

## HCS 604.03 “Molecular Methods” Module 2 – Library Screening

### How to identify an address:

The membrane is divided into 6 fields (diagram provided). Each field contains 384 squares. The 384 squares represent the row and column identification of the BAC. Within each square there are 16 positions where 8 clones are spotted in duplicate (diagram). The pattern of the spotted clones will generate the plate address of the BAC. To identify your clone, please follow the directions below.

The most complicated part about identifying a clone address is that consecutive plates are not spotted into each field. The 384 well plates are spotted onto the membrane with plates 1-6 spotted into fields 1-6 respectively (duplication pattern 1, see diagram). Since there is a total of 6 fields on the membrane, the cycle will continue with the next six consecutive plates (plates 7 through 12) again being spotted into fields 1 through 6 respectively, but in a different duplication pattern (duplication pattern 2, see diagram). This gridding cycle will continue until all the plates have been spotted.

1. The library name and filter number is used to orient the membrane. Place the membrane with the label facing up and on the right-hand side as shown in the diagram. (The colonies are on the same side of the filter as the label)
2. Identify the field number of the hybridizing colonies. The spacing of colonies is slightly wider between the fields.
3. Identify the well location (I have included a grid to help locate well positions) and identify the well position (e.g. L18)
4. Identify the plate number. This is accomplished by determining the orientation of the duplicate spots (duplication pattern in the diagram) and referring to the table inside each field in the figure.
5. Libraries which have more than one filter (48 plates) will also need to decode the plate number based on the filter. Plates 1-48 are spotted on filterA, 49-96 on filterB, etc. Identify the plate number and well location as described above and record the filter letter. Go to the conversion table and read down the column corresponding to the filter letter and read across the plate number identified from the filter. The intersection is the actual plate number of the clone.

Example: If you have horizontal spots, they could either be duplication position 4 or 8 (from the duplication pattern). They are distinguished by the closeness of the spots and position in the pattern. Assume it is position 4 in field 3. Read down the table in field 3 of the diagram to pos4 and read the plate number as 21. If you are reading filterB, identify the library plate number from row 21 and the column labeled FilterB of the library filter plate decoder. The library plate number is 69. Once the plate number is determined, identify the well location either by using the supplied grid or counting the rows and columns.

# 4 X 4 Pattern

Six Fields

Serial Number

Filter Label

Field 2

Field 6

Field 3

A1

pos1=plate 2  
pos2=plate 8  
pos3=plate 14  
pos4=plate 20  
pos5=plate 26  
pos6=plate 32  
pos7=plate 38  
pos8=plate 44

P24

A1

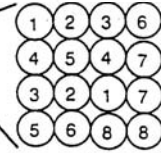
pos1=plate 6  
pos2=plate 12  
pos3=plate 18  
pos4=plate 24  
pos5=plate 30  
pos6=plate 36  
pos7=plate 42  
pos8=plate 48

P24

A1

pos1=plate 3  
pos2=plate 9  
pos3=plate 15  
pos4=plate 21  
pos5=plate 27  
pos6=plate 33  
pos7=plate 39  
pos8=plate 45

P24



Duplication Pattern

Pen spots

A1

pos1=plate 4  
pos2=plate 10  
pos3=plate 16  
pos4=plate 22  
pos5=plate 28  
pos6=plate 34  
pos7=plate 40  
pos8=plate 46

P24

A1

pos1=plate 1  
pos2=plate 7  
pos3=plate 13  
pos4=plate 19  
pos5=plate 25  
pos6=plate 31  
pos7=plate 37  
pos8=plate 43

P24

A1

pos1=plate 5  
pos2=plate 11  
pos3=plate 17  
pos4=plate 23  
pos5=plate 29  
pos6=plate 35  
pos7=plate 41  
pos8=plate 47

P24

Field 4

Field 1

Field 5

Plate # from filter	Library Plate Number							
	Filter A	Filter B	Filter C	Filter D	Filter E	Filter F	Filter G	Filter H
1	1	49	97	145	193	241	289	337
2	2	50	98	146	194	242	290	338
3	3	51	99	147	195	243	291	339
4	4	52	100	148	196	244	292	340
5	5	53	101	149	197	245	293	341
6	6	54	102	150	198	246	294	342
7	7	55	103	151	199	247	295	343
8	8	56	104	152	200	248	296	344
9	9	57	105	153	201	249	297	345
10	10	58	106	154	202	250	298	346
11	11	59	107	155	203	251	299	347
12	12	60	108	156	204	252	300	348
13	13	61	109	157	205	253	301	349
14	14	62	110	158	206	254	302	350
15	15	63	111	159	207	255	303	351
16	16	64	112	160	208	256	304	352
17	17	65	113	161	209	257	305	353
18	18	66	114	162	210	258	306	354
19	19	67	115	163	211	259	307	355
20	20	68	116	164	212	260	308	356
21	21	69	117	165	213	261	309	357
22	22	70	118	166	214	262	310	358
23	23	71	119	167	215	263	311	359
24	24	72	120	168	216	264	312	360
25	25	73	121	169	217	265	313	361
26	26	74	122	170	218	266	314	362
27	27	75	123	171	219	267	315	363
28	28	76	124	172	220	268	316	364
29	29	77	125	173	221	269	317	365
30	30	78	126	174	222	270	318	366
31	31	79	127	175	223	271	319	367
32	32	80	128	176	224	272	320	368
33	33	81	129	177	225	273	321	369
34	34	82	130	178	226	274	322	370
35	35	83	131	179	227	275	323	371
36	36	84	132	180	228	276	324	372
37	37	85	133	181	229	277	325	373
38	38	86	134	182	230	278	326	374
39	39	87	135	183	231	279	327	375
40	40	88	136	184	232	280	328	376
41	41	89	137	185	233	281	329	377
42	42	90	138	186	234	282	330	378
43	43	91	139	187	235	283	331	379
44	44	92	140	188	236	284	332	380
45	45	93	141	189	237	285	333	381
46	46	94	142	190	238	286	334	382
47	47	95	143	191	239	287	335	383
48	48	96	144	192	240	288	336	384