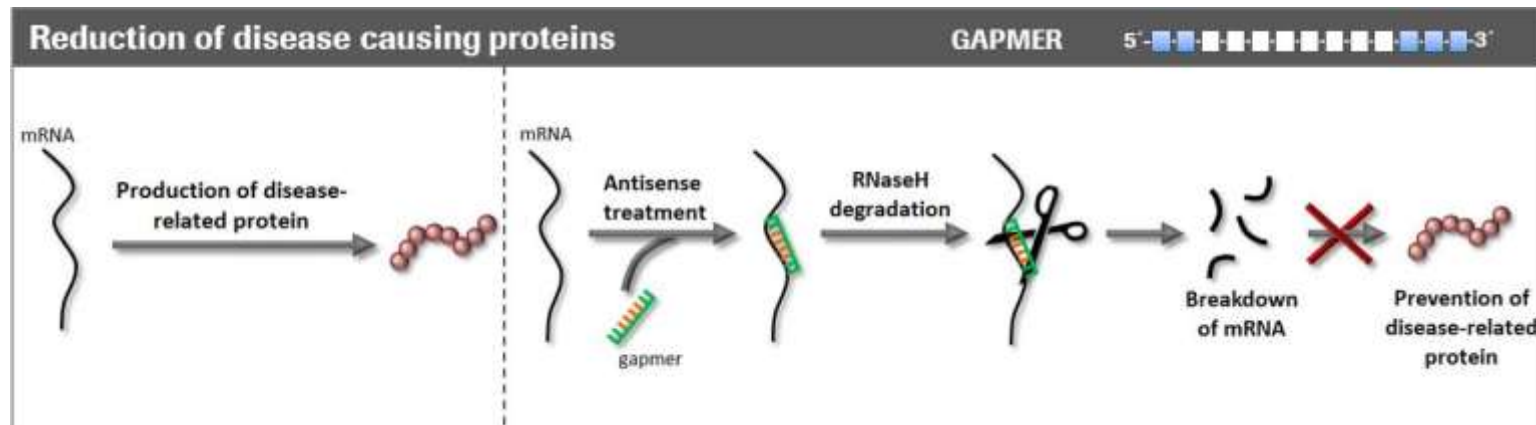

HELM in Action: Complex Oligonucleotides
Pistoia Conference, London, 13-Mar-2019

Bernhard Schirm, Matteo Cassotti, Dennis Hansen

RMR Details



- 2014: Roche acquires Santaris Pharma A/S to introduce a new modality: RMR
- RMR designs, synthesizes and screens chemically-modified SSOs exploiting the LNA platform
- LNA-modified oligonucleotides target RNA molecules to downregulate translation to proteins or modify protein sequences by splice-switching

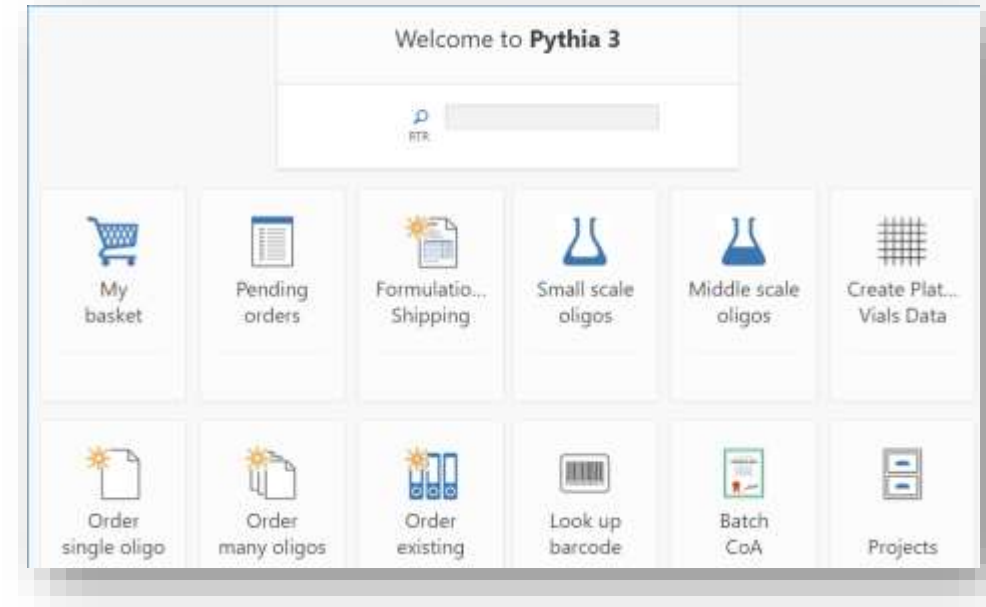


Example of mechanism of RNA degradation via gapmers

Pythia

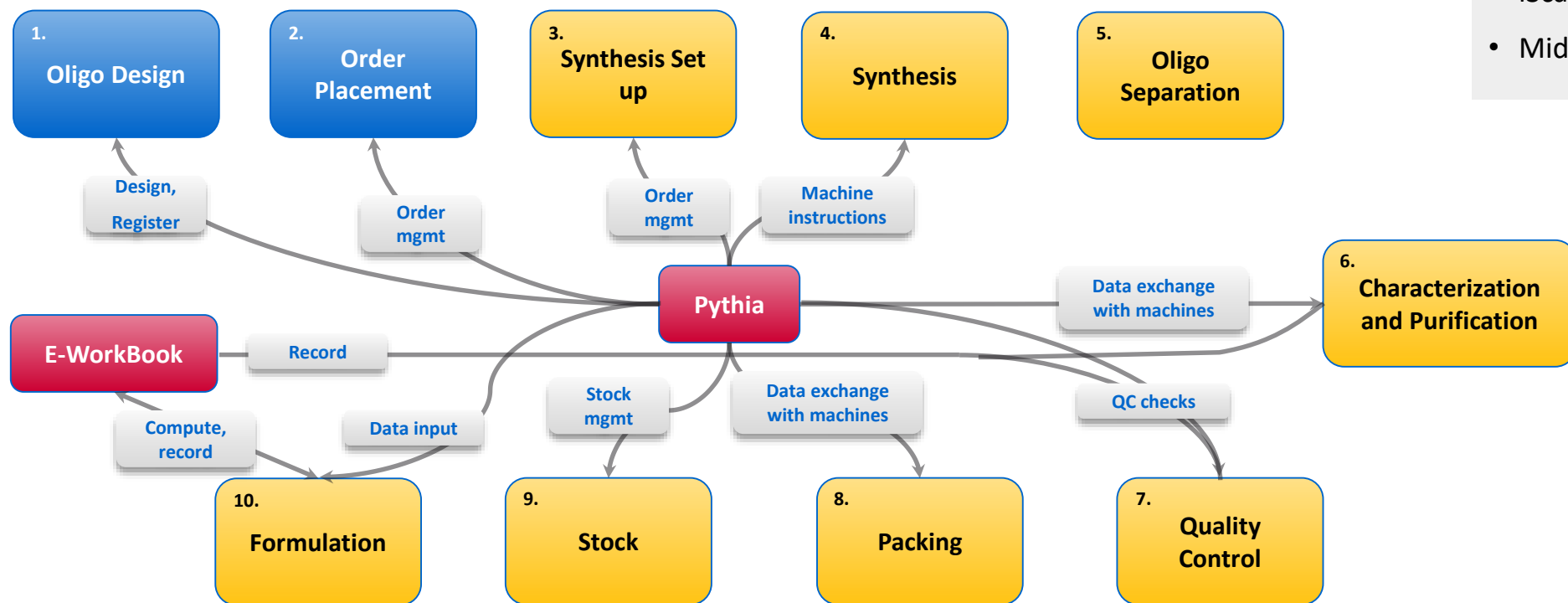
Pythia is the central software system for:

- Registration of oligonucleotides, batches and containers
- Storage of calculated properties (MW, Ext. coef.)
- Synthesis workflow:
 - data exchange with machines
 - calculations of volumes for dilutions, concentrations, in-out of specifications
- Formulation and shipment workflow:
 - check against stock
 - dependencies with synthesis orders
 - generation of documents for shipment
- Planning of activities:
 - Gantt charts with synthesis, formulation and shipment orders
 - Additional activities (eg. planned maintenance of machines)



Lab workflows

High level workflows



Synthesis Scales

The chemical synthesis at RICC is divided into three different scales:

- Small scale (250-1000 μ M)
- iScale (10-100 mg dry powder)
- Middle scale (mg to g dry powder)

HELM Centricity

Pythia

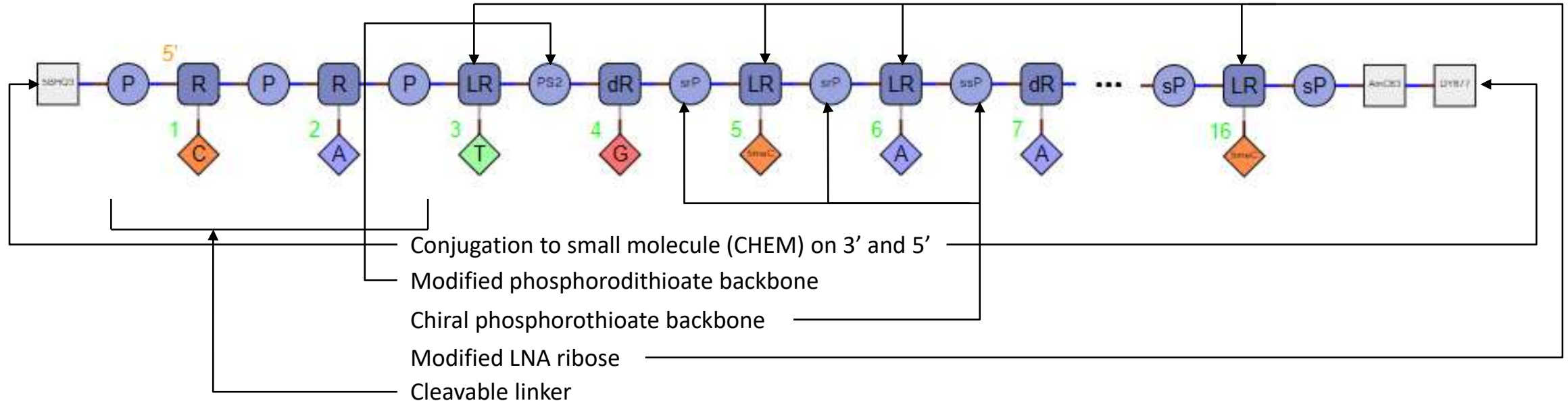
- Central software system in RICC for oligonucleotide management
- In production since 2015 in its current form
- 2018: full support of HELM 2.0

Everything in Pythia is now HELM-based:

- Ordering of oligos
- Calculation of properties
- Machine instructions
- Generation of sequences for bioinformatics predictions and mapping to genome (separate application - Mímir)

Pythia

Complex oligonucleotides



CHEM1 { [5BHQ3] } |

RNA1 { P . R (C) P . R (A) P . [LR] (T) [PS2] . [dR] (G) [srP] . [LR] ([5meC]) [srP] . [LR] (A) [ssP] . [dR] (A) . . .
 [sP] . [LR] ([5meC]) [sP] . [LR] ([5meC]) [sP] } |

CHEM2 { [AmC63] } |

CHEM3 { [DY677] }

\$CHEM1, RNA1, 1 : R2 - 1 : R1 | RNA1, CHEM2, 49 : R2 - 1 : R1 | CHEM2, CHEM3, 1 : R2 - 1 : R1 \$\$\$V2.0

Registration of monomers and oligonucleotides

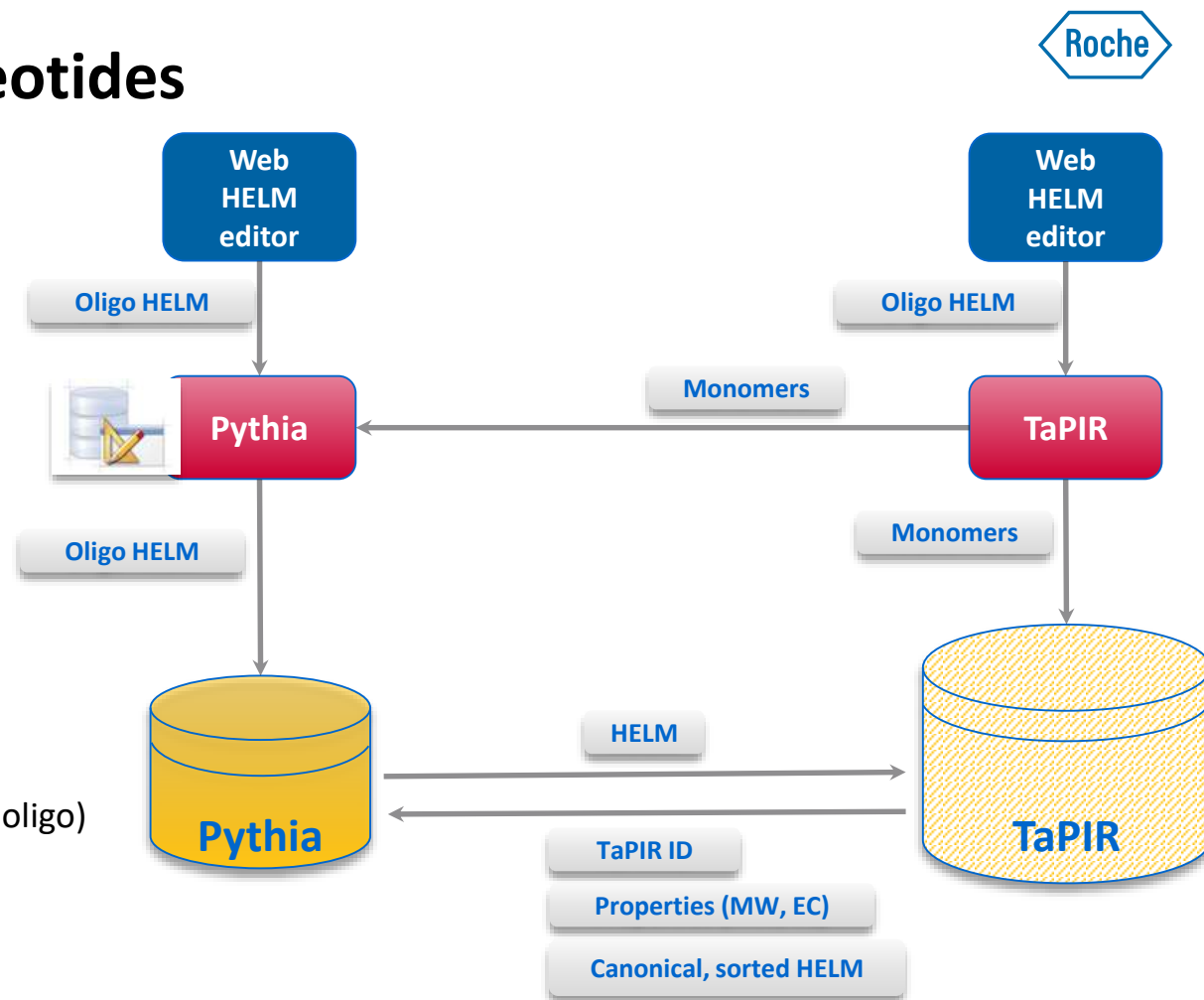
Connections and data flow

Monomers

- Registered in TaPIR UI
- Sync'ed to Pythia with molfile for Pythia's web HELM editor

Oligonucleotides

- TaPIR:
 - HELM entered (or built) in TaPIR Web HELM editor
- Pythia:
 - HELM entered (or built) in Pythia's Web HELM editor (order single oligo)
 - HELM created manually or from sequences via Shiny app from Oligoinformatics and entered in order many oligos¹ or csv upload²
 - HELM strings sent to TaPIR for:
 - Validation
 - Uniqueness check
 - Registration
 - Property calculations (MW, Na-salt MW, Ext. Coef., canonical and sorted HELMs) via micro-services
 - Oligo and batch registration is asynchronous, but concept TaPIR ID sent immediately to Pythia



¹supports up to 100-200 oligos

² Supports up to 2000-3000 oligos

Pythia screenshots

Oligo details page

Calculated properties, ID and link to Oligoinformatics application (Mimir)

Structure and sequences
(using Pistoia's JavaScript HELM Editor)

Batches

Oligo Information for TapirID RTR147661

Calculated Molar Weight	1609.94 g/mol	TapIR ID	RTR147661
No-Salt MW	1741.83 g/mol	SRN	
Calculated Extinction Coefficient	50.4 mM ⁻¹ cm ⁻¹	Alias	
Length	5'	Conjugated	no

[Order this Oligo](#)
[Look up in Mimir](#)
[Update from TapIR](#)

Basic Info

Designed by	Matteo Cassotti
Created at	2019-03-05
Oligo ID	125871
Is partner?	no (Roche)

Structure

[Mol File](#)
[ID File](#)
[vHELM](#)

HELM: `RNA1(RIA)PR(U)PR(C)PR(G)PR(U)P)SSSSV2.0`

Natural Analogue Seq: 5'-AUGGU-3'

Chemical Formula: $C_{27}H_{49}N_5O_{11}P_5$

Legacy Values

HELM Sketch

Batches

Q Go Actions

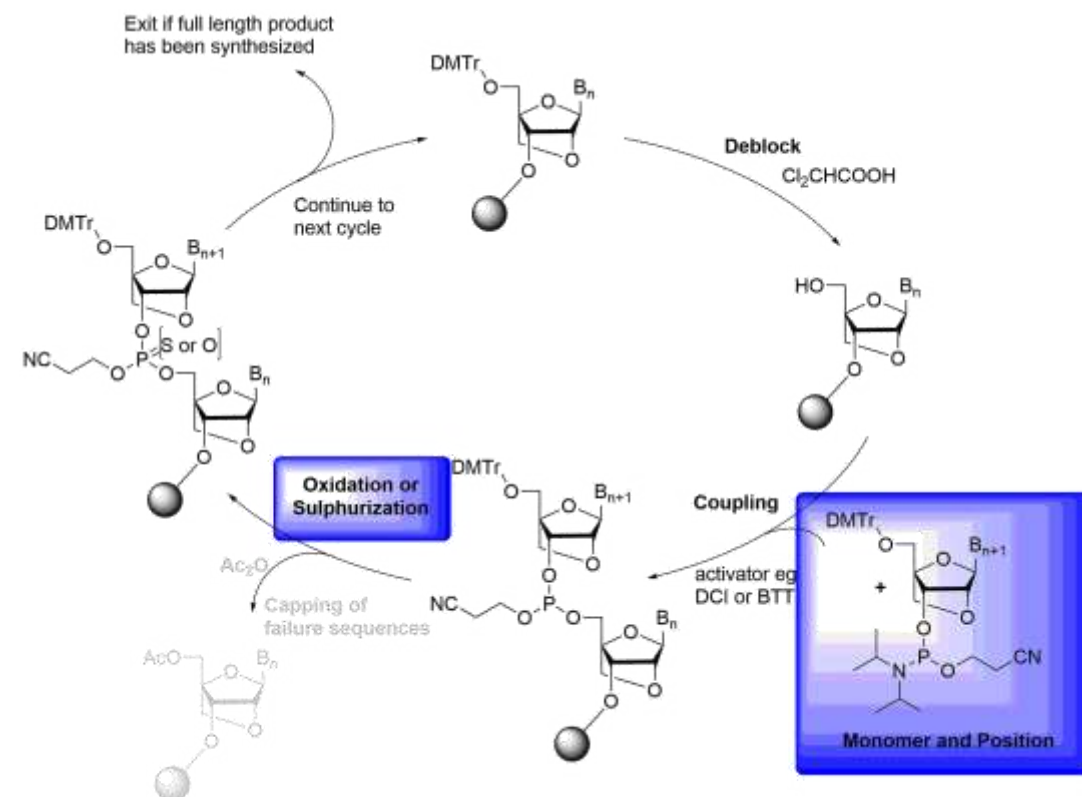
Tapir ID	Batch (Legacy Name)	Username	Project	Amount	Stock (mg)	Status	Identity check	Target
RTR147661-001	RTR147661-001	Matteo Cassotti	maskintest	5	-	Pending	-	
RTR147661-003	RTR147661-003	Matteo Cassotti	maskintest	5	-	Cancelled	-	

1 - 2 of 2

Machine instructions

- Combination of monomers represented via HELM are assigned a specific representation for the synthesizer machine.
- Machine representation corresponds to a position on the instrument containing the reagents for a specific step/cycle of the synthesis

Final HELM	Representation	Ambiguous ...	HELM 1	HELM 2	HELM 3	CHEM (in co
[Ge]NAC_C3]P	(M10)			[Ge]NAC_C3]	P	
[HEG][sP]	(M2)*			[HEG]	sp	
[HEG]P	(M2)			[HEG]	P	
[LR][A][PS2]	(M2)*		LR	A	PS2	
[LR][A][ssP]	(M2)*		LR	A	ssP	
[LR][A][srP]	(M3)*		LR	A	srP	
[LR][A]	(A)		LR	A		
[LR][A][sP]	(A)*		LR	A	sP	
[LR][A]P	(A)		LR	A	P	
[LR][G][PS2]	(M13)*		LR	G	PS2	
[LR][G][ssP]	(M14)*		LR	G	ssP	
[LR][G][srP]	(M13)*		LR	G	srP	

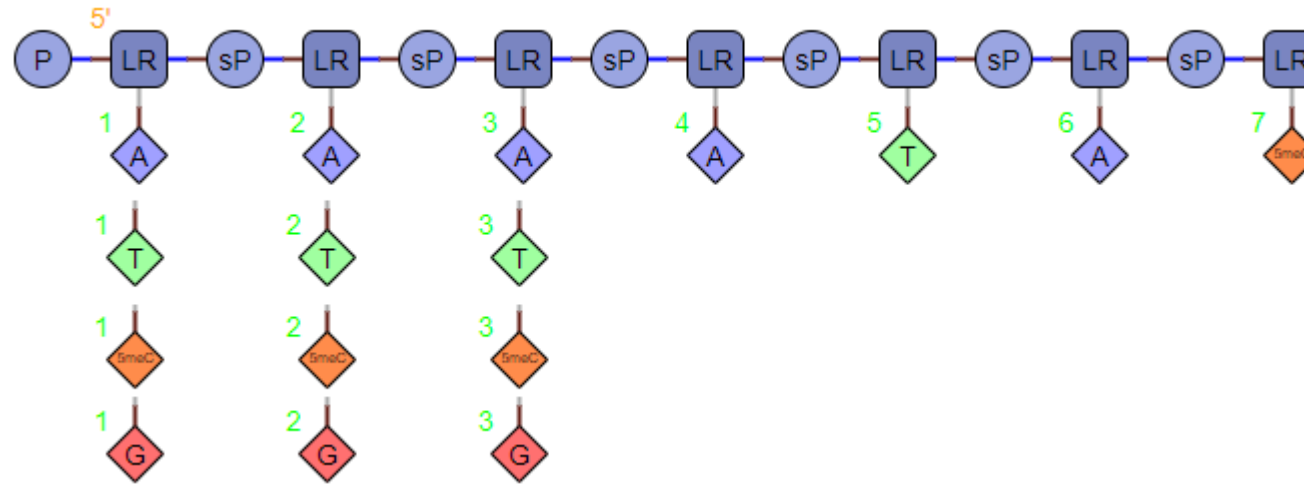


Ambiguous oligonucleotides

- Ambiguous oligos contain a mixture of different monomers at given positions (it can be a mixture of nucleobases, sugars, backbones or a combination of them)
- In order to make it simpler for users and avoid several different HELM strings for the same ambiguous oligo, we use substitutions

Ambiguous Monomers														
<input type="text" value="Search: All Text Columns"/> <input type="button" value="Go"/> <input type="button" value="Actions"/> <input type="button" value="Edit"/> <input type="button" value="Save"/> <input type="button" value="Add Row"/> <input type="button" value="Reset"/>														
<input type="checkbox"/>		Id	Name	Nucleobase	Helm	Display name	Available	Comments	Modifications	Created by	Created on	Updated by	Updated on	Ambig substitut...
<input checked="" type="checkbox"/>		485	[Ld-R](A)P	A	[Ld-R](A)P	[A [^] {DNA/LNA}]	1	1:1 mixture of LR ...	3	PYTHIA	2018-06-18	fichtner	2017-11-14	(({LR})(A)P+[dR](A)P)
<input type="checkbox"/>		800	[LR](N)P	-	[LR](N)P	N [^] {o}	1	1:1:1:1 mixture of...	-	PYTHIA	2018-06-18	fichtner	2017-11-14	(({LR})(A)P+[LR](T)...
<input type="checkbox"/>		801	[LR](N)[sP]	-	[LR](N)[sP]	N [^] {o}	1	1:1:1:1 mixture of...	-	PYTHIA	2018-06-18	gerwien	2017-11-30	(({LR})(A)[sP]+[LR](...
<input type="checkbox"/>		802	[LR](R)[sP]	-	[LR](R)[sP]	R [^] {o}	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	gerwien	2017-11-30	(({LR})(A)[sP]+[LR](...
<input type="checkbox"/>		803	[LR](Y)[sP]	-	[LR](Y)[sP]	Y [^] {o}	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	gerwien	2017-11-30	(({LR})(T)[sP]+[LR](...
<input type="checkbox"/>		804	[dR](N)[sP]	-	[dR](N)[sP]	n	1	1:1:1:1 mixture of...	-	PYTHIA	2018-06-18	gerwien	2017-11-30	(({dR})(A)[sP]+[dR](...
<input type="checkbox"/>		805	[dR](R)[sP]	-	[dR](R)[sP]	r	1	1:1 mixture of dR...	-	PYTHIA	2018-06-18	fichtner	2017-11-30	(({dR})(A)[sP]+[dR](...
<input type="checkbox"/>		806	[dR](Y)[sP]	-	[dR](Y)[sP]	y	1	1:1 mixture of dR...	-	PYTHIA	2018-06-18	gerwien	2017-11-30	(({dR})(T)[sP]+[dR](...
<input type="checkbox"/>		809	[Ld-R](A)[sP]	A	[Ld-R](A)[sP]	[A [^] {DNA/LNA}]	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	gerwien	2017-12-01	(({LR})(A)[sP]+[dR](...
<input type="checkbox"/>		810	[Ld-R](G)[sP]	G	[Ld-R](G)[sP]	[G [^] {DNA/LNA}]	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	gerwien	2017-12-01	(({LR})(G)[sP]+[dR](...
<input type="checkbox"/>		811	[Ld-R](T)[sP]	T	[Ld-R](T)[sP]	[T [^] {DNA/LNA}]	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	gerwien	2017-12-01	(({dR})(T)[sP]+[LR](...
<input type="checkbox"/>		812	[Ld-R](C)[sP]	C	[Ld-R](C)[sP]	[C [^] {DNA/LNA}]	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	gerwien	2017-12-01	(({LR})([5meC])[sP]...
<input type="checkbox"/>		706	[Ld-R](G)P	G	[Ld-R](G)P	[G [^] {DNA/LNA}]	1	1:1 mixture of LR ...	3	PYTHIA	2018-06-18	fichtner	2017-11-14	(({LR})(G)P+[dR](G)P)
<input type="checkbox"/>		725	[Ld-R](T)P	T	[Ld-R](T)P	[T [^] {DNA/LNA}]	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	fichtner	2017-11-14	(({dR})(T)P+[LR](T)P)
<input type="checkbox"/>		726	[Ld-R](C)P	C	[Ld-R](C)P	[C [^] {DNA/LNA}]	1	1:1 mixture of LR ...	-	PYTHIA	2018-06-18	fichtner	2017-11-14	(({LR})([5meC])P+[...

Ambiguous oligonucleotides



HELM input:

```
RNA1 { [5FAM] P . [LR] (N) [sP] . [LR] (N) [sP] . [LR] (N) [sP] . [LR] (A) [sP] . [LR] (T) [sP] . [LR] (A) [sP] . [LR] ([5meC]) }$$$$V2.0
```

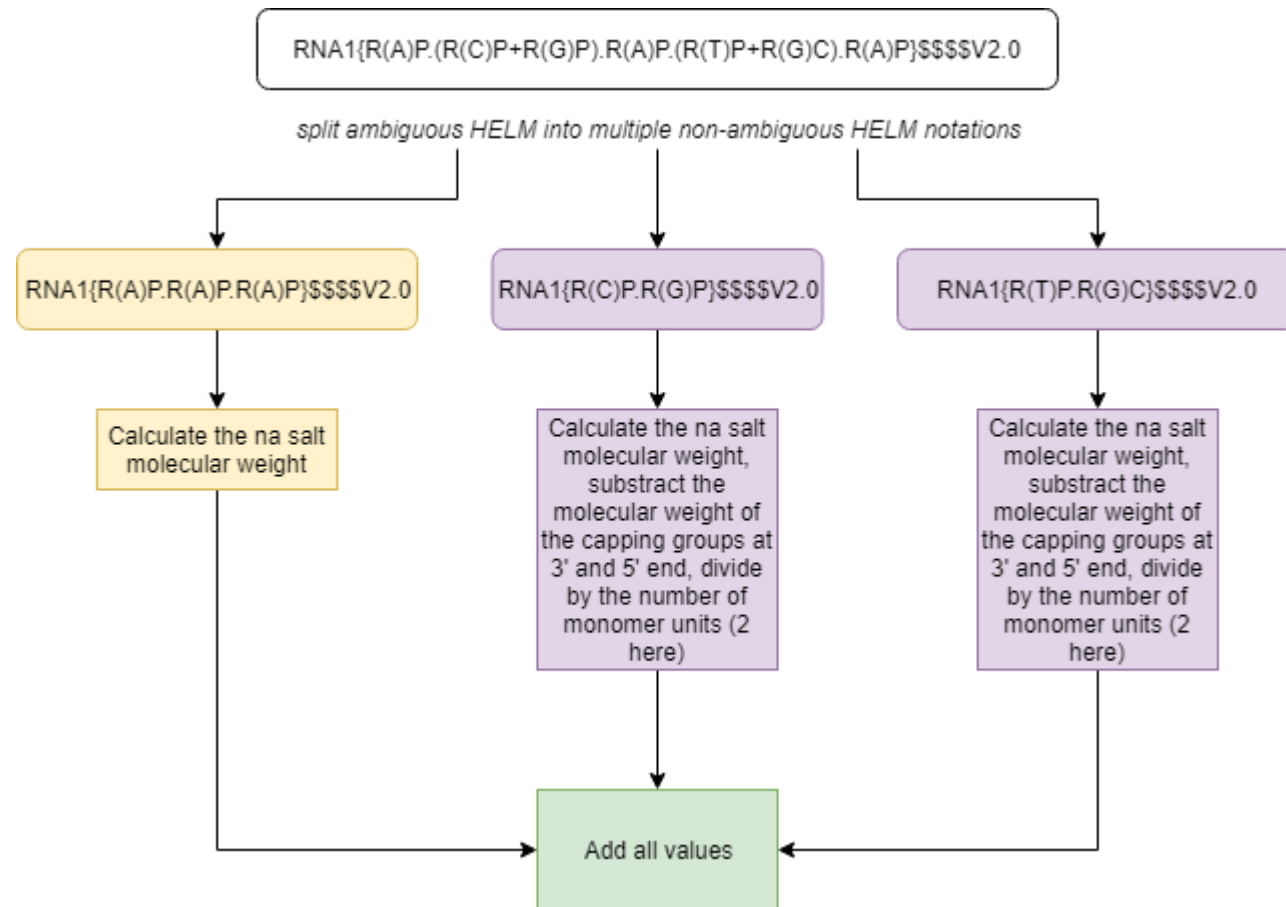
HELM canonical:

```
RNA1 { [5FAM] P .
([LR] (A) [sP] + [LR] (T) [sP] + [LR] ([5meC]) [sP] + [LR] (G) [sP]) .
([LR] (A) [sP] + [LR] (T) [sP] + [LR] ([5meC]) [sP] + [LR] (G) [sP]) .
([LR] (A) [sP] + [LR] (T) [sP] + [LR] ([5meC]) [sP] + [LR] (G) [sP]) .
[LR] (A) [sP] . [LR] (T) [sP] . [LR] (A) [sP] . [LR] ([5meC]) }$$$$V2.0
```

Ambiguous oligonucleotides

Calculation of properties

- Calculate average MW/ext. Coeff. for the ambiguous part of the HELM
- Add to total value



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- Markus Weisser

***Doing now what patients need
next***