Multiplex detection

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SEVENTH FRAMEWORK PROGRAMME

Overview

- Introduction
- Technology
- Results
- Conclusions

Introduction: Platform choice

- Platform technologies used:
  - ELISA
  - TaqMan

Introduction: Platform choice

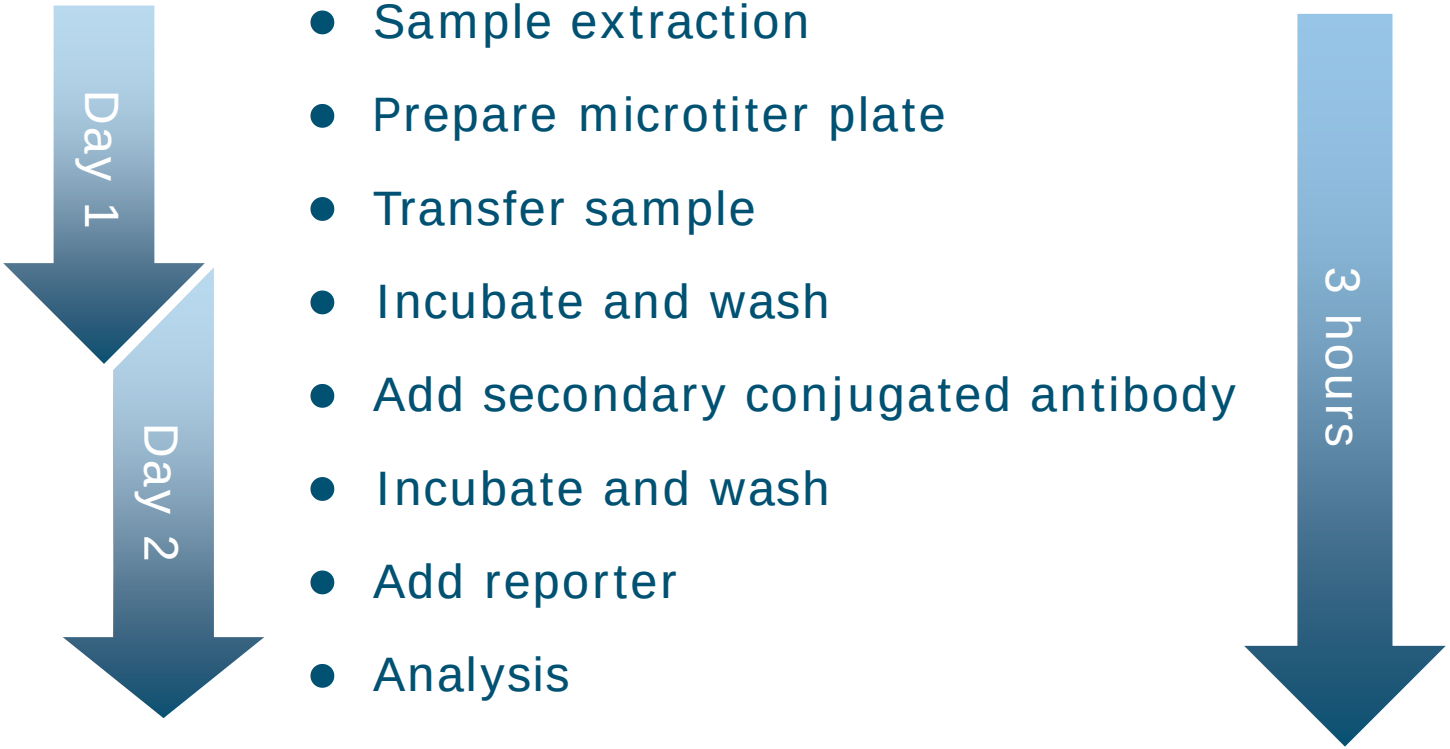
- ELISA: Antibody based
  - AgBio working horse for testing
    - Can ELISA be improved?
      - Increase target range
      - Decrease cost per data point
- DNA/RNA based
  - TaqMan; widely used
    - Can TaqMan be improved?
      - Increase target numbers
      - Decrease cost per data point

Introduction: Platform choice

Target	ELISA/IF	TaqMan
Small molecules		
Protein		
Virus		
Bacteria		
Fungi		

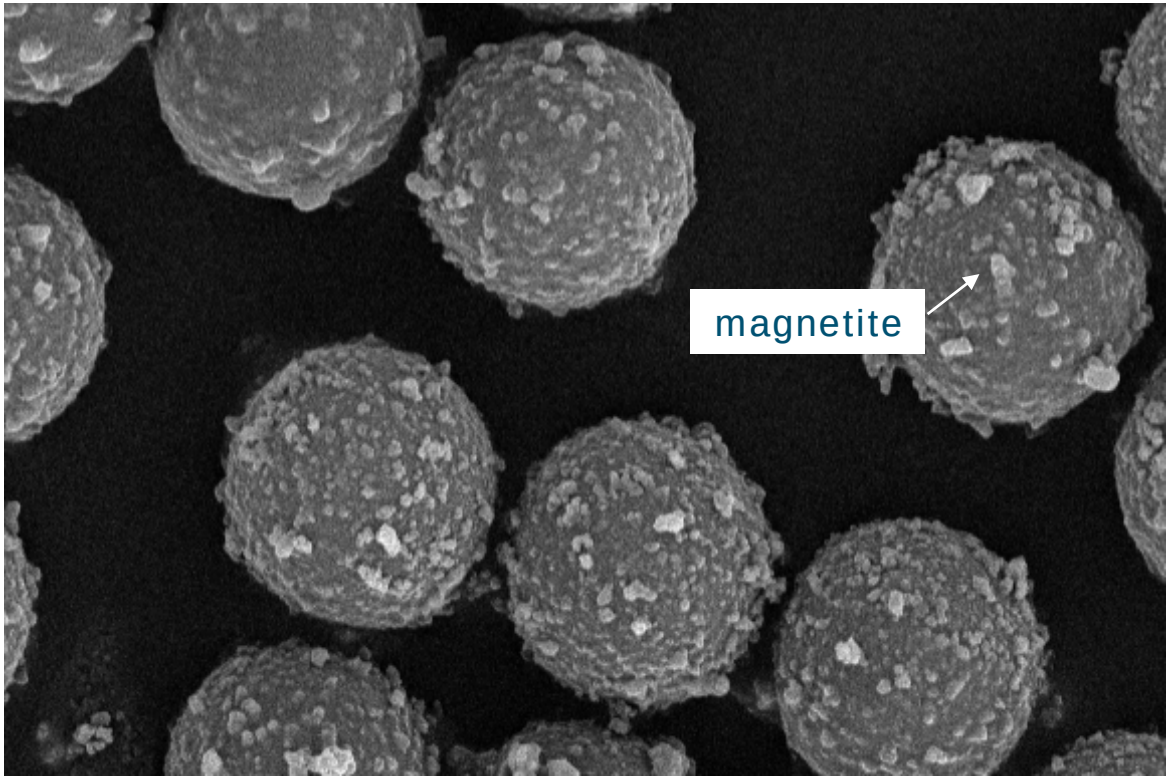
Technology: workflow ELISA and xMAP

- Workflow (single target)



- Sample extraction
- Prepare microtiter plate
- Transfer sample
- Incubate and wash
- Add secondary conjugated antibody
- Incubate and wash
- Add reporter
- Analysis

Technology: MagPlex Microspheres



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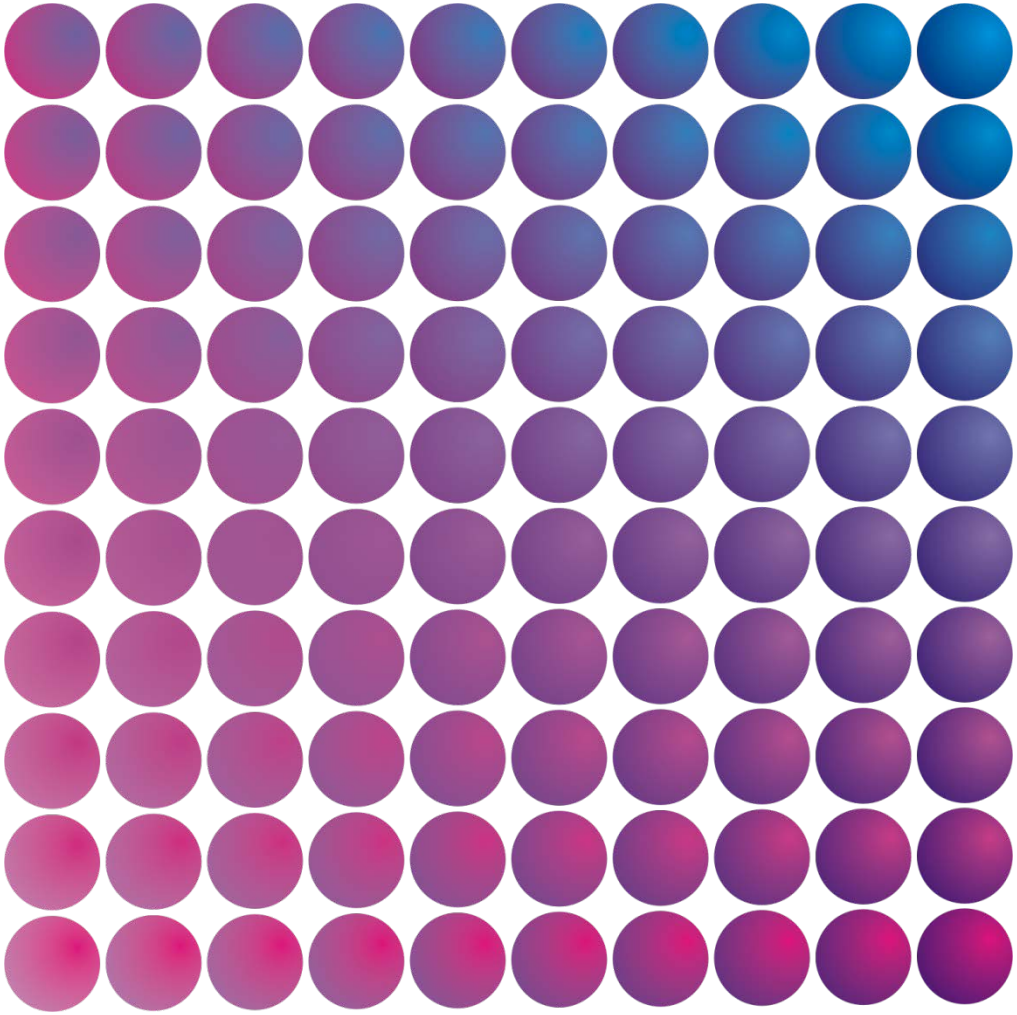
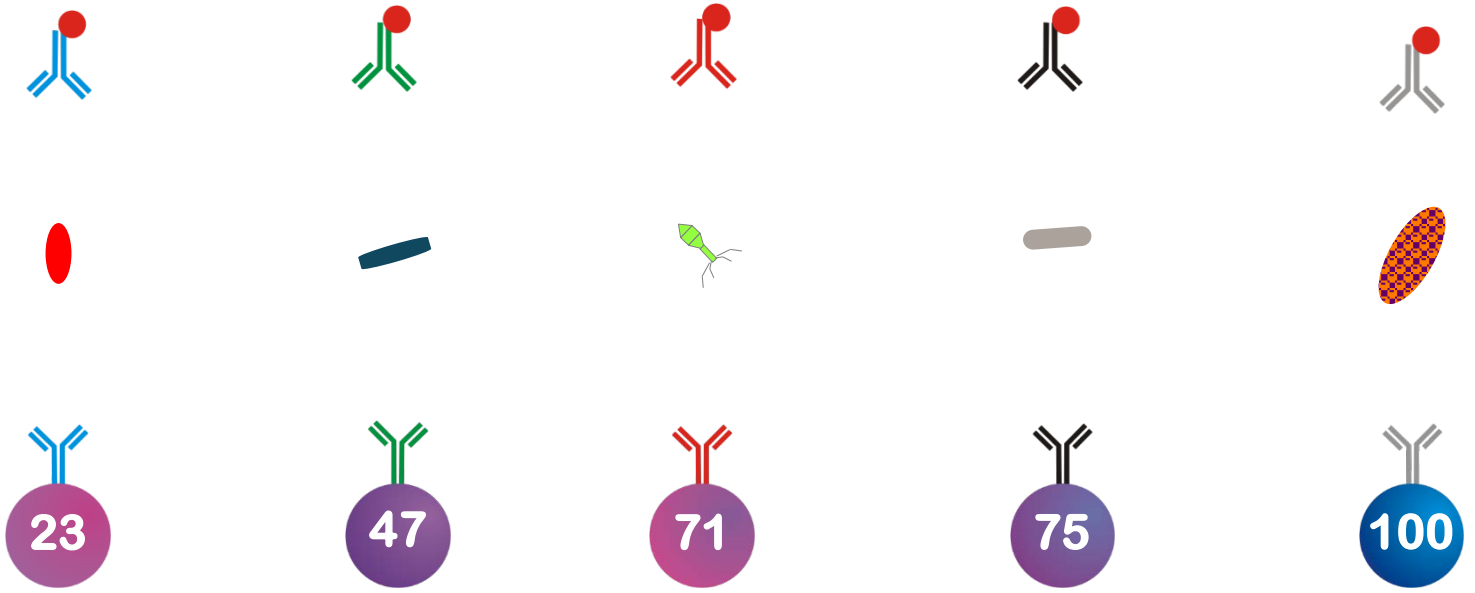
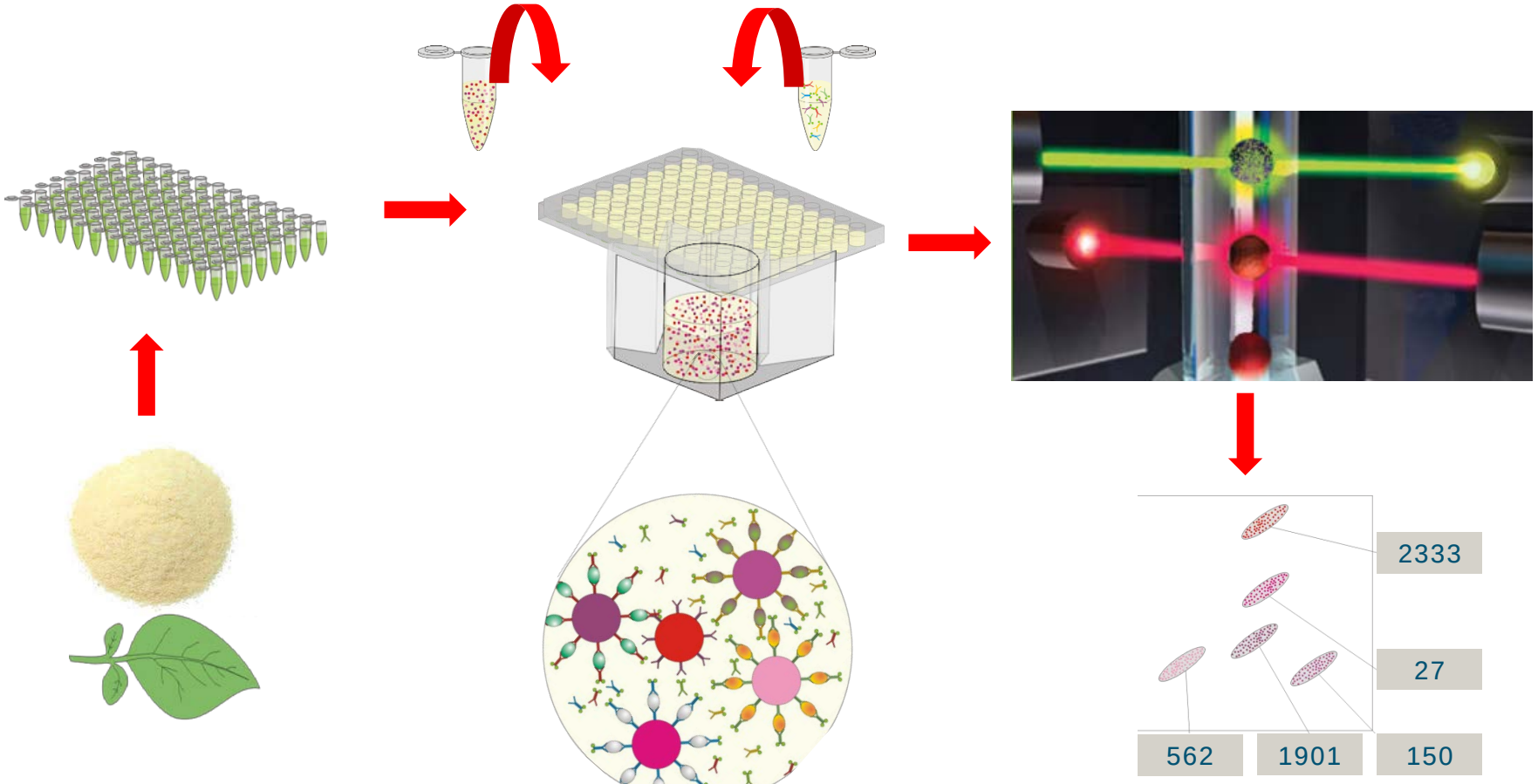
Technology: bead array



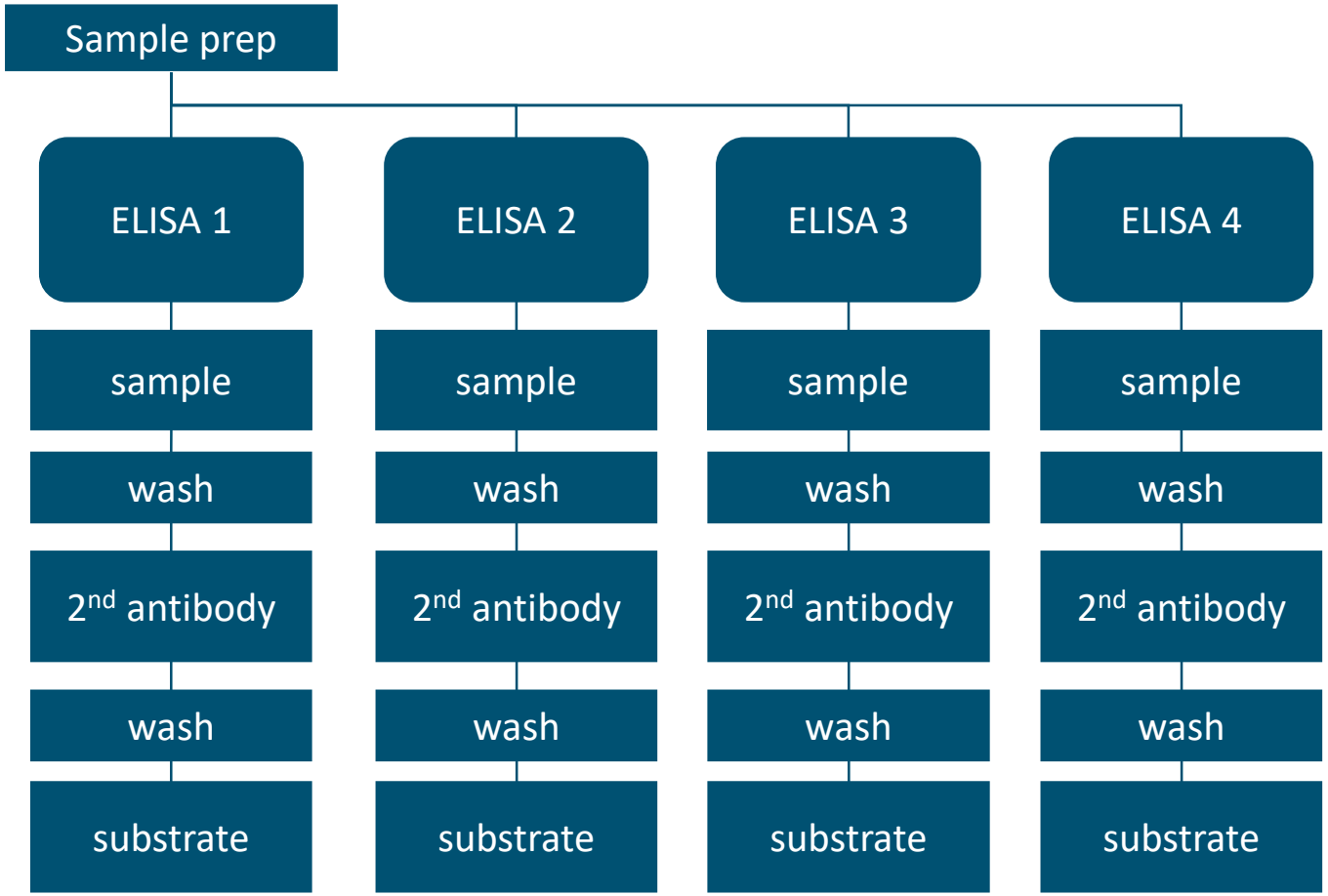
Technology: bead array



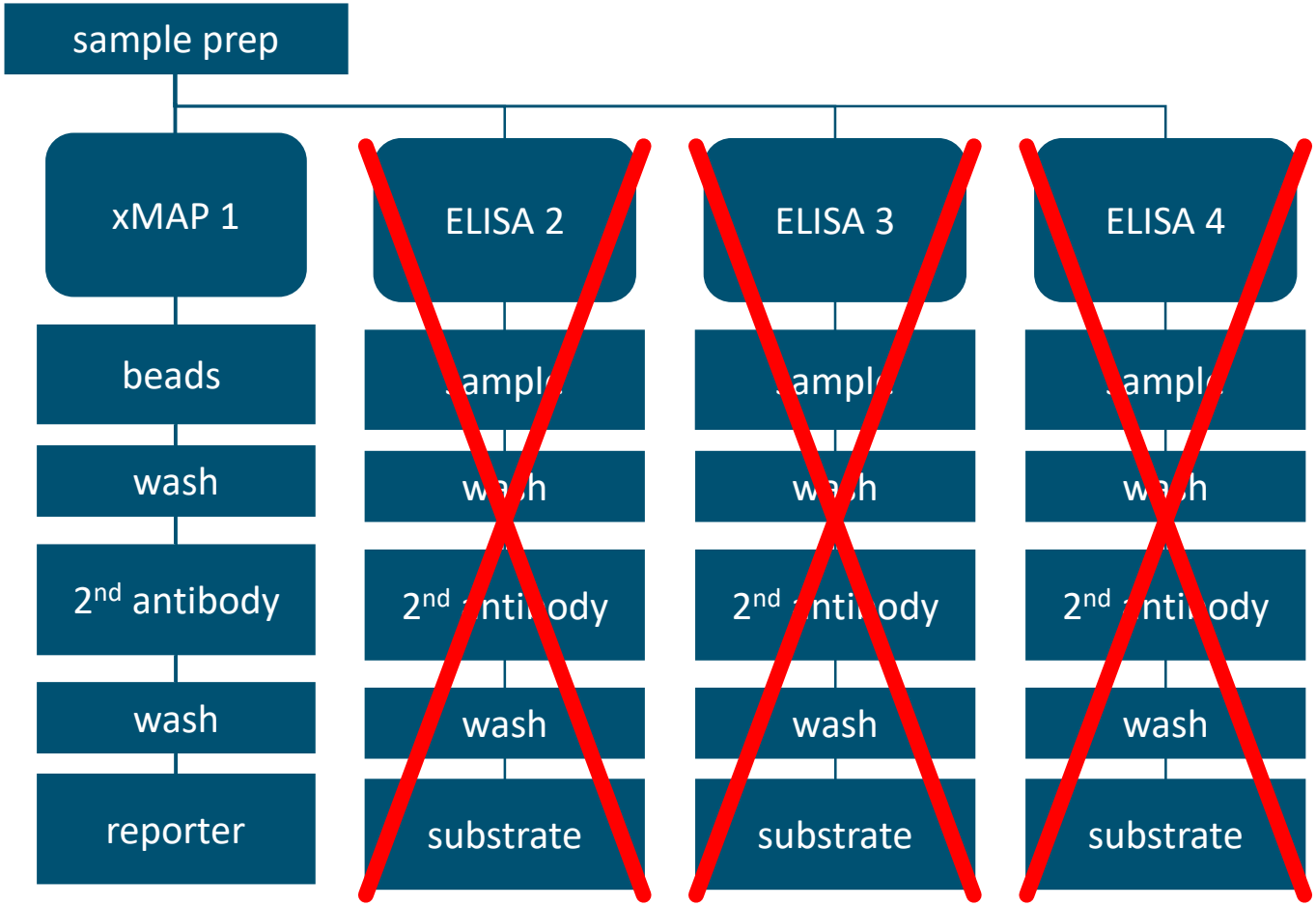
Procedure xMAP



Platform choice



Platform choice



Results xMAP: Carnation 6-plex

Sample	CERV	CNFV	CRSV	CVMV	CarMV	CLV	CIRV
NC	57.0	428.0	46.0	57.1	53.0	67.0	44.0
NC	60.0	518.0	42.5	55.5	42.0	57.0	87.0
CRSV	71.0	3110.0	15845.0	99.0	83.0	6449.0	49.5
CVMV	48.0	2499.5	75.0	4593.0	91.0	11126.0	61.5
CRSV	68.0	3385.0	16910.0	101.0	78.0	6326.0	46.0
CNFV	134.5	3720.0	3356.0	1570.0	11688.0	14405.5	66.0
CNFV	233.0	3770.5	163.5	3657.0	15551.0	22675.0	35.0
CarMV	68.5	6202.5	70.5	87.0	10210.5	10243.5	64.0
NC	51.0	631.0	61.0	49.0	62.5	86.0	50.0
NC	70.0	469.0	56.0	64.5	50.0	72.0	64.0
CRSV	174.0	1335.0	17444.0	112.0	137.0	6666.0	46.0
CVMV	81.0	2245.0	105.0	5543.0	73.0	9829.0	90.0
CRSV	73.0	4211.0	183.0	86.0	59.0	4133.0	53.0
CNFV	114.0	2349.5	117.0	1044.0	14422.0	14868.5	44.0
CNFV	335.0	2599.0	75.0	2036.0	11690.5	1111.0	64.0
CarMV	71.0	6334.0	119.5	104.0	26046.0	11675.0	66.0
CVMV	90.5	4212.0	72.0	2074.0	15037.0	4379.0	40.5
CarMV	116.0	2589.5	101.5	113.0	17662.0	13196.0	53.0

Sample	CERV	CNFV	CRSV	CVMV	CarMV	CLV	CIRV
CLV	74.0	1659.5	10533.5	100.0	17231.0	9296.5	66.0
CLV	126.0	4410.5	11166.0	151.0	16880.0	13187.0	93.0
CVMV	80.0	4602.0	5838.5	4368.0	12318.0	8399.5	54.0
CarMV	48.0	111.0	48.0	52.0	6604.0	59.0	46.0
CERV	3232.0	165.0	57.0	73.0	100.0	62.0	59.0
CLV	73.5	78.5	55.0	72.0	83.0	11105.0	70.0
CVMV	73.0	4651.0	60.5	1913.0	16567.5	16162.5	72.0
CarMV	54.0	1262.0	56.8	87.5	17607.5	14421.0	46.5
CLV	68.0	1369.0	10093.0	86.5	14560.0	14263.5	51.0
CLV	86.0	4104.5	11926.0	106.0	15751.0	9217.0	83.0
CVMV	67.0	6630.5	75.0	5570.0	14442.5	9072.5	76.0
CNFV	38.5	356.8	47.0	78.0	135.5	29.0	80.0
CRSV	53.0	70.0	15667.0	104.0	49.0	56.0	34.0
CVMV	55.0	118.0	99.0	474.5	77.0	82.0	63.0
Gezond	53.5	791.0	51.0	68.0	44.0	65.5	50.5
CIRV	267.5	372.0	224.0	312.0	352.0	281.0	277.0

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Results xMAP: Tomato seed

Sample	TMV	ToMV O3	ToMV D	CMV A	CMV S4	PepMV 1066	PepMV Chili
ToMV	42.5	48.5	81.0	22.0	13.5	6.0	25.0
TMV + ToMV	79.5	69.0	190.0	23.0	16.0	26.0	19.0
TMV + ToMV +PepMV	94.5	75.0	199.5	21.0	20.0	171.0	146.0
TMV + ToMV +PepMV	71.0	75.0	156.0	15.0	21.5	90.0	100.0
TMV + ToMV	108.0	87.5	248.0	4.0	33.5	25.0	20.0
TMV + ToMV + CMV	108.0	85.5	172.0	106.0	91.0	11.0	24.5
Healthy	35.0	29.5	17.0	24.0	15.0	23.5	22.0
Healthy	16.0	17.0	29.5	19.0	21.0	17.5	28.0
Healthy	27.5	31.0	36.0	33.0	52.5	25.5	30.0
Healthy	14.0	30.0	10.0	22.0	54.5	23.0	20.0
Healthy	13.5	15.0	37.0	16.5	41.5	17.0	29.0
Healthy	25.0	22.0	28.0	22.0	25.0	34.5	22.0
Postive control spike	1484.0	850.5	1560.5	95.0	197.5	56.0	51.0
No bead control	NA	NA	NA	NA	NA	NA	NA
Treshold	48.0	45.3	58.4	39.8	85.6	42.6	38.1

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TMV + ToMV +PepMV	94.5	75.0	199.5	21.0	20.0	171.0	146.0
TMV + ToMV +PepMV	71.0	75.0	156.0	15.0	21.5	90.0	100.0
TMV + ToMV	108.0	87.5	248.0	4.0	33.5	25.0	20.0
TMV + ToMV + CMV	108.0	85.5	172.0	106.0	91.0	11.0	24.5
Healthy	35.0	29.5	17.0	24.0	15.0	23.5	22.0
Healthy	16.0	17.0	29.5	19.0	21.0	17.5	28.0
Healthy	27.5	31.0	36.0	33.0	52.5	25.5	30.0
Healthy	14.0	30.0	10.0	22.0	54.5	23.0	20.0
Healthy	13.5	15.0	37.0	16.5	41.5	17.0	29.0
Healthy	25.0	22.0	28.0	22.0	25.0	34.5	22.0
Postive control spike	1484.0	850.5	1560.5	95.0	197.5	56.0	51.0
No bead control	NA	NA	NA	NA	NA	NA	NA
Treshold	48.0	45.3	58.4	39.8	85.6	42.6	38.1

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Summary xMAP

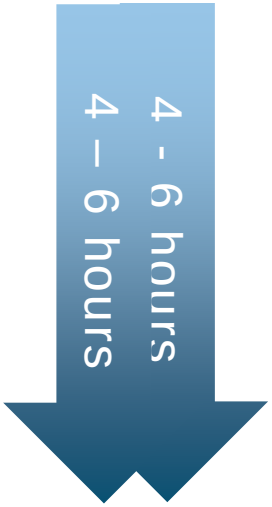
- Comparable work flow as ELISA
- Faster
- Decrease in labour and consumable costs

Introduction: Platform choice

Target	ELISA/IF	TaqMan
Small molecules	✓	✓
Protein	✓	
Virus	✓	✓
Bacteria	✓	✓
Fungi		✓

TaqMan

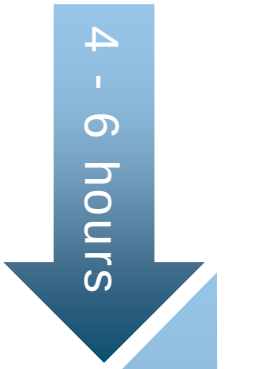
- Workflow 1- 4 targets



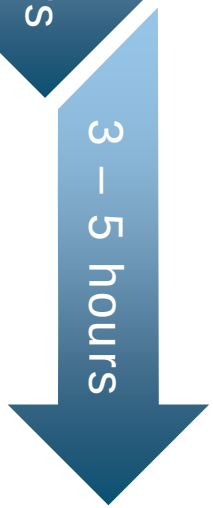
- RNA extraction
- cDNA synthesis
- Add primers and probe(s)
- Run TaqMan
  - 30 – 40 cycli
- Analysis

xTAG technology

- Workflow 2 – 50 targets



- RNA extraction
- cDNA synthesis
- Multiplexed PCR



- Remove excess primers, amplicons and dNTPs
- Use purified amplicons as template for TSPE
- Linear TSPE reactions (incorporation of biotin-dCTP)
- Microsphere hybridization
- Add streptavidin-*R*-phycoerythrin
- Analysis

xTAG technology

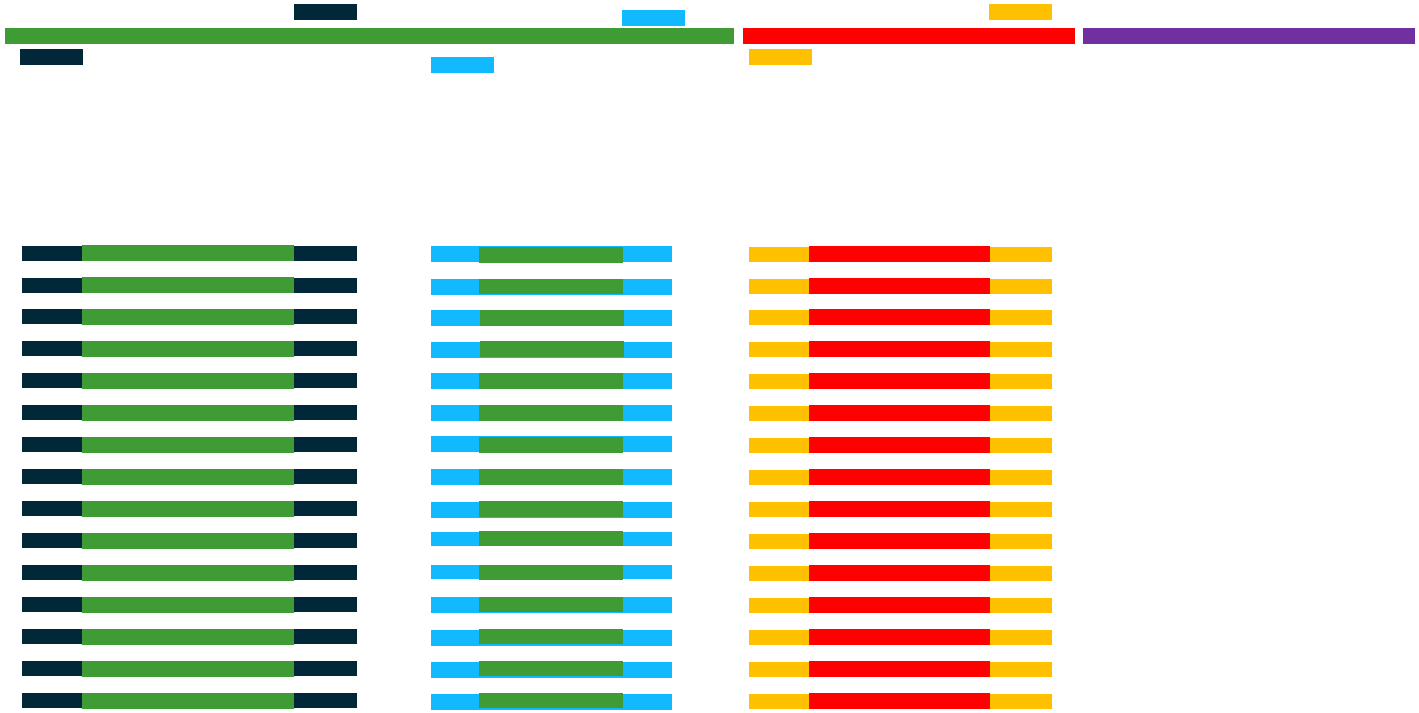
- Design ‘outer’ primers
  - Multiplex amplification
  - Conserved region
- Design TSPE primers
  - Linear amplification
  - Variable region

xTAG technology: Target amplification

Outer primer spacing: 500-2000 nucleotides

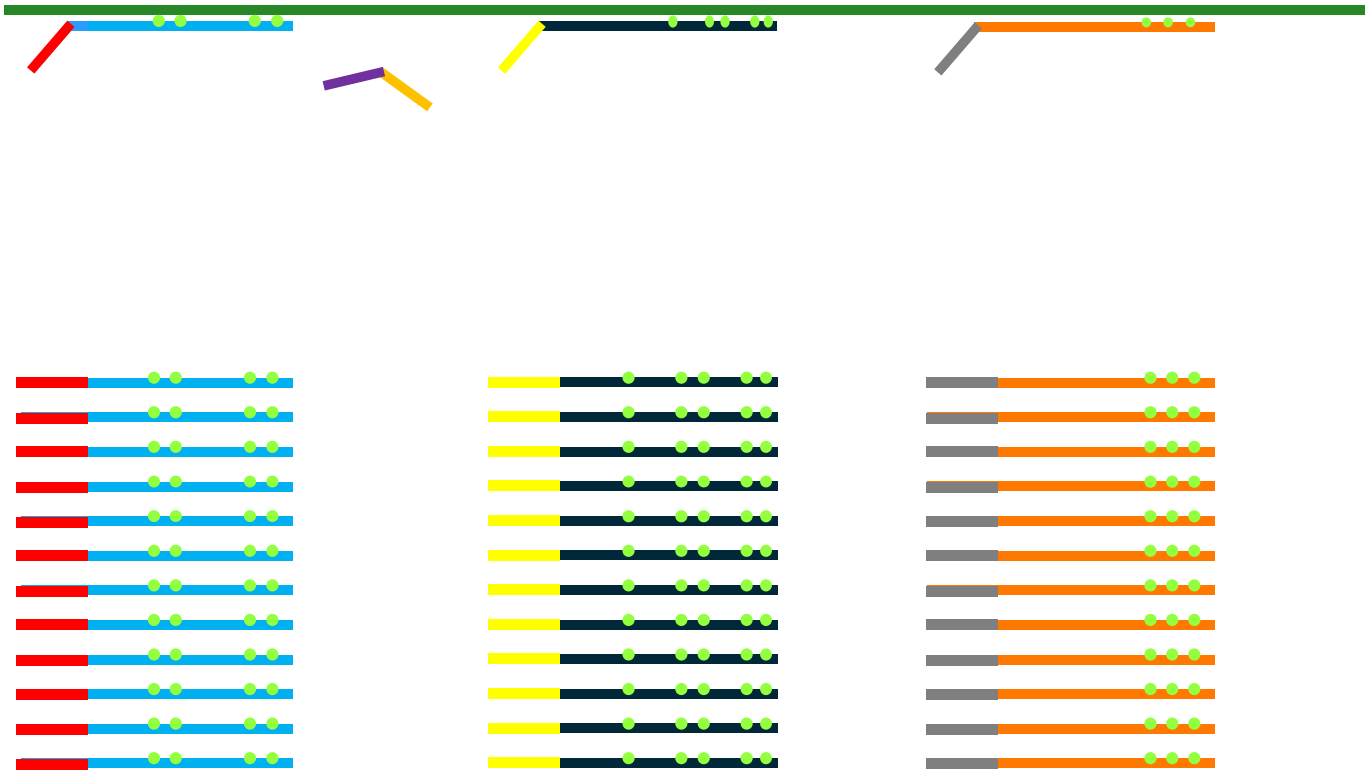
xTAG technology: Target amplification

Pre amplification of targets



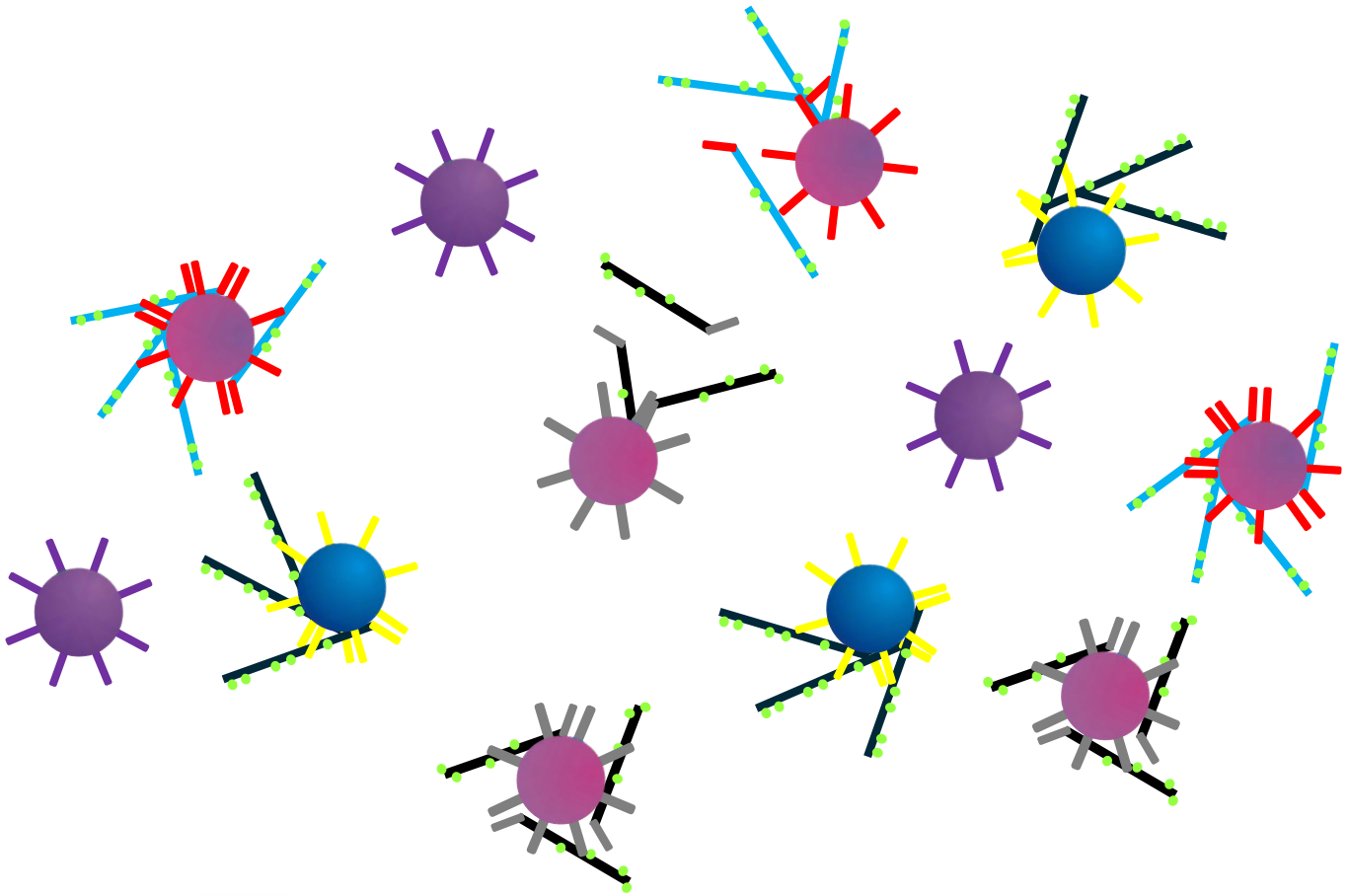
xTAG technology: TSPE (extension step)

Incorporation of (biotin)-dCTP

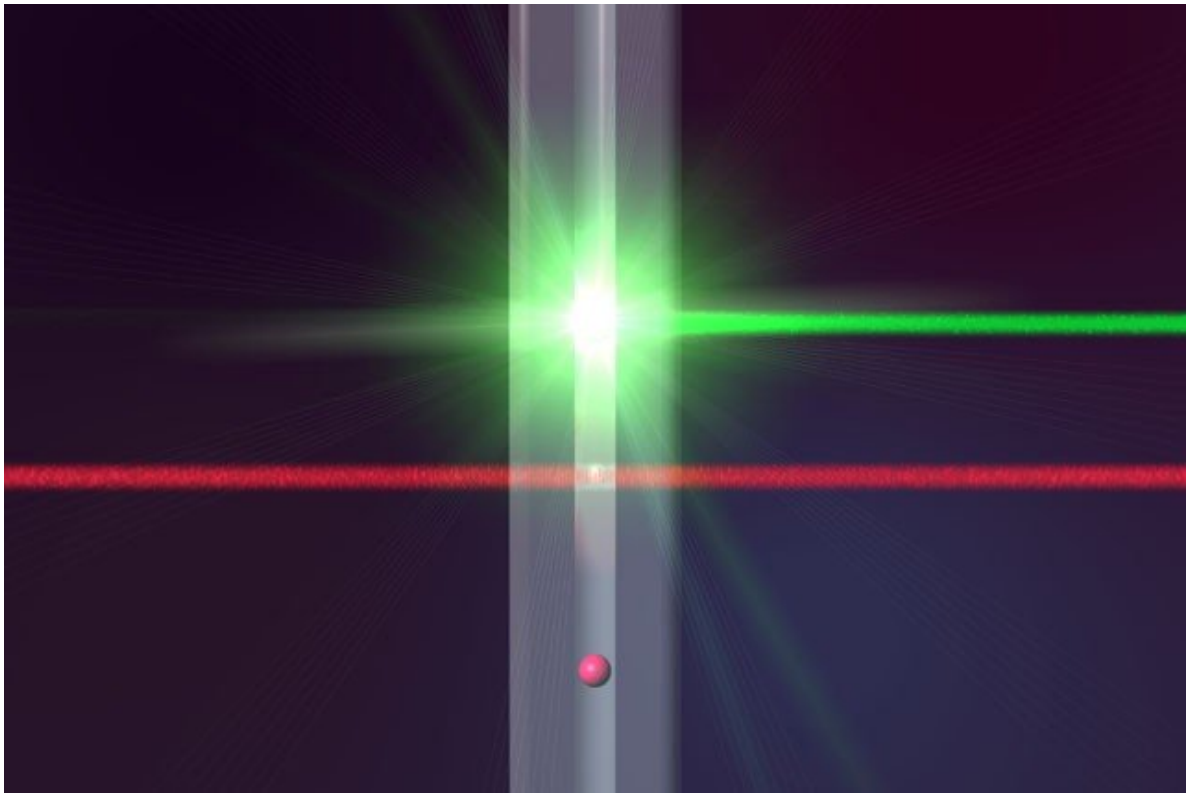


xTAG technology: TSPE (hybridization)

Hybridization of TAG to Luminex bead mix



xTAG technology : detection



Detection of individual bead addresses
+ reporter fluoresence

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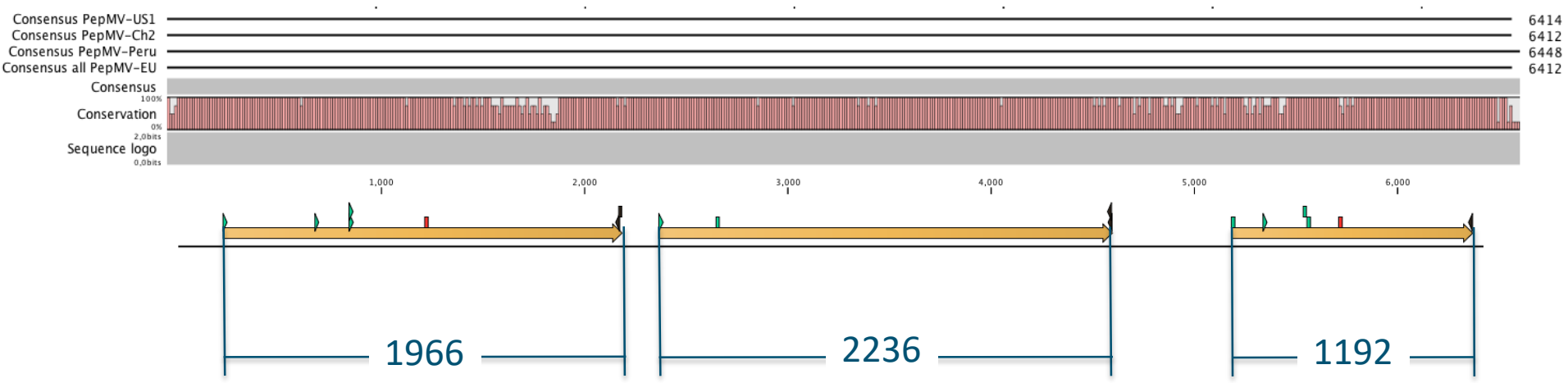
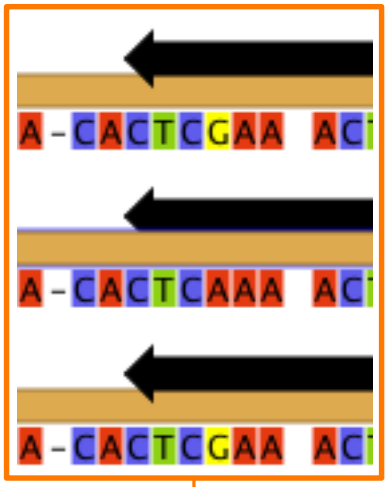
PepMV sequence data

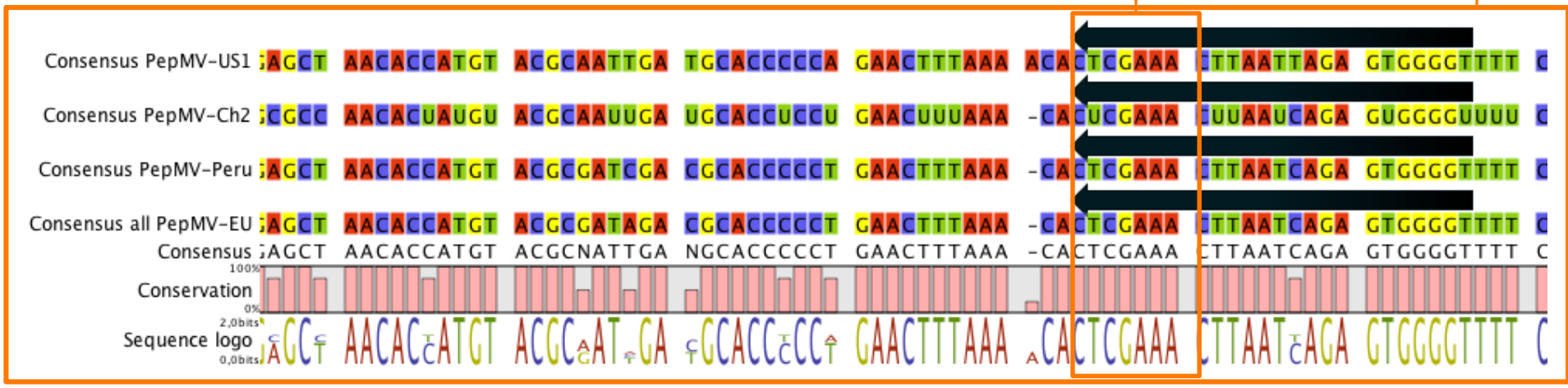


Design outer primers

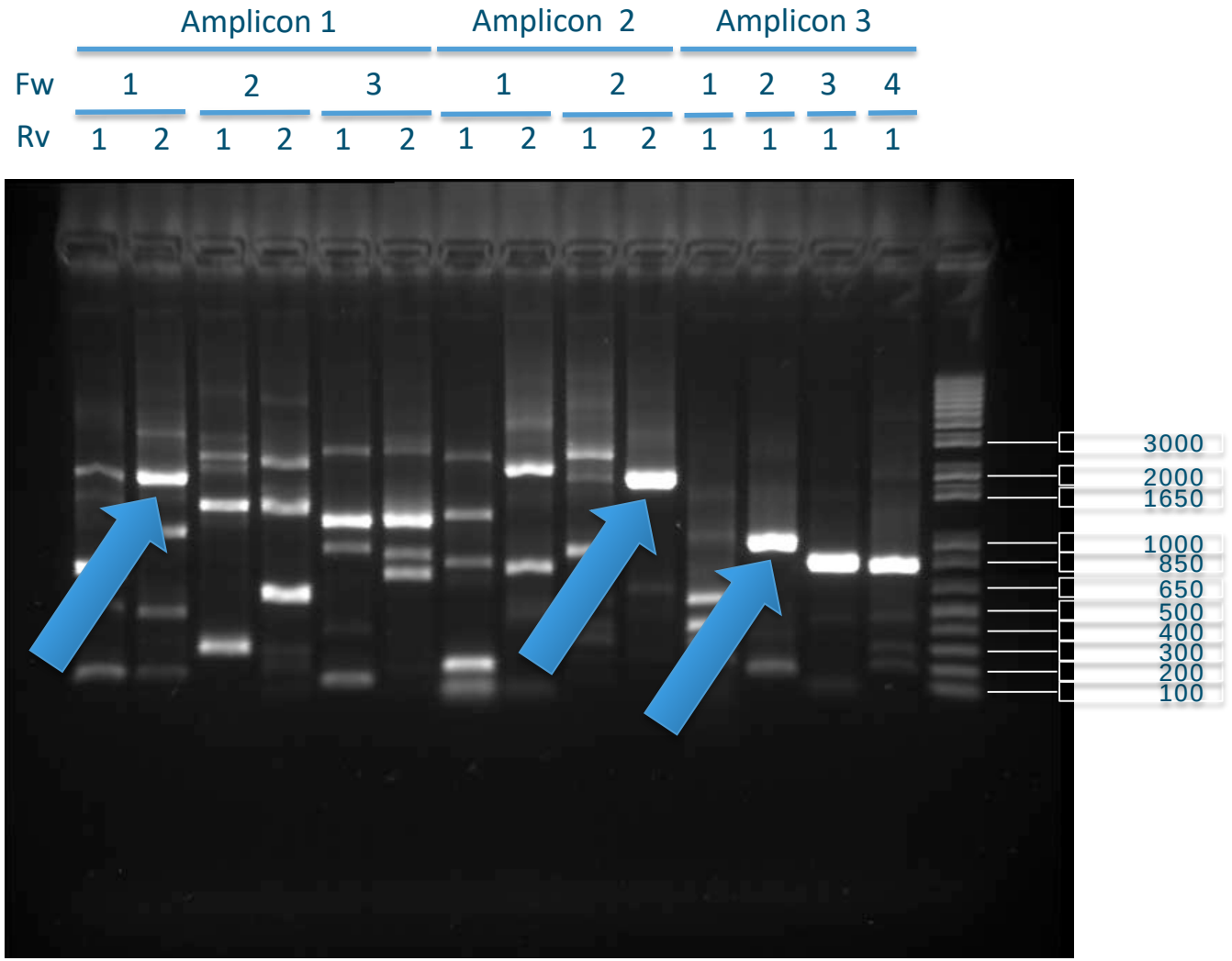
Name	Sequence
PepMV Fw1-1	AyTATGAAGChGACACCCTwGAGA
PepMV Fw1-2	AAyTACAAyGAAGAAGGyTTTGA
PepMV Fw1-3	GCCAAyCAyCTyTTCCTyTTCCAA
PepMV Rv1-1	TCTTCyTGTrGCATTTTGA
PepMV Rv1-2	CTTCyTGTrGCATTTTGAAwGCAA
PepMV Fw2-1	AACATTTGArAAAGCwATGATTCA
PepMV Fw2-2	GTTTACAGTGAGAAAGAAGG
PepMV Rv2-1	CCrGAyCCACTwGGATATTGATTC
PepMV Rv2-2	CAAGGrCTyGGTGAwCCTATAACT
PepMV Fw3-1	ACCCCACTCTyATTAAGTTTrGAG
PepMV Fw3-2	ATGTCCTCATACTyATCTTCATTC
PepMV Fw3-3	rArGCAATmAACTTCTCCCC
PepMV Fw3-4	CCyTGGAACGGGTTAAGTTTTC
PepMV Rv3-1	ACCCCACTCTy*ATTAAGTTTr*GAG



(*: y = C or T; r = A or G)

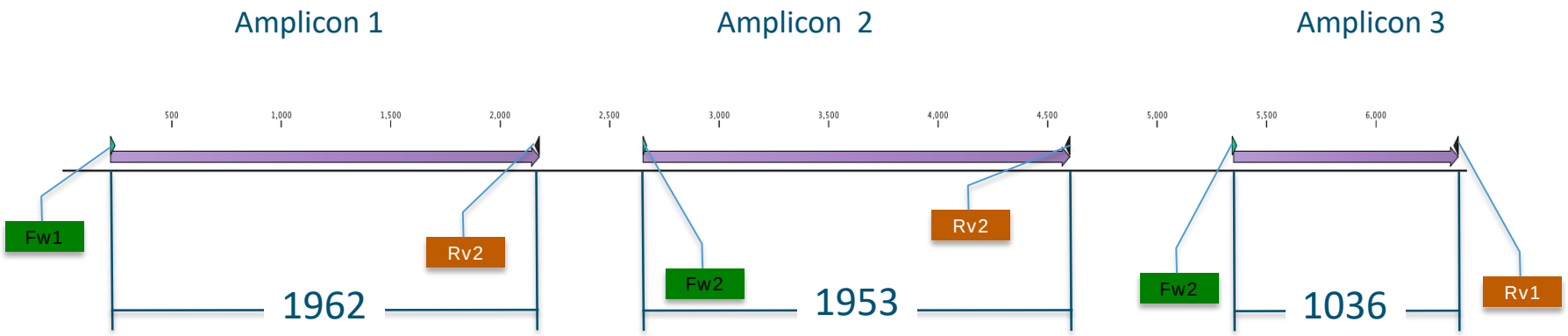


Test outer primers



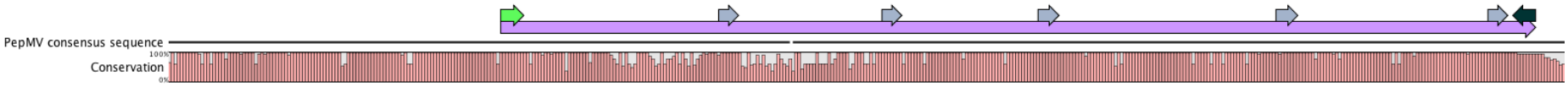
Selected outer primers

Name	Sequence
PepMV Fw1-1	AyTATGAAGChGACACCCTwGAGA
PepMV Rv1-2	CTTCyTGTrGCATTTTGAAwGCAA
PepMV Fw2-2	GTTTACAGTGAGAAAGAAGG
PepMV Rv2-2	CAAGGrCTyGGTGAwCCTATAACT
PepMV Fw3-2	ATGTCCTCATACTyATCTTCATTC
PepMV Rv3-1	ACCCCACTCTyATTAAGTTTrGAG



Design TSPE

PepMV Fw3-2	ATGTCCTCATACTyATCTTCATTC
PepMV Rv3-1	ACCCCACTCTyATTAAGTTTrGAG



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PepMV strain specific TSPE

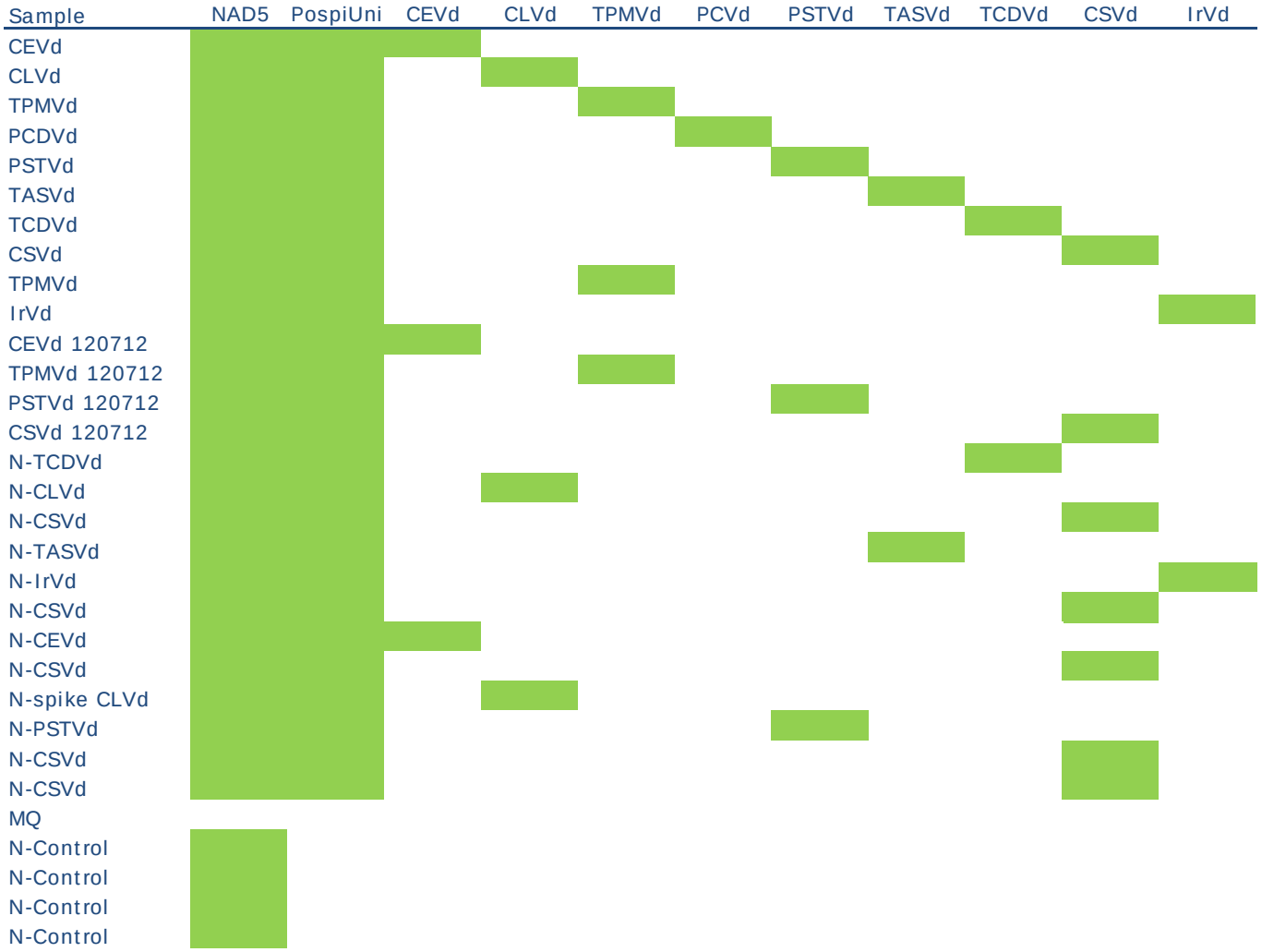
Sample	Generic Fw3-5	Ch2 Fw 1	EU Fw 1	Peru Fw1	US1 Fw1
PepMV Ch2	47522	8568	745	517	503
PepMV Ch2	48283	10182	675	489	709
PepMV Ch2	34597	9815	670	461	599
PepMV Ch2	27327	9651	677	519	516
PC (EU)	45698	12816	2969	835	618
NC	530	894	671	483	689

Sample	Gen Fw3-5	Ch2 Fw2	EU Fw2	Peru Fw2	US1 Fw2
PepMV Ch2	48814	27050	761	578	707
PepMV Ch2	48699	20195	709	671	687
PepMV Ch2	32624	15534	691	530	679
PepMV Ch2	22988	6724	678	539	700
PC (EU)	46483	1154	23057	2595	742
NC	511	970	545	397	665

PepMV strain specific TSPE

	Generic	EU Fw2	Ch2 Fw2	US1 Fw2	Peru Fw1
Ch2	3169	816	3122	909	728
Ch2	6324	769	1802	800	608
Ch2	4253	676	1552	909	687
EU new	78553	69471	1270	2399	4298
EU 1066	73856	73660	1159	2921	4445
NC	1163	865	814	786	701
Peru	8582	770	820	754	2983
US1	63297	195	1284	90635	728
Ch2	70556	2087	49576	1258	1139
Peru purple	3790	786	697	697	4441

Pospiviroid xTAG test (various plant material)



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Pospiviroid xTAG test (various plant material)

Sample	NAD5	PospiUni	CEVd	CLVd	TPMVd	PCVd	PSTVd	TASVd	TCDVd	CSVd	IrVd
CEVd	25679	52936	42541	186	380	298	171	2038	6236	172	390
CLVd	32780	201	23	195	265	566	91	3505	150	211	487
TPMVd	17725	21754	104	171	13961	382	320	1550	483	167	386
PCDVd	40987	2372	61	245	340	2425	150	3311	172	88	562
PSTVd	23642	16183	93	198	191	467	7794	1625	288	193	287
TASVd	23596	21317	123	391	239	476	209	22859	172	177	520
TCDVd	29558	2970	62	216	342	404	97	2736	1445	109	457
CSVd	10057	1001	55	77	108	161	100	623	247	415	264
TPMVd	8101	17403	85	180	7759	339	636	1366	87	149	409
IrVd	14937	24079	121	187	409	427	489	1783	423	133	28821
CEVd 120712	1154	3137	940	116	147	71	115	548	1144	111	166
TPMVd 120712	439	5707	95	73	12539	79	159	174	127	108	210
PSTVd 120712	746	5036	73	61	157	87	10274	159	196	86	208
CSVd 120712	96	86	83	77	108	73	101	53	80	27	294
N-TCDVd	29443	26013	147	265	272	665	339	2954	6108	237	558
N-CLVd	16828	3745	114	8296	173	389	121	1357	204	94	352
N-CSVd	12792	3745	76	134	178	237	98	803	130	720	242
N-TASVd	38789	43416	160	589	364	561	835	41689	268	239	664
N-IrVd	41609	62522	115	263	459	711	741	2660	455	118	63881
N-CSVd	22836	33471	106	218	622	611	183	1980	228	13718	448
N-CEVd	17541	20327	12413	194	1880	426	139	1664	1170	120	343
N-CSVd	38455	66045	28	274	527	629	321	2154	286	31595	543
N-spike CLVd	58345	1264	132	2328	680	1081	142	4707	578	158	1013
N-PSTVd	32550	43554	158	199	725	671	29616	3064	539	350	895
N-CSVd	29662	54259	121	188	484	569	221	1872	217	25690	466
N-CSVd	39514	51565	191	303	540	868	252	4931	340	11473	783
MQ	26	23	48	37	67	106	65	80	49	69	168
N-Control	44083	845	112	243	370	687	155	2965	207	143	668
N-Control	51933	703	115	170	316	689	62	2311	179	119	577
N-Control	41252	878	136	283	609	671	150	3971	253	185	742
N-Control	61464	903	159	349	409	879	76	5250	206	111	792

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Pospiviroid xTAG test (various plant material)

Sample	NAD5	PospiUni	CEVd	CLVd	TPMVd	PCVd	PSTVd	TASVd	TCDVd	CSVd	IrVd
CEVd	25679	52936	42541	186	380	298	171	2038	6236	172	390
CLVd	32780	201	23	195	265	566	91	3505	150	211	487
TPMVd	17725	21754	104	171	13961	382	320	1550	483	167	386
PCDVd	40987	2372	61	245	340	2425	150	3311	172	88	562
PSTVd	23642	16183	93	198	191	467	7794	1625	288	193	287
TASVd	23596	21317	123	391	239	476	209	22859	172	177	520
TCDVd	29558	2970	62	216	342	404	97	2736	1445	109	457
CSVd	10057	1001	55	77	108	161	100	623	247	415	264
TPMVd	8101	17403	85	180	7759	339	636	1366	87	149	409
IrVd	14937	24079	121	187	409	427	489	1783	423	133	28821
CEVd 120712	1154	3137	940	116	147	71	115	548	1144	111	166
TPMVd 120712	439	5707	95	73	12539	79	159	174	127	108	210
PSTVd 120712	746	5036	73	61	157	87	10274	159	196	86	208
CSVd 120712	96	86	83	77	108	73	101	53	80	27	294
N-TCDVd	29443	26013	147	265	272	665	339	2954	6108	237	558
N-CLVd	16828	3745	114	8296	173	389	121	1357	204	94	352
N-CSVd	12792	3745	76	134	178	237	98	803	130	720	242
N-TASVd	38789	43416	160	589	364	561	835	41689	268	239	664
N-IrVd	41609	62522	115	263	459	711	741	2660	455	118	63881
N-CSVd	22836	33471	106	218	622	611	183	1980	228	13718	448
N-CEVd	17541	20327	12413	194	1880	426	139	1664	1170	120	343
N-CSVd	38455	66045	28	274	527	629	321	2154	286	31595	543
N-spike CLVd	58345	1264	132	2328	680	1081	142	4707	578	158	1013
N-PSTVd	32550	43554	158	199	725	671	29616	3064	539	350	895
N-CSVd	29662	54259	121	188	484	569	221	1872	217	25690	466
N-CSVd	39514	51565	191	303	540	868	252	4931	340	11473	783
MQ	26	23	48	37	67	106	65	80	49	69	168
N-Control	44083	845	112	243	370	687	155	2965	207	143	668
N-Control	51933	703	115	170	316	689	62	2311	179	119	577
N-Control	41252	878	136	283	609	671	150	3971	253	185	742
N-Control	61464	903	159	349	409	879	76	5250	206	111	792

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Clavibacter xTAG

- TSPE primer concentration: 0.25µM each

Sample	ALL gyrB FW1	ALL gyrB FW2	Cmi recA FW1	Cmm recA FW2	Cmn recA FW2	Cms recA FW3	Cmt recA FW1
CMI 2404	36397.0	33304.0	22583.5	1831.0	1822.5	1865.0	1918.0
CMM 5842	32992.5	25445.5	1558.0	6831.0	2120.0	2623.0	2418.0
CMN 3521	29138.5	31458.0	1798.0	877.0	5169.0	2255.0	1405.0
CMS 1875	37424.5	27996.0	1360.0	1973.5	1769.0	30962.0	1699.0
CMT 3496	22641.0	17772.0	1607.0	3325.0	1621.0	1900.5	12255.0
LAL 26313	20587.0	20252.5	1439.0	1721.0	1661.5	1439.5	1540.0
SAP 7575	25665.0	23795.5	1970.0	2008.0	1771.0	1394.0	1944.0
Water	2380.5	1710.0	1745.0	1780.0	1909.0	1746.0	1612.0
Water	1718.0	849.0	877.5	1250.0	1169.0	1099.0	1138.0

Conclusions

- Luminex technology is versatile
  - Wide range of applications
    - Immunological testing
      - DAS
      - Inhibition
    - DNA/RNA
      - ssRNA
      - dsDNA

Conclusions

- xMAP
  - Results are comparable to ELISA
  - Faster
  - Multiplex
    - Reduction of labour- and consumable costs
  - Reliable
    - Internal control
    - Repeated measurements
  - Improved signal to noise ratio

Conclusions

- xTAG
  - Multiple Loci Geno Typing
    - Several targets for one sequence
  - Multiplex
  - Up until now a two step reaction

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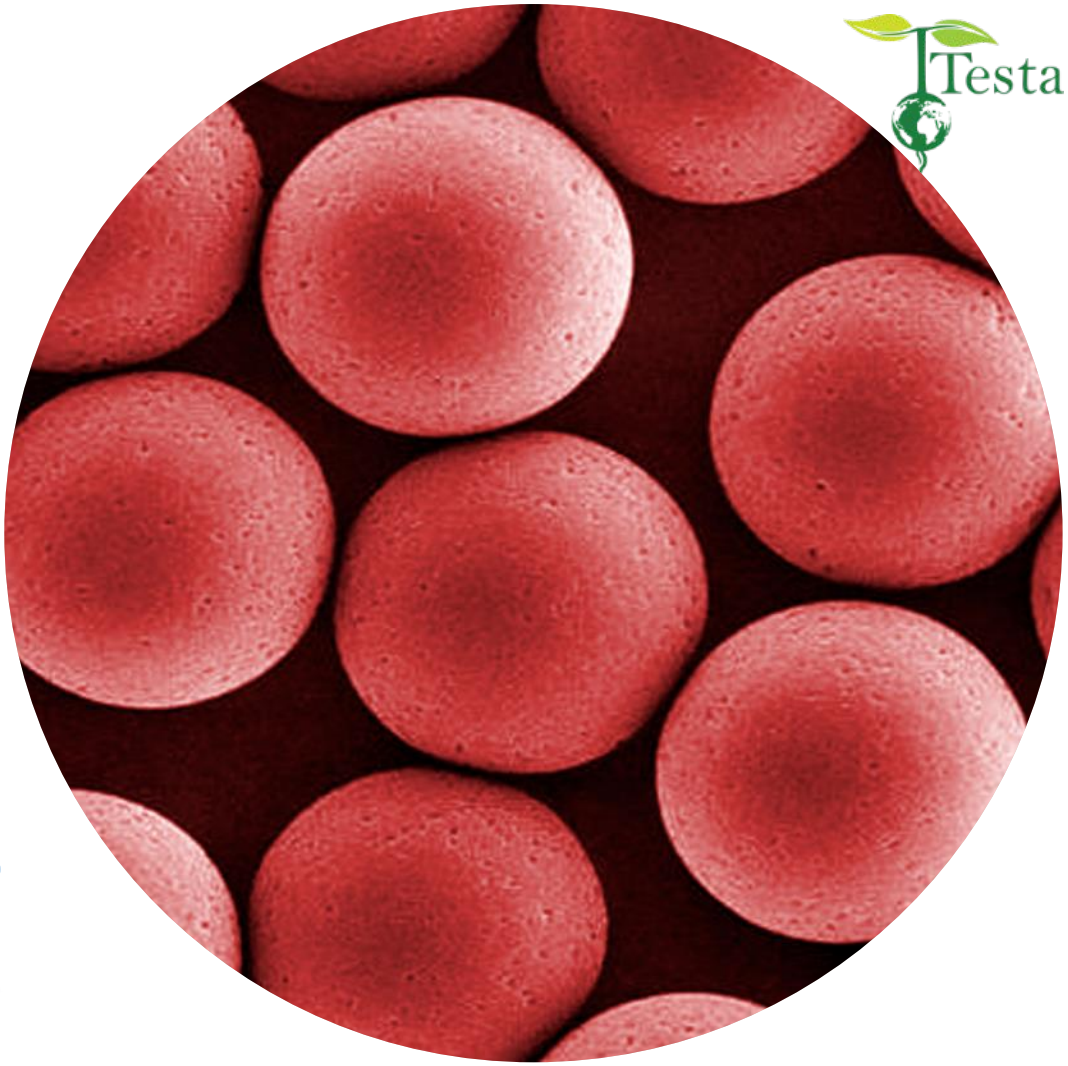

QUESTIONS?

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