

# *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*

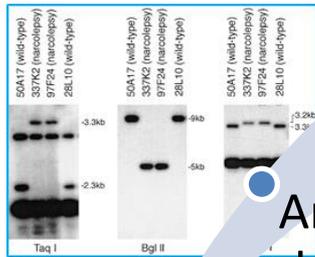
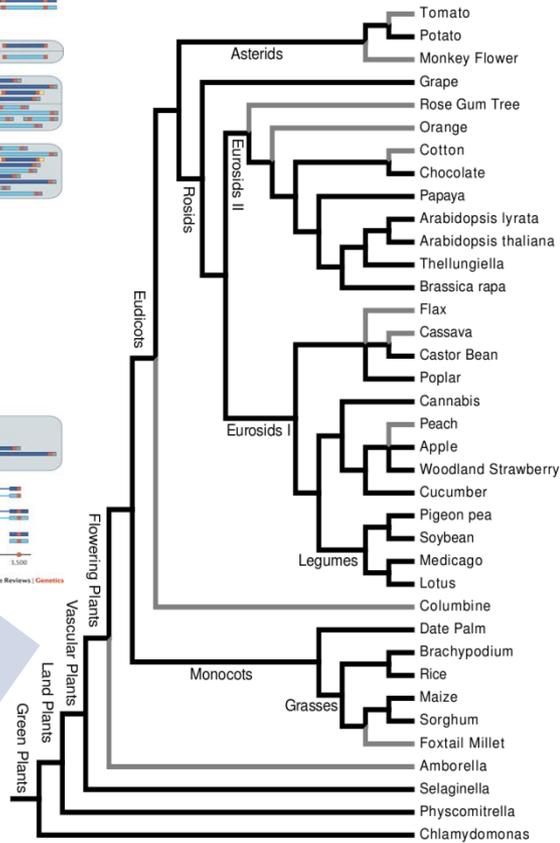
*\*\*IBBA-CNR, Milano*

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

*Firenze, 28 novembre 2013*

# TRACCIABILITA' MOLECOLARE

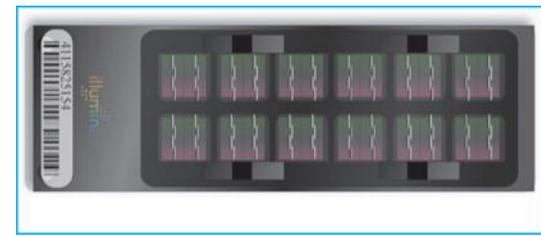
*..in prodotti di filiera...*



Anni '00:  
centinaia di  
marcatori

Anni '90:  
decine di  
marcatori

Oggi:  
migliaia di  
marcatori,  
genomi



TRACCIABILITA' MOLECOLARE



Specie

varietà

microbioma



TRACCIABILITA' MOLECOLARE



Specie

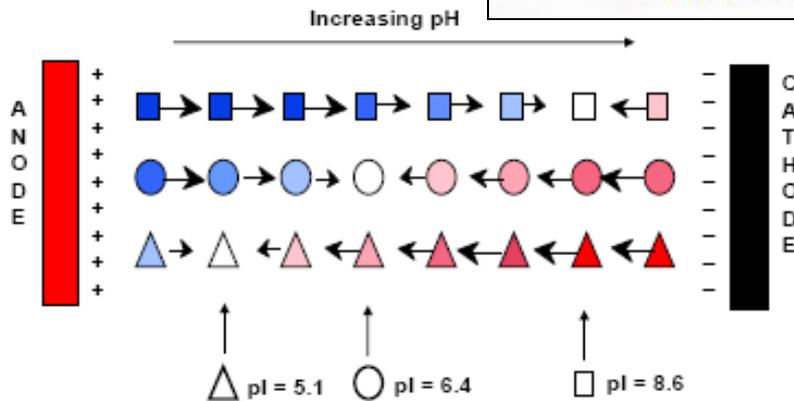
varietà

microbioma



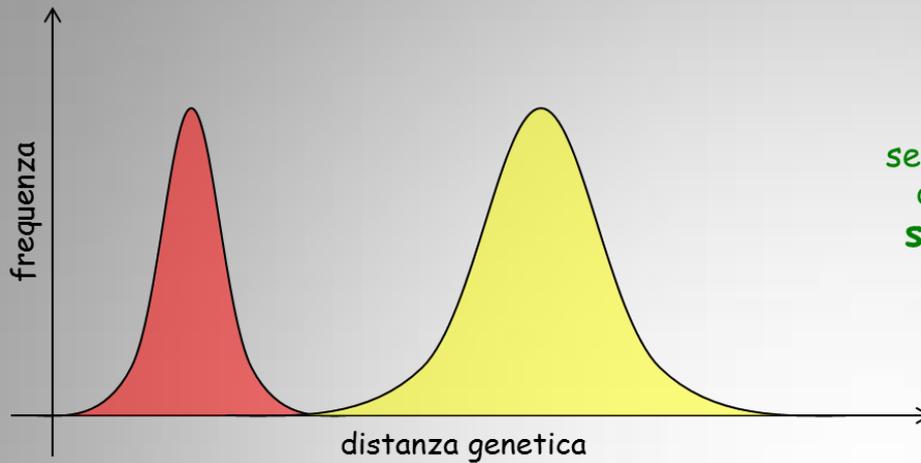
# Tracciabilità di specie e qualità

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

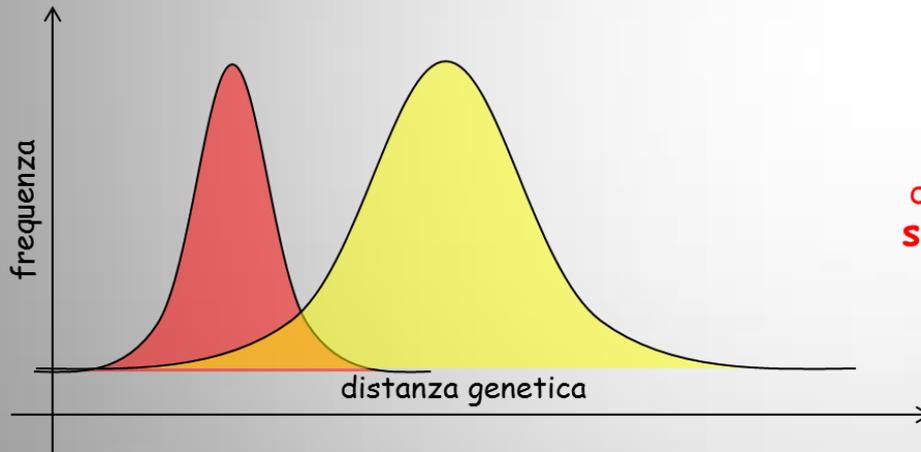


Metodo ufficiale = IEF proteine  
di riserva della cariosside

# 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

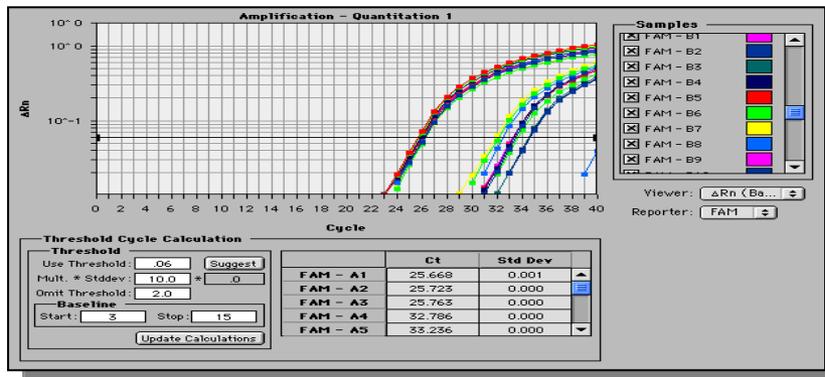
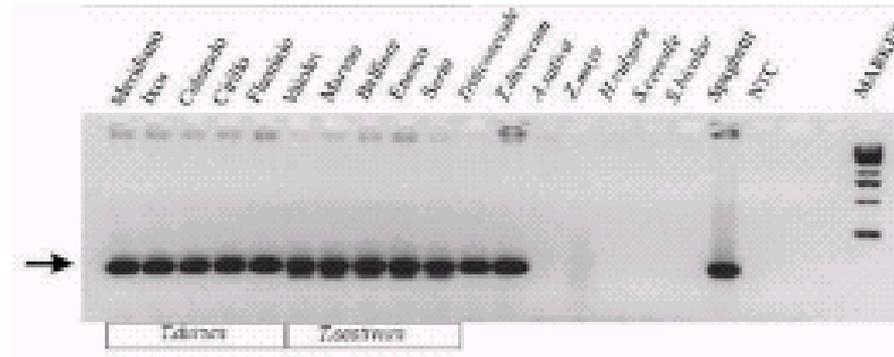
Paul D. N. Hebert\*, Alina Cywinska, Shelley L. Ball  
and Jeremy R. deWaard

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 bioidentification 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



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

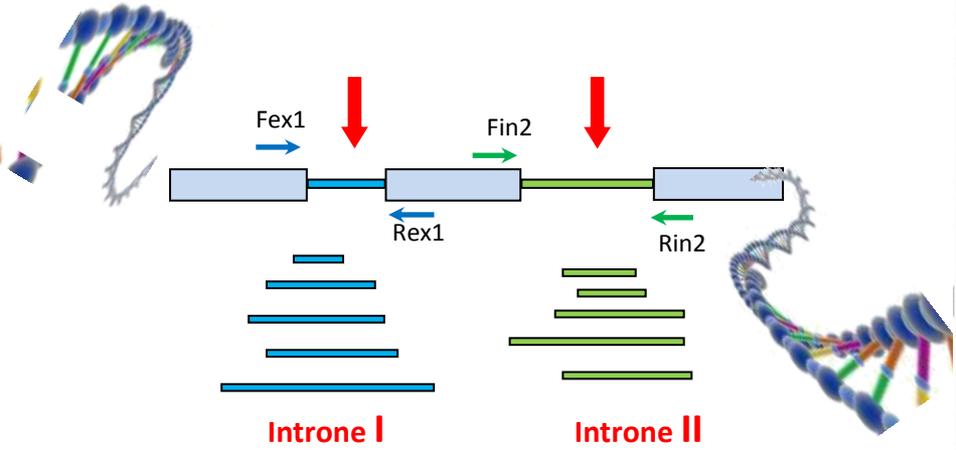
Valeria Terzi<sup>a,\*</sup>, Mauro Malnati<sup>b</sup>, Martino Barbarera<sup>c</sup>, A. Michele Stanca<sup>a</sup>, Primetta Faccioli<sup>d</sup>

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

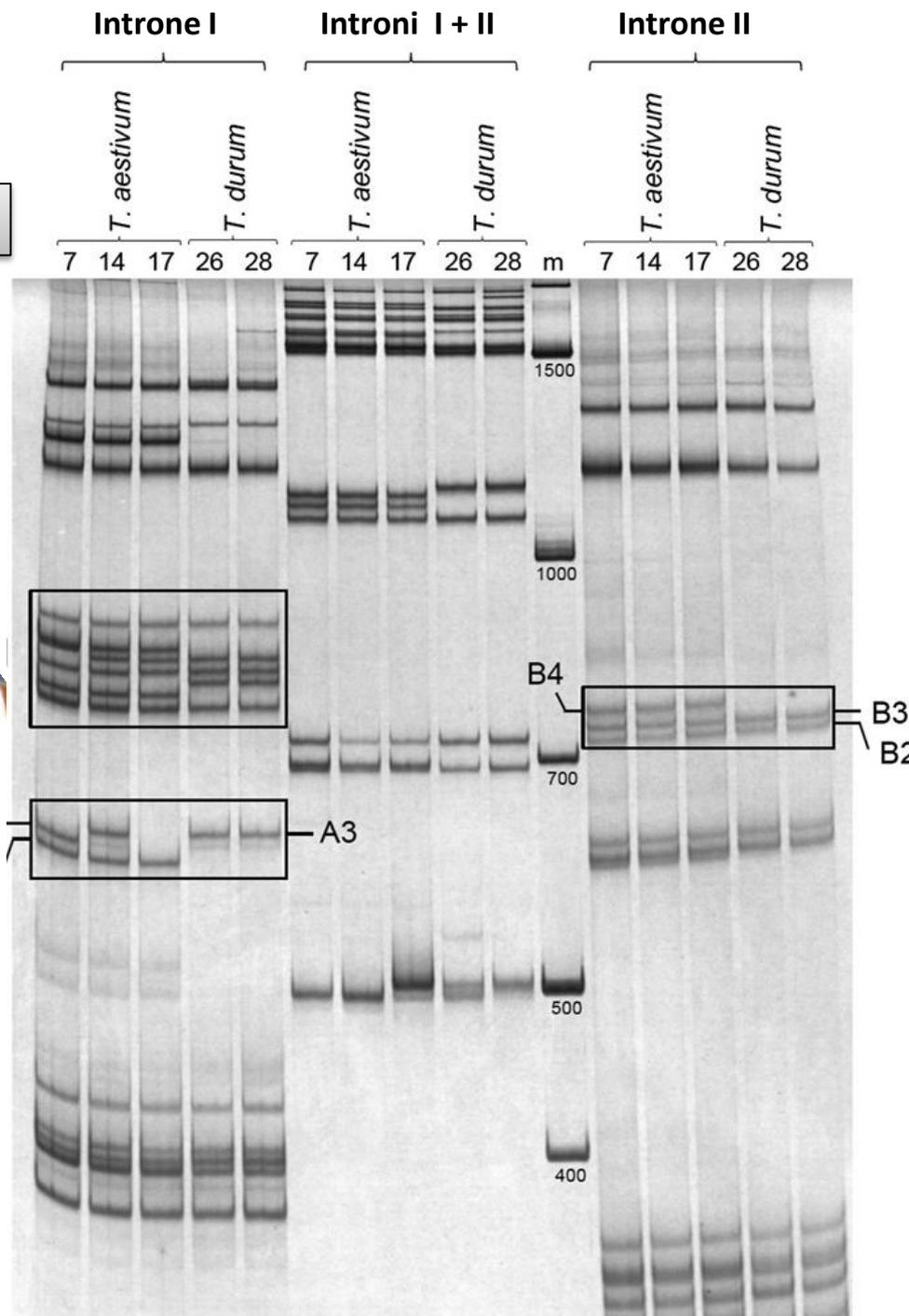
Received 5 August 2002; revised 7 November 2002; accepted 22 November 2002

**Il Metodo TBP (Tubulin-Based-Polymorphism)**

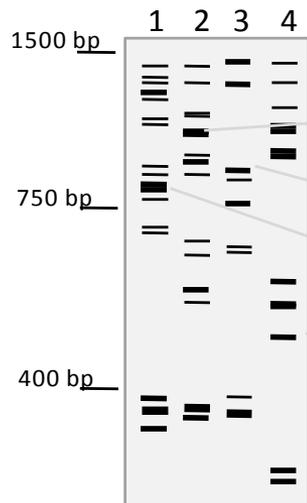
**La Famiglia delle  $\beta$ -tubuline [5-20]**



**1. TBP amplification**



## 2. TBP profile on PAGE



```

GTATACCCTCCCGCGTGGATCTGGACTTTTCCCTTCTCCCGAAATGGATCTCGAATG 60
ATTGGGGCGGTGTGATCTCTCCCTTCTCTGTGTAAAGCGACTTGTAGATGCT 120
ATTAGATTAC TGGGTTTTCGCGCTGTAGACTCTGGCGTTTCTCTCCCTTCCGAGGAG 180
TGGTCCGCGTGGTTCGAACTGTAGACTCTGGACTCCAGCACTACCAGGATCAGGATACA 240
CTGTCCATTATGCTTGTCTACTTGACAGAGAGAGCGTTTGGATGGTTATGTGGATCTA 300
ATC GCGGAGATCAGAGAAATAGCGGCATGGATTCGATATATGTCTGCTCCGTTGTGTTC 360
CTC GTGTATGATAGATCARTCTGATTCCTCCGCTTCTACGCGTATGATGATCTGTG 420
CATTAAGTGTGTTTTCAGGTGAGGCTTCCAGGATGATCTGTGGTGCATACTATGT 480
GTGAATTCATGTTTAAAGTAGTCTTCCACTCTTACTACTCTCTGAGATAAGTA 540
TGTAGTAAATCTCAAAGAGTCAAAAGCATCGGTAGAGTAGCATGTCTGTGCGATTT 600
CTCAAGCACCAGTAGGATGCTTATGTGTGTGTGGATCAAGCATCAGTAGAGTAGCA 660
TGC TTGA TGTGATTCCAGCAGCAGTAGGACTGCTTAAAGTGTGTGTGTAGCAAG 720
CATCGGTGATGAGATGCTGATGTTGTGTGTGAGCAGCAGTGTGATGATGATGCT 780
TGATGTATTCATATGTGCAAGTTTCCCTGATGTGTGTGTGTGCTGACCATCAAAAC 840
ATAACACTACTGTAGTTATCCGCTCACCTGTTTATCTGATGTAACTGAAATGAGATCC 900
AAATTAACCTATGTTTTCATCTACCACAG 930
    
```

```

GTATTTGAATCGAA TTAATTTGGTGA TAGTTTGAATC CTATGGCAGCCTTACATGT 60
ATTAAATTCAGTTTTGGATATAGAAACAGCCACTTACTGTGTGTGAGATTTGAAATGTGT 120
TGGCTACAGTATGTATTTCCGCCATGTATGCGATGATCCGCTGGCATCTTCTGTT 180
AGTATGCATCTTTTTTATTTGCGCATGATTC TTGTGTGTAATGAGGCACTAGTTGGAA 240
AACCTGCATCTTGGAGCACTGGGTGTATATTCAGAGTTAGTA CTAGGCTCTCTCTGTC 300
ATTGTTTTGTATTTGCTGATGGCACTCATTGCCATATCTGGTGAATTA TCTGATA 360
GTAGACATCTAGCA TAAC TACTGATTTAGCTATGCTTCTCATCTATTTGTGTGT 420
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GCATCTTTTATGTTTTTCAG 500
    
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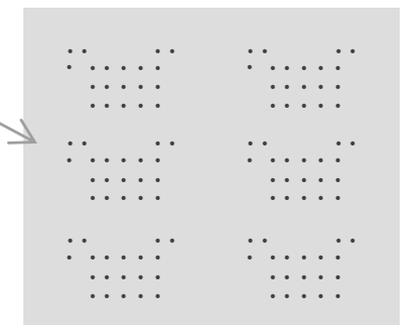
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GTATACCCTCCCGCGTGGATCTGGACTTTTCCCTTCTCCCGAAATGGATCTCGAATG 60
ATTTTGGGGCGGTGTGATCTGGCTGCCCTTCTTCTGTGTAAAGCGACTTGTAGATGCT 120
ATTAGATTAC TGGGTTTTCGCGCTGTAGACTCTGGCGTTTCTCTCCCTTCCGAGGAG 180
TGGTCCGCGTGGTTCGAACTGTAGACTCTGGACTCCAGCACTACCAGGATCAGGATACA 240
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```

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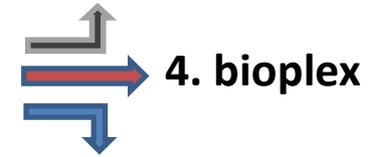
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TTTATTGGCAGTATTACTTGTGTTGTGAATGAGGCACTAGTTGGAA 240
GACAACTTGGTATTA TCAAGAGTAGTACTAGGCTCTCTGCTTGC 300
TTGTCATGGCACTGATTGCTTCCATATCTGTGTGAATTA TCTGATA 360
ATTAAC TACTGATATGACCTATGCTGCTTTCATCTATTTGTGTGT 420
TATTTATATTCAGGATTA CCTCTGTCCAGTTGAAATCATTATTTAA 480
TTTTCA G 500
    
```

Casazza et al. Journal of Cereal science 56 (2012) 733-740



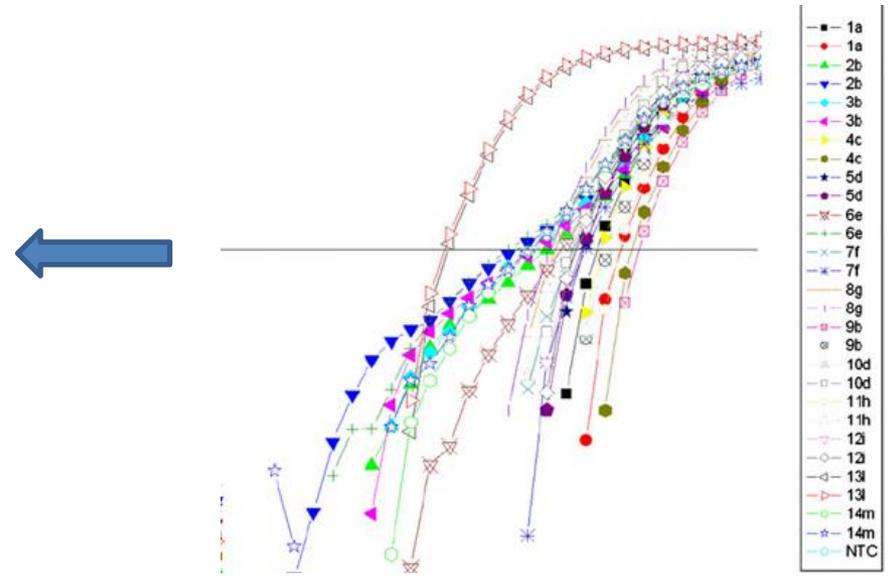
## 3. Diagnostic band sequencing and probe design

## 4. Array preparation

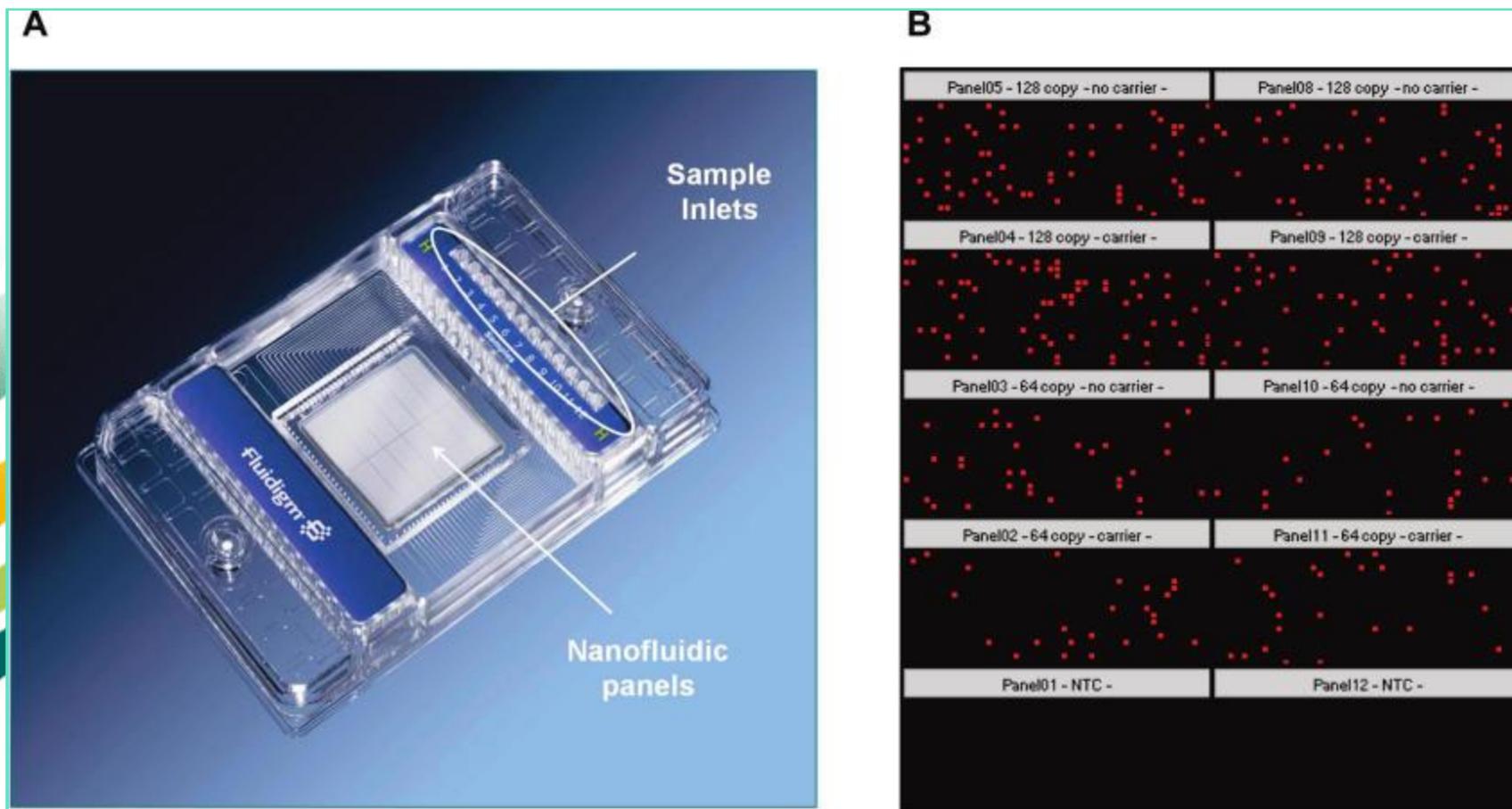


SAMPLE	Mean Ct	Soft wheat amount %
1	32.07	0.4
2	28.05	5.9
3	28.24	5.2
4	32.49	0.3
5	30.66	1.0
6	28.01	6.1
7	30.60	1.0
8	29.21	2.7
9	32.87	0.2
10	29.89	1.7
11	32.30	0.3
12	30.41	1.2
13	23.73	100
14	28.07	5.9

## 4. RT-PCR

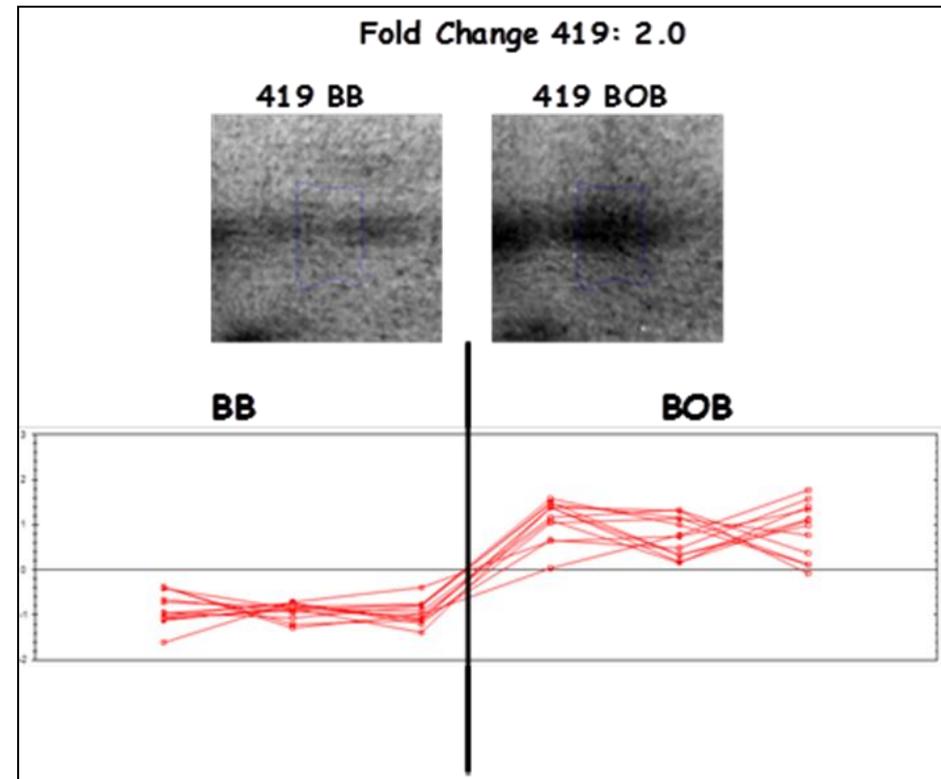


# Un nuovo modo di fare qPCR: microfluidic digital PCR (dPCR)



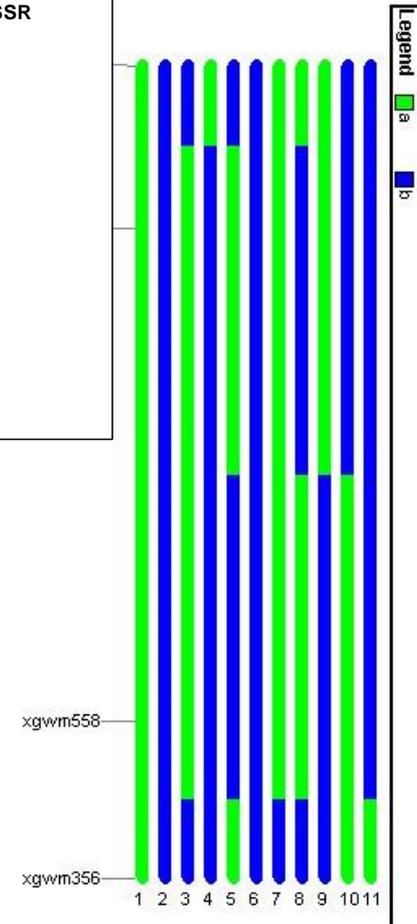
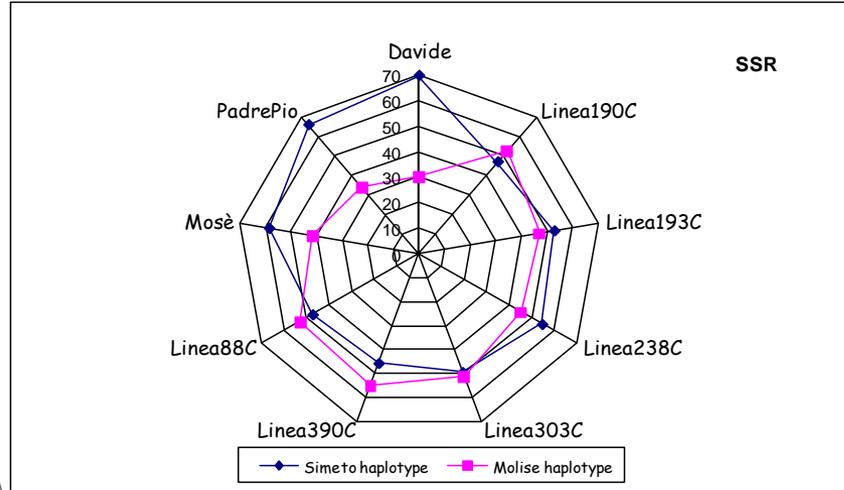
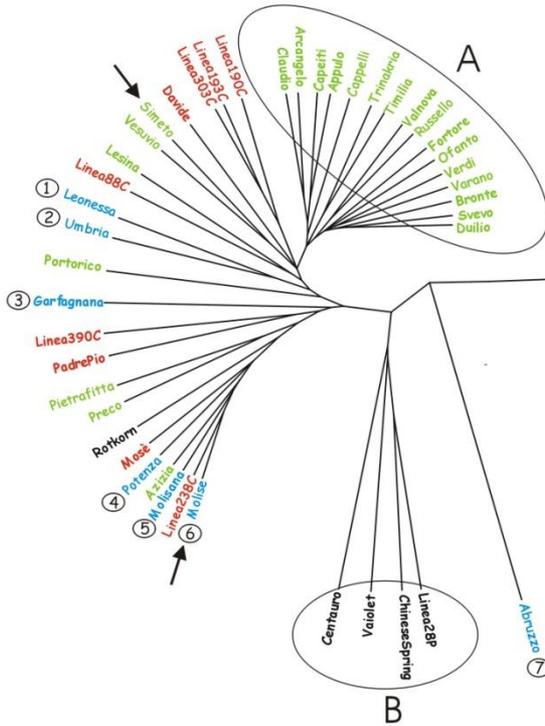
Sanders et al, Anal Chem, 2011

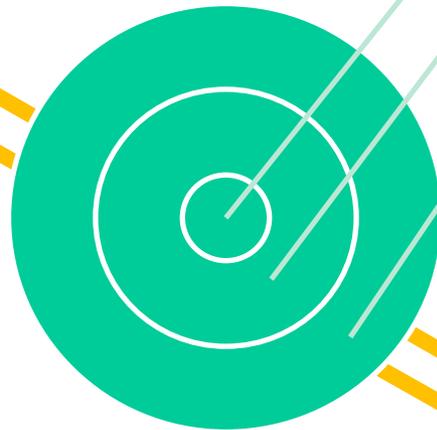
# Analisi del proteoma del pane per la tracciabilità di specie



**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





Specie

**GENOTIPI**

microbi  
oma



# 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  
 Covariante: Istituto sperimentale per la Cerealicoltura - Roma, Italia

**CARATTERI VEGETATIVI**

Portamento a fave acrobatiche  
 semi-rasato  
 Stelo:  
 eretto  
 Pigiocostatura anticimica delle  
 auricole:  
 media  
 Guaina:  
 +Densità minore  
 glaucoscienza, eretta

**CARATTERI DELLA SPIGA**

Tipa:  
 distesa  
 Aristareo:  
 pennato  
 Sagittatura delle ariste:  
 medio  
 Portamento:  
 acrobatico  
 Compattazione:  
 fitta  
 Glucoscomi:  
 medio o medio debole  
 Forma delle spighe: atriili:  
 subrotati  
 Lunghezza dell'articollo basale  
 dei rachidi:  
 corto  
 Lunghezza delle glume in rapporto  
 alla glumella inferiore:  
 più corta

**CARATTERI DEL SEME**

Vestito/manto:  
 eretto  
 Lunghezza dei peli della rachide:  
 lunga  
 Sagittatura delle glumelle:  
 arioso  
 Villosoità del sacco:  
 arioso  
 Arriccioni sulle nervature:  
 pennato

**CARATTERI FENOLOGICI**

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

ARDA

**ARDA**

CORNEL



## Protein chip

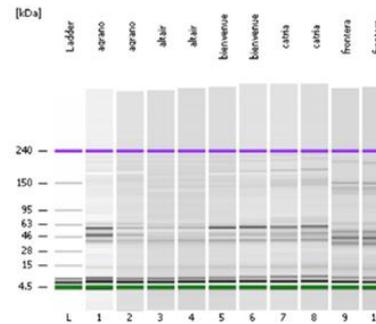
2100 expert\_Protein 230\_DE72901269\_2008-11-06\_11-06-50.xad

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.xay  
 Title: Protein Analysis 14 - 230 kDa  
 Version: 3.1  
 Assay Comments: © Copyright 2003 - 2008 Agilent Technologies, Inc.

**Chip Information:**

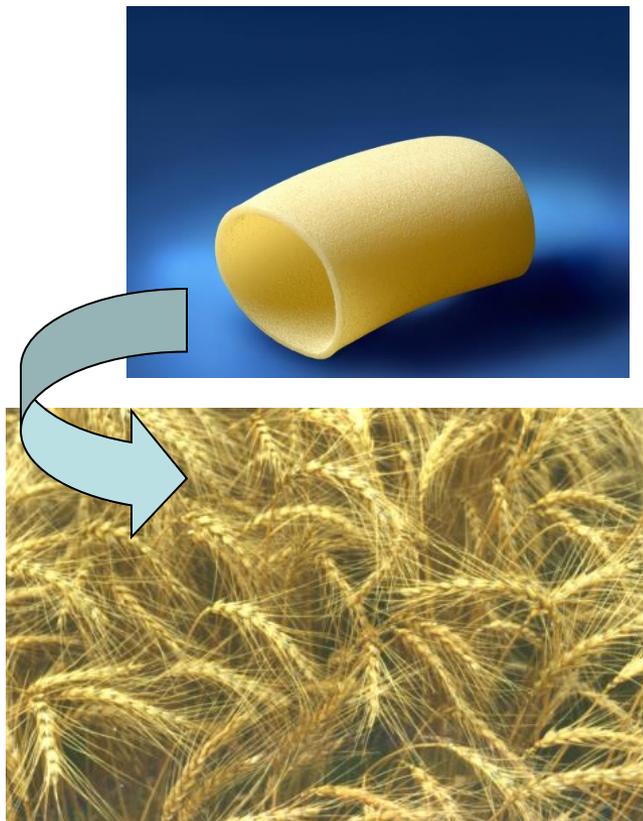
Chip Lot:  
 Reagent Kit Lot:  
 Chip Comments:



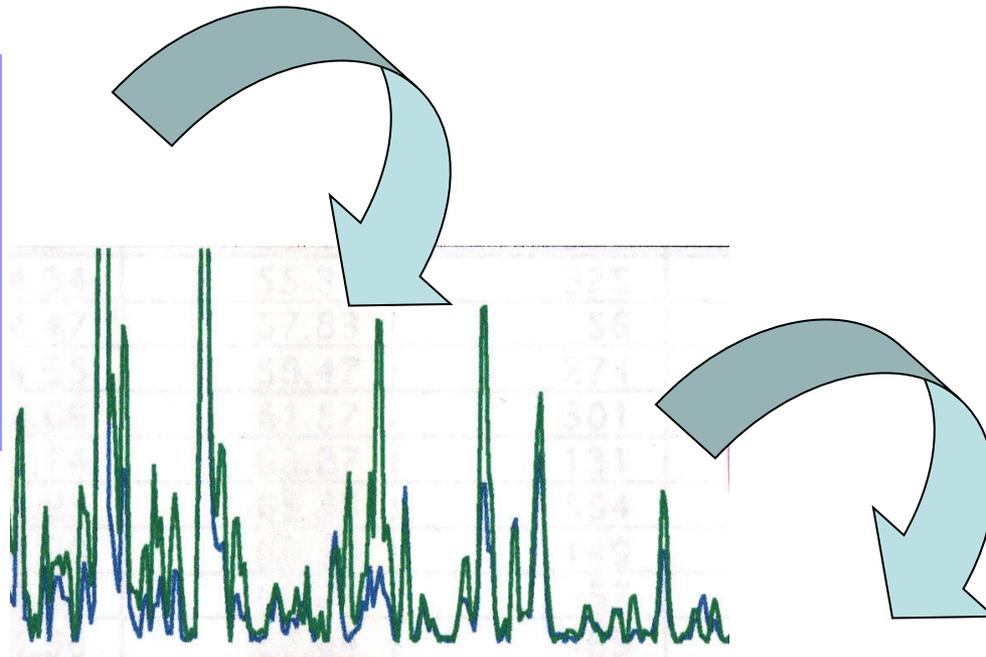
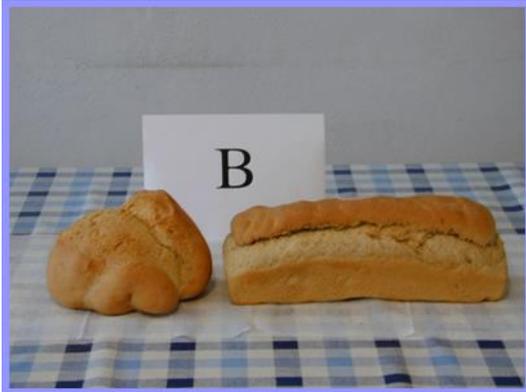
## ORIGINAL PAPER

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

### DNA-based analysis for authenticity assessment of monovarietal pasta



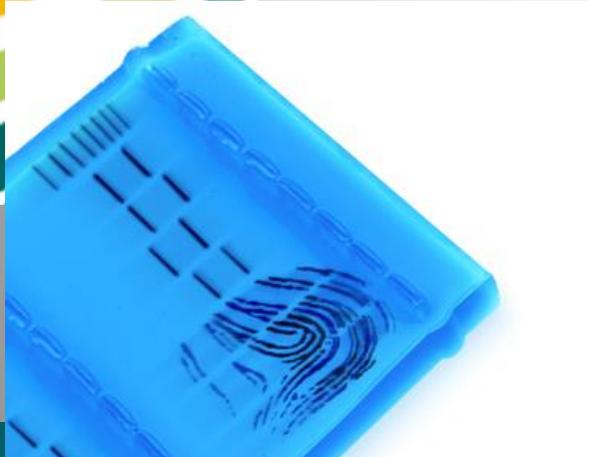
# Tracciabilità di varietà



“Passaporto molecolare” per certificazione di autenticità varietale

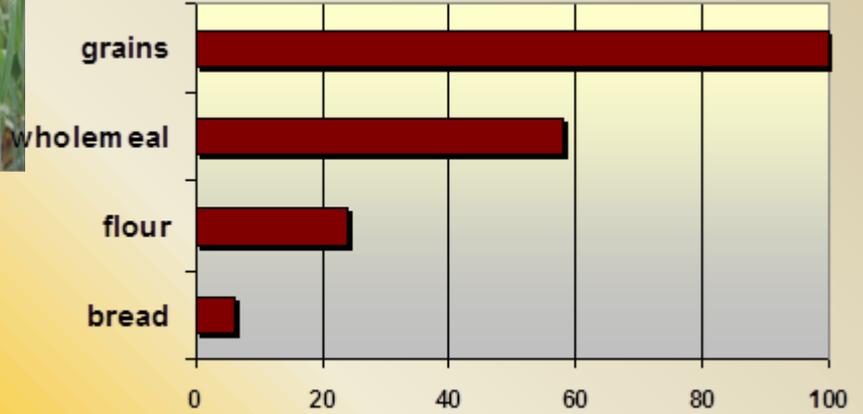
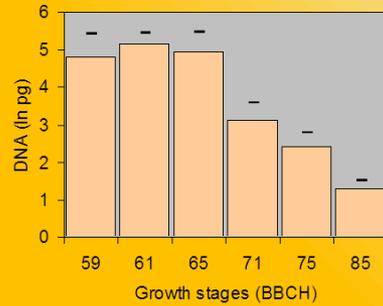
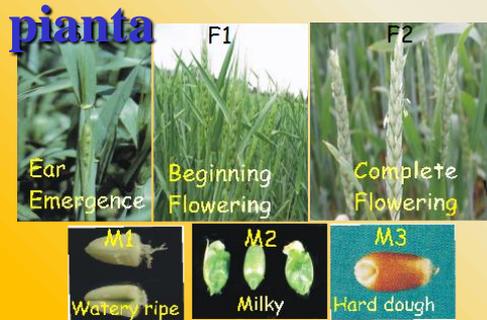


TRACCIABILITA' MOLECOLARE

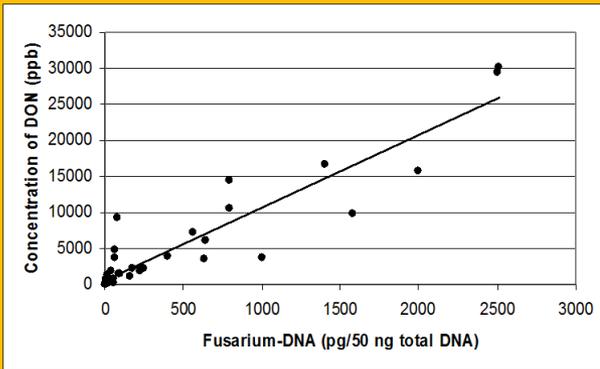


# Tracciabilità DNA-based di *Fusarium* lungo la catena di produzione del pane

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					



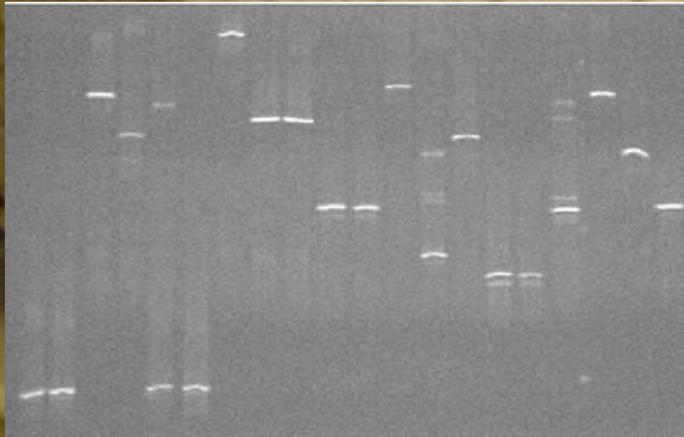
International Journal of Food Science and Technology  
 Original article  
**Fusarium DNA traceability along the bread production chain**  
 Valeria Trenti,<sup>1\*</sup> Caterina Marzola,<sup>2</sup> Pinella Pascale,<sup>3</sup> Nadia Piacchi,<sup>4</sup> Vittorio Rossi,<sup>4</sup> Mariacristina Capalini,<sup>2</sup> Maria Corbelli,<sup>2</sup> Diego Scuderi,<sup>2</sup> & Giovanni Di Gregori<sup>1</sup>  
 1 C.R.A. - Centro Nazionale Cereali, Via San Pietro 102, 20097 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 Nazionale 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.



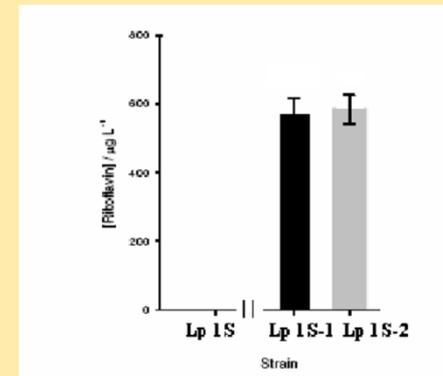
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1177F	5'GAGTACGacCGCAAGgt	16S V6
1258F	5'CATGTGGTTTAATTCTGAAGCaAC	16S V6
V6F	5'TCGAtGCAACGCGAAGAA	16S V6
1046R	5'CGACAACCATGCAGCACCT	16S V6
1391R	5'GACGGGCGGTGtGTaCA	16S V6
1731R	5'CGGGAACGTATTCAACCGCGGC	16S V6
1504R	5'TTTGTCAACCGCA	16S V6
1538R	5'TAAGGGGCATGATGATTTGACG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

DGGE

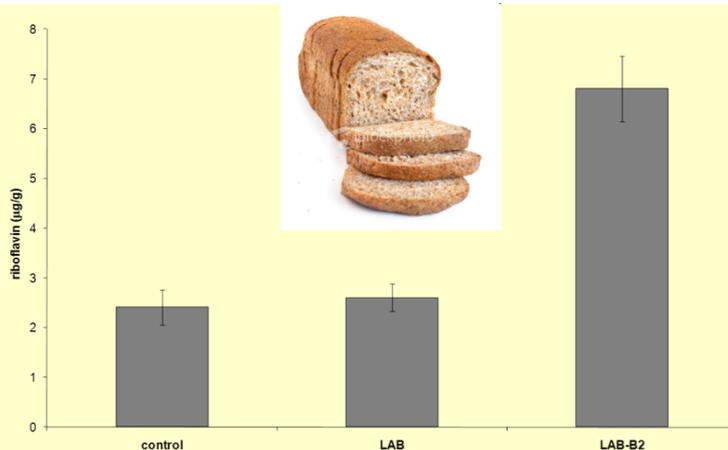
Sequenziamento

# Riborich (Riboflavin enriched bread and pasta)

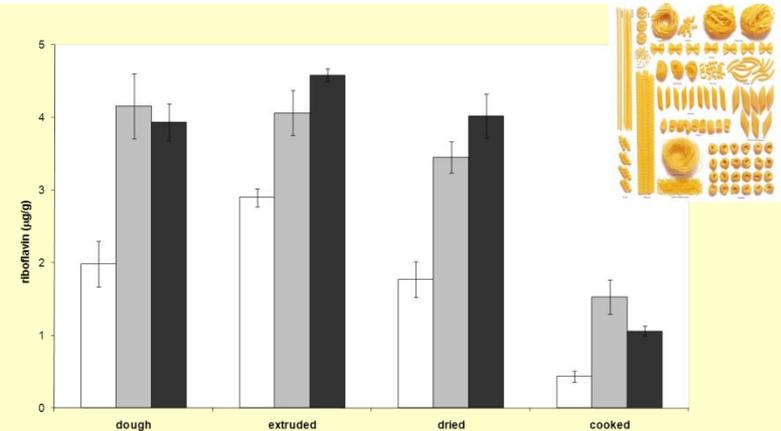
## Identification of (sourdough) Lactic Acid Bacteria overproducing riboflavin



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 ± 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 ± standard deviations for three replicates.

## Biotechnological Production of Vitamin B2-Enriched Bread and Pasta

Vittorio Capozzi,<sup>†</sup> Valeria Menga,<sup>§</sup> Anna Maria Digesù,<sup>§</sup> Pasquale De Vita,<sup>§</sup> Douwe van Sinderen,<sup>#</sup>  
Luigi Cattivelli,<sup>‡</sup> Clara Fares,<sup>§</sup> and Giuseppe Spano<sup>\*,†</sup>

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<sup>1,2</sup>, Pasquale Russo<sup>1,2</sup>, Mariagiovanna Fragasso<sup>2</sup>, Pasquale De Vita<sup>2</sup>, Daniela Fiocco<sup>4</sup> and Giuseppe Spano<sup>1\*</sup>*

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!*