‘Internal development’ validation of EuroForMix

from version 4.2

Numerical tests automatically checked using *testthat* (R-package)
Manual checks for GUI results

Carried out regarding euroformix R-package release

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Test files available at https://github.com/oyvble/euroformix/tree/master/tests/testthat

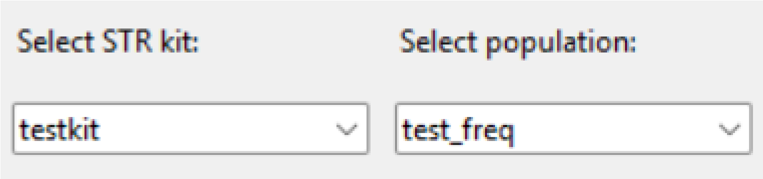
Checking R-functions depending on C++

Interpretation function:

- contLikMLE: Obtain maximum likelihood value/parameter estimates
- validMLEmodel: Calculates cumulative probabilities (of PHs)
- deconvolve: Calculates posterior probabilities of unknown genotypes
- contLikINT: Bayesian approach (integration)
- contLikMCMC: MCMC simulation

Example data

- Constructing the testkit (subset of SGMplus)
  - Two dyes only (blue and yellow)
  - TH01 renamed to 'TH 01' (white space in names)



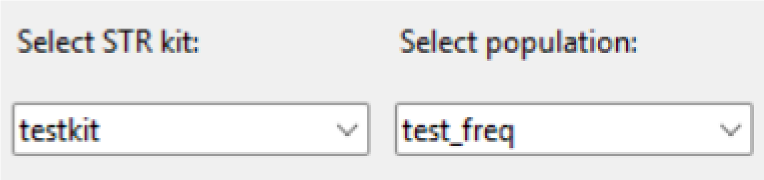
Select STR kit: testkit

Select population: test_freq

- Two example data sets which includes backward and forward stutters
  1. 1 contributor, 1 replicate
  2. 2 contributors, 2 replicates
- One example data set including SNPs.
  - 2 contributors, 2 replicates
- One example data set including MPS-STR.
- Data available in R-package installation folder **euroformix*examples***

Settings for CE data

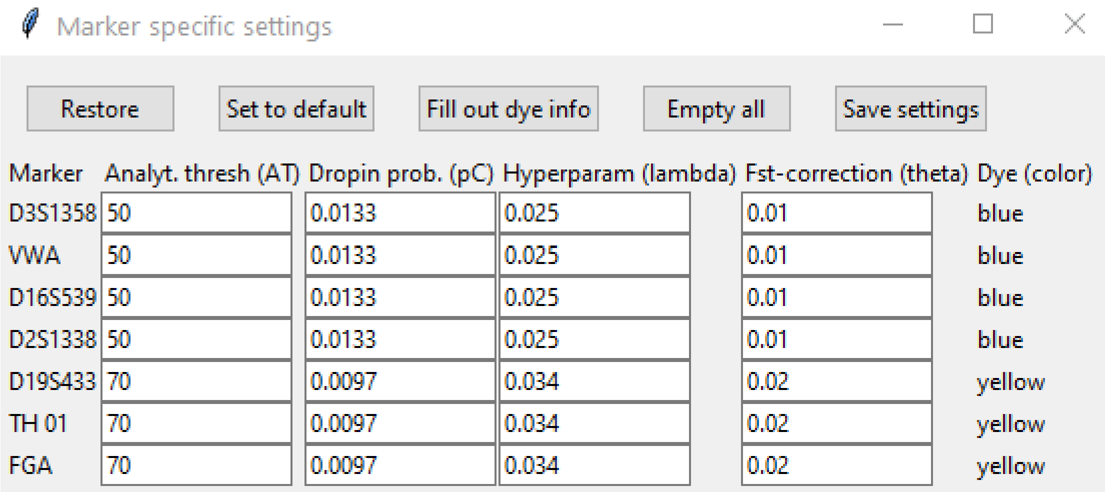
- Selected kit: testkit



Select STR kit: testkit

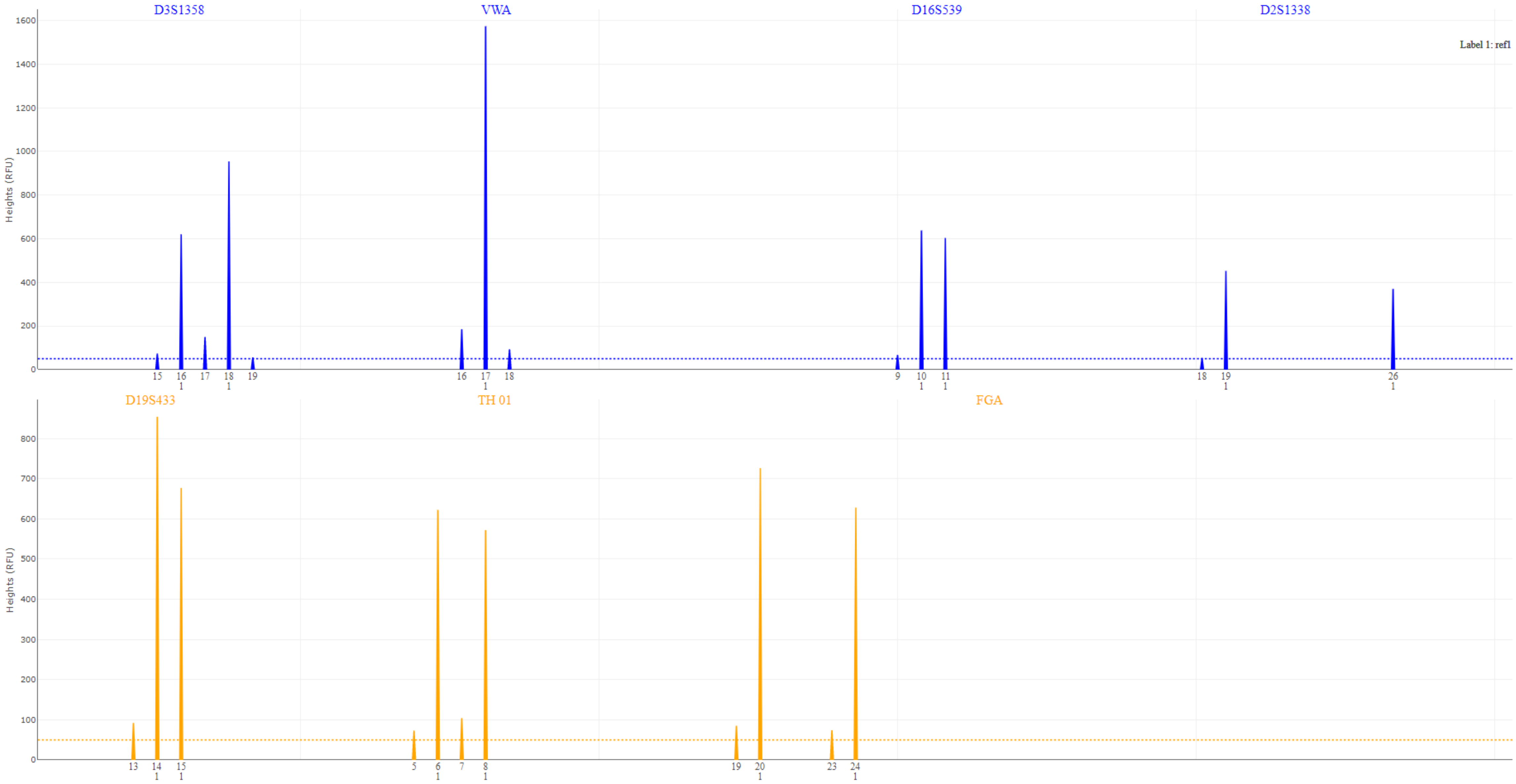
Select population: test_freq

- Dye specific settings:



Marker	Analyt. thresh (AT)	Dropin prob. (pC)	Hyperparam (lambda)	Fst-correction (theta)	Dye (color)
D3S1358	50	0.0133	0.025	0.01	blue
VWA	50	0.0133	0.025	0.01	blue
D16S539	50	0.0133	0.025	0.01	blue
D2S1338	50	0.0133	0.025	0.01	blue
D19S433	70	0.0097	0.034	0.02	yellow
TH 01	70	0.0097	0.034	0.02	yellow
FGA	70	0.0097	0.034	0.02	yellow

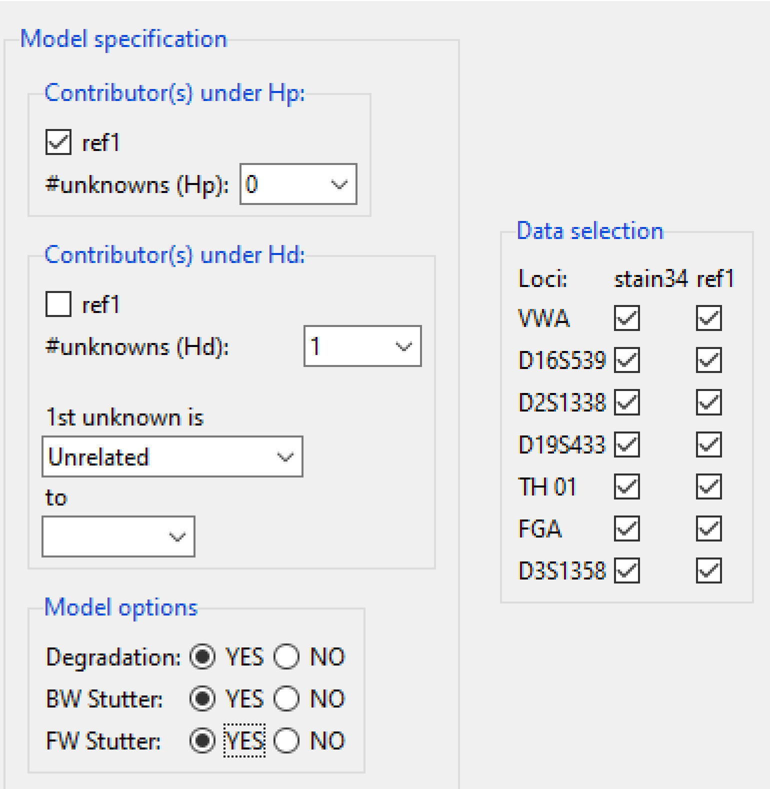
Example data 1: stain34



Propositions tested for dataset 1: *test_logLik1contr*

- Hyp 1: ref1 is contributor
- Hyp 2: 1 (unrelated) unknown is contributor, ref1 is known non-contributor
- Hyp 3: 1 unknown sibling of ref1 is contributor, ref1 is known non-contributor

GUI check Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ ref1

#unknowns (Hp): 0

Contributor(s) under Hd:

☐ ref1

#unknowns (Hd): 1

1st unknown is

Unrelated

to

Model options

Degradation: ● YES ○ NO

BW Stutter: ● YES ○ NO

FW Stutter: ● YES ○ NO

Data selection

Loci:	stain34	ref1
VWA	☑	☑
D16S539	☑	☑
D2S1338	☑	☑
D19S433	☑	☑
TH 01	☑	☑
FGA	☑	☑
D3S1358	☑	☑

Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	1.000	0.000
P.H.expectation	921.892	39.706
P.H.variability	0.099	0.014
Degrad. slope	0.771	0.036
BWstutt-prop.	0.104	0.010
FWstutt-prop.	0.051	0.010

Maximum Likelihood value

logLik= -155.34

adj.loglik= -160.34

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	1.000	0.000
P.H.expectation	921.892	39.706
P.H.variability	0.099	0.014
Degrad. slope	0.771	0.036
BWstutt-prop.	0.104	0.010
FWstutt-prop.	0.051	0.010

Maximum Likelihood value

logLik= -134.1

adj.loglik= -139.1

Further Action

Joint LR

LR=1.69e9

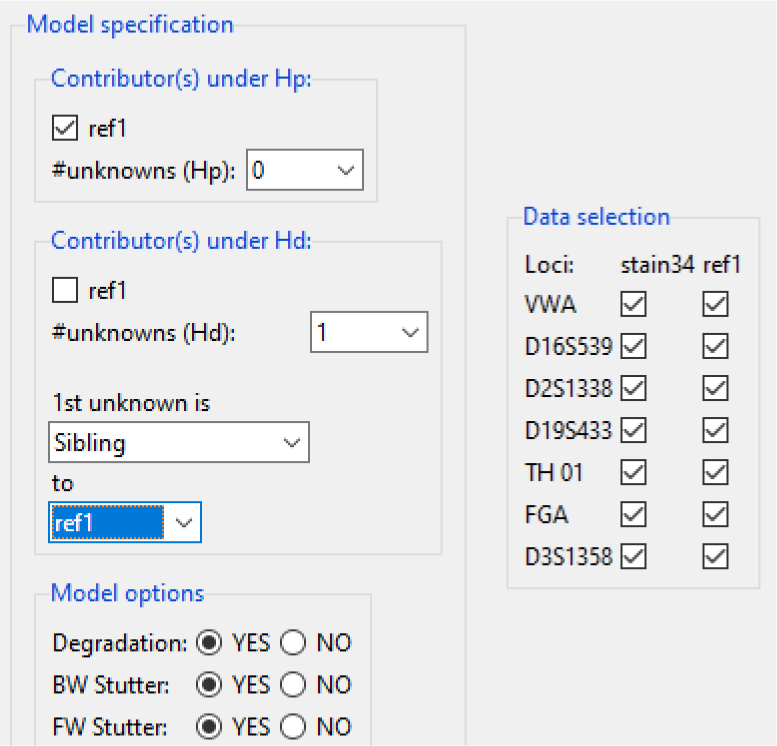
log10LR=9.23

Upper boundary=9.23

LR per-marker

Marker	LR	log10LR
VWA	9.5	0.98
D16S539	26.0	1.42
D2S1338	141.3	2.15
D19S433	7.3	0.86
TH 01	22.8	1.36
FGA	20.5	1.31
D3S1358	14.3	1.16

GUI check Hp: Hyp 1 vs Hd: Hyp 3



Model specification

Contributor(s) under Hp:

☑ ref1

#unknowns (Hp): 0

Contributor(s) under Hd:

☐ ref1

#unknowns (Hd): 1

1st unknown is Sibling

to ref1

Model options

Degradation: ● YES ○ NO

BW Stutter: ● YES ○ NO

FW Stutter: ● YES ○ NO

Data selection

Loci:	stain34	ref1
VWA	☑	☑
D16S539	☑	☑
D2S1338	☑	☑
D19S433	☑	☑
TH 01	☑	☑
FGA	☑	☑
D3S1358	☑	☑

Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	1.000	0.000
P.H.expectation	921.892	39.706
P.H.variability	0.099	0.014
Degrad. slope	0.771	0.036
BWstutt-prop.	0.104	0.010
FWstutt-prop.	0.051	0.010

Maximum Likelihood value

logLik= -141.24

adj.loglik= -146.24

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	1.000	0.000
P.H.expectation	921.892	39.706
P.H.variability	0.099	0.014
Degrad. slope	0.771	0.036
BWstutt-prop.	0.104	0.010
FWstutt-prop.	0.051	0.010

Maximum Likelihood value

logLik= -134.1

adj.loglik= -139.1

Further Action

Joint LR

LR=1.26e3

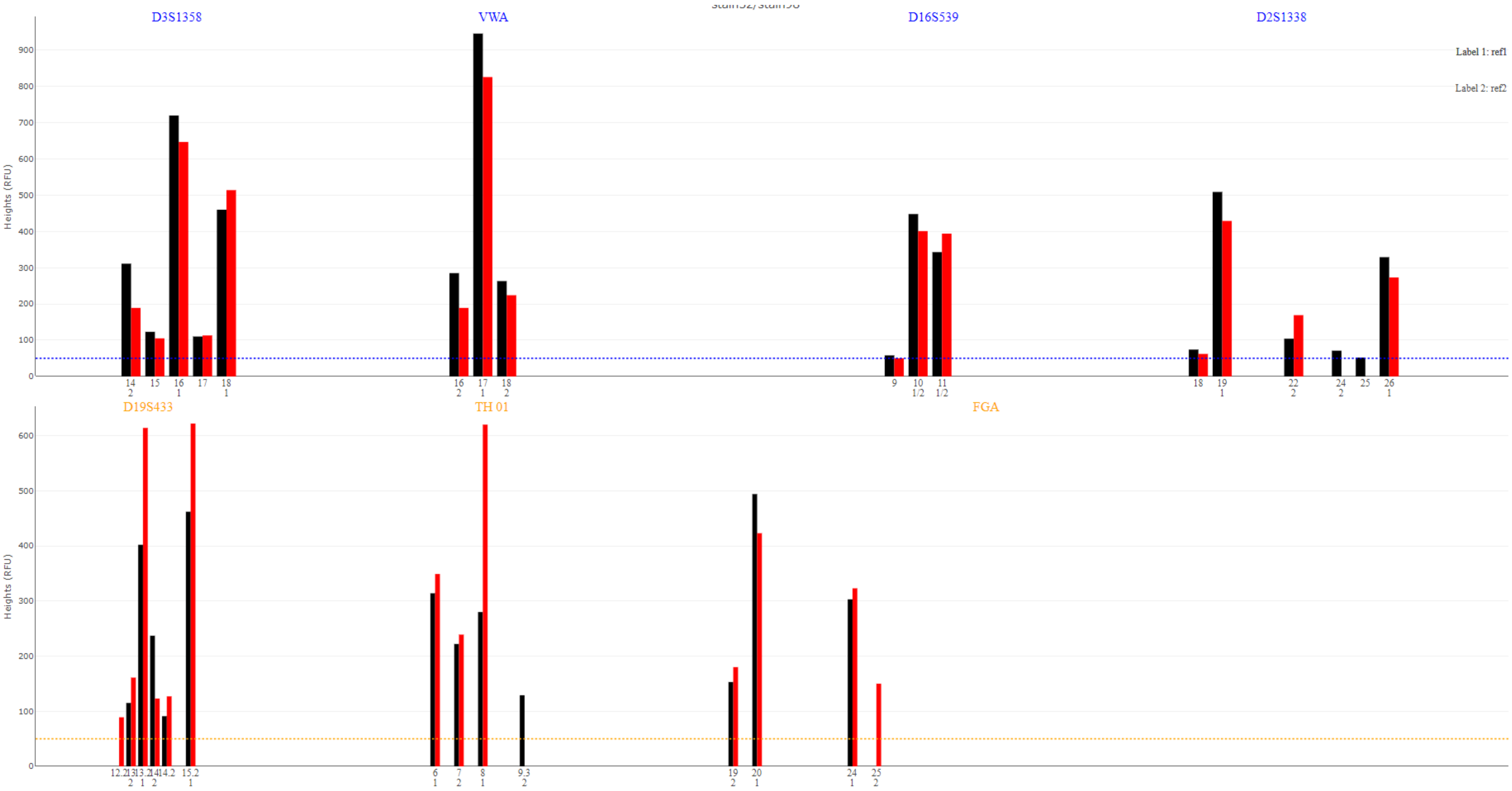
log10LR=3.1

Upper boundary=9.23

LR per-marker

Marker	LR	log10LR
VWA	2.3	0.36
D16S539	2.8	0.45
D2S1338	3.5	0.54
D19S433	2.4	0.37
TH 01	2.9	0.47
FGA	2.9	0.47
D3S1358	2.7	0.44

Example data 2: stain52/stain98



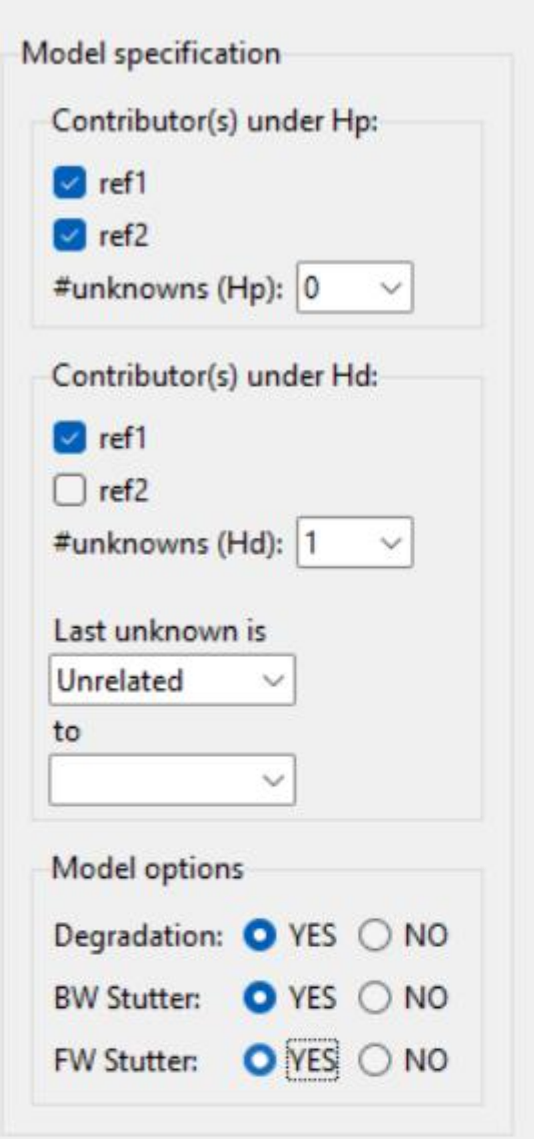
Propositions tested for dataset 2: *test_logLik2contr2Rep*

- Hyp 1: ref1 + ref2 are contributors

- Hyp2: ref1 + 1 (unrelated) unknown are contributors, ref2 is known non-contributor

- Hyp3: ref1 + 1 related (sibling) unknown of ref2 are contributors, ref2 is known non-contributor

GUI check Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ ref1

☑ ref2

#unknowns (Hp): 0

Contributor(s) under Hd:

☑ ref1

☐ ref2

#unknowns (Hd): 1

Last unknown is

Unrelated

to

Model options

Degradation: ● YES ○ NO

BW Stutter: ● YES ○ NO

FW Stutter: ● YES ○ NO

Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.763	0.022
Mix-prop. C2	0.237	0.022
P.H.expectation	796.620	38.254
P.H.variability	0.148	0.017
Degrad. slope	0.740	0.040
BWstutt-prop.	0.119	0.018
FWstutt-prop.	0.050	0.020

Maximum Likelihood value

logLik= -349.57

adj.loglik= -355.57

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.796	0.016
Mix-prop. C2	0.204	0.016
P.H.expectation	794.831	38.655
P.H.variability	0.151	0.014
Degrad. slope	0.747	0.040
BWstutt-prop.	0.119	0.015
FWstutt-prop.	0.055	0.017

Maximum Likelihood value

logLik= -330.46

adj.loglik= -336.46

Further Action

Joint LR

LR=1.99e8

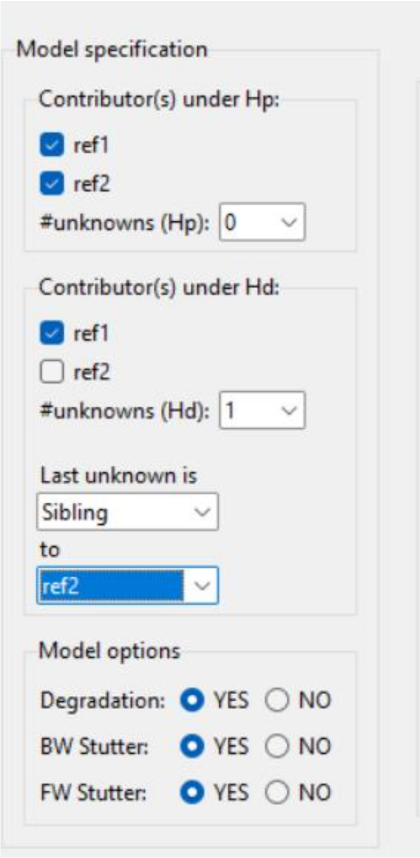
log10LR=8.3

Upper boundary=9.67

LR per-marker

Marker	LR	log10LR
VWA	7.1	0.85
D16S539	15.1	1.18
D2S1338	164.9	2.22
D19S433	7.2	0.85
TH 01	6.3	0.80
FGA	64.7	1.81
D3S1358	3.9	0.59

GUI check Hp: Hyp 1 vs Hd: Hyp 3



Model specification

Contributor(s) under Hp:

☑ ref1

☑ ref2

#unknowns (Hp): 0

Contributor(s) under Hd:

☑ ref1

☐ ref2

#unknowns (Hd): 1

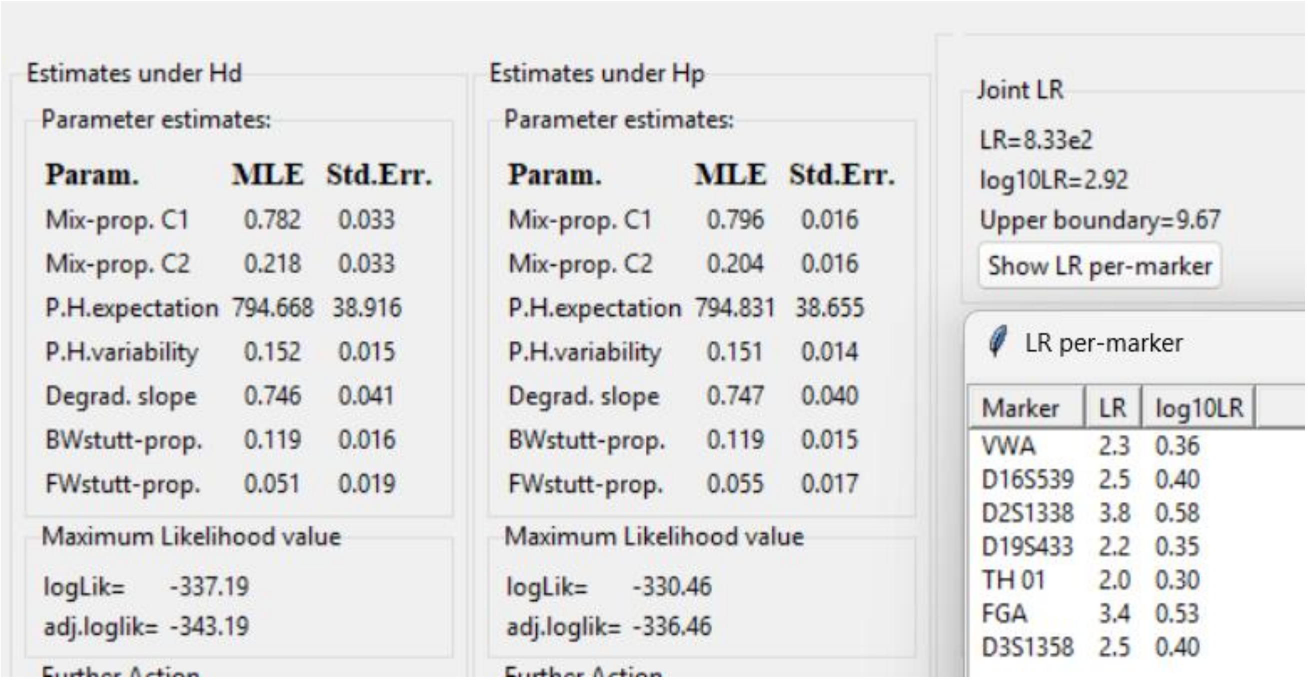
Last unknown is Sibling to ref2

Model options

Degradation: ● YES ○ NO

BW Stutter: ● YES ○ NO

FW Stutter: ● YES ○ NO



Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.782	0.033
Mix-prop. C2	0.218	0.033
P.H.expectation	794.668	38.916
P.H.variability	0.152	0.015
Degrad. slope	0.746	0.041
BWstutt-prop.	0.119	0.016
FWstutt-prop.	0.051	0.019

Maximum Likelihood value

logLik= -337.19

adj.loglik= -343.19

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.796	0.016
Mix-prop. C2	0.204	0.016
P.H.expectation	794.831	38.655
P.H.variability	0.151	0.014
Degrad. slope	0.747	0.040
BWstutt-prop.	0.119	0.015
FWstutt-prop.	0.055	0.017

Maximum Likelihood value

logLik= -330.46

adj.loglik= -336.46

Joint LR

LR=8.33e2

log10LR=2.92

Upper boundary=9.67

LR per-marker

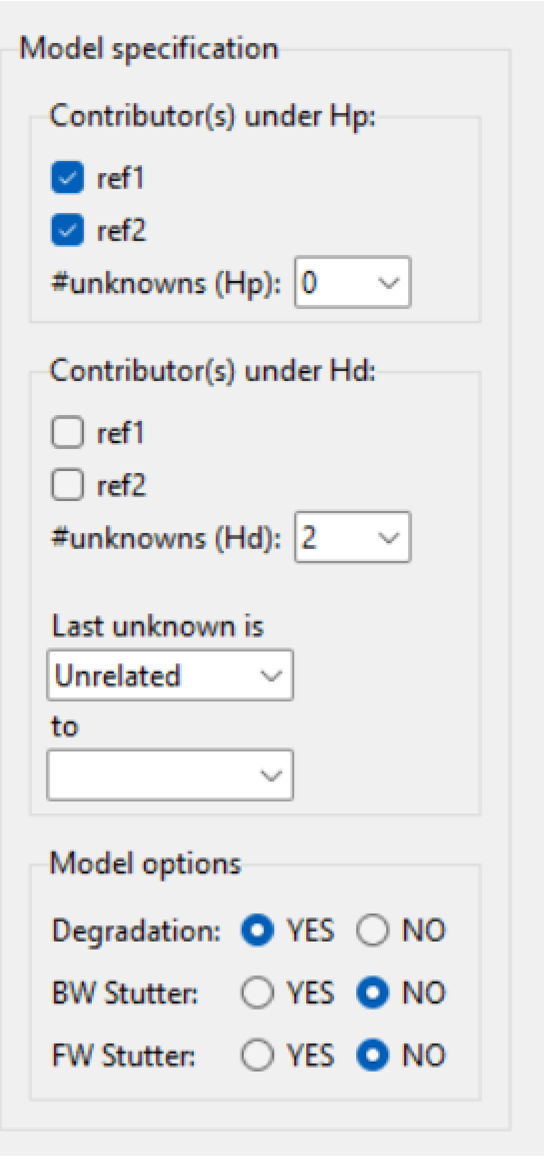
Marker	LR	log10LR
VWA	2.3	0.36
D16S539	2.5	0.40
D2S1338	3.8	0.58
D19S433	2.2	0.35
TH 01	2.0	0.30
FGA	3.4	0.53
D3S1358	2.5	0.40

Propositions tested for dataset 2’ *test_logLik2contr1RepNoStutter*

- Considering 1 replicate (stain52 only)
- Stutter models are turned off, degrad still turned on.

- Hyp 1: ref1 and ref2 are contributor
- Hyp 2: 2 (unrelated) unknowns are contributors, ref1 and ref2 are known non-contributors

GUI check   Hd: Hyp 1 versus Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ ref1
☑ ref2
#unknowns (Hp): 0

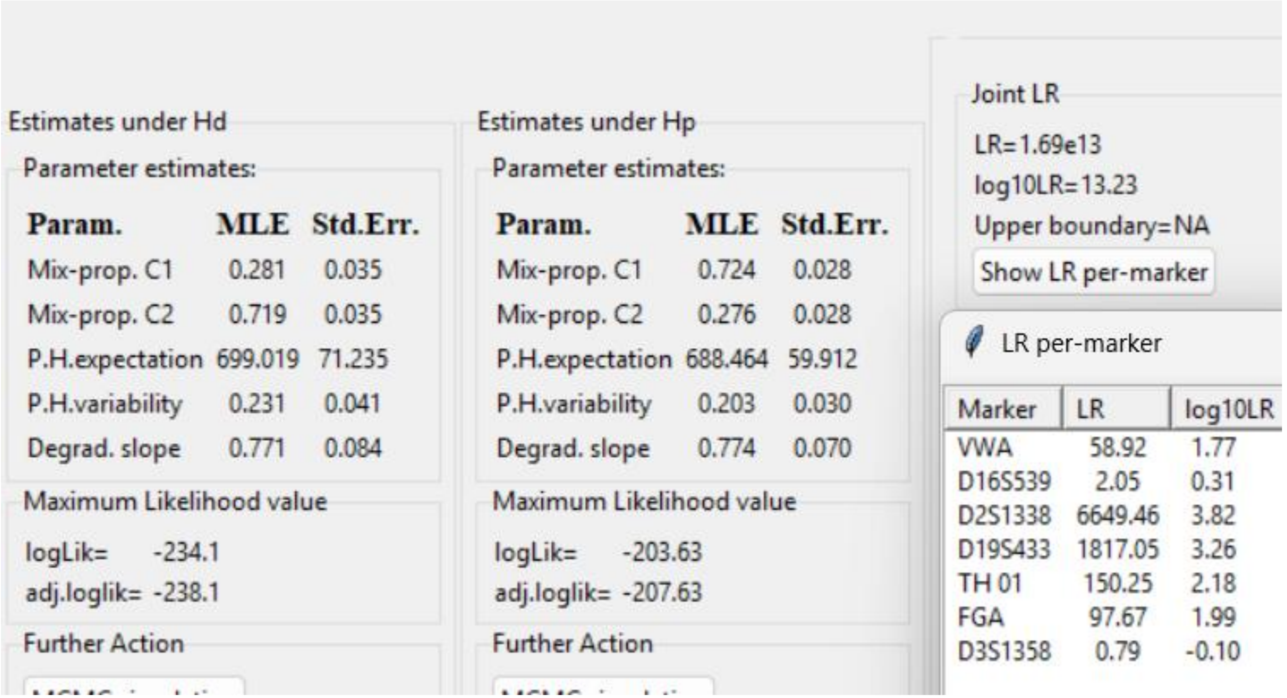
Contributor(s) under Hd:

☐ ref1
☐ ref2
#unknowns (Hd): 2

Last unknown is
Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ○ YES ● NO
FW Stutter: ○ YES ● NO



Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.281	0.035
Mix-prop. C2	0.719	0.035
P.H.expectation	699.019	71.235
P.H.variability	0.231	0.041
Degrad. slope	0.771	0.084

Maximum Likelihood value

logLik= -234.1
adj.loglik= -238.1

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.724	0.028
Mix-prop. C2	0.276	0.028
P.H.expectation	688.464	59.912
P.H.variability	0.203	0.030
Degrad. slope	0.774	0.070

Maximum Likelihood value

logLik= -203.63
adj.loglik= -207.63

Further Action

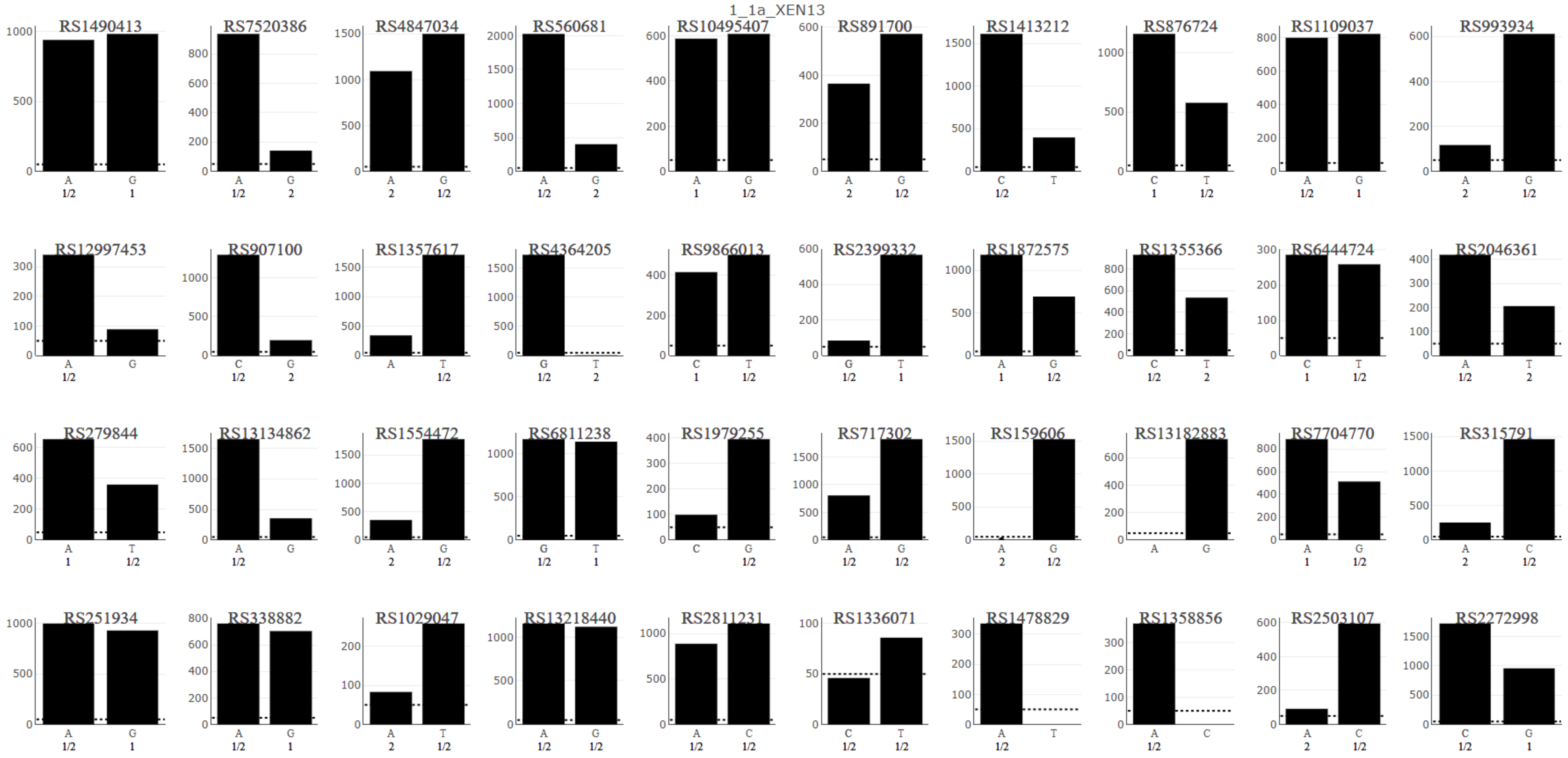
Joint LR

LR=1.69e13
log10LR=13.23
Upper boundary=NA

LR per-marker

Marker	LR	log10LR
VWA	58.92	1.77
D16S539	2.05	0.31
D2S1338	6649.46	3.82
D19S433	1817.05	3.26
TH 01	150.25	2.18
FGA	97.67	1.99
D3S1358	0.79	-0.10

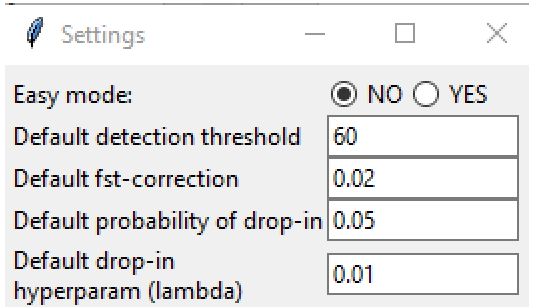
Example data 3: 1_1a_XEN13/ 1_1b_XEN13 (SNPs)



Propositions tested for dataset 3 (SNPs): *test_logLik2contr2RepSNP*

Note: Profiles P1+P3 are missing markers RS13182883 and RS576261 (substituting with additional unknown)

Global settings for all markers:



Easy mode: ● NO ○ YES

Default detection threshold 60

Default fst-correction 0.02

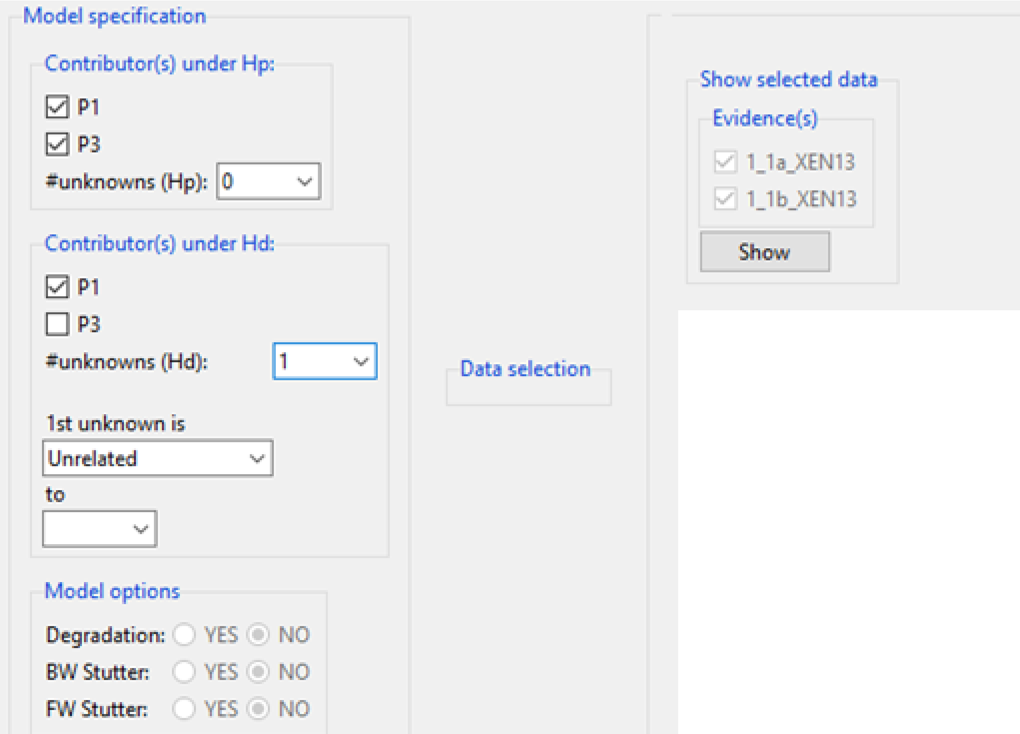
Default probability of drop-in 0.05

Default drop-in hyperparam (lambda) 0.01

Using **both** replicates

- Hyp 1: P1 + P3 are contributors
- Hyp2: P1 + 1 (unrelated) unknown are contributors, P3 is known non-contributor

GUI check Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:
- ☑ P1
- ☑ P3

#unknowns (Hp): 0

Contributor(s) under Hd:
- ☑ P1
- ☐ P3

#unknowns (Hd): 1

1st unknown is Unrelated

to

Model options

Degradation: ○ YES ● NO

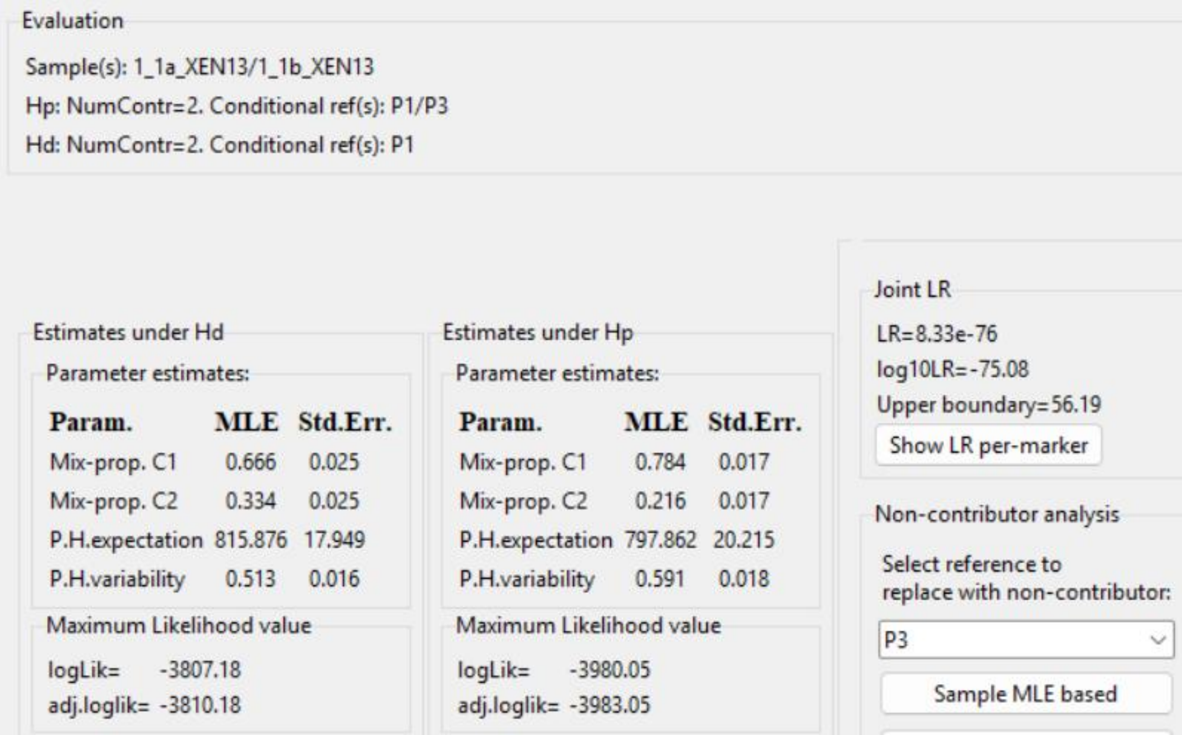
BW Stutter: ○ YES ● NO

FW Stutter: ○ YES ● NO

Data selection

Show selected data

Evidence(s)
- ☑ 1_1a_XEN13
- ☑ 1_1b_XEN13



Evaluation

Sample(s): 1_1a_XEN13/1_1b_XEN13

Hp: NumContr=2. Conditional ref(s): P1/P3

Hd: NumContr=2. Conditional ref(s): P1

Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.666	0.025
Mix-prop. C2	0.334	0.025
P.H.expectation	815.876	17.949
P.H.variability	0.513	0.016

Maximum Likelihood value

logLik= -3807.18

adj.loglik= -3810.18

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	0.784	0.017
Mix-prop. C2	0.216	0.017
P.H.expectation	797.862	20.215
P.H.variability	0.591	0.018

Maximum Likelihood value

logLik= -3980.05

adj.loglik= -3983.05

Joint LR

LR=8.33e-76

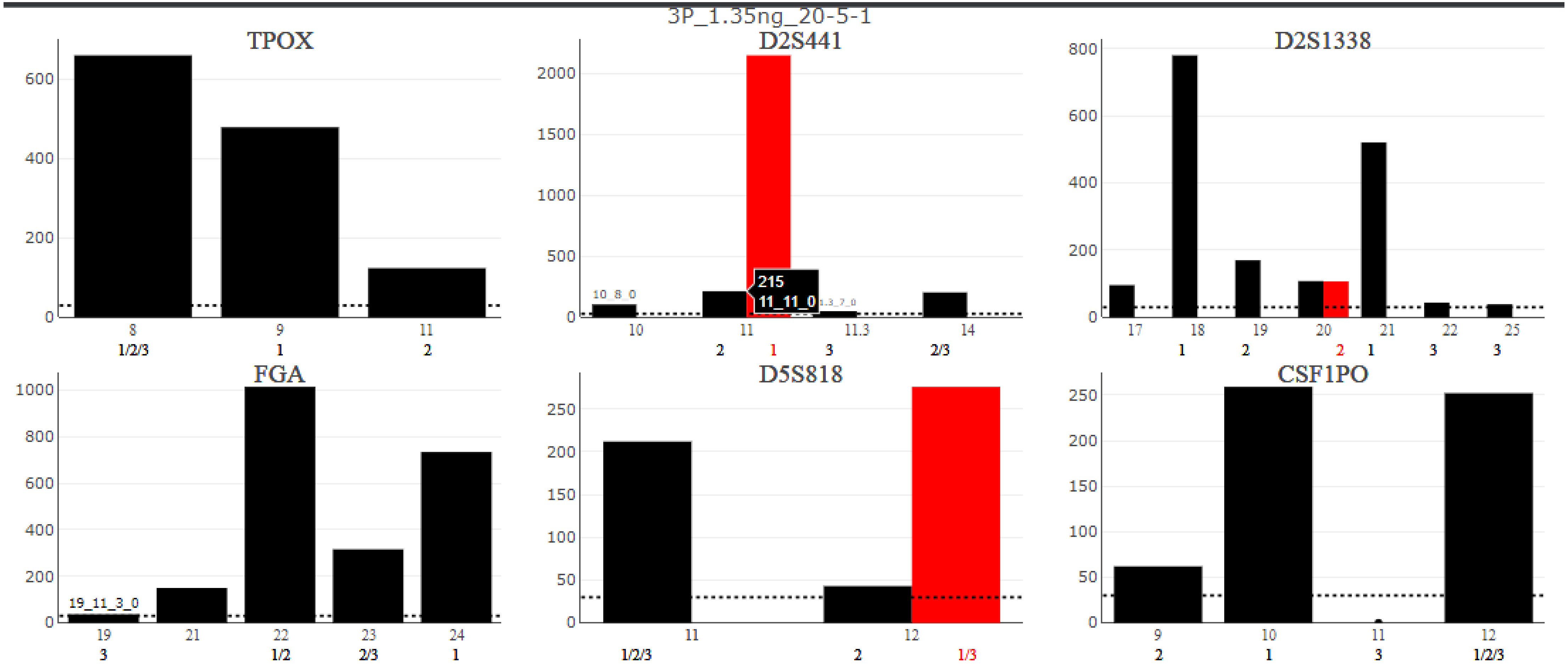
log10LR=-75.08

Upper boundary=56.19

Non-contributor analysis

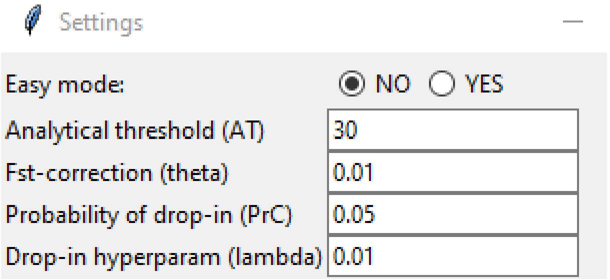
Select reference to replace with non-contributor: P3

Example data 4: 3P_1.35ng_20-5-1 (MPS LUS+)



Propositions tested for dataset 4 (MPS-LUS+): *test_logLik3contr2RepLUS*

Global settings for all markers:

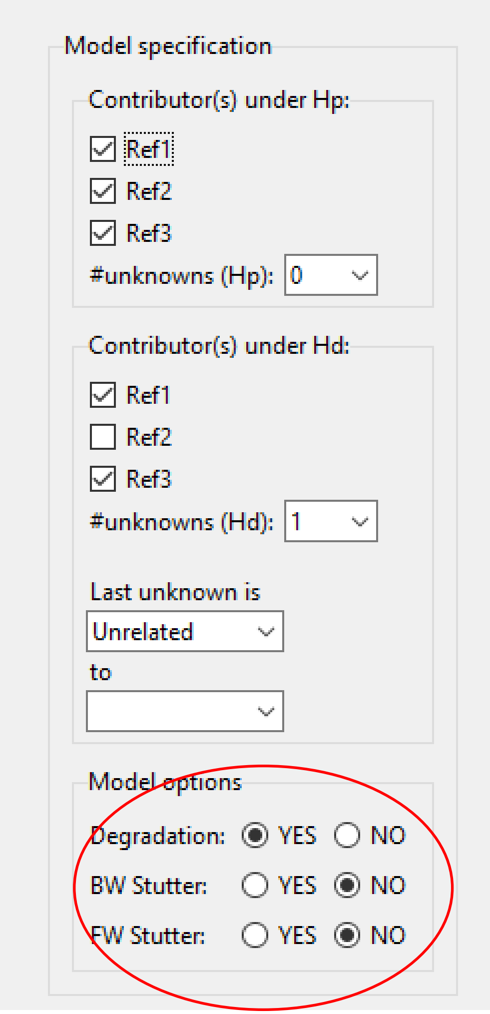


Easy mode: ● NO ○ YES
Analytical threshold (AT) 30
Fst-correction (theta) 0.01
Probability of drop-in (PrC) 0.05
Drop-in hyperparam (lambda) 0.01

Using **only** replicate
3P_1.35ng_20-5-1

- Hyp 1: Ref1 + Ref2 + Ref3 are contributors
- Hyp2: Ref1 + Ref3 + 1 (unrelated) unknown are contributors, Ref2 is a known non-contributor

GUI check   Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ Ref1
☑ Ref2
☑ Ref3
#unknowns (Hp): 0

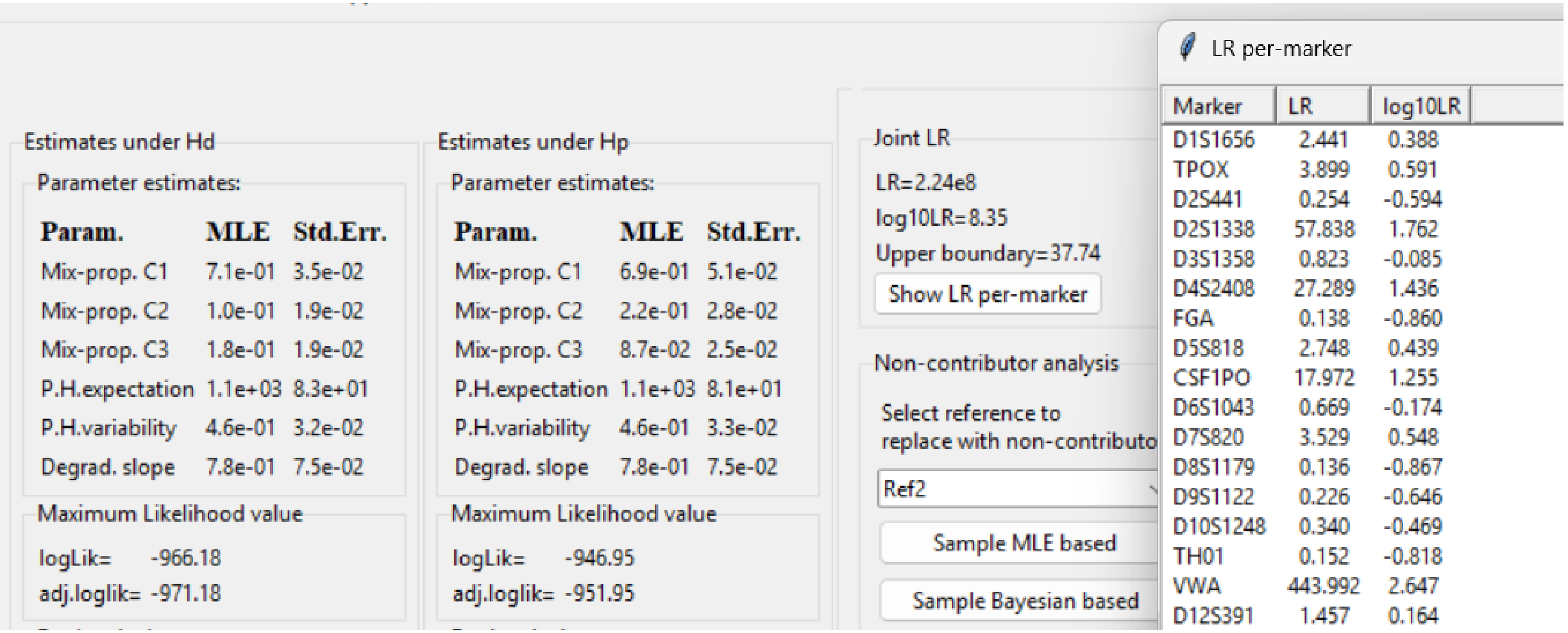
Contributor(s) under Hd:

☑ Ref1
☐ Ref2
☑ Ref3
#unknowns (Hd): 1

Last unknown is
Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ○ YES ● NO
FW Stutter: ○ YES ● NO



Estimates under Hd

Parameter estimates:

Param.	**MLE**	**Std.Err.**
Mix-prop. C1	7.1e-01	3.5e-02
Mix-prop. C2	1.0e-01	1.9e-02
Mix-prop. C3	1.8e-01	1.9e-02
P.H.expectation	1.1e+03	8.3e+01
P.H.variability	4.6e-01	3.2e-02
Degrad. slope	7.8e-01	7.5e-02

Maximum Likelihood value

logLik= -966.18
adj.loglik= -971.18

Estimates under Hp

Parameter estimates:

Param.	**MLE**	**Std.Err.**
Mix-prop. C1	6.9e-01	5.1e-02
Mix-prop. C2	2.2e-01	2.8e-02
Mix-prop. C3	8.7e-02	2.5e-02
P.H.expectation	1.1e+03	8.1e+01
P.H.variability	4.6e-01	3.3e-02
Degrad. slope	7.8e-01	7.5e-02

Maximum Likelihood value

logLik= -946.95
adj.loglik= -951.95

Joint LR

LR=2.24e8
log10LR=8.35
Upper boundary=37.74

Non-contributor analysis

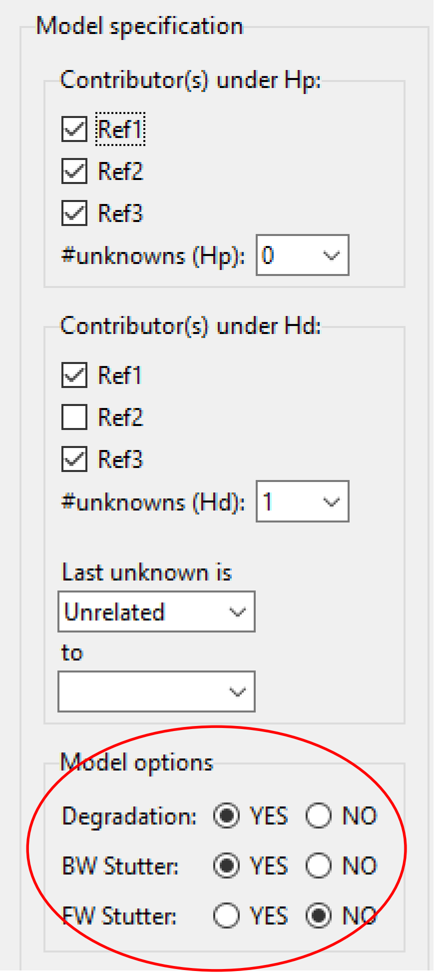
Select reference to
replace with non-contributo

Ref2

LR per-marker

Marker	LR	log10LR
D1S1656	2.441	0.388
TPOX	3.899	0.591
D2S441	0.254	-0.594
D2S1338	57.838	1.762
D3S1358	0.823	-0.085
D4S2408	27.289	1.436
FGA	0.138	-0.860
D5S818	2.748	0.439
CSF1PO	17.972	1.255
D6S1043	0.669	-0.174
D7S820	3.529	0.548
D8S1179	0.136	-0.867
D9S1122	0.226	-0.646
D10S1248	0.340	-0.469
TH01	0.152	-0.818
VWA	443.992	2.647
D12S391	1.457	0.164

GUI check Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ Ref1
☑ Ref2
☑ Ref3
#unknowns (Hp): 0

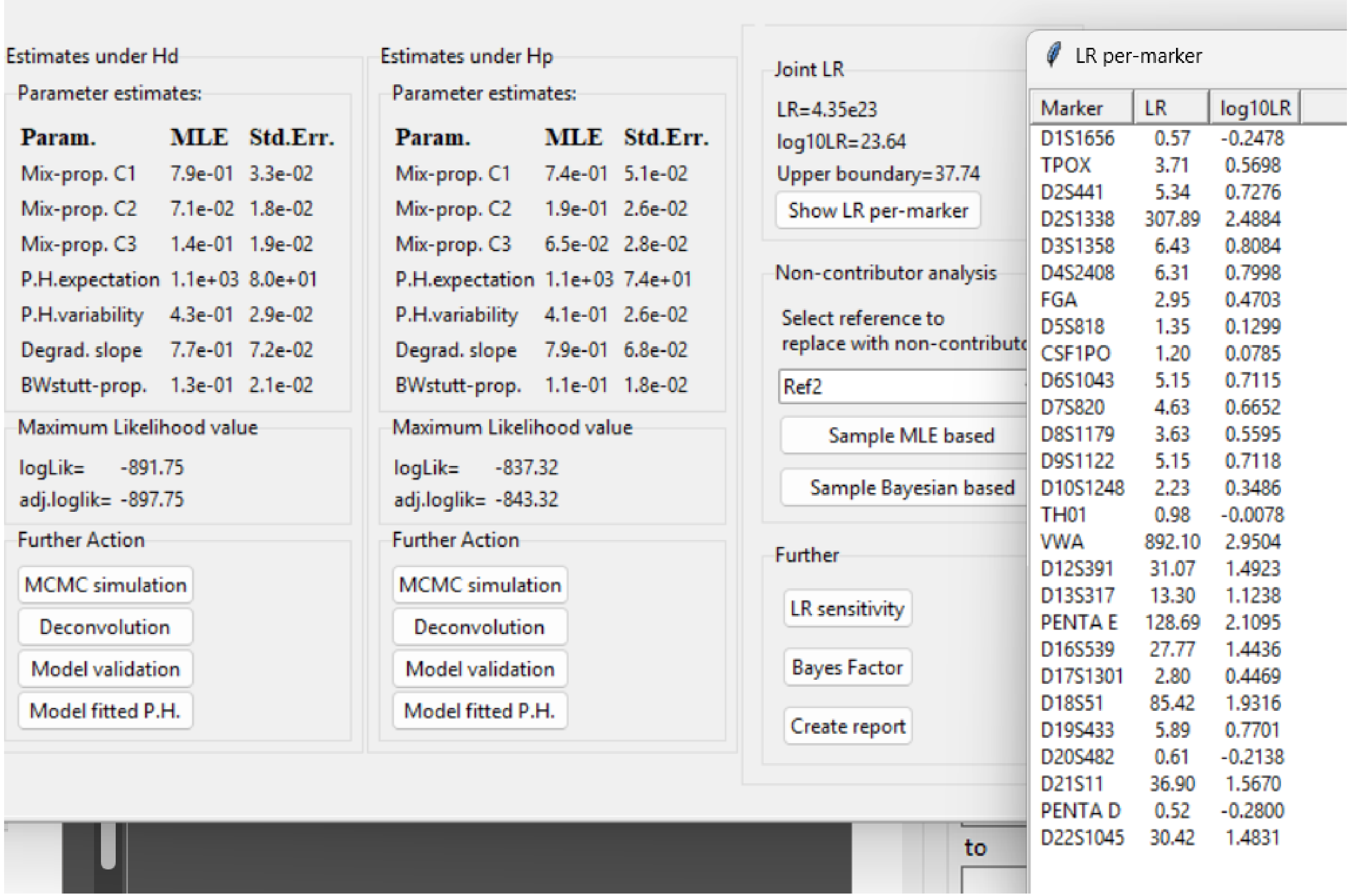
Contributor(s) under Hd:

☑ Ref1
☐ Ref2
☑ Ref3
#unknowns (Hd): 1

Last unknown is
Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ● YES ○ NO
FW Stutter: ○ YES ● NO



Estimates under Hd

Parameter estimates:

Param.	**MLE**	**Std.Err.**
Mix-prop. C1	7.9e-01	3.3e-02
Mix-prop. C2	7.1e-02	1.8e-02
Mix-prop. C3	1.4e-01	1.9e-02
P.H.expectation	1.1e+03	8.0e+01
P.H.variability	4.3e-01	2.9e-02
Degrad. slope	7.7e-01	7.2e-02
BWstutt-prop.	1.3e-01	2.1e-02

Maximum Likelihood value

logLik= -891.75
adj.loglik= -897.75

Further Action

Estimates under Hp

Parameter estimates:

Param.	**MLE**	**Std.Err.**
Mix-prop. C1	7.4e-01	5.1e-02
Mix-prop. C2	1.9e-01	2.6e-02
Mix-prop. C3	6.5e-02	2.8e-02
P.H.expectation	1.1e+03	7.4e+01
P.H.variability	4.1e-01	2.6e-02
Degrad. slope	7.9e-01	6.8e-02
BWstutt-prop.	1.1e-01	1.8e-02

Maximum Likelihood value

logLik= -837.32
adj.loglik= -843.32

Further Action

Joint LR

LR=4.35e23
log10LR=23.64
Upper boundary=37.74

Non-contributor analysis

Select reference to
replace with non-contributo

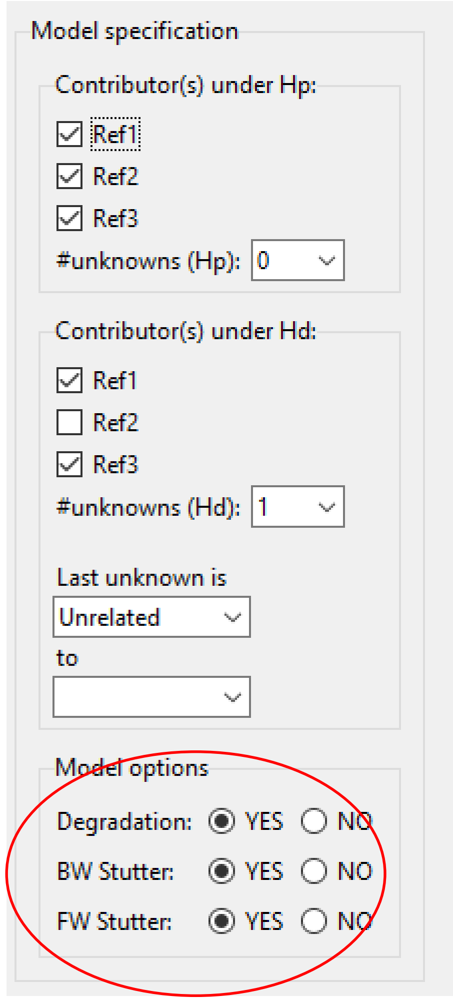
Ref2

Further

LR per-marker

Marker	LR	log10LR
D1S1656	0.57	-0.2478
TPOX	3.71	0.5698
D2S441	5.34	0.7276
D2S1338	307.89	2.4884
D3S1358	6.43	0.8084
D4S2408	6.31	0.7998
FGA	2.95	0.4703
D5S818	1.35	0.1299
CSF1PO	1.20	0.0785
D6S1043	5.15	0.7115
D7S820	4.63	0.6652
D8S1179	3.63	0.5595
D9S1122	5.15	0.7118
D10S1248	2.23	0.3486
TH01	0.98	-0.0078
VWA	892.10	2.9504
D12S391	31.07	1.4923
D13S317	13.30	1.1238
PENTA E	128.69	2.1095
D16S539	27.77	1.4436
D17S1301	2.80	0.4469
D18S51	85.42	1.9316
D19S433	5.89	0.7701
D20S482	0.61	-0.2138
D21S11	36.90	1.5670
PENTA D	0.52	-0.2800
D22S1045	30.42	1.4831

GUI check Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ Ref1
☑ Ref2
☑ Ref3
#unknowns (Hp): 0

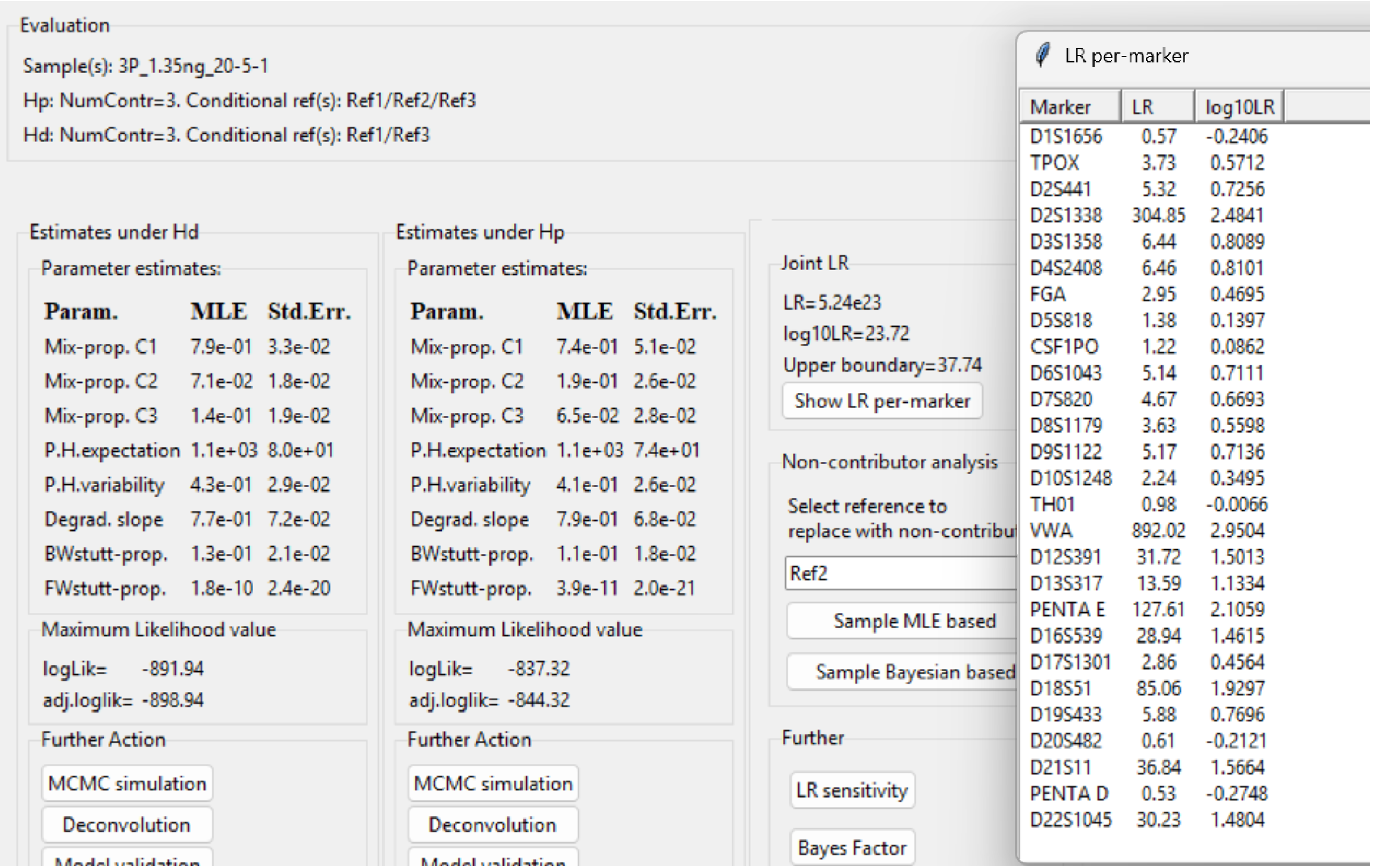
Contributor(s) under Hd:

☑ Ref1
☐ Ref2
☑ Ref3
#unknowns (Hd): 1

Last unknown is Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ● YES ○ NO
FW Stutter: ● YES ○ NO



Evaluation

Sample(s): 3P_1.35ng_20-5-1
Hp: NumContr=3. Conditional ref(s): Ref1/Ref2/Ref3
Hd: NumContr=3. Conditional ref(s): Ref1/Ref3

Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	7.9e-01	3.3e-02
Mix-prop. C2	7.1e-02	1.8e-02
Mix-prop. C3	1.4e-01	1.9e-02
P.H.expectation	1.1e+03	8.0e+01
P.H.variability	4.3e-01	2.9e-02
Degrad. slope	7.7e-01	7.2e-02
BWstutt-prop.	1.3e-01	2.1e-02
FWstutt-prop.	1.8e-10	2.4e-20

Maximum Likelihood value

logLik= -891.94
adj.loglik= -898.94

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	7.4e-01	5.1e-02
Mix-prop. C2	1.9e-01	2.6e-02
Mix-prop. C3	6.5e-02	2.8e-02
P.H.expectation	1.1e+03	7.4e+01
P.H.variability	4.1e-01	2.6e-02
Degrad. slope	7.9e-01	6.8e-02
BWstutt-prop.	1.1e-01	1.8e-02
FWstutt-prop.	3.9e-11	2.0e-21

Maximum Likelihood value

logLik= -837.32
adj.loglik= -844.32

Further Action

Joint LR

LR=5.24e23
log10LR=23.72
Upper boundary=37.74

Non-contributor analysis

Select reference to replace with non-contribu

Ref2

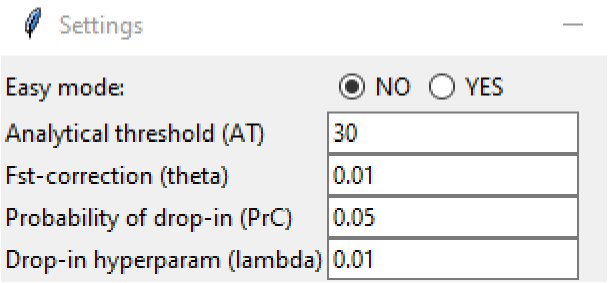
Further

LR per-marker

Marker	LR	log10LR
D1S1656	0.57	-0.2406
TPOX	3.73	0.5712
D2S441	5.32	0.7256
D2S1338	304.85	2.4841
D3S1358	6.44	0.8089
D4S2408	6.46	0.8101
FGA	2.95	0.4695
D5S818	1.38	0.1397
CSF1PO	1.22	0.0862
D6S1043	5.14	0.7111
D7S820	4.67	0.6693
D8S1179	3.63	0.5598
D9S1122	5.17	0.7136
D10S1248	2.24	0.3495
TH01	0.98	-0.0066
VWA	892.02	2.9504
D12S391	31.72	1.5013
D13S317	13.59	1.1334
PENTA E	127.61	2.1059
D16S539	28.94	1.4615
D17S1301	2.86	0.4564
D18S51	85.06	1.9297
D19S433	5.88	0.7696
D20S482	0.61	-0.2121
D21S11	36.84	1.5664
PENTA D	0.53	-0.2748
D22S1045	30.23	1.4804

Propositions tested for dataset 4 (MPS-LUS+): *test_logLik3contr2RepLUS*

Global settings for all markers:



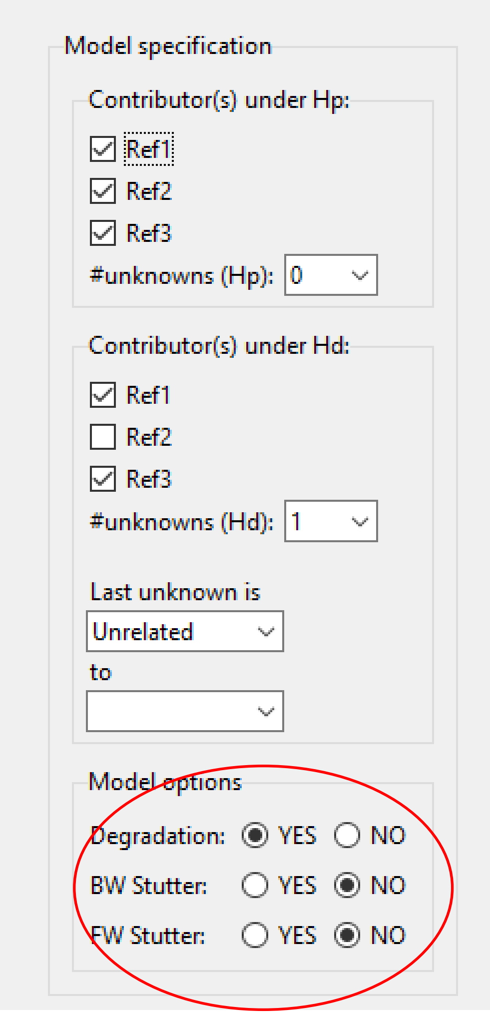
Easy mode: ● NO ○ YES

Analytical threshold (AT)	30
Fst-correction (theta)	0.01
Probability of drop-in (PrC)	0.05
Drop-in hyperparam (lambda)	0.01

Using **Both** replicates

- Hyp 1: Ref1 + Ref2 + Ref3 are contributors
- Hyp2: Ref1 + Ref3 + 1 (unrelated) unknown are contributors, Ref2 is a known non-contributor

GUI check Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ Ref1
☑ Ref2
☑ Ref3
#unknowns (Hp): 0

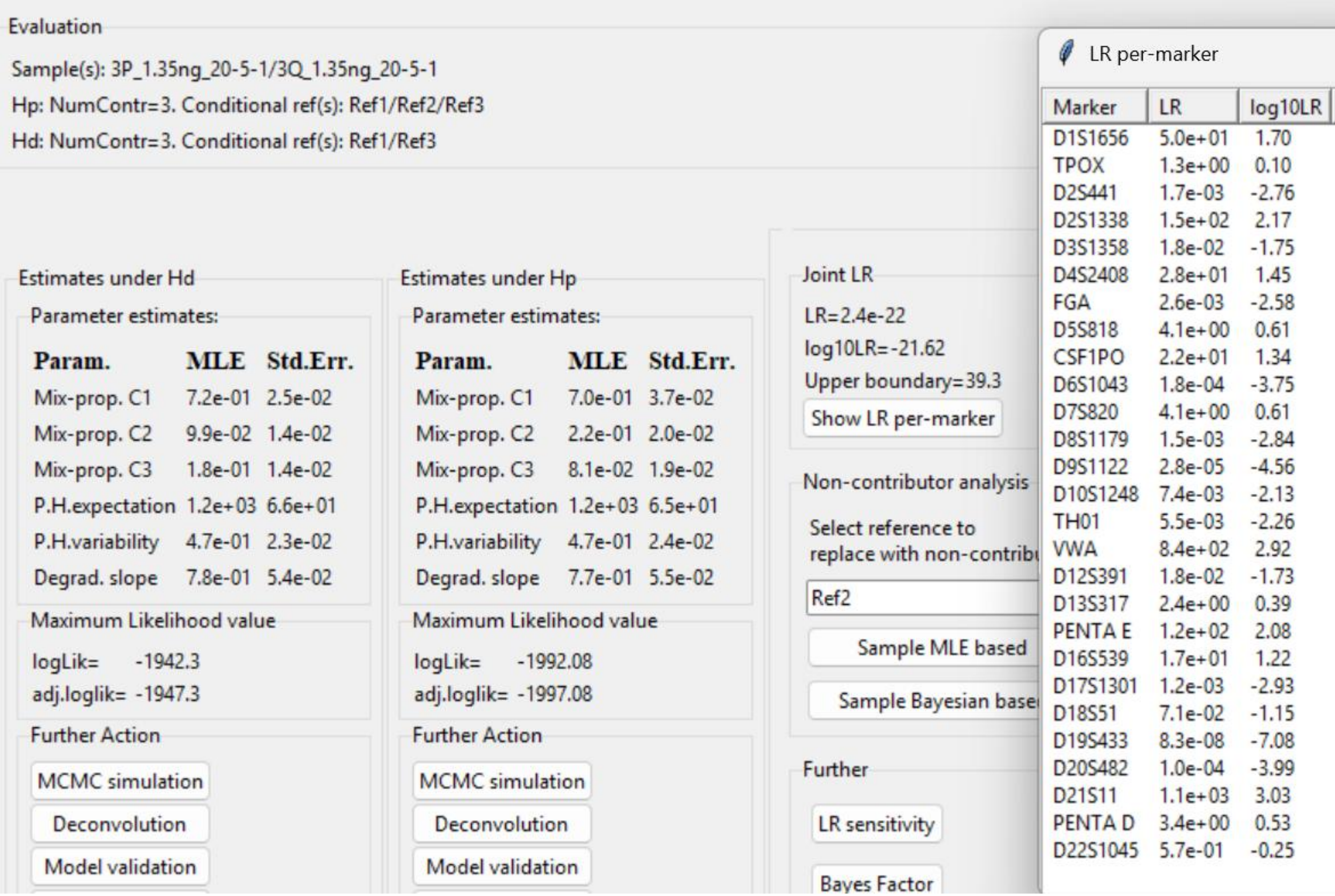
Contributor(s) under Hd:

☑ Ref1
☐ Ref2
☑ Ref3
#unknowns (Hd): 1

Last unknown is
Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ○ YES ● NO
FW Stutter: ○ YES ● NO



Evaluation

Sample(s): 3P_1.35ng_20-5-1/3Q_1.35ng_20-5-1
Hp: NumContr=3. Conditional ref(s): Ref1/Ref2/Ref3
Hd: NumContr=3. Conditional ref(s): Ref1/Ref3

Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	7.2e-01	2.5e-02
Mix-prop. C2	9.9e-02	1.4e-02
Mix-prop. C3	1.8e-01	1.4e-02
P.H.expectation	1.2e+03	6.6e+01
P.H.variability	4.7e-01	2.3e-02
Degrad. slope	7.8e-01	5.4e-02

Maximum Likelihood value

logLik= -1942.3
adj.loglik= -1947.3

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	7.0e-01	3.7e-02
Mix-prop. C2	2.2e-01	2.0e-02
Mix-prop. C3	8.1e-02	1.9e-02
P.H.expectation	1.2e+03	6.5e+01
P.H.variability	4.7e-01	2.4e-02
Degrad. slope	7.7e-01	5.5e-02

Maximum Likelihood value

logLik= -1992.08
adj.loglik= -1997.08

Further Action

Joint LR

LR=2.4e-22
log10LR=-21.62
Upper boundary=39.3

Non-contributor analysis

Select reference to
replace with non-contribu

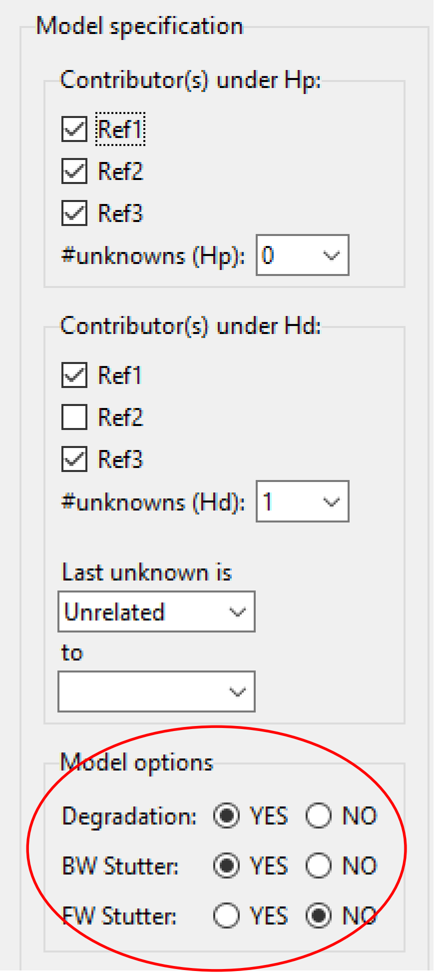
Ref2

Further

LR per-marker

Marker	LR	log10LR
D1S1656	5.0e+01	1.70
TPOX	1.3e+00	0.10
D2S441	1.7e-03	-2.76
D2S1338	1.5e+02	2.17
D3S1358	1.8e-02	-1.75
D4S2408	2.8e+01	1.45
FGA	2.6e-03	-2.58
D5S818	4.1e+00	0.61
CSF1PO	2.2e+01	1.34
D6S1043	1.8e-04	-3.75
D7S820	4.1e+00	0.61
D8S1179	1.5e-03	-2.84
D9S1122	2.8e-05	-4.56
D10S1248	7.4e-03	-2.13
TH01	5.5e-03	-2.26
VWA	8.4e+02	2.92
D12S391	1.8e-02	-1.73
D13S317	2.4e+00	0.39
PENTA E	1.2e+02	2.08
D16S539	1.7e+01	1.22
D17S1301	1.2e-03	-2.93
D18S51	7.1e-02	-1.15
D19S433	8.3e-08	-7.08
D20S482	1.0e-04	-3.99
D21S11	1.1e+03	3.03
PENTA D	3.4e+00	0.53
D22S1045	5.7e-01	-0.25

GUI check   Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ Ref1
☑ Ref2
☑ Ref3
#unknowns (Hp): 0

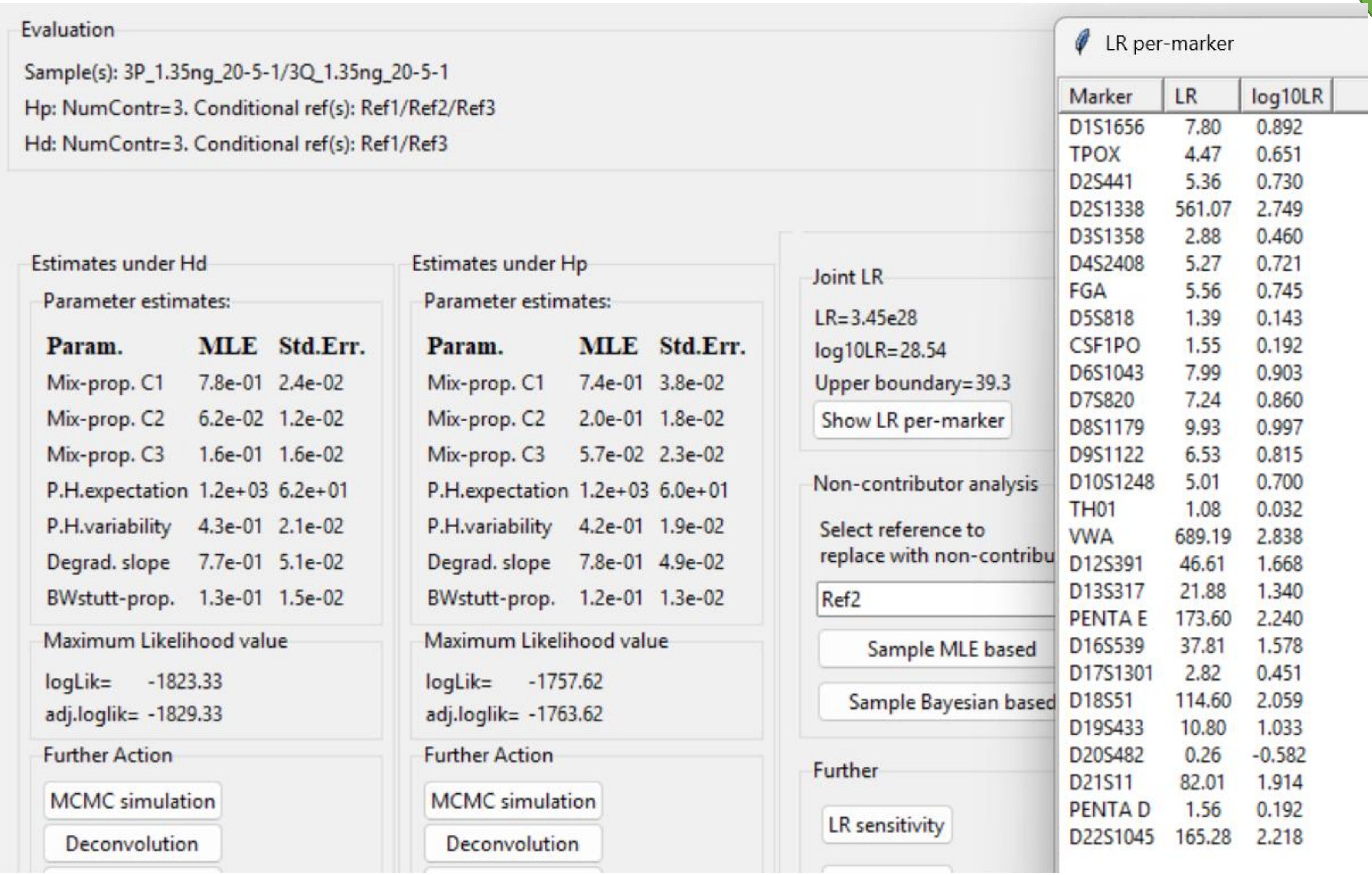
Contributor(s) under Hd:

☑ Ref1
☐ Ref2
☑ Ref3
#unknowns (Hd): 1

Last unknown is
Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ● YES ○ NO
FW Stutter: ○ YES ● NO



Evaluation

Sample(s): 3P_1.35ng_20-5-1/3Q_1.35ng_20-5-1
Hp: NumContr=3. Conditional ref(s): Ref1/Ref2/Ref3
Hd: NumContr=3. Conditional ref(s): Ref1/Ref3

Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	7.8e-01	2.4e-02
Mix-prop. C2	6.2e-02	1.2e-02
Mix-prop. C3	1.6e-01	1.6e-02
P.H.expectation	1.2e+03	6.2e+01
P.H.variability	4.3e-01	2.1e-02
Degrad. slope	7.7e-01	5.1e-02
BWstutt-prop.	1.3e-01	1.5e-02

Maximum Likelihood value

logLik= -1823.33
adj.loglik= -1829.33

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	7.4e-01	3.8e-02
Mix-prop. C2	2.0e-01	1.8e-02
Mix-prop. C3	5.7e-02	2.3e-02
P.H.expectation	1.2e+03	6.0e+01
P.H.variability	4.2e-01	1.9e-02
Degrad. slope	7.8e-01	4.9e-02
BWstutt-prop.	1.2e-01	1.3e-02

Maximum Likelihood value

logLik= -1757.62
adj.loglik= -1763.62

Further Action

Joint LR

LR=3.45e28
log10LR=28.54
Upper boundary=39.3

Non-contributor analysis

Select reference to replace with non-contribu

Ref2

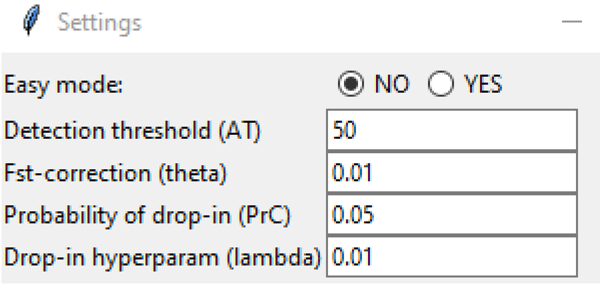
Further

LR per-marker

Marker	LR	log10LR
D1S1656	7.80	0.892
TPOX	4.47	0.651
D2S441	5.36	0.730
D2S1338	561.07	2.749
D3S1358	2.88	0.460
D4S2408	5.27	0.721
FGA	5.56	0.745
D5S818	1.39	0.143
CSF1PO	1.55	0.192
D6S1043	7.99	0.903
D7S820	7.24	0.860
D8S1179	9.93	0.997
D9S1122	6.53	0.815
D10S1248	5.01	0.700
TH01	1.08	0.032
VWA	689.19	2.838
D12S391	46.61	1.668
D13S317	21.88	1.340
PENTA E	173.60	2.240
D16S539	37.81	1.578
D17S1301	2.82	0.451
D18S51	114.60	2.059
D19S433	10.80	1.033
D20S482	0.26	-0.582
D21S11	82.01	1.914
PENTA D	1.56	0.192
D22S1045	165.28	2.218

Additional test

ESX17 profile with partial references



Settings

Easy mode: ● NO ○ YES

Detection threshold (AT): 50

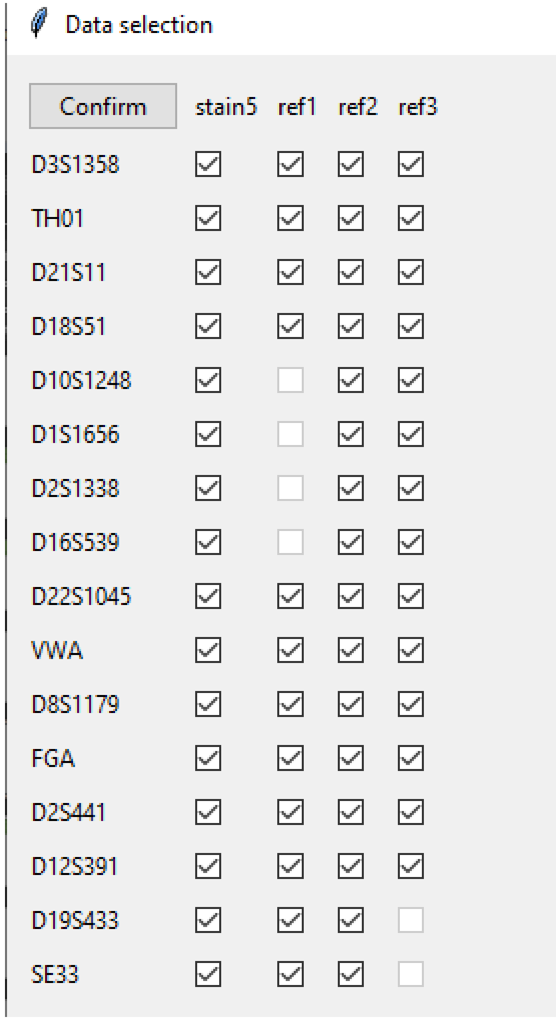
Fst-correction (theta): 0.01

Probability of drop-in (PrC): 0.05

Drop-in hyperparam (lambda): 0.01

Using **only stain5**

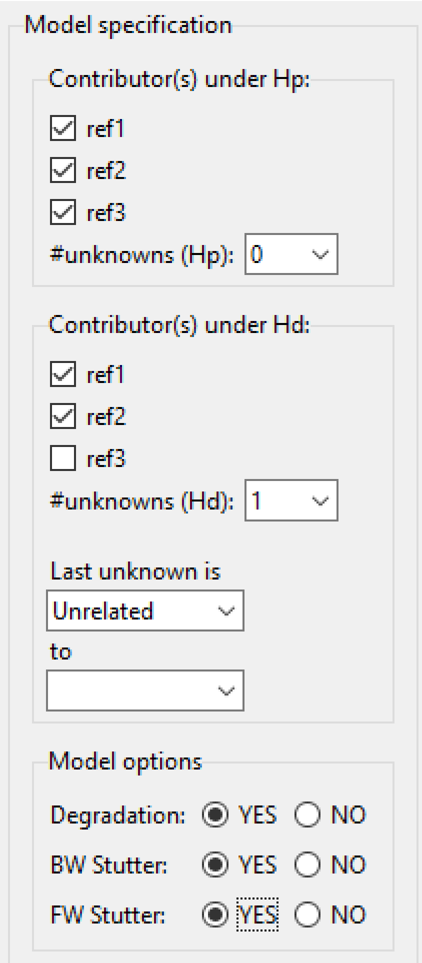
- Hyp 1: Ref1 + Ref2 + Ref3 are contributors
- Hyp2: Ref1 + Ref2 + 1 (unrelated) unknown are contributors, Ref3 is a known non-contributor



Data selection

Confirm	stain5	ref1	ref2	ref3
D3S1358	☑	☑	☑	☑
TH01	☑	☑	☑	☑
D21S11	☑	☑	☑	☑
D18S51	☑	☑	☑	☑
D10S1248	☑	☐	☑	☑
D1S1656	☑	☐	☑	☑
D2S1338	☑	☐	☑	☑
D16S539	☑	☐	☑	☑
D22S1045	☑	☑	☑	☑
VWA	☑	☑	☑	☑
D8S1179	☑	☑	☑	☑
FGA	☑	☑	☑	☑
D2S441	☑	☑	☑	☑
D12S391	☑	☑	☑	☑
D19S433	☑	☑	☑	☐
SE33	☑	☑	☑	☐

GUI check   Hp: Hyp 1 vs Hd: Hyp 2



Model specification

Contributor(s) under Hp:

☑ ref1
☑ ref2
☑ ref3
#unknowns (Hp): 0

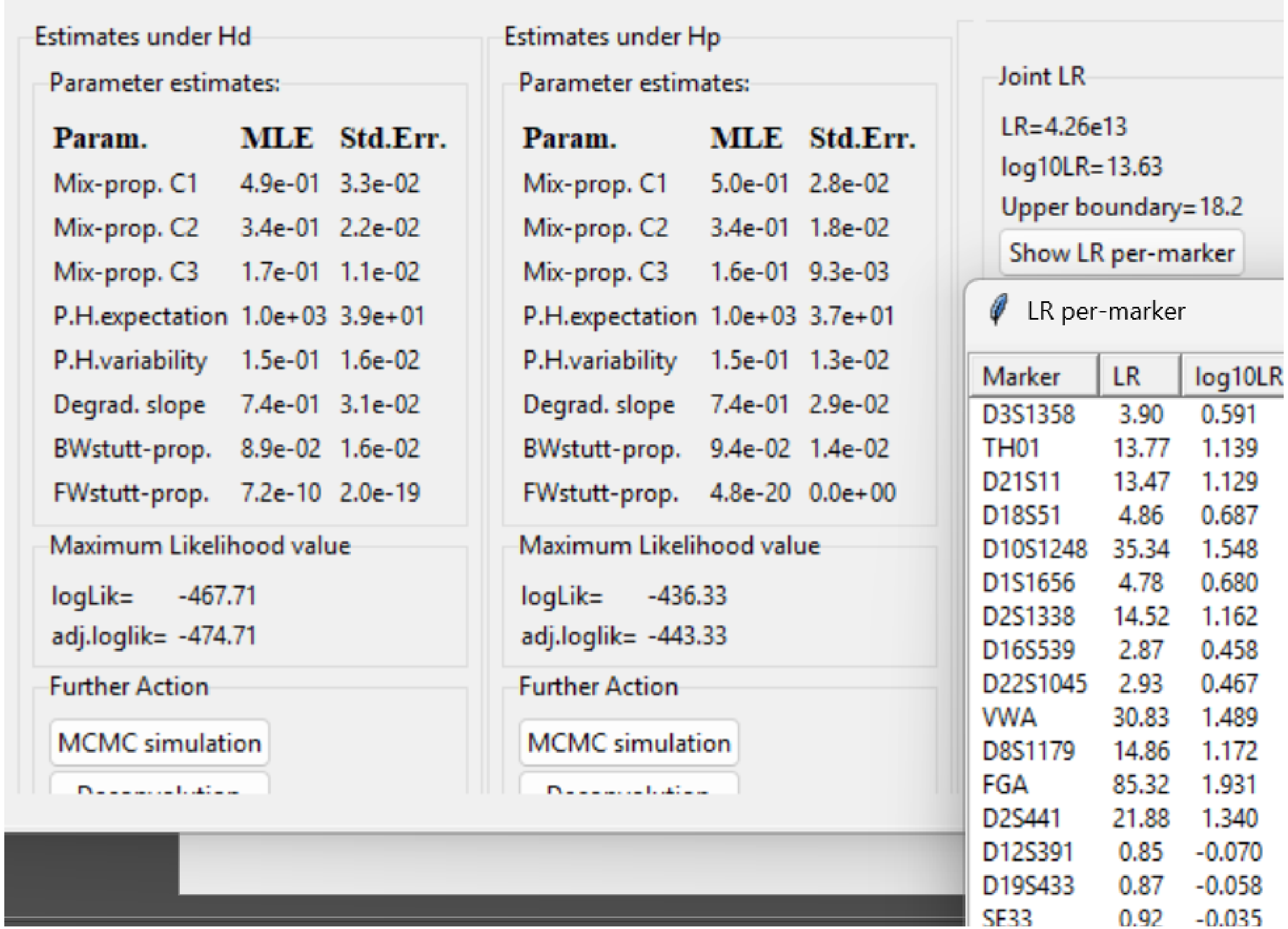
Contributor(s) under Hd:

☑ ref1
☑ ref2
☐ ref3
#unknowns (Hd): 1

Last unknown is Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ● YES ○ NO
FW Stutter: ● YES ○ NO



Estimates under Hd

Parameter estimates:

Param.	**MLE**	**Std.Err.**
Mix-prop. C1	4.9e-01	3.3e-02
Mix-prop. C2	3.4e-01	2.2e-02
Mix-prop. C3	1.7e-01	1.1e-02
P.H.expectation	1.0e+03	3.9e+01
P.H.variability	1.5e-01	1.6e-02
Degrad. slope	7.4e-01	3.1e-02
BWstutt-prop.	8.9e-02	1.6e-02
FWstutt-prop.	7.2e-10	2.0e-19

Maximum Likelihood value

logLik= -467.71
adj.loglik= -474.71

Further Action

Estimates under Hp

Parameter estimates:

Param.	**MLE**	**Std.Err.**
Mix-prop. C1	5.0e-01	2.8e-02
Mix-prop. C2	3.4e-01	1.8e-02
Mix-prop. C3	1.6e-01	9.3e-03
P.H.expectation	1.0e+03	3.7e+01
P.H.variability	1.5e-01	1.3e-02
Degrad. slope	7.4e-01	2.9e-02
BWstutt-prop.	9.4e-02	1.4e-02
FWstutt-prop.	4.8e-20	0.0e+00

Maximum Likelihood value

logLik= -436.33
adj.loglik= -443.33

Further Action

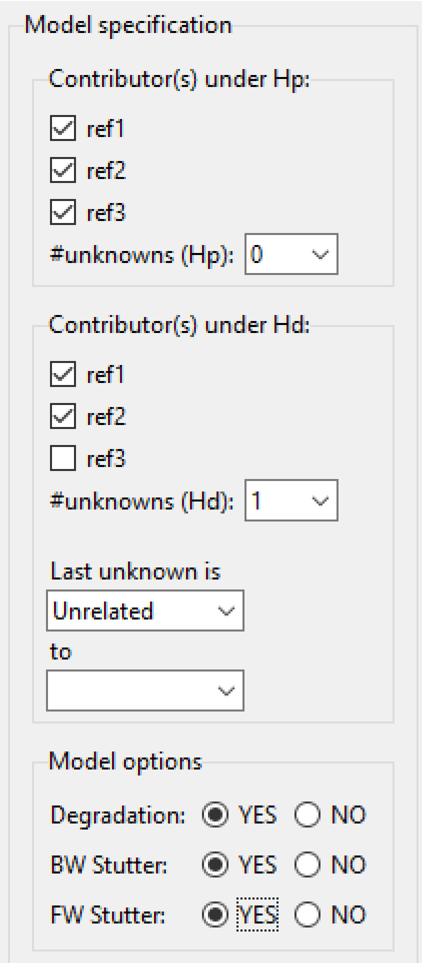
Joint LR

LR=4.26e13
log10LR=13.63
Upper boundary=18.2

LR per-marker

Marker	LR	log10LR
D3S1358	3.90	0.591
TH01	13.77	1.139
D21S11	13.47	1.129
D18S51	4.86	0.687
D10S1248	35.34	1.548
D1S1656	4.78	0.680
D2S1338	14.52	1.162
D16S539	2.87	0.458
D22S1045	2.93	0.467
VWA	30.83	1.489
D8S1179	14.86	1.172
FGA	85.32	1.931
D2S441	21.88	1.340
D12S391	0.85	-0.070
D19S433	0.87	-0.058
SE33	0.92	-0.035

GUI check   Again but with all replicates



Model specification

Contributor(s) under Hp:

☑ ref1
☑ ref2
☑ ref3
#unknowns (Hp): 0

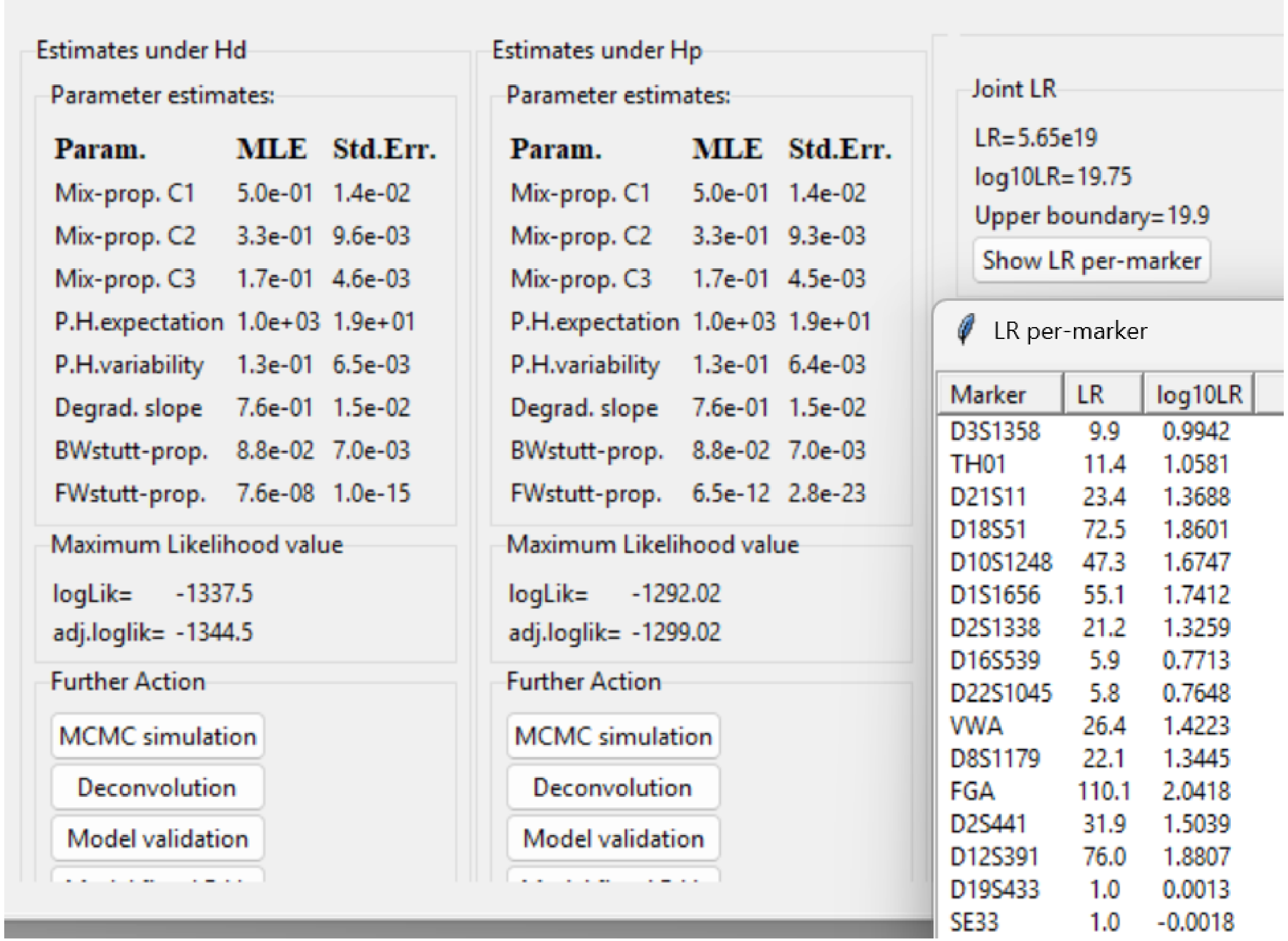
Contributor(s) under Hd:

☑ ref1
☑ ref2
☐ ref3
#unknowns (Hd): 1

Last unknown is
Unrelated
to

Model options

Degradation: ● YES ○ NO
BW Stutter: ● YES ○ NO
FW Stutter: ● YES ○ NO



Estimates under Hd

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	5.0e-01	1.4e-02
Mix-prop. C2	3.3e-01	9.6e-03
Mix-prop. C3	1.7e-01	4.6e-03
P.H.expectation	1.0e+03	1.9e+01
P.H.variability	1.3e-01	6.5e-03
Degrad. slope	7.6e-01	1.5e-02
BWstutt-prop.	8.8e-02	7.0e-03
FWstutt-prop.	7.6e-08	1.0e-15

Maximum Likelihood value

logLik= -1337.5
adj.loglik= -1344.5

Further Action

Estimates under Hp

Parameter estimates:

Param.	MLE	Std.Err.
Mix-prop. C1	5.0e-01	1.4e-02
Mix-prop. C2	3.3e-01	9.3e-03
Mix-prop. C3	1.7e-01	4.5e-03
P.H.expectation	1.0e+03	1.9e+01
P.H.variability	1.3e-01	6.4e-03
Degrad. slope	7.6e-01	1.5e-02
BWstutt-prop.	8.8e-02	7.0e-03
FWstutt-prop.	6.5e-12	2.8e-23

Maximum Likelihood value

logLik= -1292.02
adj.loglik= -1299.02

Further Action

Joint LR

LR=5.65e19
log10LR=19.75
Upper boundary=19.9

LR per-marker

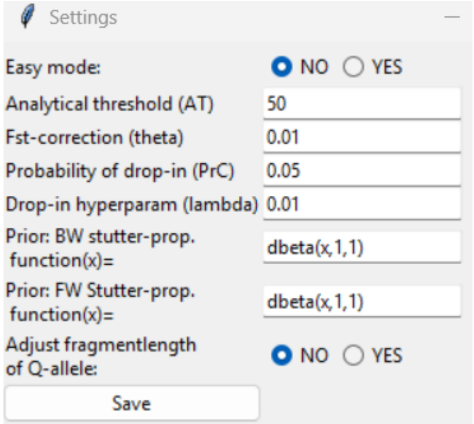
Marker	LR	log10LR
D3S1358	9.9	0.9942
TH01	11.4	1.0581
D21S11	23.4	1.3688
D18S51	72.5	1.8601
D10S1248	47.3	1.6747
D1S1656	55.1	1.7412
D2S1338	21.2	1.3259
D16S539	5.9	0.7713
D22S1045	5.8	0.7648
VWA	26.4	1.4223
D8S1179	22.1	1.3445
FGA	110.1	2.0418
D2S441	31.9	1.5039
D12S391	76.0	1.8807
D19S433	1.0	0.0013
SE33	1.0	-0.0018

Tests for Tri-alleles (**test_triAlleles.R**)

All tests are calculated with logLik, Deconvolution and MLE-validation

- Value of logLik and cumulative probs in validation.
- Deconvolution shows genotype with triAllele
- Assume NOC=2, condition on both contributors

Kit=testkit


Settings

Easy mode: ● NO ○ YES
Analytical threshold (AT): 50
Fst-correction (theta): 0.01
Probability of drop-in (PrC): 0.05
Drop-in hyperparam (lambda): 0.01
Prior: BW stutter-prop. function(x)= dbeta(x,1,1)
Prior: FW Stutter-prop. function(x)= dbeta(x,1,1)
Adjust fragmentlength of Q-allele: ● NO ○ YES

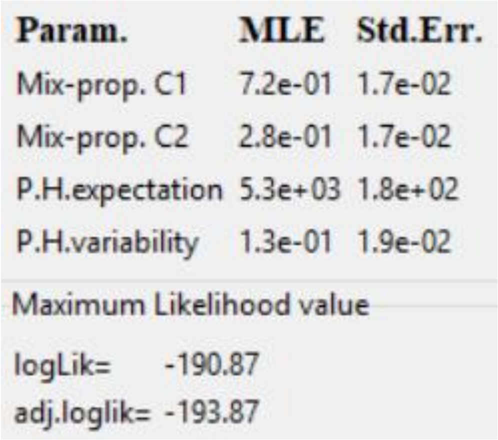
Tests:

1) 1 triAllele for Ref1
2) 2 triAlleles: 1 for Ref 1 and 1 for Ref 2 (different marker)
3) 3 triAlleles: 2 for Ref 1 and 1 for Ref 2 (different markers)
4) 4 triAlleles: Same as above but a shared triAllele for one marker
5) A dropout triAllele: Ref1 has a triAllele that is not in Evidence
6) 1 triAllele for Ref1 (2 replicates, degradation + backward and forward stutter model)
7) 1 triAllele for Ref1 (2 replicates, degradation + only backward stutter model)

- (1-5) are with one replicate, without degradation, and no stutter models

GUI check

1)

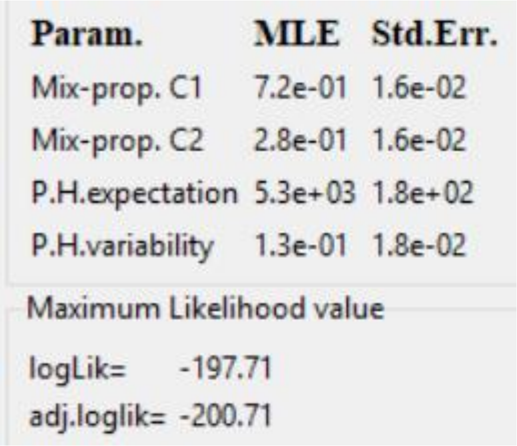


Param.	MLE	Std.Err.
Mix-prop. C1	7.2e-01	1.7e-02
Mix-prop. C2	2.8e-01	1.7e-02
P.H.expectation	5.3e+03	1.8e+02
P.H.variability	1.3e-01	1.9e-02

Maximum Likelihood value

logLik= -190.87
adj.loglik= -193.87

2)

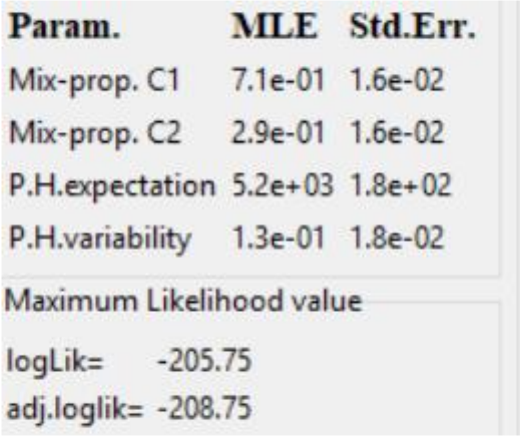


Param.	MLE	Std.Err.
Mix-prop. C1	7.2e-01	1.6e-02
Mix-prop. C2	2.8e-01	1.6e-02
P.H.expectation	5.3e+03	1.8e+02
P.H.variability	1.3e-01	1.8e-02

Maximum Likelihood value

logLik= -197.71
adj.loglik= -200.71

3)

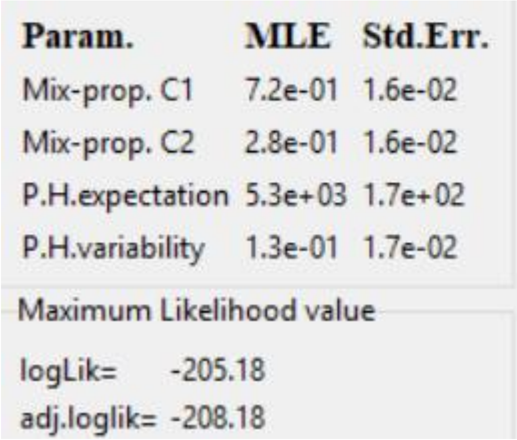


Param.	MLE	Std.Err.
Mix-prop. C1	7.1e-01	1.6e-02
Mix-prop. C2	2.9e-01	1.6e-02
P.H.expectation	5.2e+03	1.8e+02
P.H.variability	1.3e-01	1.8e-02

Maximum Likelihood value

logLik= -205.75
adj.loglik= -208.75

4)

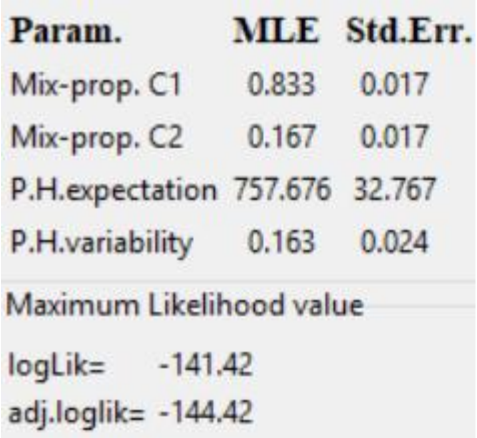


Param.	MLE	Std.Err.
Mix-prop. C1	7.2e-01	1.6e-02
Mix-prop. C2	2.8e-01	1.6e-02
P.H.expectation	5.3e+03	1.7e+02
P.H.variability	1.3e-01	1.7e-02

Maximum Likelihood value

logLik= -205.18
adj.loglik= -208.18

5)

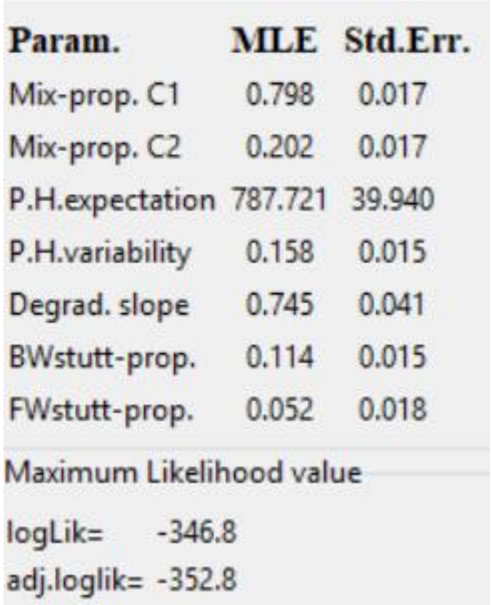


Param.	MLE	Std.Err.
Mix-prop. C1	0.833	0.017
Mix-prop. C2	0.167	0.017
P.H.expectation	757.676	32.767
P.H.variability	0.163	0.024

Maximum Likelihood value

logLik= -141.42
adj.loglik= -144.42

6)

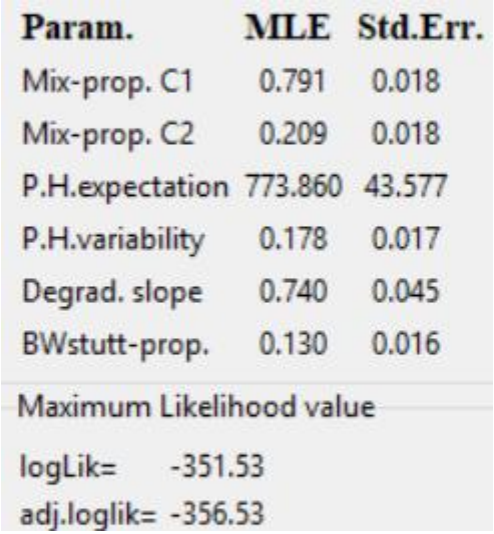


Param.	MLE	Std.Err.
Mix-prop. C1	0.798	0.017
Mix-prop. C2	0.202	0.017
P.H.expectation	787.721	39.940
P.H.variability	0.158	0.015
Degrad. slope	0.745	0.041
BWstutt-prop.	0.114	0.015
FWstutt-prop.	0.052	0.018

Maximum Likelihood value

logLik= -346.8
adj.loglik= -352.8

7)



Param.	MLE	Std.Err.
Mix-prop. C1	0.791	0.018
Mix-prop. C2	0.209	0.018
P.H.expectation	773.860	43.577
P.H.variability	0.178	0.017
Degrad. slope	0.740	0.045
BWstutt-prop.	0.130	0.016

Maximum Likelihood value

logLik= -351.53
adj.loglik= -356.53

Testing the **qualitative** model (tested for dataset 2, using both replicates)

Different drop-out probability models are tested:

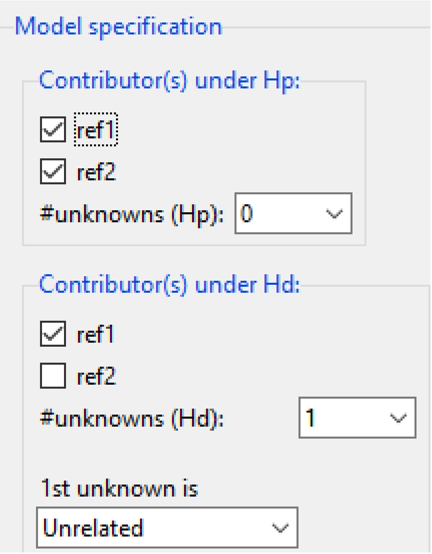
- Hyp 1: Ref1 + Ref2, common drop-out parameter
- Hyp 2: Ref1 + Ref2, Ref1 drop-out parameter set to 0.
- Hyp 3: Ref1 + Ref2, 2 drop-out parameters

Ref2 known non-contr for hyps Hyp ‘4-6’:

- Hyp 4: Ref1 + 1U, common drop-out parameter.
- Hyp 5: Ref1 + 1U, Ref1 drop-out parameter set to 0.
- Hyp 6: Ref1 + 1U, 2 drop-out parameters

GUI check (Qual model)

Only common drop-out parameter implemented in GUI


Model specification

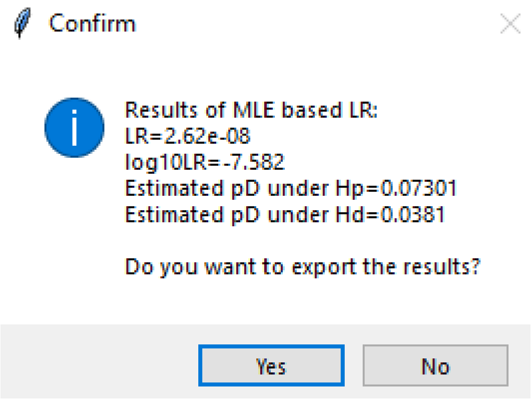
Contributor(s) under Hp:

☑ ref1
☑ ref2
#unknowns (Hp): 0

Contributor(s) under Hd:

☑ ref1
☐ ref2
#unknowns (Hd): 1

1st unknown is
Unrelated



Results of MLE based LR:
LR=2.62e-08
log10LR=-7.582
Estimated pD under Hp=0.07301
Estimated pD under Hd=0.0381

Do you want to export the results?

Hyp 1 (Hp) : R1+R2 (common drop-out parameter):
logLik = -95.1168543762792
d = 0.0730126, 0.0730126

Hyp 4 (Hd): R1+1U (common drop-out parameter):
logLik = -77.6593191143117
d = 0.03809141, 0.03809141

log10LR = -7.581711

Additional: NFI validation data (Fusion 6C)

- Shared validation data (multi-laboratory comparison) from NFI is used for comparing the results from EFM:
  - https://www.forensicinstitute.nl/research-and-innovation/documents/publications/2020/06/29/dnaxs_dnastatistx-test-data-nfi
- Using same marker settings for obtaining comparable results.
- A R-script is run to check for compatible results.

Additional validation of EFM GUI

- The GUI is manually inspected for concise results whenever new versions are released – based on following data:
  - The output from the manual (tutorial data)
    - Also covers the Bayesian approach
  - Workshop material:
    - ENFSI exercises (ENFSI1 and ENFSI2)
    - EFMexercise26