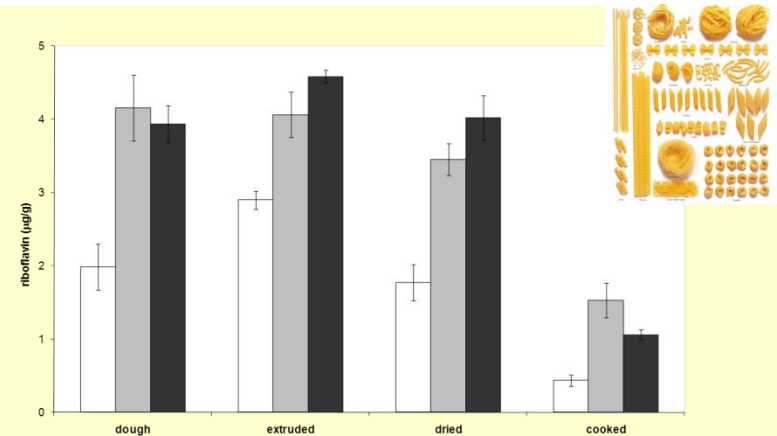
Identification of (sourdough) Lactic Acid Bacteria overproducing riboflavin



Riboflavin produced by *Lactobacillus plantarum* Lp 1S-1 and Lp 1S-2 strains was determined in the cell free supernatant.



Riboflavin in bread trials. Final riboflavin levels in bread inoculated with baking yeast cultures (control), with *L. plantarum* WCFS1 (LAB), and with selected riboflavin-overproducing derivatives (LAB-B2). Data are means \pm standard deviations for three replicates.



The concentration of riboflavin at specific steps of the pasta-making process using PR22D89 semolina. Vitamin B2 content is presented for samples taken after kneading (dough), extrusion (extruded), drying (dried), and after cooking (cooked). All trials were subjected to a pre-fermentation step of 16 hours. White bar charts indicate the dough humidity at 42%, without microbial inoculation; grey bar charts and black bar charts indicate the dough inoculated with selected riboflavin-overproducing derivatives, with respectively 42 % and 60 % humidity level. Data are means \pm standard deviations for three replicates.

Biotechnological Production of Vitamin B2-Enriched Bread and Pasta

Vittorio Capozzi,[†] Valeria Menga,[§] Anna Maria Digesù,[§] Pasquale De Vita,[§] Douwe van Sinderen,[#]
Luigi Cattivelli,[‡] Clara Fares,[§] and Giuseppe Spano^{*,†}

frontiers in
MICROBIOLOGY

MINI REVIEW ARTICLE
published: xx March 2012
doi: 10.3389/fmicb.2012.00004



Biotechnology and pasta-making: lactic acid bacteria as a new driver of innovation

Vittorio Capozzi^{1,2}, Pasquale Russo^{1,2}, Mariagiovanna Fragasso², Pasquale De Vita², Daniela Fiocco⁴ and Giuseppe Spano^{1}*

Appl Microbiol Biotechnol
DOI 10.1007/s00253-012-4440-2

MINI-REVIEW

Lactic acid bacteria producing B-group vitamins: a great potential for functional cereals products

Vittorio Capozzi • Pasquale Russo •
María Teresa Dueñas • Paloma López • Giuseppe Spano

Grazie per l'attenzione!