Supplementary Information: A DIY guide to building and using a benchtop centrifuge force microscope

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Supplementary Note 1 – Achieving the desired force

The force applied in the CFM is relatively straightforward to calculate, however there are a few subtleties and factors to consider when designing and setting up your experiments. The physics derives from Newton's law, where the force F = ma, where m is the mass and a is acceleration. The centripetal acceleration of a body in uniform circular motion is given as $a = v^2/R$, where v is the instantaneous velocity and R is the radius of rotation. Noting that the instantaneous velocity can be related to rotational frequency f by $v = 2\pi fR$, we derive $F = 4\pi^2 mf^2 R$. For a bead in a liquid, we must consider the effective mass to be the mass of the bead minus the mass of the displaced liquid to account for buoyancy. By considering the volume of a sphere ($4\pi r^3/3$) with radius r and the density of the bead and the liquid we derive the final force: $F = (16/3)\pi^3 r^3 f^2 R(\rho_{bead}-\rho_{liquid})$. In this equation, f has units of revolutions/s – to convert to RPM we have to use f = RPM/60. This would simplify to $F = (\pi^3/675)r^3(RPM)^2 R(\rho_{bead}-\rho_{liquid})$, using SI units. To simplify to convenient units where r is in microns, ρ is in g/cm^3 and F is in pN, we get $F\ (pN) = 4.6x10^{-5}\ [r\ (\mu m)]^3 * [R\ (m)] * (\rho_{bead}-\rho_{liquid}) * RPM^2$.

From the force equation, we can see that F is dependent linearly on R and $(\rho_{bead}-\rho_{liquid})$, dependent on the square of rotational speed, and dependent on the cube of the bead radius. These relationships give us a great range of forces and often many different options on how to achieve a particular force. Let's consider each variable independently. First the radius of rotation R – this parameter has the least flexibility and is set by the mechanical design, and for most cases will not be considered as a variable. For the build described here, we measured this as 0.119 m. Assuming a measurement accuracy of 1 mm, The force uncertainty from this parameter is < 1%.

Next, we will consider the density term. While the density of available bead materials varies by less than a factor of 4, the "effective" density term can vary by nearly a factor of 100. This effect is well illustrated by the difference between PMMA and polystyrene – the density of PMMA is only about 14% higher than polystyrene but its effective density during centrifugation is about 400% higher. If using less dense material like polystyrene, it becomes very important to accurately determine (to 3+ significant digits) both the bead density and the liquid density to avoid large force uncertainties. Even variations in the liquid density due to salt content or temperature can affect the force in this case. Considering all of these factors, our recommendation for most applications is to use magnetic core microspheres or a heavier plastic microspheres such as PMMA or melamine.

Bead material	**Bead density (g/cm^3)**	**$\rho_{bead}-\rho_{water}$ (g/cm^3)**	**Typical uncertainty**
Polystyrene	1.05-1.06	0.04 - 0.06	~20%
PMMA	1.2	0.2	~5%
Melamine	1.5	0.5	~2%
Magnetic core polystyrene	1.6	0.6	~2%
Silica	2.0 - 2.6	1.0 - 1.6	<1%
Aluminum oxide	3.95	2.95	<1%

Next we consider bead radius, which is arguably the most important parameter for determining force range due to the cubic dependence of force. The recommended range for practical experiments in the CFM is between 1 and 25 micron diameter particles. It is possible to work outside of this range, but smaller becomes difficult to resolve and has extremely low forces, and larger reduces the ability to multiplex effectively and has extremely high forces. We typically use 2.8 micron diameter magnetic core particles to achieve forces in the range of ~0.8 – 20 pN at reasonable rotation speeds from ~300 – 1500 RPM. Due to the sensitivity of force to the particle size, the forces can be shifted up or down by an order of magnitude by increasing or decreasing the particle size by a factor of ~2. It is important to keep in mind when choosing beads that the size distribution of beads is likely to be the dominant source of force uncertainty. For this reason we recommend choosing beads that are highly uniform in size. A 3% deviation in size will cause almost a 10% deviation in the force.

Finally, we will consider the rotational speed, which will be the main parameter to control the force during the experiment. Since the force is dependent on the square of the rotational speed, a 10-fold increase in speed will result in a 100-fold increase in force. For our centrifuge, the minimum set speed is 300 RPM so the effective force range will be set by the square of your max speed divided by 300. A 3000 RPM max speed will get you a force range of 100-fold from minimum force, while a 2000 RPM max will give 44-fold and 1500 RPM max will give 25-fold. In some cases it is desirable to control the force in real time or to apply force ramps. The force can be ramped by accelerating slowly using the different acceleration profiles on the centrifuge. Alternatively, it is possible to make different rotation programs using either a custom board available from Thermo Fisher that enables computer control, or by using newer programmable centrifuges. A table of expected forces for the CFM as built using different types of beads is shown below.

Speed (RPM)			**Force (pN)**		
	2.8 µm dyna	**3 µm poly**	**3 µm silica**	**1 µm silica**	**10 µm silica**
300	0.81	0.08	2.49	0.09	92.37
450	1.83	0.19	5.61	0.21	207.84
600	3.24	0.33	9.98	0.37	369.50
750	5.07	0.52	15.59	0.58	577.34
900	7.30	0.75	22.45	0.83	831.36
1050	9.94	1.02	30.55	1.13	1131.58
1200	12.98	1.33	39.91	1.48	1477.98
1350	16.43	1.68	50.51	1.87	1870.57
1500	20.28	2.08	62.35	2.31	2309.34
1650	24.54	2.51	75.45	2.79	2794.31
1800	29.20	2.99	89.79	3.33	3325.46
1950	34.27	3.51	105.38	3.90	3902.79
2100	39.74	4.07	122.21	4.53	4526.31
2250	45.63	4.68	140.29	5.20	5196.02
2400	51.91	5.32	159.62	5.91	5911.92
2550	58.60	6.01	180.20	6.67	6674.00

2700	65.70	6.73	202.02	7.48	7482.27
2850	73.20	7.50	225.09	8.34	8336.73
3000	81.11	8.31	249.41	9.24	9237.38

There are a few subtle modifications to the equation that we typically do not include. First, the radius R will change depending on the swing angle of the bucket. Second, the mass of the bead applies a force down due to gravity which increases the overall force and changes the overall force direction. It has previously been shown [34] that even slow centrifugation at 300 RPM causes the bucket to tilt an angle of ~81˚, and increases to 83˚ at 1800 RPM. In this range the sine of the angle is ~0.99, which would correspond to a small fraction of a millimeter in the change of R, <<1%. Even at that slow speed of 300 RPM, the acceleration is equivalent to about 12 g's, so an additional 1g acting perpendicular only causes a deviation in force of also <<1%. Since the deviations are so small even for the worst-case scenario of slow rotation, we do not typically consider these extra factors. Even the relatively small uncertainties of the measurement due to bead size and density far out shadow the minor adjustments due to bead weight and incomplete bucket rotation.

Supplementary Note 2 – using other objectives

The protocol is written for a Nikon finite conjugate objective, but the CFM can be used with a variety of RMS threaded objectives. This Nikon objective (as well as most finite conjugate objectives) has a mechanical tube length of 160 mm, which corresponds to an optical tube length of 150 mm which is measured from the objective flange to the image plane. In our design, the actual optical tube length is measured at 144 mm, which means that the true magnification is multiplied by the ratio of 144/150 or about 0.96. So for a 40x objective we are getting about a 38x actual magnification.

We have also frequently used Olympus Plan Achromat infinity corrected objectives. Two variations to the protocol for the Olympus objectives are 1) extra steps needed to remove the outer casing, and 2) the additional "tube" lens needed to form an image with infinity conjugate lens.

Removing the outer casing of the Olympus objective is somewhat challenging due to the strong connection (evidently with thread lock adhesive) and the limited area to grip with pliers. It is difficult to do without damaging at least the casing of the objective, so proceed with caution. Our strategy has been to heat the casing using a hotplate or digital dry bath in the 80-100C range for about 15 or 20 minutes to loosen the thread lock glue. Then carefully pick up the objective from the casing with tongue and groove pliers (pump pliers). Using a second set of tongue and groove pliers (Grainger 46MW60 recommended), adjust the pliers so that they cannot accidentally slip and crush the RMS threads on the objective. Then carefully grip the thin brass ring above the threads and below the casing and unscrew the objective from the casing. If it is still too difficult, we have had some success in carefully applying acetone to the interface between the brass housing and casing to try to weaken the thread glue, and repeating cycles of acetone and heating to weaken the connection.

To add the focusing lens, we put it in the flange directly behind the objective. Starting from the fully built CFM module, remove the mirror housing from the top. Into the flange above the objective, place the focusing lens (we suggest achromatic doublet AC254-125-A from Thorlabs) with the flatter side facing away from the objective and use a retaining ring to lock into place. Then you can reattach the mirror housing and it is ready for use.

Three important notes for this configuration: 1) The actual magnification will be less than the printed magnification due to the shorter tube lens focal length (125 mm vs. 180 mm), 2) you should rebalance since the mass and center of mass will have changed, and 3) care should be taken to avoid letting the lens fall out if the 1.5" tube containing the objective is removed. If the 1.5" tube needs to be removed, it can be done upside down so that the lens rests on the retaining ring, or the tube lens should be removed first.

Using other objectives should still be fine, with the main considerations being the mass, the magnification, and thread compatibility. Different manufacturers use different threads and also use different tube lens focal lengths, which can change both the physical integration as well as the imaging characteristics.

Supplementary Note 3 – Advanced counterbalancing

Counterbalancing is a critical step, and can be tricky to get right. The most straightforward counterbalance is a duplicate CFM, which was the strategy used by LeFevre et. al. [40]. For those not wanting to make a duplicate or planning frequent design alterations, an adjustable counterbalance is necessary. The simple counterbalance described in the main protocol is well suited for the exact CFM design we've provided, but sometimes more flexibility is needed.

For those planning to modify the CFM and requiring more adjustability in the counterbalance, we have provided 3D models for an adjustable counterbalance that we sometimes use. This design consists of two components, a base and a top piece that screw together. The top piece has receptacles to house large coins (e.g. US quarters) to match the mass. The threaded design allows up and down movement of the mass to set the center of mass to match the CFM module. The threads are interrupted by vertical holes which allow locking of the thread using a physical obstruction in one or more holes (e.g. a pipette tip or wooden dowel).

The general approach from steps 39-43 should be used, except that the threaded internal piece will adjust the center of mass rather than rearranging coins. For very fine balancing, it is possible to measure the vibrations of the centrifuge at a few variations of height and pick the best one.

Supplementary Note 4 – Camera and bandwidth considerations

In this note, we will discuss the choice of camera, camera parameters, and WiFi bandwidth. As previously stated, our bandwidth is limited to 100 Mbps by the LAN port on the WiFi router. Experimental goals will determine how to use that bandwidth, and how to tradeoff frame resolution and frame rate. Most of our experiments and the one described in this protocol are relatively long and so we have favored a higher resolution camera (~6 MP) with a low framerate (~1 fps). Even in the Blackfly line of cameras which are interchangeable in our design, there are resolutions ranging from 0.4 MP to 24.5 MP, which would offer maximum frame rates at 100 Mbps ranging from ~30 fps to ~0.5 fps, respectively.

It is worth noting that maximum theoretical bandwidth and sustained real world bandwidth are not the same [36]. If your experimental transfer rate is close to the maximum bandwidth, then it will be common to lose some data (lost data will appear as sections of black pixels in the image). In our experience, this problem tends to be worse at high centrifuge speeds (>1500 RPM) and tends to come in bursts where there are many perfect frames interspersed with a few very bad frames. There are a few practical steps we will discuss to consider to optimize your data transfer: 1) Tweak your frame rate, 2) Optimize your WiFi signal, and 3) Optimize your camera's ethernet parameters.

The first consideration is changing your frame rate. For most cases where frame rate is not critical, reducing the frame rate so that you are using about half of the maximum bandwidth is the easiest way to get trouble-free performance. In cases where this is acceptable, then other optimizations below are likely to be unnecessary (and we mostly operate without them). If not, however, you can further optimize your WiFi signal with a few strategies. First, you can attach an external adhesive antenna (i.e. Digikey 001-0034-ND) to the router on the CFM. Such an antenna plugs in to a small receptacle on the bottom back side of the router board, opposite the metal prong that sticks out on the front side. The antenna part itself is adhesive, and when placed at or near the top of the mirror housing will improve the transmission. Second, you can optimize the placement of the WiFi antenna attached to the computer. For best results, you can remove physical barriers by unscrewing and removing the central window from the centrifuge and by removing the plastic housing of the WiFi antenna. The bandwidth can be highly dependent upon position and orientation of the antenna, so measuring the bandwidth in real time is the best way to optimize placement. This can be done by streaming at full frame rate while the centrifuge is running at your highest required speed, and observing the quality of the frames or the measured transfer rate in the task manager. Third, you can minimize interference from other external sources, and optionally select specific uncrowded WiFi channels in the router settings. There are various WiFi network analysis tools to determine which sections of the WiFi spectrum are crowded or uncrowded.

The ethernet parameters in the camera settings can also be important, particularly the packet resend parameters. If you are operating near the maximum bandwidth, trying to recover lost packets can take up extra bandwidth and cause a downward spiral and potentially a camera timeout. If you are operating further from the maximum bandwidth, enabling resends can help recover some of the lost packets if they occur relatively infrequently. It can be useful to observe the number of lost and recovered packets in NIMAX during streaming to understand how these ethernet parameters can influence the data transfer rate.

Supplementary Table 1. 3D printed parts

	Name	Purpose	Mass (g)	Time (hrs)	Filename(s)
A	Mirror housing	Houses turning mirrors	10.9	1.7	CFM mirror housing.stl
B	Optics housing	Houses main optical assembly and electronics	31.3	5.0	CFM optics housing.stl
C	Base	Houses illumination system and battery	34.5	5.3	CFM base.stl
D	Simple counterbalance	Provide counterbalance for CFM as built	120.0	10.5	Simple counterbalance.stl
	Optional extras				
E	Adjustable balance base	Support for counterbalance	86	8.5 (fast)	Adjustable balance base.stl
F	Adjustable balance top	Provide mass and position adjustment for counterbalance	49	5.2 (fast)	Adjustable balance top.stl
G	CFM inverted holder	Holding the CFM inverted for experimental prep	43	4.3 (fast)	CFM cradle.stl
H	CFM assembly	Full 3D model for making modifications	--	--	CFM holder_2023 v22.f3d

Supplementary Table 2. List of oligonucleotide sequences.

Name	Sequence	Length
Backbone sequences (5′-3′)		
1. 5'Biotin	(5' 2x bio) AACATCCAATAAATCATACAGGCAAGGCAAAGAATTAGCA	40
2	AAATTAAGCAATAAAGCCTC	20
3	AGAGCATAAAGCTAAATCGGTTGTACCAAAAACATTATGACCCTGTAATACTTTTGCGGG	60
4	AGAAGCCTTTATTTCAACGCAAGGATAAAAATTTTTAGAACCCTCATATATTTTAAATGC	60
5	AATGCCTGAGTAATGTGTAGGTAAAGATTCAAAAGGGTGAGAAAGGCCGGAGACAGTCAA	60
6	ATCACCATCAATATGATATTCAACCGTTCTAGCTGATAAATTAATGCCGGAGAGGGTAGC	60
7	TATTTTTGAGAGATCTACAAAGGCTATCAGGTCATTGCCTGAGAGTCTGGAGCAAACAAG	60
8	AGAATCGATGAACGGTAATCGTAAAACTAGCATGTCAATCATATGTACCCCGGTTGATAA	60
9	TCAGAAAAGCCCCAAAAACAGGAAGATTGTATAAGCAAATATTTAAATTGTAAACGTTAA	60
10	TATTTTGTTAAAATTCGCATTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGA	60
11	ACGCCATCAAAAATAATTCGCGTCTGGCCTTCCTGTAGCCAGCTTTCATCAACATTAAAT	60
12	GTGAGCGAGTAACAACCCGTCGGATTCTCCGTGGGAACAAACGGCGGATTGACCGTAATG	60
13	GGATAGGTCACGTTGGTGTAGATGGGCGCATCGTAACCGTGCATCTGCCAGTTTGAGGGG	60
14	ACGACGACAGTATCGGCCTCAGGAAGATCGCACTCCAGCCAGCTTTCCGGCACCGCTTCT	60
15	GGTGCCGGAAACCAGGCAAAGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGG	60
16	CGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGG	60
17	CGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGT	60
18	GCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC	60
19	GTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAA	60
20	CATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCAC	60
21	ATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCA	60
22	TTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCCAGGGTGGTTT	60
23	TTCTTTTCACCAGTGAGACGGGCAACAGCTGATTGCCCTTCACCGCCTGGCCCTGAGAGA	60
24	GTTGCAGCAAGCGGTCCACGCTGGTTTGCCCCAGCAGGCGAAAATCCTGTTTGATGGTGG	60
25	TTCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGCCCGAGATAGGGTTGAGTGT	60
26	TGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCG	60
27	AAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACCATCACCCAAATCAAGTTTTTT	60
28	GGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCCGATTTAGAGC	60
29	TTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGG	60
30	CGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCT	60
31	TAATGCGCCGCTACAGGGCGCGTACTATGGTTGCTTTGACGAGCACGTATAACGTGCTTT	60
32	CCTCGTTAGAATCAGAGCGGGAGCTAAACAGGAGGCCGATTAAAGGGATTTTAGACAGGA	60
33	ACGGTACGCCAGAATCCTGAGAAGTGTTTTTATAATCAGTGAGGCCACCGAGTAAAAGAG	60
34	TCTGTCCATCACGCAAATTAACCGTTGTAGCAATACTTCTTTGATTAGTAATAACATCAC	60
35	TTGCCTGAGTAGAAGAACTCAAACTATCGGCCTTGCTGGTAATATCCAGAACAATATTAC	60
36	CGCCAGCCATTGCAACAGGAAAAACGCTCATGGAAATACCTACATTTTGACGCTCAATCG	60
37	TCTGAAATGGATTATTTACATTGGCAGATTCACCAGTCACACGACCAGTAATAAAAGGGA	60
38	CATTCTGGCCAACAGAGATAGAACCCTTCTGACCTGAAAGCGTAAGAATACGTGGCACAG	60
39	ACAATATTTTTGAATGGCTATTAGTCTTTAATGCGCGAACTGATAGCCCTAAAACATCGC	60
40	CATTAAAAATACCGAACGAACCACCAGCAGAAGATAAAACAGAGGTGAGGCGGTCAGTAT	60

41	TAACACCGCCTGCAACAGTGCCACGCTGAGAGCCAGCAGCAAATGAAAAATCTAAAGCAT	60
42	CACCTTGCTGAACCTCAAATATCAAACCCTCAATCAATATCTGGTCAGTTGGCAAATCAA	60
43	CAGTTGAAAGGAATTGAGGAAGGTTATCTAAAATATCTTTAGGAGCACTAACAACTAATA	60
44	GATTAGAGCCGTCAATAGATAATACATTTGAGGATTTAGAAGTATTAGACTTTACAAACA	60
45	ATTCGACAACTCGTATTAAATCCTTTGCCCGAACGTTATTAATTTTAAAAGTTTGAGTAA	60
46	CATTATCATTTTGCGGAACAAAGAAACCACCAGAAGGAGCGGAATTATCATCATATTCCT	60
47	GATTATCAGATGATGGCAATTCATCAATATAATCCTGATTGTTTGGATTATACTTCTGAA	60
48	TAATGGAAGGGTTAGAACCTACCATATCAAAATTATTTGCACGTAAAACAGAAATAAAGA	60
49	AATTGCGTAGATTTTCAGGTTTAACGTCAGATGAATATACAGTAACAGTACCTTTTACAT	60
50	CGGGAGAAACAATAACGGATTCGCCTGATTGCTTTGAATACCAAGTTACAAAATCGCGCA	60
51	GAGGCGAATTATTCATTTCAATTACCTGAGCAAAAGAAGATGATGAAACAAACATCAAGA	60
52	AAACAAAATTAATTACATTTAACAATTTCATTTGAATTACCTTTTTTAATGGAAACAGTA	60
53	CATAAATCAATATATGTGAGTGAATAACCTTGCTTCTGTAAATCGTCGCTATTAATTAAT	60
54	TTTCCCTTAGAATCCTTGAAAACATAGCGATAGCTTAGATTAAGACGCTGAGAAGAGTCA	60
55	ATAGTGAATTTATCAAAATCATAGGTCTGAGAGACTACCTTTTTAACCTCCGGCTTAGGT	60
56	TGGGTTATATAACTATATGTAAATGCTGATGCAAATCCAATCGCAAGACAAAGAACGCGA	60
57	GAAAACTTTTTCAAATATATTTTAGTTAATTTCATCTTCTGACCTAAATTTAATGGTTTG	60
58	AAATACCGACCGTGTGATAAATAAGGCGTTAAATAAGAATAAACACCGGAATCATAATTA	60
59	CTAGAAAAAGCCTGTTTAGTATCATATGCGTTATACAAATTCTTACCAGTATAAAGCCAA	60
60	CGCTCAACAGTAGGGCTTAATTGAGAATCGCCATATTTAACAACGCCAACATGTAATTTA	60
61	GGCAGAGGCATTTTCGAGCCAGTAATAAGAGAATATAAAGTACCGACAAAAGGTAAAGTA	60
62	ATTCTGTCCAGACGACGACAATAAACAACATGTTCAGCTAATGCAGAACGCGCCTGTTTA	60
63	TCAACAATAGATAAGTCCTGAACAAGAAAAATAATATCCCATCCTAATTTACGAGCATGT	60
64	AGAAACCAATCAATAATCGGCTGTCTTTCCTTATCATTCCAAGAACGGGTATTAAACCAA	60
65	GTACCGCACTCATCGAGAACAAGCAAGCCGTTTTTATTTTCATCGTAGGAATCATTACCG	60
66	CGCCCAATAGCAAGCAAATCAGATATAGAAGGCTTATCCGGTATTCTAAGAACGCGAGGC	60
67	GTTTTAGCGAACCTCCCGACTTGCGGGAGGTTTTGAAGCCTTAAATCAAGATTAGTTGCT	60
68	ATTTTGCACCCAGCTACAATTTTATCCTGAATCTTACCAACGCTAACGAGCGTCTTTCCA	60
69	GAGCCTAATTTGCCAGTTACAAAATAAACAGCCATATTATTTATCCCAATCCAAATAAGA	60
70	AACGATTTTTTGTTTAACGTCAAAAATGAAAATAGCAGCCTTTACAGAGAGAATAACATA	60
71	AAAACAGGGAAGCGCATTAGACGGGAGAATTAACTGAACACCCTGAACAAAGTCAGAGGG	60
72	TAATTGAGCGCTAATATCAGAGAGATAACCCACAAGAATTGAGTTAAGCCCAATAATAAG	60
73	AGCAAGAAACAATGAAATAGCAATAGCTATCTTACCGAAGCCCTTTTTAAGAAAAGTAAG	60
74	CAGATAGCCGAACAAAGTTACCAGAAGGAAACCGAGGAAACGCAATAATAACGGAATACC	60
75	CAAAAGAACTGGCATGATTAAGACTCCTTATTACGCAGTATGTTAGCAAACGTAGAAAAT	60
76	ACATACATAAAGGTGGCAACATATAAAAGAAACGCAAAGACACCACGGAATAAGTTTATT	60
77	TTGTCACAATCAATAGAAAATTCATATGGTTTACCAGCGCCAAAGACAAAAGGGCGACAT	60
78	TCAACCGATTGAGGGAGGGAAGGTAAATATTGACGGAAATTATTCATTAAAGGTGAATTA	60
79	TCACCGTCACCGACTTGAGCCATTTGGGAATTAGAGCCAGCAAAATCACCAGTAGCACCA	60
80	TTACCATTAGCAAGGCCGGAAACGTCACCAATGAAACCATCGATAGCAGCACCGTAATCA	60
81	GTAGCGACAGAATCAAGTTTGCCTTTAGCGTCAGACTGTAGCGCGTTTTCATCGGCATTT	60
82	TCGGTCATAGCCCCCTTATTAGCGTTTGCCATCTTTTCATAATCAAAATCACCGGAACCA	60
83	GAGCCACCACCGGAACCGCCTCCCTCAGAGCCGCCACCCTCAGAACCGCCACCCTCAGAG	60
84	CCACCACCCTCAGAGCCGCCACCAGAACCACCACCAGAGCCGCCGCCAGCATTGACAGGA	60

85	GGTTGAGGCAGGTCAGACGATTGGCCTTGATATTCACAAACAAATAAATCCTCATTAAAG	60
86	CCAGAATGGAAAGCGCAGTCTCTGAATTTACCGTTCCAGTAAGCGTCATACATGGCTTTT	60
87	GATGATACAGGAGTGTACTGGTAATAAGTTTTAACGGGGTCAGTGCCTTGAGTAACAGTG	60
88	CCCGTATAAACAGTTAATGCCCCCTGCCTATTTCGGAACCTATTATTCTGAAACATGAAA	60
89	GTATTAAGAGGCTGAGACTCCTCAAGAGAAGGATTAGGATTAGCGGGGTTTTGCTCAGTA	60
90	CCAGGCGGATAAGTGCCGTCGAGAGGGTTGATATAAGTATAGCCCGGAATAGGTGTATCA	60
91	CCGTACTCAGGAGGTTTAGTACCGCCACCCTCAGAACCGCCACCCTCAGAACCGCCACCC	60
92	TCAGAGCCACCACCCTCATTTTCAGGGATAGCAAGCCCAATAGGAACCCATGTACCGTAA	60
93	CACTGAGTTTCGTCACCAGTACAAACTACAACGCCTGTAGCATTCCACAGACAGCCCTCA	60
94	TAGTTAGCGTAACGATCTAAAGTTTTGTCGTCTTTCCAGACGTTAGTAAATGAATTTTCT	60
95	GTATGGGATTTTGCTAAACAACTTTCAACAGTTTCAGCGGAGTGAGAATAGAAAGGAACA	60
96	ACTAAAGGAATTGCGAATAATAATTTTTTCACGTTGAAAATCTCCAAAAAAAAAGGCTCCA	60
97	AAAGGAGCCTTTAATTGTATCGGTTTATCAGCTTGCTTTCGAGGTGAATTTCTTAAACAG	60
98	CTTGATACCGATAGTTGCGCCGACAATGACAACAACCATCGCCCACGCATAACCGATATA	60
99	TTCGGTCGCTGAGGCTTGCAGGGAGTTAAAGGCCGCTTTTGCGGGATCGTCACCCTCAGC	60
100	AGCGAAAGACAGCATCGGAACGAGGGTAGCAACGGCTACAGAGGCTTTGAGGACTAAAGA	60
101	CTTTTTCATGAGGAAGTTTCCATTAAACGGGTAAAATACGTAATGCCACTACGAAGGCAC	60
102	CAACCTAAAACGAAAGAGGCAAAAGAATACACTAAAACACTCATCTTTGACCCCCAGCGA	60
103	TTATACCAAGCGCGAAACAAAGTACAACGGAGATTTGTATCATCGCCTGATAAATTGTGT	60
104	CGAAATCCGCGACCTGCTCCATGTTACTTAGCCGGAACGAGGCGCAGACGGTCAATCATA	60
105	AGGGAACCGAACTGACCAACTTTGAAAGAGGACAGATGAACGGTGTACAGACCAGGCGCA	60
106	TAGGCTGGCTGACCTTCATCAAGAGTAATCTTGACAAGAACCGGATATTCATTACCCAAA	60
107	TCAACGTAACAAAGCTGCTCATTCAGTGAATAAGGCTTGCCCTGACGAGAAACACCAGAA	60
108	CGAGTAGTAAATTGGGCTTGAGATGGTTTAATTTCAACTTTAATCATTGTGAATTACCTT	60
109	ATGCGATTTTAAGAACTGGCTCATTATACCAGTCAGGACGTTGGGAAGAAAAATCTACGT	60
110	TAATAAAACGAACTAACGGAACAACATTATTACAGGTAGAAAGATTCATCAGTTGAGATT	60
111	TAGGAATACCACATTCAACTAATGCAGATACATAACGCCAAAAGGAATTACGAGGCATAG	60
112	TAAGAGCAACACTATCATAACCCTCGTTTACCAGACGACGATAAAAACCAAAATAGCGAG	60
113	AGGCTTTTGCAAAAGAAGTTTTGCCAGAGGGGGTAATAGTAAAATGTTTAGACTGGATAG	60
114	CGTCCAATACTGCGGAATCGTCATAAATATTCATTGAATCCCCCTCAAATGCTTTAAACA	60
115	GTTCAGAAAACGAGAATGACCATAAATCAAAAATCAGGTCTTTACCCTGACTATTATAGT	60
116	CAGAAGCAAAGCGGATTGCATCAAAAAGATTAAGAGGAAGCCCGAAAGACTTCAAATATC	60
117	GCGTTTTAATTCGAGCTTCAAAGCGAACCAGACCGGAAGCAAACTCCAACAGGTCAGGAT	60
118	TAGAGAGTACCTTTAATTGCTCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGC	60
119	TTAATTGCTGAATATAATGCTGTAGCTCAACATGTTTTAAATATGCAACTAAAGTACGGT	60
120	GTCTGGAAGTTTCATTCCATATAACAGTTGATTCCCAATTCTGCGAACGAGTAGATTTAG	60
121	TTTGACCATTAGATACATTTCGCAAATGGTCAATAACCTGTTTAGCTAT	49
122	ATTTTCATTTGGGGCGCGAGCTGAAAAGGT	30
Sequence used in specific combination for each construct (5′-3′)		
OH-A	GGCATCAATTCTACTAATAGTAGTAGCATTCCGTGCCTGTGAACGAGCTGCCCCATGGCA	60
OH-T	GGCATCAATTCTACTAATAGTAGTAGCATTCCGTGCCTGTGAACGAGCTGCCCCATGGCT	60
T: Sp-A-C	CCGCTGCATTTAGCCATGGGGCAGCTCGTTCACAGGCACGG	41
A: G-T	TGCAGCGGTGCCATGGGGCAGCTCGTTCACAGGCACGG	38