SMART 2.0 Operation Manual

Data: 2023.05.24

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1. Software installation

1.1 Operating Environment

Operating System: Windows 7, Windows 10, Windows 11

System digits: 64-bit

1.2 Hardware Requirements

CPU: X86-based

Memory: 16GB or more

Disk space: 20G or more

1.3 Installation steps

a) Download smart_2.0.zip.

b) Copy smart 2.0.zip to the installation path, such as: C:\, and unzip it.

c) Open C:\smart_2.0\GenerateCmd\

d) Double-click the file 'get-C2V.exe' to complete the installation.

e) Open C:\smart 2.0, double-click the Smart icon to launch it.

2. Data input format

2.1 Data Processing

SMART can directly process the offline data from genetic analyzers, or process the electrophoresis graphs in fluorescence mapping software. During the process, OL peaks, penetration peaks, sharp peaks, and artificially identified insertion peaks need to be removed. Note that SMART has already modeled the shadow peaks at the minus one position (backward) and plus one position (forward) of the allele, so there is no need to delete the forward and backward shadow peaks. However, other types of shadow peaks, such as those at the minus two position or plus two position, need to be deleted. For some specific loci (e.g., SE33 locus), shadow peaks that are two base positions shorter may also need to be deleted.

After the graph processing is completed, the graph data is exported as a text file (TXT format) in the fluorescence mapping software (taking GenemapperID-X

software as an example).

2.2 Data Types

The input data for SMART includes two types:

(1) Data files of mixed DNA samples, consisting of five main parts: sample name, Marker name, allele name, size of the allele, and corresponding peak height of the allele.

Sample		Marker	Allele 1	Allele 2	Allele 3	Allele 4			Allele 7	Allele 8						Size 6		Size 8	Size 9							Height 7 Height 8 Height
test	D8S1179	9 9	10	12	13	14	15	16			125.64	129.66	137.82	141.99	146.18	150.3	154.44			171	3665	119	1953	114	306	4086
test	D21S11	29	30	31	32						203.01	207.01	210.93	215.01						448	639	92	782			
test	D7S820	7	8	10	11	12					258.32	262.29	270.35	274.29	278.37					69	1500	551	283	1918		
test	CSF1PO	9	11	12							315.02	323.43	327.46							253	169	1567				
test	D3S1358	3 14	15	16	17	18					118.35	122.27	126.24	130.26	134.43					432	5393	516	3926	572		
test	TH01	6	7	8	9	9.3					168.83	172.9	176.98	181.1	184.17					103	6507	100	2657	6223		
test	D13S317	7 7	8	9	11	12	13				211.15	215.13	219.2	227.26	231.31	235.36				54	2074	269	51	629	1236	
test	D16S539	9.9	10	11							267,46	271.51	275.45							730	2829	3416				
test	D2S1338	3 17	18	19	20	23	24				313.16	317.44	321.6	325.63	337.6	341.61				85	1329	1444	113	74	173	
test	D19S433		12.2	13	13.2	14	14.2	15			112.51	114.58	116.52	118.55	120,49	122.56	124.45			461	186	7057	2399	6183	213	2232
test	vWA	14	15	16	17						164.35	168.19	172.25	176.34						1394	104	1783	296			
test	TPOX		8	9	11								233.11							53	2270	1986	338			
test	D18551		13	14	15	16	17						289.29		297.32	301.25				72	1476	3696	138	1672	3754	
test	AMEL		v	14	15	10	**					110.92	209.29	200.00	201.32	30123				1962	2424	3030	130	1072	3734	
test	D55818		12	13								153.06	157.21							139	1150	1273				
test	FGA		21	22	23	24							232.99	227.06	241.17					672	163	2999	1468	3740		
test	FOA	20	21	22	23	24					224.91	226.95	252.99	237.00	291.17					0/2	103	2999	1408	3/40		

Figure 2.1 Data files of mixed DNA samples

(2) Data files of single-person DNA samples, which are STR typing files for a single individual. These are generally STR typing files for suspects or typing files for known contributors of mixed samples. The file format is the same as the mixed DNA file format.

M	farker .	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	Allele 7	Allele 8	Allele 9	Size 1	Size 2	Size 3	Size 4	Size 5	Size 6	Size 7	Size 8	Size 9	Height 1 Height	2 Height 3 Height 4 Height 5 Height 6 Height 7 Height 8 Height 9
D	8S1179	9	10									125.64	129.66							171	3665
D.	21511	29										203.01								448	
D	75820	7	8									258.32	262.29							69	1500
C!	SF1PO	9										315.02								253	
D	3S1358	14	15									118.35	122.27							432	5393
TH	H01	6	7									168.83	172.9							103	6507
D	13S317	7	8									211.15	215.13							54	2074
D	16S539	9										267.46								730	
D.	251338	17	18									313.16	317.44							85	1329
D	195433	12										112.51								461	
V۱	WA	14	15									164.35	168.19							1394	104
TE	POX	7	8									225.02	229.06							53	2270
D	18851	12										281.28								72	
A	MEL	X	Υ									104.92	110.92							1962	2424
D	55818	11										148.92								139	
FC	GA .	20	21									224.91	228.95							672	163

Figure 2.2 Sample of single DNA sample data file

3. Steps for using SMART

3.1 Create a new project.

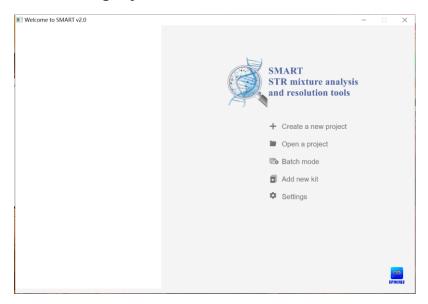


Figure 3.1 Main interface of SMART

Click on "Create a new projet" to create a new task.

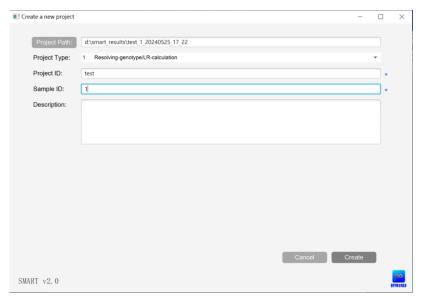
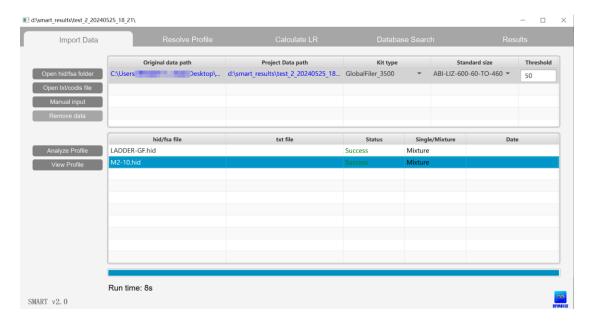


Figure 3.2 Creat a new project

Enter Project ID and Sample ID. Then, click on "Create".

3.2 Input raw data



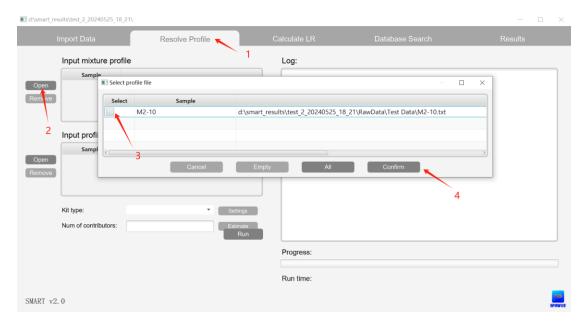
Click on "Open hid/fsa folder" and choose a folder that contains the fluorescence data files to be analyzed (files with the ".hid" suffix). This folder should contain at least one file named "Ladder". Then click on "Analyze Profile".

3.3 Profile processing



Process the profile, delete spurious peaks except allelic peaks and stutter peaks, and click save after processing.

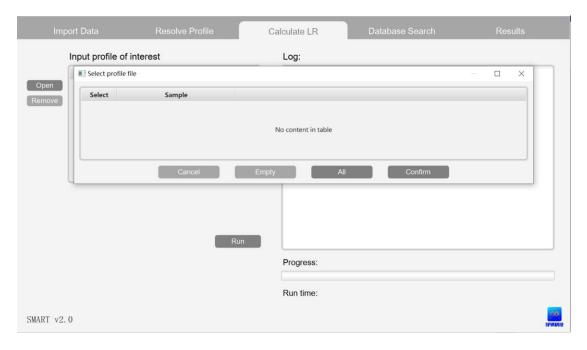
3.4 Resolve Profile



- 1. Click on "Resolve Profile"
- 2. Click on "Open"
- 3. Select the mixed file that has just been processed. If there are parallel amplification files, multiple parallel amplification files can be selected and inputted simultaneously.
 - 4. Click on "Confirm"

If there are known contributors in the mixed samples, they can be added by clicking on "Input profile of known contributor". Then choose "Kit type" and input "num of contributors". Finally click on "Run".

3.5 Calculate LR

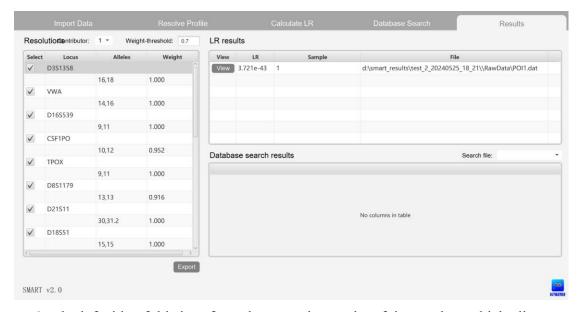


After running "Resolve Profile", in order to calculate LR, click "Calculate LR", add the POI's genotype, and then click "Run".

4. View results

4.1 View results in smart

Click on "Results".



On the left side of this interface, there are the results of the resolve, which allows

you to view the genotyping results of different contributors (Contributor 1~N) at different loci that exceed the specified threshold.

On the upper right side of this interface, there are the results of the Likelihood Ratio (LR) calculations. Each row represents the LR calculation result for a particular Person of Interest (POI), including information such as the LR value and the sample name.

4.2 View results in pdf

The detailed calculation results of resolution are located under

*/smart_results/ProjectID_SampleID_time/Reports/Resolve.pdf.

The detailed calculation results of LR are located under

*/smart_results/ProjectID_SampleID_time/Reports/ LR_POI1.pdf.