

Strategy for molecular taxonomy of Forest Genetic Resources in the Mediterranean area

A. Technical Data

1. Title of the *Mobility grant* (reflecting the method/tool/approach to be learnt)

Strengthening conservation: a key issue for adaptation of marginal/ peripheral populations of forest trees to climate change in Europe (MaP-FGR)

2. Dates of the visit

From 07/10/2013 to 21/10/2013

3. Granted person's name, title/affiliation and contact information

- Dr. Lamia Hamrouni Bel Hadj Brahim
- Chargé de recherche
- Laboratoire d'Ecologie Forestière
- hamrounilam@yahoo.fr
- Phone : 00(216)21269226
- Fax : 00(216)71717951

B. Description of the work carried out during the Mobility grant

Lamia Hamrouni, Research at INRGREF was invited by the Research Centre of Avignon - Ecology Research Unit of Mediterranean Forests whose objective is the establishment of common protocols for genetic studies of population Mediterranean woody species, further analysis of data acquired in common, the consultation document databases. Similarly, the preparation of future collaboration on the topic of genetic resources.

B.1. Introduction:

This mission trip will aim to analyze the genetic diversity of ecological and economic important forest resources in Tunisia, including through of sustainable development plans involving the Tunisian rural society. The task will aim to learn new information for the (population genetics and technique of barcode) molecular methods, their analysis, with particular emphasis on differentiation existing between populations from the center of the distribution area and marginal populations.

B.2. Methodology

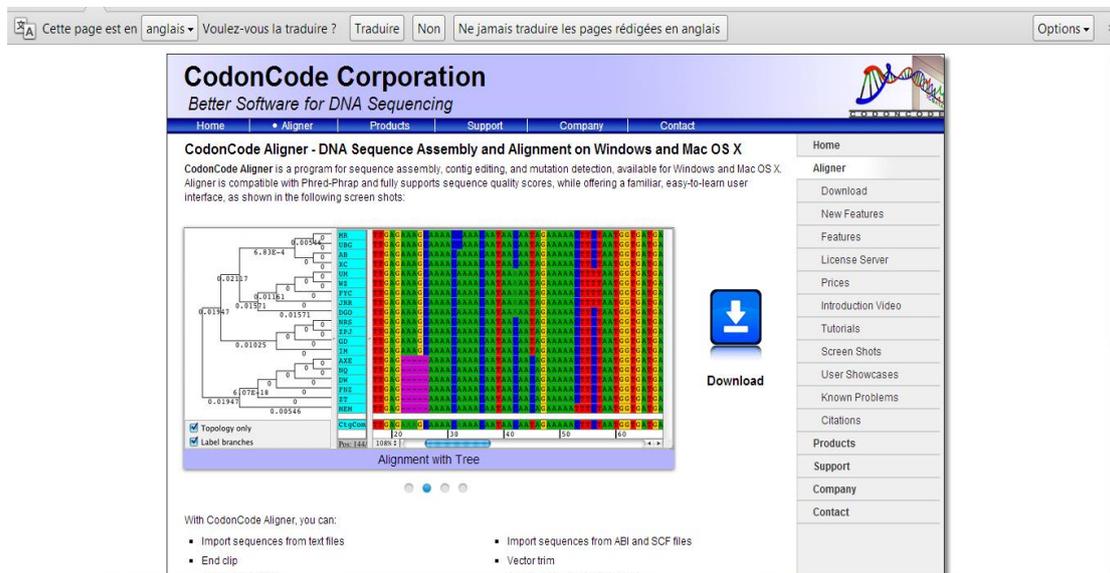
Within the framework of the activities of research of Research Centre of Avignon - Ecology Research Unit of Mediterranean Forests, we develop research projects of methods who allow the identification, the characterization and the selection of the forest species from the center of the distribution area and marginal populations using molecular tools and use of statistical software to analyze the database of molecular sequencing.

During the course of 15 days we used several logiciels statistics that provide analysis the genetic diversity of populations of various forest tree species and above all that adapt well in marginal areas to identify these species to establish a database that characterizes Mediterranean forest tree species.

The statistical software Aligner programs used are the following

B.2.1. CodonCode Aligner

CodonCode Aligner is a program for sequence assembly, contig editing, and mutation detection, available for Windows and Mac OS X. Aligner is compatible with Phred-Phrap and fully supports sequence quality scores, while offering a familiar, easy-to-learn user interface.

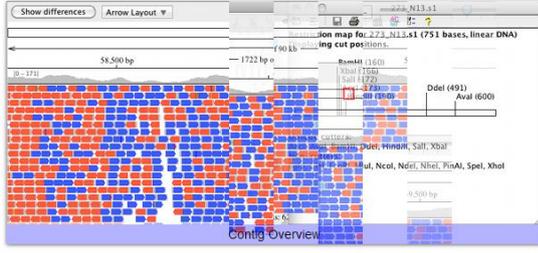


CodonCode Corporation
Better Software for DNA Sequencing

Home • **Aligner** • Products • Support • Company • Contact

CodonCode Aligner - DNA Sequence Assembly and Alignment on Windows and Mac OS X

CodonCode Aligner is a program for sequence assembly, contig editing, and mutation detection, available for Windows and Mac OS X. Aligner is compatible with Phred-Phrap and fully supports sequence quality scores, while offering a familiar, easy-to-learn user interface, as shown in the following screen shots:



Download

With CodonCode Aligner, you can:

- Import sequences from text files
- End clip
- Import sequences from ABI and SCF files
- Vector trim

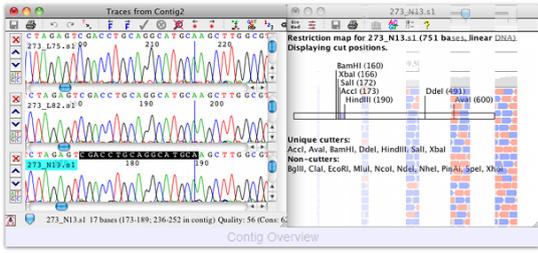
Home
Aligner
Download
New Features
Features
License Server
Prices
Introduction Video
Tutorials
Screen Shots
User Showcases
Known Problems
Citations
Products
Support
Company
Contact

CodonCode Corporation
Better Software for DNA Sequencing

Home • **Aligner** • Products • Support • Company • Contact

CodonCode Aligner - DNA Sequence Assembly and Alignment on Windows and Mac OS X

CodonCode Aligner is a program for sequence assembly, contig editing, and mutation detection, available for Windows and Mac OS X. Aligner is compatible with Phred-Phrap and fully supports sequence quality scores, while offering a familiar, easy-to-learn user interface, as shown in the following screen shots:



Download

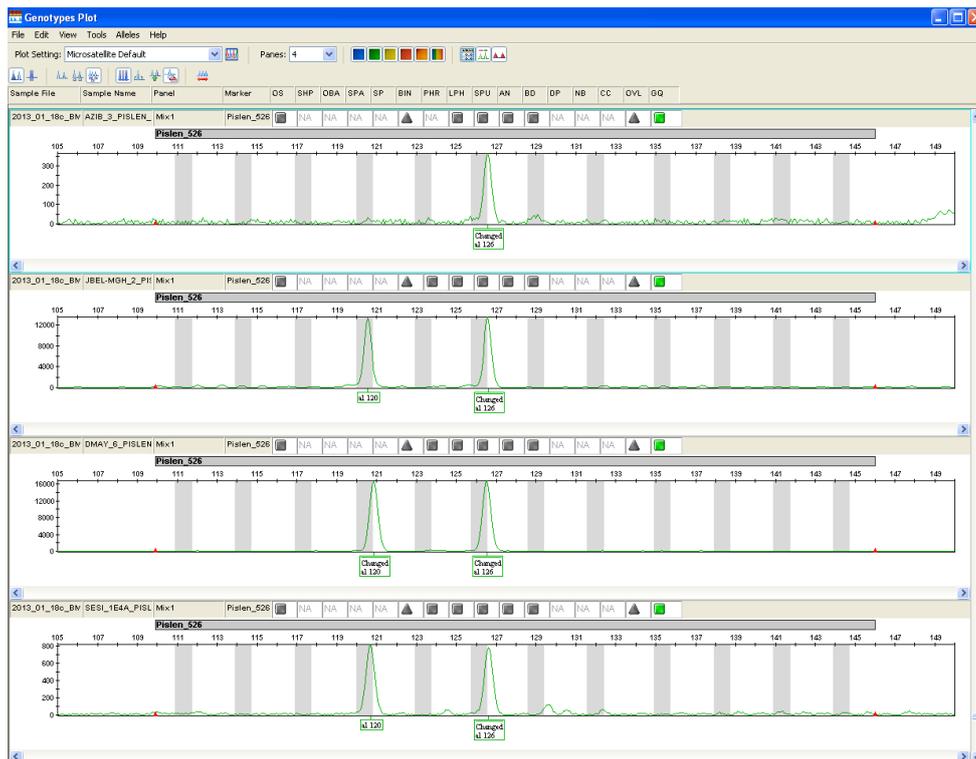
With CodonCode Aligner, you can:

- Import sequences from text files
- End clip
- Import sequences from ABI and SCF files
- Vector trim

Home
Aligner
Download
New Features
Features
License Server
Prices
Introduction Video
Tutorials
Screen Shots
User Showcases
Known Problems
Citations
Products
Support
Company
Contact

B.2.2. GeneMapper

GeneMapper Software is a flexible genotyping software package that provides DNA sizing and quality allele calls for all Life Technologies electrophoresis-based genotyping systems. This software specializes in multiapplication functionality, including amplified fragment length polymorphism (AFLP), loss of heterozygosity (LOH), microsatellite, and SNP genotyping analysis.



B.2.3. Gene Alex

GenAIEx: Genetic Analysis in Excel is a cross-platform package for population genetic analyses that runs within Microsoft Excel. GenAIEx offers analysis of diploid codominant, haploid and binary genetic loci and DNA sequences. Both frequency-based (F-statistics, heterozygosity, HWE, population assignment, relatedness) and distance-based (AMOVA, PCoA, Mantel tests, multivariate spatial autocorrelation) analyses are provided. New features include calculation of new estimators of population structure: $G'(ST)$, $G''(ST)$, Jost's $D(est)$ and $F'(ST)$ through AMOVA, Shannon Information analysis, linkage disequilibrium analysis for biallelic data and novel heterogeneity tests for spatial autocorrelation analysis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R		
1		45																		
2	Sapin	Voles	Genalex_Pyr	Arbo	Arque	Aspe	Aulu	Aure	Bagn	Baro	Bele	Beth	Biel	Bolq	Borc	Bout	Call	Camp	Cani	Caut
3	Amel	Aran																		
4																				
5		0.000																		
6		0.287	0.000																	
7		0.373	0.044	0.000																
8		0.086	0.197	0.275	0.000															
9		0.319	0.436	0.465	0.343	0.000														
10		0.112	0.191	0.274	0.097	0.341	0.000													
11		0.222	0.047	0.065	0.175	0.357	0.186	0.000												
12		0.238	0.037	0.057	0.156	0.400	0.120	0.057	0.000											
13		0.191	0.038	0.058	0.133	0.362	0.107	0.041	0.020	0.000										
14		0.081	0.215	0.322	0.059	0.416	0.074	0.224	0.167	0.147	0.000									
15		0.101	0.160	0.234	0.072	0.326	0.050	0.165	0.113	0.097	0.061	0.000								
16		0.287	0.044	0.038	0.216	0.386	0.203	0.061	0.045	0.043	0.231	0.185	0.000							
17		0.081	0.243	0.342	0.070	0.368	0.094	0.198	0.197	0.164	0.071	0.079	0.256	0.000						
18		0.434	0.057	0.036	0.324	0.453	0.241	0.064	0.065	0.094	0.406	0.289	0.058	0.407	0.000					
19		0.134	0.093	0.129	0.091	0.385	0.058	0.094	0.051	0.045	0.085	0.054	0.093	0.110	0.181	0.000				
20		0.090	0.207	0.297	0.064	0.376	0.068	0.206	0.141	0.122	0.033	0.051	0.216	0.065	0.368	0.075	0.000			
21		0.108	0.219	0.322	0.089	0.344	0.094	0.201	0.181	0.149	0.094	0.070	0.252	0.080	0.384	0.128	0.064	0.000		
22		0.070	0.242	0.340	0.080	0.356	0.096	0.205	0.200	0.164	0.074	0.089	0.269	0.058	0.409	0.136	0.076	0.072	0.000	
23		0.239	0.029	0.053	0.182	0.375	0.176	0.028	0.036	0.025	0.207	0.159	0.038	0.219	0.059	0.089	0.190	0.212	0.216	
24		0.061	0.234	0.338	0.055	0.324	0.092	0.205	0.187	0.160	0.051	0.069	0.265	0.060	0.390	0.113	0.061	0.081	0.038	
25		0.182	0.193	0.274	0.117	0.455	0.102	0.253	0.133	0.162	0.075	0.079	0.199	0.138	0.355	0.085	0.086	0.152	0.160	
26		0.099	0.187	0.272	0.071	0.372	0.061	0.206	0.135	0.132	0.052	0.199	0.076	0.346	0.068	0.071	0.109	0.099		
27		0.067	0.190	0.289	0.040	0.348	0.067	0.191	0.142	0.125	0.044	0.060	0.210	0.065	0.337	0.090	0.052	0.067	0.057	
28		0.110	0.192	0.268	0.087	0.388	0.099	0.203	0.142	0.132	0.033	0.080	0.192	0.096	0.355	0.085	0.047	0.086	0.098	
29		0.071	0.228	0.327	0.068	0.293	0.076	0.210	0.175	0.152	0.070	0.079	0.238	0.066	0.363	0.090	0.055	0.090	0.067	
30		0.060	0.204	0.297	0.055	0.338	0.058	0.192	0.147	0.131	0.039	0.051	0.204	0.046	0.364	0.066	0.038	0.081	0.050	
31		0.206	0.047	0.070	0.142	0.363	0.120	0.051	0.033	0.022	0.153	0.106	0.046	0.160	0.103	0.062	0.148	0.157	0.185	
32		0.222	0.027	0.055	0.162	0.392	0.130	0.037	0.026	0.018	0.179	0.115	0.037	0.191	0.074	0.057	0.157	0.178	0.188	
33		0.312	0.091	0.092	0.253	0.439	0.251	0.085	0.119	0.081	0.317	0.250	0.080	0.299	0.094	0.178	0.288	0.270	0.253	
34		0.373	0.044	0.033	0.276	0.458	0.272	0.065	0.060	0.061	0.329	0.258	0.035	0.347	0.043	0.138	0.297	0.342	0.361	

B.2.4. Structure analyse

STRUCTURE is a program for using multi-locus genotype data to investigate population structure. Its uses include inferring the presence of distinct populations, assigning individuals to populations, studying hybrid zones, identifying migrants and admixed individuals, and estimating population allele frequencies in situations where many individuals are migrants or admixed. It can be applied to most of the commonly-used genetic markers, including microsatellites, RFLPs and SNPs

B.2.5. Micro Cheker

MICRO-CHECKER is a Windows-based software package for Windows 98 SE, 2000 and XP (not yet tested on NT or ME), which can test the genotyping of microsatellite data from diploid populations. The program aids the identification of various genotyping errors, and can also detect typographic errors. **MICRO-CHECKER** estimates the frequency of null alleles at a locus using a series of algorithms. Importantly, **MICRO-CHECKER** can also adjust allele and genotype frequencies of the amplified alleles, which allows the data to be used in further population genetic analysis, for instance with GenePop, Arlequin or Fstat.

C. Conclusion

Through this course we have developed several techniques of analysis Statistics to study the genetic diversity of populations of various forestry species and establish a relationship between the genetic history and geographical distribution of species and especially for marginal population groups who have interesting characters for Mediterranean area.

Also during the mission, we discussed several issues with the different people we met and agreed on the following points:

- Establish a framework agreement between Research Centre of Avignon - Ecology Research Unit of Mediterranean Forests and INRGREF;
- Establish a bilateral project between the Research Centre of Avignon - Ecology Research Unit of Mediterranean Forests and Forest Ecology Laboratory (Plant Biotechnology);
- Exchange visits of researchers and students in training from Master Research Centre of Avignon - Ecology Research Unit and INRGREF.