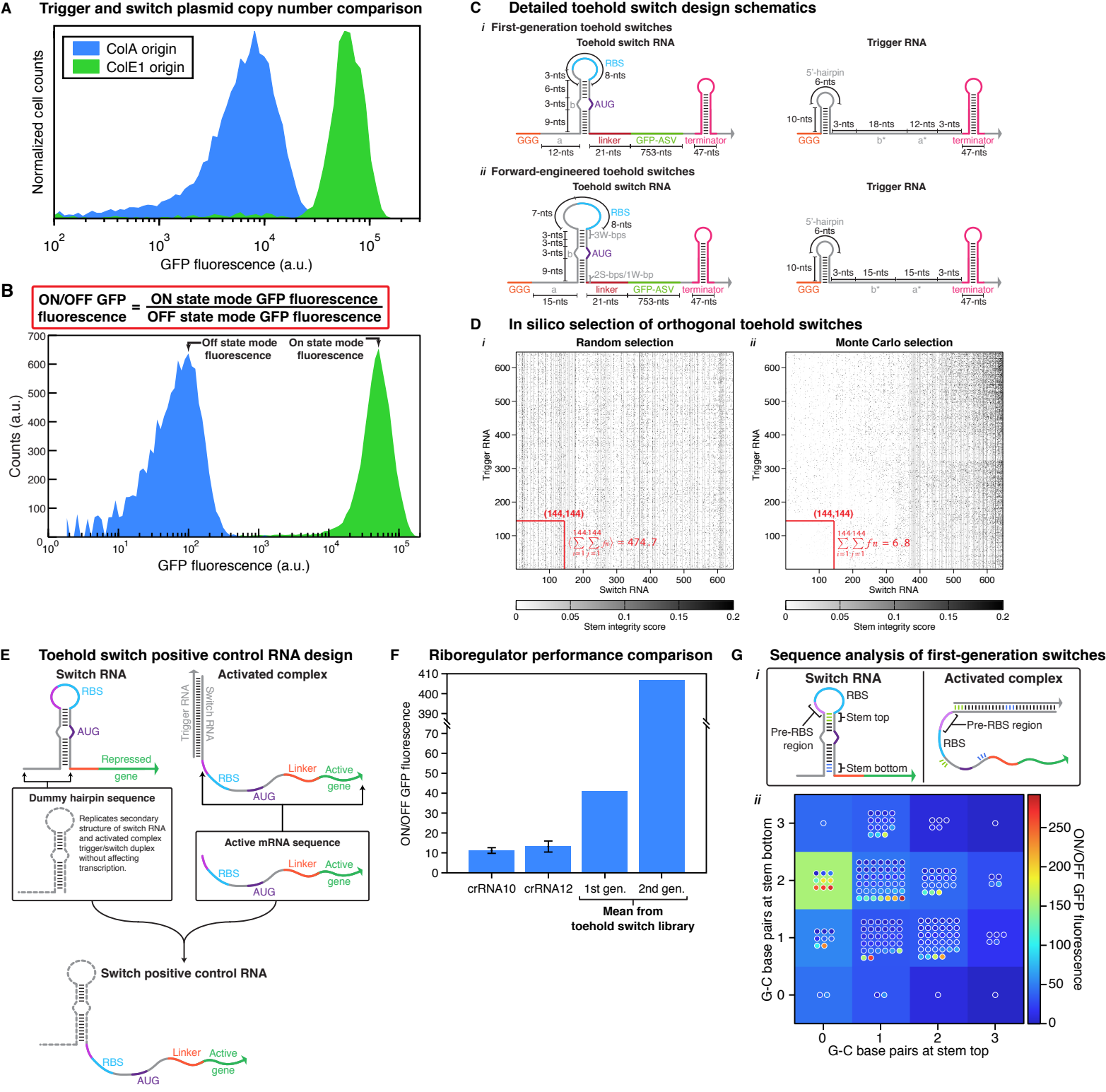
A
Trigger and switch plasmid copy number comparison
ColA origin
ColE1 origin
Normalized cell counts
GFP fluorescence (a.u.)
B
ON/OFF GFP fluorescence = ON state mode GFP fluorescence / OFF state mode GFP fluorescence
Off state mode fluorescence
On state mode fluorescence
Counts (a.u.)
GFP fluorescence (a.u.)
C
Detailed toehold switch design schematics
i First-generation toehold switches
Toehold switch RNA
Trigger RNA
RBS
AUG
GGG
linker
GFP-ASV
terminator
5'-hairpin
ii Forward-engineered toehold switches
Toehold switch RNA
Trigger RNA
D
In silico selection of orthogonal toehold switches
i Random selection
ii Monte Carlo selection
Trigger RNA
Switch RNA
(144,144)
Stem integrity score
E
Toehold switch positive control RNA design
Switch RNA
Activated complex
Trigger RNA
Switch RNA
RBS
AUG
Repressed gene
Linker
Active gene
Dummy hairpin sequence
Replicates secondary structure of switch RNA and activated complex trigger/switch duplex without affecting transcription.
Active mRNA sequence
Switch positive control RNA
F
Riboregulator performance comparison
ON/OFF GFP fluorescence
crRNA10
crRNA12
1st gen.
2nd gen.
Mean from toehold switch library
G
Sequence analysis of first-generation switches
i
Switch RNA
Activated complex
RBS
Stem top
Pre-RBS region
Stem bottom
Pre-RBS region
RBS
ii
G-C base pairs at stem bottom
G-C base pairs at stem top
ON/OFF GFP fluorescence
H
Toehold switch performance with different output proteins
i
ON/OFF cerulean fluorescence
ii
ON/OFF sfGFP fluorescence
iii
ON/OFF venus fluorescence
iv
ON/OFF mCherry fluorescence
1
3
80L27
Toehold switch number