



inov3PT
SEED POTATO
FOR THE FUTURE

Potato variety identification in the seed certification process using SSR markers

Improvement of the official method in France

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Context

Single sequence repeat (SSR) markers are essential tools to fingerprint varieties for their identification. Six coordinated French partners are using the same procedure which is officially used in the seed potato certification scheme in case of doubt or fraud, and to manage genetic resources. We were supported by the French Ministry of Agriculture through the research project IdEvol (2019-2023) to improve the way of resolving the markers. We assessed the suitability of the Qiagen QIAxcel Advanced capillary gel electrophoresis for our purposes.

Why change the process?

What were the points of focus?

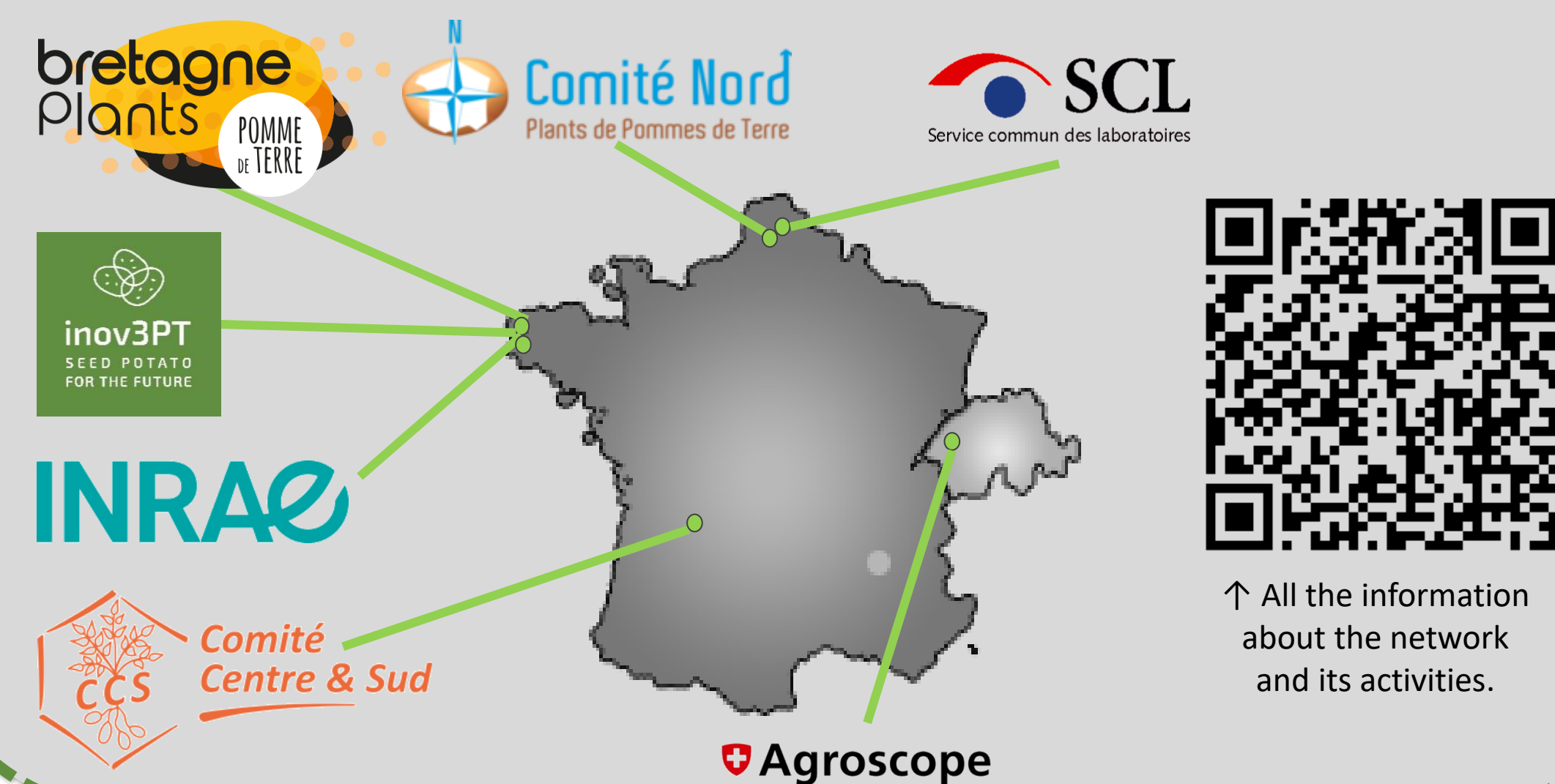
Reasons for this change were:

- ↑ throughput analysis
- ↓ the use of chemicals and toxic products and the costs of recycling
- ↓ tedious and repetitive lab processes

The use of a semi automatic sequencing machine should solve most of these issues. However, the resolutive power is expected to impact the profiles readability and we want to keep on using the huge amount of data stored in the IdeAle database. We had a previous experience of markers' transferability onto Li-Cor and capillary sequencing systems (Marhadour *et al.*, 2014).

A network of 7 partners

7 partners are operating using a common set of markers. The network is coordinated by inov3PT.



Methodology and main results

Former and future systems



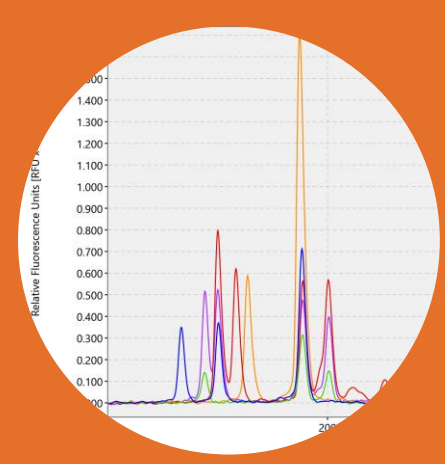
Current official system:
AgNO₃ + polyacrylamide gel



New system: QIAxcel
Advanced (QXL)

Transferability of the current markers and identification of new ones

- 8 « historic » SSR markers (Tab. 1)
- 15 new candidate SSR markers assessed



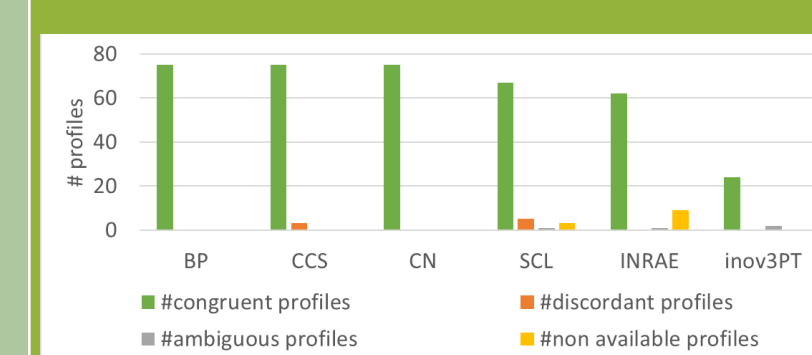
Markers transferability assessment

- 350 varieties split between the 6 French partners
- 8-12 reference varieties
- QXL = 2700 profiles generated
- Comparison to the IdeAle database
- Congruence scores



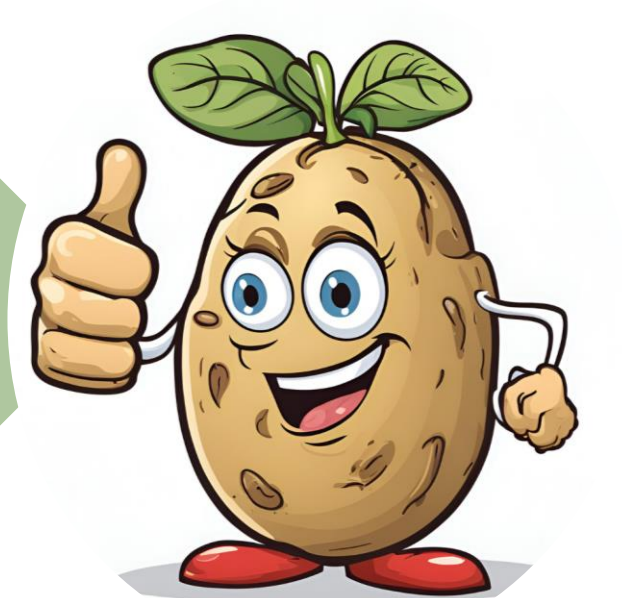
Main results

- 9 markers well adapted to the new system
- 6/8 from the ancient kit (94 to 97% of congruence)
- 3 new markers



Internal ring test 2023 successful

- 12 unknown DNA samples to identify
- 8 analyzed in each of the lab of 5 partners
- 100% of the samples identified in each lab



Towards a new official method

The kit we are currently using

The official kit consists in 8 SSR markers resolved using high resolutive polyacrylamide gel electrophoresis followed by a silver staining step (Tab. 1). 6/8 markers were equally scorable on the new QIAxcel Advanced system. 15 new markers were tested and 3 of them were chosen during the project to complement the new kit. Marker STM5148 was previously occasionally used in one lab using polyacrylamide electrophoresis.

Marker*	Former System	New system	SSR motif	Chr.	#alleles	Molecular weight QXL (bp)
SSR1			(TCAC)n	8	17	200-228
STM2005			(CTGTTG)n and/ or (GA)27	11	8	167-195
LEMALX			(ATT)n	5	5	121-133
STM1097			(CGTTT)n	7	9	230-280
STM2020			(TAA)n	1	11	160-200
STGBSS			(TCT)n	8	13	≈ 120
STM5136			(AGA)5	1	13	226-257
STM5140			(AAT)n	4	8	175-197
STM5148			(GAA)17	5	15**	397-481
STM2028			TAC5.. (TA)3... (CAT)3	12	7**	289-410
STI028			(CAA)imp	11	8**	165-204

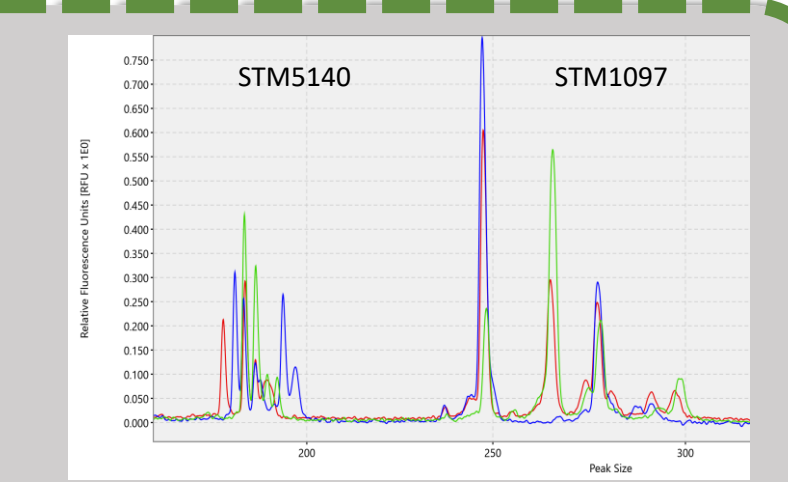
Tab. 1: Characteristics of the SSR markers used in the procedure.

- Fully operational
- Not used by all partners
- Not operational

* references of the markers : SSR1 (Kawchuk *et al.* 1996), STM2005 (Milbourne *et al.* 1998; Maras *et al.* 2017); LEMALX, STM1097 and STM2020 (Milbourne *et al.* 1998), STGBSS (Provan *et al.* 1996), STM5136, STM2028, STM5148 (Reid and Kerr, 2007), STM5140 (Ghislain *et al.* 2004), STI028 (Feingold *et al.* 2005).
** data acquisition in progress

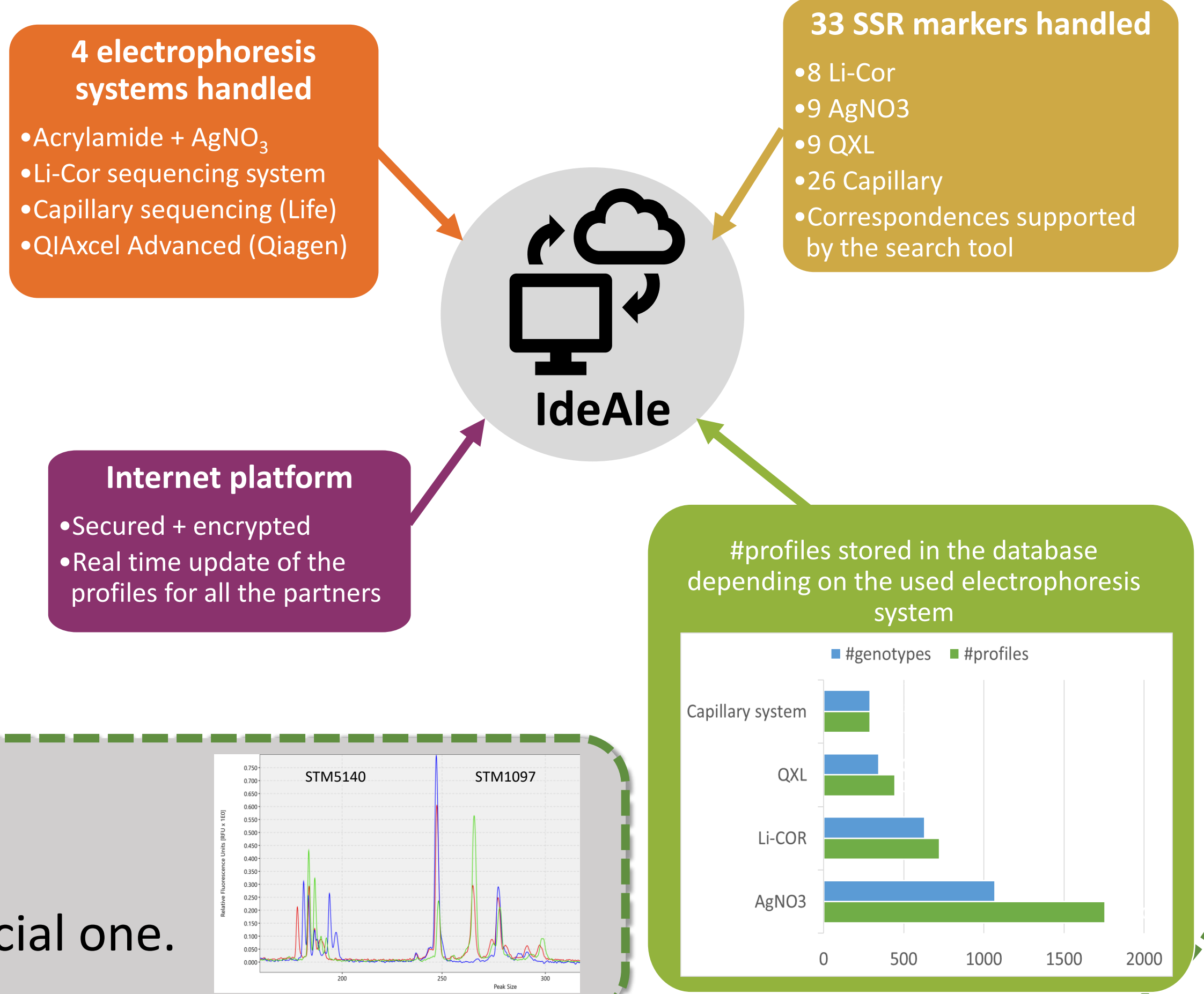
Prospects

- Markers multiplexing in progress.
- New procedure about to replace the official one.



A common database

IdeAle central database, supported and maintained by FN3PT, was built during a former project supported by the French Ministry of Agriculture.



Addresses

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References

Marhadour, S., C. Dargier, F. Esnault, N. Laversin, A. Méar, M. Perramant and Y. Le Hingrat (2014). "Construction d'une base de données multi-utilisateurs pour améliorer la gestion des empreintes génétiques des variétés de pomme de terre produites en plants en France." *Innovations agronomiques* **35**: 161-172.



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