



**POLYSKOPE**  
**L A B S**

# Polyskope 1.0 Multiplex Pathogen Detection Assay

## User Guide

Test for the real-time simultaneous PCR detection of *E.coli* O157 STEC, *Salmonella* spp. and *Listeria monocytogenes* in food and environmental samples

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## I. INTRODUCTION

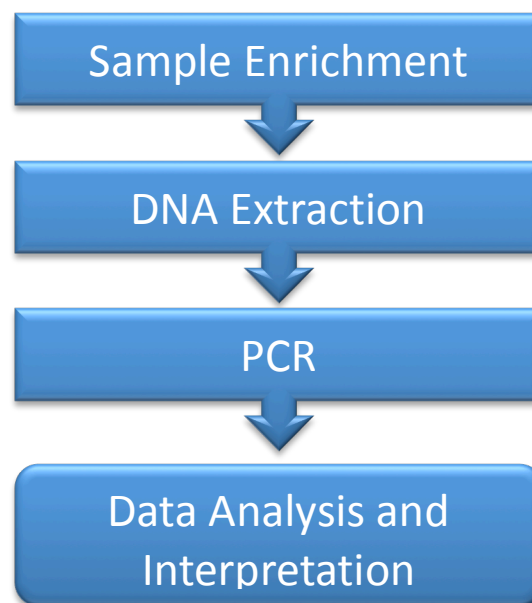
Conventional bacteriological detection methods are often long and labor intensive. In comparison, Polyskope 1.0 is a simple and rapid qualitative test, allowing the detection of specific DNA sequences unique to *E.coli* O157 STEC, *Salmonella* spp. and *Listeria monocytogenes* found in environmental samples and food products. Using real-time polymerase chain reaction (PCR), specific DNA sequences are amplified and detected simultaneously by means of fluorescent probes. Up to 94 samples can be processed, with a minimized risk of contamination and an easy to use procedure. The intended users of this kit are trained laboratory personnel who are performing tests to detect *E.coli* O157 STEC, *Salmonella* spp. and *Listeria monocytogenes* (also known as “The Big Three”). The use of this test allows results to be obtained within a few hours following enrichment of a sample.

## II. THE Polyskope 1.0. TECHNOLOGY

The Polyskope 1.0 kit is a test based on gene amplification and detection by real-time PCR. Ready-to-use PCR reagents contain oligonucleotides (primers and probes) specific to the “Big Three” pathogens as well as DNA polymerase and nucleotides. PCR is a well-established technique used to rapidly generate profuse copies of target DNA. During the PCR reaction, cycles of heating and cooling promote DNA denaturation, followed by primers binding to specific target regions. The DNA polymerase then recognizes these primers and utilizes deoxynucleotide triphosphates (dNTPs) to extend the DNA, creating copies of the target DNA, called amplicons. Next, specific probes are used to detect the DNA during the amplification, by hybridizing to the amplicons. These probes are bound to a fluorophore which fluoresces only when hybridized to the correct target sequence. In the

absence of target DNA, no fluorescence will be detected. As the amplicons increase with each round of amplification, fluorescence intensity also increases. At the annealing step of each PCR cycle, the detector measures this fluorescence and the associated software plots the fluorescence intensity versus number of cycles. This method allows a simple determination of the presence, or absence, of up to five targets in a single reaction. An unrelated DNA "internal control" is included in the reaction mix. This control is amplified with a specific probe at the same time as the other probe target DNA sequences and detected by a specific fluorophore. It allows for the validation of any negative result. . Polyskope 1.0 is specifically designed to detect pathogenic bacteria capable of human infection. The oligonucleotides are targeted to specific pathogen-related genes that are present in these bacteria and distinguish them from closely related non-pathogenic bacteria.

The **PolySkope 1.0 method** allows the simultaneous detection of *E.coli* O157 STEC, *Salmonella* spp. and *Listeria monocytogenes* in environmental samples and food products previously enriched by culture in Polyskope Multiplex Enrichment Media (PMEM). It includes the following 4 main steps:



### III. KIT COMPONENTS

The Polyskope 1.0 kit contains sufficient reagents for 96 tests.

Reference ID	Reagent	Quantity Provided
A	Amplification Mix	2 tubes ( 2 X 1 ml)
B	Probes	1 tube (.66 ml)
C	Lysis Component 1	1 bottle (7.5 ml)
D	Lysis Component 2	1 bottle (7.5 ml)
E	Lysis Component 3, Beads	1 bottle (13.2 g)
F	PCR positive control	1 tube (.25 ml)
G	PCR negative control	1 tube (.25 ml)

### IV. SHELF LIFE AND STORAGE

Once received, the kit must be stored between +2 ° C and +8° C. Reagents stored between at this temperature can be used until the expiration date indicated on the reagent tube. Shelf life of lysis buffer is 1 month once mixed with lysis beads.

### V. MATERIAL REQUIRED BUT NOT SUPPLIED

#### Equipment

- "Stomacher" masticator or equivalent for homogenizing test samples
- 37°C incubator for microbiological enrichment of sample
- Agitator-thermomixer for deepwell plates, such as a "Thermomixer" capable of 100° C ± 5° C and shaking at 1300 RPM
- Vortex apparatus
- Magnetic stir plate
- 1 µl, 20 µl, 200 µl and 1000 µl micropipette tips
- Combi-tip pipettes or equivalent repeat pipettors
- Real-time PCR system, e.g. Quantstudio 5, CFX96 or CFX96 Deep Well systems

## Supplies

- PMEM (available in 500 g to 2.5 kg quantities)
- Stomacher bag with integral mesh filter
- Environmental swabs and environmental sponges
- 1 ml deepwell plate
- Pre-pierced sealing film, such as "X-Pierce™ Sealing Films"
- 200 µl wide opening tips for lysis buffer transfer
- 96-well white, sterile PCR plates
- 96-well clear film plate sealer
- Sterile filter tips, adaptable to 20 µl, 200 µl and 1000 µl micropipettes
- Sterile 2.5 ml Eppendorf or similar tubes
- Powder-free gloves.
- Sterile distilled water
- Bleach 5%
- Decontaminating agent such as DNA AWAY® or RNase AWAY®

## VI. PRECAUTIONS AND RECOMMENDATIONS FOR BEST RESULTS

- This test must be performed by trained personnel
- Food samples and enrichment cultures must be handled as possibly infectious material and disposed of according to local rules and regulations
- Any potentially infectious material should be autoclaved before disposal
- The quality of results depends on strict compliance with the following Good Laboratory Practices (for example the EN ISO 7218 standard), especially concerning PCR
- The laboratory equipment (pipettes, tubes, etc.) must not circulate from one work station to another
- It is critical to use a positive control and a negative control for each series of amplification reactions
- Do not use reagents past expiration date
- Vortex reagents from the kit before using them to ensure homogeneity
- Periodically verify the accuracy and precision of pipettes, as well as correct functioning of the instruments
- Change gloves often, especially if contaminated
- Clean work spaces periodically with at least 5% bleach and a decontaminating agent like DNA AWAY on specialized equipment
- Use powder-free gloves and avoid handling and writing on caps of tubes as this will interfere with data acquisition

## **Instructions for media preparation**

- 1.) Using aseptic technique, carefully weigh 100 grams of Polyskope PMEM and pour into a sterile one liter flask
- 2.) Add 1 Liter of distilled water and place on heated stir plate
- 3.) Agitate and heat until boiling for one minute
- 4.) Remove from stir plate and autoclave at 121°C for 15 minutes
- 5.) Store at room temperature away from light until ready for use, then pre-heat to 37°C immediately before use

## **VII. PROTOCOL**

It is strongly recommended to read the entire protocol before starting the test

### **A. Sample Enrichment**

Ensure that the enrichment media is at the appropriate incubation temperature (37° C) before use

- 1.) Using an appropriate scale, weigh 25 grams\* of sample to be tested and place in a stomacher bag with incorporated mesh filter
  - 2.) Place sample bag on scale and pour 225 ml of Polyskope PMEM into filter bag
  - 3.) Place filter bag in stomacher and homogenize at 130 RPM for 30 seconds. Fold top of filter bag over at least 3 times and close
  - 4.) Incubate without shaking, for 23 ±1 hour at 37°C before lysis and PCR analysis
- \* Test portions other than 25 g have not been tested

### **B. DNA Extraction**

- General recommendations:
- Turn on the thermomixer heat block before starting the extraction and preheat it to 65° C
- In general, avoid shaking the enrichment bag and collecting large fragments of food debris. For food samples with a fatty supernatant, collect the sample just below this layer
- Open tubes and wells carefully to avoid any possible cross contamination
- Cool the deepwell plate before pipetting directly through the pre-pierced sealing film
  
- Reconstitute the final lysis buffer solution as follows:
  - Carefully pour all the contents from reagent C (Lysis Component 1) into reagent D (Lysis Component 2) and mix thoroughly. Next, carefully pour the contents from reagent E (Lysis Component 3, beads) into the mixed lysis buffer components
  - Use consumables with a wide enough tip to allow pipetting of the homogenized lysis reagent
  - The lysis reagent mixed with lysis beads (reagents C + D + E) has a shelf life of 1 month, when stored at 4° C.
- Before every use, gently agitate the lysis reagent by hand first to resuspend the resin. Then repeat pipette rapidly, in order to keep the lysis buffer in suspension while pipetting from the lysis bottle into the deepwell block.

## **Lysis Protocol**

- 1.) Aliquot 150 µl of homogenized lysis reagent (reagents C + D + E) into the wells of a deepwell block
- 2.) Add 50 µl of decanted, enriched sample. Mix by repeat pipetting and seal the deepwell block with the pre-pierced sealing film
- 3.) Incubate deepwell block in the heat block at 65° C for 15 minutes, shaking at 1400 RPM. Secure deepwell block with lab tape if necessary.
- 4.) Remove block from thermomixer and adjust temperature to 95°C. After thermomixer has achieved proper temperature, reinsert block and shake at 1400 RPM for an additional 10 minutes.

Note: If you choose to temporarily stop the procedure, this is the recommended stopping point. The supernatant can be stored for up to 1 year at -20°C. Always allow the block to thaw completely before use.

### C. Real-time PCR mix preparation

1.) Prepare PCR mixture containing the amplification solution (reagent A) and the fluorescent probes (reagent B) depending on the number of samples and controls to analyze (at least one positive and one negative control must be included for each PCR run). Use the pipetting table in appendix to find the correct volumes to use for each reagent

- After preparation, the PCR mix (reagent A + B) must be used immediately. It is stable for only 1 hour maximum at 2°C-8°C

3.) Pipette 19 µl of this PCR mix into each well according to your plate setup

4.) Add 1 µl of sample or reagent F (negative control) or reagent E (positive control). Hermetically seal the wells of the plate by lightly applying pressure after the plastic film is in place. It is important to avoid bubbles at the bottom of the wells by pipetting carefully. If necessary, to eliminate any bubbles, centrifuge the sealed PCR plate (quick spin)

5.) Place the plate in the thermal cycler. Be sure to place the plate correctly: A1 well at the upper left corner. Close the reaction module

#### PCR Start

To start the PCR run, follow instructions in the real-time PCR system user guide for Polyskope 1.0 on the Quantstudio 5

### D. Data Analysis

Data can be analyzed directly at the end of the PCR run or at a later time by opening the stored data file

#### Interpreting Results

Once the data analysis parameters have been set, results are interpreted by analyzing the C<sub>q</sub> values of each sample (the cycle at which the amplification curve crosses the threshold).

#### Controls

Before interpreting sample results, it is necessary to verify the positive and negative controls. For the experiment to be valid, the controls must have the following results, as summarized in the table below, otherwise the PCR reaction needs to be repeated.



Test	Target Probe Detection	Internal Control Detection
Positive Control	$14 < Cq < 40$	$14 < Cq < 40$
Negative Control	$Cq = N/A$	$14 < Cq < 40$

## Samples

A positive signal must have a Cq value  $\geq 10$  for any of the target (non-internal control) fluorophore. If the Cq value is below 10, verify that as raw data the curve is a regular amplification curve (with a flat base line, followed by a rapid increase of fluorescence and then a flattening out). If the curve seems correct, it may be considered a positive sample. If there is no Cq value ( $Cq = N/A$ ) for the target fluorophores or the curve is not a typical amplification curve, the internal control for that sample must then be analyzed:

- This sample is considered as a negative sample if there is no Cq value in any target fluorophore channels and the internal control has a  $Cq \geq 14$
- Should the internal control also not have a Cq value ( $Cq = N/A$ ), this likely indicates an inhibition of the PCR reaction. The sample needs to be diluted (perform a 1 /10 dilution in distilled sterile water using 10  $\mu$ l of lysate in 90  $\mu$ l sterile water, then use 1  $\mu$ l of the dilution) and the PCR repeated
- Should the Cq value for the internal control be  $< 14$  it is not possible to interpret the result. Verify that the threshold was correctly placed, or that the curve as raw data is a regular amplification curve. If the curve does not have a characteristic shape, it will be necessary to repeat the PCR test

Interpretation of sample results is summarized in the following table:

Target Probe (Any fluorophore)	Internal Control Detection	Interpretation
$Cq \geq 10$	$Cq \geq 14$	Positive
$Cq = N/A$	$Cq \geq 14$	Negative
$Cq = N/A$	$Cq = N/A$	Inhibition

The software indicates a Cq value of N/A (not applicable) when the fluorescence of a sample does not rise significantly above the background noise, and hence does not cross the threshold. If results of negative and positive controls differ from those in the table above, it is necessary to repeat the PCR. It should be noted that a 1:10 dilution of the lysate sample must be tested also if both the internal control and target fluorophores give a result of N/A.

## Confirmation of positive results

In the context of the food safety regulations, all positive sample results need to be confirmed in one of three ways using Bacteriological Analytical Manual (BAM) or AOAC-approved methods:

1.) Using classic tests described in the BAM

2.) Streaking for isolation on a chromogenic medium. The protocol for these validated chromogenic methods used for confirmation should start from the PMEM enrichment broth. The presence of characteristic pathogen colonies is sufficient to confirm the presence of pathogens

3.) Using any other certified method based on a principle different from that used in the Polyskope 1.0. PCR test. The validated protocol of this second method must be followed entirely. In the event of results that are not in agreement between Polyskope 1.0 and one of the confirmation options listed above, the laboratory should follow the necessary steps to ensure the validity of their results. It is possible to store the enriched PMEM at 2-8°C, for 72 hours maximum following the incubation at 37° C, before carrying out the confirmation.

## VIII. APPENDIX A

### 3-in-1 Probe Mixing Guideline

When using the all-in-one version of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (µls)	Reagent B (µls)
1	5	14
2	10	28
3	15	42
4	20	56
5	28	77
6	33	92
7	39	108
8	44	123
9	50	139
10	55	154
11	61	169
12	66	185
13	72	200
14	77	216
15	83	231
16	88	246
17	94	262
18	99	277
19	105	293
20	110	308
21	116	323
22	121	339
23	127	354
24	132	370
25	138	385

# of Reactions	Reagent A (µls)	Reagent B (µls)
26	143	400
27	149	416
28	154	431
29	160	447
30	165	462
31	171	477
32	176	493
33	182	508
34	187	524
35	193	539
36	198	554
37	204	570
38	209	585
39	215	601
40	220	616
41	226	631
42	231	647
43	237	662
44	242	678
45	248	693
46	253	708
47	259	724
48	264	739
49	270	755
50	275	770

## VIII. APPENDIX A

### 3-in-1 Probe Mixing Guideline

When using the all-in-one version of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (µls)	Reagent B (µls)
51	281	785
52	286	801
53	292	816
54	297	832
55	303	847
56	308	862
57	314	878
58	319	893
59	325	909
60	330	924
61	336	939
62	341	955
63	347	970
64	352	986
65	358	1001
66	363	1016
67	369	1032
68	374	1047
69	380	1063
70	385	1078
71	391	1093
72	396	1109
73	402	1124
74	407	1140
75	413	1155

# of Reactions	Reagent A (µls)	Reagent B (µls)
76	418	1170
77	424	1186
78	429	1201
79	435	1217
80	440	1232
81	446	1247
82	451	1263
83	457	1278
84	462	1294
85	468	1309
86	473	1324
87	479	1340
88	484	1355
89	490	1371
90	495	1386
91	501	1401
92	506	1417
93	512	1432
94	517	1448
95	523	1463
96	528	1478

## VIII. APPENDIX B

### Single-Probe Mixing Guideline

When using one of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (µls)	Reagent B: Single Probe (µls)	Sterile H2O (µls)
1	5	5	9
2	10	9	19
3	15	14	28
4	20	18	37
5	28	25	51
6	33	30	61
7	39	36	71
8	44	41	81
9	50	46	91
10	55	51	102
11	61	56	112
12	66	61	122
13	72	66	132
14	77	71	142
15	83	76	152
16	88	81	163
17	94	86	173
18	99	91	183
19	105	97	193
20	110	102	203
21	116	107	213
22	121	112	224
23	127	117	234
24	132	122	244
25	138	127	254

**VIII. APPENDIX B****Single-Probe Mixing Guideline**

When using one of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

<b># of Reactions</b>	<b>Reagent A (µls)</b>	<b>Reagent B: Single Probe (µls)</b>	<b>Sterile H2O (µls)</b>
26	143	132	264
27	149	137	274
28	154	142	285
29	160	147	295
30	165	152	305
31	171	158	315
32	176	163	325
33	182	168	335
34	187	173	346
35	193	178	356
36	198	183	366
37	204	188	376
38	209	193	386
39	215	198	396
40	220	203	407
41	226	208	417
42	231	213	427
43	237	219	437
44	242	224	447
45	248	229	457
46	253	234	468
47	259	239	478
48	264	244	488
49	270	249	498
50	275	254	508

## VIII. APPENDIX B

### Single-Probe Mixing Guideline

When using one of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (µls)	Reagent B: Single Probe (µls)	Sterile H2O (µls)
51	281	259	518
52	286	264	529
53	292	269	539
54	297	274	549
55	303	280	559
56	308	285	569
57	314	290	579
58	319	295	590
59	325	300	600
60	330	305	610
61	336	310	620
62	341	315	630
63	347	320	640
64	352	325	650
65	358	330	661
66	363	335	671
67	369	340	681
68	374	346	691
69	380	351	701
70	385	356	711
71	391	361	722
72	396	366	732
73	402	371	742
74	407	376	752
75	413	381	762

**VIII. APPENDIX B****Single-Probe Mixing Guideline**

When using one of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

<b># of Reactions</b>	<b>Reagent A (µls)</b>	<b>Reagent B: Single Probe (µls)</b>	<b>Sterile H2O (µls)</b>
76	418	386	772
77	424	391	783
78	429	396	793
79	435	401	803
80	440	407	813
81	446	412	823
82	451	417	833
83	457	422	844
84	462	427	854
85	468	432	864
86	473	437	874
87	479	442	884
88	484	447	894
89	490	452	905
90	495	457	915
91	501	462	925
92	506	468	935
93	512	473	945
94	517	478	955
95	523	483	966
96	528	488	976



## VIII. APPENDIX C

### Two-Probe Mixing Guideline

When using two out of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (µls)	Reagent B: Probe 1 or 2 or 3 (µls)	Reagent B: Probe 1 or 2 or 3 (µls)	Sterile H2O (µls)
1	5	5	5	4
2	10	9	9	9
3	15	14	14	14
4	20	18	18	18
5	28	25	25	25
6	33	30	30	30
7	39	36	36	36
8	44	41	41	41
9	50	46	46	46
10	55	51	51	51
11	61	56	56	56
12	66	61	61	61
13	72	66	66	66
14	77	71	71	71
15	83	76	76	76
16	88	81	81	81
17	94	86	86	86
18	99	91	91	91
19	105	97	97	97
20	110	102	102	102
21	116	107	107	107
22	121	112	112	112
23	127	117	117	117
24	132	122	122	122
25	138	127	127	127

## VIII. APPENDIX C

### Two-Probe Mixing Guideline

When using two out of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (µls)	Reagent B: Probe 1 or 2 or 3 (µls)	Reagent B: Probe 1 or 2 or 3 (µls)	Sterile H2O (µls)
26	143	132	132	132
27	149	137	137	137
28	154	142	142	142
29	160	147	147	147
30	165	152	152	152
31	171	158	158	158
32	176	163	163	163
33	182	168	168	168
34	187	173	173	173
35	193	178	178	178
36	198	183	183	183
37	204	188	188	188
38	209	193	193	193
39	215	198	198	198
40	220	203	203	203
41	226	208	208	208
42	231	213	213	213
43	237	219	219	219
44	242	224	224	224
45	248	229	229	229
46	253	234	234	234
47	259	239	239	239
48	264	244	244	244
49	270	249	249	249
50	275	254	254	254

## VIII. APPENDIX C

### Two-Probe Mixing Guideline

When using two out of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (μls)	Reagent B: Probe 1 or 2 or 3 (μls)	Reagent B: Probe 1 or 2 or 3 (μls)	Sterile H2O (μls)
51	281	259	259	259
52	286	264	264	264
53	292	269	269	269
54	297	274	274	274
55	303	280	280	280
56	308	285	285	285
57	314	290	290	290
58	319	295	295	295
59	325	300	300	300
60	330	305	305	305
61	336	310	310	310
62	341	315	315	315
63	347	320	320	320
64	352	325	325	325
65	358	330	330	330
66	363	335	335	335
67	369	340	340	340
68	374	346	346	346
69	380	351	351	351
70	385	356	356	356
71	391	361	361	361
72	396	366	366	366
73	402	371	371	371
74	407	376	376	376
75	413	381	381	381

## VIII. APPENDIX C

### Two-Probe Mixing Guideline

When using two out of the three probes of Polyskope 1.0, refer to the following tables to ensure an accurate ratio of reagents when making the PCR mix

# of Reactions	Reagent A (µls)	Reagent B: Probe 1 or 2 or 3 (µls)	Reagent B: Probe 1 or 2 or 3 (µls)	Sterile H2O (µls)
76	418	386	386	386
77	424	391	391	391
78	429	396	396	396
79	435	401	401	401
80	440	407	407	407
81	446	412	412	412
82	451	417	417	417
83	457	422	422	422
84	462	427	427	427
85	468	432	432	432
86	473	437	437	437
87	479	442	442	442
88	484	447	447	447
89	490	452	452	452
90	495	457	457	457
91	501	462	462	462
92	506	468	468	468
93	512	473	473	473
94	517	478	478	478
95	523	483	483	483
96	528	488	488	488

## **PolySkope 1.0 Multiplex Assay Quick Reference Worksheet**

### **PolySkope Media Preparation**

100 g PMEM Media into 1 Liter Water



Boil 1 minute & mix Thoroughly



Autoclave @ 121°C for 15 minutes

### **PolySkope Lysis Buffer Preparation**

Pour contents (7.5 mL) of Reagent Bottle C (Lysis Component 1) into Reagent Bottle D (7.5 mL)



Pour contents (13.2g) of Reagent Bottle E (beads) into Reagent Bottle D (15 mL lysis solution)



Mix thoroughly before transfer to keep in suspension

## PolySkoPE Sample Preparation

225 ml pre-heated (37°C) PMEM media + 25 g sample



Stomach at 130 RPM for 30 seconds



Incubate @ 37°C for 22±2 hours



Add 150 µL lysis buffer to deepwell block



Transfer 50 µL sample from sample bag to deepwell block



Place block on thermomixer @ 65°C for 15 minutes @ 1400 RPM



Remove deepwell block, adjust thermomixer to 95°C



Shake deepwell block @ 95°C for 10 minutes @ 1400 RPM



Remove deepwell block, cool to room temperature before use



Transfer required volume sample lysate for analysis (ensure no lysis beads transferred)

POLYSKOPE LABS  
755 Research Pkwy.  
Oklahoma City, OK 73104  
1.800.525.4081  
405.633.0540  
[info@polyskopelabs.com](mailto:info@polyskopelabs.com)  
[www.polyskopelabs.com](http://www.polyskopelabs.com)