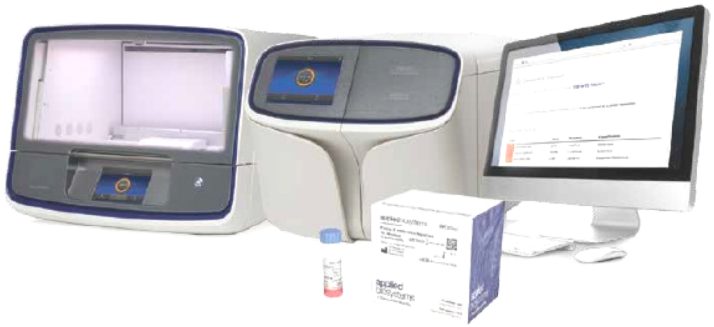
Automation of the Precision ID

NGS System for routine use



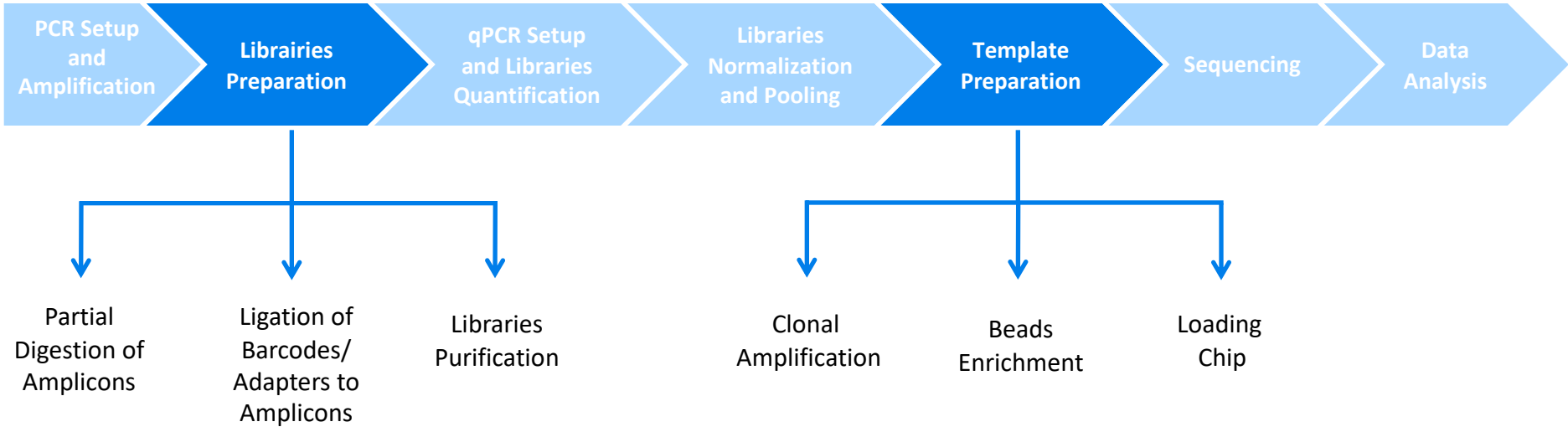
Collaboration and Aim

- Collaboration

- Aim

Develop a fully automated procedure for SNP, STR and Sequencing analyses with the Precision ID NGS System and the Ion Torrent Technology from Thermo Fisher Scientific, and with the MicroLab STAR Line from Hamilton

Workflow


PCR Setup and Amplification
Librairies Preparation
qPCR Setup and Libraries Quantification
Libraries Normalization and Pooling
Template Preparation
Sequencing
Data Analysis
Partial Digestion of Amplicons
Ligation of Barcodes/ Adapters to Amplicons
Libraries Purification
Clonal Amplification
Beads Enrichment
Loading Chip

Semi-automated procedure

PCR Setup and Amplification → Librairies Preparation → qPCR Setup and Libraries Quantification → Libraries Normalization and Pooling → Template Preparation → Sequencing → Data Analysis

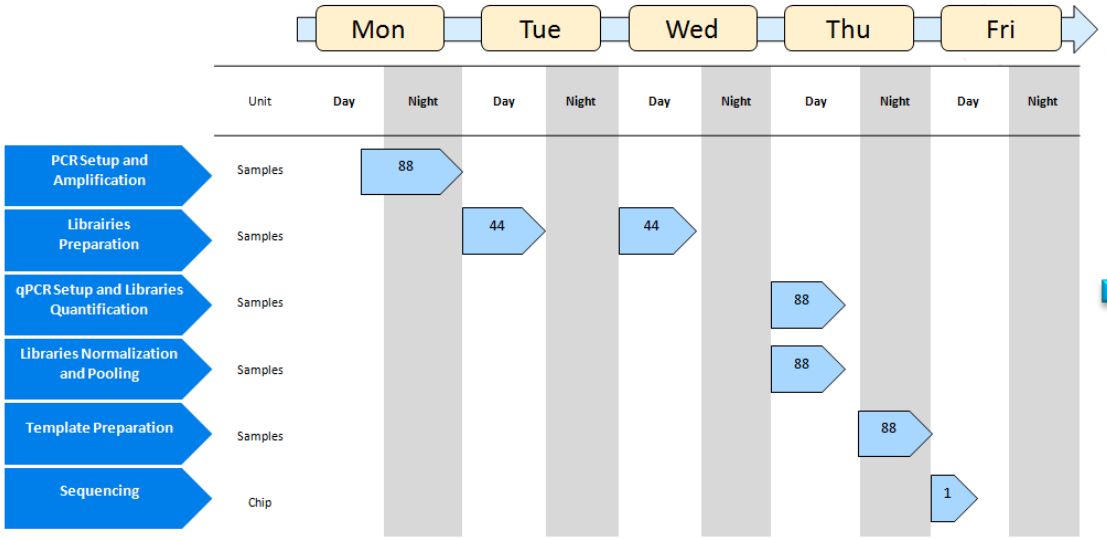
Manual (PCR setup) + Manual + Manual (PCR setup) + Manual | Automatic | Automatic | Automatic







Duration of semi-automated procedure

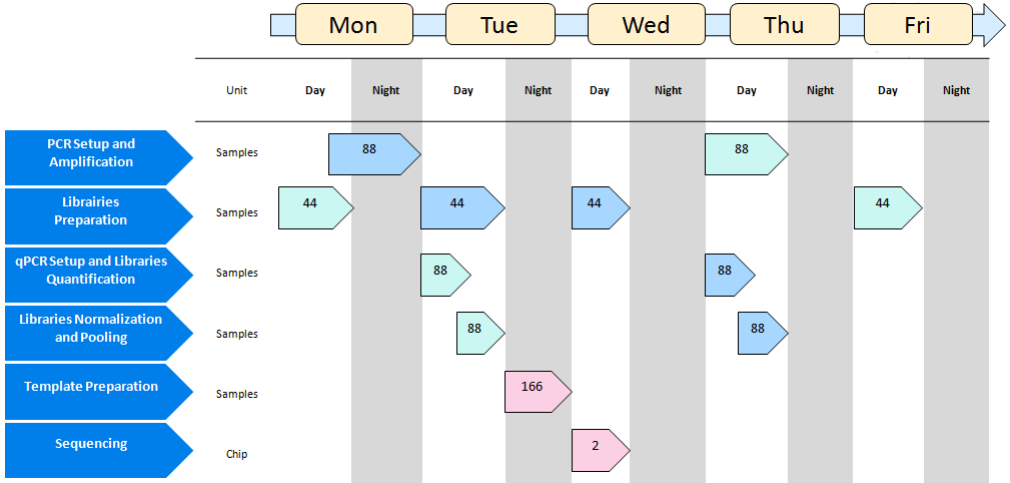


4 days to analyse 88 samples

over a 5 days period

7 days to analyse 166 samples

over a 8 days period



Semi-automated procedure

PCR Setup and Amplification → **Librairies Preparation** → qPCR Setup and Libraries Quantification → Libraries Normalization and Pooling → Template Preparation → Sequencing → Data Analysis

Many manual steps, no traceability, time-consuming and error risk

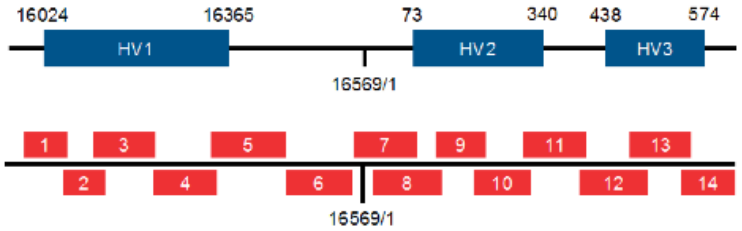
AUTOMATION

Precision ID panels

- SNP Panels
  - Precision ID Identity Panel, 1 amplification per sample ⇨ 1 library per sample
  - Precision ID Ancestry Panel, 1 amplification per sample ⇨ 1 library per sample

- STR Panel
  - Precision ID GlobalFiler™ NGS STR Panel v2, 1 amplification per sample ⇨ 1 library per sample

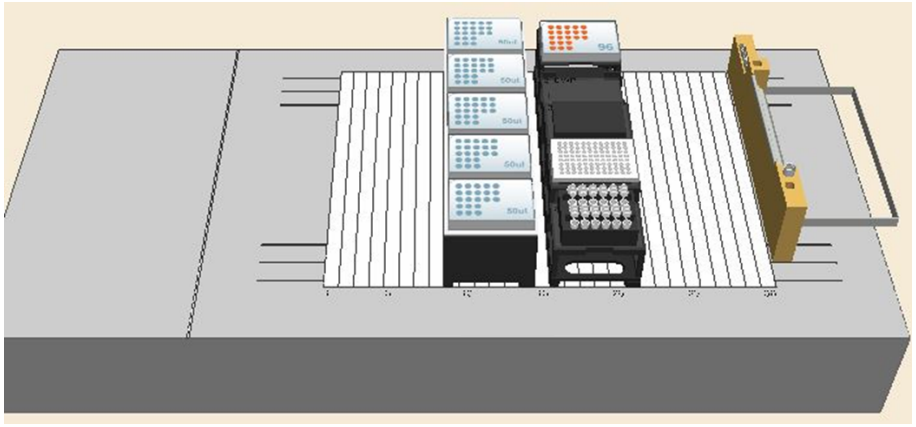
- Sequencing Panel (HV1/2/3 regions of mtDNA)
  - Precision ID mtDNA Control Region Panel, 2 amplifications per sample ⇨ 1 or 2 libraries per sample
  - 3 analyses methods:
    - Full : 2 amplifications per sample (20µl) ⇨ 2 libraries per sample
    - 2in1 : 2 amplifications per sample (20µl), pooling of 10µl of each amplification ⇨ 1 library per sample
    - Conservative : 2 amplifications per sample (10µl), pooling of these 2 amplifications ⇨ 1 library per sample



Automation pre-PCR

PCR Setup and Amplification → Librairies Preparation → qPCR Setup and Libraries Quantification → Libraries Normalization and Pooling → Template Preparation → Sequencing → Data Analysis

- 3 Analyses (SNP, STR, Seq)→ 1 program
- Program
  - PCR mix dispensing
  - Samples dispensing
- 2 plate maps



SNP, STR

From 1 to 88 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	/
B	2	10	18	26	34	42	50	58	66	74	82	/
C	3	11	19	27	35	43	51	59	67	75	83	/
D	4	12	20	28	36	44	52	60	68	76	84	/
E	5	13	21	29	37	45	53	61	69	77	85	/
F	6	14	22	30	38	46	54	62	70	78	86	/
G	7	15	23	31	39	47	55	63	71	79	87	/
H	8	16	24	32	40	48	56	64	72	80	88	/

mtDNA (3 methods)

From 1 to 48 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	1	9	17	25	33	41
B	2	10	18	26	34	42	2	10	18	26	34	42
C	3	11	19	27	35	43	3	11	19	27	35	43
D	4	12	20	28	36	44	4	12	20	28	36	44
E	5	13	21	29	37	45	5	13	21	29	37	45
F	6	14	22	30	38	46	6	14	22	30	38	46
G	7	15	23	31	39	47	7	15	23	31	39	47
H	8	16	24	32	40	48	8	16	24	32	40	48

Amplification 1 | Amplification 2

From 49 to 88 samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	/
B	2	10	18	26	34	42	50	58	66	74	82	/
C	3	11	19	27	35	43	51	59	67	75	83	/
D	4	12	20	28	36	44	52	60	68	76	84	/
E	5	13	21	29	37	45	53	61	69	77	85	/
F	6	14	22	30	38	46	54	62	70	78	86	/
G	7	15	23	31	39	47	55	63	71	79	87	/
H	8	16	24	32	40	48	56	64	72	80	88	/

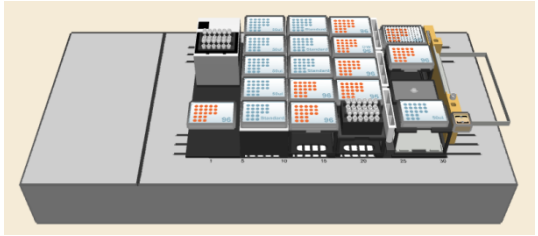
Amplification 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9	17	25	33	41	49	57	65	73	81	/
B	2	10	18	26	34	42	50	58	66	74	82	/
C	3	11	19	27	35	43	51	59	67	75	83	/
D	4	12	20	28	36	44	52	60	68	76	84	/
E	5	13	21	29	37	45	53	61	69	77	85	/
F	6	14	22	30	38	46	54	62	70	78	86	/
G	7	15	23	31	39	47	55	63	71	79	87	/
H	8	16	24	32	40	48	56	64	72	80	88	/

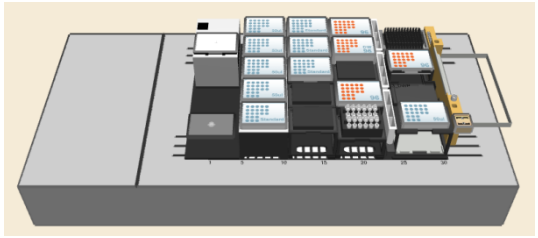
Amplification 2

Automation post-PCR

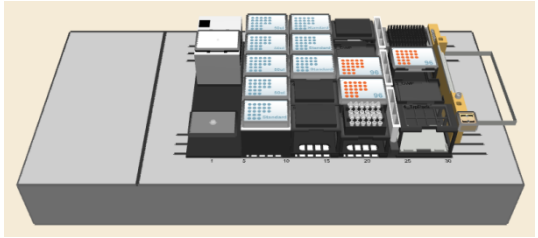
PCR Setup and Amplification → Librairies Preparation → qPCR Setup and Libraries Quantification → Libraries Normalization and Pooling → Template Preparation → Sequencing → Data Analysis



Libraries Preparation



Libraries qPCR setup



Libraries Normalization & Pooling

Automation post-PCR

- mtDNA and SNP libraries can be prepared together but STR libraries must be prepared separately

- 4 steps :
  1. Pooling of amplicons (mtDNA CR panel)
  2. Partial digestion of amplicons
  3. Ligation of barcodes/adapters to amplicons
  4. Libraries purification

- 2 worklists generate manually or by a LIMS
  1. Samples worklist (.csv file)
  2. Barcode/adapter worklist (.csv file)

Samples worklist

SampleID	SampleBarcode	SamplePositionID	SampleComment
370110302124	PLT_1	A1	P16-06566
TN00016084	PLT_1	B1	P16-06566
TP00012741	PLT_1	C1	P16-06566
370110302125	PLT_1	D1	P16-06566
370110302126	PLT_1	E1	P16-06566
370110302127	PLT_1	F1	P16-06566
370110302128	PLT_1	G1	P16-06566
370110302129	PLT_1	H1	P16-06566
370110302130	PLT_1	A2	P16-06566
370110302131	PLT_1	B2	P16-06566
370110302132	PLT_1	C2	P16-06566
370110302133	PLT_1	D2	P16-06566
370110302134	PLT_1	E2	P16-06566

Adapter/barcode worklist

AdapterBarcode	AdapterPositionID
IonXpress_001	A1
IonXpress_002	B1
IonXpress_003	C1
IonXpress_004	D1
IonXpress_005	E1
IonXpress_006	F1
IonXpress_007	G1
IonXpress_008	H1
IonXpress_009	A2
IonXpress_010	B2
IonXpress_011	C2
IonXpress_012	D2
IonXpress_013	E2

Automation post-PCR

PCR Setup and Amplification → Librairies Preparation → **qPCR Setup and Libraries Quantification** → Libraries Normalization and Pooling → Template Preparation → Sequencing → Data Analysis

- 3 steps :
  1. Libraries and standard dilution
  2. PCR mix dispensing
  3. Libraries and standard dispensing
- 1 samples worklist (.csv file)
- Samples file (.txt) for 7500 <u>generate automatically by the program</u>

Samples worklist

SampleID	SampleBarcode	SamplePositionID	SampleComment	AdapterBarcode	AdapterPositionID
370110302124	PLT_1	A1	P16-06566	IonXpress_002	B1
TN00016084	PLT_1	B1	P16-06566	IonXpress_003	C1
TP00012741	PLT_1	C1	P16-06566	IonXpress_004	D1
370110302125	PLT_1	D1	P16-06566	IonXpress_005	E1
370110302126	PLT_1	E1	P16-06566	IonXpress_006	F1
370110302127	PLT_1	F1	P16-06566	IonXpress_007	G1
370110302128	PLT_1	G1	P16-06566	IonXpress_008	H1
370110302129	PLT_1	H1	P16-06566	IonXpress_009	A2
370110302130	PLT_1	A2	P16-06566	IonXpress_010	B2
370110302131	PLT_1	B2	P16-06566	IonXpress_011	C2
370110302132	PLT_1	C2	P16-06566	IonXpress_012	D2
370110302133	PLT_1	D2	P16-06566	IonXpress_013	E2
370110302134	PLT_1	E2	P16-06566	IonXpress_014	F2

Samples file for 7500

```
* Block Type = 96alum
* Chemistry = TAQMAN
* Experiment File Name = E:\QlibFrJ171116.eds
* Experiment Run End Time = 2017-11-16 16:51:19 PM CET
* Instrument Type = sds7500
* Passive Reference = ROX

[Sample Setup]
```

Well	Sample Name	Sample Color	Biogroup Name	Biogroup Color	Target Name	Target Color	Task	Reporter	Quencher	Quantity	Comments
A1	STD-1	RGB(132,193,241)			Q_Library	RGB(139,189,249)	STANDARD	FAM	NFQ-MGB	6.8	
A2	370110302124	RGB(132,193,241)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A3	370110302130	RGB(96,255,160)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A4	370110302138	RGB(213,244,165)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A5	370110302146	RGB(253,138,88)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A6	370110302154	RGB(255,204,153)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A7	370110302162	RGB(180,255,0)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A8	370110302170	RGB(247,255,168)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A9	370110302178	RGB(223,221,142)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A10	370110302186	RGB(168,255,222)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A11	370110305001	RGB(132,193,241)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		
A12	TAMPpos	RGB(96,255,160)			Q_Library	RGB(139,189,249)	UNKNOWN	FAM	NFQ-MGB		

Automation post-PCR

PCR Setup and Amplification → Librairies Preparation → qPCR Setup and Libraries Quantification → **Libraries Normalization and Pooling** → Template Preparation → Sequencing → Data Analysis

- 3 steps :
  1. Data file import from 7500 and automatic dilution factor determination for each library
  2. Libraries dilution
  3. Libraries pooling

Data file from 7500

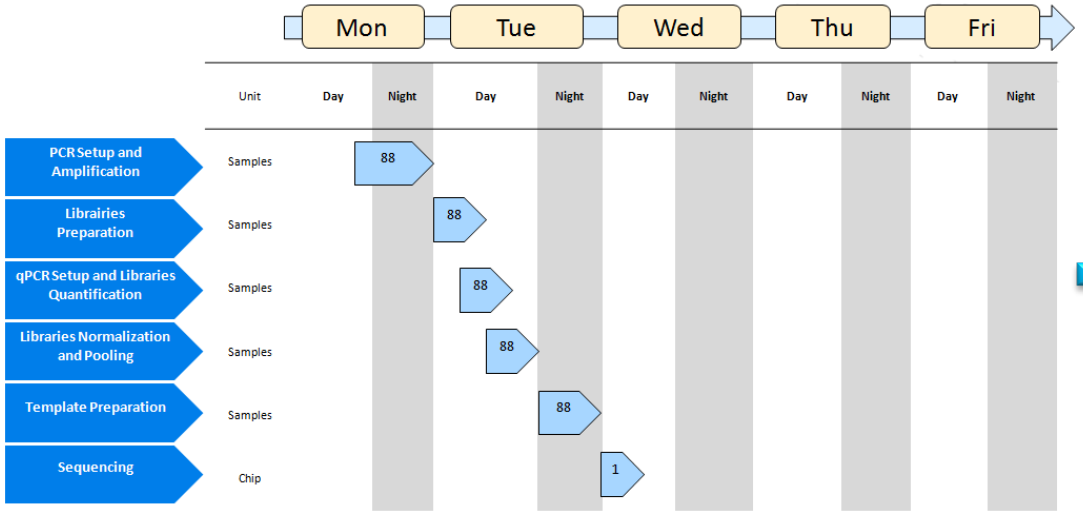
```
* Block Type = 96alum
* Chemistry = TAQMAN
* Experiment File Name = C:\Applied Biosystems\7500\experiments\QlibFrJ171116.eds
* Experiment Run End Time = 2017-11-16 16:51:19 PM CET
* Instrument Type = sds7500
* Passive Reference = ROX

[Results]
```

Well	Sample Name	Target Name	Task	Reporter	Quencher	CN,	CN, Mean	CN, SD	Quantity	Quantity Mean	Quantity SD	Automatic Ct Threshold	Ct Threshold	Automatic Baseline	Baseline Start	Baseline End	Comments	NOISE	SPIKE	EXPFAIL	CTFAIL
A1	STD-1	Q_Library	STANDARD	FAM	NFQ-MGB	12.8041	12.7845	0.0277	6.8			false	0.2	true	3	8		N	N	N	N
A2	370110302124	Q_Library	UNKNOWN	FAM	NFQ-MGB	19.376	19.376		0.0952	0.0952		false	0.2	true	3	14		N	N	N	N
A3	370110302130	Q_Library	UNKNOWN	FAM	NFQ-MGB	14.1767	14.1767		2.725	2.725		false	0.2	true	3	9		N	N	N	N
A4	370110302138	Q_Library	UNKNOWN	FAM	NFQ-MGB	12.9825	12.9825		5.8878	5.8878		false	0.2	true	3	8		N	N	N	N
A5	370110302146	Q_Library	UNKNOWN	FAM	NFQ-MGB	13.8894	13.8894		3.2799	3.2799		false	0.2	true	3	9		N	N	N	N
A6	370110302154	Q_Library	UNKNOWN	FAM	NFQ-MGB	15.3726	15.3726		1.2598	1.2598		false	0.2	true	3	10		N	N	N	N
A7	370110302162	Q_Library	UNKNOWN	FAM	NFQ-MGB	13.6714	13.6714		3.7751	3.7751		false	0.2	true	3	9		N	N	N	N
A8	370110302170	Q_Library	UNKNOWN	FAM	NFQ-MGB	14.08	14.08		2.9003	2.9003		false	0.2	true	3	9		N	N	N	N
A9	370110302178	Q_Library	UNKNOWN	FAM	NFQ-MGB	14.6439	14.6439		2.0159	2.0159		false	0.2	true	3	10		N	N	N	N
A10	370110302186	Q_Library	UNKNOWN	FAM	NFQ-MGB	15.1232	15.1232		1.4797	1.4797		false	0.2	true	3	10		N	N	N	N
A11	370110305001	Q_Library	UNKNOWN	FAM	NFQ-MGB	18.35	18.35		0.1846	0.1846		false	0.2	true	3	13		N	N	N	N
A12	TAMPpos	Q_Library	UNKNOWN	FAM	NFQ-MGB	13.0954	13.0954		5.4742	5.4742		false	0.2	true	3	8		N	N	N	N

- Pool of libraries ready for the run template (pooling of different pools is possible but make sure that there are no two different samples with the same barcode)

Duration of automated procedure

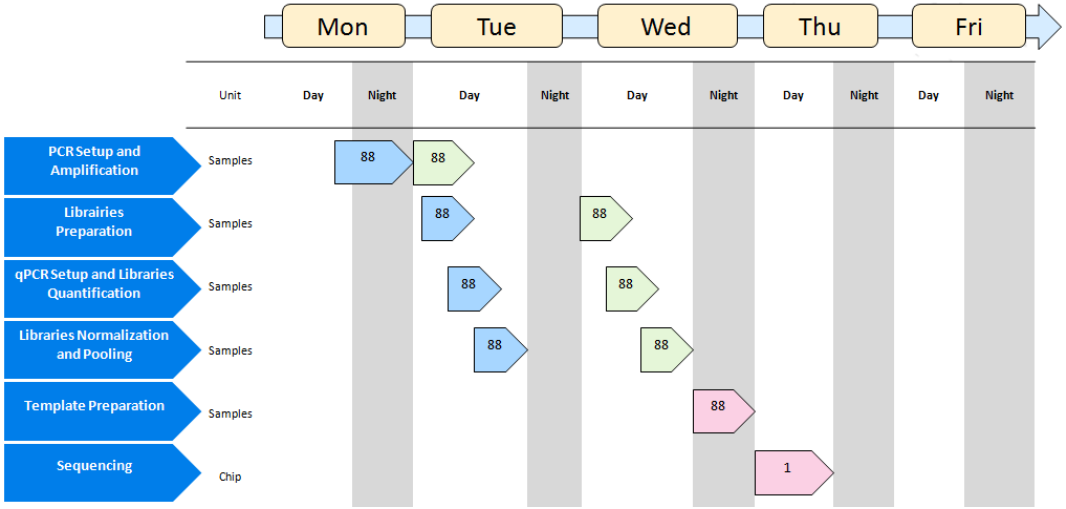


2 days to analyse 88 samples

over a 3 days period (vs 4 days over a 5 days period)

3.5 days to analyse 166 samples

over a 4 days period (vs 7 days over a 8 days period)



Automated procedure test

- 429 hair analysed in mtDNA (Precision ID mtDNA Control Region panel)
  - 200 hair analysed with the semi-automated precedure
  - 229 hair analysed with the automated precedure
- Lysis and DNA extraction with the Crime PrepAdem kit from Ademetch
- mtDNA quantification by an inhouse method
- mtDNA normalization to 50 mtDNA copies/µl
- All positive samples in mtDNA (QmtDNA ≥ 2.5 copies/µl) have been analysed in MPS with the conservative method (7.5 to 150 mtDNA copies/amplification and 26 PCR cycles)
- Templates preparation ⇨ Ion Chef, and Sequencing ⇨ Ion S5
- Sequencing data analysis ⇨ mtDNA plugin

Results

	First Analysis	Second Analysis	Total
Procedure	Semi-automatic	Automatic	/
Number of samples	200	229	429
Chip size used for sequencing	520	530	/
Number of samples per chip	48	80	/
Mean number of reads	85 326	113 904	/
minimum reads	44 783	36 476	/
maximum reads	130 323	184 016	/
Sequencing quality (≥Q20)	91%	90%	91%
Mean rate of loading chip	70%	73%	72%
Mean rate of polyclonal	33%	35%	34%
Mean rate of aligned bases	96%	95%	96%
Mean raw accuracy	98,5%	98,5%	98,5%
Number of positive samples in mtDNA quantification	189/200 (95%)	210/229 (92%)	399/429 (93%)
Number of positive samples in sequencing	**189/200 (95%)**	**208/229 (91%)**	**397/429 (93%)**

Conclusion

- Semi-automated procedure and automated procedure : same performance
- Automation onto Hamilton STARlet significantly reduces the preparation time of libraries with a full traceability and without error risk
- Only one automated procedure for SNP, STR and Sequencing analyses
- Programs flexibility (from 1 to 88 libraries prepared at the same time)
- Very high success rate with the semi-automated procedure and the automated procedure by using the Precision ID mtDNA Control Region panel and the Ion Torrent technology, higher than with the conventional procedure
- Validation of the automated procedure in progress for SNP, STR and Sequencing analyses

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