Kerstin Spirohn Boutros Lab
April 2009

2ND PCR WITH TAG PRIMER

Protocol for the re-amplification of PCR templates to be used in *in vitro* transcription reactions for RNAi libraries. The protocol is optimized and tag primers are arranged for re-amplifications of the library **Heidelberg2**.

PCR reactions are performed in PCR plates from Biozym (cat# 710875) using Biorad Tetrad PCR machines.

Pre-load PCR plates with 3ul of 1st PCR (1:3 diluted) and 10ul T7 tag primer (2uM) TS1-TS4.

Add 87ul PCR pre-mix containing per well:

	Per 100µl reaction
H_2O	72µl
Primer TU (10µM)	2µl
10xBuffer (Qiagen, cat# 201205)	10µl
dNTP 10mM (Fermentas, cat# R0182)	2µl
Taq (Qiagen, cat# 201205)	0.3µl

PCR Cycle Program:

Step 1 94°C 2:00min
Step 2 94°C 0:30min
Step 3 57°C 1:00 min
Step 4 72°C 1:00 min
Step 5 72°C 2:00min
Step 6 4°C hold

35 cycles (steps 2-4)

Tag primer layout (96-well plate format):

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	2	3	4	1	2	3	4	1	2	3	4
B	3	4	1	2	3	4	1	2	3	4	1	2
C	1	2	3	4	1	2	3	4	1	2	3	4
D	3	4	1	2	3	4	1	2	3	4	1	2
E	1	2	3	4	1	2	3	4	1	2	3	4
F	3	4	1	2	3	4	1	2	3	4	1	2
G	1	2	3	4	1	2	3	4	1	2	3	4
H	3	4	1	2	3	4	1	2	3	4	1	2

Tag primer sequence (T7 underlined):

TU TAATACGACTCACTATAGGGtggcgcccctagatg
TS1 TAATACGACTCACTATAGGGcgacgcccgctgata
TS2 TAATACGACTCACTATAGGGtaggtctagccccgc
TS3 TAATACGACTCACTATAGGGcgcatgtagcctgcc
TS4 TAATACGACTCACTATAGGGtagcctccctagcgc

Notes: